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Van Der Werf et al.

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(54) **STRAIN OF SARS-ASSOCIATED CORONAVIRUS AND APPLICATIONS THEREOF**

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(21) Appl. No.: **12/754,908**

(22) Filed: **Apr. 6, 2010**

(65) **Prior Publication Data**

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Related U.S. Application Data

(60) Division of application No. 10/581,356, filed on Feb. 8, 2007, now Pat. No. 7,736,850, which is a continuation of application No. PCT/FR2004/003106, filed on Dec. 2, 2004.

(30) **Foreign Application Priority Data**

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Dec. 2, 2003 (FR) 03 14152

(51) **Int. Cl.**
C12Q 1/70 (2006.01)
G01N 33/53 (2006.01)
G01N 33/542 (2006.01)
G01N 33/00 (2006.01)

(52) **U.S. Cl.** **435/5**; 435/7.1; 435/7.9; 435/7.92; 435/7.94; 435/7.95

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

The invention relates to a novel strain of severe acute respiratory syndrome (SARS)-associated coronavirus, resulting from a sample collected in Hanoi (Vietnam), reference number 031589, nucleic acid molecules originating from the genome of same, proteins and peptides coded by said nucleic acid molecules and, more specifically, protein N and the applications thereof, for example, as diagnostic reagents and/or as a vaccine.

8 Claims, 116 Drawing Sheets

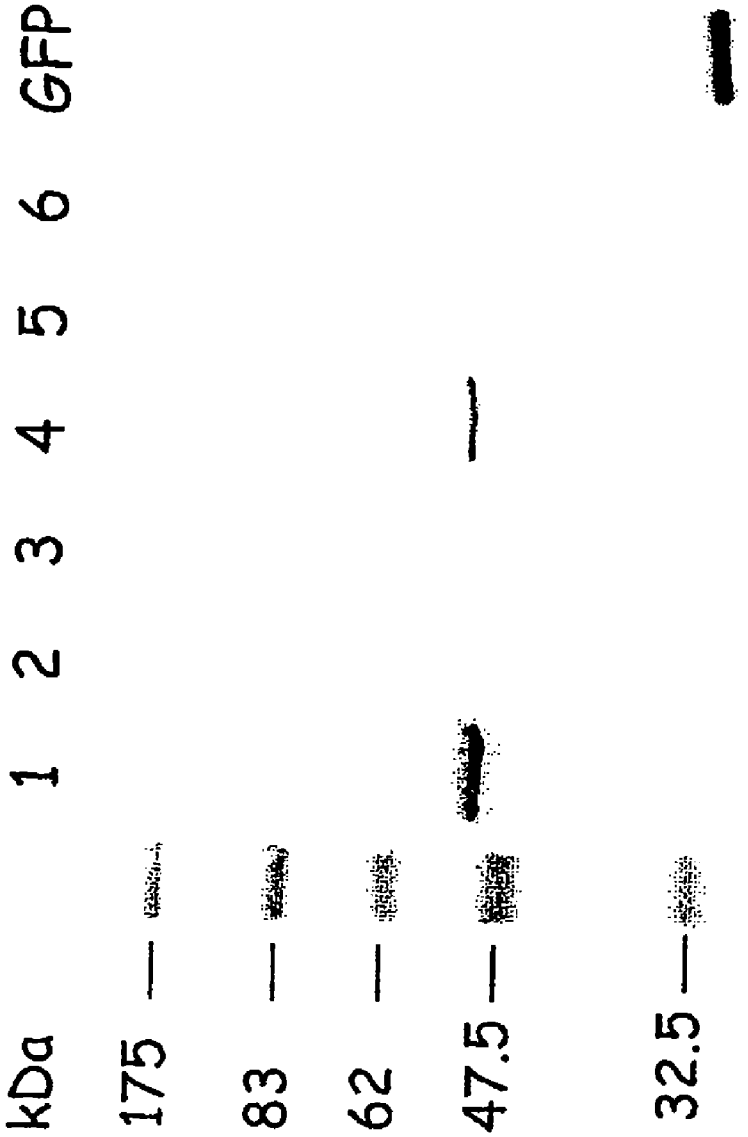


FIGURE 1

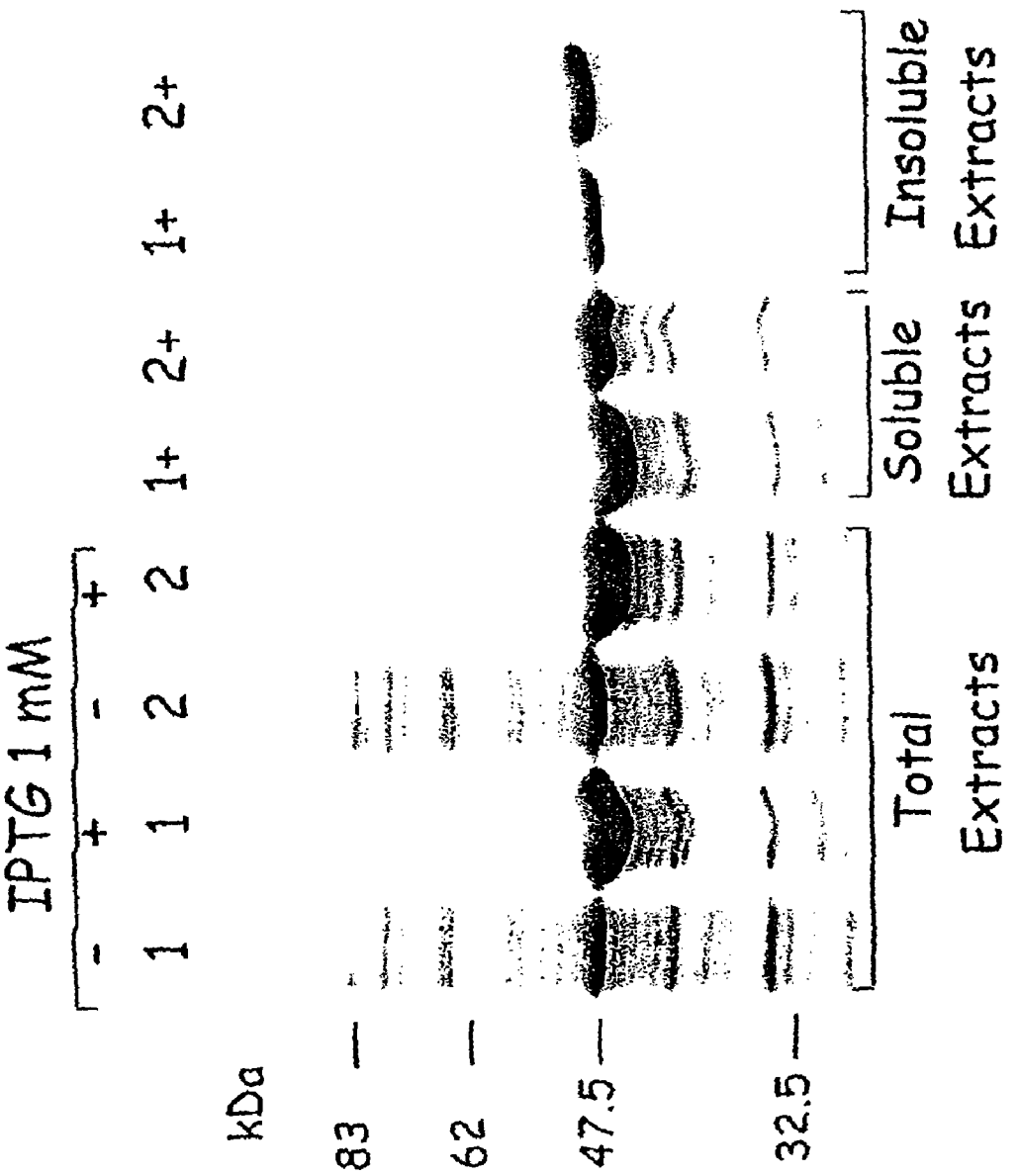

IPTG 1 mM
- + - +
1 1 2 2 1+ 2+ 1+ 2+
kDa
83
62
47.5
32.5
Total Extracts
Soluble Extracts
Insoluble Extracts

FIGURE 2

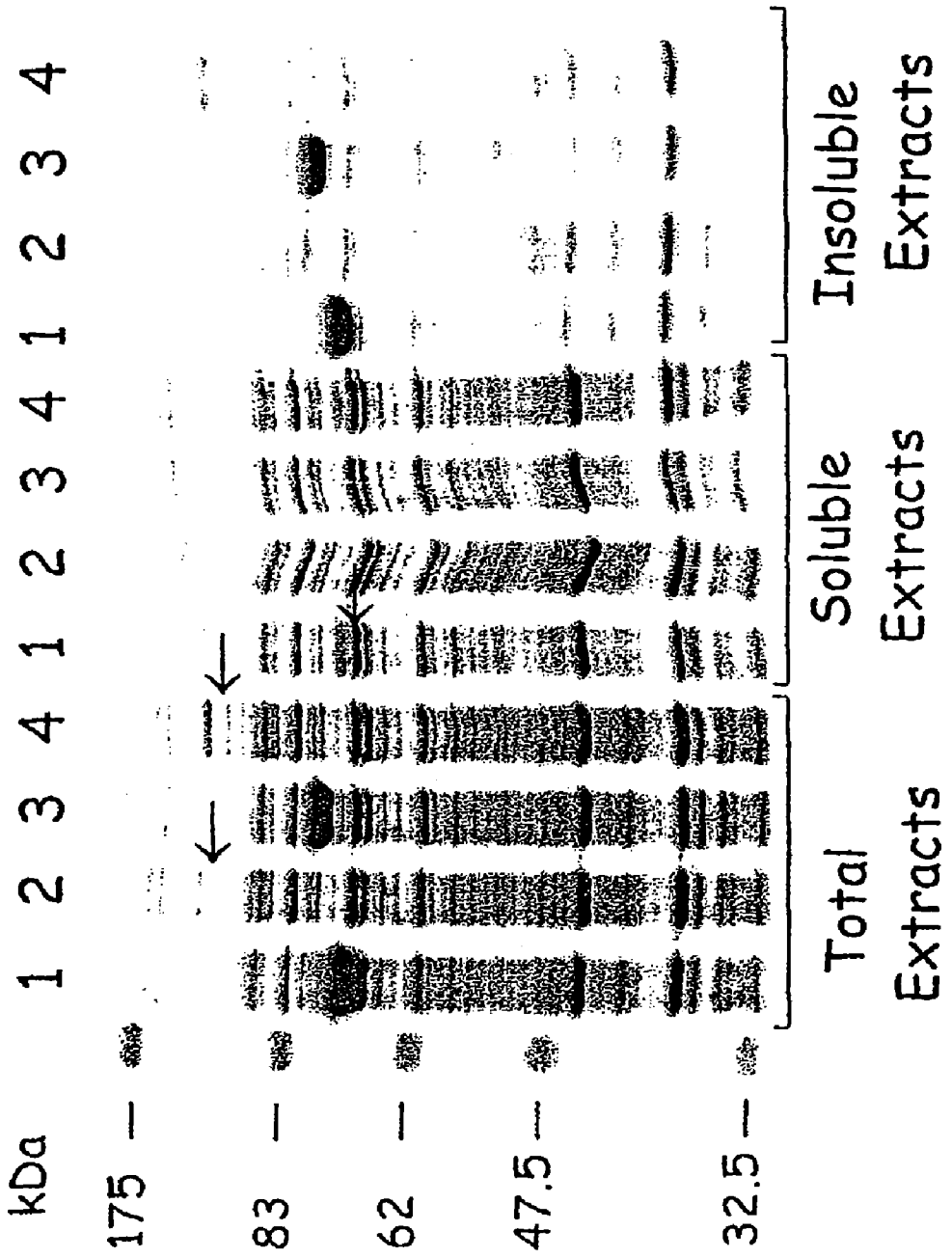

kDa
175
83
62
47.5
32.5
1 2 3 4 1 2 3 4 1 2 3 4
Total Extracts
Soluble Extracts
Insoluble Extracts

FIGURE 3

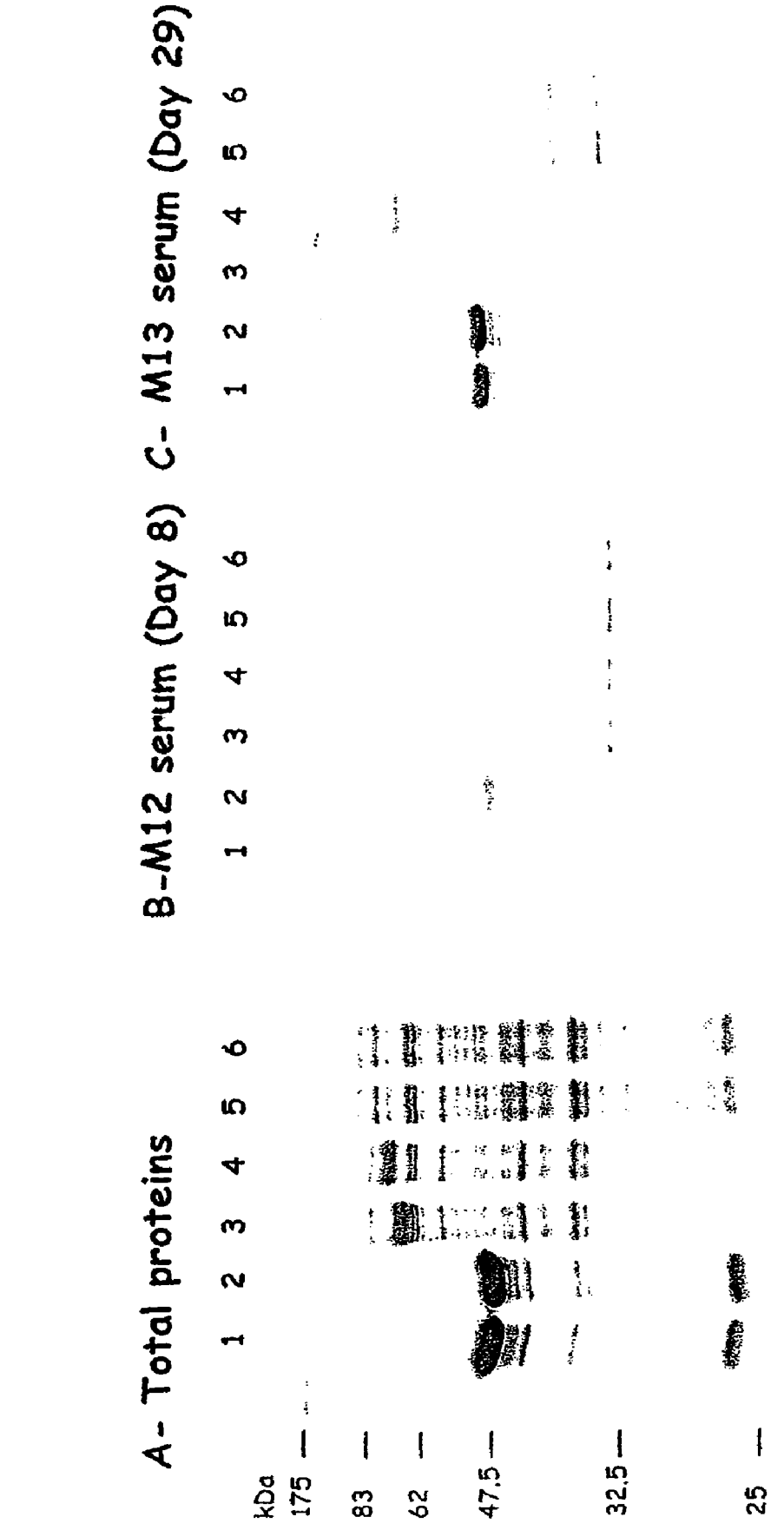

A- Total proteins
1 2 3 4 5 6
kDa
175
83
62
47.5
32.5
25
10 µl extract deposit
B-M12 serum (Day 8)
1 2 3 4 5 6
2 µl extract deposit
C- M13 serum (Day 29)
1 2 3 4 5 6
2 µl extract deposit

FIGURE 4

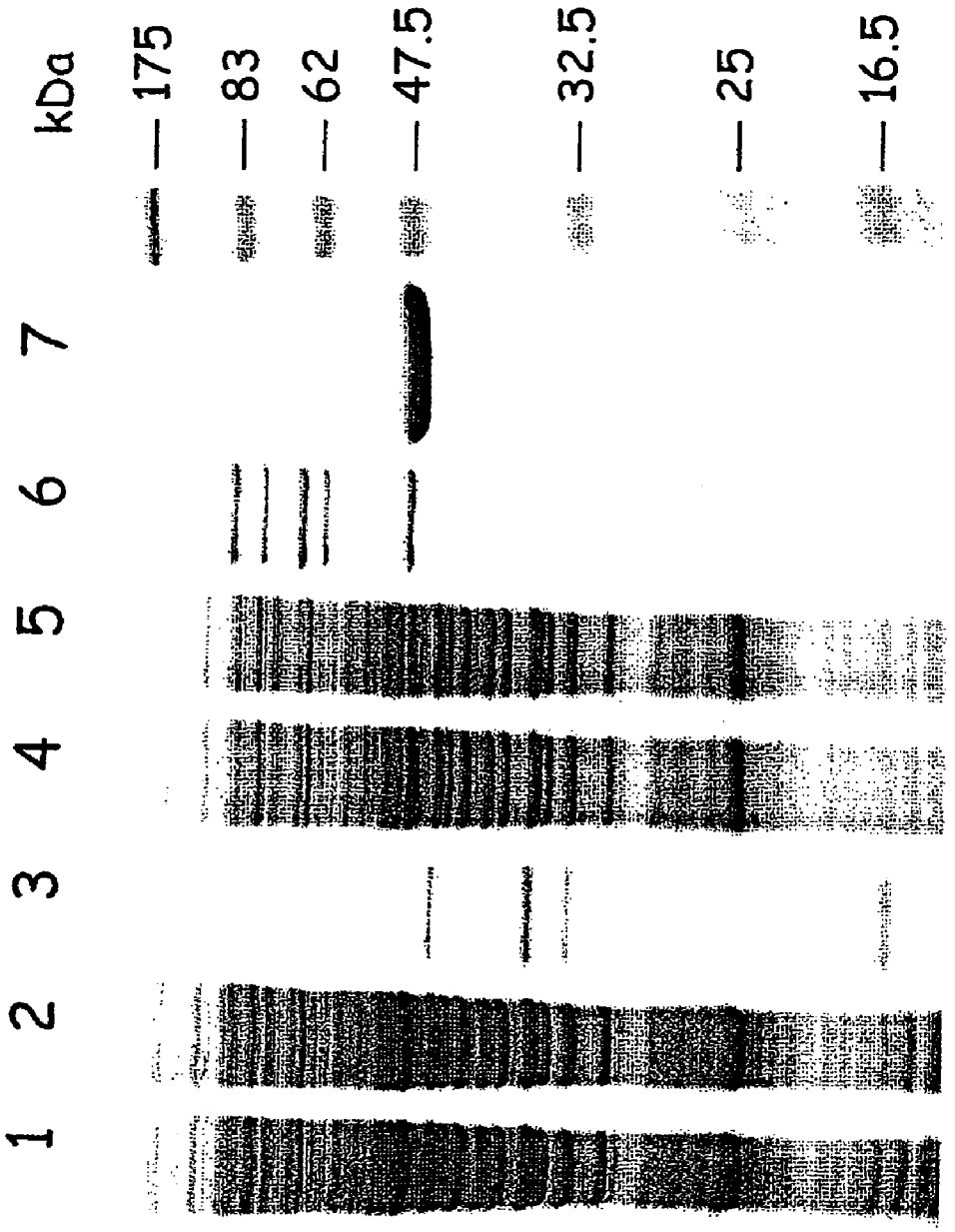


FIGURE 5

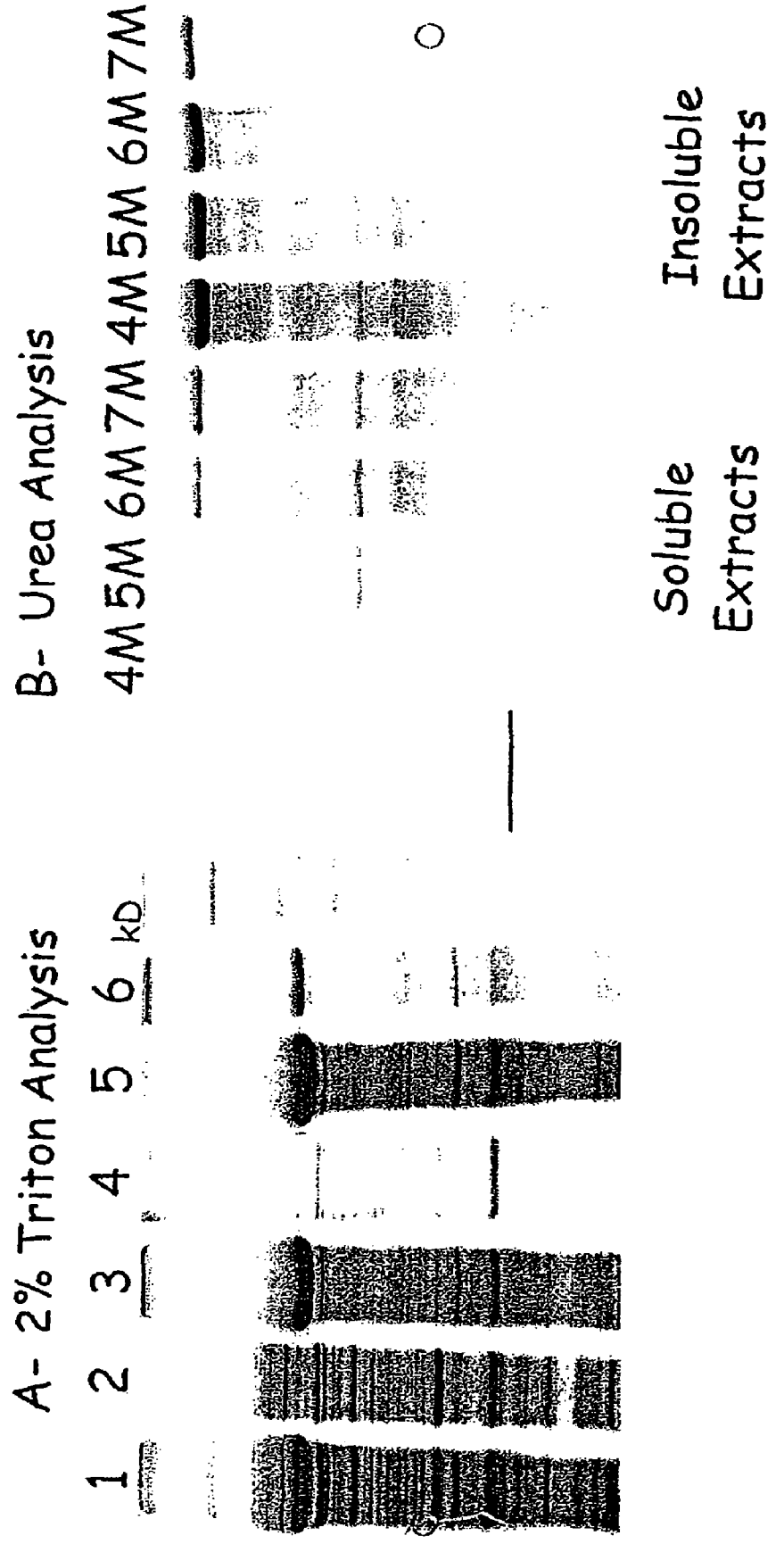

A- 2% Triton Analysis
1 2 3 4 5 6 kD
B- Urea Analysis
4M 5M 6M 7M 4M 5M 6M 7M
Soluble Extracts
Insoluble Extracts

FIGURE 6

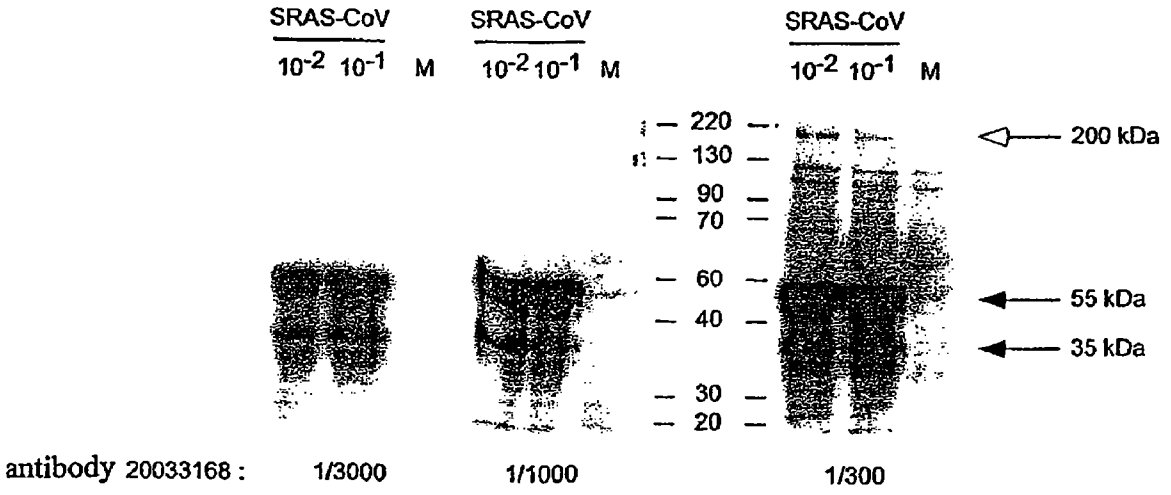

SRAS-CoV
10-2 10-1 M
SRAS-CoV
10-2 10-1 M
SRAS-CoV
10-2 10-1 M
220
130
90
70
60
40
30
20
200 kDa
55 kDa
35 kDa
antibody 20033168 :
1/3000
1/1000
1/300

FIGURE 7

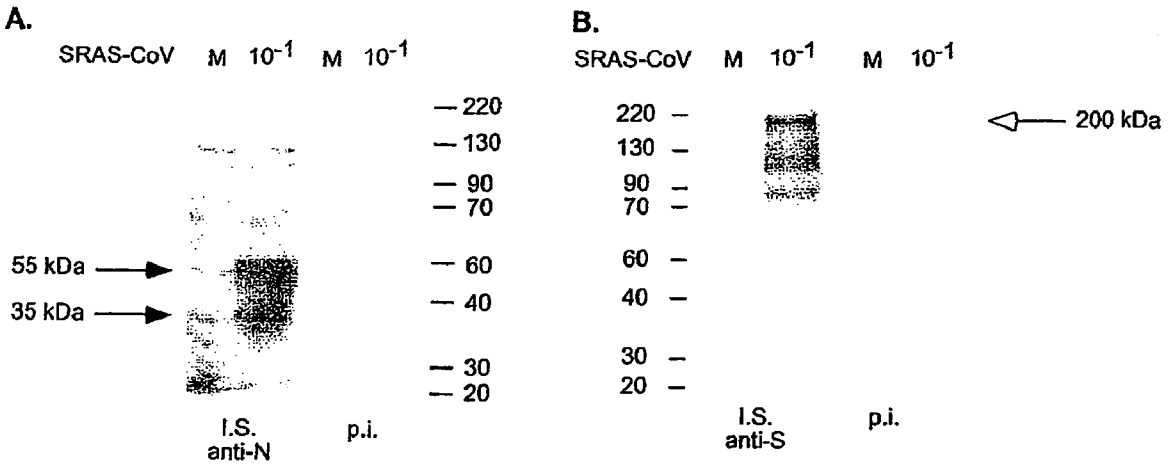
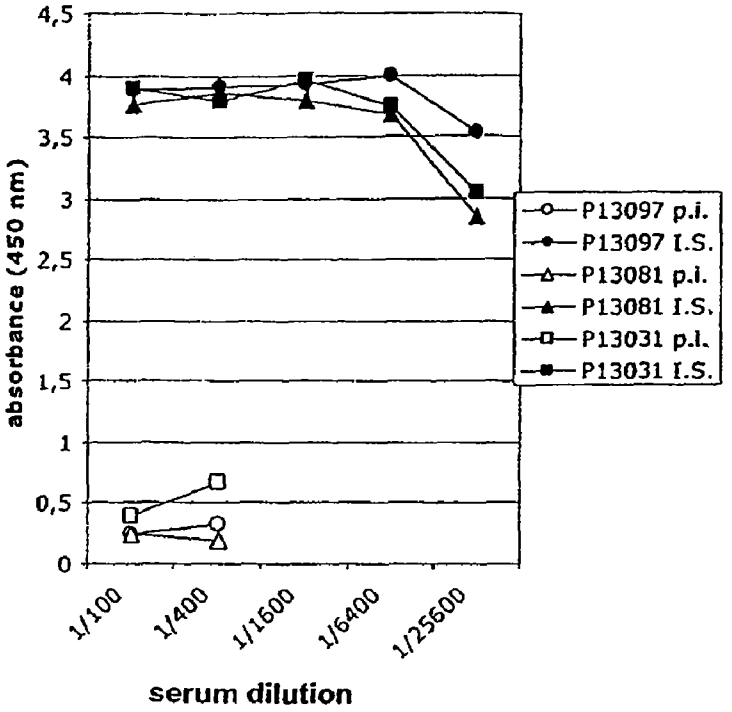

A.
SRAS-CoV
M
10-1
M
10-1
220
130
90
70
60
40
30
20
55 kDa
35 kDa
I.S. anti-N
p.i.
B.
SRAS-CoV
M
10-1
M
10-1
220
130
90
70
60
40
30
20
200 kDa
I.S. anti-S
p.i.

FIGURE 8

A


4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
absorbance (450 nm)
1/100
1/400
1/1600
1/6400
1/25600
serum dilution
P13097 p.i.
P13097 I.S.
P13081 p.i.
P13081 I.S.
P13031 p.i.
P13031 I.S.

B

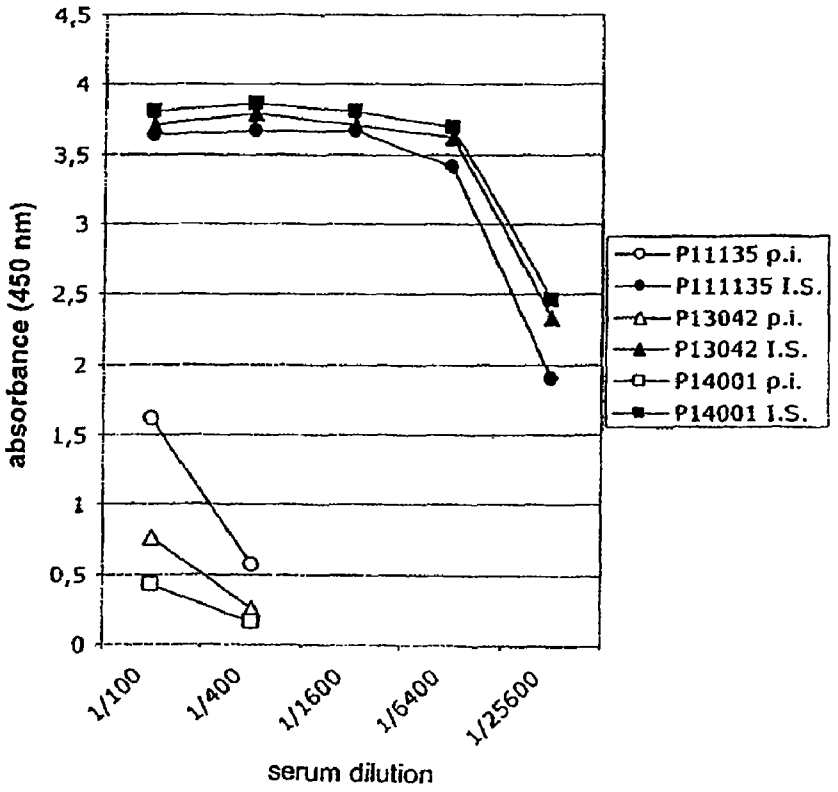
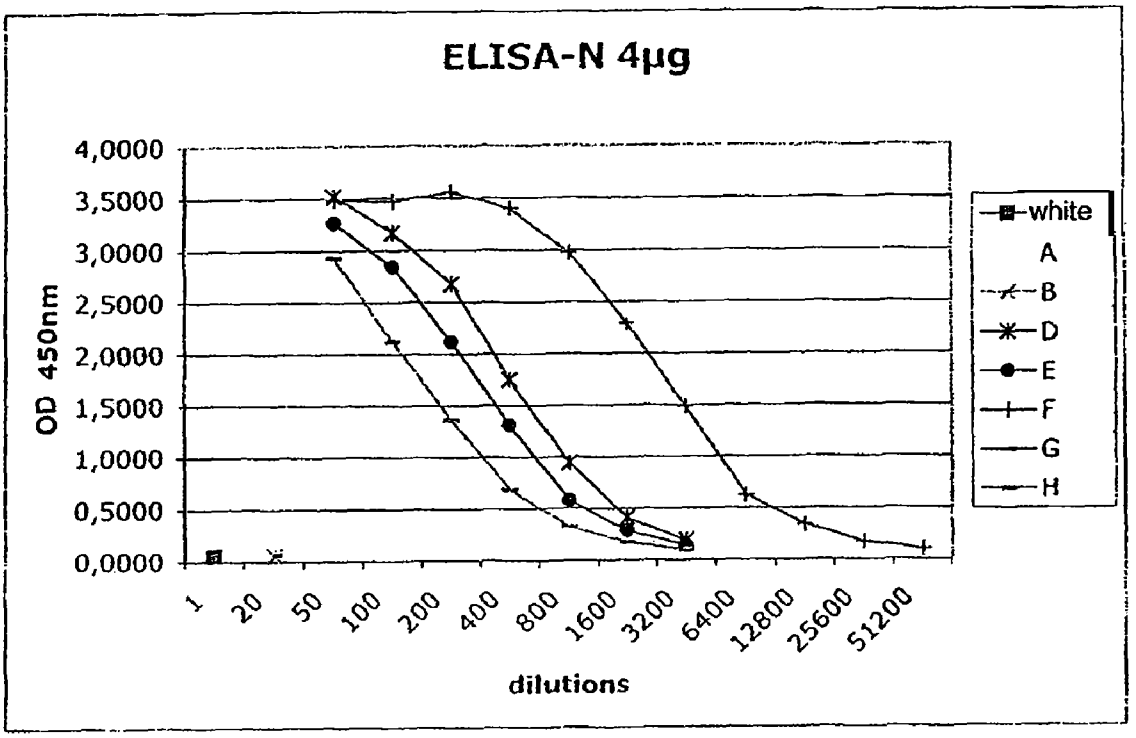

4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
absorbance (450 nm)
1/100
1/400
1/1600
1/6400
1/25600
serum dilution
P11135 p.i.
P111135 I.S.
P13042 p.i.
P13042 I.S.
P14001 p.i.
P14001 I.S.

FIGURE 9


ELISA-N 4µg
OD 450nm
4,0000
3,5000
3,0000
2,5000
2,0000
1,5000
1,0000
0,5000
0,0000
1
20
50
100
200
400
800
1600
3200
6400
12800
25600
51200
dilutions
white
A
B
D
E
F
G
H

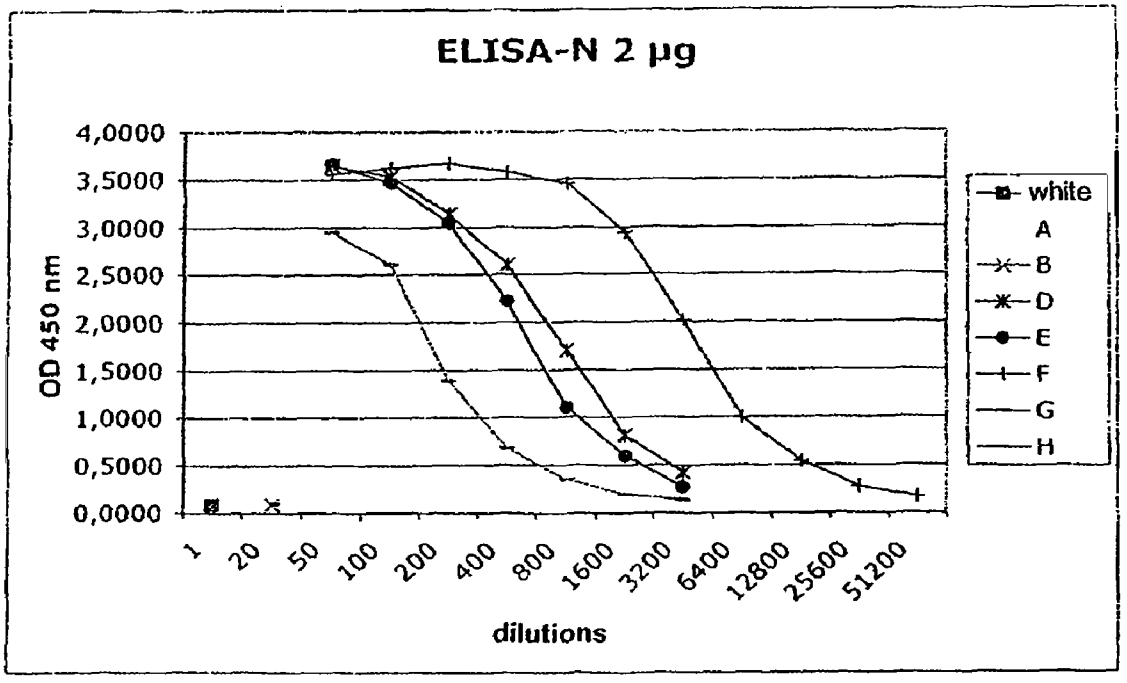

ELISA-N 2 µg
OD 450 nm
4,0000
3,5000
3,0000
2,5000
2,0000
1,5000
1,0000
0,5000
0,0000
1
20
50
100
200
400
800
1600
3200
6400
12800
25600
51200
dilutions
white
A
B
D
E
F
G
H

FIGURE 10a

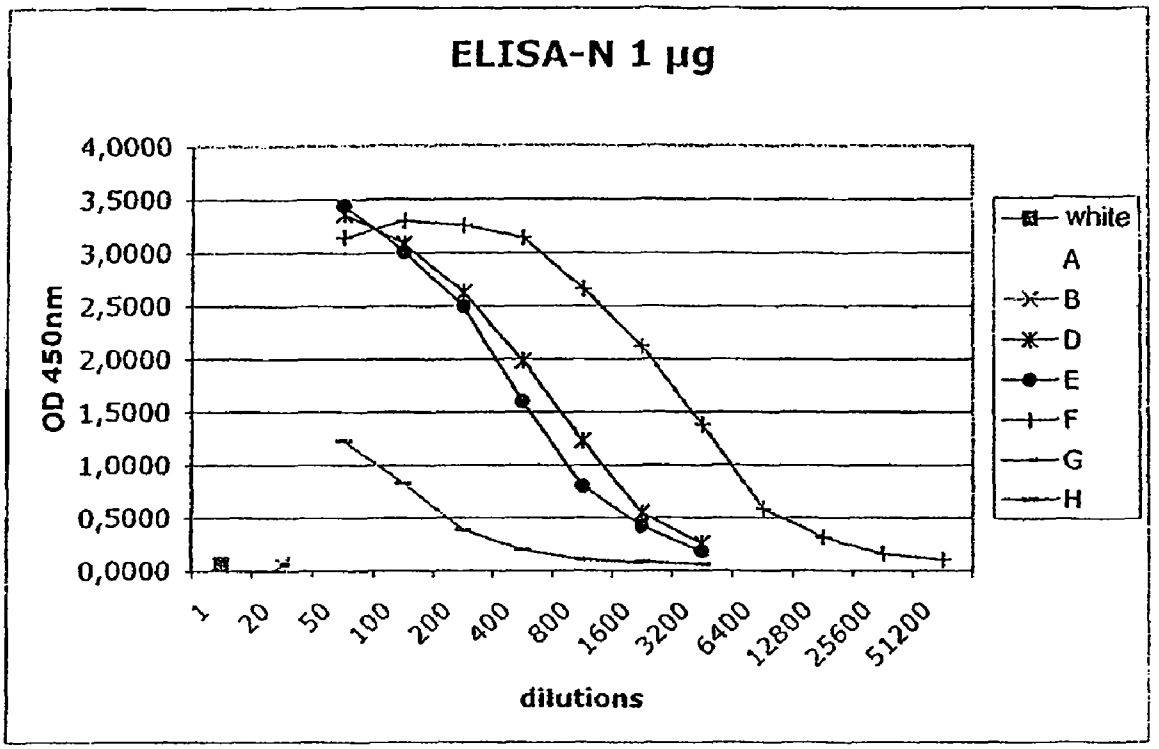

ELISA-N 1 µg
OD 450nm
4,0000
3,5000
3,0000
2,5000
2,0000
1,5000
1,0000
0,5000
0,0000
1
20
50
100
200
400
800
1600
3200
6400
12800
25600
51200
dilutions
white
A
B
D
E
F
G
H

FIGURE 10b

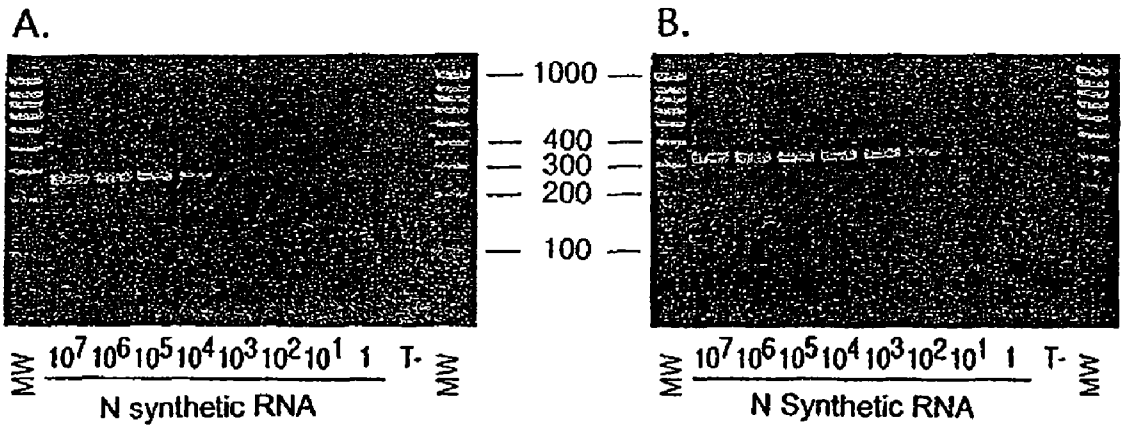

A.
B.
1000
400
300
200
100
MW
10^7 10^6 10^5 10^4 10^3 10^2 10^1 1 T-
MW
N synthetic RNA
MW
10^7 10^6 10^5 10^4 10^3 10^2 10^1 1 T-
MW
N Synthetic RNA

FIGURE 11

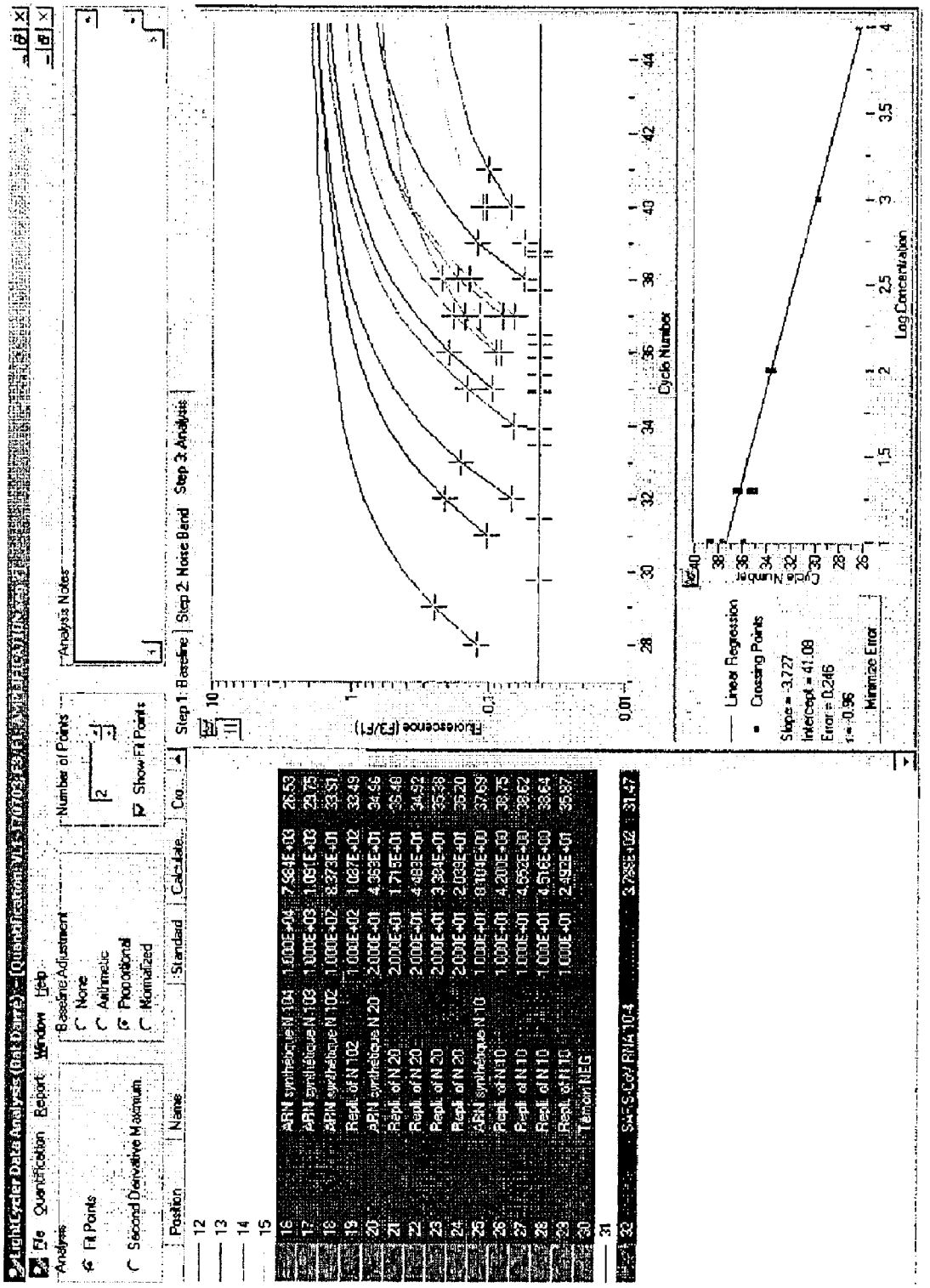

LightCycler Data Analysis (Bat Diane) - [Quantification Results]
File Quantification Report Window Help
Analysis
Fit Points
Second Derivative Maximum
Baseline Adjustment
None
Arithmetic
Proportional
Normalized
Number of Points
Show Fit Points
Analysis Notes
Step 1: Baseline
Step 2: Noise Band
Step 3: Analysis
Fluorescence (F2/F1)
Cycle Number
Linear Regression
Crossing Points
Slope = -3.727
Intercept = 41.08
Error = 0.246
r = -0.96
Minimize Error
Log Concentration
Position
Name
Standard
Calculate
Cq
ARN synthétique N 104
ARN synthétique N 103
ARN synthétique N 102
Repl. of N 102
ARN synthétique N 20
Repl. of N 20
Repl. of N 20
Repl. of N 20
Repl. of N 20
ARN synthétique N 10
Repl. of N 10
Repl. of N 10
Repl. of N 10
Repl. of N 10
Témoin NEG
SWS-Cov-RNA 10-4

FIGURE 12

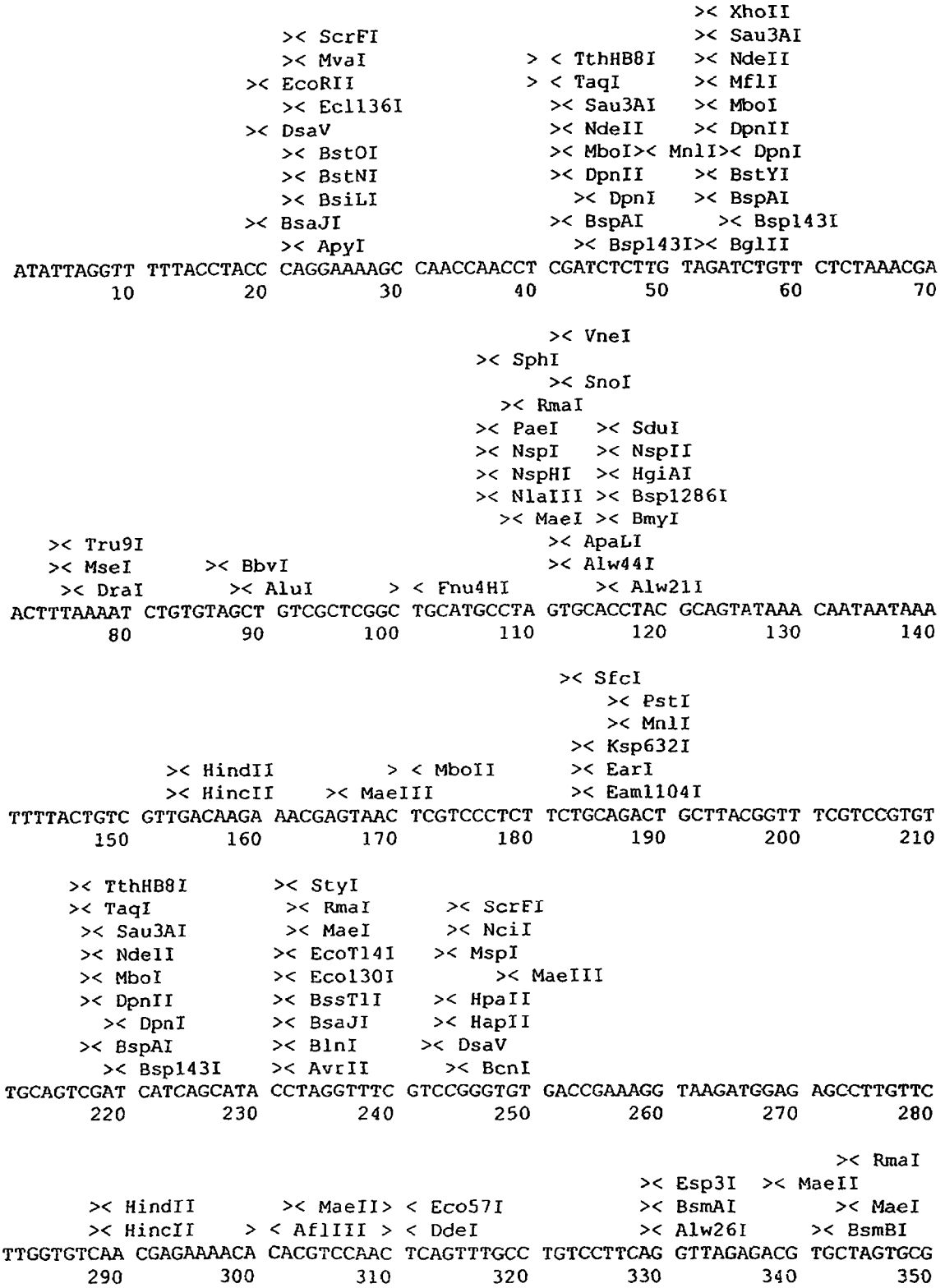

>< XhoII
>< ScrFI >< Sau3AI
>< MvaI > < TthHB8I >< NdeII
>< EcoRII > < TaqI >< MflI
>< Ecl136I >< Sau3AI >< MboI
>< DsaV >< NdeII >< DpnII
>< BstOI >< MboI>< MnlI>< DpnI
>< BstNI >< DpnII >< BstYI
>< BsiLI >< DpnI >< BspAI
>< BsaJI >< BspAI >< Bsp143I
>< ApyI >< Bsp143I>< BglII
ATATTAGGTT TTTACCTACC CAGGAAAAGC CAACCAACCT CGATCTCTTG TAGATCTGTT CTCTAAACGA
10 20 30 40 50 60 70
>< VneI
>< SphI
>< SnoI
>< RmaI
>< PaeI >< SduI
>< NspI >< NspII
>< NspHI >< HgiAI
>< NlaIII >< Bsp1286I
>< MaeI >< BmyI
>< Tru9I >< ApaLI
>< MseI >< BbvI >< Alw44I
>< DraI >< AluI > < Fnu4HI >< Alw21I
ACTTTAAAAT CTGTGTAGCT GTCGCTCGGC TGCATGCCTA GTGCACCTAC GCAGTATAAA CAATAATAAA
80 90 100 110 120 130 140
>< SfcI
>< PstI
>< MnlI
>< Ksp632I
>< HindII > < MboII >< EarI
>< HincII >< MaeIII >< Eam1104I
TTTTACTGTC GTTGACAAGA AACGAGTAAC TCGTCCCTCT TCTGCAGACT GCTTACGGTT TCGTCCGTGT
150 160 170 180 190 200 210
>< TthHB8I >< StyI
>< TaqI >< RmaI >< ScrFI
>< Sau3AI >< MaeI >< NciI
>< NdeII >< EcoT14I >< MspI
>< MboI >< Eco130I >< MaeIII
>< DpnII >< BssT1I >< HpaII
>< DpnI >< BsaJI >< HapII
>< BspAI >< BlnI >< DsaV
>< Bsp143I >< AvrII >< BcnI
TGCAGTCGAT CATCAGCATA CCTAGGTTTC GTCCGGGTGT GACCGAAAGG TAAGATGGAG AGCCTTGTTC
220 230 240 250 260 270 280
>< RmaI
>< Esp3I >< MaeII
>< HindII >< MaeII> < Eco57I >< BsmAI >< MaeI
>< HincII > < AflIII > < DdeI >< Alw26I >< BsmBI
TTGGTGTCAA CGAGAAAACA CACGTCCAAC TCAGTTTGCC TGTCCTTCAG GTTAGAGACG TGCTAGTGCG
290 300 310 320 330 340 350

FIGURE 13.1

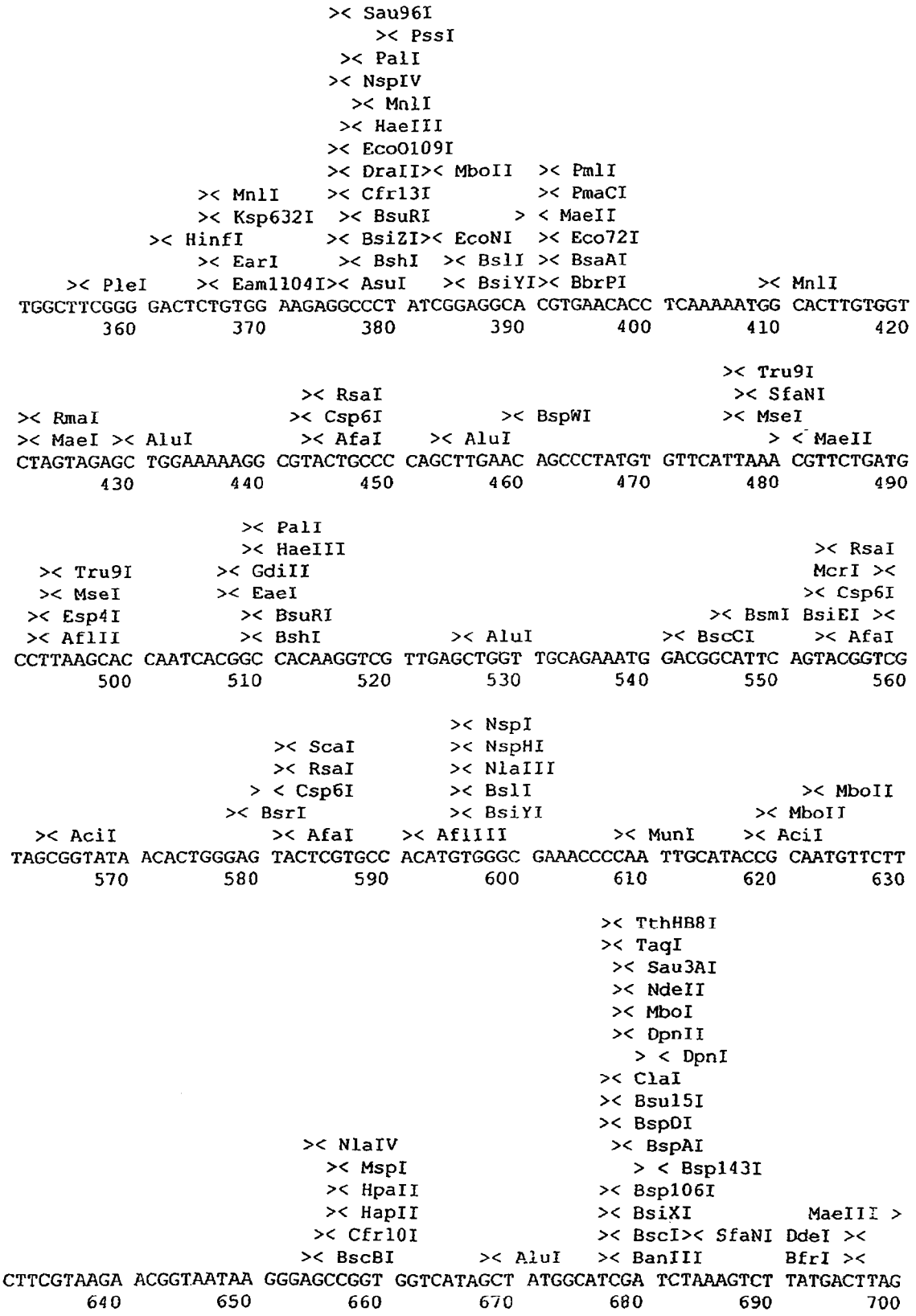

>< Sau96I
>< PssI
>< PalI
>< NspIV
>< MnlI
>< HaeIII
>< Eco0109I
>< DraII>< MboII >< PmlI
>< MnlI >< Cfr13I >< PmaCI
>< Ksp632I >< BsuRI > < MaeII
>< HinfI >< BsiZI>< EcoNI >< Eco72I
>< EarI >< BshI >< BslI >< BsaAI
>< PleI >< Eam1104I>< AsuI >< BsiYI>< BbrPI >< MnlI
TGGCTTCGGG GACTCTGTGG AAGAGGCCCT ATCGGAGGCA CGTGAACACC TCAAAAATGG CACTTGTGGT
360 370 380 390 400 410 420
>< Tru9I
>< SfaNI
>< RsaI
>< RmaI >< Csp6I >< BspWI >< MseI
>< MaeI >< AluI >< AfaI >< AluI > < MaeII
CTAGTAGAGC TGGAAAAAGG CGTACTGCCC CAGCTTGAAC AGCCCTATGT GTTCATTAAA CGTTCTGATG
430 440 450 460 470 480 490
>< PalI
>< HaeIII
>< RsaI
>< Tru9I >< GdiII McrI ><
>< MseI >< EaeI >< Csp6I
>< Esp4I >< BsuRI >< BsmI BsiEI ><
>< AflII >< BshI >< AluI >< BscCI >< AfaI
CCTTAAGCAC CAATCACGGC CACAAGGTCG TTGAGCTGGT TGCAGAAATG GACGGCATTC AGTACGGTCG
500 510 520 530 540 550 560
>< NspI
>< ScaI >< NspHI
>< RsaI >< NlaIII
> < Csp6I >< BslI >< MboII
>< BsrI >< BsiYI >< MboII
>< AciI >< AfaI >< AflIII >< MunI >< AciI
TAGCGGTATA ACACTGGGAG TACTCGTGCC ACATGTGGGC GAAACCCCAA TTGCATACCG CAATGTTCTT
570 580 590 600 610 620 630
>< TthHB8I
>< TaqI
>< Sau3AI
>< NdeII
>< MboI
>< DpnII
> < DpnI
>< ClaI
>< Bsu15I
>< BspDI
>< NlaIV >< BspAI
>< MspI > < Bsp143I
>< HpaII >< Bsp106I
>< HapII >< BsiXI MaeIII >
>< Cfr10I >< BscI>< SfaNI DdeI ><
>< BscBI >< AluI >< BanIII BfrI ><
CTTCGTAAGA ACGGTAATAA GGGAGCCGGT GGTCATAGCT ATGGCATCGA TCTAAAGTCT TATGACTTAG
640 650 660 670 680 690 700

FIGURE 13.2

```
                    >< Sau3AI
                    >< NdeII
                    >< MboI
                 >< HphI                                                VneI ><
                    >< DpnII                                            SnoI ><
                    >< BspAI                                         > < NlaIII
                 >< AlwI>< DpnI                                  >< DdeI   ApaLI ><
          >< AluI     >< Bsp143I            >< MboII  >< BsrI       Alw44I ><
GTGACGAGCT TGGCACTGAT CCCATTGAAG ATTATGAACA AAACTGGAAC ACTAAGCATG GCAGTGGTGC
       710        720        730        740        750        760        770

                          >< SstI
                          >< SduI
                          >< SacI
                          >< NspII
                        >< MnlI                                        Sau96I ><
                          >< HgiAI                           >< TthHB8I    PalI ><
><  SduI                  >< Eco24I                          >< TaqI     NspIV ><
><  NspII                >< Ecl136II                       > < SalI      HaeIII ><
><  HgiAI                  >< Bsp1286I                     > < RtrI      Cfr13I ><
    >< DraIII              >< BmyI                            >< HindII   BsuRI ><
><  Bsp1286I               >< BanII                           >< HincII  BsiZI ><
><  BmyI                   >< Alw21I                            >< BsgI    BshI ><
><  Alw21I               >< AluI         >< MaeIII         >< AccI      AsuI ><
ACTCCGTGAA CTCACTCGTG AGCTCAATGG AGGTGCAGTC ACTCGCTATG TCGACAACAA TTTCTGTGGC
       780        790        800        810        820        830        840

                                                    >< ThaI
                                                 >< ThaI
                                                    >< MvnI
                                                 >< MvnI
          > < RsaI                               >< HinP1I
          > < NlaIV                              >< Hin6I          > < VneI
              >< KpnI                              >< HhaI         > < SnoI
        >< Eco64I                                  >< CfoI               >< SduI
         >< Csp6I                                  >< BstUI       NspII ><
          > < BscBI                             >< BstUI         HgiAI ><
        >< BanI                                    >< Bsp50I   Bsp1286I ><
        >< Asp718                               >< Bsp50I              >< BmyI
          > < AfaI                                 >< AciI         > < ApaLI
        >< AccB1I                                  >< AccII        > < Alw44I
        >< Acc65I        >< MnlI   >< SfaNI     >< AccII        Alw21I ><
CCAGATGGGT ACCCTCTTGA TTGCATCAAA GATTTTCTCG CACGCGCGGG CAAGTCAATG TGCACTCTTT
       850        860        870        880        890        900        910

                          >< TthHB8I
                       >< TthHB8I
                          >< TaqI
                       >< TaqI
                          >< MnlI
                       >< Ksp632I                                NlaIII ><
                       >< HinfI>< PleI                           >< NlaIII
                       >< Eam1104I        >< MboII   >< MaeIII        EcoRII ><
                       >< EarI  > < BbvI>< AccI >< Fnu4HI             DsaV ><
CCGAACAACT TGATTACATC GAGTCGAAGA GAGGTGTCTA CTGCTGCCGT GACCATGAGC ATGAAATTGC
       920        930        940        950        960        970        980

                                                    >< TthHB8I
                                                    >< TaqI
                                                    >< SfuI
                                                    >< NspV>< Tru9I
><  ScrFI     >< HinP1I                             >< LspI>< MseI
```

FIGURE 13.3

```
>< MvaI      >< Hin6I                >< SduI      >< Csp45I
>< Ecl136I     >< HhaI               >< NspII     >< BstBI
>< BstOI        >< HaeII             >< HgiAI     >< Bsp119I
>< BstNI      >< Eco47III            >< Bsp1286I  >< BsiCI
>< BsiLI       >< CfoI               >< BmyI      >< Bpu14I
>< ApyI >< DdeI >< Bsp143II >< AluI   >< Alw21I    >< AsuII
CTGGTTCACT GAGCGCTCTG ATAAGAGCTA CGAGCACCAG ACACCCTTCG AAATTAAGAG TGCCAAGAAA
       990       1000       1010       1020       1030       1040       1050

                                                  >< Tru9I
                          >< BsmI                 >< MseI
                       >< BscCI                        > < MnlI
TTTGACACTT TCAAAGGGGA ATGCCCAAAG TTTGTGTTTC CTCTTAACTC AAAAGTCAAA GTCATTCAAC
      1060       1070       1080       1090       1100       1110       1120

  >< PmlI
  >< PmaCI
 >< MaeII
  >< Eco72I
  >< BsaAI                              >< NlaIII            >< RsaI
  >< BbrPI                                      >< Bst1107I >< Csp6I
 >< AflIII      >< MnlI>< DdeI                 >< AccI       >< AfaI
CACGTGTTGA AAAGAAAAAG ACTGAGGGTT TCATGGGGCG TATACGCTCT GTGTACCCTG TTGCATCTCC
      1130       1140       1150       1160       1170       1180       1190

 >< SfaNI
       >< MaeIII           >< AccI                                  NlaIII ><
ACAGGAGTGT AACAATATGC ACTTGTCTAC CTTGATGAAA TGTAATCATT GCGATGAAGT TTCATGGCAG
      1200       1210       1220       1230       1240       1250       1260

                                                                    >< SinI
                                                                    >< Sau96I
                                                                   PssI ><
                                                                     >< Psp5II
                                                                    >< PpuMI
                                                                    >< NspIV
                                                                     >< NspHII
                                                                    >< Eco47I
                                                                    >< DraII
                                                                    >< Cfr13I
                                                                    >< BsiZI
                                                                    >< Bme18I
                                                                    >< AvaII
                                                                    >< AsuI
>< MaeII                                                  EcoO109I ><AflIII >
ACGTGCGACT TTCTGAAAGC CACTTGTGAA CATTGTGGCA CTGAAAATTT AGTTATTGAA GGACCTACTA
      1270       1280       1290       1300       1310       1320       1330

                                                                  Van91I ><
                                                                      SinI ><
       >< RsaI                                                     Sau96I ><
  >< NspI                                                         PflMI ><
       >< NlaIV                                                     NspIV ><
  >< NlaIII                                                         NspHII >
  >< NspHI>< KpnI                                                  Eco47I ><
     >< Eco64I                                                     Cfr13I ><
      >< Csp6I                                                    BslI ><
       >< BscBI                                                     BsiZI ><
     >< BanI                                                      BsiYI ><
     >< Asp718                                                     Bme18I ><
       >< AfaI                                                     AvaII ><
     >< AccBlI                                                      AsuI ><
```

FIGURE 13. 4

```
      >< Acc65I          >< SfcI            >< NlaIII         AccB7I ><
CATGTGGGTA CCTACCTACT AATGCTGTAG TGAAAATGCC ATGTCCTGCC TGTCAAGACC CAGAGATTGG
      1340       1350       1360       1370       1380       1390       1400

                                                    >< TthHB8I
                                                    >< TaqI>< MnlI
                                                       >< HinfI
  >< DdeI                                          >< PleI    >< AciI
ACCTGAGCAT AGTGTTGCAG ATTATCACAA CCACTCAAAC ATTGAAACTC GACTCCGCAA GGGAGGTAGG
      1410       1420       1430       1440       1450       1460       1470

 >< RmaI                                                          NlaIV ><
    >< MnlI                                                            >< BsrI
 >< MaeI                  >< BbvI        >< Fnu4HI                BscBI ><
ACTAGATGTT TTGGAGGCTG TGTGTTTGCC TATGTTGGCT GCTATAATAA GCGTGCCTAC TGGGTTCCTC
      1480       1490       1500       1510       1520       1530       1540

                                                                  XhoII ><
                                                                 Sau3AI ><
                                                                  NdeII ><
                                                                   MflI ><
                                                 >< MaeIII          MboI ><
                          >< PalI                      >< Eco31I      DpnII ><
                          >< HaeIII                 >< BsrI       >< MnlI DpnI >
   >< RmaI                >< BsuRI   >< BsrI           >< BsmAI        BstYI ><
      >< MnlI       > < DdeI     >< BspWI             >< BsaI>< HphI  BspAI ><
   >< MaeI                  >< BshI>< BglI             >< Alw26I       Bspl43I >
GTGCTAGTGC TGATATTGGC TCAGGCCATA CTGGCATTAC TGGTGACAAT GTGGAGACCT TGAATGAGGA
      1550       1560       1570       1580       1590       1600       1610

                             > < Tru9I
                             > < MseI
                          >< MaeII    >< Tru9I
                                >< HpaI                          > < MnlI
                                >< HindII                        > < Ksp632I
                  >< HinfI >< PleI >< HincII                     > < EarI
     >< AlwI  >< DdeI     >< AflIII   >< MseI                   > < Eam1104I
TCTCCTTGAG ATACTGAGTC GTGAACGTGT TAACATTAAC ATTGTTGGCG ATTTTCATTT GAATGAAGAG
      1620       1630       1640       1650       1660       1670       1680

      >< MboII                                                       PleI ><
            >< BstXI      >< SfaNI                                 > < HinfI
GTTGCCATCA TTTTGGCATC TTTCTCTGCT TCTACAAGTG CCTTTATTGA CACTATAAAG AGTCTTGATT
      1690       1700       1710       1720       1730       1740       1750

                                                    >< StyI
                                                 >< MaeIII
                                                    >< EcoT14I
                                  >< PleI           >< Eco130I
                                   >< MaeIII        >< BssT1I     BslI ><
                           >< HinfI>< AciI          >< BsaJI     BsiYI ><
ACAAGTCTTT CAAAACCATT GTTGAGTCCT GCGGTAACTA TAAAGTTACC AAGGGAAAGC CCGTAAAAGG
      1760       1770       1780       1790       1800       1810       1820

                        >< Sau3AI              >< Van91I
                        >< NdeII               >< PflMI
                        >< MboI                >< DraIII
                        >< DpnII               >< BslI
                          >< DpnI >< Tru9I     >< BsiYI
                        >< BspAI  >< MseI           >< BbvI          >< MnlI
                          >< Bspl43I           >< AccB7I     Fnu4HI ><
```

FIGURE 13.5

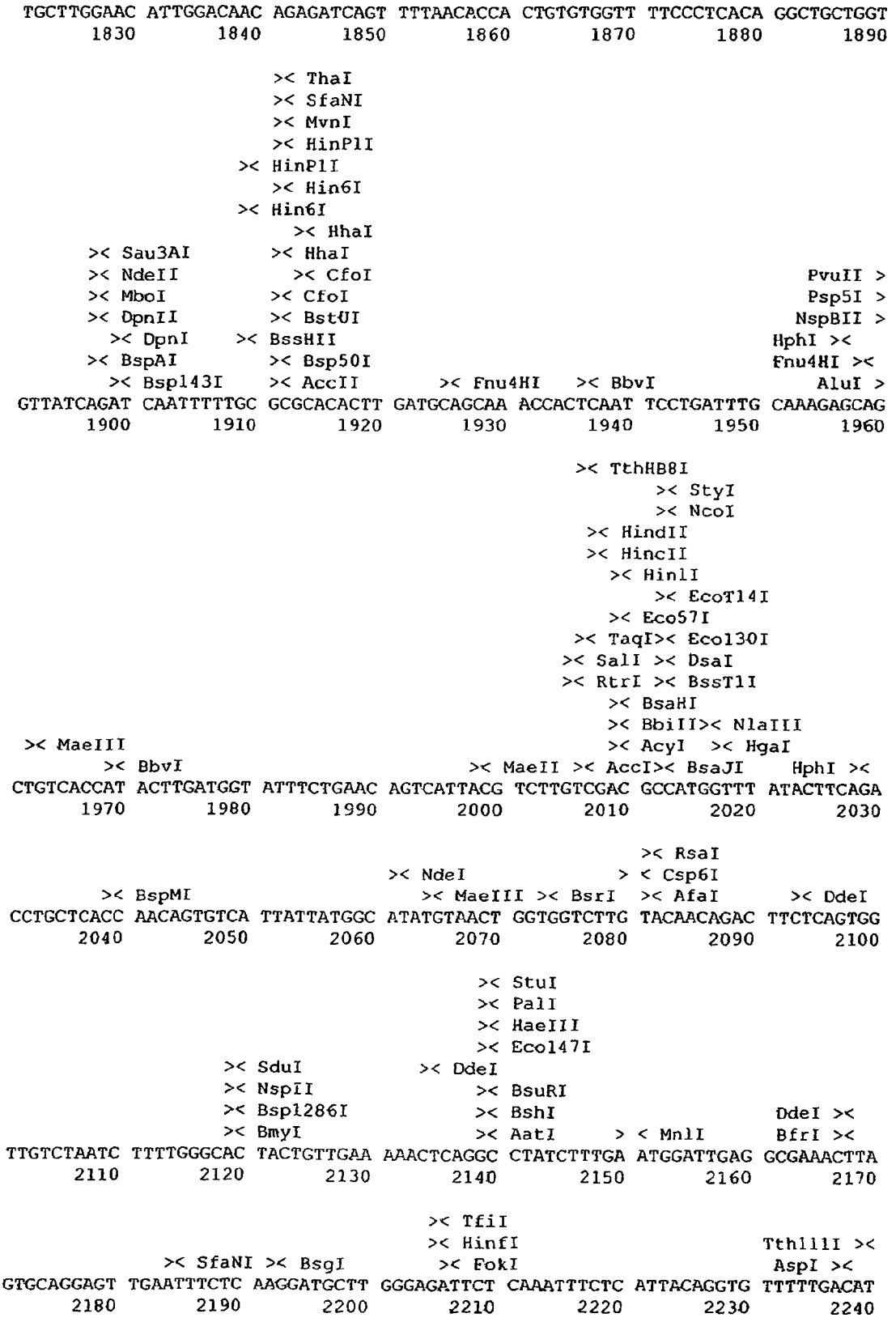

TGCTTGGAAC ATTGGACAAC AGAGATCAGT TTTAACACCA CTGTGTGGTT TTCCCTCACA GGCTGCTGGT
1830 1840 1850 1860 1870 1880 1890
>< ThaI
>< SfaNI
>< MvnI
>< HinP1I
>< HinP1I
>< Hin6I
>< Hin6I
>< HhaI
>< Sau3AI
>< HhaI
>< NdeII
>< CfoI
PvuII >
>< MboI
>< CfoI
Psp5I >
>< DpnII
>< BstUI
NspBII >
>< DpnI
>< BssHII
HphI ><
>< BspAI
>< Bsp50I
Fnu4HI ><
>< Bsp143I
>< AccII
>< Fnu4HI
>< BbvI
AluI >
GTTATCAGAT CAATTTTTGC GCGCACACTT GATGCAGCAA ACCACTCAAT TCCTGATTTG CAAAGAGCAG
1900 1910 1920 1930 1940 1950 1960
>< TthHB8I
>< StyI
>< NcoI
>< HindII
>< HincII
>< HinlI
>< EcoT14I
>< Eco57I
>< TaqI>< Eco130I
>< SalI >< DsaI
>< RtrI >< BssT1I
>< BsaHI
>< BbiII>< NlaIII
>< MaeIII
>< AcyI >< HgaI
>< BbvI
>< MaeII >< AccI>< BsaJI
HphI ><
CTGTCACCAT ACTTGATGGT ATTTCTGAAC AGTCATTACG TCTTGTCGAC GCCATGGTTT ATACTTCAGA
1970 1980 1990 2000 2010 2020 2030
>< RsaI
>< NdeI
> < Csp6I
>< BspMI
>< MaeIII >< BsrI
>< AfaI
>< DdeI
CCTGCTCACC AACAGTGTCA TTATTATGGC ATATGTAACT GGTGGTCTTG TACAACAGAC TTCTCAGTGG
2040 2050 2060 2070 2080 2090 2100
>< StuI
>< PalI
>< HaeIII
>< Eco147I
>< SduI
>< DdeI
>< NspII
>< BsuRI
>< Bsp1286I
>< BshI
DdeI ><
>< BmyI
>< AatI
> < MnlI
BfrI ><
TTGTCTAATC TTTTGGGCAC TACTGTTGAA AAACTCAGGC CTATCTTTGA ATGGATTGAG GCGAAACTTA
2110 2120 2130 2140 2150 2160 2170
>< TfiI
>< HinfI
Tth111I ><
>< SfaNI >< BsgI
>< FokI
AspI ><
GTGCAGGAGT TGAATTTCTC AAGGATGCTT GGGAGATTCT CAAATTTCTC ATTACAGGTG TTTTTGACAT
2180 2190 2200 2210 2220 2230 2240

FIGURE 13.6

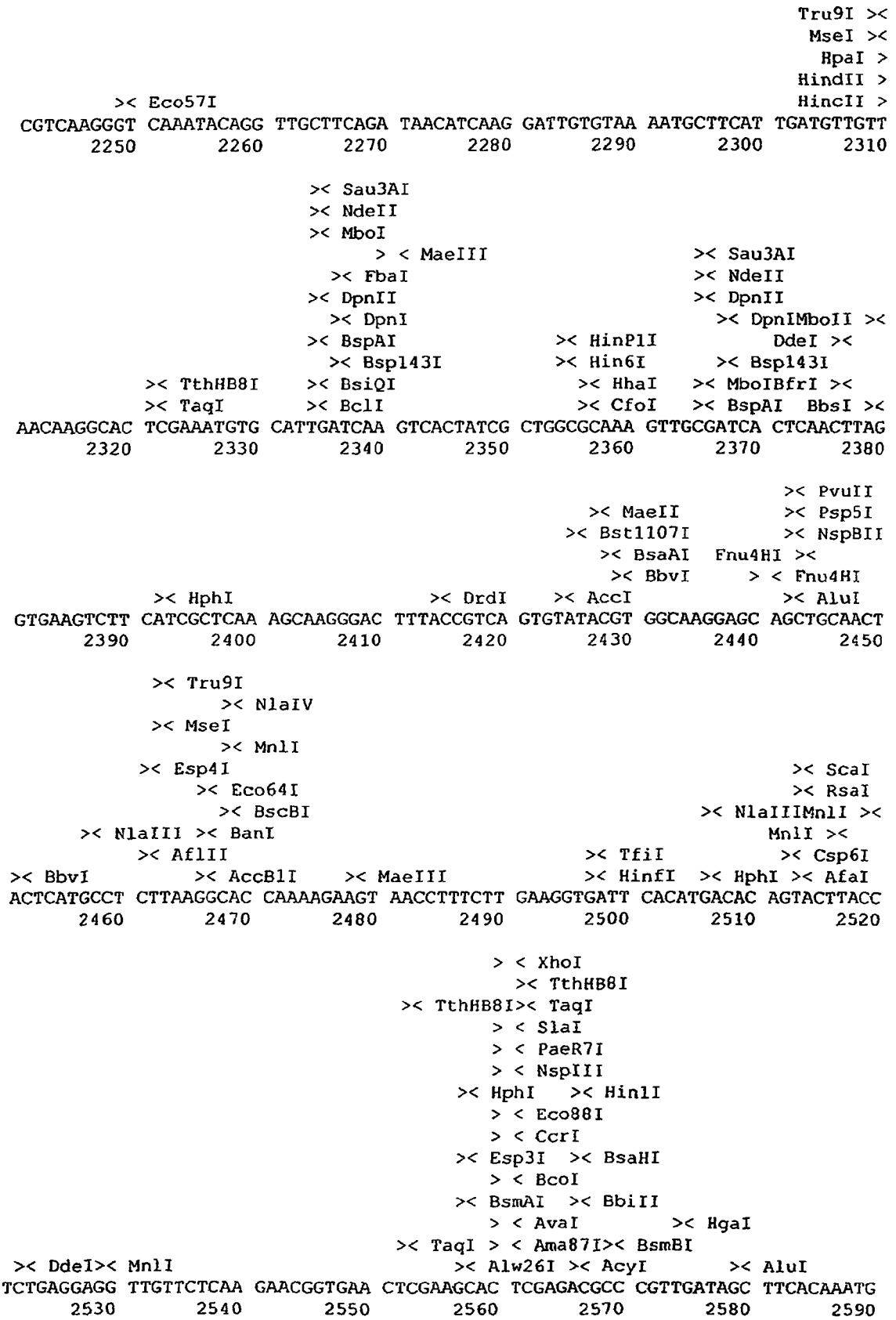

Tru9I ><
MseI ><
HpaI >
HindII >
HincII >
>< Eco57I
CGTCAAGGGT CAAATACAGG TTGCTTCAGA TAACATCAAG GATTGTGTAA AATGCTTCAT TGATGTTGTT
2250 2260 2270 2280 2290 2300 2310
>< Sau3AI
>< NdeII
>< MboI
> < MaeIII
>< Sau3AI
>< FbaI
>< NdeII
>< DpnII
>< DpnII
>< DpnI
>< DpnIMboII ><
>< BspAI
>< HinP1I
DdeI ><
>< Bsp143I
>< Hin6I
>< Bsp143I
>< TthHB8I
>< BsiQI
>< HhaI
>< MboIBfrI ><
>< TaqI
>< BclI
>< CfoI
>< BspAI BbsI ><
AACAAGGCAC TCGAAATGTG CATTGATCAA GTCACTATCG CTGGCGCAAA GTTGCGATCA CTCAACTTAG
2320 2330 2340 2350 2360 2370 2380
>< PvuII
>< MaeII
>< Psp5I
>< Bst1107I
>< NspBII
>< BsaAI
Fnu4HI ><
>< BbvI
> < Fnu4HI
>< HphI
>< DrdI
>< AccI
>< AluI
GTGAAGTCTT CATCGCTCAA AGCAAGGGAC TTTACCGTCA GTGTATACGT GGCAAGGAGC AGCTGCAACT
2390 2400 2410 2420 2430 2440 2450
>< Tru9I
>< NlaIV
>< MseI
>< MnlI
>< Esp4I
>< ScaI
>< Eco64I
>< RsaI
>< BscBI
>< NlaIIIMnlI ><
>< NlaIII >< BanI
MnlI ><
>< AflII
>< TfiI
>< Csp6I
>< BbvI
>< AccB1I
>< MaeIII
>< HinfI
>< HphI >< AfaI
ACTCATGCCT CTTAAGGCAC CAAAAGAAGT AACCTTTCTT GAAGGTGATT CACATGACAC AGTACTTACC
2460 2470 2480 2490 2500 2510 2520
> < XhoI
>< TthHB8I
>< TthHB8I>< TaqI
> < SlaI
> < PaeR7I
> < NspIII
>< HphI >< Hin1I
> < Eco88I
> < CcrI
>< Esp3I >< BsaHI
> < BcoI
>< BsmAI >< BbiII
> < AvaI
>< HgaI
>< TaqI > < Ama87I>< BsmBI
>< DdeI>< MnlI
>< Alw26I >< AcyI
>< AluI
TCTGAGGAGG TTGTTCTCAA GAACGGTGAA CTCGAAGCAC TCGAGACGCC CGTTGATAGC TTCACAAATG
2530 2540 2550 2560 2570 2580 2590

FIGURE 13.7

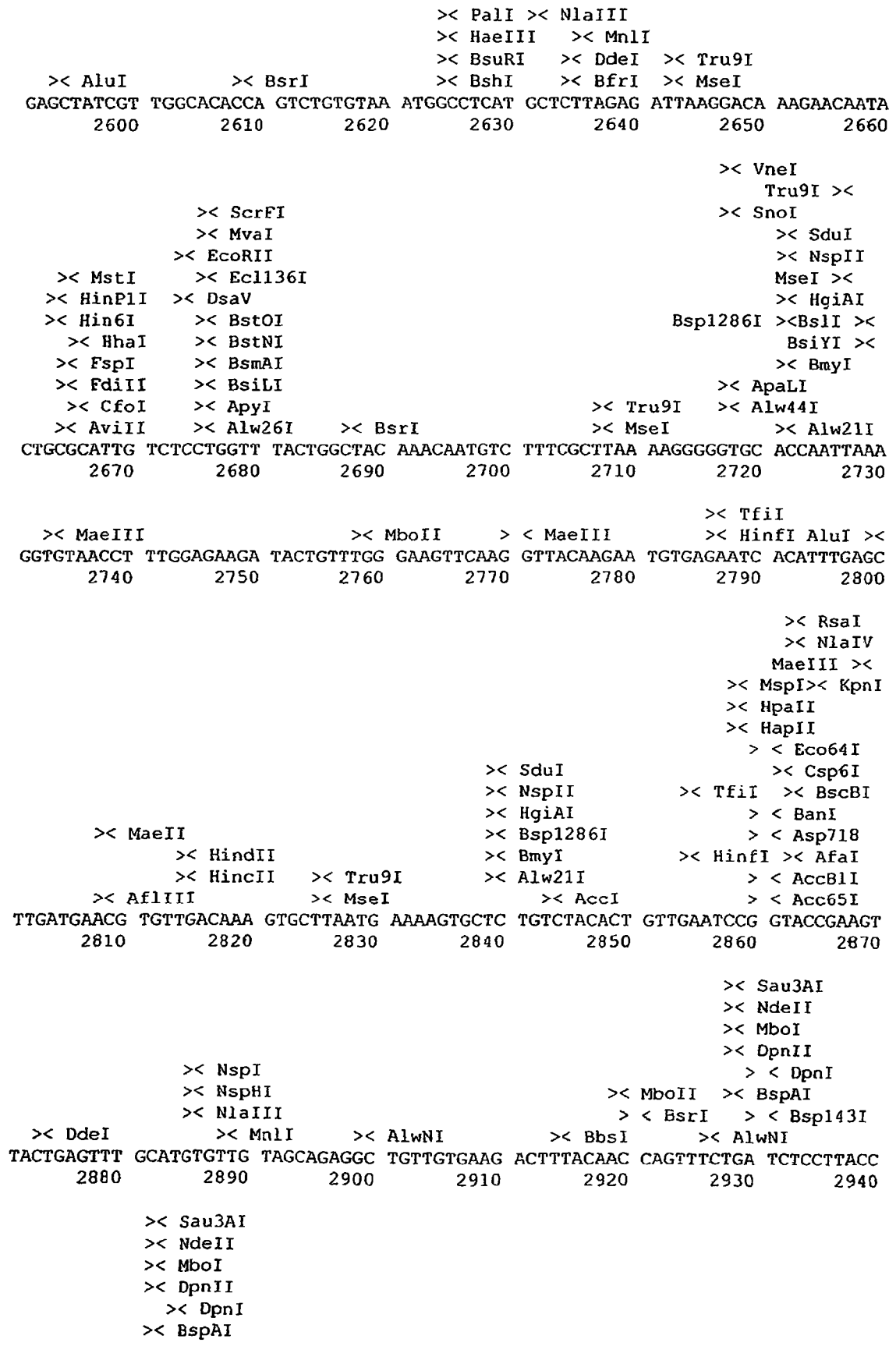

>< PalI >< NlaIII
>< HaeIII >< MnlI
>< BsuRI >< DdeI >< Tru9I
>< AluI >< BsrI >< BshI >< BfrI >< MseI
GAGCTATCGT TGGCACACCA GTCTGTGTAA ATGGCCTCAT GCTCTTAGAG ATTAAGGACA AAGAACAATA
2600 2610 2620 2630 2640 2650 2660
>< VneI
Tru9I ><
>< ScrFI
>< MvaI
>< EcoRII
>< SnoI
>< SduI
>< NspII
MseI ><
>< MstI >< Ecl136I
>< HinP1I >< DsaV
>< Hin6I >< BstOI
>< HgiAI
Bsp1286I ><BsiI ><
BsiYI ><
>< HhaI >< BstNI
>< FspI >< BsmAI
>< FdiII >< BsiLI
>< BmyI
>< ApaLI
>< CfoI >< ApyI >< Tru9I >< Alw44I
>< AviII >< Alw26I >< BsrI >< MseI >< Alw21I
CTGCGCATTG TCTCCTGGTT TACTGGCTAC AAACAATGTC TTTCGCTTAA AAGGGGGTGC ACCAATTAAA
2670 2680 2690 2700 2710 2720 2730
>< TfiI
>< MaeIII >< MboII > < MaeIII >< HinfI AluI ><
GGTGTAACCT TTGGAGAAGA TACTGTTTGG GAAGTTCAAG GTTACAAGAA TGTGAGAATC ACATTTGAGC
2740 2750 2760 2770 2780 2790 2800
>< RsaI
>< NlaIV
MaeIII ><
>< MspI>< KpnI
>< HpaII
>< HapII
> < Eco64I
>< Csp6I
>< SduI
>< NspII >< TfiI >< BscBI
>< HgiAI
> < BanI
>< MaeII >< Bsp1286I > < Asp718
>< HindII >< BmyI >< HinfI >< AfaI
>< HincII >< Tru9I >< Alw21I > < Acc65I
>< AflIII >< MseI >< AccI > < AccBII
TTGATGAACG TGTTGACAAA GTGCTTAATG AAAAGTGCTC TGTCTACACT GTTGAATCCG GTACCGAAGT
2810 2820 2830 2840 2850 2860 2870
>< Sau3AI
>< NdeII
>< MboI
>< DpnII
> < DpnI
>< NspI
>< NspHI
>< NlaIII
>< MboII >< BspAI
> < BsrI > < Bsp143I
>< DdeI >< MnlI >< AlwNI >< BbsI >< AlwNI
TACTGAGTTT GCATGTGTTG TAGCAGAGGC TGTTGTGAAG ACTTTACAAC CAGTTTCTGA TCTCCTTACC
2880 2890 2900 2910 2920 2930 2940
>< Sau3AI
>< NdeII
>< MboI
>< DpnII
>< DpnI
>< BspAI

FIGURE 13.8

```
      >< NlaIII>< Bsp143I            >< AluI     >< SfaNI
 AACATGGGTA TTGATCTTGA TGAGTGGAGT GTAGCTACAT TCTACTTATT TGATGATGCT GGTGAAGAAA
       2950        2960        2970        2980        2990        3000        3010

                                                    >< SfaNI
                                                  >< MnlI
      >< MboII       >< GsuI                      >< Ksp632I          >< MnlI
            >< BsaAI                              >< EarI           > < MboII
   >< HphI  >< MaeII>< BpmI          >< MnlI    >< Eam1104I     >< MboII
 ACTTTTCATC ACGTATGTAT TGTTCCTTTT ACCCTCCAGA TGAGGAAGAA GAGGACGATG CAGAGTGTGA
       3020        3030        3040        3050        3060        3070        3080

                                          > < RsaI
                                       >< RsaI
                                    >< NlaIII
                                       >< MnlI                    >< FokI
                                          >< Csp6I                Eco31I ><
                                      >< Csp6I             >< MamI  BsmAI ><
                  >< MboII                 > < AfaI      >< BsiBI  BsaI ><
               >< MboII                 >< AfaI          >< BsaBIAlw26I ><
 GGAAGAAGAA ATTGATGAAA CCTGTGAACA TGAGTACGGT ACAGAGGATG ATTATCAAGG TCTCCCTCTG
       3090        3100        3110        3120        3130        3140        3150

         >< NlaIV>< PvuII>< XmnI
       >< Eco64I >< Psp5I     >< TthHB8I
     >< MnlI >< DdeI          >< TaqI      >< MnlI             >< MboII
         >< BscBI>< NspBII  >< MnlI       >< Ksp632I       >< MboII  >< MboII
       >< BanI          >< MnlI           >< EarI                >< BsrI
       >< AccBlI >< AluI >< Asp700I       >< Eam1104I >< MboII>< BbsI
 GAATTTGGTG CCTCAGCTGA AACAGTTCGA GTTGAGGAAG AAGAAGAGGA AGACTGGCTG GATGATACTA
       3160        3170        3180        3190        3200        3210        3220

                                                       >< Tru9I
   >< FokI                                             >< MseI    >< Eco57I
 >< DdeI                                            >< BsrI>< MboII  BsrI ><
 CTGAGCAATC AGAGATTGAG CCAGAACCAG AACCTACACC TGAAGAACCA GTTAATCAGT TTACTGGTTA
       3230        3240        3250        3260        3270        3280        3290

  >< Tru9I                                  >< MnlI
  >< MseI                    >< Tru9I    >< HindII>< Tru9I        >< DraIII
   >< DraI                   >< MseI     >< HincII>< MseI         >< BspWI
 TTTAAAACTT ACTGACAATG TTGCCATTAA ATGTGTTGAC ATCGTTAAGG AGGCACAAAG TGCTAATCCT
       3300        3310        3320        3330        3340        3350        3360

                                                          >< VneI
                                                          >< SnoI
                                                              > < SduI
                                                              > < NspII
                                                              > < HgiAI
                                                              > < Bsp1286I
                                                              > < BmyI
                                                          >< ApaLI
              >< HphI                      > < NlaIII       >< Alw44I
   >< BbvI        >< Fnu4HI                    >< BspMI          > < Alw21I
 ATGGTGATTG TAAATGCTGC TAACATACAC CTGAAACATG GTGGTGGTGT AGCAGGTGCA CTCAACAAGG
       3370        3380        3390        3400        3410        3420        3430

                                                       >< Sau96I
                                                        >< PalI
                                                       >< NspIV
                                                        >< HaeIII
          >< NlaIV                                     >< Cfr13I
```

FIGURE 13.9

```
        >< Eco64I                                                    >< BsuRI
           >< BscBI                                  > < Tru9I     >< BsiZI
        >< BanI                                      > < MseI      >< BshI      >< MnlI
        >< AccB1I>< NlaIII                                  >< AluI >< AsuI >< MnlI
CAACCAATGG TGCCATGCAA AAGGAGAGTG ATGATTACAT TAAGCTAAAT GGCCCTCTTA CAGTAGGAGG
      3440       3450       3460       3470       3480       3490       3500

                                                             >< SinI
                                                             >< Sau96I
                                                             >< NspIV
                                                          >< NspHI>< NspHII
                                                             >< Eco47I
                                                             >< Cfr13I
                                                          >< NlaIII    >< BspMI
                                                             >< BsiZI
                                                             >< Bme18I
                                                             >< AvaII MnlI ><
                                    > < DdeI              >< NspI>< AsuI FokI ><
GTCTTGTTTG CTTTCTGGAC ATAATCTTGC TAAGAAGTGT CTGCATGTTG TTGGACCTAA CCTAAATGCA
      3510       3520       3530       3540       3550       3560       3570

                     > < Tru9I
                  >< HphI> < MseI
                        >< Esp4I
                     >< AluI         > < NdeI
                        >< AflII>< Fnu4HI   >< BbvI
GGTGAGGACA TCCAGCTTCT TAAGGCAGCA TATGAAAATT TCAATTCACA GGACATCTTA CTTGCACCAT
      3580       3590       3600       3610       3620       3630       3640

                                                                      RsaI ><
                                                                    Csp6I ><
                     >< Eco57I                        >< BcgI          AfaI ><
TGTTGTCAGC AGGCATATTT GGTGCTAAAC CACTTCAGTC TTTACAAGTG TGCGTGCAGA CGGTTCGTAC
      3650       3660       3670       3680       3690       3700       3710

    >< BsgI                        >< BspMI
       >< BcgI/a                >< AluI                    >< NlaIII
ACAGGTTTAT ATTGCAGTCA ATGACAAAGC TCTTTATGAG CAGGTTGTCA TGGATTATCT TGATAACCTG
      3720       3730       3740       3750       3760       3770       3780

                                                          >< MnlI
    >< RmaI          > < MnlI      >< NlaIV          >< TfiI   >< MboII
    >< MaeI       >< Eco57I        >< BscBI          >< HinfI    >< DdeI
AAGCCTAGAG TGGAAGCACC TAAACAAGAG GAGCCACCAA ACACAGAAGA TTCCAAAACT GAGGAGAAAT
      3790       3800       3810       3820       3830       3840       3850

                                       >< Tru9I
                                             >< StuI
                                             >< PalI
                                       >< MseI   >< MnlI     >< MaeIII
                                             >< HaeIII       >< Eco0651
                                             >< Eco147I      >< Eco91I
     >< RsaI                                 >< BsuRI              BstXI ><
    >< Csp6I       >< TthHB8I                >< BshI         >< BstPI
     >< AfaI       >< TaqI                   >< AatI         >< BstEII
CTGTCGTACA GAAGCCTGTC GATGTGAAGC CAAAAATTAA GGCCTGCATT GATGAGGTTA CCACAACACT
      3860       3870       3880       3890       3900       3910       3920

                                                                    TfiI ><
                                                                  NlaIII ><
                                                                   HinfI ><
       >< DdeI                                    >< EcoRV   >< HindIII
```

FIGURE 13.10

```
  >< BsrI     >< MboII      >< MaeIII           >< Eco32I    >< AluI
GGAAGAAACT AAGTTTCTTA CCAATAAGTT ACTCTTGTTT GCTGATATCA ATGGTAAGCT TTACCATGAT
      3930       3940       3950       3960       3970       3980       3990

           >< NspI
           >< NspHI
           >< NlaIII               >< SfaNI
         >< MnlI                      > < EcoNI
           >< DdeI                  >< MboII >< BslI          > < NlaIII
 >< DdeI     >< BfrI             >< HphI     >< BsiYI         >< FokI
TCTCAGAACA TGCTTAGAGG TGAAGATATG TCTTTCCTTG AGAAGGATGC ACCTTACATG GTAGGTGATG
      4000       4010       4020       4030       4040       4050       4060

     >< SpeI
      >< RmaI
      >< MaeI    >< EcoRV>< HphI                        >< SfaNI
     >< HphI     >< Eco32I                      >< MnlI         >< DdeI
TTATCACTAG TGGTGATATC ACTTGTGTTG TAATACCCTC CAAAAAGGCT GGTGGCACTA CTGAGATGCT
      4070       4080       4090       4100       4110       4120       4130

                                                    >< ScrFI
                                                 >< RsaI
                                                    >< MvaI
                                                  >< EcoRII
                                                    >< Ecl136I
                                                  >< DsaV
                                                >< Csp6I >< EcoNI
                                                    >< BstOI
                                                    >< BstNI
                                                    >< BsiLI
                                                  >< BsaJI
                                                >< BsaAI    >< BslI
                          >< MboII              >< MaeII>< ApyI
      >< AluI          >< BsrI                    >< AfaI   >< BsiYI
CTCAAGAGCT TTGAAGAAAG TGCCAGTTGA TGAGTATATA ACCACGTACC CTGGACAAGG ATGTGCTGGT
      4140       4150       4160       4170       4180       4190       4200

                           >< Tru9I
                           >< MseI
                  >< DdeI     >< Esp4I                        >< RsaI
 >< MnlI               >< BspWI                             >< Csp6I
 >< FokI         >< AluI      >< AflII              >< Eco57I >< AfaI
TATACACTTG AGGAAGCTAA GACTGCTCTT AAGAAATGCA AATCTGCATT TTATGTACTA CCTTCAGAAG
      4210       4220       4230       4240       4250       4260       4270

                                       >< ScrFI
                                       >< MvaI
                                     >< EcoRII
                    >< XmnI            >< Ecl136I            NlaIII ><
        > < Ksp632I     >< RmaI      >< DsaV                Ksp632I ><
        > < EarI   > < TfiI>< MboII    >< BstOI                      >< EarI
        > < Eam1104I    >< MaeI        >< BstNI             Eam1104I ><
        > < DdeI   > < HinfI           >< BsiLI                  BsmAI ><
     >< BspWI      >< Asp700I          >< ApyI                  Alw26I ><
CACCTAATGC TAAGGAAGAG ATTCTAGGAA CTGTATCCTG GAATTTGAGA GAAATGCTTG CTCATGCTGA
      4280       4290       4300       4310       4320       4330       4340

           >< VspI          >< Zsp2I
           >< Tru9I     >< Ppu10I
           >< MseI          >< NsiI
         >< MboII              >< NlaIII     >< FokI
               >< Eco57I    >< Mph1103I   >< FokI
```

FIGURE 13. 11

```
              >< AsnI          >< EcoT22I          >< BspWI
              >< AseI        >< AvaIII             >< BglI          >< MaeII
AGAGACAAGA AAATTAATGC CTATATGCAT GGATGTTAGA GCCATAATGG CAACCATCCA ACGTAAGTAT
      4350       4360       4370       4380       4390       4400       4410

                                  >< SfaNI
       >< Tru9I                 > < HindII      >< TfiI        >< SpeI
       >< MseI                  > < HincII>< MboII              >< RmaI
           >< MnlI                        >< DrdI >< HinfI        >< MaeI
AAAGGAATTA AAATTCAAGA GGGCATCGTT GACTATGGTG TCCGATTCTT CTTTTATACT AGTAAAGAGC
      4420       4430       4440       4450       4460       4470       4480

                                                  >< MaeIII
><  SfcI                                       >< Fnu4HI        >< MunI
     >< AluI             >< AluI                >< AciI              MaeIII ><
CTGTAGCTTC TATTATTACG AAGCTGAACT CTCTAAATGA GCCGCTTGTC ACAATGCCAA TTGGTTATGT
      4490       4500       4510       4520       4530       4540       4550

                                  >< ThaI
                                  >< MvnI
                                   >< MboII
                                  >< HinP1I
                                >< HinP1I
                                  >< Hin6I
                                >< Hin6I
                                     >< HhaI
           >< Tru9I               >< HhaI
       >< NlaIII             >< Fnu4HI
            >< MseI                  >< CfoI
               >< MnlI             >< CfoI
               >< Ksp632I          >< BstUI
               >< EarI          >< BssHII>< BspWI     >< Tru9I
               >< Eam1104I         >< Bsp50I          >< MseI
             >< BbvI               >< AccII                >< AluI     HphI ><
GACACATGGT TTTAATCTTG AAGAGGCTGC GCGCTGTATG CGTTCTCTTA AAGCTCCTGC CGTAGTGTCA
      4560       4570       4580       4590       4600       4610       4620

                  >< MaeIII
  >< SfaNI      >< AlwNI                          >< MnlI >< MnlI>< DdeI
GTATCATCAC CAGATGCTGT TACTACATAT AATGGATACC TCACTTCGTC ATCAAAGACA TCTGAGGAGC
      4630       4640       4650       4660       4670       4680       4690

                                                 >< SinI
                                                 >< Sau96I
                                                 >< NspIV
                                                  >< NspHII
><  SduI                                         >< Eco47I
><  NspII                                        >< Cfr13I
><  HgiAI                                        >< BsiZI
><  Bsp1286I                                     >< Bme18I           >< RsaI
><  BmyI                                         >< AvaII          >< Csp6I
><  Alw21I                                       >< AsuI             >< AfaI
ACTTTGTAGA AACAGTTTCT TTGGCTGGCT CTTACAGAGA TTGGTCCTAT TCAGGACAGC GTACAGAGTT
      4700       4710       4720       4730       4740       4750       4760

                                                                  > < TthHB8I
                                                                  > < TaqI
                                                               >< SduI
                                                     >< Van91I  >< NspII
              >< Tru9I                  >< RsaI    >< PflMI   >< Eco24I
              >< MseI                >< HphI       >< BslI    >< Bsp1286I
             >< Esp4I                   >< Csp6I   >< BsiYI   >< BmyI GsuI ><
```

FIGURE 13.12

```
                ><AflII  ><MaeIII       ><AfaI  ><AccB7I  ><BanIIBpmI ><
AGGTGTTGAA TTTCTTAAGC GTGGTGACAA AATTGTGTAC CACACTCTGG AGAGCCCCGT CGAGTTTCAT
      4770       4780       4790       4800       4810       4820       4830

                                                          ><Tru9I
                                                  ><PleI  ><EcoNI
                                                     ><MnlI ><BslI
                                                  ><BsmAI    ><BsiYI
 ><MnlI          ><HphI           ><HinfI><Alw26I><AciI ><MseI
CTTGACGGTG AGGTTCTTTC ACTTGACAAA CTAAAGAGTC TCTTATCCCT GCGGGAGGTT AAGACTATAA
      4840       4850       4860       4870       4880       4890       4900

                                          ><AluI                  ><NdeI
AAGTGTTCAC AACTGTGGAC AACACTAATC TCCACACACA GCTTGTGGAT ATGTCTATGA CATATGGACA
      4910       4920       4930       4940       4950       4960       4970

       ><SinI
       ><Sau96I
       ><NspIV
        ><NspHII
       ><Eco47I
       ><Cfr13I                                                 NlaIII ><
       ><BsiZI                                                ><NlaIII
       ><Bme18I                                                  > <MnlI
       ><AvaII                    ><MaeIII   ><Tru9I     ><MnlI
       ><AsuI                      ><FokI    ><MseI           ><BspHI
GCAGTTTGGT CCAACATACT TGGATGGTGC TGATGTTACA AAAATTAAAC CTCATGTAAA TCATGAGGGT
      4980       4990       5000       5010       5020       5030       5040

                                                 > <TthHB8I
                ><RsaI                           > <TaqI
                     > <RmaI          ><SnaBI            ><ScaI
                     > <MaeI         ><MaeII ><HindIII  ><RsaI
               ><Csp6I                ><Eco105I         ><Csp6I
                ><AfaI                ><BsaAI  ><AluI   ><AfaI
AAGACTTTCT TTGTACTACC TAGTGATGAC ACACTACGTA GTGAAGCTTT CGAGTACTAC CATACTCTTG
      5050       5060       5070       5080       5090       5100       5110

                    ><RsaI
                         ><NspI
                         ><NspHI
                         ><NlaIII
                    > <Csp6I      ><Tru9I                          MnlI >
                      ><AflIII    ><MseI                        BslI ><
                     ><AfaI        ><DraI                       BsiYI ><
ATGAGAGTTT TCTTGGTAGG TACATGTCTG CTTTAAACCA CACAAAGAAA TGGAAATTTC CTCAAGTTGG
      5120       5130       5140       5150       5160       5170       5180

   ><Tru9I   ><Tru9I                          ><RmaI
   ><MseI    ><MseI           ><MunI          ><MaeI                 AluI >
TGGTTTAACT TCAATTAAAT GGGCTGATAA CAATTGTTAT TTGTCTAGTG TTTTATTAGC ACTTCAACAG
      5190       5200       5210       5220       5230       5240       5250

                                                  ><SfaNI
                                                  ><SduI
                                                  ><NspII
                                                  ><Eco24I
                                                  ><Bsp1286I
                                                  ><BmyI          HphI >
                                                  ><BbvI Fnu4HI ><
                          ><MnlI                  ><BanII   ><BspWI
```

FIGURE 13.13

```
CTTGAAGTCA AATTCAATGC ACCAGCACTT CAAGAGGCTT ATTATAGAGC CCGTGCTGGT GATGCTGCTA
      5260       5270       5280       5290       5300       5310       5320

      >< VneI
      >< SnoI
           >< SduI
           >< NspII
           >< HgiAI
           >< Bsp1286I
           >< BmyI
      >< ApaLI
      >< Alw44I                                                        MboII ><
           >< Alw21I                                  >< AluI          >< HphI
ACTTTTGTGC ACTCATACTC GCTTACAGTA ATAAAACTGT TGGCGAGCTT GGTGATGTCA GAGAAACTAT
      5330       5340       5350       5360       5370       5380       5390

                    > < SphI
                    > < PaeI
                    > < NspI
                    > < NspHI >< TfiI              >< Tru9I
               >< SfcI  > < NlaIII>< HinfI         >< MseI
GACCCATCTT CTACAGCATG CTAATTTGGA ATCTGCAAAG CGAGTTCTTA ATGTGGTGTG TAAACATTGT
      5400       5410       5420       5430       5440       5450       5460

                                                      >< RsaI
                    >< Tru9I                        > < Csp6I       Esp4I >
                    >< MseI          >< AluI          >< AfaI       AflII >
GGTCAGAAAA CTACTACCTT AACGGGTGTA GAAGCTGTGA TGTATATGGG TACTCTATCT TATGATAATC
      5470       5480       5490       5500       5510       5520       5530

                                                            >< RsaI
                                                                >< MboII
                                                         >< RmaIHinfI ><
                                                            >< Csp6I
>< Tru9I                      >< SfaNI                   >< MaeI >< BbsI
>< MseI                    >< NlaIII                        >< AfaI
TTAAGACAGG TGTTTCCATT CCATGTGTGT GTGGTCGTGA TGCTACACAA TATCTAGTAC AACAAGAGTC
      5540       5550       5560       5570       5580       5590       5600

                                                         >< RsaI
   >< PleI                     > < DdeI                 >< Csp6I
  >< BsgI                   >< BspWI >< BspMI           >< AfaI
TTCTTTTGTT ATGATGTCTG CACCACCTGC TGAGTATAAA TTACAGCAAG GTACATTCTT ATGTGCGAAT
      5610       5620       5630       5640       5650       5660       5670

                                                   >< Eco31I
   >< RsaI                                          >< DdeI
        > < MaeIII                                 >< BsmAI
  >< Csp6I                                         >< BsaI            MnlI ><
   >< AfaI  >< BsrI                                >< Alw26I             HphI >
GAGTACACTG GTAACTATCA GTGTGGTCAT TACACTCATA TAACTGCTAA GGAGACCCTC TATCGTATTG
      5680       5690       5700       5710       5720       5730       5740

      >< SstI                        >< SinI
      >< SduI                        >< Sau96I
      >< SacI                        >< NspIV
      >< NspII                        >< NspHII
      >< HgiAI                  > < RsaI     >< MaeIII
      >< Eco24I                        >< Eco47I
    >< Ecl136II                        >< Cfr13I
      >< Bsp1286I                      >< BsiZI
      >< BmyI                          >< Bme18I
```

FIGURE 13. 14

```
        >< BanII                          >< AvaII
        >< Alw21I                   >< Csp6I>< AsuI
      >< AluI                        > < AfaI  >< BsrI>< AlwNI
ACGGAGCTCA CCTTACAAAG ATGTCAGAGT ACAAAGGACC AGTGACTGAT GTTTTCTACA AGGAAACATC
      5750       5760       5770       5780       5790       5800       5810

                                    >< TthHB8I
                                    >< TaqI >< MaeIII
TTACACTACA ACCATCAAGC CTGTGTCGTA TAAACTCGAT GGAGTTACTT ACACAGAGAT TGAACCAAAA
      5820       5830       5840       5850       5860       5870       5880

                                                          >< RsaI
                                                         >< Csp6I
                                                    >< SfcI >< BbvI
                  >< FokI                        >< Fnu4HI       >< AfaI
TTGGATGGGT ATTATAAAAA GGATAATGCT TACTATACAG AGCAGCCTAT AGACCTTGTA CCAACTCAAC
      5890       5900       5910       5920       5930       5940       5950

                                                             Tru9I ><
                                                               SwaI ><
                                                              MseI ><
                                                 > < NspI      MamI ><
                                                 > < NspHI     DraI ><
                                                 > < NlaIII    BsiBI ><
                                               >< AflIII       BsaBI ><
CATTACCAAA TGCGAGTTTT GATAATTTCA AACTCACATG TTCTAACACA AAATTTGCTG ATGATTTAAA
      5960       5970       5980       5990       6000       6010       6020

                                                  >< MboII
                           >< AluI      >< AluI>< MaeIII
TCAAATGACA GGCTTCACAA AGCCAGCTTC ACGAGAGCTA TCTGTCACAT TCTTCCCAGA CTTGAATGGC
      6030       6040       6050       6060       6070       6080       6090

                  >< SfcI
GATGTAGTGG CTATTGACTA TAGACACTAT TCAGCGAGTT TCAAGAAAGG TGCTAAATTA CTGCATAAGC
      6100       6110       6120       6130       6140       6150       6160

                ><  Tru9I
                      >< ScrFI
                      >< MvaI
                ><  MseI
                   >< EcoRII
                      >< Ecl136I
                   >< DsaV
                      >< BstOI
                      >< BstNI                                    MaeII ><
><  MunI              >< BsiLI                                  >< DraIII
      >< BstXI        >< ApyI              >< MaeII          >< BstXI
CAATTGTTTG GCACATTAAC CAGGCTACAA CCAAGACAAC GTTCAAACCA AACACTTGGT GTTTACGTTG
      6170       6180       6190       6200       6210       6220       6230

        > < RsaI
       >< Csp6I                                                    MboII ><
        > < AfaI>< BsrI                                             >< BbsI
TCTTTGGAGT ACAAAGCCAG TAGATACTTC AAATTCATTT GAAGTTCTGG CAGTAGAAGA CACACAAGGA
      6240       6250       6260       6270       6280       6290       6300

                         >< HindII                        >< MboII
                         >< HincII                  >< MnlI       >< Eco57I
ATGGACAATC TTGCTTGTGA AAGTCAACAA CCCACCTCTG AAGAAGTAGT GGAAAATCCT ACCATACAGA
      6310       6320       6330       6340       6350       6360       6370
```

FIGURE 13.15

```
                ><MaeIII                                          ><Tru9I
                   ><MaeII                                        ><MseI
AGGAAGTCAT AGAGTGTGAC GTGAAAACTA CCGAAGTTGT AGGCAATGTC ATACTTAAAC CATCAGATGA
      6380       6390       6400       6410       6420       6430       6440

                                    ><XhoII
                                    ><Sau3AI
                                  ><NlaIII
                                    ><NdeII
                                    ><MflI
                                    ><MboI
                                    ><DpnII
                                      ><DpnI
                                    ><BstYI
     ><Tru9I                        ><BspAI
     ><MseI                  ><BspHI ><Bsp143I><Fnu4HI
        > <MaeIII       ><MnlI ><BbvI     ><AlwI
AGGTGTTAAA GTAACACAAG AGTTAGGTCA TGAGGATCTT ATGGCTGCTT ATGTGGAAAA CACAAGCATT
      6450       6460       6470       6480       6490       6500       6510

                                  ><SauI
                               ><RmaI
                                  ><MstII
                               ><MaeI
                                  ><Eco81I
                                  ><DdeI
                                  ><CvnI
                                  ><Bsu36I
                                  ><Bse21I
                                  ><BfrI> < Tru9I
    ><Tru9I                       ><AxyI> < MseI><MunI          ><NlaIII
    ><MseI           ><AluI       ><AocI  ><DraI       ><BbvI Fnu4HI ><
ACCATTAAGA AACCTAATGA GCTTTCACTA GCCTTAGGTT TAAAAACAAT TGCCACTCAT GGTATTGCTG
      6520       6530       6540       6550       6560       6570       6580

   ><VspI     ><StyI
   ><Tru9I    ><EcoT14I                              > < DdeI
   ><MseI     ><Eco130I                                ><BslI
   ><AsnI     ><BssT1I                                 ><BsiYI
   ><AseI     ><BsaJI                                > < BfrI     ><Fnu4HI
CAATTAATAG TGTTCCTTGG AGTAAAATTT TGGCTTATGT CAAACCATTC TTAGGACAAG CAGCAATTAC
      6590       6600       6610       6620       6630       6640       6650

           ><HinP1I
           ><Hin6I                      ><Tru9I
             ><HhaI              ><MaeII><MseI
              ><DdeI              ><DraIII
 ><BbvI      ><CfoI              ><AflIII
AACATCAAAT TGCGCTAAGA GATTAGCACA ACGTGTGTTT AACAATTATA TGCCTTATGT GTTTACATTA
      6660       6670       6680       6690       6700       6710       6720

            ><RsaI          > < RsaI><XbaI
           ><Csp6I          ><Csp6I  ><RmaI
     ><MunI  ><AfaI          > < AfaI ><MaeI     ><AluI
TTGTTCCAAT TGTGTACTTT TACTAAAAGT ACCAATTCTA GAATTAGAGC TTCACTACCT ACAACTATTG
      6730       6740       6750       6760       6770       6780       6790

                                          ><VspI
                                          ><Tru9I
                                       ><NaeI
                                      ><MspI
                                          ><MseI
```

FIGURE 13. 16

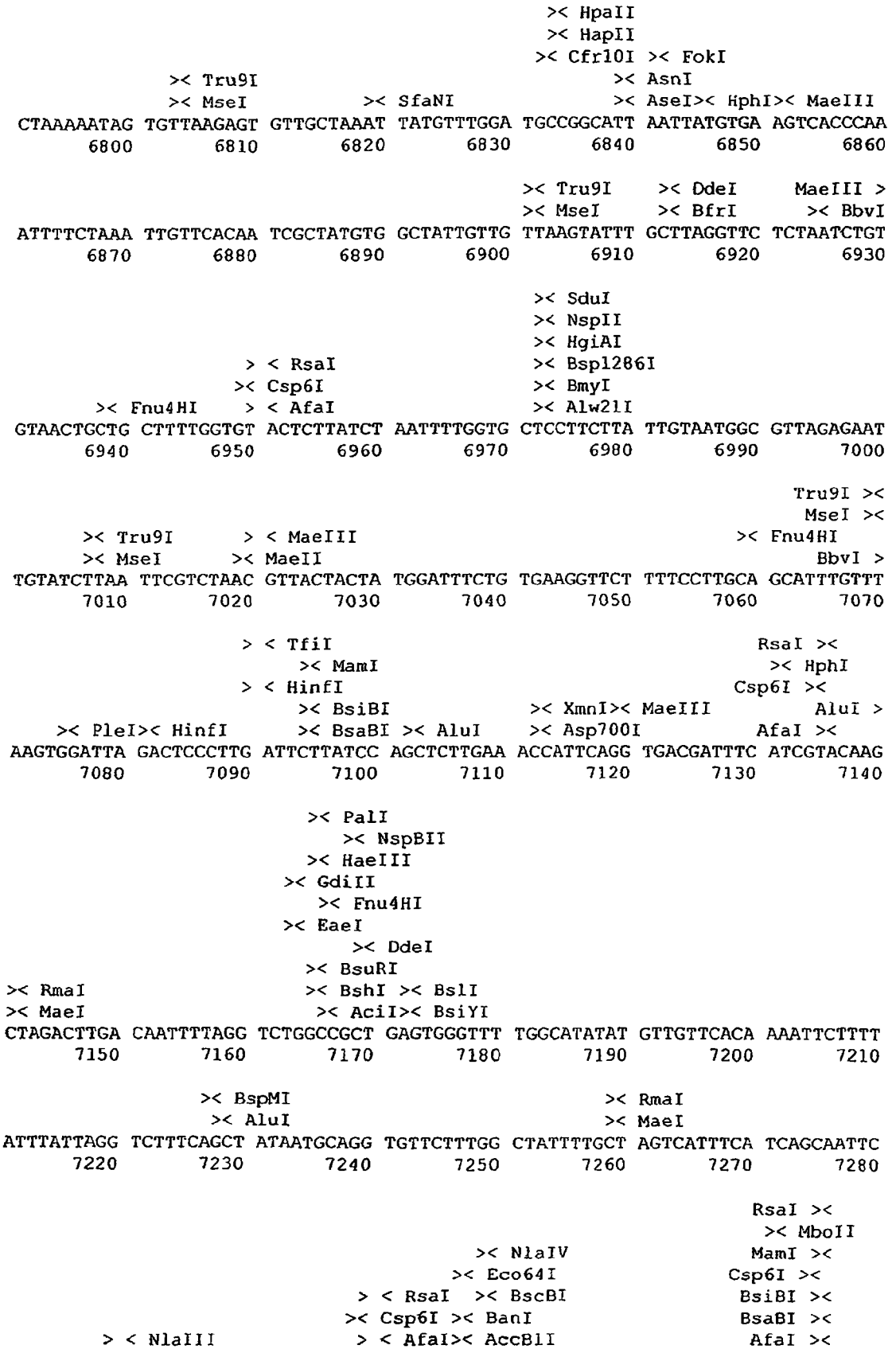

>< HpaII
>< HapII
>< Cfr10I >< FokI
>< Tru9I >< AsnI
>< MseI >< SfaNI >< AseI>< HphI>< MaeIII
CTAAAAATAG TGTTAAGAGT GTTGCTAAAT TATGTTTGGA TGCCGGCATT AATTATGTGA AGTCACCCAA
6800 6810 6820 6830 6840 6850 6860
>< Tru9I >< DdeI MaeIII >
>< MseI >< BfrI >< BbvI
ATTTTCTAAA TTGTTCACAA TCGCTATGTG GCTATTGTTG TTAAGTATTT GCTTAGGTTC TCTAATCTGT
6870 6880 6890 6900 6910 6920 6930
>< SduI
>< NspII
>< HgiAI
> < RsaI >< Bsp1286I
>< Csp6I >< BmyI
>< Fnu4HI > < AfaI >< Alw21I
GTAACTGCTG CTTTTGGTGT ACTCTTATCT AATTTTGGTG CTCCTTCTTA TTGTAATGGC GTTAGAGAAT
6940 6950 6960 6970 6980 6990 7000
Tru9I ><
MseI ><
>< Tru9I > < MaeIII >< Fnu4HI
>< MseI >< MaeII BbvI >
TGTATCTTAA TTCGTCTAAC GTTACTACTA TGGATTTCTG TGAAGGTTCT TTTCCTTGCA GCATTTGTTT
7010 7020 7030 7040 7050 7060 7070
> < TfiI RsaI ><
>< MamI >< HphI
> < HinfI Csp6I ><
>< BsiBI >< XmnI>< MaeIII AluI >
>< PleI>< HinfI >< BsaBI >< AluI >< Asp700I AfaI ><
AAGTGGATTA GACTCCCTTG ATTCTTATCC AGCTCTTGAA ACCATTCAGG TGACGATTTC ATCGTACAAG
7080 7090 7100 7110 7120 7130 7140
>< PalI
>< NspBII
>< HaeIII
>< GdiII
>< Fnu4HI
>< EaeI
>< DdeI
>< BsuRI
>< RmaI >< BshI >< BslI
>< MaeI >< AciI>< BsiYI
CTAGACTTGA CAATTTTAGG TCTGGCCGCT GAGTGGGTTT TGGCATATAT GTTGTTCACA AAATTCTTTT
7150 7160 7170 7180 7190 7200 7210
>< BspMI >< RmaI
>< AluI >< MaeI
ATTTATTAGG TCTTTCAGCT ATAATGCAGG TGTTCTTTGG CTATTTTGCT AGTCATTTCA TCAGCAATTC
7220 7230 7240 7250 7260 7270 7280
RsaI ><
>< MboII
>< NlaIV MamI ><
>< Eco64I Csp6I ><
> < RsaI >< BscBI BsiBI ><
>< Csp6I >< BanI BsaBI ><
> < NlaIII > < AfaI>< AccBII AfaI ><

FIGURE 13.17


TTGGCTCATG TGGTTTATCA TTAGTATTGT ACAAATGGCA CCCGTTTCTG CAATGGTTAG GATGTACATC
7290 7300 7310 7320 7330 7340 7350
TthHB8I ><
>< TaqI
MnlI ><
>< NdeI
>< Ksp632I
>< EarI
Ksp632I ><
>< FokI
>< MboII EarI ><
>< FokI
>< Eam1104I>< AluI>< MboII >< NlaIII Eam1104I ><
TTCTTTGCTT CTTTCTACTA CATATGGAAG AGCTATGTTC ATATCATGGA TGGTTGCACC TCTTCGACTT
7360 7370 7380 7390 7400 7410 7420
XhoII ><
Sau3AI ><
NlaIII ><
NdeII ><
MflI ><
MboI ><
>< Tha I
>< MvnI
> < Ksp632I
> < EarI
>< HinP1I
>< MluI
> < Eam1104I
>< Hin6I
>< BstUI
DpnII ><
>< HhaI
>< Bsp50I >< RsaI
BstYI ><
>< NlaIII
>< CfoI
>< AflIII >< Csp6I
>< Tru9I BspAI ><
>< BspWI >< BspWI
>< AccII >< AfaI
>< MseI BglII ><
GCATGATGTG CTATAAGCGC AATCGTGCCA CACGCGTTGA GTGTACAACT ATTGTTAATG GCATGAAGAG
7430 7440 7450 7460 7470 7480 7490
>< PalI
>< HaeIII
>< DsaI
>< MunI
>< MboII
>< BsuRI
MaeIII ><
>< DpnI
>< BshI
>< MunI
BsmAI ><
>< Bsp143I
>< MnlI
>< BsaJI >< PleI>< HinfI
Alw26I ><
ATCTTTCTAT GTCTATGCAA ATGGAGGCCG TGGCTTCTGC AAGACTCACA ATTGGAATTG TCTCAATTGT
7500 7510 7520 7530 7540 7550 7560
>< RsaI
Tru9I ><
> < Csp6I
MseI ><
>< BsrI
>< GsuI
>< MaeIIIDraI ><
>< AfaI
>< BpmI
> < BsrI
GACACATTTT GCACTGGTAG TACATTCATT AGTGATGAAG TTGCTCGTGA TTTGTCACTC CAGTTTAAAA
7570 7580 7590 7600 7610 7620 7630
>< Tha I
>< MvnI
> < HphI
HinP1I ><
>< HinP1I
>< Hin6I
>< Hin6I
HhaI ><
>< HhaI
CfoI ><
>< CfoI
>< BstUI
>< BssHII
Bsp50I ><
> < BsrI
>< AccII
GACCAATCAA CCCTACTGAC CAGTCATCGT ATATTGTTGA TAGTGTTGCT GTGAAAAATG GCGCGCTTCA
7640 7650 7660 7670 7680 7690 7700

FIGURE 13. 18


>< FokI
>< BsmAI
>< MnlI >< Alw26I >< AciI
CCTCTACTTT GACAAGGCTG GTCAAAAGAC CTATGAGAGA CATCCGCTCT CCCATTTTGT CAATTTAGAC
7710 7720 7730 7740 7750 7760 7770
>< VspI
>< Tru9I
>< MseI
>< AsnI
> < AluI >< AseI >< BcgI/a
AATTTGAGAG CTAACAACAC TAAAGGTTCA CTGCCTATTA ATGTCATAGT TTTTGATGGC AAGTCCAAAT
7780 7790 7800 7810 7820 7830 7840
>< SfcI >< PvuII
>< RsaI >< Psp5I
>< PleI >< Csp6I >< NspBII
>< HinfI >< DdeI >< BcgI >< AfaI >< AluI
GCGACGAGTC TGCTTCTAAG TCTGCTTCTG TGTACTACAG TCAGCTGATG TGCCAACCTA TTCTGTTGCT
7850 7860 7870 7880 7890 7900 7910
TthHB8I ><
TaqI ><
SalI ><
RtrI ><
>< ScaI HindII >
>< RsaI >< Tru9I HincII >
>< Csp6I >< SfaNI >< Eco57I
>< AluI >< MaeII >< AfaI >< MseI AccI ><
TGACCAAGCT CTTGTATCAG ACGTTGGAGA TAGTACTGAA GTTTCCGTTA AGATGTTTGA TGCTTATGTC
7920 7930 7940 7950 7960 7970 7980
>< Tru9I
>< MseI
> < Esp4I >< SfcI
> < AflII >< BspWI >< AluI
GACACCTTTT CAGCAACTTT TAGTGTTCCT ATGGAAAAAC TTAAGGCACT TGTTGCTACA GCTCACAGCG
7990 8000 8010 8020 8030 8040 8050
>< PvuII
>< Psp5I
>< NspBII
>< Fnu4HI
>< AluI >< BbvI >< AluI
AGTTAGCAAA GGGTGTAGCT TTAGATGGTG TCCTTTCTAC ATTCGTGTCA GCTGCCCGAC AAGGTGTTGT
8060 8070 8080 8090 8100 8110 8120
MaeIII ><
>< HindII >< BsmAI >< DdeI
>< HincII >< FokI>< Alw26I >< BfrI
TGATACCGAT GTTGACACAA AGGATGTTAT TGAATGTCTC AAACTTTCAC ATCACTCTGA CTTAGAAGTG
8130 8140 8150 8160 8170 8180 8190
>< XhoII
Sau3AI ><
>< NdeII
>< MflI
>< MboI
>< NlaIII >< HgaI
>< HinlI >< DpnII
DpnI ><

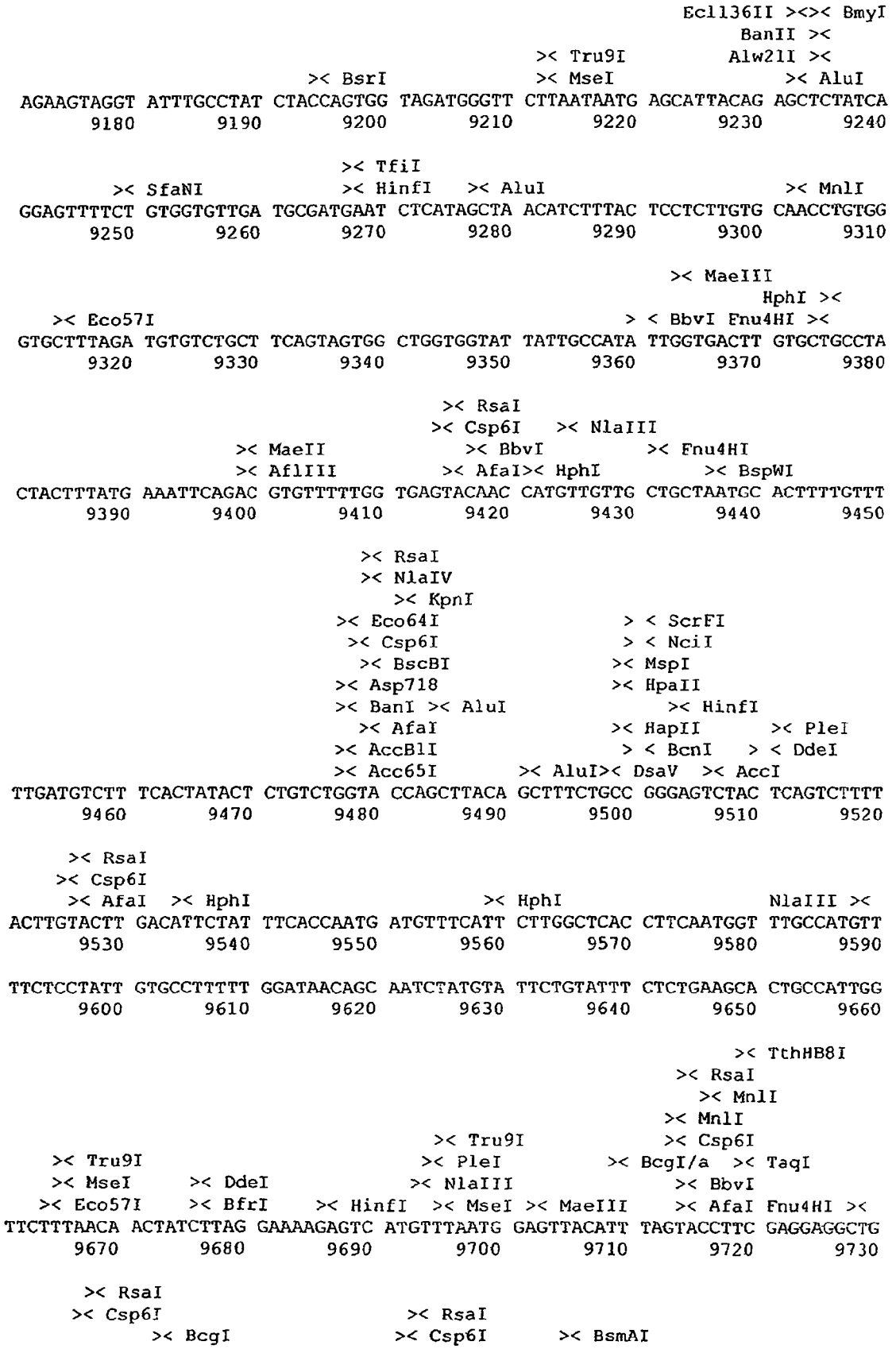
FIGURE 13.19

```
                                                                    Bsp143I ><
                                                                 >< BsaHI >< BstYI
                 >< MaeIII>< HphI                                >< BbiII >< BspAI
    >< MaeIII     >< HphI      >< NlaIII                         >< AcyI  >< BglII
 ACAGGTGACA GTTGTAACAA TTTCATGCTC ACCTATAATA AGGTTGAAAA CATGACGCCC AGAGATCTTG
       8200       8210       8220       8230       8240       8250       8260

       >< NspI
       >< NspHI
       >< NlaIII
 >< HinPlI
 >< Hin6I
   >< HhaI
   >< CfoI                                        >< BspWI  >< MaeIII
 GCGCATGTAT TGACTGTAAT GCAAGGCATA TCAATGCCCA AGTAGCAAAA AGTCACAATG TTTCACTCAT
       8270       8280       8290       8300       8310       8320       8330

                         >< NspI
                         >< NspHI        >< PvuII
                         >< NlaIII       >< Psp5I
                      >< Eam1105I        >< NspBII
                           >< BbvI        >< Fnu4HI
                    >< AflIII            >< AluI   >< BbvI        > < Fnu4HI
 CTGGAATGTA AAAGACTACA TGTCTTTATC TGAACAGCTG CGTAAACAAA TTCGTAGTGC TGCCAAGAAG
       8340       8350       8360       8370       8380       8390       8400

                                      >< RmaI
          >< MboII                    >< MaeI  >< Eam1105I
 AACAACATAC CTTTTAGACT AACTTGTGCT ACAACTAGAC AGGTTGTCAA TGTCATAACT ACTAAAATCT
       8410       8420       8430       8440       8450       8460       8470

                                                 >< Tru9I
                                                       >< PalI
                                                 >< MseI
                                                       >< HaeIII
                              >< ScaI             >< Esp4I
                              >< RsaI  >< Tru9I          >< BsuRI
                             >< Csp6I  >< MseI           >< BshI
                              >< AfaI   >< DraI    >< AflII       >< BbvI
 CACTCAAGGG TGGTAAGATT GTTAGTACTT GTTTTAAACT TATGCTTAAG GCCACATTAT TGTGCGTTCT
       8480       8490       8500       8510       8520       8530       8540

                                    >< RsaI
                                   >< Csp6I
                                >< BsrI                    >< NlaIII
   >< Fnu4HI                       >< AfaI                   >< MaeIII
 TGCTGCATTG GTTTGTTATA TCGTTATGCC AGTACATACA TTGTCAATCC ATGATGGTTA CACAAATGAA
       8550       8560       8570       8580       8590       8600       8610

                                          >< MaeIII
                                    > < MaeIII
      >< MaeIII                           >< FokI
 ATCATTGGTT ACAAAGCCAT TCAGGATGGT GTCACTCGTG ACATCATTTC TACTGATGAT TGTTTTGCAA
       8620       8630       8640       8650       8660       8670       8680

                                                                        SfcI >
        >< NspI                                                    Fnu4HI ><
        >< NspHI           >< NlaIII                                 BbvI ><
        >< NlaIII            >< HgaI       >< BstXI       >< BbvI       >< AluI
 ATAAACATGC TGGTTTTGAC GCATGGTTTA GCCAGCGTGG TGGTTCATAC AAAAATGACA AAAGCTGCCC
       8690       8700       8710       8720       8730       8740       8750
```

FIGURE 13. 20

```
                                                                  >< ScrFI
                                                    >< ScrFI        >< RsaI
                                                    >< MvaI  >< MspI
                                                  >< EcoRII  >< HpaII
                                                    >< Ecl136I>< NciI
                                                  >< DsaV    >< HapII
                                                    >< BstOI>< DsaV
                                                    >< BstNI      >< Csp6I
          >< Fnu4HI                                 >< BsiLI  >< BcnIDdeI ><
         >< AluI                                    >< ApyI       >< AfaI
TGTAGTAGCT GCTATCATTA CAAGAGAGAT TGGTTTCATA GTGCCTGGCT TACCGGGTAC TGTGCTGAGA
      8760       8770       8780       8790       8800       8810       8820

        > < MaeIII    >< HphI             >< MnlI            >< BspWI
GCAATCAATG GTGACTTCTT GCATTTTCTA CCTCGTGTTT TTAGTGCTGT TGGCAACATT TGCTACACAC
      8830       8840       8850       8860       8870       8880       8890

                                                                     Tru9I >
                                                                SfaNI ><
                                                                   >< RsaI
                                                                     MseI >
                                          >< BspWI          >< Fnu4HI >< Csp6I
                                            >< BbvI>< MnlI     >< DdeI >< AfaI
CTTCCAAACT CATTGAGTAT AGTGATTTTG CTACCTCTGC TTGCGTTCTT GCTGCTGAGT GTACAATTTT
      8900       8910       8920       8930       8940       8950       8960

                                                  > < RmaI
                                                >< MnlI
                  >< FokI                         > < MaeI
TAAGGATGCT ATGGGCAAAC CTGTGCCATA TTGTTATGAC ACTAATTTGC TAGAGGGTTC TATTTCTTAT
      8970       8980       8990       9000       9010       9020       9030

                                                                     ScrFI >
                                                                      MvaI >
                                                                 MnlI ><
                                                                  EcoRII ><
                                                                   Ecl136I >
                                                                    DsaV ><
                                                                     BstOI >
                                                  >< NlaIV           BstNI >
                                                        >< FokI      BsiLI >
     >< AluI                                        >< BscBI          ApyI >
AGTGAGCTTC GTCCAGACAC TCGTTATGTG CTTATGGATG GTTCCATCAT ACAGTTTCCT AACACTTACC
      9040       9050       9060       9070       9080       9090       9100

                                                              >< RsaI
                                                    >< SfcI         >< NspI
                                                  >< ScaI           >< NspHI
                        >< SfaNI                  >< RsaI           >< NlaIII
                    > < MaeIII                  >< Csp6I      >< NlaIII
                      >< GsuI                   >< AfaI       >< Csp6I
                      >< BpmI             >< DdeI    >< AccI   >< AfaI
TGGAGGGTTC TGTTAGAGTA GTAACAACTT TTGATGCTGA GTACTGTAGA CATGGTACAT GCGAAAGGTC
      9110       9120       9130       9140       9150       9160       9170

                                                                     >< SstI
                                                                     >< SduI
                                                                     >< SacI
                                                                NspII ><
                                                                HgiAI ><
                                                               Eco24I ><
                                                            Bsp1286I ><
```

FIGURE 13.21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BanII ><
>< Tru9I Alw21I ><
>< BsrI >< MseI >< AluI
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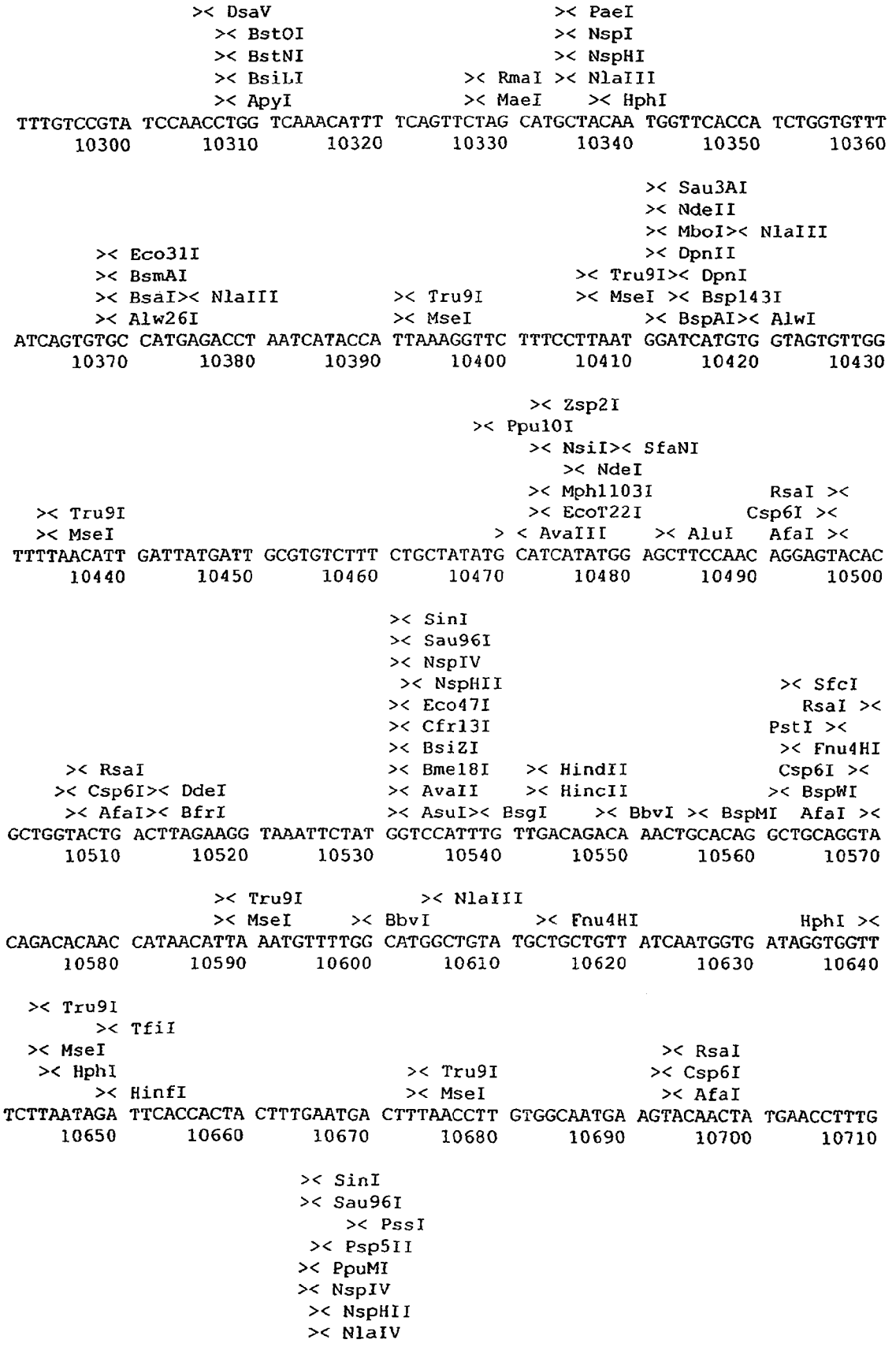
9180 9190 9200 9210 9220 9230 9240
>< TfiI
>< SfaNI >< HinfI >< AluI >< MnlI
GGAGTTTTCT GTGGTGTTGA TGCGATGAAT CTCATAGCTA ACATCTTTAC TCCTCTTGTG CAACCTGTGG
9250 9260 9270 9280 9290 9300 9310
>< MaeIII
HphI ><
>< Eco57I > < BbvI Fnu4HI ><
GTGCTTTAGA TGTGTCTGCT TCAGTAGTGG CTGGTGGTAT TATTGCCATA TTGGTGACTT GTGCTGCCTA
9320 9330 9340 9350 9360 9370 9380
>< RsaI
>< Csp6I >< NlaIII
>< MaeII >< BbvI >< Fnu4HI
>< AflIII >< AfaI>< HphI >< BspWI
CTACTTTATG AAATTCAGAC GTGTTTTTGG TGAGTACAAC CATGTTGTTG CTGCTAATGC ACTTTTGTTT
9390 9400 9410 9420 9430 9440 9450
>< RsaI
>< NlaIV
>< KpnI
>< Eco64I > < ScrFI
>< Csp6I > < NciI
>< BscBI >< MspI
>< Asp718 >< HpaII
>< BanI >< AluI >< HinfI
>< AfaI >< HapII >< PleI
>< AccBlI > < BcnI > < DdeI
>< Acc65I >< AluI>< DsaV >< AccI
TTGATGTCTT TCACTATACT CTGTCTGGTA CCAGCTTACA GCTTTCTGCC GGGAGTCTAC TCAGTCTTTT
9460 9470 9480 9490 9500 9510 9520
>< RsaI
>< Csp6I
>< AfaI >< HphI >< HphI NlaIII ><
ACTTGTACTT GACATTCTAT TTCACCAATG ATGTTTCATT CTTGGCTCAC CTTCAATGGT TTGCCATGTT
9530 9540 9550 9560 9570 9580 9590
TTCTCCTATT GTGCCTTTTT GGATAACAGC AATCTATGTA TTCTGTATTT CTCTGAAGCA CTGCCATTGG
9600 9610 9620 9630 9640 9650 9660
>< TthHB8I
>< RsaI
>< MnlI
>< MnlI
>< Tru9I >< Csp6I
>< Tru9I >< PleI >< BcgI/a >< TaqI
>< MseI >< DdeI >< NlaIII >< BbvI
>< Eco57I >< BfrI >< HinfI >< MseI >< MaeIII >< AfaI Fnu4HI ><
TTCTTTAACA ACTATCTTAG GAAAAGAGTC ATGTTTAATG GAGTTACATT TAGTACCTTC GAGGAGGCTG
9670 9680 9690 9700 9710 9720 9730
>< RsaI
>< Csp6I
>< RsaI
>< BcgI >< Csp6I >< BsmAI

FIGURE 13.22

```
       >< AfaI                    >< AfaI      >< Alw26I
CTTTGTGTAC CTTTTTGCTC AACAAGGAAA TGTACCTAAA ATTGCGTAGC GAGACACTGT TGCCACTTAC
      9740       9750       9760       9770       9780       9790       9800

                                                    >< NlaIV
                                  >< RsaI              >< DdeI
                                 >< Csp6I            >< BscBI
                                  >< AfaI              >< BfrI   AluI ><
ACAGTATAAC AGGTATCTTG CTCTATATAA CAAGTACAAG TATTTCAGTG GAGCCTTAGA TACTACCAGC
      9810       9820       9830       9840       9850       9860       9870

                  >< Fnu4HI
                        >< DdeI
            >< Fnu4HI   >< BfrI
   >< BbvI   >< AluI  >< BbvI                          >< DdeI >< AlwNI
TATCGTGAAG CAGCTTGCTG CCACTTAGCA AAGGCTCTAA ATGACTTTAG CAACTCAGGT GCTGATGTTC
      9880       9890       9900       9910       9920       9930       9940

                                        >< SfcI                    >< BsmI
                                             >< PstI          >< BscCI
TCTACCAACC ACCACAGACA TCAATCACTT CTGCTGTTCT GCAGAGTGGT TTTAGGAAAA TGGCATTCCC
      9950       9960       9970       9980       9990      10000      10010

                          >< RsaI
                         >< NlaIII
                               >< MaeIII
                          >< Csp6I                    >< Tru9I
                           >< AfaI                    >< MseI
GTCAGGCAAA GTTGAAGGGT GCATGGTACA AGTAACCTGT GGAACTACAA CTCTTAATGG ATTGTGGTTG
     10020      10030      10040      10050      10060      10070      10080

                                                                 XhoII ><
                                                                Sau3AI ><
                                                  >< Tru9I      NdeII ><
                                                ><  NspI        MflI ><
                                                ><  NspHI       MboI ><
                          >< NspI               ><  NlaIII      DpnII ><
            >< FokI       >< NspHI                >< MseI      BstYI ><
           >< Bst1107I    >< NlaIII                 >< MboII  BspAI ><
          >< AccI      >< AflIII                 > < BbsI      BglII ><
GATGACACAG TATACTGTCC AAGACATGTC ATTTGCACAG CAGAAGACAT GCTTAATCCT AACTATGAAG
     10090      10100      10110      10120      10130      10140      10150

                                                                  PalI >
                                                                  MscI >
                                                                 HaeIII >
                                                                 EaeI ><
                                                                  BsuRI >
><  DpnI >< MboII                                                 BshI >
><  Bsp143I                 >< AluI                               BalI >
ATCTGCTCAT TCGCAAATCC AACCATAGCT TTCTTGTTCA GGCTGGCAAT GTTCAACTTC GTGTTATTGG
     10160      10170      10180      10190      10200      10210      10220

                       >< DdeI> < Tru9I
                       >< BfrI> < MseI              >< DdeI
CCATTCTATG CAAAATTGTC TGCTTAGGCT TAAAGTTGAT ACTTCTAACC CTAAGACACC CAAGTATAAA
     10230      10240      10250      10260      10270      10280      10290

                 >< ScrFI
                 >< MvaI
               >< EcoRII
                 >< Ecl136I                  >< SphI
```

FIGURE 13.23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>< BstOI
>< BstNI
>< BsiLI
>< ApyI
>< PaeI
>< NspI
>< NspHI
>< RmaI >< NlaIII
>< MaeI >< HphI
TTTGTCCGTA TCCAACCTGG TCAAACATTT TCAGTTCTAG CATGCTACAA TGGTTCACCA TCTGGTGTTT
10300 10310 10320 10330 10340 10350 10360
>< Sau3AI
>< NdeII
>< MboI>< NlaIII
>< Eco31I
>< DpnII
>< BsmAI
>< Tru9I>< DpnI
>< BsaI>< NlaIII
>< Tru9I
>< MseI >< Bsp143I
>< Alw26I
>< MseI
>< BspAI>< AlwI
ATCAGTGTGC CATGAGACCT AATCATACCA TTAAAGGTTC TTTCCTTAAT GGATCATGTG GTAGTGTTGG
10370 10380 10390 10400 10410 10420 10430
>< Zsp2I
>< Ppu10I
>< NsiI>< SfaNI
>< NdeI
>< Mph1103I
RsaI ><
>< Tru9I
>< MseI
>< EcoT22I
Csp6I ><
> < AvaIII >< AluI AfaI ><
TTTTAACATT GATTATGATT GCGTGTCTTT CTGCTATATG CATCATATGG AGCTTCCAAC AGGAGTACAC
10440 10450 10460 10470 10480 10490 10500
>< SinI
>< Sau96I
>< NspIV
>< NspHII
>< SfcI
RsaI ><
>< Eco47I
>< Cfr13I
PstI ><
>< BsiZI
>< Fnu4HI
>< RsaI
>< Bme18I >< HindII
Csp6I ><
>< Csp6I>< DdeI
>< AvaII >< HincII
>< BspWI
>< AfaI>< BfrI
>< AsuI>< BsgI >< BbvI >< BspMI AfaI ><
GCTGGTACTG ACTTAGAAGG TAAATTCTAT GGTCCATTTG TTGACAGACA AACTGCACAG GCTGCAGGTA
10510 10520 10530 10540 10550 10560 10570
>< Tru9I
>< NlaIII
>< MseI
>< BbvI
>< Fnu4HI
HphI ><
CAGACACAAC CATAACATTA AATGTTTTGG CATGGCTGTA TGCTGCTGTT ATCAATGGTG ATAGGTGGTT
10580 10590 10600 10610 10620 10630 10640
>< Tru9I
>< TfiI
>< MseI
>< RsaI
>< HphI
>< Tru9I
>< Csp6I
>< HinfI
>< MseI
>< AfaI
TCTTAATAGA TTCACCACTA CTTTGAATGA CTTTAACCTT GTGGCAATGA AGTACAACTA TGAACCTTTG
10650 10660 10670 10680 10690 10700 10710
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>< Psp5II
>< PpuMI
>< NspIV
>< NspHII
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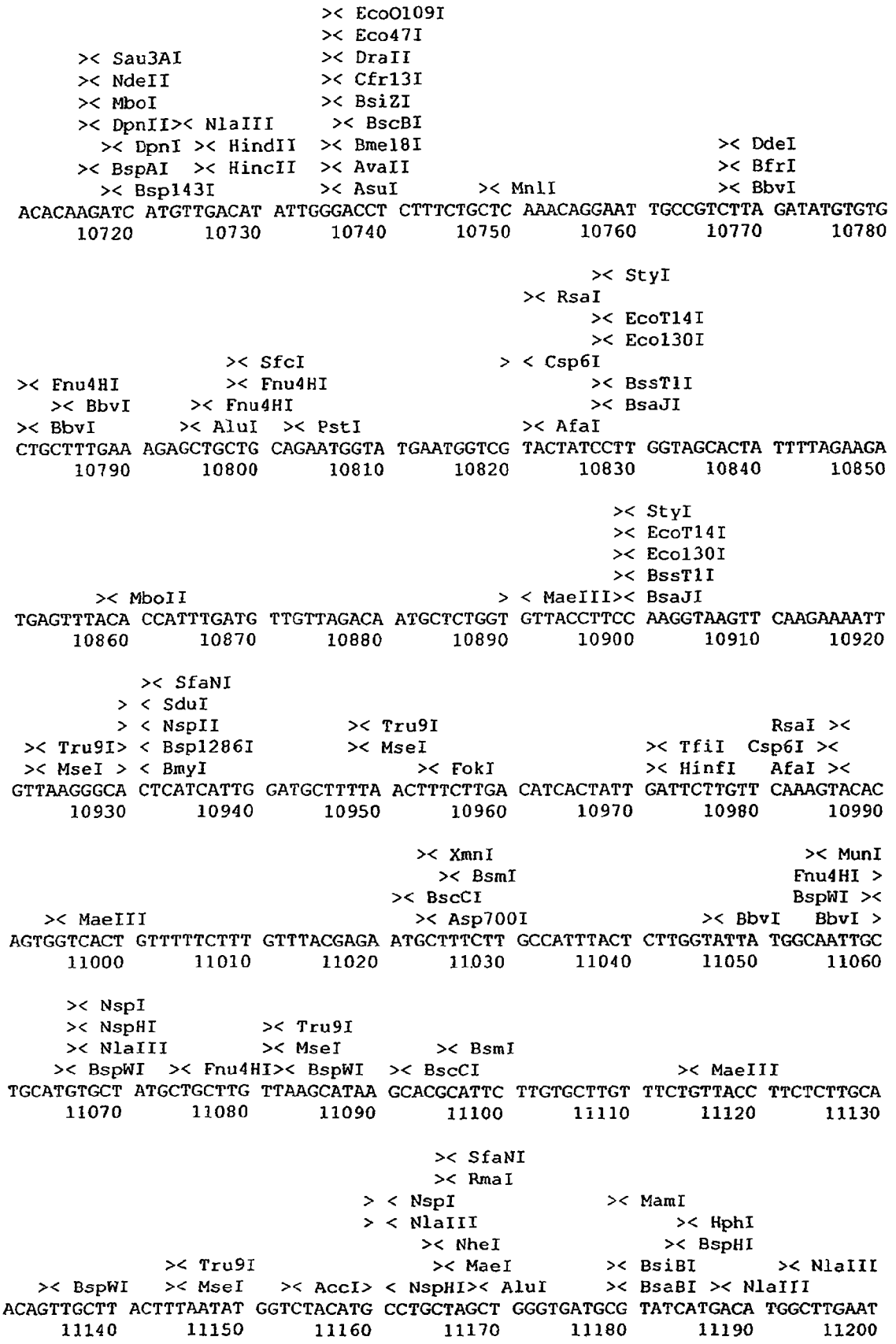
FIGURE 13. 24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>< NdeII >< Cfr13I
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>< DpnII>< NlaIII >< BscBI
>< DpnI >< HindII >< Bme18I >< DdeI
>< BspAI >< HincII >< AvaII >< BfrI
>< Bsp143I >< AsuI >< MnlI >< BbvI
ACACAAGATC ATGTTGACAT ATTGGGACCT CTTTCTGCTC AAACAGGAAT TGCCGTCTTA GATATGTGTG
10720 10730 10740 10750 10760 10770 10780
>< StyI
>< RsaI
>< EcoT14I
>< Eco130I
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>< Fnu4HI >< Fnu4HI >< BssT1I
>< BbvI >< Fnu4HI >< BsaJI
>< BbvI >< AluI >< PstI >< AfaI
CTGCTTTGAA AGAGCTGCTG CAGAATGGTA TGAATGGTCG TACTATCCTT GGTAGCACTA TTTTAGAAGA
10790 10800 10810 10820 10830 10840 10850
>< StyI
>< EcoT14I
>< Eco130I
>< BssT1I
>< MboII > < MaeIII>< BsaJI
TGAGTTTACA CCATTTGATG TTGTTAGACA ATGCTCTGGT GTTACCTTCC AAGGTAAGTT CAAGAAAATT
10860 10870 10880 10890 10900 10910 10920
>< SfaNI
> < SduI
> < NspII >< Tru9I RsaI ><
>< Tru9I> < Bsp1286I >< MseI >< TfiI Csp6I ><
>< MseI > < BmyI >< FokI >< HinfI AfaI ><
GTTAAGGGCA CTCATCATTG GATGCTTTTA ACTTTCTTGA CATCACTATT GATTCTTGTT CAAAGTACAC
10930 10940 10950 10960 10970 10980 10990
>< XmnI >< MunI
>< BsmI Fnu4HI >
>< BscCI BspWI ><
>< MaeIII >< Asp700I >< BbvI BbvI >
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>< NspI
>< NspHI >< Tru9I
>< NlaIII >< MseI >< BsmI
>< BspWI >< Fnu4HI>< BspWI >< BscCI >< MaeIII
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11070 11080 11090 11100 11110 11120 11130
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>< RmaI
> < NspI >< MamI
> < NlaIII >< HphI
>< NheI >< BspHI
>< Tru9I >< MaeI >< BsiBI >< NlaIII
>< BspWI >< MseI >< AccI> < NspHI>< AluI >< BsaBI >< NlaIII
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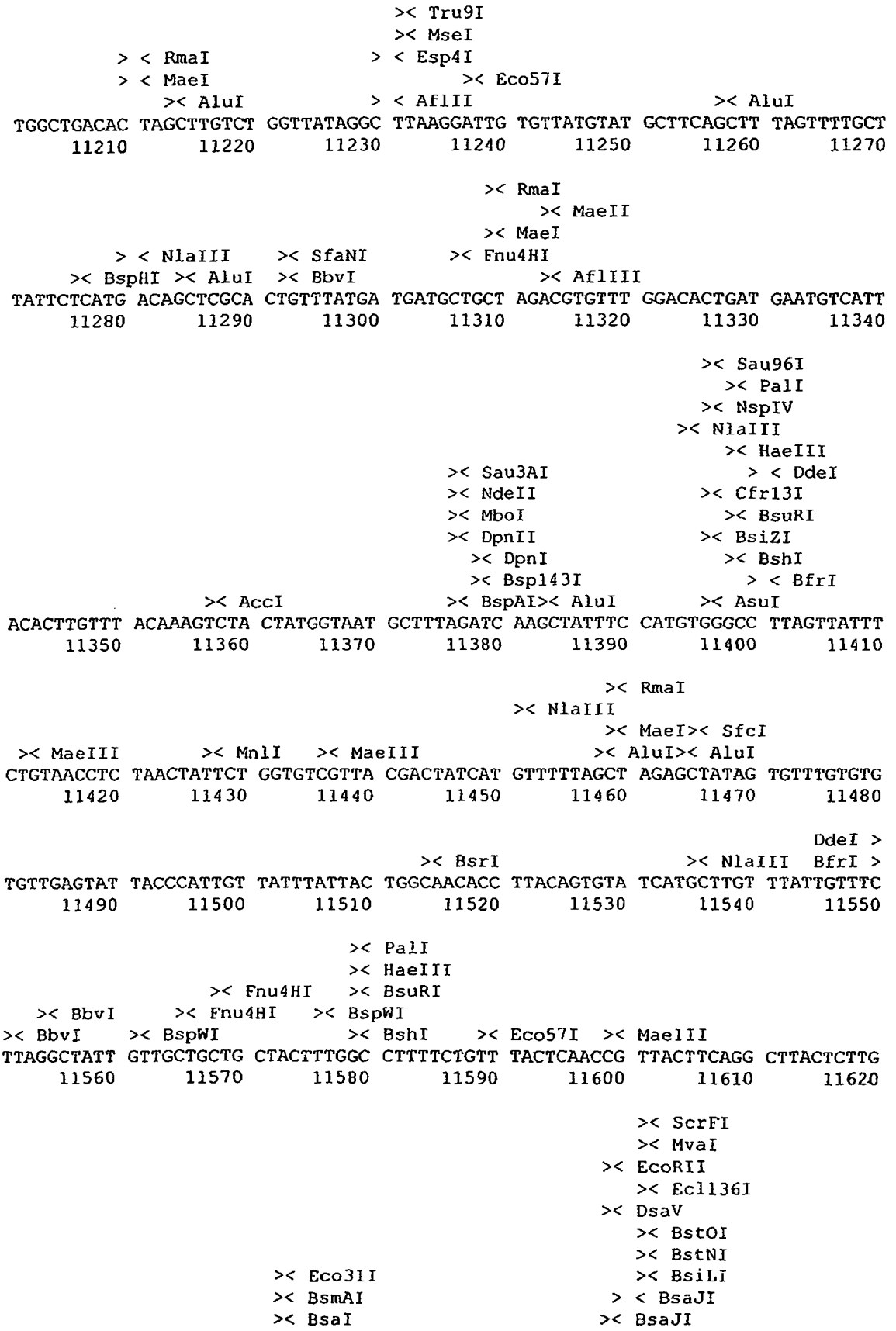
11140 11150 11160 11170 11180 11190 11200

FIGURE 13.25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11210 11220 11230 11240 11250 11260 11270
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>< MaeII
>< MaeI
> < NlaIII
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>< Fnu4HI
>< BspHI >< AluI
>< BbvI
>< AflIII
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11280 11290 11300 11310 11320 11330 11340
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>< PalI
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>< Sau3AI
> < DdeI
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>< MboI
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>< DpnII
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>< DpnI
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>< AsuI
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11350 11360 11370 11380 11390 11400 11410
>< RmaI
>< NlaIII
>< MaeI>< SfcI
>< MaeIII
>< MnlI
>< MaeIII
>< AluI>< AluI
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11420 11430 11440 11450 11460 11470 11480
DdeI >
>< BsrI
>< NlaIII BfrI >
TGTTGAGTAT TACCCATTGT TATTTATTAC TGGCAACACC TTACAGTGTA TCATGCTTGT TTATTGTTTC
11490 11500 11510 11520 11530 11540 11550
>< PalI
>< HaeIII
>< Fnu4HI
>< BsuRI
>< BbvI
>< Fnu4HI
>< BspWI
>< BbvI
>< BspWI
>< BshI
>< Eco57I
>< MaeIII
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11560 11570 11580 11590 11600 11610 11620
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>< MvaI
>< EcoRII
>< Ecl136I
>< DsaV
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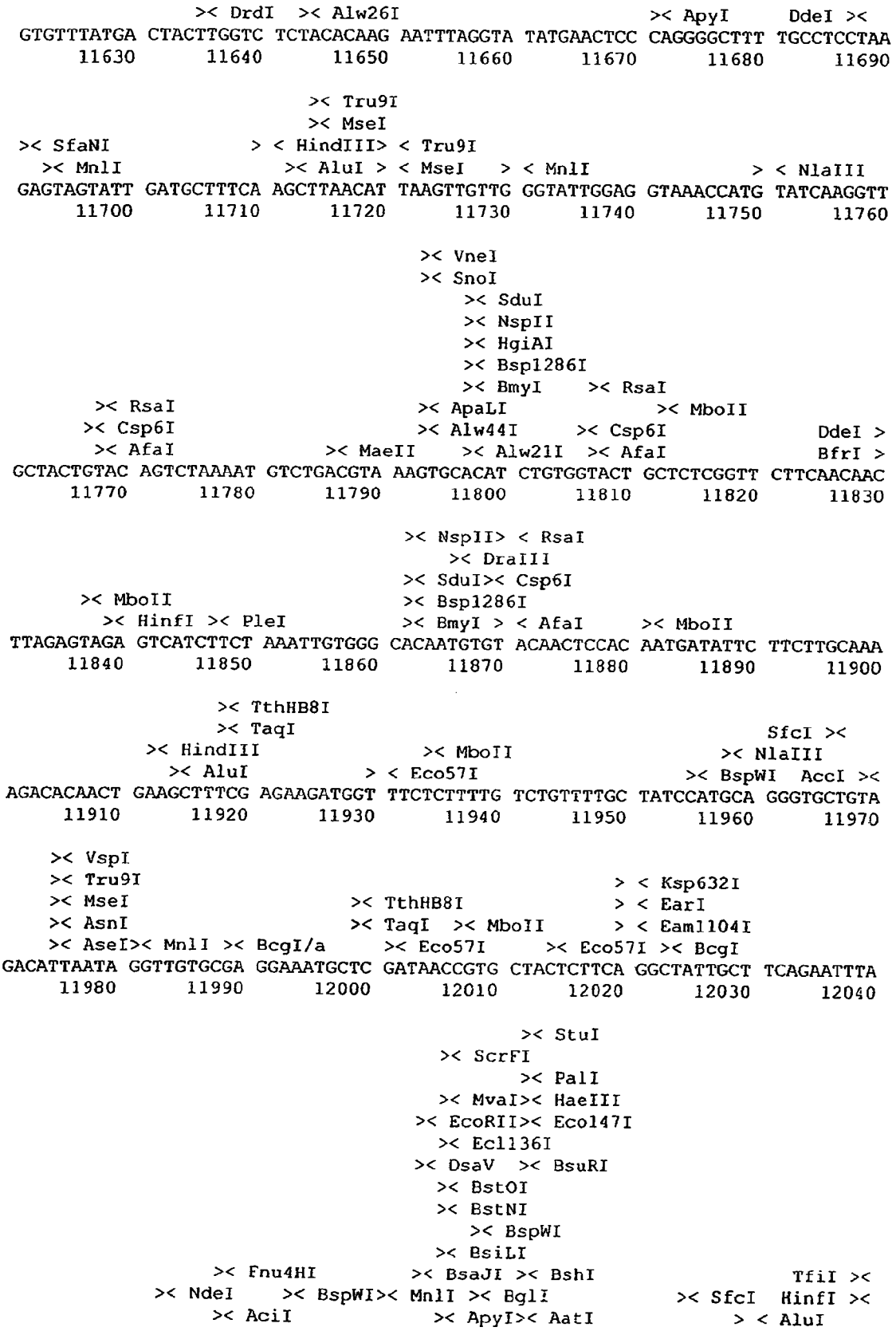
>< BsaJI

FIGURE 13.26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>< MseI
>< SfaNI > < HindIII> < Tru9I
>< MnlI >< AluI > < MseI > < MnlI > < NlaIII
GAGTAGTATT GATGCTTTCA AGCTTAACAT TAAGTTGTTG GGTATTGGAG GTAAACCATG TATCAAGGTT
11700 11710 11720 11730 11740 11750 11760
>< VneI
>< SnoI
>< SduI
>< NspII
>< HgiAI
>< Bsp1286I
>< BmyI >< RsaI
>< RsaI >< ApaLI >< MboII
>< Csp6I >< Alw44I >< Csp6I DdeI >
>< AfaI >< MaeII >< Alw21I >< AfaI BfrI >
GCTACTGTAC AGTCTAAAAT GTCTGACGTA AAGTGCACAT CTGTGGTACT GCTCTCGGTT CTTCAACAAC
11770 11780 11790 11800 11810 11820 11830
>< NspII> < RsaI
>< DraIII
>< SduI>< Csp6I
>< MboII >< Bsp1286I
>< HinfI >< PleI >< BmyI > < AfaI >< MboII
TTAGAGTAGA GTCATCTTCT AAATTGTGGG CACAATGTGT ACAACTCCAC AATGATATTC TTCTTGCAAA
11840 11850 11860 11870 11880 11890 11900
>< TthHB8I
>< TaqI
SfcI ><
>< HindIII >< MboII >< NlaIII
>< AluI > < Eco57I >< BspWI AccI ><
AGACACAACT GAAGCTTTCG AGAAGATGGT TTCTCTTTTG TCTGTTTTGC TATCCATGCA GGGTGCTGTA
11910 11920 11930 11940 11950 11960 11970
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>< MseI >< TthHB8I > < EarI
>< AsnI >< TaqI >< MboII > < Eam1104I
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11980 11990 12000 12010 12020 12030 12040
>< StuI
>< ScrFI
>< PalI
>< MvaI>< HaeIII
>< EcoRII>< Eco147I
>< Ecl136I
>< DsaV >< BsuRI
>< BstOI
>< BstNI
>< BspWI
>< BsiLI
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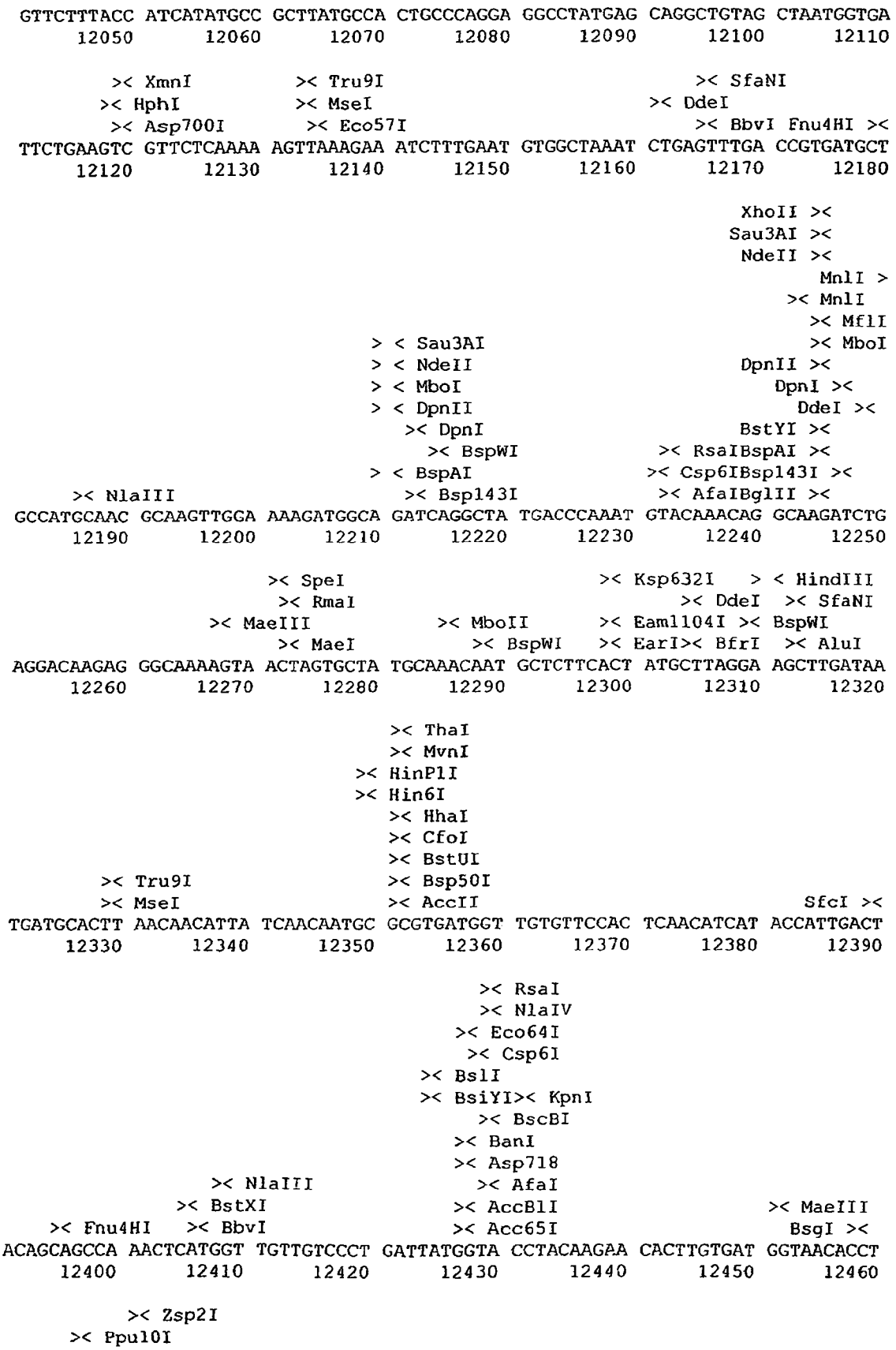
>< AciI >< ApyI>< AatI > < AluI

FIGURE 13. 27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>< HphI
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>< Tru9I
>< MseI
>< Eco57I
>< SfaNI
>< DdeI
>< BbvI Fnu4HI ><
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12120 12130 12140 12150 12160 12170 12180
XhoII ><
Sau3AI ><
NdeII ><
MnlI >
>< MnlI
>< MflI
>< MboI
> < Sau3AI
> < NdeII
> < MboI
> < DpnII
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DpnI ><
DdeI ><
>< DpnI
>< BspWI
BstYI ><
>< RsaIBspAI ><
> < BspAI
>< Csp6IBsp143I ><
>< NlaIII
>< Bsp143I
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GCCATGCAAC GCAAGTTGGA AAAGATGGCA GATCAGGCTA TGACCCAAAT GTACAAACAG GCAAGATCTG
12190 12200 12210 12220 12230 12240 12250
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>< RmaI
>< MaeIII
>< MaeI
>< MboII
>< BspWI
>< Ksp632I
>< Eam1104I
>< EarI
> < HindIII
>< DdeI
>< SfaNI
>< BspWI
>< BfrI
>< AluI
AGGACAAGAG GGCAAAAGTA ACTAGTGCTA TGCAAACAAT GCTCTTCACT ATGCTTAGGA AGCTTGATAA
12260 12270 12280 12290 12300 12310 12320
>< ThaI
>< MvnI
>< HinP1I
>< Hin6I
>< HhaI
>< CfoI
>< BstUI
>< Bsp50I
>< AccII
>< Tru9I
>< MseI
SfcI ><
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12330 12340 12350 12360 12370 12380 12390
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>< NlaIV
>< Eco64I
>< Csp6I
>< BslI
>< BsiYI
>< KpnI
>< BscBI
>< BanI
>< Asp718
>< AfaI
>< AccBlI
>< Acc65I
>< NlaIII
>< BstXI
>< Fnu4HI
>< BbvI
>< MaeIII
BsgI ><
ACAGCAGCCA AACTCATGGT TGTTGTCCCT GATTATGGTA CCTACAAGAA CACTTGTGAT GGTAACACCT
12400 12410 12420 12430 12440 12450 12460
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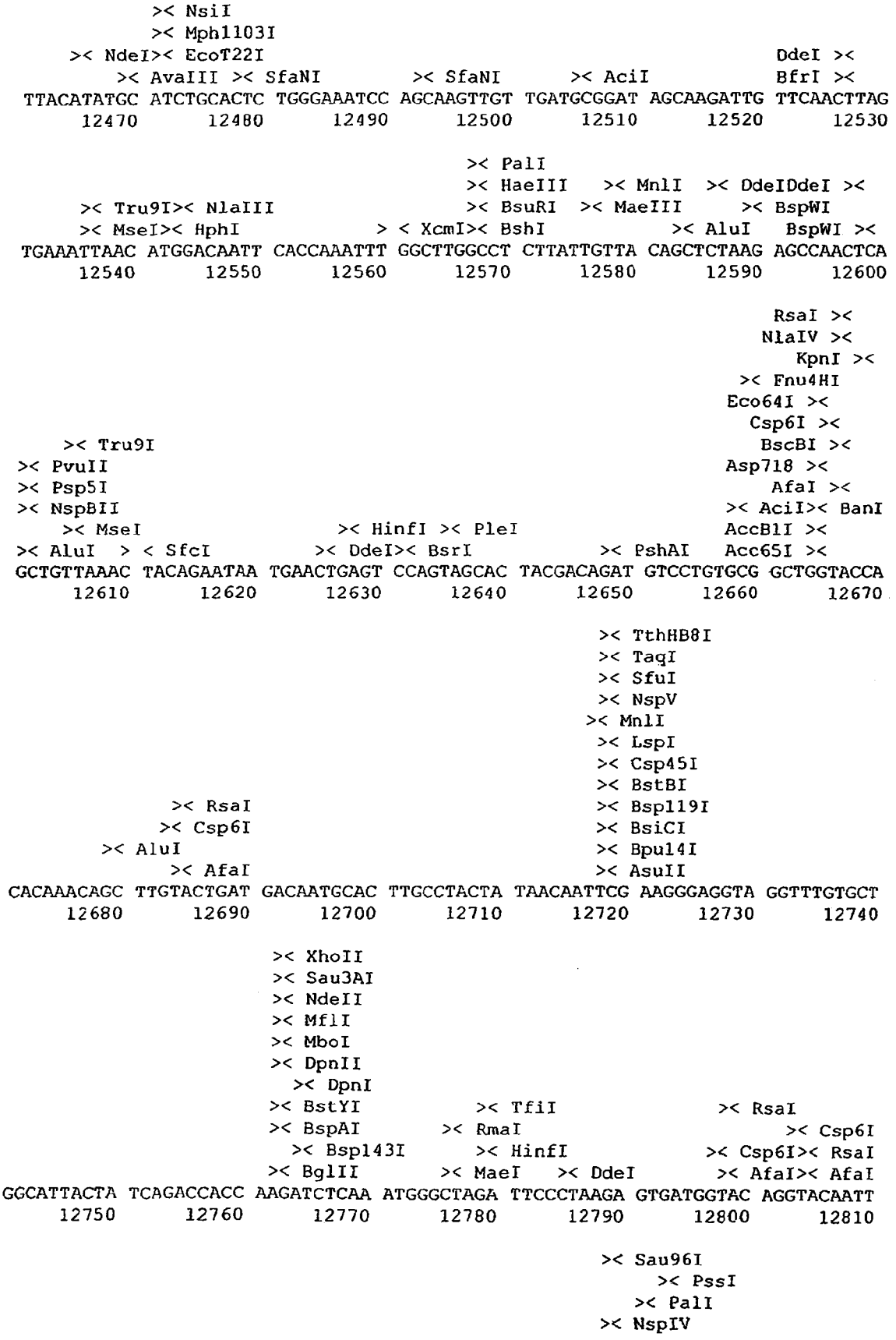
FIGURE 13. 28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12470 12480 12490 12500 12510 12520 12530
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> < XcmI>< BshI
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12540 12550 12560 12570 12580 12590 12600
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NlaIV ><
KpnI ><
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Eco64I ><
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>< Psp5I
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>< NspBII
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>< MseI
>< HinfI >< PleI
AccB1I ><
>< AluI > < SfcI
>< DdeI>< BsrI
>< PshAI
Acc65I ><
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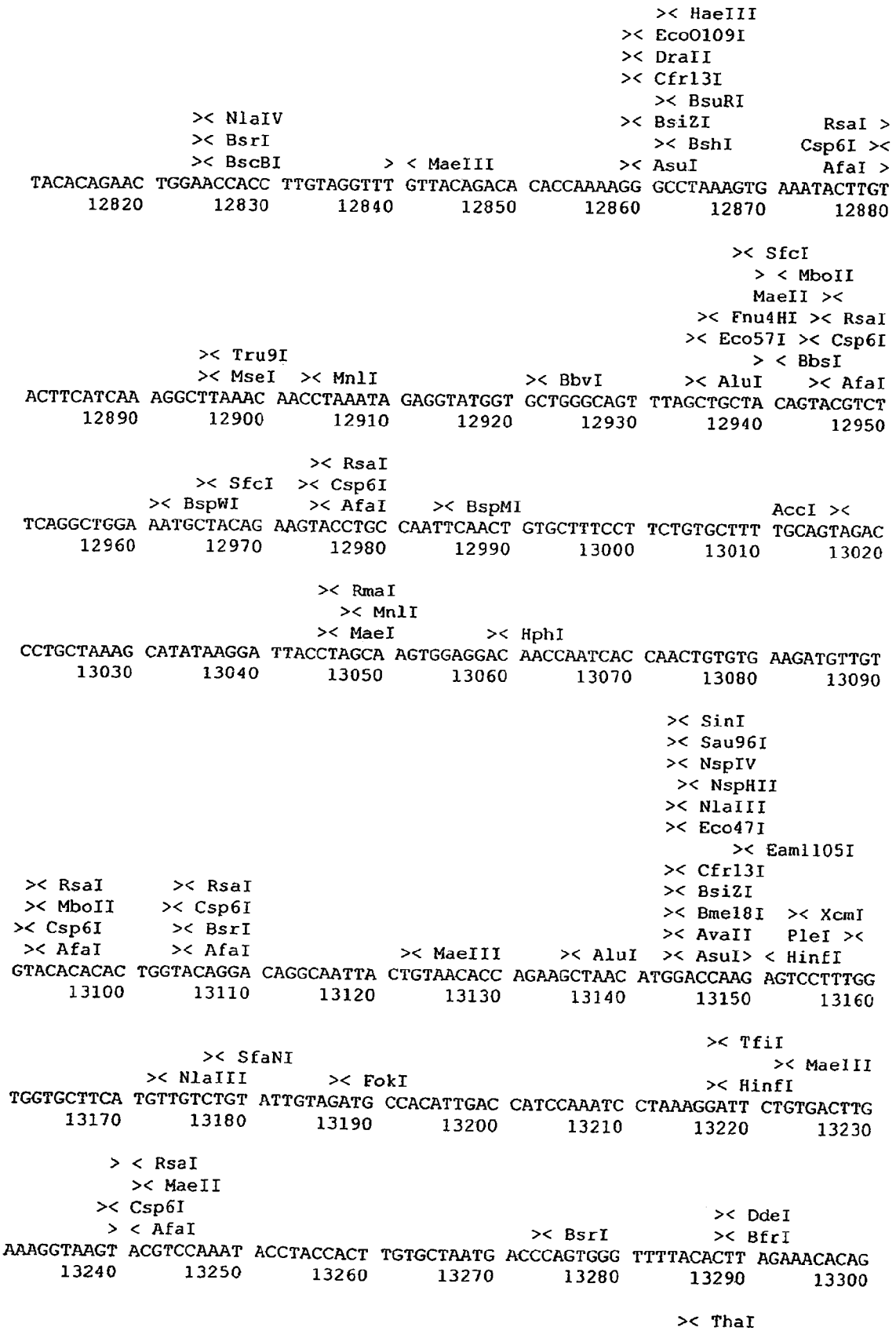
>< TthHB8I
>< TaqI
>< SfuI
>< NspV
>< MnlI
>< LspI
>< Csp45I
>< BstBI
>< RsaI
>< Bsp119I
>< Csp6I
>< BsiCI
>< AluI
>< Bpu14I
>< AfaI
>< AsuII
CACAAACAGC TTGTACTGAT GACAATGCAC TTGCCTACTA TAACAATTCG AAGGGAGGTA GGTTTGTGCT
12680 12690 12700 12710 12720 12730 12740
>< XhoII
>< Sau3AI
>< NdeII
>< MflI
>< MboI
>< DpnII
>< DpnI
>< BstYI
>< TfiI
>< RsaI
>< BspAI
>< RmaI
>< Csp6I
>< Bsp143I
>< HinfI
>< Csp6I>< RsaI
>< BglII
>< MaeI >< DdeI
>< AfaI>< AfaI
GGCATTACTA TCAGACCACC AAGATCTCAA ATGGGCTAGA TTCCCTAAGA GTGATGGTAC AGGTACAATT
12750 12760 12770 12780 12790 12800 12810
>< Sau96I
>< PssI
>< PalI
>< NspIV

FIGURE 13.29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RsaI >
Csp6I ><
AfaI >
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> < MboII
MaeII ><
>< Fnu4HI >< RsaI
>< Eco57I >< Csp6I
> < BbsI
>< Tru9I
>< MseI >< MnlI
>< BbvI
>< AluI
>< AfaI
ACTTCATCAA AGGCTTAAAC AACCTAAATA GAGGTATGGT GCTGGGCAGT TTAGCTGCTA CAGTACGTCT
12890 12900 12910 12920 12930 12940 12950
>< RsaI
>< SfcI >< Csp6I
>< BspWI >< AfaI >< BspMI
AccI ><
TCAGGCTGGA AATGCTACAG AAGTACCTGC CAATTCAACT GTGCTTTCCT TCTGTGCTTT TGCAGTAGAC
12960 12970 12980 12990 13000 13010 13020
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>< MnlI
>< MaeI
>< HphI
CCTGCTAAAG CATATAAGGA TTACCTAGCA AGTGGAGGAC AACCAATCAC CAACTGTGTG AAGATGTTGT
13030 13040 13050 13060 13070 13080 13090
>< SinI
>< Sau96I
>< NspIV
>< NspHII
>< NlaIII
>< Eco47I
>< Eam1105I
>< Cfr13I
>< BsiZI
>< Bme18I >< XcmI
>< AvaII PleI ><
>< RsaI
>< MboII
>< Csp6I
>< AfaI
>< RsaI
>< Csp6I
>< BsrI
>< AfaI
>< MaeIII
>< AluI
>< AsuI> < HinfI
GTACACACAC TGGTACAGGA CAGGCAATTA CTGTAACACC AGAAGCTAAC ATGGACCAAG AGTCCTTTGG
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>< TfiI
>< SfaNI
>< MaeIII
>< NlaIII
>< FokI
>< HinfI
TGGTGCTTCA TGTTGTCTGT ATTGTAGATG CCACATTGAC CATCCAAATC CTAAAGGATT CTGTGACTTG
13170 13180 13190 13200 13210 13220 13230
> < RsaI
>< MaeII
>< Csp6I
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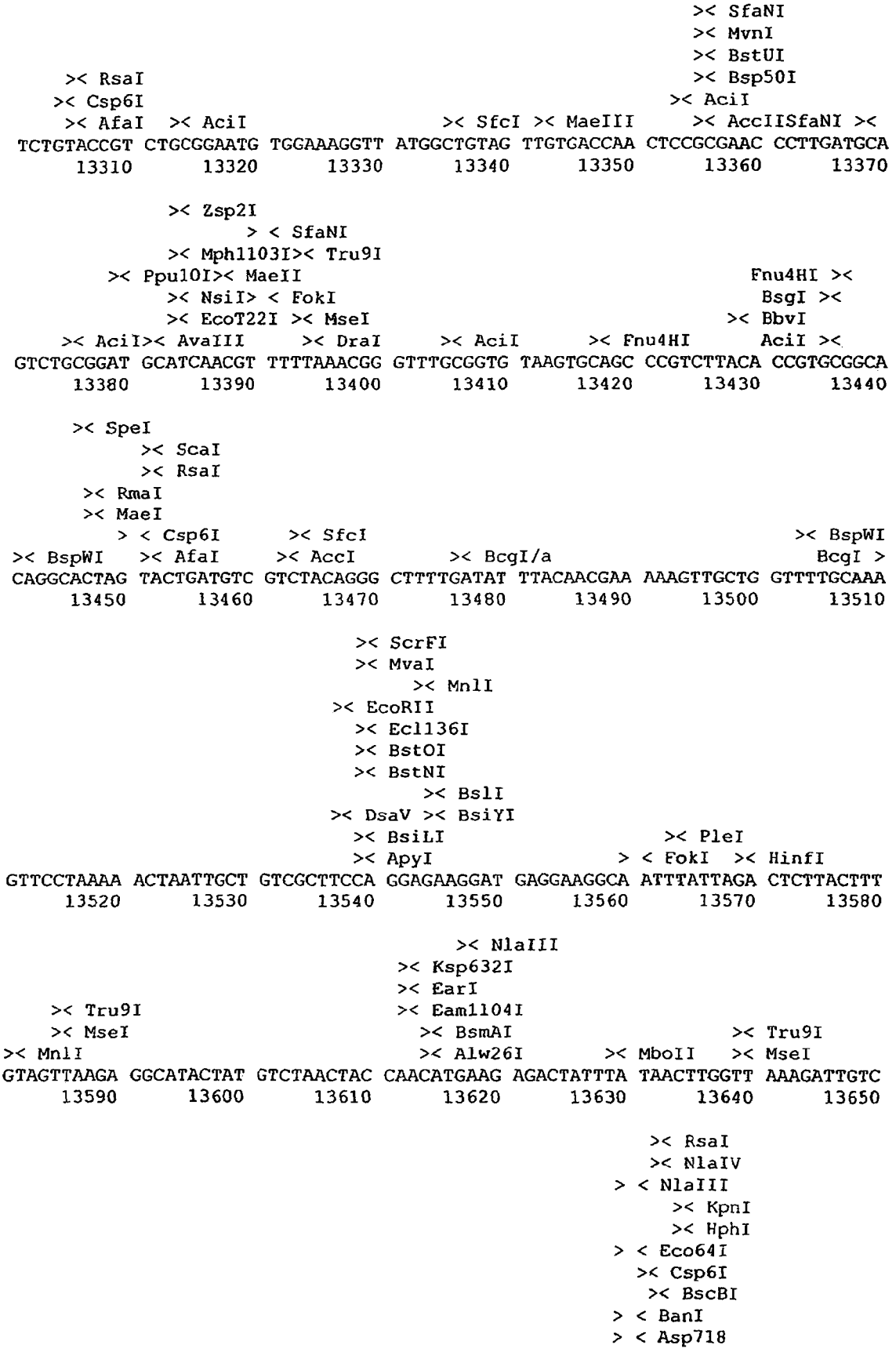
>< BsrI
>< DdeI
>< BfrI
AAAGGTAAGT ACGTCCAAAT ACCTACCACT TGTGCTAATG ACCCAGTGGG TTTTACACTT AGAAACACAG
13240 13250 13260 13270 13280 13290 13300
>< ThaI

FIGURE 13.30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> < SfaNI
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>< NsiI> < FokI BsgI ><
>< EcoT22I >< MseI >< BbvI
>< AciI>< AvaIII >< DraI >< AciI >< Fnu4HI AciI ><
GTCTGCGGAT GCATCAACGT TTTTAAACGG GTTTGCGGTG TAAGTGCAGC CCGTCTTACA CCGTGCGGCA
13380 13390 13400 13410 13420 13430 13440
>< SpeI
>< ScaI
>< RsaI
>< RmaI
>< MaeI
> < Csp6I >< SfcI >< BspWI
>< BspWI >< AfaI >< AccI >< BcgI/a BcgI >
CAGGCACTAG TACTGATGTC GTCTACAGGG CTTTTGATAT TTACAACGAA AAAGTTGCTG GTTTTGCAAA
13450 13460 13470 13480 13490 13500 13510
>< ScrFI
>< MvaI
>< MnlI
>< EcoRII
>< Ecl136I
>< BstOI
>< BstNI
>< BslI
>< DsaV >< BsiYI
>< BsiLI >< PleI
>< ApyI > < FokI >< HinfI
GTTCCTAAAA ACTAATTGCT GTCGCTTCCA GGAGAAGGAT GAGGAAGGCA ATTTATTAGA CTCTTACTTT
13520 13530 13540 13550 13560 13570 13580
>< NlaIII
>< Ksp632I
>< EarI
>< Tru9I >< Eam1104I
>< MseI >< BsmAI >< Tru9I
>< MnlI >< Alw26I >< MboII >< MseI
GTAGTTAAGA GGCATACTAT GTCTAACTAC CAACATGAAG AGACTATTTA TAACTTGGTT AAAGATTGTC
13590 13600 13610 13620 13630 13640 13650
>< RsaI
>< NlaIV
> < NlaIII
>< KpnI
>< HphI
> < Eco64I
>< Csp6I
>< BscBI
> < BanI
> < Asp718

FIGURE 13.31

```
                                                  >< MaeIII  >< AfaI
   >< NspBII                                                > < AccBlI MaeII ><
   >< AciI          >< NlaIII                               > < Acc65I > < HgaI
 CAGCGGTTGC TGTCCATGAC TTTTTCAAGT TTAGAGTAGA TGGTGACATG GTACCACATA TATCACGTCA
      13660      13670      13680      13690      13700      13710      13720

                                                      >< MnlI
                                                  >< MaeII
 GCGTCTAACT AAATACACAA TGGCTGATTT AGTCTATGCT CTACGTCATT TTGATGAGGG TAATTGTGAT
      13730      13740      13750      13760      13770      13780      13790

    >< Tru9I
    >< MseI         >< MaeIII >< MunI
 ACATTAAAAG AAATACTCGT CACATACAAT TGCTGTGATG ATGATTATTT CAATAAGAAG GATTGGTATG
      13800      13810      13820      13830      13840      13850      13860

                             >< ThaI
                             >< MvnI
                           >< MluI
                             >< BstUI                        >< RsaI
                             >< Bsp50I                         >< HphI
           >< TfiI         >< AflIII        >< DdeI        >< Csp6I  Tru9I ><
           >< HinfI          >< AccII       >< BfrI          >< AfaI   MseI ><
 ACTTCGTAGA GAATCCTGAC ATCTTACGCG TATATGCTAA CTTAGGTGAG CGTGTACGCC AATCATTATT
      13870      13880      13890      13900      13910      13920      13930

                                                                      XhoII >
                                                                     Sau3AI >
                                                                      NdeII >
                                                                       MflI >
         > < SfaNI                                        >< RsaI      MboI >
        >< RsaI                                         > < Csp6I      DpnII >
       >< Csp6I                                       >< BspWI          BstYI >
        >< AfaI         >< SfaNI                        >< AfaI         BspAI >
 AAAGACTGTA CAATTCTGCG ATGCTATGCG TGATGCAGGC ATTGTAGGCG TACTGACATT AGATAATCAG
      13940      13950      13960      13970      13980      13990      14000

                                                    > < ScrFI
                                                    > < MvaI
                                                        >< Fnu4HI
                                                  >< EcoRII
                                                    > < Ecl136I
                                                    > < BstOI
                                                    > < BstNI
    >< Tru9I                              >< RsaI           >< BslI
    >< MseI          >< RsaI                > < HphI        >< BsiYI
 >< DpnI           >< Csp6I              >< Csp6I       > < BsiLI
 >< Bspl43I         >< BsrI                > < BbvI    > < ApyI
       >< AlwI     >< AfaI                >< AfaI    >< DsaV  >< AciI
 GATCTTAATG GGAACTGGTA CGATTTCGGT GATTTCGTAC AAGTAGCACC AGGCTGCGGA GTTCCTATTG
      14010      14020      14030      14040      14050      14060      14070

                                                                    >< SfaNI
                                                  >< RmaI            > < HinfI
                             >< MamI            >< MnlI        >< Fnu4HIPleI ><
   >< TfiI     >< SfaNI      >< BsiBI            >< MaeI          >< DdeI
   >< HinfI      >< FokI     >< BsaBI          >< BbvI        >< BspWI NdeI ><
 TGGATTCATA TTACTCATTG CTGATGCCCA TCCTCACTTT GACTAGGGCA TTGGCTGCTG AGTCCCATAT
      14080      14090      14100      14110      14120      14130      14140

     >< Sau3AI
     >< NdeII
```

FIGURE 13.32

```
        >< MboI
       >< MamI
        >< DpnII                                                    Tth111I ><
          >< DpnI                                                   MboII ><
             >< BspWI                                          >< Ksp632I
        >< BspAI                                               >< Eam1104I
          >< Bsp143I          >< XcmI                              >< BsmAI
       >< BsiBI               >< Tru9I                        >< EarI  AspI ><
       >< BsaBI >< FokI       >< MseI                            >< Alw26I
GGATGCTGAT CTCGCAAAAC CACTTATTAA GTGGGATTTG CTGAAATATG ATTTTACGGA AGAGAGACTT
     14150      14160      14170      14180      14190      14200      14210

                              > < SinI
                              > < Sau96I
                              > < NspIV
                                >< NspHII
         >< TthHB8I             >< NlaIV
         >< TaqI               >< FokI
              >< McrI           > < Eco47I
           > < Ksp632I          > < Cfr13I
           > < EarI             > < BsiZI
           > < Eam1104I     >< SspI>< BscBI
         >< BsmAI       > < Tru9I  > < Bme18I
><MboII    >< BsiEI> < MseI   > < AvaII                    >< Tru9I
       >< Alw26I       >< DraI  > < AsuI            >< MunI  >< MseI
TGTCTCTTCG ACCGTTATTT TAAATATTGG GACCAGACAT ACCATCCCAA TTGTATTAAC TGTTTGGATG
     14220      14230      14240      14250      14260      14270      14280

                                                                 SinI ><
                                                                Sau96I ><
                                                                 NspIV ><
                                                                 NspHII >
                                                                Eco47I ><
                                                                Cfr13I ><
                                                                 BsiZI ><
                                                                Bme18I ><
                                 >< Tru9I                        AvaII ><
         >< FokI                 >< MseI                         AsuI ><
ATAGGTGTAT CCTTCATTGT GCAAACTTTA ATGTGTTATT TTCTACTGTG TTTCCACCTA CAAGTTTTGG
     14290      14300      14310      14320      14330      14340      14350

   >< SpeI
    >< RmaI
    >< MaeI       >< SspI                                  >< BsrI
ACCACTAGTA AGAAAAATAT TTGTAGATGG TGTTCCTTTT GTTGTTTCAA CTGGATACCA TTTTCGTGAG
     14360      14370      14380      14390      14400      14410      14420

                                                 >< ThaI>< Esp3I
                                                     >< DdeI
                                                 >< BstUI
           >< RsaI                               >< Bsp50I   >< BsmBI
   >< HinfI >< PleI                              >< MvnI>< BsmAI
        > < Csp6I                        >< HgaI>< AluI     >< Alw26I
          >< AfaI                           >< FokI  >< AccII          > < BbvI
TTAGGAGTCG TACATAATCA GGATGTAAAC TTACATAGCT CGCGTCTCAG TTTCAAGGAA CTTTTAGTGT
     14430      14440      14450      14460      14470      14480      14490

                     >< Zsp2I
                       >< SphI
                  >< Ppu10I
                       >< PaeI
                       >< NspI
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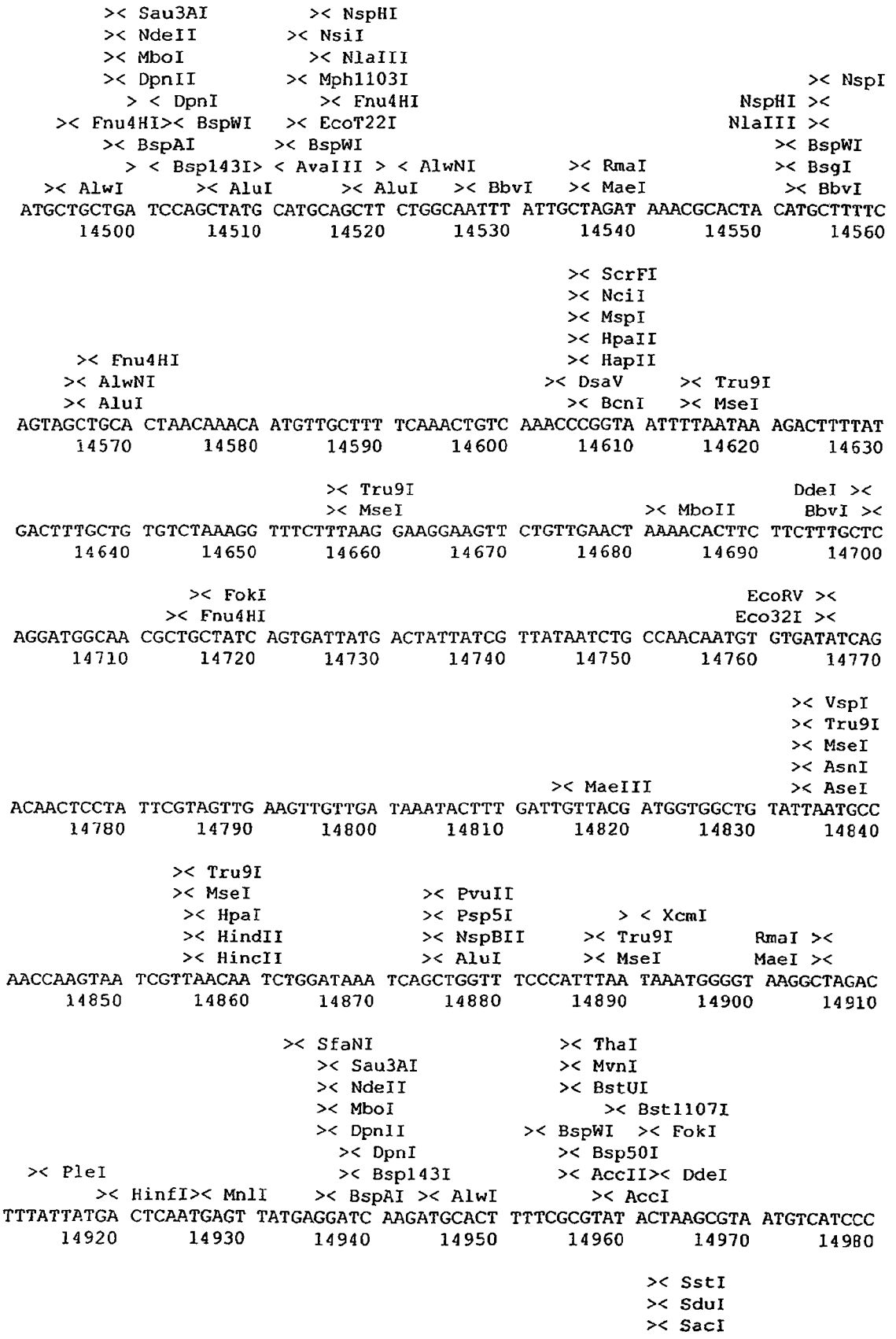
FIGURE 13.33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>< BsgI
>< AlwI
>< AluI
>< AluI
>< BbvI
>< MaeI
>< BbvI
ATGCTGCTGA TCCAGCTATG CATGCAGCTT CTGGCAATTT ATTGCTAGAT AAACGCACTA CATGCTTTTC
14500 14510 14520 14530 14540 14550 14560
>< ScrFI
>< NciI
>< MspI
>< HpaII
>< Fnu4HI
>< HapII
>< AlwNI
>< DsaV
>< Tru9I
>< AluI
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14570 14580 14590 14600 14610 14620 14630
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14640 14650 14660 14670 14680 14690 14700
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14710 14720 14730 14740 14750 14760 14770
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>< AsnI
>< MaeIII
>< AseI
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14780 14790 14800 14810 14820 14830 14840
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>< MseI
>< PvuII
>< HpaI
>< Psp5I
> < XcmI
>< HindII
>< NspBII
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RmaI ><
>< HincII
>< AluI
>< MseI
MaeI ><
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>< HinfI>< MnlI
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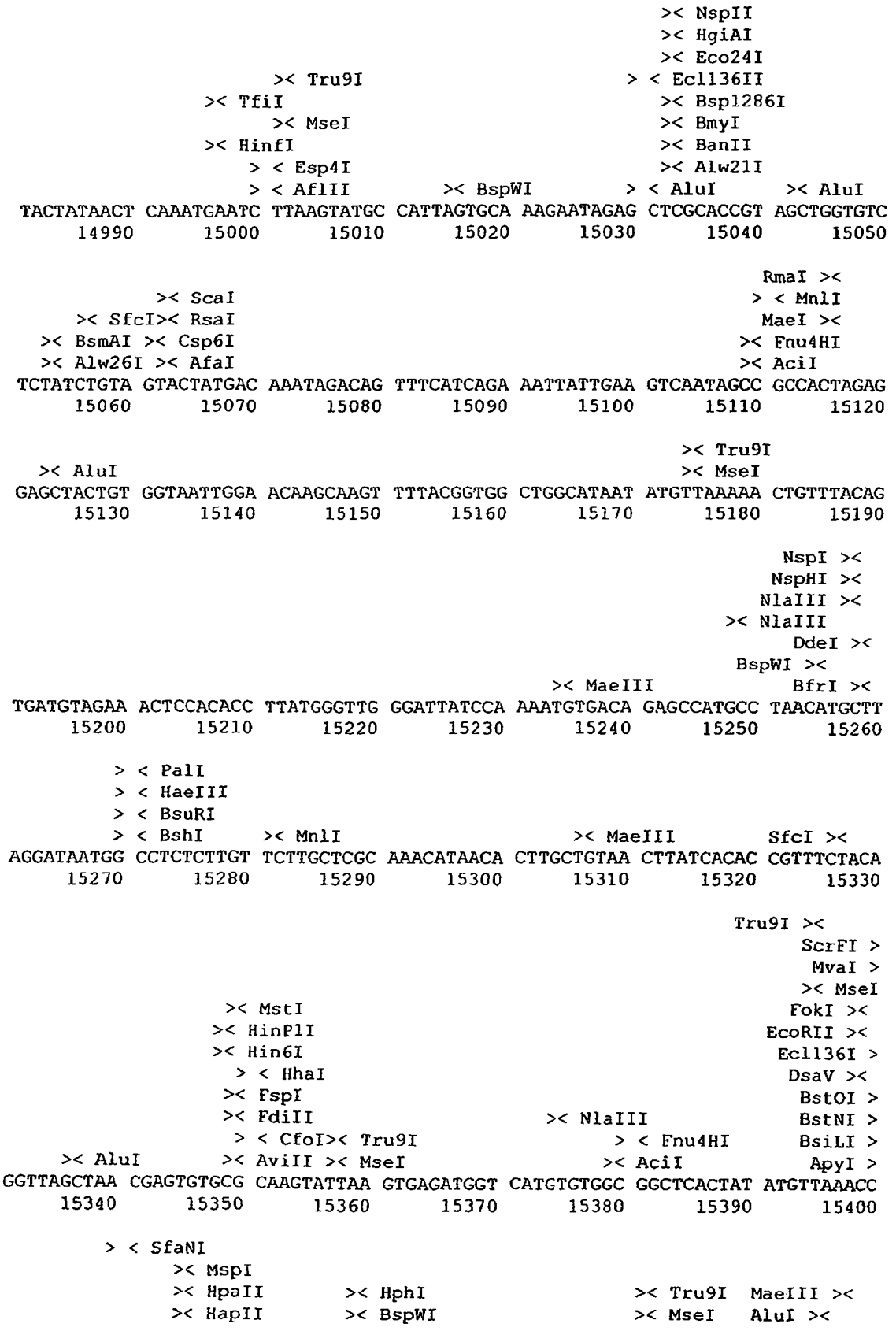
>< SduI
>< SacI

FIGURE 13.34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DdeI ><
BspWI ><
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SfcI ><
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15270 15280 15290 15300 15310 15320 15330
Tru9I ><
ScrFI >
MvaI >
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>< MstI
FokI ><
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>< FdiII
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>< AviII >< MseI
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15340 15350 15360 15370 15380 15390 15400
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>< HphI
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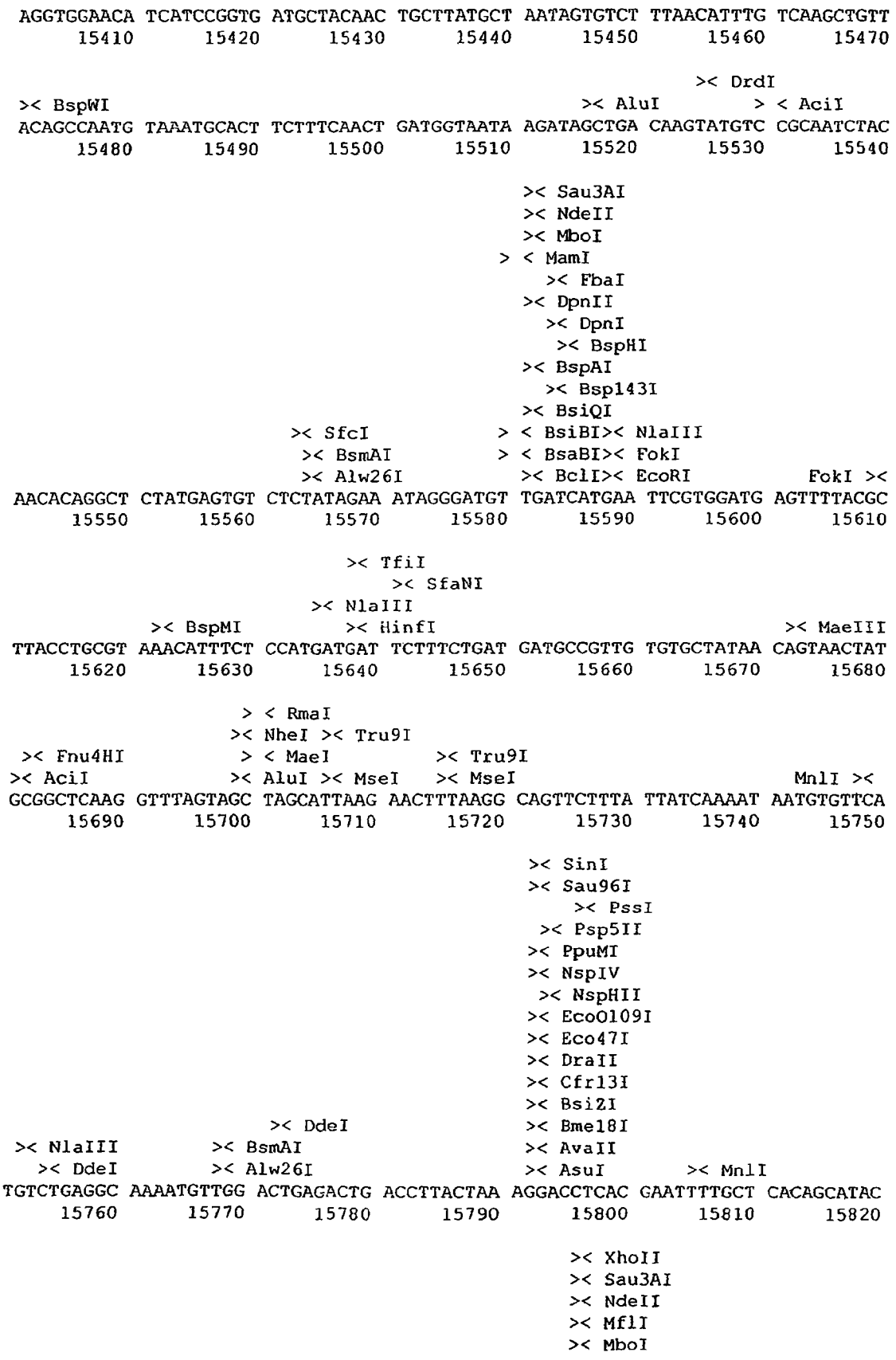
FIGURE 13.35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>< BspWI >< AluI > < AciI
ACAGCCAATG TAAATGCACT TCTTTCAACT GATGGTAATA AGATAGCTGA CAAGTATGTC CGCAATCTAC
15480 15490 15500 15510 15520 15530 15540
>< Sau3AI
>< NdeII
>< MboI
> < MamI
>< FbaI
>< DpnII
>< DpnI
>< BspHI
>< BspAI
>< Bsp143I
>< BsiQI
>< SfcI > < BsiBI>< NlaIII
>< BsmAI > < BsaBI>< FokI
>< Alw26I >< BclI>< EcoRI FokI ><
AACACAGGCT CTATGAGTGT CTCTATAGAA ATAGGGATGT TGATCATGAA TTCGTGGATG AGTTTTACGC
15550 15560 15570 15580 15590 15600 15610
>< TfiI
>< SfaNI
>< NlaIII
>< BspMI >< HinfI >< MaeIII
TTACCTGCGT AAACATTTCT CCATGATGAT TCTTTCTGAT GATGCCGTTG TGTGCTATAA CAGTAACTAT
15620 15630 15640 15650 15660 15670 15680
> < RmaI
>< NheI >< Tru9I
>< Fnu4HI > < MaeI >< Tru9I
>< AciI >< AluI >< MseI >< MseI MnlI ><
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15690 15700 15710 15720 15730 15740 15750
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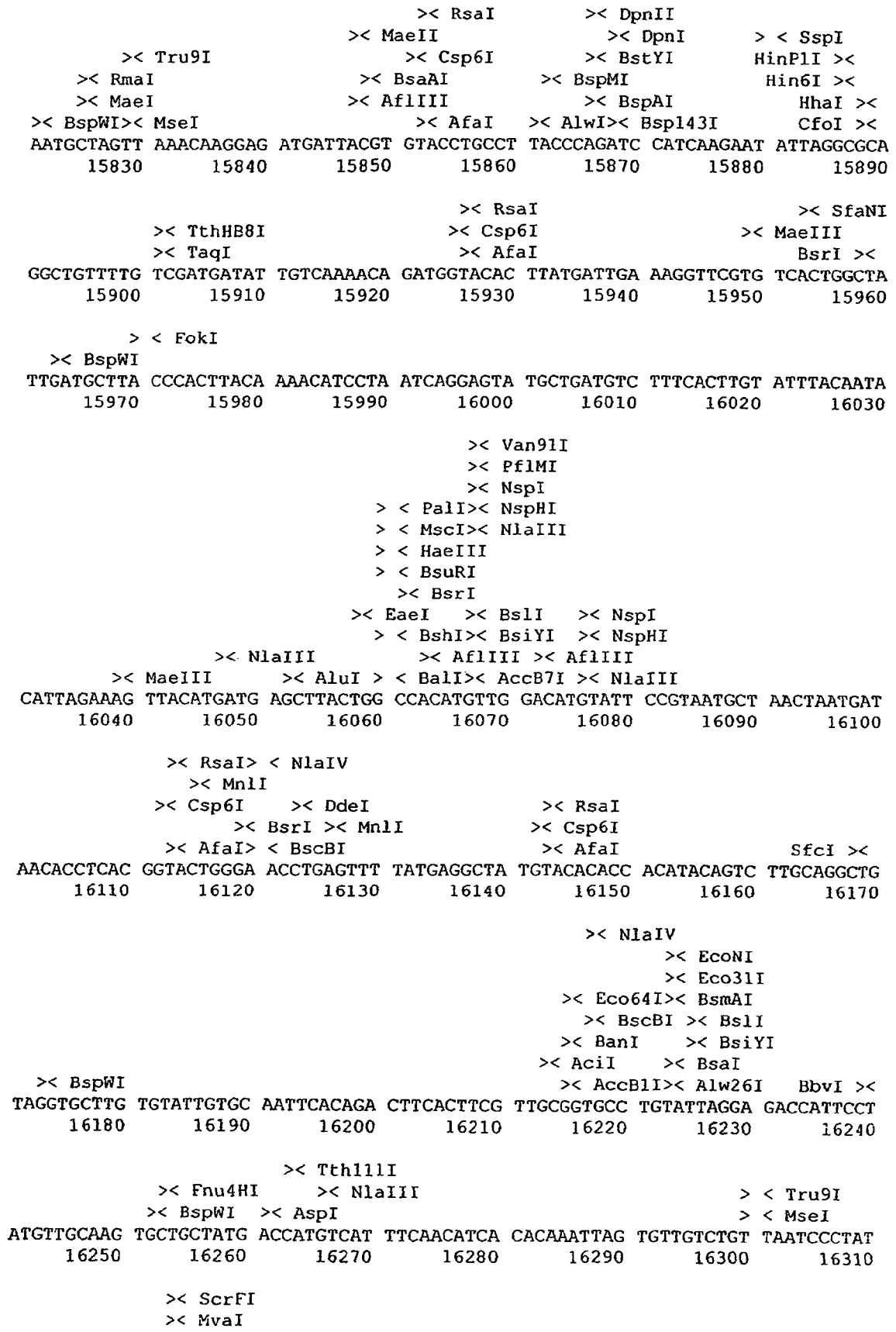
>< MboI

FIGURE 13. 36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>< Tru9I >< Csp6I >< BstYI HinPlI ><
>< RmaI >< BsaAI >< BspMI Hin6I ><
>< MaeI >< AflIII >< BspAI HhaI ><
>< BspWI>< MseI >< AfaI >< AlwI>< Bsp143I CfoI ><
AATGCTAGTT AAACAAGGAG ATGATTACGT GTACCTGCCT TACCCAGATC CATCAAGAAT ATTAGGCGCA
15830 15840 15850 15860 15870 15880 15890
>< RsaI >< SfaNI
>< TthHB8I >< Csp6I >< MaeIII
>< TaqI >< AfaI BsrI ><
GGCTGTTTTG TCGATGATAT TGTCAAAACA GATGGTACAC TTATGATTGA AAGGTTCGTG TCACTGGCTA
15900 15910 15920 15930 15940 15950 15960
> < FokI
>< BspWI
TTGATGCTTA CCCACTTACA AAACATCCTA ATCAGGAGTA TGCTGATGTC TTTCACTTGT ATTTACAATA
15970 15980 15990 16000 16010 16020 16030
>< Van91I
>< PflMI
>< NspI
> < PalI>< NspHI
> < MscI>< NlaIII
> < HaeIII
> < BsuRI
>< BsrI
>< EaeI >< BslI >< NspI
> < BshI>< BsiYI >< NspHI
>< NlaIII >< AflIII >< AflIII
>< MaeIII >< AluI > < BalI>< AccB7I >< NlaIII
CATTAGAAAG TTACATGATG AGCTTACTGG CCACATGTTG GACATGTATT CCGTAATGCT AACTAATGAT
16040 16050 16060 16070 16080 16090 16100
>< RsaI> < NlaIV
>< MnlI
>< Csp6I >< DdeI >< RsaI
>< BsrI >< MnlI >< Csp6I
>< AfaI> < BscBI >< AfaI SfcI ><
AACACCTCAC GGTACTGGGA ACCTGAGTTT TATGAGGCTA TGTACACACC ACATACAGTC TTGCAGGCTG
16110 16120 16130 16140 16150 16160 16170
>< NlaIV
>< EcoNI
>< Eco31I
>< Eco64I>< BsmAI
>< BscBI >< BslI
>< BanI >< BsiYI
>< AciI >< BsaI
>< BspWI >< AccB1I>< Alw26I BbvI ><
TAGGTGCTTG TGTATTGTGC AATTCACAGA CTTCACTTCG TTGCGGTGCC TGTATTAGGA GACCATTCCT
16180 16190 16200 16210 16220 16230 16240
>< Tth111I
>< Fnu4HI >< NlaIII > < Tru9I
>< BspWI >< AspI > < MseI
ATGTTGCAAG TGCTGCTATG ACCATGTCAT TTCAACATCA CACAAATTAG TGTTGTCTGT TAATCCCTAT
16250 16260 16270 16280 16290 16300 16310
>< ScrFI
>< MvaI

FIGURE 13. 37

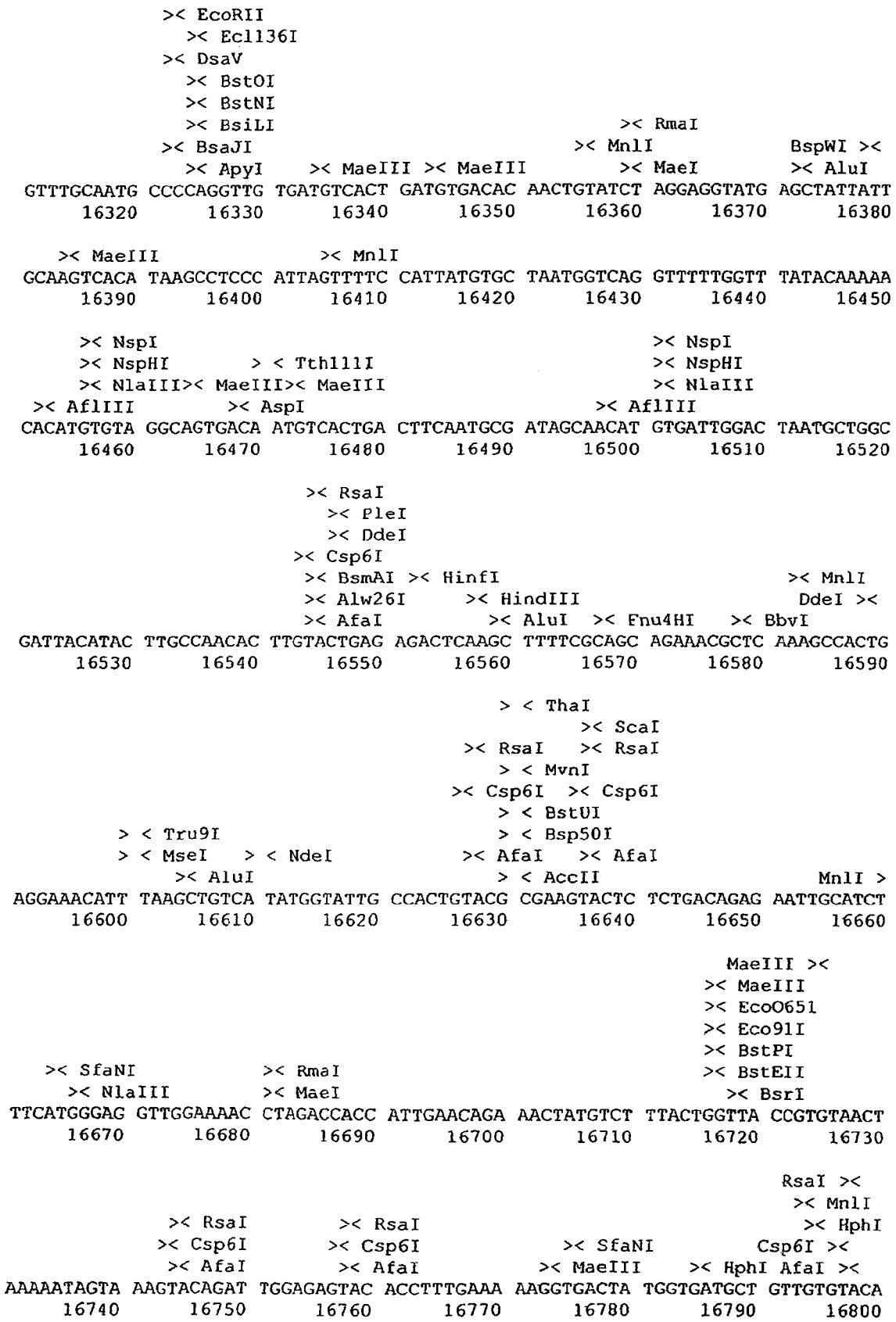

>< EcoRII
>< Ecl136I
>< DsaV
>< BstOI
>< BstNI
>< BsiLI
>< RmaI
>< BsaJI
>< MnlI
BspWI ><
>< ApyI
>< MaeIII >< MaeIII
>< MaeI
>< AluI
GTTTGCAATG CCCCAGGTTG TGATGTCACT GATGTGACAC AACTGTATCT AGGAGGTATG AGCTATTATT
16320 16330 16340 16350 16360 16370 16380
>< MaeIII
>< MnlI
GCAAGTCACA TAAGCCTCCC ATTAGTTTTC CATTATGTGC TAATGGTCAG GTTTTTGGTT TATACAAAAA
16390 16400 16410 16420 16430 16440 16450
>< NspI
>< NspHI
> < Tth111I
>< NlaIII>< MaeIII>< MaeIII
>< NspI
>< NspHI
>< NlaIII
>< AflIII
>< AspI
>< AflIII
CACATGTGTA GGCAGTGACA ATGTCACTGA CTTCAATGCG ATAGCAACAT GTGATTGGAC TAATGCTGGC
16460 16470 16480 16490 16500 16510 16520
>< RsaI
>< PleI
>< DdeI
>< Csp6I
>< BsmAI >< HinfI
>< MnlI
>< Alw26I
>< HindIII
DdeI ><
>< AfaI
>< AluI >< Fnu4HI >< BbvI
GATTACATAC TTGCCAACAC TTGTACTGAG AGACTCAAGC TTTTCGCAGC AGAAACGCTC AAAGCCACTG
16530 16540 16550 16560 16570 16580 16590
> < ThaI
>< ScaI
>< RsaI >< RsaI
> < MvnI
>< Csp6I >< Csp6I
> < BstUI
> < Tru9I
> < Bsp50I
> < MseI > < NdeI
>< AfaI >< AfaI
>< AluI
> < AccII
MnlI >
AGGAAACATT TAAGCTGTCA TATGGTATTG CCACTGTACG CGAAGTACTC TCTGACAGAG AATTGCATCT
16600 16610 16620 16630 16640 16650 16660
MaeIII ><
>< MaeIII
>< EcoO651
>< Eco91I
>< BstPI
>< SfaNI
>< RmaI
>< BstEII
>< NlaIII
>< MaeI
>< BsrI
TTCATGGGAG GTTGGAAAAC CTAGACCACC ATTGAACAGA AACTATGTCT TTACTGGTTA CCGTGTAACT
16670 16680 16690 16700 16710 16720 16730
RsaI ><
>< MnlI
>< RsaI
>< RsaI
>< HphI
>< Csp6I
>< Csp6I
>< SfaNI
Csp6I ><
>< AfaI
>< AfaI
>< MaeIII
>< HphI AfaI ><
AAAAATAGTA AAGTACAGAT TGGAGAGTAC ACCTTTGAAA AAGGTGACTA TGGTGATGCT GTTGTGTACA
16740 16750 16760 16770 16780 16790 16800

FIGURE 13. 38

```
     >< RsaI                                    >< HphI
    >< Csp6I                                        >< HindII          DdeI ><
     >< AfaI                                        >< HincII          BfrI ><
GAGGTACTAC GACATACAAG TTGAATGTTG GTGATTACTT TGTGTTGACA TCTCACACTG TAATGCCACT
     16810      16820      16830      16840      16850      16860      16870

   >< VneI
   >< SnoI
      >< SduI
      >< NspII
      >< HgiAI                 > < SduI
 >< DraIII                     > < NspII
      >< Bsp1286I              > < HgiAI
      >< BmyI             >< BspWI  >< DraIII          >< RsaI
  >< ApaLI    >< RmaI           > < Bsp1286I          >< Csp6I
  >< Alw44I   >< MaeI           > < BmyI          >< BsrI
      >< Alw21I                 > < Alw21I             >< AfaI      DdeI >
TAGTGCACCT ACTCTAGTGC CACAAGAGCA CTATGTGAGA ATTACTGGCT TGTACCCAAC ACTCAACATC
     16880      16890      16900      16910      16920      16930      16940

                                                                 StyI ><
                                                                    SinI >
                                                                  Sau96I >
                                                                   NspIV >
                                                              EcoT14I ><
                                                                   Eco47I >
                                                              Eco130I ><
                                                           >< ScaI  Cfr13I >
                                                                BssT1I ><
                                                     >< SphI >< RsaI   BsiZI >
                                                     >< PaeI       BsaJI ><
                                                     >< NlaIII        Bme18I >
             >< RmaI                                 >< NspI>< Csp6I   AvaII >
             >< MaeI                                 >< NspHI>< AfaI    AsuI >
TCAGATGAGT TTTCTAGCAA TGTTGCAAAT TATCAAAAGG TCGGCATGCA AAAGTACTCT ACACTCCAAG
     16950      16960      16970      16980      16990      17000      17010

      >< ScrFI
           >< RsaI
      >< MvaI
     >< EcoRII
      >< Ecl136I
         > < Csp6I
      >< BstOI
      >< BstNI
  >< XcmI   >< BslI
>< NspHII   >< BsiYI
      >< BsiLI
      >< ApyI    >< BsrI
   >< DsaV>< AfaI  > < HinfI>< PleI
GACCACCTGG TACTGGTAAG AGTCATTTTG CCATCGGACT TGCTCTCTAT TACCCATCTG CTCGCATAGT
     17020      17030      17040      17050      17060      17070      17080

                  >< SfaNI
            >< SphI     >< PvuII
            >< PaeI     >< Psp5I
            >< NspI     >< NspBII
            >< NspHI  >< Fnu4HI                    > < Tru9I
  >< Bst1107I       > < NlaIII>< BspWI                    >< SspI
 >< AccI    >< NlaIII   >< AluI   >< BbvI            > < MseI
GTATACGGCA TGCTCTCATG CAGCTGTTGA TGCCCTATGT GAAAAGGCAT TAAAATATTT GCCCATAGAT
     17090      17100      17110      17120      17130      17140      17150
```

FIGURE 13.39

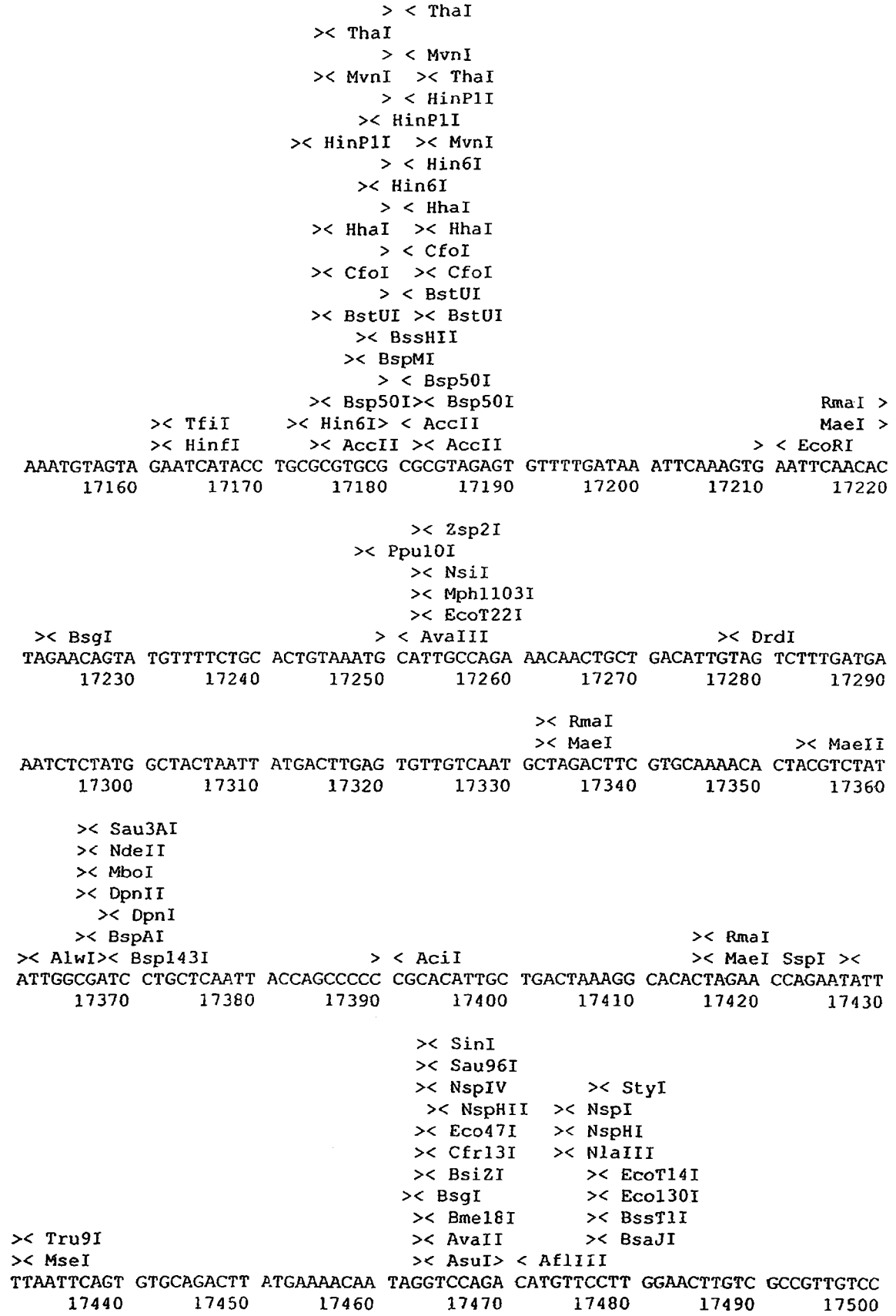

> < ThaI
>< ThaI
> < MvnI
>< MvnI >< ThaI
> < HinP1I
>< HinP1I
>< HinP1I >< MvnI
> < Hin6I
>< Hin6I
> < HhaI
>< HhaI >< HhaI
> < CfoI
>< CfoI >< CfoI
> < BstUI
>< BstUI >< BstUI
>< BssHII
>< BspMI
> < Bsp50I
>< Bsp50I>< Bsp50I
RmaI >
MaeI >
>< TfiI
>< HinfI
>< Hin6I> < AccII
>< AccII >< AccII
> < EcoRI
AAATGTAGTA GAATCATACC TGCGCGTGCG CGCGTAGAGT GTTTTGATAA ATTCAAAGTG AATTCAACAC
17160 17170 17180 17190 17200 17210 17220
>< Zsp2I
>< Ppu10I
>< NsiI
>< Mph1103I
>< EcoT22I
>< BsgI
> < AvaIII
>< DrdI
TAGAACAGTA TGTTTTCTGC ACTGTAAATG CATTGCCAGA AACAACTGCT GACATTGTAG TCTTTGATGA
17230 17240 17250 17260 17270 17280 17290
>< RmaI
>< MaeI
>< MaeII
AATCTCTATG GCTACTAATT ATGACTTGAG TGTTGTCAAT GCTAGACTTC GTGCAAAACA CTACGTCTAT
17300 17310 17320 17330 17340 17350 17360
>< Sau3AI
>< NdeII
>< MboI
>< DpnII
>< DpnI
>< BspAI
>< RmaI
>< AlwI>< Bspl43I
> < AciI
>< MaeI SspI ><
ATTGGCGATC CTGCTCAATT ACCAGCCCCC CGCACATTGC TGACTAAAGG CACACTAGAA CCAGAATATT
17370 17380 17390 17400 17410 17420 17430
>< SinI
>< Sau96I
>< NspIV
>< StyI
>< NspHII
>< NspI
>< Eco47I
>< NspHI
>< Cfr13I
>< NlaIII
>< BsiZI
>< EcoT14I
>< BsgI
>< Eco130I
>< Bme18I
>< BssT1I
>< Tru9I
>< AvaII
>< BsaJI
>< MseI
>< AsuI> < AflIII
TTAATTCAGT GTGCAGACTT ATGAAAACAA TAGGTCCAGA CATGTTCCTT GGAACTTGTC GCCGTTGTCC
17440 17450 17460 17470 17480 17490 17500

FIGURE 13. 40

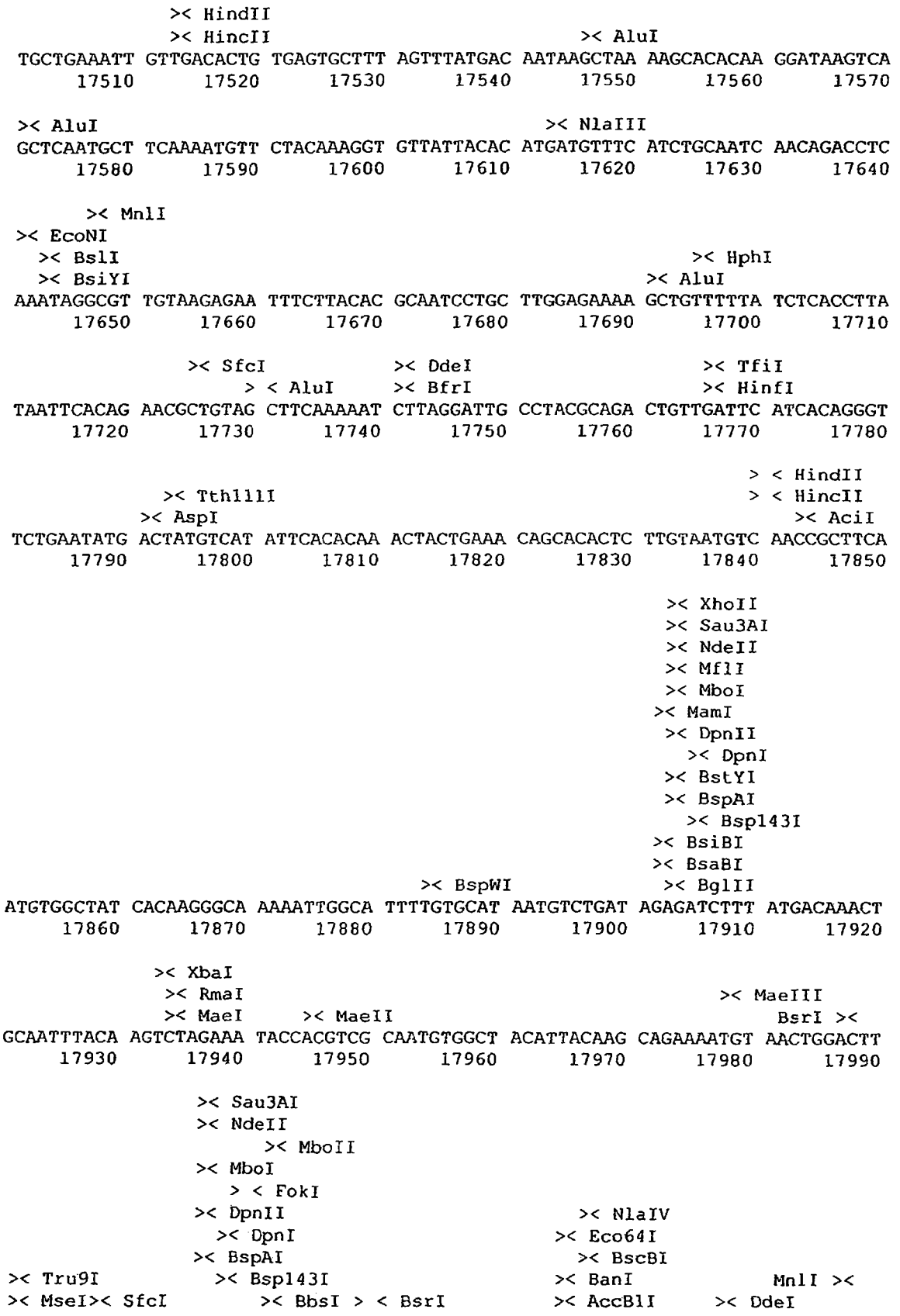

>< HindII
>< HincII
>< AluI
TGCTGAAATT GTTGACACTG TGAGTGCTTT AGTTTATGAC AATAAGCTAA AAGCACACAA GGATAAGTCA
17510 17520 17530 17540 17550 17560 17570
>< AluI
>< NlaIII
GCTCAATGCT TCAAAATGTT CTACAAAGGT GTTATTACAC ATGATGTTTC ATCTGCAATC AACAGACCTC
17580 17590 17600 17610 17620 17630 17640
>< MnlI
>< EcoNI
>< BslI
>< BsiYI
>< HphI
>< AluI
AAATAGGCGT TGTAAGAGAA TTTCTTACAC GCAATCCTGC TTGGAGAAAA GCTGTTTTTA TCTCACCTTA
17650 17660 17670 17680 17690 17700 17710
>< SfcI
> < AluI
>< DdeI
>< BfrI
>< TfiI
>< HinfI
TAATTCACAG AACGCTGTAG CTTCAAAAAT CTTAGGATTG CCTACGCAGA CTGTTGATTC ATCACAGGGT
17720 17730 17740 17750 17760 17770 17780
> < HindII
> < HincII
>< Tth111I
>< AspI
>< AciI
TCTGAATATG ACTATGTCAT ATTCACACAA ACTACTGAAA CAGCACACTC TTGTAATGTC AACCGCTTCA
17790 17800 17810 17820 17830 17840 17850
>< XhoII
>< Sau3AI
>< NdeII
>< MflI
>< MboI
>< MamI
>< DpnII
>< DpnI
>< BstYI
>< BspAI
>< Bsp143I
>< BsiBI
>< BsaBI
>< BspWI
>< BglII
ATGTGGCTAT CACAAGGGCA AAAATTGGCA TTTTGTGCAT AATGTCTGAT AGAGATCTTT ATGACAAACT
17860 17870 17880 17890 17900 17910 17920
>< XbaI
>< RmaI
>< MaeI
>< MaeII
>< MaeIII
BsrI ><
GCAATTTACA AGTCTAGAAA TACCACGTCG CAATGTGGCT ACATTACAAG CAGAAAATGT AACTGGACTT
17930 17940 17950 17960 17970 17980 17990
>< Sau3AI
>< NdeII
>< MboII
>< MboI
> < FokI
>< DpnII
>< DpnI
>< BspAI
>< NlaIV
>< Eco64I
>< BscBI
>< Tru9I
>< Bsp143I
>< BanI
MnlI ><
>< MseI>< SfcI
>< BbsI > < BsrI
>< AccBlI
>< DdeI

FIGURE 13. 41

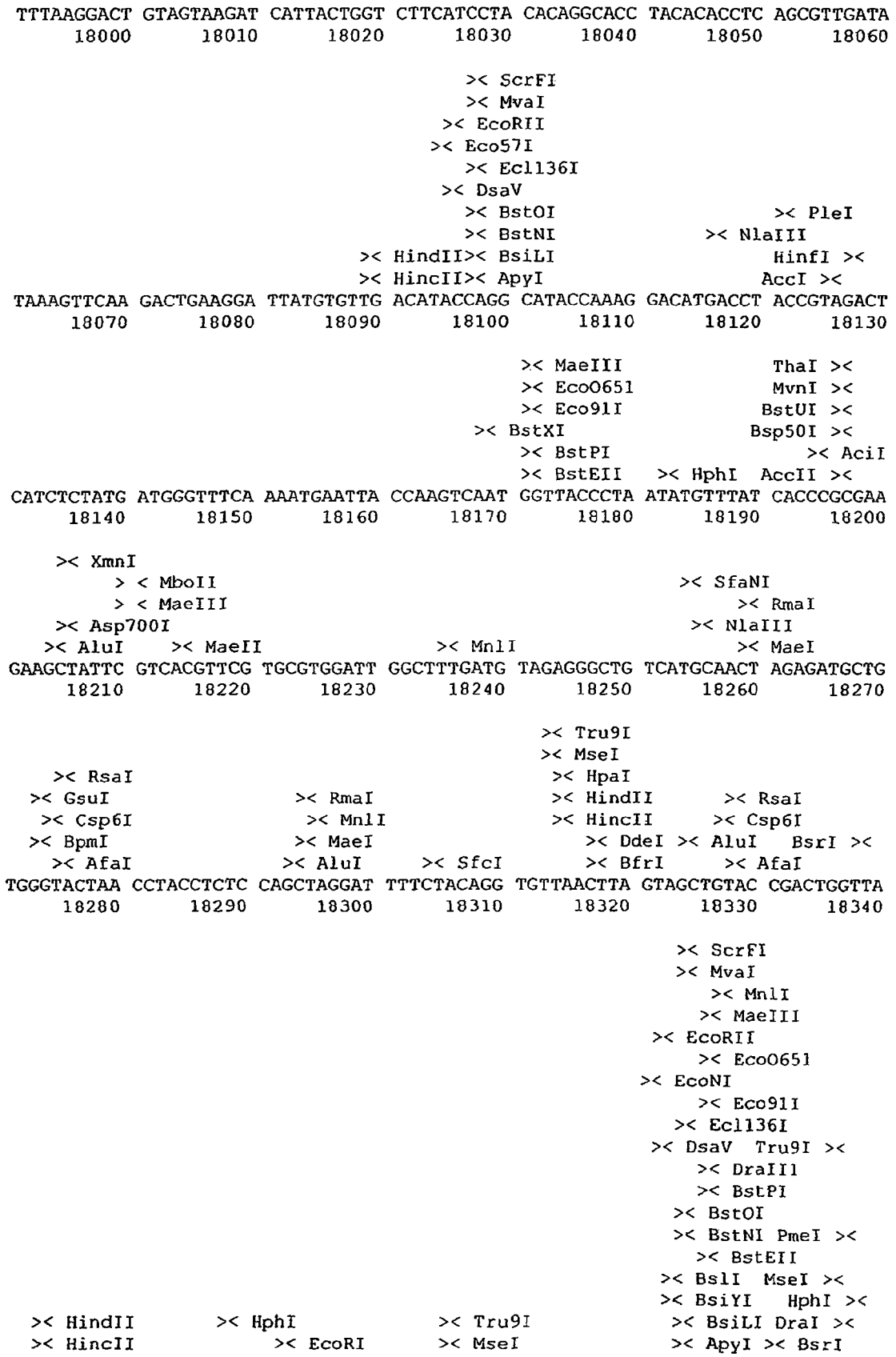

TTTAAGGACT GTAGTAAGAT CATTACTGGT CTTCATCCTA CACAGGCACC TACACACCTC AGCGTTGATA
18000 18010 18020 18030 18040 18050 18060
>< ScrFI
>< MvaI
>< EcoRII
>< Eco57I
>< Ecl136I
>< DsaV
>< BstOI
>< BstNI
>< PleI
>< NlaIII
>< HindII >< BsiLI
>< HincII >< ApyI
HinfI ><
AccI ><
TAAAGTTCAA GACTGAAGGA TTATGTGTTG ACATACCAGG CATACCAAAG GACATGACCT ACCGTAGACT
18070 18080 18090 18100 18110 18120 18130
>< MaeIII
>< Eco0651
>< Eco91I
>< BstXI
>< BstPI
>< BstEII
ThaI ><
MvnI ><
BstUI ><
Bsp50I ><
>< AciI
>< HphI AccII ><
CATCTCTATG ATGGGTTTCA AAATGAATTA CCAAGTCAAT GGTTACCCTA ATATGTTTAT CACCCGCGAA
18140 18150 18160 18170 18180 18190 18200
>< XmnI
> < MboII
> < MaeIII
>< Asp700I
>< AluI >< MaeII
>< MnlI
>< SfaNI
>< RmaI
>< NlaIII
>< MaeI
GAAGCTATTC GTCACGTTCG TGCGTGGATT GGCTTTGATG TAGAGGGCTG TCATGCAACT AGAGATGCTG
18210 18220 18230 18240 18250 18260 18270
>< Tru9I
>< MseI
>< RsaI
>< GsuI
>< Csp6I
>< BpmI
>< AfaI
>< RmaI
>< MnlI
>< MaeI
>< AluI
>< SfcI
>< HpaI
>< HindII
>< HincII
>< DdeI
>< BfrI
>< RsaI
>< Csp6I
>< AluI
>< AfaI
BsrI ><
TGGGTACTAA CCTACCTCTC CAGCTAGGAT TTTCTACAGG TGTTAACTTA GTAGCTGTAC CGACTGGTTA
18280 18290 18300 18310 18320 18330 18340
>< ScrFI
>< MvaI
>< MnlI
>< MaeIII
>< EcoRII
>< Eco0651
>< EcoNI
>< Eco91I
>< Ecl136I
>< DsaV Tru9I ><
>< DraIII
>< BstPI
>< BstOI
>< BstNI PmeI ><
>< BstEII
>< BslI MseI ><
>< BsiYI HphI ><
>< BsiLI DraI ><
>< ApyI >< BsrI
>< HindII
>< HincII
>< HphI
>< EcoRI
>< Tru9I
>< MseI

FIGURE 13.42

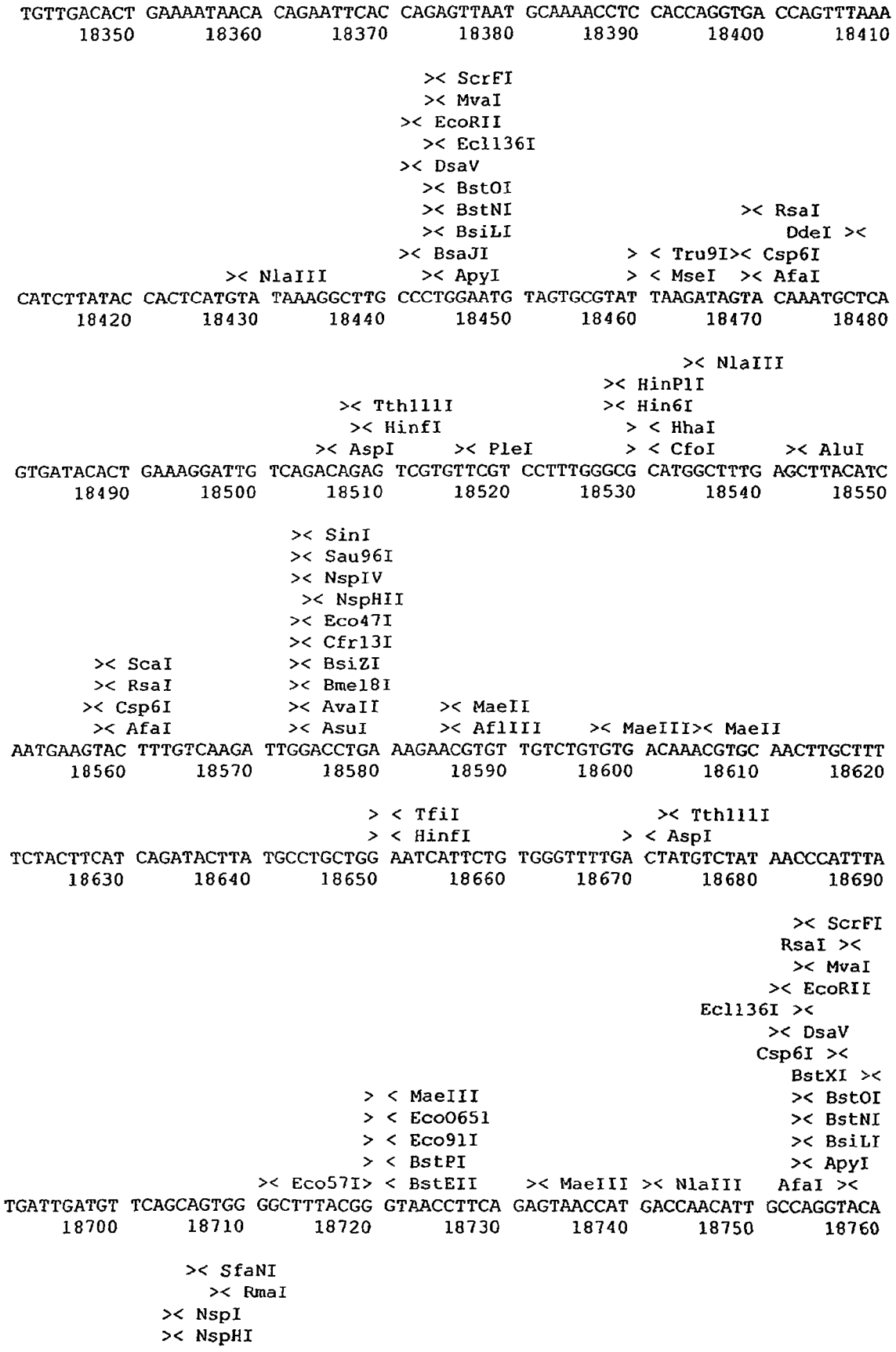

TGTTGACACT GAAAATAACA CAGAATTCAC CAGAGTTAAT GCAAAACCTC CACCAGGTGA CCAGTTTAAA
18350 18360 18370 18380 18390 18400 18410
>< ScrFI
>< MvaI
>< EcoRII
>< Ecl136I
>< DsaV
>< BstOI
>< BstNI
>< BsiLI
>< BsaJI
>< RsaI
DdeI ><
> < Tru9I>< Csp6I
> < MseI >< AfaI
>< NlaIII
>< ApyI
CATCTTATAC CACTCATGTA TAAAGGCTTG CCCTGGAATG TAGTGCGTAT TAAGATAGTA CAAATGCTCA
18420 18430 18440 18450 18460 18470 18480
>< NlaIII
>< HinPlI
>< Hin6I
>< Tth111I
>< HinfI
> < HhaI
>< AspI >< PleI
> < CfoI >< AluI
GTGATACACT GAAAGGATTG TCAGACAGAG TCGTGTTCGT CCTTTGGGCG CATGGCTTTG AGCTTACATC
18490 18500 18510 18520 18530 18540 18550
>< SinI
>< Sau96I
>< NspIV
>< NspHII
>< Eco47I
>< Cfr13I
>< ScaI
>< BsiZI
>< RsaI
>< Bme18I
>< Csp6I
>< AvaII
>< MaeII
>< AfaI
>< AsuI
>< AflIII
>< MaeIII>< MaeII
AATGAAGTAC TTTGTCAAGA TTGGACCTGA AAGAACGTGT TGTCTGTGTG ACAAACGTGC AACTTGCTTT
18560 18570 18580 18590 18600 18610 18620
> < TfiI
> < HinfI
>< Tth111I
> < AspI
TCTACTTCAT CAGATACTTA TGCCTGCTGG AATCATTCTG TGGGTTTTGA CTATGTCTAT AACCCATTTA
18630 18640 18650 18660 18670 18680 18690
>< ScrFI
RsaI ><
>< MvaI
>< EcoRII
Ecl136I ><
>< DsaV
Csp6I ><
BstXI ><
> < MaeIII
> < Eco0651
> < Eco91I
> < BstPI
>< BstOI
>< BstNI
>< BsiLI
>< ApyI
>< Eco57I> < BstEII >< MaeIII >< NlaIII AfaI ><
TGATTGATGT TCAGCAGTGG GGCTTTACGG GTAACCTTCA GAGTAACCAT GACCAACATT GCCAGGTACA
18700 18710 18720 18730 18740 18750 18760
>< SfaNI
>< RmaI
>< NspI
>< NspHI

FIGURE 13.43

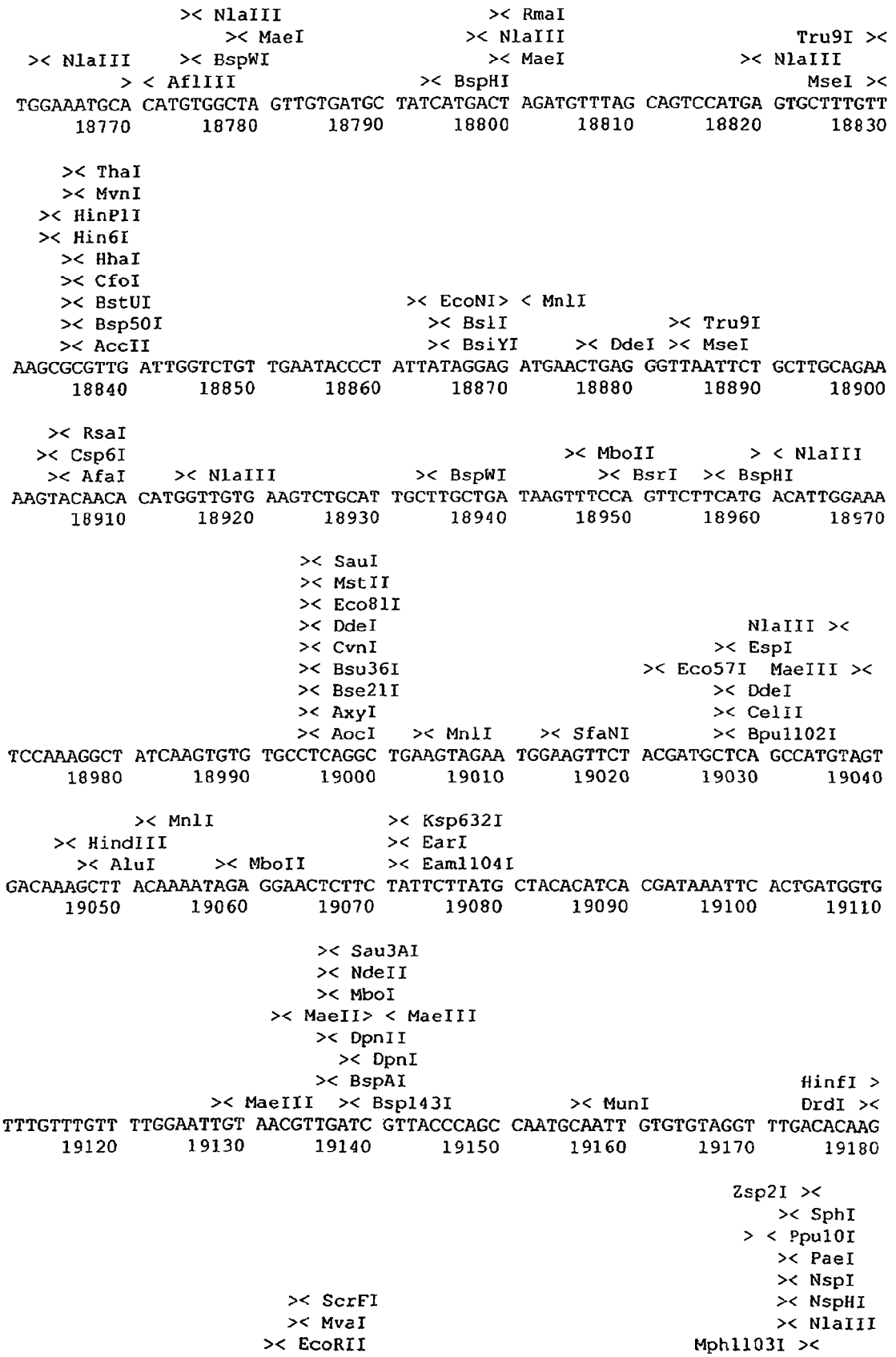

>< NlaIII
>< MaeI
>< NlaIII
>< BspWI
>< MaeI
Tru9I ><
>< NlaIII
> < AflIII
>< BspHI
MseI ><
TGGAAATGCA CATGTGGCTA GTTGTGATGC TATCATGACT AGATGTTTAG CAGTCCATGA GTGCTTTGTT
18770 18780 18790 18800 18810 18820 18830
>< ThaI
>< MvnI
>< HinP1I
>< Hin6I
>< HhaI
>< CfoI
>< BstUI
>< EcoNI> < MnlI
>< Bsp50I
>< BslI
>< Tru9I
>< AccII
>< BsiYI
>< DdeI >< MseI
AAGCGCGTTG ATTGGTCTGT TGAATACCCT ATTATAGGAG ATGAACTGAG GGTTAATTCT GCTTGCAGAA
18840 18850 18860 18870 18880 18890 18900
>< RsaI
>< Csp6I
>< MboII
> < NlaIII
>< AfaI
>< NlaIII
>< BspWI
>< BsrI >< BspHI
AAGTACAACA CATGGTTGTG AAGTCTGCAT TGCTTGCTGA TAAGTTTCCA GTTCTTCATG ACATTGGAAA
18910 18920 18930 18940 18950 18960 18970
>< SauI
>< MstII
>< Eco81I
>< DdeI
NlaIII ><
>< CvnI
>< EspI
>< Bsu36I
>< Eco57I MaeIII ><
>< Bse21I
>< DdeI
>< AxyI
>< CelII
>< AocI
>< MnlI
>< SfaNI
>< Bpu1102I
TCCAAAGGCT ATCAAGTGTG TGCCTCAGGC TGAAGTAGAA TGGAAGTTCT ACGATGCTCA GCCATGTAGT
18980 18990 19000 19010 19020 19030 19040
>< MnlI
>< Ksp632I
>< HindIII
>< EarI
>< AluI
>< MboII
>< Eam1104I
GACAAAGCTT ACAAAATAGA GGAACTCTTC TATTCTTATG CTACACATCA CGATAAATTC ACTGATGGTG
19050 19060 19070 19080 19090 19100 19110
>< Sau3AI
>< NdeII
>< MboI
>< MaeII> < MaeIII
>< DpnII
>< DpnI
>< BspAI
HinfI >
>< MaeIII
>< Bspl43I
>< MunI
DrdI ><
TTTGTTTGTT TTGGAATTGT AACGTTGATC GTTACCCAGC CAATGCAATT GTGTGTAGGT TTGACACAAG
19120 19130 19140 19150 19160 19170 19180
Zsp2I ><
>< SphI
> < Ppu10I
>< PaeI
>< NspI
>< ScrFI
>< NspHI
>< MvaI
>< NlaIII
>< EcoRII
Mph1103I ><

FIGURE 1344

```
                          >< Ecl136I                          >< GsuI
                         >< DsaV                              EcoT22I ><
                          >< BstOI                                  >< BsmI
                          >< BstNI                             >< BscCI
                          >< BsiLI                            >< BpmI >< NsiI
       >< PleI            >< ApyI                                  >< AvaIII
AGTCTTGTCA AACTTGAACT TACCAGGCTG TGATGGTGGT AGTTTGTATG TGAATAAGCA TGCATTCCAC
     19190      19200      19210      19220      19230      19240      19250

                                    >< Tru9I
                                         > < MunI
          >< TthHB8I                >< MseI
>< BcgI/a  >< TaqI                   >< DraI
      >< AluI                        >< BcgI
ACTCCAGCTT TCGATAAAAG TGCATTTACT AATTTAAAGC AATTGCCTTT CTTTTACTAT TCTGATAGTC
     19260      19270      19280      19290      19300      19310      19320

             >< PleI                                              SfaNI ><
              >< NlaIII                                             >< MaeII
            >< BsmAI                                          BsaAI ><
     >< HinfI>< Alw26I                                       AflIII ><
CTTGTGAGTC TCATGGCAAA CAAGTAGTGT CGGATATTGA TTATGTTCCA CTCAAATCTG CTACGTGTAT
     19330      19340      19350      19360      19370      19380      19390

                                                                  Zsp2I >
                                                            >< ScaI
                                                             Ppu10I ><
                                                            >< RsaINsiI >
                                                               Mph1103I >
                                                          >< SfaNIEcoT22I >
                                                    > < RsaI >< Csp6I
                                                   >< Csp6I      AvaIII ><
                                             >< NlaIII> < AfaI  >< AfaI
TACACGATGC AATTTAGGTG GTGCTGTTTG CAGACACCAT GCAAATGAGT ACCGACAGTA CTTGGATGCA
     19400      19410      19420      19430      19440      19450      19460

      >< FokI
TATAATATGA TGATTTCTGC TGGATTTAGC CTATGGATTT ACAAACAATT TGATACTTAT AACCTGTGGA
     19470      19480      19490      19500      19510      19520      19530

          >< ScrFI
          >< MvaI
            >< MaeIII
       >< EcoRII
          >< Ecl136I
       >< DsaV
          >< BstOI
          >< BstNI
          >< BsiLI                                  >< Tru9I
          >< ApyI                                   >< MseI
ATACATTTAC CAGGTTACAG AGTTTAGAAA ATGTGGCTTA TAATGTTGTT AATAAAGGAC ACTTTGATGG
     19540      19550      19560      19570      19580      19590      19600

   >< SgrAI
     >< NaeI
    >< MspI                  > < VspI
    >< HpaII                 > < Tru9I
    >< HapII                 > < MseI
   >< Cfr10I                 > < AsnI
         >< BspWI            > < AseI
ACACGCCGGC GAAGCACCTG TTTCCATCAT TAATAATGCT GTTTACACAA AGGTAGATGG TATTGATGTG
     19610      19620      19630      19640      19650      19660      19670
```

FIGURE 13. 45

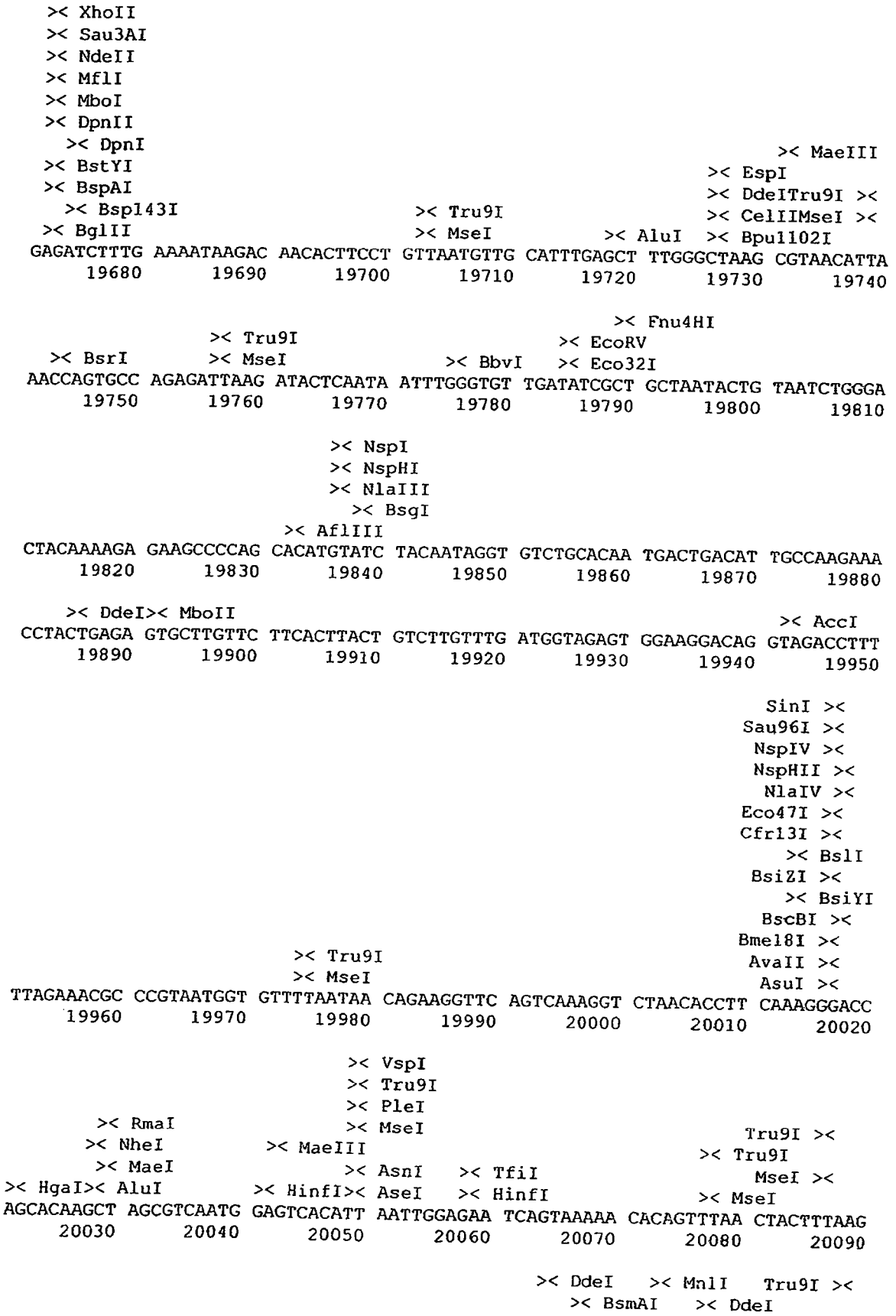

>< XhoII
>< Sau3AI
>< NdeII
>< MflI
>< MboI
>< DpnII
>< DpnI
>< BstYI
>< BspAI
>< Bsp143I
>< BglII
>< Tru9I
>< MseI
>< AluI
>< MaeIII
>< EspI
>< DdeITru9I ><
>< CelIIMseI ><
>< Bpu1102I
GAGATCTTTG AAAATAAGAC AACACTTCCT GTTAATGTTG CATTTGAGCT TTGGGCTAAG CGTAACATTA
19680 19690 19700 19710 19720 19730 19740
>< Fnu4HI
>< Tru9I
>< EcoRV
>< BsrI
>< MseI
>< BbvI
>< Eco32I
AACCAGTGCC AGAGATTAAG ATACTCAATA ATTTGGGTGT TGATATCGCT GCTAATACTG TAATCTGGGA
19750 19760 19770 19780 19790 19800 19810
>< NspI
>< NspHI
>< NlaIII
>< BsgI
>< AflIII
CTACAAAAGA GAAGCCCCAG CACATGTATC TACAATAGGT GTCTGCACAA TGACTGACAT TGCCAAGAAA
19820 19830 19840 19850 19860 19870 19880
>< DdeI>< MboII
>< AccI
CCTACTGAGA GTGCTTGTTC TTCACTTACT GTCTTGTTTG ATGGTAGAGT GGAAGGACAG GTAGACCTTT
19890 19900 19910 19920 19930 19940 19950
SinI ><
Sau96I ><
NspIV ><
NspHII ><
NlaIV ><
Eco47I ><
Cfr13I ><
>< BslI
BsiZI ><
>< BsiYI
BscBI ><
Bme18I ><
>< Tru9I
>< MseI
AvaII ><
AsuI ><
TTAGAAACGC CCGTAATGGT GTTTTAATAA CAGAAGGTTC AGTCAAAGGT CTAACACCTT CAAAGGGACC
19960 19970 19980 19990 20000 20010 20020
>< VspI
>< Tru9I
>< PleI
>< RmaI
>< MseI
>< NheI
>< MaeIII
Tru9I ><
>< Tru9I
>< MaeI
>< AsnI
>< TfiI
MseI ><
>< HgaI>< AluI
>< HinfI>< AseI
>< HinfI
>< MseI
AGCACAAGCT AGCGTCAATG GAGTCACATT AATTGGAGAA TCAGTAAAAA CACAGTTTAA CTACTTTAAG
20030 20040 20050 20060 20070 20080 20090
>< DdeI
>< MnlI
Tru9I ><
>< BsmAI
>< DdeI

FIGURE 1346

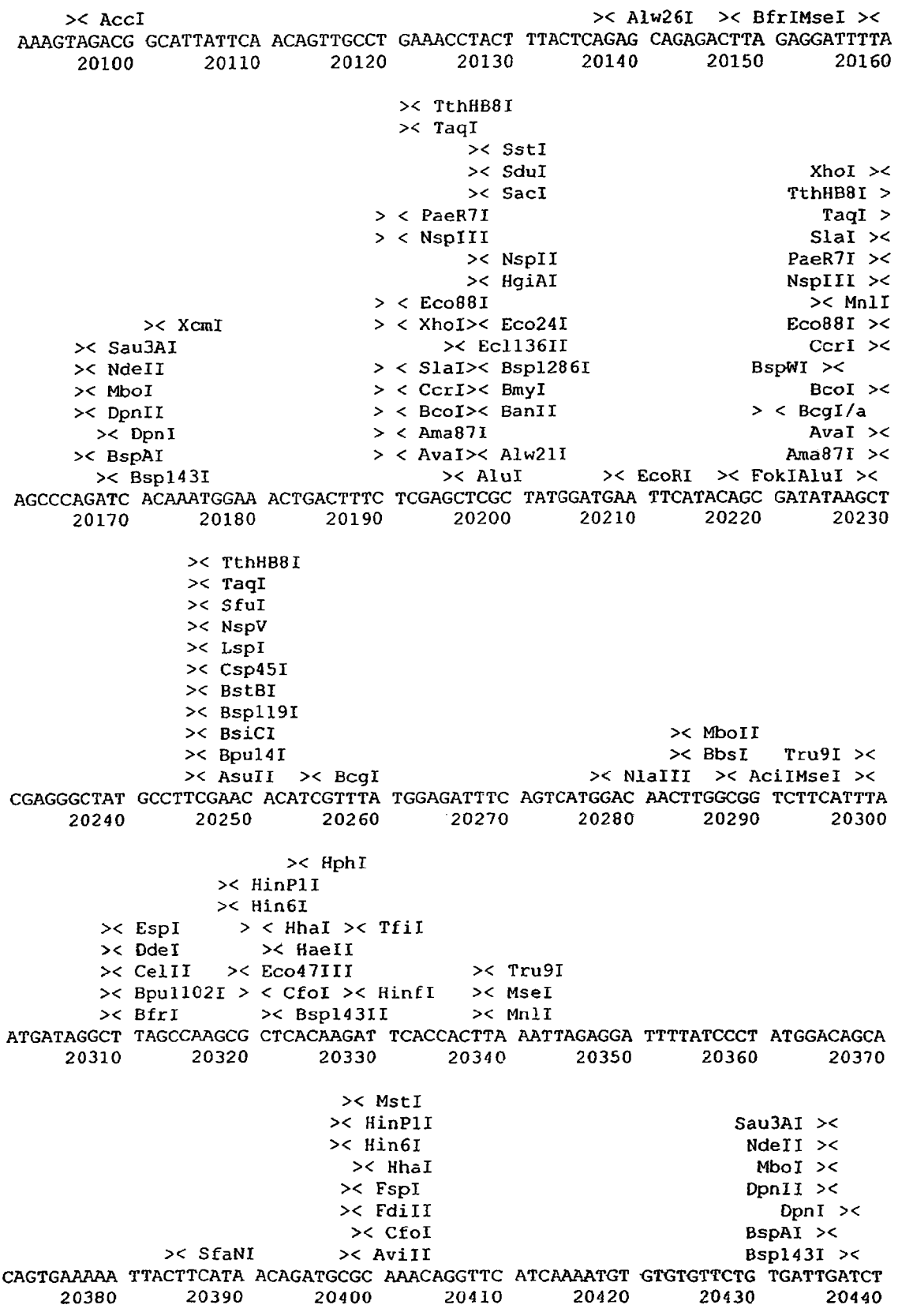

>< AccI
>< Alw26I >< BfrIMseI ><
AAAGTAGACG GCATTATTCA ACAGTTGCCT GAAACCTACT TTACTCAGAG CAGAGACTTA GAGGATTTTA
20100 20110 20120 20130 20140 20150 20160
>< TthHB8I
>< TaqI
>< SstI
>< SduI
XhoI ><
>< SacI
TthHB8I >
> < PaeR7I
TaqI >
> < NspIII
SlaI ><
>< NspII
PaeR7I ><
>< HgiAI
NspIII ><
> < Eco88I
>< MnlI
>< XcmI
> < XhoI>< Eco24I
Eco88I ><
>< Sau3AI
>< Ecl136II
CcrI ><
>< NdeII
> < SlaI>< Bspl286I
BspWI ><
>< MboI
> < CcrI>< BmyI
BcoI ><
>< DpnII
> < BcoI>< BanII
> < BcgI/a
>< DpnI
> < Ama87I
AvaI ><
>< BspAI
> < AvaI>< Alw21I
Ama87I ><
>< Bsp143I
>< AluI >< EcoRI >< FokIAluI ><
AGCCCAGATC ACAAATGGAA ACTGACTTTC TCGAGCTCGC TATGGATGAA TTCATACAGC GATATAAGCT
20170 20180 20190 20200 20210 20220 20230
>< TthHB8I
>< TaqI
>< SfuI
>< NspV
>< LspI
>< Csp45I
>< BstBI
>< Bspl19I
>< BsiCI
>< MboII
>< Bpu14I
>< BbsI Tru9I ><
>< AsuII >< BcgI
>< NlaIII >< AciIMseI ><
CGAGGGCTAT GCCTTCGAAC ACATCGTTTA TGGAGATTTC AGTCATGGAC AACTTGGCGG TCTTCATTTA
20240 20250 20260 20270 20280 20290 20300
>< HphI
>< HinPlI
>< Hin6I
>< EspI > < HhaI >< TfiI
>< DdeI >< HaeII
>< CelII >< Eco47III >< Tru9I
>< Bpu1102I > < CfoI >< HinfI >< MseI
>< BfrI >< Bsp143II >< MnlI
ATGATAGGCT TAGCCAAGCG CTCACAAGAT TCACCACTTA AATTAGAGGA TTTTATCCCT ATGGACAGCA
20310 20320 20330 20340 20350 20360 20370
>< MstI
>< HinPlI
Sau3AI ><
>< Hin6I
NdeII ><
>< HhaI
MboI ><
>< FspI
DpnII ><
>< FdiII
DpnI ><
>< CfoI
BspAI ><
>< SfaNI >< AviII
Bsp143I ><
CAGTGAAAAA TTACTTCATA ACAGATGCGC AAACAGGTTC ATCAAAATGT GTGTGTTCTG TGATTGATCT
20380 20390 20400 20410 20420 20430 20440
>< TthHB8I

FIGURE 13.47

```
                  >< Tth111I
                     >< TaqI
               >< AspI            > < MaeIII                          MaeIII ><
TTTACTTGAT GACTTTGTCG AGATAATAAA GTCACAAGAT TTGTCAGTGA TTTCAAAAGT GGTCAAGGTT
     20450      20460      20470      20480      20490      20500      20510

                                                        >< NspI
                                                        >< NspHI
                                                        >< NlaIII
                                                           >< FokI
 >< MunI                        > < NlaIII          >< AflIII
ACAATTGACT ATGCTGAAAT TTCATTCATG CTTTGGTGTA AGGATGGACA TGTTGAAACC TTCTACCCAA
     20520      20530      20540      20550      20560      20570      20580

                               >< SfaNI
                               >< ScrFI
                               >< MvaI
                              >< EcoRII
                               >< Ecl136I
                              >< DsaV
                               >< BstOI            >< SfaNI
                               >< BstNI                  >< RsaI  BspWI ><
                               >< BsiLI                > < Csp6I        BsmI >
                  >< BspWI     >< ApyI                   >< AfaI   BscCI ><
AACTACAAGC AAGTCAAGCG TGGCAACCAG GTGTTGCGAT GCCTAACTTG TACAAGATGC AAAGAATGCT
     20590      20600      20610      20620      20630      20640      20650

 >< Eco57I >< MaeIII                          >< HphI
TCTTGAAAAG TGTGACCTTC AGAATTATGG TGAAAATGCT GTTATACCAA AAGGAATAAT GATGAATGTC
     20660      20670      20680      20690      20700      20710      20720

                                                    > < RsaI
                                                   >< Csp6I
       >< Bst1107I           >< Tru9I            >< AluI
      >< AccI                >< MseI                > < AfaINlaIII ><
GCAAAGTATA CTCAACTGTG TCAATACTTA AATACACTTA CTTTAGCTGT ACCCTACAAC ATGAGAGTTA
     20730      20740      20750      20760      20770      20780      20790

                                     >< ScrFI
                                        >< RsaI
                                     >< MvaI
                                   >< EcoRII  >< NspBII
                                     >< Ecl136I       >< SduI
                                        > < Csp6I     >< NspII
                                     >< BstOI >< PvuII>< HgiAI
                                     >< BstNI        >< DdeI
                                     >< BsiLI >< Psp5I>< Bsp1286I
                                     >< ApyI  >< AluI >< BmyI
                                   >< DsaV>< AfaI     >< Alw21I
TTCACTTTGG TGCTGGCTCT GATAAAGGAG TTGCACCAGG TACAGCTGTG CTCAGACAAT GGTTGCCAAC
     20800      20810      20820      20830      20840      20850      20860

                        >< XhoII
                           >< Tru9I
                        >< Sau3AI
                        >< NdeII
                  >< TthHB8I   >< MseI
                        >< MflI
                        >< MboI
                       >< MamI
                        >< DpnII
                   >< TfiI >< DpnI
```

FIGURE 13. 48

```
                        >< BstYI                          > < TfiI
                        >< BspAI                          > < HinfI
                    >< HinfI>< Bsp143I                >< Esp3I          >< Tru9I
                        >< BsiBI          >< Tth111I   >< BsmBI       >< MseI
                        >< BsaBI                      >< BsmAI        > < BsmAI
    >< BsrI      >< TaqI >< BglII     >< AspI     >< Alw26I  >< HgaI> < Alw26I
TGGCACACTA CTTGTCGATT CAGATCTTAA TGACTTCGTC TCCGACGCAG ATTCTACTTT AATTGGAGAC
     20870      20880      20890      20900      20910      20920      20930

                                                                 >< StyI
                                                                      >< SinI
                                                                      >< Sau96I
                                  > < SinI                          >< RmaI
                                  > < Sau96I                            >< NspIV
                                      >< PssI                     NspHII ><
                                    >< Psp5II                       >< MaeI
                                  > < PpuMI                         >< EcoT14I
                                  > < NspIV                             >< Eco47I
                                    >< NspHII                       >< Eco130I
                                    >< NlaIV                              >< Cfr13I
                                  > < Eco0109I                      >< BssT1I
                                  > < Eco47I                            >< BsiZI
                                  > < DraII                         >< BsaJI
                                  > < Cfr13I                            >< Bme18I
                                  > < BsiZI                         >< BlnI
                                    >< BscBI                        >< AvrII
          >< RsaI                 > < Bme18I                            >< AvaII
        > < Csp6I                 > < AvaII                             >< AsuI
          >< AfaI                 > < AsuI                              AflIII ><
TGTGCAACAG TACATACGGC TAATAAATGG GACCTTATTA TTAGCGATAT GTATGACCCT AGGACCAAAC
     20940      20950      20960      20970      20980      20990      21000

  >< NspI
  >< NspHI
  >< NlaIII >< PleI                                                   RmaI ><
 >< MaeIII        >< HinfI                                            MaeI ><
ATGTGACAAA AGAGAATGAC TCTAAAGAAG GGTTTTTCAC TTATCTGTGT GGATTTATAA AGCAAAAACT
     21010      21020      21030      21040      21050      21060      21070

    >< ScrFI
    >< MvaI
  >< EcoRII
    >< Ecl136I
  >< DsaV
    >< BstOI                                                        Sau96I >
    >< BstNI                                                         NspIV >
    >< BsiLI                                                        Cfr13I >
   >< BsaJI                                                          BsiZI >
  >< BsaJI     >< SfcI                >< BsmI          >< BsmI       AsuI >
    >< ApyI         > < AluI       >< BscCI       >< BscCIHindIII ><>< AluI
AGCCCTGGGT GGTTCTATAG CTGTAAAGAT AACAGAGCAT TCTTGGAATG CTGACCTTTA CAAGCTTATG
     21080      21090      21100      21110      21120      21130      21140

                                                 >< Zsp2I
                                              >< Ppu10I
 >< PalI                                         >< NsiI
 >< HaeIII                                       >< Mph1103I    Tru9I ><
 >< BsuRI                    >< MaeIII           >< EcoT22I          >< MseI
 >< BshI      >< NlaIII>< AluI     >< BcgI     >< AvaIII >< SfaNIBcgI/a ><
GGCCATTTCT CATGGTGGAC AGCTTTTGTT ACAAATGTAA ATGCATCATC ATCGGAAGCA TTTTTAATTG
     21150      21160      21170      21180      21190      21200      21210
```

FIGURE 13.49

```
                                                                    >< Zsp2I
                                                                      >< SphI
                                                                 >< Ppu10I
                                                                      >< PaeI
                                                                      >< NspI
                                                                      >< NspHI
                                                                    >< NsiI
                                                                      >< NlaIII
                                                                  > < NlaIII
                                                                     >< Mph1103I
                                                                     >< EcoT22I
                                                                  > < AvaIII      >< MnlI
GGGCTAACTA TCTTGGCAAG CCGAAGGAAC AAATTGATGG CTATACCATG CATGCTAACT ACATTTTCTG
     21220      21230      21240      21250      21260      21270      21280

                                                                          Tru9I ><
                    >< MboII                                               >< Tru9I
                     >< GsuI                                              MseI ><
                     >< BsrI                                              >< MseI
                     >< BpmI                                               MnlI ><
                    >< BbsI                                  >< NlaIII     >< MnlI
GAGGAACACA AATCCTATCC AGTTGTCTTC CTATTCACTC TTTGACATGA GCAAATTTCC TCTTAAATTA
     21290      21300      21310      21320      21330      21340      21350

                         >< Tru9I
                         >< MseI
                        >< Esp4I> < TfiI
                         >< BsmAI                          Ksp632I ><
                         >< Alw26I                    >< MboII      >< EarI
                        >< AflII> < HinfI                 Eam1104I ><
AGAGGAACTG CTGTAATGTC TCTTAAGGAG AATCAAATCA ATGATATGAT TTATTCTCTT CTGGAAAAAG
     21360      21370      21380      21390      21400      21410      21420

                                                          >< Tru9I
                                                          >< MseI
                                                           >< HindII
                                                           >< HincII
                                                           >< HpaI AflIII >
GTAGGCTTAT CATTAGAGAA AACAACAGAG TTGTGGTTTC AAGTGATATT CTTGTTAACA ACTAAACGAA
     21430      21440      21450      21460      21470      21480      21490

                                                          >< VneI
                                                          >< SnoI
                                                              >< SduI
                                                              >< NspII
                                                        >< HpaII
                                                              >< HgiAI
                                                        >< HapII
                                                       >< Cfr10I
                                                              >< Bsp1286I
                                                        >< MspI>< BmyI
   >< NspI                          >< SpeI                  >< ApaLI
   >< NspHI                          >< RmaI                 >< Alw44I
   >< NlaIII                         >< MaeI  >< MaeIII  >< AgeI >< Alw21I
CATGTTTATT TTCTTATTAT TTCTTACTCT CACTAGTGGT AGTGACCTTG ACCGGTGCAC CACTTTTGAT
     21500      21510      21520      21530      21540      21550      21560

         > < AluI                    >< MnlI
GATGTTCAAG CTCCTAATTA CACTCAACAT ACTTCATCTA TGAGGGGGGT TTACTATCCT GATGAAATTT
     21570      21580      21590      21600      21610      21620      21630

  >< Sau3AI
```

FIGURE 13. 50

```
   >< NdeII
   >< MboI
   >< DpnII
     >< DpnI          >< Tru9I
   >< BspAI           >< MseI  > < MboII
     >< Bsp143I           >< DdeI                          >< MaeIII
TTAGATCAGA CACTCTTTAT TTAACTCAGG ATTTATTTCT TCCATTTTAT TCTAATGTTA CAGGGTTTCA
     21640      21650      21660      21670      21680      21690      21700

     >< VspI
     >< Tru9I
     >< MseI
     >< AsnI                              >< Tru9I          >< FokI
     >< AseI  >< MaeII                    >< MseI >< BbvI       > < Fnu4HI
TACTATTAAT CATACGTTTG GCAACCCTGT CATACCTTTT AAGGATGGTA TTTATTTTGC TGCCACAGAG
     21710      21720      21730      21740      21750      21760      21770

                    >< BslI
               >< DsaI>< BsiYI               >< NlaIII
               >< BsaJI                           > < MaeIII
AAATCAAATG TTGTCCGTGG TTGGGTTTTT GGTTCTACCA TGAACAACAA GTCACAGTCG GTGATTATTA
     21780      21790      21800      21810      21820      21830      21840

                                  >< NspI
><  Tru9I                         >< NspHI
><  MseI                         >< NlaIII
 >< HphI                        >< MaeIII       >< MaeIII
TTAACAATTC TACTAATGTT GTTATACGAG CATGTAACTT TGAATTGTGT GACAACCCTT TCTTTGCTGT
     21850      21860      21870      21880      21890      21900      21910

        >< StyI                                    >< Zsp2I
             >< NlaIII                                >< Tru9I
        >< NcoI >< RsaI                          >< Ppu10I   TthHB8I ><
        >< EcoT14I                                  >< NsiI          >< TaqI
        >< Eco130I                                     >< MseI     SfaNI ><
        >< DsaI>< Csp6I                             >< Mph1103I      RsaI ><
        >< BssT1I                          >< TthHB8I >< EcoT22I     Csp6I ><
        >< BsaJI>< AfaI                    >< TaqI  >< AvaIII          AfaI ><
TTCTAAACCC ATGGGTACAC AGACACATAC TATGATATTC GATAATGCAT TTAATTGCAC TTTCGAGTAC
     21920      21930      21940      21950      21960      21970      21980

                                              >< Tru9I
                                              >< MseI
                                               >< DraI
ATATCTGATG CCTTTTCGCT TGATGTTTCA GAAAAGTCAG GTAATTTTAA ACACTTACGA GAGTTTGTGT
     21990      22000      22010      22020      22030      22040      22050

                                                                  >< Sau3AI
                                                                  >< NdeII
                                                                  >< MboI
                                                                  >< DpnII
><  Tru9I                                                           >< DpnI
><  MseI                                                          >< BspAI
 >< DraI                                          >< SfcI     Bsp143I ><
TTAAAAATAA AGATGGGTTT CTCTATGTTT ATAAGGGCTA TCAACCTATA GATGTAGTTC GTGATCTACC
     22060      22070      22080      22090      22100      22110      22120

                                              >< Tru9I
        >< Tru9I              > < Tru9I       >< MseI
        >< MseI               > < MseI        >< MnlI
TTCTGGTTTT AACACTTTGA AACCTATTTT TAAGTTGCCT CTTGGTATTA ACATTACAAA TTTTAGAGCC
     22130      22140      22150      22160      22170      22180      22190
```

FIGURE 13.51

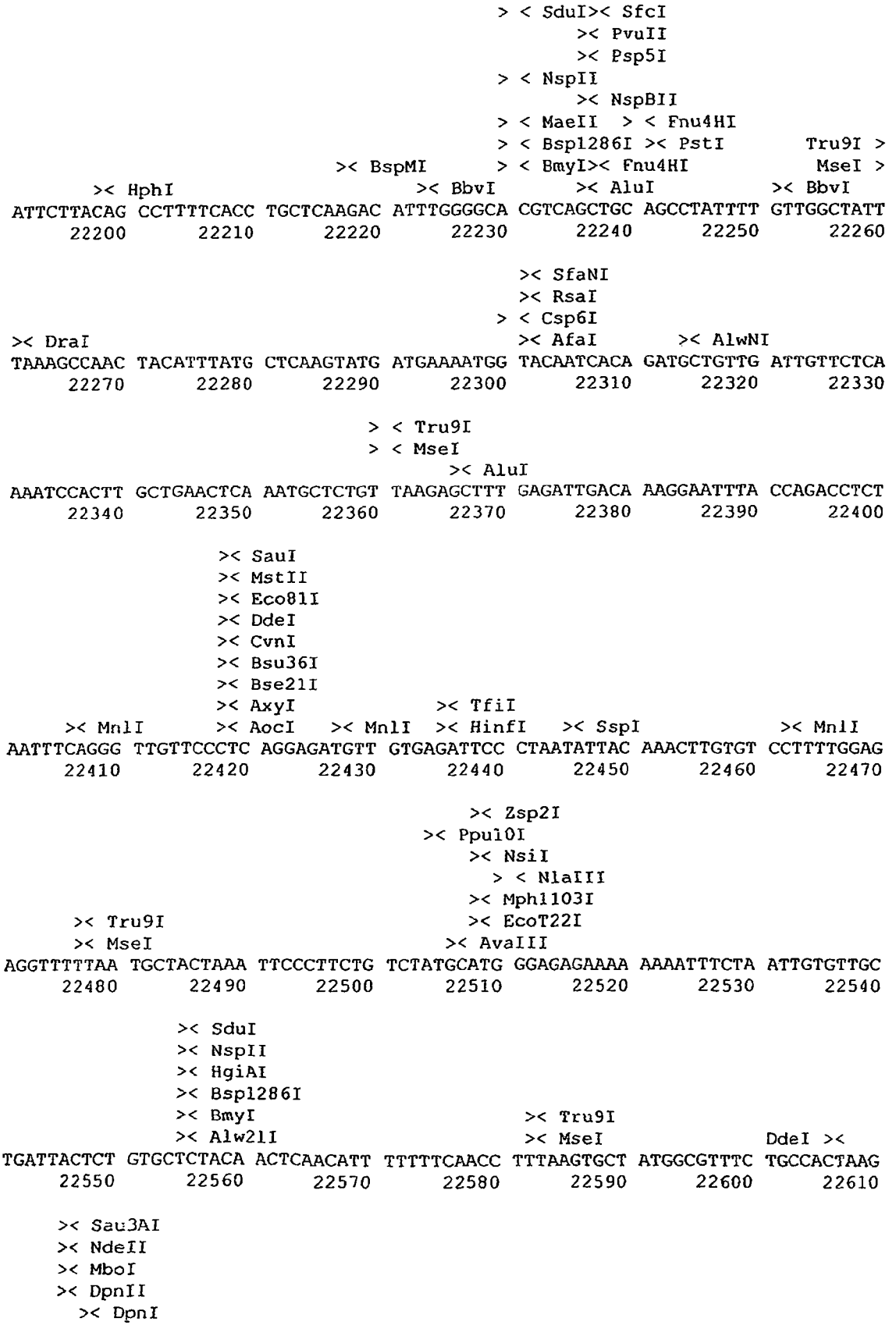

> < SduI>< SfcI
>< PvuII
>< Psp5I
> < NspII
>< NspBII
> < MaeII > < Fnu4HI
> < Bsp1286I >< PstI Tru9I >
>< BspMI > < BmyI>< Fnu4HI MseI >
>< HphI >< BbvI >< AluI >< BbvI
ATTCTTACAG CCTTTTCACC TGCTCAAGAC ATTTGGGGCA CGTCAGCTGC AGCCTATTTT GTTGGCTATT
22200 22210 22220 22230 22240 22250 22260
>< SfaNI
>< RsaI
> < Csp6I
>< DraI >< AfaI >< AlwNI
TAAAGCCAAC TACATTTATG CTCAAGTATG ATGAAAATGG TACAATCACA GATGCTGTTG ATTGTTCTCA
22270 22280 22290 22300 22310 22320 22330
> < Tru9I
> < MseI
>< AluI
AAATCCACTT GCTGAACTCA AATGCTCTGT TAAGAGCTTT GAGATTGACA AAGGAATTTA CCAGACCTCT
22340 22350 22360 22370 22380 22390 22400
>< SauI
>< MstII
>< Eco81I
>< DdeI
>< CvnI
>< Bsu36I
>< Bse21I
>< AxyI >< TfiI
>< MnlI >< AocI >< MnlI >< HinfI >< SspI >< MnlI
AATTTCAGGG TTGTTCCCTC AGGAGATGTT GTGAGATTCC CTAATATTAC AAACTTGTGT CCTTTTGGAG
22410 22420 22430 22440 22450 22460 22470
>< Zsp2I
>< Ppu10I
>< NsiI
> < NlaIII
>< Mph1103I
>< Tru9I >< EcoT22I
>< MseI >< AvaIII
AGGTTTTTAA TGCTACTAAA TTCCCTTCTG TCTATGCATG GGAGAGAAAA AAAATTTCTA ATTGTGTTGC
22480 22490 22500 22510 22520 22530 22540
>< SduI
>< NspII
>< HgiAI
>< Bsp1286I
>< BmyI >< Tru9I
>< Alw21I >< MseI DdeI ><
TGATTACTCT GTGCTCTACA ACTCAACATT TTTTTCAACC TTTAAGTGCT ATGGCGTTTC TGCCACTAAG
22550 22560 22570 22580 22590 22600 22610
>< Sau3AI
>< NdeII
>< MboI
>< DpnII
>< DpnI

FIGURE 13.52

```
      >< BspAI                          >< TfiI
        >< Bsp143I                      >< HinfI
TTGAATGATC TTTGCTTCTC CAATGTCTAT GCAGATTCTT TTGTAGTCAA GGGAGATGAT GTAAGACAAA
     22620      22630      22640      22650      22660      22670      22680

       >< ScrFI
       >< MvaI
   >< HinP1I
   >< Hin6I
     >< HhaI
      >< HaeII
     >< EcoRII
       >< Ecl136I
     >< DsaV
     >< CfoI
       >< BstOI
       >< BstNI
      >< Bsp143II
       >< BsiLI
       >< ApyI       > < BsrI                                        >< NlaIII
TAGCGCCAGG ACAAACTGGT GTTATTGCTG ATTATAATTA TAAATTGCCA GATGATTTCA TGGGTTGTGT
     22690      22700      22710      22720      22730      22740      22750

               >< SfaNI
              >< RmaI                                                 OdeI ><
              >< MaeI                          >< BsrI                BfrI ><
CCTTGCTTGG AATACTAGGA ACATTGATGC TACTTCAACT GGTAATTATA ATTATAAATA TAGGTATCTT
     22760      22770      22780      22790      22800      22810      22820

                >< Sau96I
                 >< PalI
                >< NspIV
          > < HindIII
                 >< HaeIII
                >< Eco0109I
                >< DraII
             >< DdeI
                >< Cfr13I
                 >< BsuRI
                >< BsiZI
                 >< BshI
            >< BfrI  >< PssI
      >< NlaIII  >< AsuI>< BsmAI
            >< AluI     >< Alw26I                                     BspWI ><
AGACATGGCA AGCTTAGGCC CTTTGAGAGA GACATATCTA ATGTGCCTTT CTCCCCTGAT GGCAAACCTT
     22830      22840      22850      22860      22870      22880      22890

                                    >< Tru9I
                                  >< PalI
                                  >< MscI
                                  >< HaeIII
                                 >< EaeI>< MseI
               >< Tru9I           >< BsuRI
               >< MseI            >< BshI
                >< BspMI          >< BalI                                BsrI ><
GCACCCCACC TGCTCTTAAT TGTTATTGGC CATTAAATGA TTATGGTTTT TACACCACTA CTGGCATTGG
     22900      22910      22920      22930      22940      22950      22960

                                                                Sau96I ><
                                                             >< PalINspIV ><
                                                          > < MspI   NspHII ><
                                                             >< HaeIII
```

FIGURE 13.53

```
                                                                  > < HpaII Eco47I ><
                                                                      >< DsaI
                                                                  > < HapII Cfr13I ><
                                                                      >< BsuRISinI ><
                                                                    >< GdiII BsiZI ><
                                >< ScaI                                 >< BsaJI
                                >< RsaI                  >< Tru9I   >< EaeI Bme18I ><
                               >< Csp6I                  >< MseI >< Cfr10I   AvaII ><
                                >< AfaI                   >< DraI     >< BshI AsuI ><
CTACCAACCT TACAGAGTTG TAGTACTTTC TTTTGAACTT TTAAATGCAC CGGCCACGGT TTGTGGACCA
     22970      22980      22990      23000      23010      23020      23030

                                                       >< Tru9I          >< RsaI
                                                     >< Tru9I           >< Csp6I
                                                       >< PleI           BsrI ><
                          > < Tru9I                    >< MseI          >< BsrI
                          > < MseI>< BsrI            >< MseI     >< HinfI   >< AfaI
AAATTATCCA CTGACCTTAT TAAGAACCAG TGTGTCAATT TTAATTTTAA TGGACTCACT GGTACTGGTG
     23040      23050      23060      23070      23080      23090      23100

  >< Tru9I                                             >< PalI
  >< MseI                                              >< HaeIII
   >< MboII                                          >< GdiII
   >< HpaI                                           >< EaeI
   >< HindII                                           >< BsuRI            TfiI ><
   >< HincII                                           >< BshI            HinfI ><
TGTTAACTCC TTCTTCAAAG AGATTTCAAC CATTTCAACA ATTTGGCCGT GATGTTTCTG ATTTCACTGA
     23110      23120      23130      23140      23150      23160      23170

         > < XhoII
       >< TthHB8I
       >< TaqI
         > < Sau3AI
         > < NdeII
         > < MflI
         > < MboI
         > < DpnII
            >< DpnI
         > < BstYI
         > < BspAI              > < SspI
    >< AlwI >< Bsp143I            >< HphI
TTCCGTTCGA GATCCTAAAA CATCTGAAAT ATTAGACATT TCACCTTGCT CTTTTGGGGG TGTAAGTGTA
     23180      23190      23200      23210      23220      23230      23240

        >< ScrFI
        >< MvaI
      >< EcoRII
        >< Ecl136I                                              >< Tru9I
      >< DsaV                                                   >< MseI
        >< BstOI                                                 >< HpaI
        >< BstNI                                                 >< HindII
        >< BsiLI                                          >< Eco57I
        >< ApyI                                        >< BsgI      >< HincII
ATTACACCTG GAACAAATGC TTCATCTGAA GTTGCTGTTC TATATCAAGA TGTTAACTGC ACTGATGTTT
     23250      23260      23270      23280      23290      23300      23310

                >< Sau3AI
              >< NlaIII
                >< NdeII
                >< MboI
                >< DpnII
                  >< DpnI                 >< HinP1I
```

FIGURE 13. 54

```
            >< BspWI                    >< Hin6I
                 >< BspAI                 > < HhaI              PleI ><
 >< SfcI           >< Bsp143I      >< AluI> < CfoI          >< BsrI
 CTACAGCAAT TCATGCAGAT CAACTCACAC CAGCTTGGCG CATATATTCT ACTGGAAACA ATGTATTCCA
      23320      23330      23340      23350      23360      23370      23380

                                       >< TthHB8I
                                       >< TaqI
                                      >< SalI
                                      >< RtrI
                                      >< NspI
                              >< EspI  >< NspHI
                              >< DdeI  >< NlaIII
                              >< CelII   >< HindII
                              >< Bpu1102I>< HincII
 >< HinfI                    >< AluI    >< AccI
 GACTCAAGCA GGCTGTCTTA TAGGAGCTGA GCATGTCGAC ACTTCTTATG AGTGCGACAT TCCTATTGGA
      23390      23400      23410      23420      23430      23440      23450

                                         > < SnaBI
                                               >< ScaI
                                               >< RsaI
                                                 >< RmaI
                                              >< MaeII >< MaeI
                                               > < Eco105I
                 >< RmaI                            >< Csp6I
                   >< MaeIII                      > < BsaAI
 >< AluI        >< MaeI                               >< AfaI
 GCTGGCATTT GTGCTAGTTA CCATACAGTT TCTTTATTAC GTAGTACTAG CCAAAAATCT ATTGTGGCTT
      23460      23470      23480      23490      23500      23510      23520

                               >< MunI
 ATACTATGTC TTTAGGTGCT GATAGTTCAA TTGCTTACTC TAATAACACC ATTGCTATAC CTACTAACTT
      23530      23540      23550      23560      23570      23580      23590

                                                                    RsaI ><
                                                                 >< MnlI
                                                                     Csp6I ><
                 >< SfcI                                               AfaI ><
 TTCAATTAGC ATTACTACAG AAGTAATGCC TGTTTCTATG GCTAAAACCT CCGTAGATTG TAATATGTAC
      23600      23610      23620      23630      23640      23650      23660

            > < TfiI
            > < HinfI
        >< AciI                                          > < AluI
 ATCTGCGGAG ATTCTACTGA ATGTGCTAAT TTGCTTCTCC AATATGGTAG CTTTTGCACA CAACTAAATC
      23670      23680      23690      23700      23710      23720      23730

 >< VneI
     >< SduI
     >< NspII
     >< HgiAI                                    >< PmlI
 >< SnoI>< DdeI                  >< Sau3AI       >< PmaCI
     >< Bsp1286I                 >< NdeII       >< MaeII
     >< BmyI                     >< MboI         >< Eco72I
     >< BbvI                        >< DpnI      >< BsaAI
 >< ApaLI                           >< Bsp143I   >< BbrPI
 >< Alw44I                       >< DpnII >< AlwI
     >< Alw21I       >< Fnu4HI   >< BspAI    >< AflIII
 GTGCACTCTC AGGTATTGCT GCTGAACAGG ATCGCAACAC ACGTGAAGTG TTCGCTCAAG TCAAACAAAT
      23740      23750      23760      23770      23780      23790      23800
```

FIGURE 13.55

```
 >< RsaI
>< Csp6I                              >< Tru9I
 >< AfaI                 >< SspI       >< MseI          >< SspI
GTACAAAACC CCAACTTTGA AATATTTTGG TGGTTTTAAT TTTTCACAAA TATTACCTGA CCCTCTAAAG
     23810      23820      23830      23840      23850      23860      23870

 >< MnlI
>< MnlI                               >< Tru9I   >< SfaNI  >< HphI  NlaIII ><
    >< DdeI   >< MnlI                 >< MseI >< MaeIII          BspHI ><
CCAACTAAGA GGTCTTTTAT TGAGGACTTG CTCTTTAATA AGGTGACACT CGCTGATGCT GGCTTCATGA
     23880      23890      23900      23910      23920      23930      23940

                                        >< XhoII
                                        >< Sau3AI
                  >< StyI             >< RmaI
                   >< RmaI              >< NdeII
                   >< MaeI              >< MflI
                  >< EcoT14I            >< MboI          >< MstI
                  >< Eco130I          >< MaeI           >< HinP1I
                  >< BssT1I   >< VspI   >< DpnII        >< Hin6I
                   >< BsmI          >< HphI> < DpnI       >< HhaI
                >< BscCI        >< Tru9I   >< BstYI       >< FspI
                  >< BsaJI      >< MseI    >< BspAI       >< FdiII
                  >< B1nI       >< AsnI      > < Bsp143I    >< CfoI
                  >< AvrII      >< AseI    >< BglII       >< AviII
AGCAATATGG CGAATGCCTA GGTGATATTA ATGCTAGAGA TCTCATTTGT GCGCAGAAGT TCAATGGACT
     23950      23960      23970      23980      23990      24000      24010

                                                          >< RmaIRsaI ><
                        >< MnlI          >< Fnu4HI      >< Fnu4HI Csp6I ><
                  >< BspWI  >< BbvI      >< BbvI    >< BspWI  >< MaeIAfaI ><
TACAGTGTTG CCACCTCTGC TCACTGATGA TATGATTGCT GCCTACACTG CTGCTCTAGT TAGTGGTACT
     24020      24030      24040      24050      24060      24070      24080

                              >< MboII
                               >< HinP1I
                               >< Hin6I
                                  >< HhaI
                                   >< HaeII
                                   >< Fnu4HI >< Ksp632I
                                  >< CfoI    >< EarI
                           >< FokI  >< BspWI   >< Eam1104I
                     >< BbvI          >< Bsp143II
GCCACTGCTG GATGGACATT TGGTGCTGGC GCTGCTCTTC AAATACCTTT TGCTATGCAA ATGGCATATA
     24090      24100      24110      24120      24130      24140      24150

                                                                 Tru9I ><
                                                                  MseI ><
                  >< MaeIII
GGTTCAATGG CATTGGAGTT ACCCAAAATG TTCTCTATGA GAACCAAAAA CAAATCGCCA ACCAATTTAA
     24160      24170      24180      24190      24200      24210      24220

                                                                MaeII ><
                           >< TfiI                            >< Fnu4HI
                           >< HinfI                 >< BbvI     >< AluI
CAAGGCGATT AGTCAAATTC AAGAATCACT TACAACAACA TCAACTGCAT TGGGCAAGCT GCAAGACGTT
     24230      24240      24250      24260      24270      24280      24290

 >< Tru9I
 >< MseI
  >< HpaI                                    >< DdeI
  >< HindII    >< BsmI >< Tru9I       >< Tru9I >< BfrI
  >< HincII>< BscCI    >< MseI        >< MseI      >< AluI
```

FIGURE 13. 56

```
GTTAACCAGA ATGCTCAAGC ATTAAACACA CTTGTTAAAC AACTTAGCTC TAATTTTGGT GCAATTTCAA
     24300      24310      24320      24330      24340      24350      24360

                        >< ThaI
                        >< SpoI
                        >< NruI
                        >< MvnI
                        >< BstUI       >< TthHB8I
                        >< Bsp68I      >< TaqI      >< RsaI
              >< EcoRV  >< Bsp50I      >< MnlI     >< Csp6I         >< Tru9I
              >< Eco32I >< AccII >< MnlI     >< AciI>< AfaI         >< MseI
GTGTGCTAAA TGATATCCTT TCGCGACTTG ATAAAGTCGA GGCGGAGGTA CAAATTGACA GGTTAATTAC
     24370      24380      24390      24400      24410      24420      24430

                             >< MaeIII >< BbvI         >< Fnu4HI   BbvI ><
AGGCAGACTT CAAAGCCTTC AAACCTATGT AACACAACAA CTAATCAGGG CTGCTGAAAT CAGGGCTTCT
     24440      24450      24460      24470      24480      24490      24500

           >< Fnu4HI                                          >< HindII
       >< BspWI             >< DdeI                          >< HincII
GCTAATCTTG CTGCTACTAA AATGTCTGAG TGTGTTCTTG GACAATCAAA AAGAGTTGAC TTTTGTGGAA
     24510      24520      24530      24540      24550      24560      24570

                                                              > < NspI
                                                              > < NspHI
                                                              > < NlaIII
                                                             >< MaeIII
                                                 >< NlaIII             >< MaeII
                                                >< MboII              >< FokI
                                     >< Fnu4HI  >< BbsI               BsaAI ><
                                          >< AciI>< BbvI          >< AflIII
AGGGCTACCA CCTTATGTCC TTCCCACAAG CAGCCCCGCA TGGTGTTGTC TTCCTACATG TCACGTATGT
     24580      24590      24600      24610      24620      24630      24640

       >< ScrFI
       >< MvaI
      >< EcoRII
       >< Ecl136I
       >< BstOI
       >< BstNI               >< HinP1I
    >< MnlI >< BslI           >< Hin6I
     >< DsaV>< BsiYI             >< HhaI
       >< BsiLI                   >< HaeII
     >< BsaJI>< HphI             >< CfoI          >< NlaIII
       >< ApyI                    >< Bsp143II >< BspHI                EcoNI ><
GCCATCCCAG GAGAGGAACT TCACCACAGC GCCAGCAATT TGTCATGAAG GCAAAGCATA CTTCCCTCGT
     24650      24660      24670      24680      24690      24700      24710

    >< MnlI
>< BslI          >< Tru9I
>< BsiYI         >< MseI               >< MnlI
GAAGGTGTTT TTGTGTTTAA TGGCACTTCT TGGTTTATTA CACAGAGGAA CTTCTTTTCT CCACAAATAA
     24720      24730      24740      24750      24760      24770      24780

                       >< DdeI                          >< Tru9I
                          >< BsmAI                         >< SfaNI
   >< SfcI                >< Alw26I                       >< MseIAlwI ><
TTACTACAGA CAATACATTT GTCTCAGGAA ATTGTGATGT CGTTATTGGC ATCATTAACA ACACAGTTTA
     24790      24800      24810      24820      24830      24840      24850

><  Sau3AI
><  NdeII
```

FIGURE 13.57

```
><MboI          ><PleI                                  >< ScaI
><DpnII       ><MnlI          > < Ksp632I               > < RsaI
  ><DpnI      ><DdeI  ><HinfI                       ><MboII
><BspAI        ><BspWI       > < Eam1104I          ><Csp6I
  ><Bsp143I       ><AluI     > < EarI   > < AluI   > < AfaI   > < HphI
TGATCCTCTG CAACCTGAGC TTGACTCATT CAAAGAAGAG CTGGACAAGT ACTTCAAAAA TCATACATCA
     24860      24870      24880      24890      24900      24910      24920

        ><Sau3AI
        ><NdeII
        ><MboI
       ><MamI
        ><DpnII
           ><DpnI
        ><BspAI
           ><Bsp143I
       ><BsiBI                  ><Tru9I         ><HindII
        ><BsaBI                 ><MseI          ><HincII         AciI ><
CCAGATGTTG ATCTTGGCGA CATTTCAGGC ATTAACGCTT CTGTCGTCAA CATTCAAAAA GAAATTGACC
     24930      24940      24950      24960      24970      24980      24990

                        ><Tru9I
                             > < TfiI
              ><MnlI       ><SwaI
     ><EcoNI             ><MseI
       ><BslI                 > < HinfI
><MnlI><BsiYI             ><DraI
GCCTCAATGA GGTCGCTAAA AATTTAAATG AATCACTCAT TGACCTTCAA GAATTGGGAA AATATGAGCA
     25000      25010      25020      25030      25040      25050      25060

             ><StyI
            ><PalI
            ><HaeIII
             ><EcoT14I
             ><Eco130I
            ><BsuRI
             ><BssT1I                                          NlaIII ><
    ><Tru9I><BshI                                              MaeIII ><
    ><MseI  ><BsaJI                                                  ><BstXI
ATATATTAAA TGGCCTTGGT ATGTTTGGCT CGGCTTCATT GCTGGACTAA TTGCCATCGT CATGGTTACA
     25070      25080      25090      25100      25110      25120      25130

                                                 > < SphI
                                                 > < PaeI
                  ><SpeI                         > < NspI
                   > < RmaI                      > < NspHI
                 ><NlaIII                        > < NlaIII
                   > < MaeI                   ><MnlI><BbvI Fnu4HI ><
ATCTTGCTTT GTTGCATGAC TAGTTGTTGC AGTTGCCTCA AGGGTGCATG CTCTTGTGGT TCTTGCTGCA
     25140      25150      25160      25170      25180      25190      25200

                       ><FokI
                    ><DdeI
><MnlI ><PleI><HinfI  ><BsrI
AGTTTGATGA GGATGACTCT GAGCCAGTTC TCAAGGGTGT CAAATTACAT TACACATAAA CGAACTTATG
     25210      25220      25230      25240      25250      25260      25270

                          ><Sau3AI
                          ><NdeII
                          ><MboI
                          ><DpnII
                            > < DpnI
```

FIGURE 13.58

```
                                  >< BspAI
                                    > < Bsp143I
                               >< BsgI       >< AlwI    >< BsrI             BspWI >
GATTTGTTTA TGAGATTTTT TACTCTTGGA TCAATTACTG CACAGCCAGT AAAAATTGAC AATGCTTCTC
     25280      25290      25300      25310      25320      25330      25340

       >< ScaI
       >< RsaI
      >< Csp6I     >< SfcI
       >< AfaI    >< NlaIII        >< AciI              >< MnlI      FokI >
CTGCAAGTAC TGTTCATGCT ACAGCAACGA TACCGCTACA AGCCTCACTC CCTTTCGGAT GGCTTGTTAT
     25350      25360      25370      25380      25390      25400      25410

                                  > < HinP1I
                                  > < Hin6I
                                     >< HhaI                          RmaI ><
                                       >< HaeII       >< HinP1I        NheI ><
                                     >< Eco47III      >< Hin6I          MaeI ><
                                      >< CfoI           >< HhaI    Fnu4HI ><
                  >< BspWI             >< Bsp143II      >< CfoI        AluI ><
TGGCGTTGCA TTTCTTGCTG TTTTTCAGAG CGCTACCAAA ATAATTGCGC TCAATAAAAG ATGGCAGCTA
     25420      25430      25440      25450      25460      25470      25480

      >< EcoNI
        >< BslI
        >< BsiYI                                  >< MaeIII
     >< BbvI      >< BsrI  >< BbvI        > < Fnu4HI              BbvI ><
GCCCTTTATA AGGGCTTCCA GTTCATTTGC AATTTACTGC TGCTATTTGT TACCATCTAT TCACATCTTT
     25490      25500      25510      25520      25530      25540      25550

                                                                   Zsp2I ><
                                                                Ppu10I ><
        > < SfcI        >< HinP1I                                  NsiI ><
             >< PstI    >< Hin6I     >< RsaI                     Mph1103I ><
        > < Fnu4HI        >< HhaI   >< Csp6I                     EcoT22I ><
  >< BspMI   >< MnlI       >< CfoI    >< AfaI      >< MnlI       AvaIII ><
TGCTTGTCGC TGCAGGTATG GAGGCGCAAT TTTTGTACCT CTATGCCTTG ATATATTTTC TACAATGCAT
     25560      25570      25580      25590      25600      25610      25620

     >< SfaNI
        >< NspI
        >< NspHI
        >< NlaIII                                                 >< SfaNI
CAACGCATGT AGAATTATTA TGAGATGTTG GCTTTGTTGG AAGTGCAAAT CCAAGAACCC ATTACTTTAT
     25630      25640      25650      25660      25670      25680      25690

                                                  >< Bst1107I
                                                 >< AccI   MaeIII ><
GATGCCAACT ACTTTGTTTG CTGGCACACA CATAACTATG ACTACTGTAT ACCATATAAC AGTGTCACAG
     25700      25710      25720      25730      25740      25750      25760

                                                                   >< MboII
                                     >< HphI                     BstXI ><
   >< MunI >< MaeIII  >< MaeIII        >< Eco57I                >< BbsI MnlI >
ATACAATTGT CGTTACTGAA GGTGACGGCA TTTCAACACC AAAACTCAAA GAAGACTACC AAATTGGTGG
     25770      25780      25790      25800      25810      25820      25830

                                              >< RsaI
                                                > < NlaIII
                                               >< HphI
                                  >< Tru9I >< Tth111I>< Csp6I
    >< DdeI       >< DdeI  >< MseI>< AspI      >< AfaI
```

FIGURE 13.59

```
TTATTCTGAG GATAGGCACT CAGGTGTTAA AGACTATGTC GTTGTACATG GCTATTTCAC CGAAGTTTAC
     25840      25850      25860      25870      25880      25890      25900

                                                                  Tru9I ><
         > < HinfI>< PleI                  >< BsrI                  MseI ><
     >< AluI >< AccI       >< SfcI  >< AlwNI       >< MboII         HindIII >
TACCAGCTTG AGTCTACACA AATTACTACA GACACTGGTA TTGAAAATGC TACATTCTTC ATCTTTAACA
     25910      25920      25930      25940      25950      25960      25970

                                        > < TthHB8I
      >< Tru9I                          > < TaqI       >< Ksp632I
      >< MseI                           > < MboII      >< EarI BspWI ><
 >< AluI                             >< Eco57I          >< Eam1104I AlwI ><
AGCTTGTTAA AGACCCACCG AATGTGCAAA TACACACAAT CGACGGCTCT TCAGGAGTTG CTAATCCAGC
     25980      25990      26000      26010      26020      26030      26040

   >< XhoII
   >< Sau3AI
     >< NlaIV
   >< NdeII
   >< MflI
   >< MboI
   >< DpnII
     >< DpnI
   >< BstYI
   >< BstI
   >< BspAI
     >< Bsp143I                                                    RsaI ><
     >< BscBI                          >< RmaI                   Csp6I ><
   >< BamHI >< AlwI                    >< MaeI                     AfaI ><
AATGGATCCA ATTTATGATG AGCCGACGAC GACTACTAGC GTGCCTTTGT AAGCACAAGA AAGTGAGTAC
     26050      26060      26070      26080      26090      26100      26110

                                        > < Tru9I
                                      >< RsaI
                                        > < MseI
                                     >< MboII
        > < RsaI                     >< MaeII             >< RsaI
       >< Csp6I                    >< Csp6I     >< Tru9I >< Csp6I
        > < AfaI                    >< AfaI      >< MseI   >< AfaI
GAACTTATGT ACTCATTCGT TTCGGAAGAA ACAGGTACGT TAATAGTTAA TAGCGTACTT CTTTTTCTTG
     26120      26130      26140      26150      26160      26170      26180

                                                ><  TthHB8I
                                                ><  TaqI
                ><  RmaI                    ><  HinP1I          > < RsaI
                  > < MaeIII                ><  Hin6I          Fnu4HI ><
                ><  MaeI    ><  RmaI          ><  HhaI         ><  Csp6I
                ><  FokI    ><  MaeI          ><  CfoI ><  BbvI  > < AfaI
CTTTCGTGGT ATTCTTGCTA GTCACACTAG CCATCCTTAC TGCGCTTCGA TTGTGTGCGT ACTGCTGCAA
     26190      26200      26210      26220      26230      26240      26250

                                                     ><  Tru9I
    ><  Tru9I                                      ><  ThaI
    ><  MseI                                       ><  MvnI
><  SspI ><  MaeII                                      ><  MseI
     ><  HpaI                                       ><  BstUI          Ksp632I >
     ><  HindII                          ><  MaeII   ><  Bsp50I   ><  MboII EarI >
     ><  HincII                            ><  AccI ><  AccII         Eam1104I >
TATTGTTAAC GTGAGTTTAG TAAAACCAAC GGTTTACGTC TACTCGCGTG TTAAAAATCT GAACTCTTCT
     26260      26270      26280      26290      26300      26310      26320
```

FIGURE 13.60

```
            >< Sau3AI
            >< NdeII
            >< MboI
            >< DpnII
         >< MboII>< DpnI
       >< XmnI >< BspAI> < Eco57I                                    >< Tru9I
       >< Asp700I>< Bsp143I                                          >< MseI
GAAGGAGTTC CTGATCTTCT GGTCTAAACG AACTAACTAT TATTATTATT CTGTTTGGAA CTTTAACATT
     26330      26340      26350      26360      26370      26380      26390

                                                        >< ScrFI
                                                        >< MvaI
                                                      >< EcoRII
                                                        >< Ecl136I
                                                      >< DsaV  NlaIV ><
                        >< RsaI                         >< BstOI
                             >< MnlI          >< Tru9I    >< BstNI   RmaI ><
                          >< Csp6I            >< MseI     >< BsiLI   MaeI ><
          > < NlaIII     >< AfaI           > < AluI      >< ApyIBscBI ><
GCTTATCATG GCAGACAACG GTACTATTAC CGTTGAGGAG CTTAAACAAC TCCTGGAACA ATGGAACCTA
     26400      26410      26420      26430      26440      26450      26460

                          >< ScrFI
                       >< RmaI
                          >< MvaI
                       >< MaeI
                          >< EcoRII
                            >< Ecl136I
                          >< DsaV
                            >< BstOI
                            >< BstNI
                            >< BsiLI
                            >< ApyI  >< MaeIII
GTAATAGGTT TCCTATTCCT AGCCTGGATT ATGTTACTAC AATTTGCCTA TTCTAATCGG AACAGGTTTT
     26470      26480      26490      26500      26510      26520      26530

                                          >< PalI
                                          >< MscI
                                       >< MnlI  >< MaeIII
                                          >< HaeIII
                                       >< EaeI
                                          >< BsuRI
                                           >< BsrI
  >< RsaI                              >< BspWI
 >< Csp6I   >< HindIII                    >< BshI
  >< AfaI     >< AluI                     >< BalI          >< BbvI Fnu4HI ><
TGTACATAAT AAAGCTTGTT TTCCTCTGGC TCTTGTGGCC AGTAACACTT GCTTGTTTTG TGCTTGCTGC
     26540      26550      26560      26570      26580      26590      26600

            >< VspI
            >< Tru9I
            >< MseI              >< HphI
   >< SfcI  >< AsnI           >< BsrI
  >< AccI   >< AseI>< MaeIII>< AciI
TGTCTACAGA ATTAATTGGG TGACTGGCGG GATTGCGATT GCAATGGCTT GTATTGTAGG CTTGATGTGG
     26610      26620      26630      26640      26650      26660      26670

><  EspI
      >< Eco57I
><  DdeI
><  CelII                                  >< RsaI
><  Bpu1102I                               >< Csp6I
```

FIGURE 13.61

```
><BfrI                                    ><AfaI
   ><AluI                                    ><AciI                  MboII >
CTTAGCTACT TCGTTGCTTC CTTCAGGCTG TTTGCTCGTA CCCGCTCAAT GTGGTCATTC AACCCAGAAA
     26680      26690      26700      26710      26720      26730      26740

                          ><ScrFI
                          ><NciI
                         ><MspI
                         ><HpaII
                         ><HapII
                        ><DsaV><MnlI
                          ><BslI
                          ><BsiYI
                         ><BsaJI ><MunI          > <XcmI
                          ><BcnI     ><MaeIII  ><AciI  ><NlaIII
CAAACATTCT TCTCAATGTG CCTCTCCGGG GGACAATTGT GACCAGACCG CTCATGGAAA GTGAACTTGT
     26750      26760      26770      26780      26790      26800      26810

                                                                Tru9I ><
                                                                    SinI >
                                                                  Sau96I >
                                                                   PpuMI >
                                                                   NspIV >
                                                                 MseI ><
                                                               ><MaeIII
           ><Sau3AI                                    > <RmaI ><HaeII
           ><NdeII                          ><PalI     > <MaeI     EcoO109I >
           ><MboI                            ><MspI          ><HinP1IEco47I >
             ><FbaI                          ><HpaII   ><StyI><Hin6I  DraII >
           ><DpnII                           ><HapII   ><EcoT14I      Cfr13I >
             ><DpnI                         ><HaeIII   ><Eco130I><Bsp143II
           ><BspAI                        ><GdiII      ><BssT1I        BsiZI >
             ><Bsp143I                    ><EaeI       ><BsaJI         Bme18I >
           ><BsiQI                          ><BsuRI    ><BlnI  ><HhaI AvaII >
           ><BclI     ><MaeIII              ><BshI     ><AvrII ><CfoI  AsuI >
CATTGGTGCT GTGATCATTC GTGGTCACTT GCGAATGGCC GGACACTCCC TAGGGCGCTG TGACATTAAG
     26820      26830      26840      26850      26860      26870      26880

              ><Sau3AI
              ><NdeII
              ><MboI
              ><DpnII
                ><DpnI
   ><PssI ><BspMI
><Psp5II     ><BspAI                    ><XmnI
><NspHII       ><Bsp143I                ><Asp700I  > <HgaI    Fnu4HI ><
GACCTGCCAA AAGAGATCAC TGTGGCTACA TCACGAACGC TTTCTTATTA CAAATTAGGA GCGTCGCAGC
     26890      26900      26910      26920      26930      26940      26950

            ><TfiI
            ><HinfI
           ><BbvI                                                 > <Tru9I
       ><BbvI            ><Fnu4HI  ><AciI                       > <MseI
GTGTAGGCAC TGATTCAGGT TTTGCTGCAT ACAACCGCTA CCGTATTGGA AACTATAAAT TAAATACAGA
     26960      26970      26980      26990      27000      27010      27020

     ><MspI                          ><RsaI
     ><HpaII                       ><RmaI
     ><HapII                         ><Csp6I
   ><Cfr10I                        ><MaeI><BcgI                    HindII ><
   ><BcgI/a          ><SspI          ><AfaI ><MaeIII               HincII ><
```

FIGURE 13.62

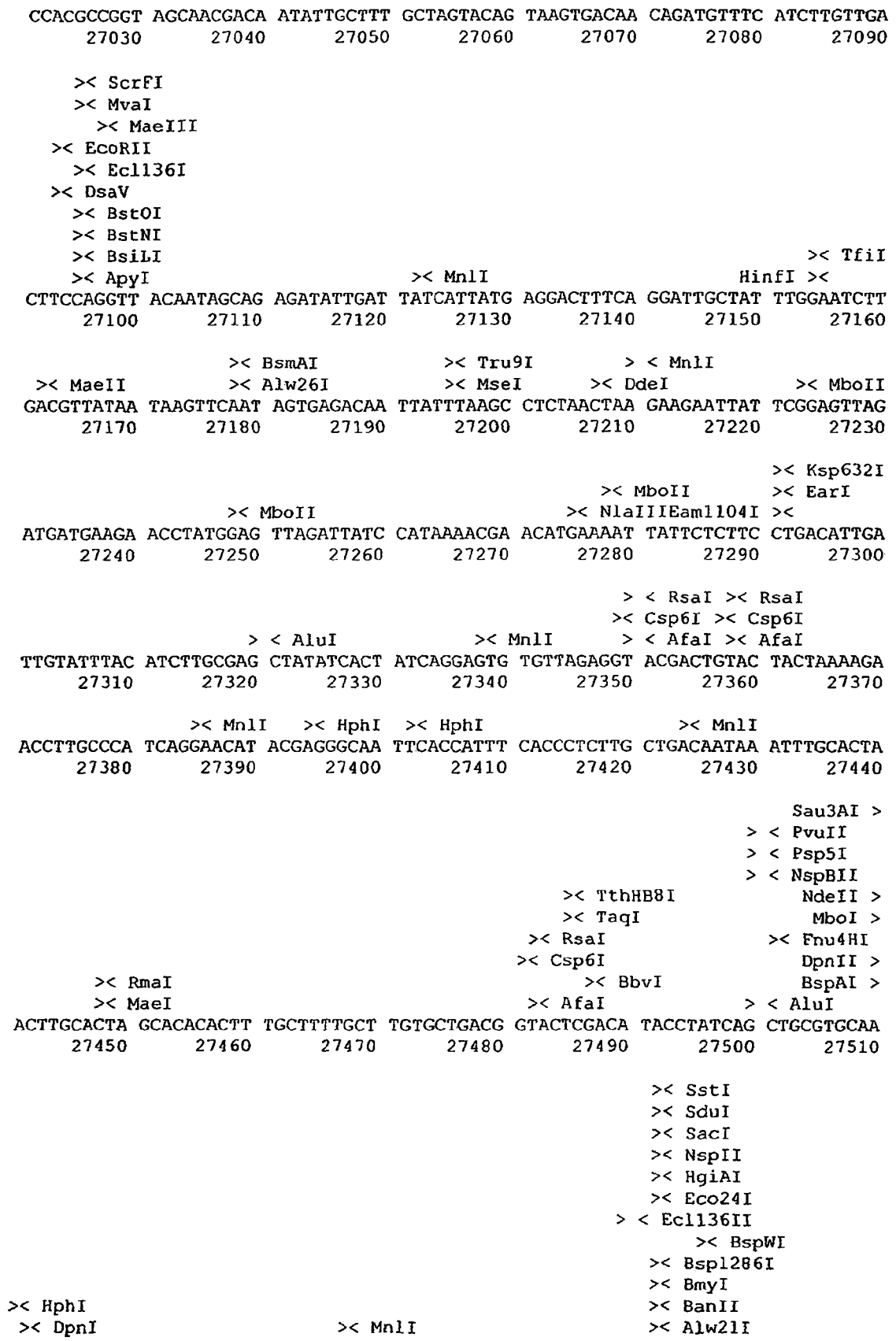

CCACGCCGGT AGCAACGACA ATATTGCTTT GCTAGTACAG TAAGTGACAA CAGATGTTTC ATCTTGTTGA
27030 27040 27050 27060 27070 27080 27090
>< ScrFI
>< MvaI
>< MaeIII
>< EcoRII
>< Ecl136I
>< DsaV
>< BstOI
>< BstNI
>< BsiLI
>< TfiI
>< ApyI
>< MnlI
HinfI ><
CTTCCAGGTT ACAATAGCAG AGATATTGAT TATCATTATG AGGACTTTCA GGATTGCTAT TTGGAATCTT
27100 27110 27120 27130 27140 27150 27160
>< BsmAI
>< Tru9I
> < MnlI
>< MaeII
>< Alw26I
>< MseI
>< DdeI
>< MboII
GACGTTATAA TAAGTTCAAT AGTGAGACAA TTATTTAAGC CTCTAACTAA GAAGAATTAT TCGGAGTTAG
27170 27180 27190 27200 27210 27220 27230
>< Ksp632I
>< MboII
>< EarI
>< MboII
>< NlaIIIEaml104I ><
ATGATGAAGA ACCTATGGAG TTAGATTATC CATAAAACGA ACATGAAAAT TATTCTCTTC CTGACATTGA
27240 27250 27260 27270 27280 27290 27300
> < RsaI >< RsaI
>< Csp6I >< Csp6I
> < AluI
>< MnlI
> < AfaI >< AfaI
TTGTATTTAC ATCTTGCGAG CTATATCACT ATCAGGAGTG TGTTAGAGGT ACGACTGTAC TACTAAAAGA
27310 27320 27330 27340 27350 27360 27370
>< MnlI
>< HphI
>< HphI
>< MnlI
ACCTTGCCCA TCAGGAACAT ACGAGGGCAA TTCACCATTT CACCCTCTTG CTGACAATAA ATTTGCACTA
27380 27390 27400 27410 27420 27430 27440
Sau3AI >
> < PvuII
> < Psp5I
> < NspBII
>< TthHB8I
NdeII >
>< TaqI
MboI >
>< RsaI
>< Fnu4HI
>< Csp6I
DpnII >
>< RmaI
>< BbvI
BspAI >
>< MaeI
>< AfaI
> < AluI
ACTTGCACTA GCACACACTT TGCTTTTGCT TGTGCTGACG GTACTCGACA TACCTATCAG CTGCGTGCAA
27450 27460 27470 27480 27490 27500 27510
>< SstI
>< SduI
>< SacI
>< NspII
>< HgiAI
>< Eco24I
> < Ecl136II
>< BspWI
>< Bspl286I
>< BmyI
>< HphI
>< BanII
>< DpnI
>< MnlI
>< Alw21I

FIGURE 13. 63

```
  >< Bsp143I              >< MnlI                    > < AluI    BbvI ><
 GATCAGTTTC ACCAAAACTT TTCATCAGAC AAGAGGAGGT TCAACAAGAG CTCTACTCGC CACTTTTTCT
      27520      27530      27540      27550      27560      27570      27580

                                                                  SstI ><
                                                                  SduI ><
                                                                  SacI ><
                                                                 NspII ><
                                                                 HgiAI ><
                                                                Eco24I ><
                                                             Ecl136II ><
                                                               Bsp1286I ><
                                                                   BmyI ><
             >< RmaI   >< Tru9I                                   BanII ><
             >< MaeI   >< MseI              >< Tru9I              Alw21I ><
        >< Fnu4HI            >< HphI        >< MseI               AluI ><
 CATTGTTGCT GCTCTAGTAT TTTTAATACT TTGCTTCACC ATTAAGAGAA AGACAGAATG AATGAGCTCA
      27590      27600      27610      27620      27630      27640      27650

  >< Tru9I                                                >< Tru9I
  >< MseI                                                 >< MseI
 CTTTAATTGA CTTCTATTTG TGCTTTTTAG CCTTTCTGCT ATTCCTTGTT TTAATAATGC TTATTATATT
      27660      27670      27680      27690      27700      27710      27720

                      >< XhoII
                         >< XbaI
                    > < ScrFI
                      >< Sau3AI
                          >< RmaI
                      >< NdeII
                    > < MvaI
                      >< MflI
                      >< MboI
                   >< EcoRII>< MaeI
                     > < Ecl136I
                      >< DpnII
                        >< DpnI
                      >< BstYI
                     > < BstOI
                     > < BstNI
           >< TthHB8I >< BspAI          > < RsaI
                 >< DsaV>< Bsp143I        >< MboII
                   > < BsiLI            >< Csp6I
           >< TaqI > < ApyI   > < AlwI   > < AfaI                  >< NlaIII
 TTGGTTTTCA CTCGAAATCC AGGATCTAGA AGAACCTTGT ACCAAAGTCT AAACGAACAT GAAACTTCTC
      27730      27740      27750      27760      27770      27780      27790

                                                    >< HinP1I
                                                    >< Hin6I
                                                      >< HhaI
                                                  >< RsaI >< HaeII
                                               >< SfcI     >< Eco47III
                                                    >< Csp6I>< CfoI  SfaNI ><
                                        >< NdeI       >< AfaI >< Bsp143II
 ATTGTTTTGA CTTGTATTTC TCTATGCAGT TGCATATGCA CTGTAGTACA GCGCTGTGCA TCTAATAAAC
      27800      27810      27820      27830      27840      27850      27860

            >< XhoII
            >< Sau3AI
            >< NdeII
        > < MnlI
            >< MflI
```

FIGURE 13.64

```
                ><  MboI
                ><  DpnII
                  ><  DpnI      ><  RsaI
                ><  BstYI   ><  MboII
      ><  NlaIII><  BspAI      ><  Csp6I ><  RmaI
         ><  AlwI ><  Bsp143I   ><  AfaI ><  MaeI
 CTCATGTGCT TGAAGATCCT TGTAAGGTAC AACACTAGGG GTAATACTTA TAGCACTGCT TGGCTTTGTG
     27870      27880      27890      27900      27910      27920      27930

  ><  SduI
   ><  RmaI
  ><  NspII
   ><  MaeI
  ><  HgiAI
  ><  Bsp1286I                                              ><  NspI
  ><  BmyI                                                  ><  NspHI
  ><  Alw21I                                                ><  NlaIII  ><  MaeIII
 CTCTAGGAAA GGTTTTACCT TTTCATAGAT GGCACACTAT GGTTCAAACA TGCACACCTA ATGTTACTAT
     27940      27950      27960      27970      27980      27990      28000

         >  <  XhoII
         >  <  Sau3AI >  <  Van91I                    ><  RsaI
                 ><  PvuII                           ><  NlaIV
                 ><  Psp5I                             ><  KpnI ><  NlaIII
         >  <  NdeII  >  <  PflMI                    ><  Eco64I      ><  MaeIII
         >  <  MflI><  NspBII                         ><  Csp6I><  HphI
         >  <  DpnII          ><  HinP1I              ><  BscBI      ><  Eco0651
            ><  Bsp143I      ><  Hin6I               ><  BanI ><  BspHI
         >  <  BstYI  >  <  BslI ><  HhaI  ><  RmaI   ><  Asp718      ><  Eco91I
         >  <  BspAI  >  <  BsiYI><  CfoI  ><  MaeI     ><  AfaI      ><  BstPI
         >  <  MboI><  AluI><  BspWI  ><  BspWI      ><  AccB1I      ><  BstEII
    ><  AlwI ><  DpnI >  <  AccB7I       ><  AluI    ><  Acc65I     ><  BbvI
 CAACTGTCAA GATCCAGCTG GTGGTGCGCT TATAGCTAGG TGTTGGTACC TTCATGAAGG TCACCAAACT
     28010      28020      28030      28040      28050      28060      28070

                                                                    ><  SinI
                                                                    ><  Sau96I
                                                                    ><  NspIV
                                                                 NspHII ><
                                                                   NlaIV ><
                                                                    ><  Eco47I
                                                                    ><  Cfr13I
                ><  RsaI                                            ><  BsiZI
  ><  Fnu4HI    ><  MaeII                                          BscBI ><
    ><  Esp3I    ><  Csp6I     ><  Tru9I                            ><  Bme18I
    ><  BsmAI     ><  BsmBI     ><  MseI            ><  Tru9I       ><  AvaII
    ><  Alw26I    ><  AfaI      ><  DraI            ><  MseI        ><  AsuI
 GCTGCATTTA GAGACGTACT TGTTGTTTTA AATAAACGAA CAAATTAAAA TGTCTGATAA TGGACCCCAA
     28080      28090      28100      28110      28120      28130      28140

                                    ><  SinI
                                    ><  Sau96I
                                    ><  NspIV
                                     ><  NspHII
                                       ><  NlaIV
                                    ><  Eco47I
                                    ><  Cfr13I
                ><  SduI            ><  BsiZI
                ><  NspII             ><  BscBI
                ><  Bsp1286I        ><  Bme18I
                ><  BmyI            ><  AvaII  ><  TfiI
      ><  MaeII      ><  AciI       ><  AsuI   ><  HinfI          ><  MnlI
```

FIGURE 13.65

```
TCAAACCAAC GTAGTGCCCC CCGCATTACA TTTGGTGGAC CCACAGATTC AACTGACAAT AACCAGAATG
     28150      28160      28170      28180      28190      28200      28210

                            >< HinP1I >< StyI
                               >< HaeII
                        > < PalI  >< Hin6I  >< EcoT14I
                        > < HaeIII   >< HhaI>< Eco130I
                              >< BspWI     >< BssT1I
                        > < BsuRI     >< Bsp143II
                  >< HgaI> < BshI     >< CfoI>< BsaJI    >< HgaI
GAGGACGCAA TGGGGCAAGG CCAAAACAGC GCCGACCCCA AGGTTTACCC AATAATACTG CGTCTTGGTT
     28220      28230      28240      28250      28260      28270      28280

                                                  >< TthHB8I
                                                       > < ScrFI
                                                     >< PalI
                                                  >< PaeR7I
                                                  >< NspIII
                                                       > < MvaI
                                                     >< HaeIII
                                                     >< EcoRII
                                                  >< Eco88I
                                                  >< XhoI > < Ecl136I
                                                     >< DsaV
                                                     >< BsuRI
                                                  >< SlaI > < BstOI
                                               >< MnlI>< TaqI> < BstNI
                                                     >< CcrI > < BsiLI
                                               >< HinfI     >< BshI
                                               >< TfiI>< BcoI>< BsaJI
                        >< MnlI          >< DdeI    >< AvaI > < ApyI
     >< AluI >< DdeI > < NlaIII    >< BfrI    >< Ama87I >< MnlI
CACAGCTCTC ACTCAGCATG GCAAGGAGGA ACTTAGATTC CCTCGAGGCC AGGGCGTTCC AATCAACACC
     28290      28300      28310      28320      28330      28340      28350

      >< SinI
      >< Sau96I
      >< NspIV
       >< NspHII
      >< Eco47I
      >< Cfr13I
      >< BsiZI
      >< Bme18I                > < Ksp632I
      >< AvaII                 > < Eam1104I
      >< AsuI                  > < EarI   > < AluI>< MboII        >< MaeIII
AATAGTGGTC CAGATGACCA AATTGGCTAC TACCGAAGAG CTACCCGACG AGTTCGTGGT GGTGACGGCA
     28360      28370      28380      28390      28400      28410      28420

            >< SstI
            >< SduI
            >< SacI
            >< NspII
            >< HgiAI
           >< EspI
            >< Eco24I                              >< Sau96I
          >< Ecl136II                  >< StyI     >< PalI
           >< DdeI                      >< RmaI   >< NspIV
           >< CelII                     >< MaeI    >< HaeIII
            >< Bsp1286I                >< EcoT14I >< Cfr13I
           >< Bpu1102I                 >< Eco130I  >< BsuRI
            >< BmyI                    >< BssT1I    > < BsrI
            >< BanII       >< RsaI     >< BsaJI   >< BsiZI
```

FIGURE 13.66

```
            >< Alw21I     >< Csp6I     >< BlnI     >< BshI>< HindIII
  >< HphI  >< AluI          >< AfaI     >< AvrII   >< AsuI   >< AluI
AAATGAAAGA GCTCAGCCCC AGATGGTACT TCTATTACCT AGGAACTGGC CCAGAAGCTT CACTTCCCTA
     28430      28440      28450      28460      28470      28480      28490

  >< HinP1I
  >< Hin6I
    >< HhaI
     >< HaeII
    >< CfoI                    > < MnlI       >< NlaIV
     >< Bspl43II             >< SfaNI  >< DdeI >< BscBI
CGGCGCTAAC AAAGAAGGCA TCGTATGGGT TGCAACTGAG GGAGCCTTGA ATACACCCAA AGACCACATT
     28500      28510      28520      28530      28540      28550      28560

  >< NlaIV
><  Eco64I
  >< BscBI
><  BanI
     >< AciI
><  AccB1I   >< BbvI        >< Fnu4HI                          >< MnlI
GGCACCCGCA ATCCTAATAA CAATGCTGCC ACCGTGCTAC AACTTCCTCA AGGAACAACA TTGCCAAAAG
     28570      28580      28590      28600      28610      28620      28630

                                                                  >< TthI
                                                               >< MnlI
                                                            >< MaeII >< MvnI
                                                     >< MnlI      BstUI ><
                            >< Fnu4HI             >< Ksp632I     Bsp50I ><
                           >< BspWI               >< EarI       >< BsaAI>< AciI
   >< MnlI    >< MnlI     >< AciI>< MboII        >< Eaml104I     AccII ><
GCTTCTACGC AGAGGGAAGC AGAGGCGGCA GTCAAGCCTC TTCTCGCTCC TCATCACGTA GTCGCGGTAA
     28640      28650      28660      28670      28680      28690      28700

                 >< ScrFI
                 >< MvaI
                ><  EcoRII                        >< TthHB8I
                 >< Ecl136I                             >< RmaI
                ><  DsaV>< Fnu4HI                     >< NheI
                 >< BstOI                            >< MnlI
                 >< BstNI                              >< MaeI
                 >< BsiLI                          > < BspWI
                 >< ApyI          >< BbvI       >< TaqI     >< AciI
TTCAAGAAAT TCAACTCCTG GCAGCAGTAG GGGAAATTCT CCTGCTCGAA TGGCTAGCGG AGGTGGTGAA
     28710      28720      28730      28740      28750      28760      28770

        > < ThaI
        > < MvnI
      >< HphI   >< MnlI
        > < HinP1I
        > < Hin6I
           >< HhaI
        > < BstUI     >< RmaI                                    PalI ><
        > < Bsp50I    >< MaeI                                   HaeIII ><
   >< BbvI >< CfoI>< Fnu4HI                                     BsuRI ><
        > < AccII>< BspWI              >< AluI                    BshI ><
ACTGCCCTCG CGCTATTGCT GCTAGACAGA TTGAACCAGC TTGAGAGCAA AGTTTCTGGT AAAGGCCAAC
     28780      28790      28800      28810      28820      28830      28840

                                                                  RsaI ><
       > < PalI>< MaeIII                                           >< MnlI
       > < HaeIII                  >< Fnu4HI                    MaeII ><
       > < BsuRI     >< DdeI         >< DdeI                      Csp6I ><
```

FIGURE 13.67

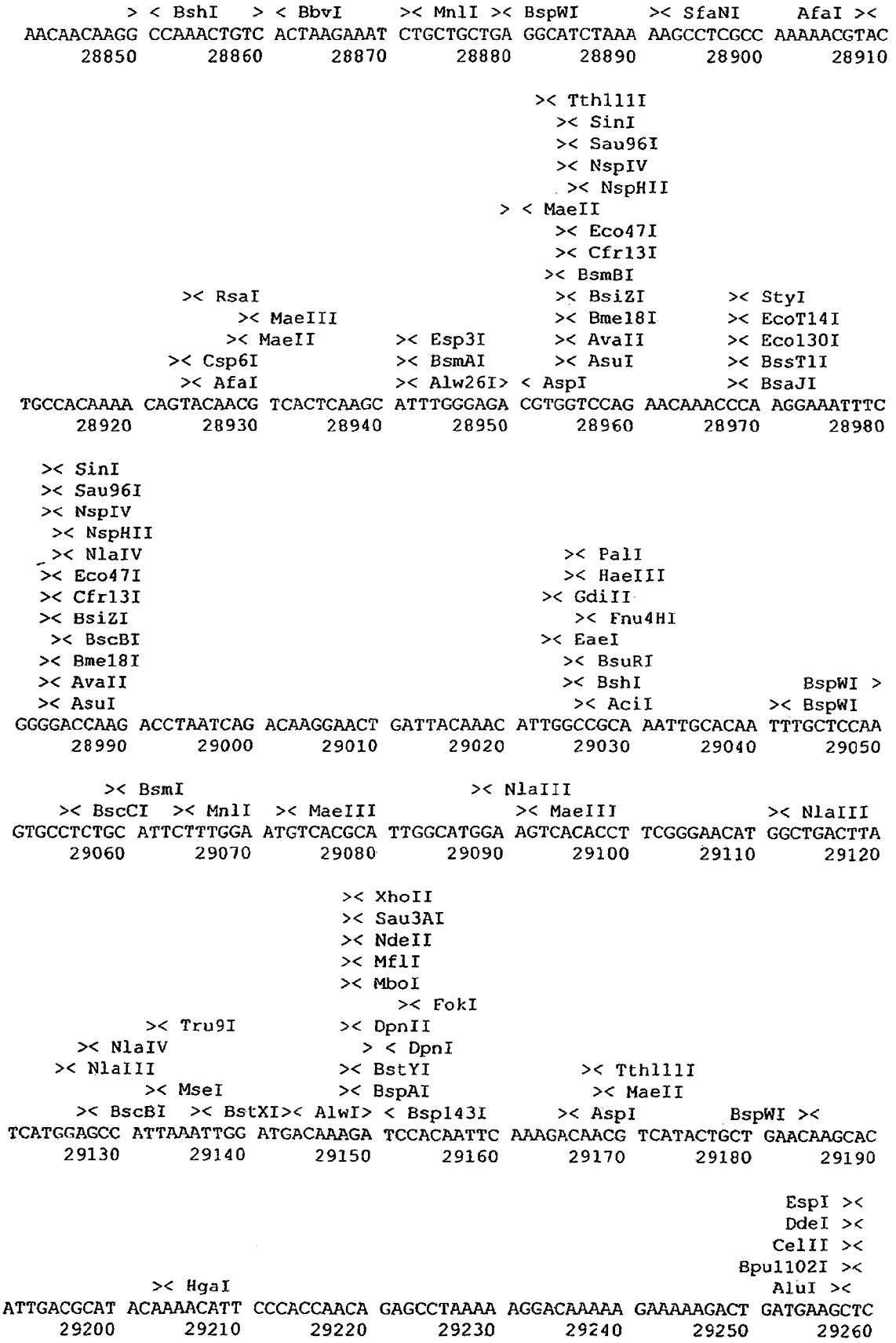

> < BshI > < BbvI >< MnlI >< BspWI >< SfaNI AfaI ><
AACAACAAGG CCAAACTGTC ACTAAGAAAT CTGCTGCTGA GGCATCTAAA AAGCCTCGCC AAAAACGTAC
28850 28860 28870 28880 28890 28900 28910
>< Tth111I
>< SinI
>< Sau96I
>< NspIV
>< NspHII
> < MaeII
>< Eco47I
>< Cfr13I
>< BsmBI
>< RsaI >< BsiZI >< StyI
>< MaeIII >< Bme18I >< EcoT14I
>< MaeII >< Esp3I >< AvaII >< Eco130I
>< Csp6I >< BsmAI >< AsuI >< BssT1I
>< AfaI >< Alw26I> < AspI >< BsaJI
TGCCACAAAA CAGTACAACG TCACTCAAGC ATTTGGGAGA CGTGGTCCAG AACAAACCCA AGGAAATTTC
28920 28930 28940 28950 28960 28970 28980
>< SinI
>< Sau96I
>< NspIV
>< NspHII
>< NlaIV
>< Eco47I
>< Cfr13I
>< BsiZI
>< BscBI
>< Bme18I
>< AvaII
>< AsuI
>< PalI
>< HaeIII
>< GdiII
>< Fnu4HI
>< EaeI
>< BsuRI
>< BshI BspWI >
>< AciI >< BspWI
GGGGACCAAG ACCTAATCAG ACAAGGAACT GATTACAAAC ATTGGCCGCA AATTGCACAA TTTGCTCCAA
28990 29000 29010 29020 29030 29040 29050
>< BsmI >< NlaIII
>< BscCI >< MnlI >< MaeIII >< MaeIII >< NlaIII
GTGCCTCTGC ATTCTTTGGA ATGTCACGCA TTGGCATGGA AGTCACACCT TCGGGAACAT GGCTGACTTA
29060 29070 29080 29090 29100 29110 29120
>< XhoII
>< Sau3AI
>< NdeII
>< MflI
>< MboI
>< FokI
>< Tru9I >< DpnII
>< NlaIV > < DpnI
>< NlaIII >< BstYI >< Tth111I
>< MseI >< BspAI >< MaeII
>< BscBI >< BstXI>< AlwI> < Bsp143I >< AspI BspWI ><
TCATGGAGCC ATTAAATTGG ATGACAAAGA TCCACAATTC AAAGACAACG TCATACTGCT GAACAAGCAC
29130 29140 29150 29160 29170 29180 29190
EspI ><
DdeI ><
CelII ><
Bpu1102I ><
AluI ><
>< HgaI
ATTGACGCAT ACAAAACATT CCCACCAACA GAGCCTAAAA AGGACAAAAA GAAAAAGACT GATGAAGCTC
29200 29210 29220 29230 29240 29250 29260

FIGURE 13.68

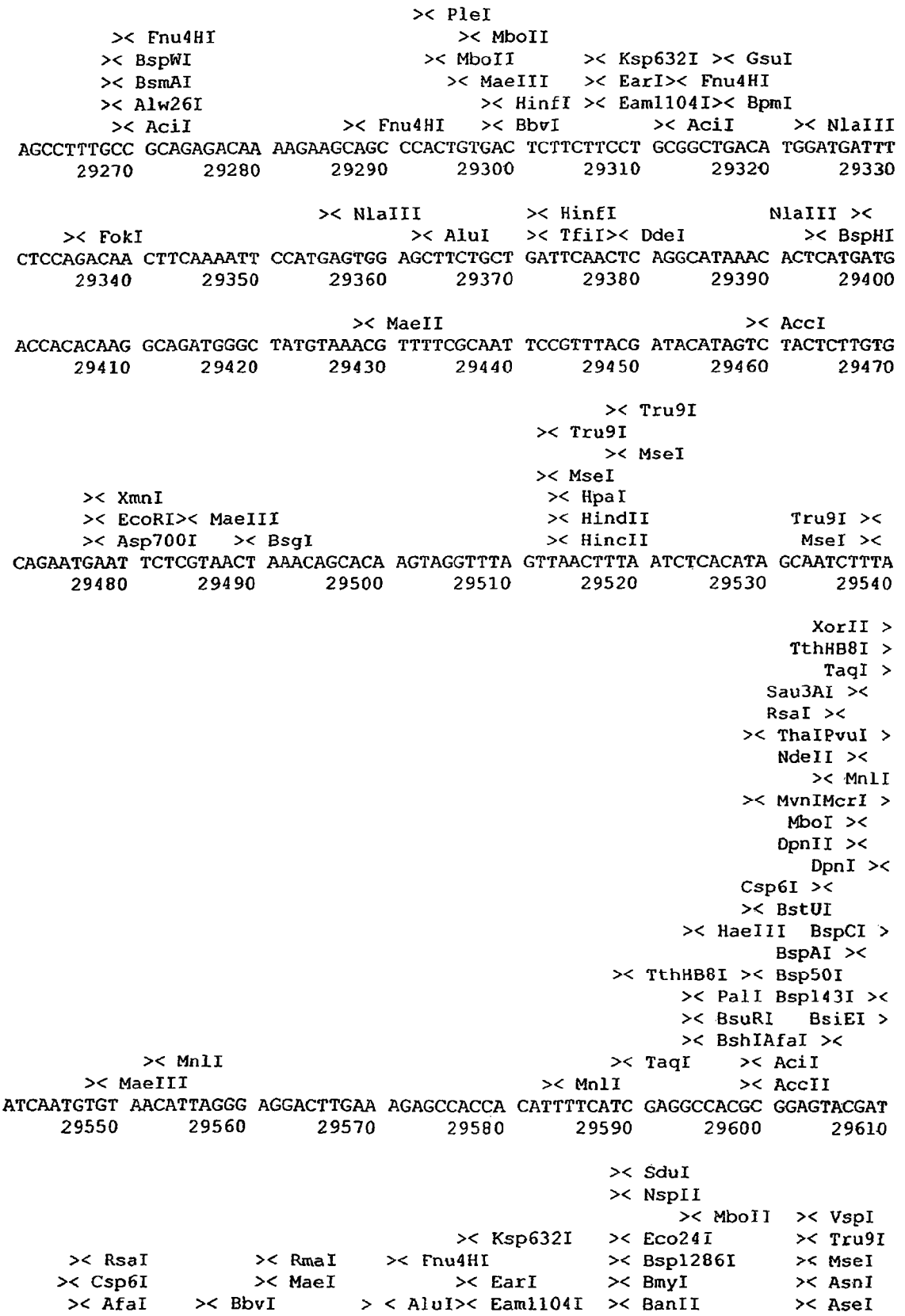

>< PleI
>< Fnu4HI
>< MboII
>< BspWI
>< MboII
>< Ksp632I >< GsuI
>< BsmAI
>< MaeIII
>< EarI>< Fnu4HI
>< Alw26I
>< HinfI >< Eam1104I>< BpmI
>< AciI
>< Fnu4HI
>< BbvI
>< AciI
>< NlaIII
AGCCTTTGCC GCAGAGACAA AAGAAGCAGC CCACTGTGAC TCTTCTTCCT GCGGCTGACA TGGATGATTT
29270 29280 29290 29300 29310 29320 29330
>< NlaIII
>< HinfI
NlaIII ><
>< FokI
>< AluI
>< TfiI>< DdeI
>< BspHI
CTCCAGACAA CTTCAAAATT CCATGAGTGG AGCTTCTGCT GATTCAACTC AGGCATAAAC ACTCATGATG
29340 29350 29360 29370 29380 29390 29400
>< MaeII
>< AccI
ACCACACAAG GCAGATGGGC TATGTAAACG TTTTCGCAAT TCCGTTTACG ATACATAGTC TACTCTTGTG
29410 29420 29430 29440 29450 29460 29470
>< Tru9I
>< Tru9I
>< MseI
>< MseI
>< XmnI
>< HpaI
>< EcoRI>< MaeIII
>< HindII
Tru9I ><
>< Asp700I >< BsgI
>< HincII
MseI ><
CAGAATGAAT TCTCGTAACT AAACAGCACA AGTAGGTTTA GTTAACTTTA ATCTCACATA GCAATCTTTA
29480 29490 29500 29510 29520 29530 29540
XorII >
TthHB8I >
TaqI >
Sau3AI ><
RsaI ><
>< ThaIPvuI >
NdeII ><
>< MnlI
>< MvnIMcrI >
MboI ><
DpnII ><
DpnI ><
Csp6I ><
>< BstUI
>< HaeIII BspCI >
BspAI ><
>< TthHB8I >< Bsp50I
>< PalI Bsp143I ><
>< BsuRI BsiEI >
>< BshIAfaI ><
>< MnlI
>< TaqI
>< AciI
>< MaeIII
>< MnlI
>< AccII
ATCAATGTGT AACATTAGGG AGGACTTGAA AGAGCCACCA CATTTTCATC GAGGCCACGC GGAGTACGAT
29550 29560 29570 29580 29590 29600 29610
>< SduI
>< NspII
>< MboII
>< VspI
>< Ksp632I
>< Eco24I
>< Tru9I
>< RsaI
>< RmaI
>< Fnu4HI
>< Bsp1286I
>< MseI
>< Csp6I
>< MaeI
>< EarI
>< BmyI
>< AsnI
>< AfaI
>< BbvI
> < AluI>< Eam1104I
>< BanII
>< AseI

FIGURE 13.69

```
CGAGGGTACA GTGAATAATG CTAGGGAGAG CTGCCTATAT GGAAGAGCCC TAATGTGTAA AATTAATTTT
     29620      29630      29640      29650      29660      29670      29680

                         >< Tru9I   >< DdeI
                         >< MseI    >< BfrI
                    >< NlaIII    > < AluI
AGTAGTGCTA TCCCCATGTG ATTTTAATAG CTTCTTAGGA GAATGACAAA AAAAAAAAAA AAAAAA
     29690      29700      29710      29720      29730      29740
```

FIGURE 13. 70

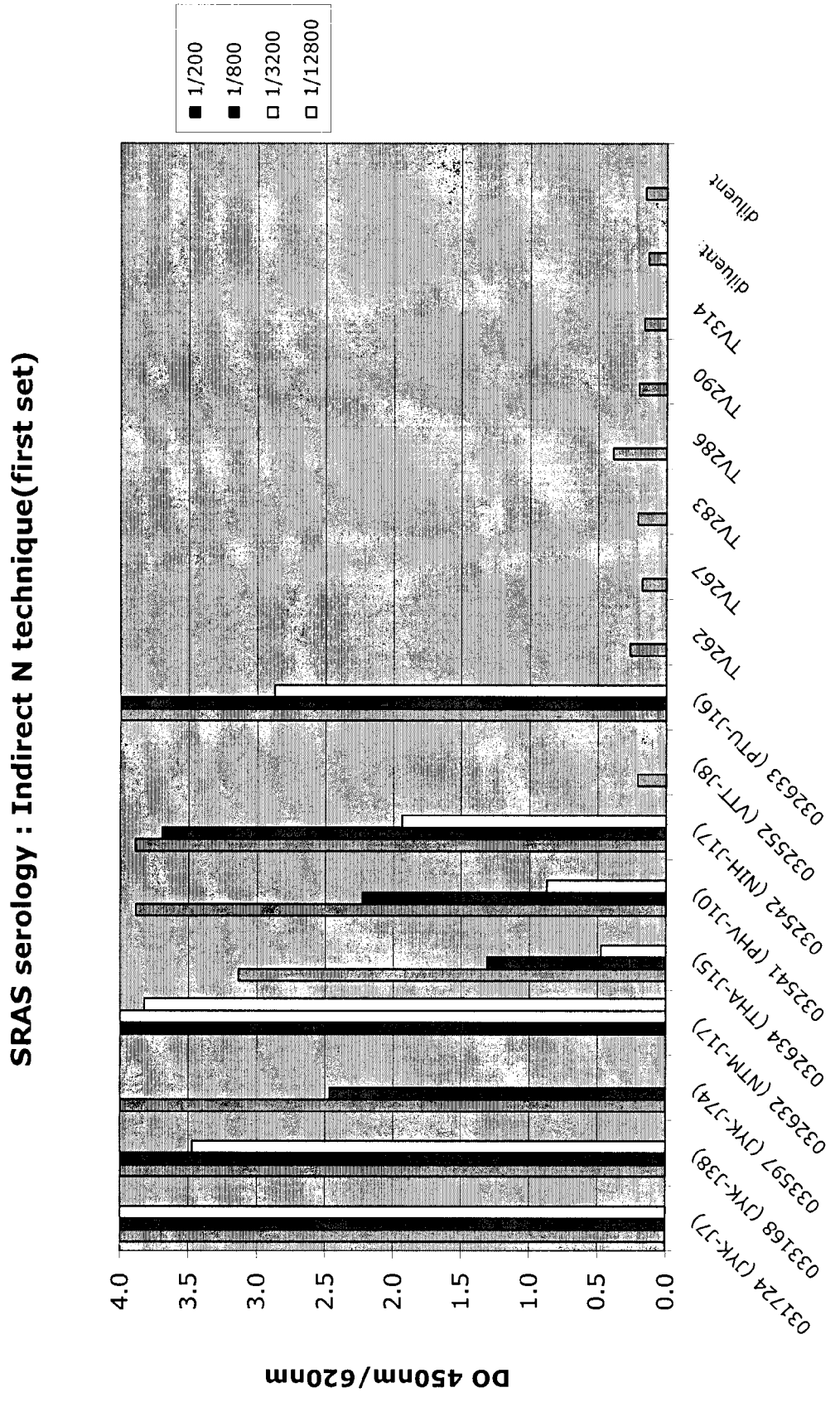

SRAS serology : Indirect N technique(first set)
1/200
1/800
1/3200
1/12800
DO 450nm/620nm
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
031724 (JYK-J7)
033168 (JYK-J38)
033597 (JYK-J74)
032632 (NTM-J17)
032634 (THA-J15)
032541 (PHV-J10)
032542 (NIH-J17)
032552 (VTT-J8)
032633 (PTU-J16)
TV262
TV267
TV283
TV286
TV290
TV314
diluent
diluent

FIGURE 14

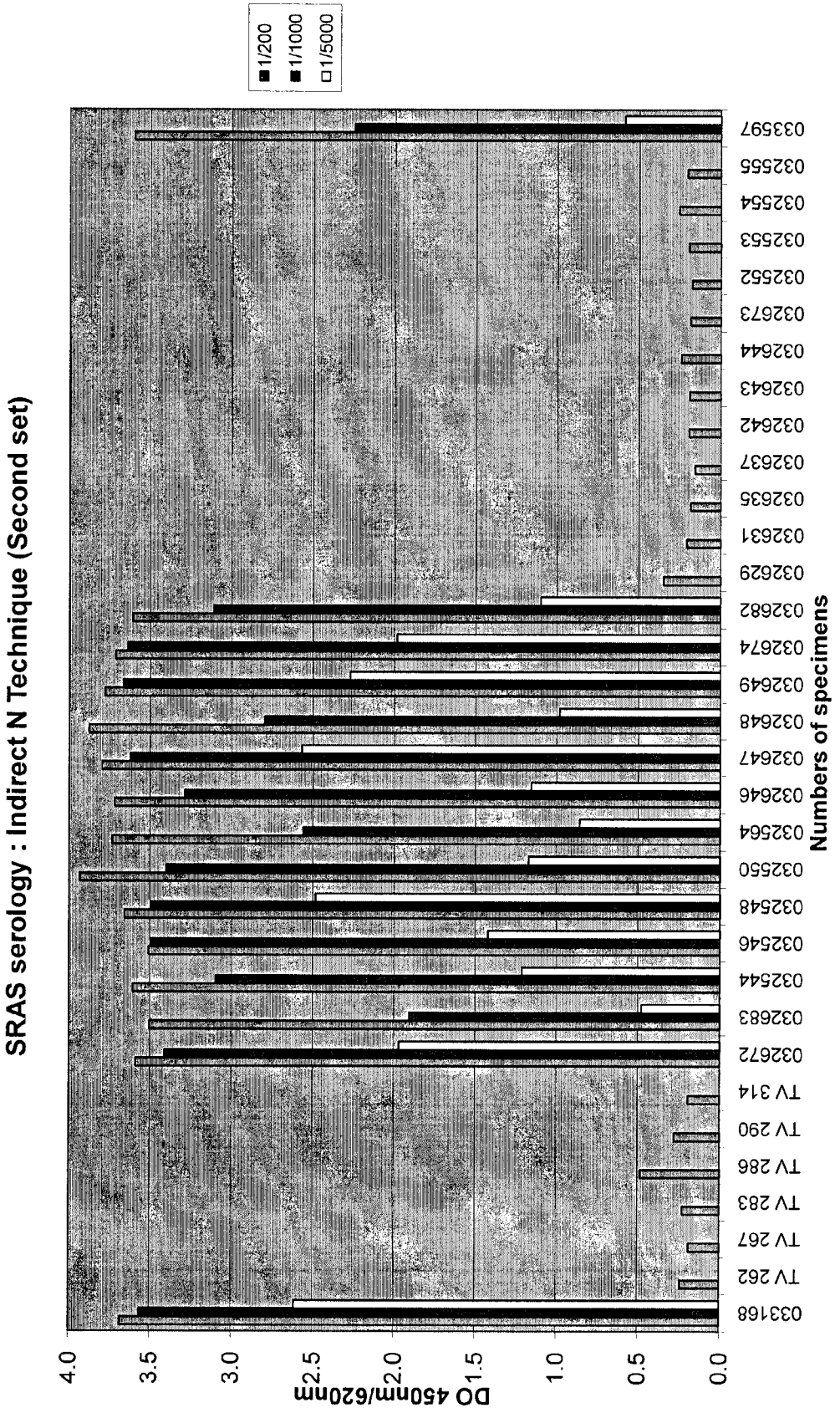

SRAS serology : Indirect N Technique (Second set)
1/200
1/1000
1/5000
DO 450nm/620nm
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Numbers of specimens
033168
TV 262
TV 267
TV 283
TV 286
TV 290
TV 314
032672
032683
032544
032546
032548
032550
032564
032646
032647
032648
032649
032674
032682
032629
032631
032635
032637
032642
032643
032644
032673
032652
032653
032654
032655
033597

FIGURE 15

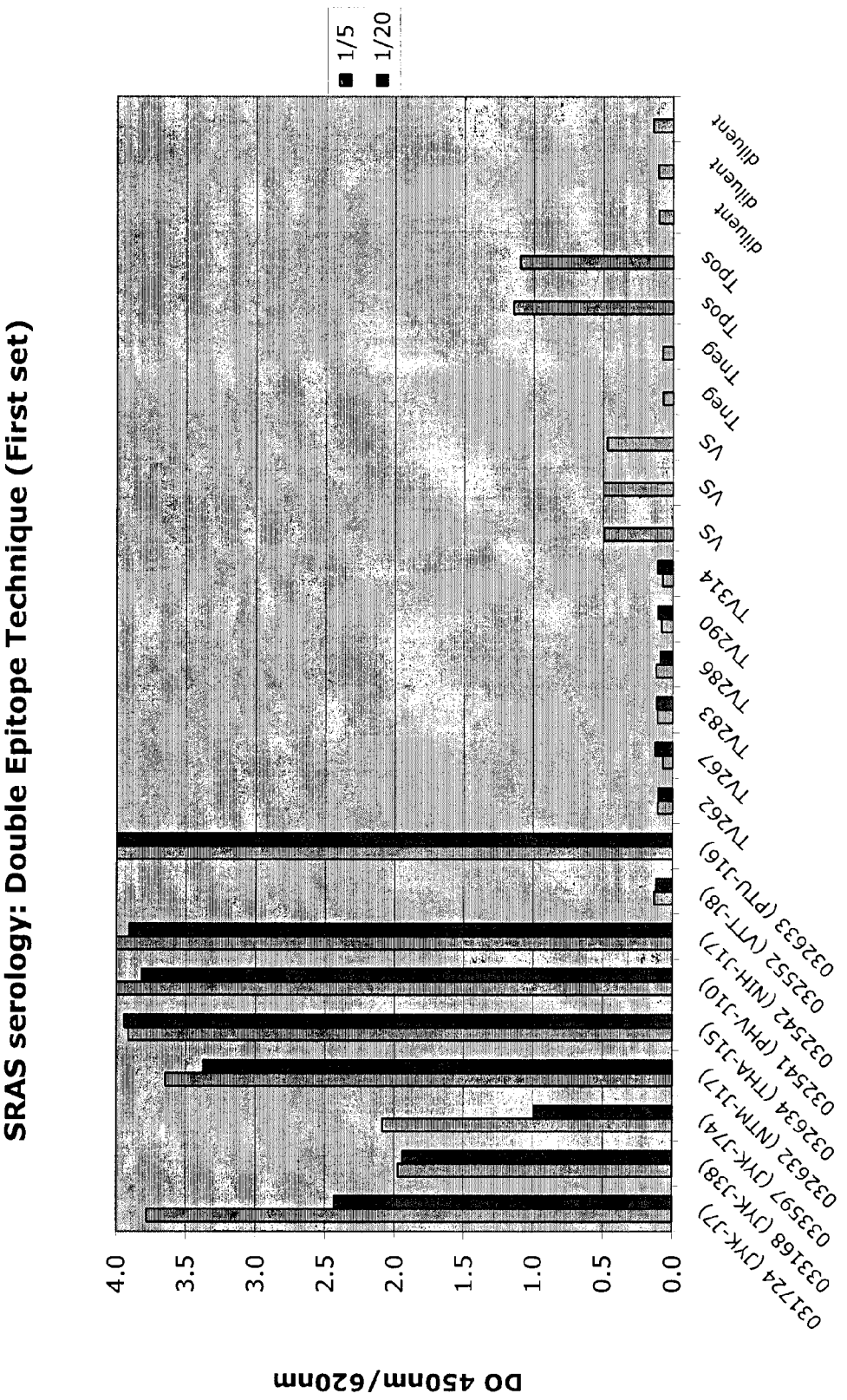

SRAS serology: Double Epitope Technique (First set)
1/5
1/20
DO 450nm/620nm
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
031724 (JYK-J7)
033168 (JYK-J38)
033597 (JYK-J74)
032632 (NTM-J17)
032634 (THA-J15)
032541 (PHV-J10)
032542 (NIH-J17)
032552 (VTT-J8)
032633 (PTU-J16)
TV262
TV267
TV283
TV286
TV290
TV314
VS
VS
VS
Tneg
Tneg
Tpos
Tpos
diluent
diluent
diluent

FIGURE 16

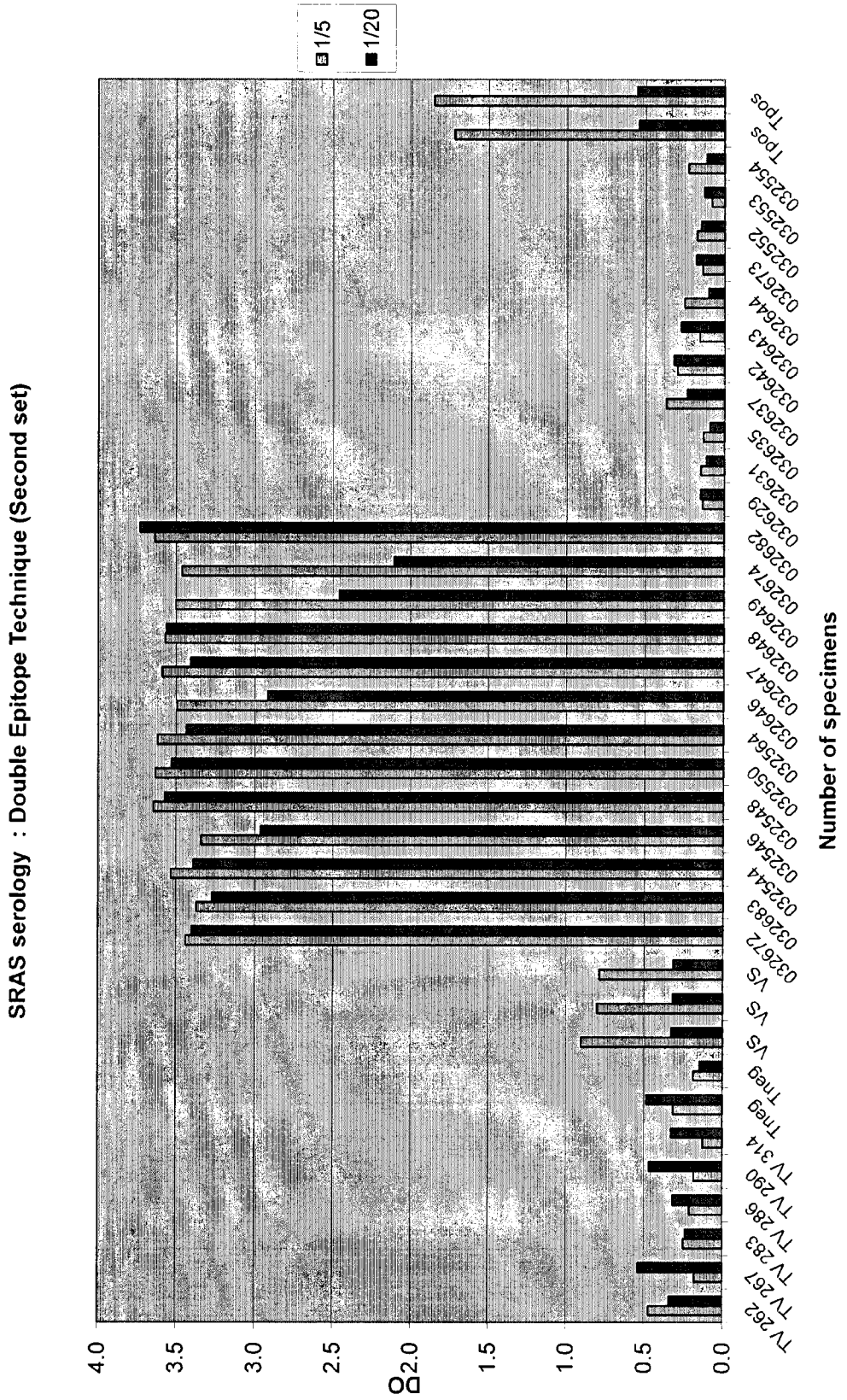

SRAS serology : Double Epitope Technique (Second set)
1/5
1/20
DO
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
TV 262
TV 267
TV 283
TV 286
TV 290
TV 314
Treg
Treg
VS
VS
VS
032672
032683
032544
032546
032548
032550
032564
032646
032647
032648
032649
032674
032682
032629
032631
032635
032637
032642
032643
032644
032673
032652
032653
032654
Tpos
Tpos
Number of specimens

FIGURE 17

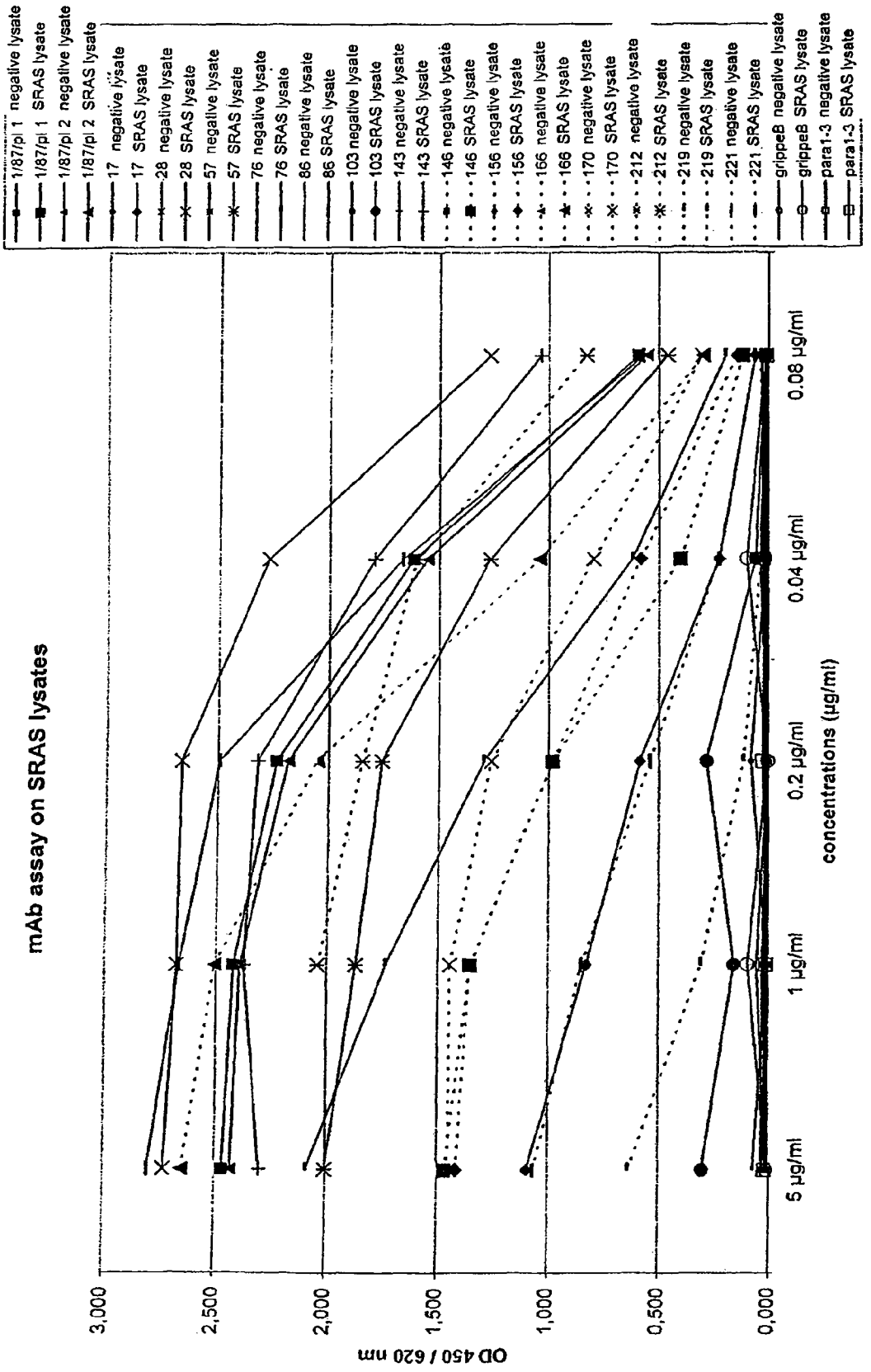

mAb assay on SRAS lysates
OD 450 / 620 nm
3,000
2,500
2,000
1,500
1,000
0,500
0,000
5 µg/ml
1 µg/ml
0.2 µg/ml
0.04 µg/ml
0.08 µg/ml
concentrations (µg/ml)
1/87/pl 1 negative lysate
1/87/pl 1 SRAS lysate
1/87/pl 2 negative lysate
1/87/pl 2 SRAS lysate
17 negative lysate
17 SRAS lysate
28 negative lysate
28 SRAS lysate
57 negative lysate
57 SRAS lysate
76 negative lysate
76 SRAS lysate
86 negative lysate
86 SRAS lysate
103 negative lysate
103 SRAS lysate
143 negative lysate
143 SRAS lysate
146 negative lysate
146 SRAS lysate
156 negative lysate
156 SRAS lysate
166 negative lysate
166 SRAS lysate
170 negative lysate
170 SRAS lysate
212 negative lysate
212 SRAS lysate
219 negative lysate
219 SRAS lysate
221 negative lysate
221 SRAS lysate
grippeB negative lysate
grippeB SRAS lysate
para1-3 negative lysate
para1-3 SRAS lysate

FIGURE 18

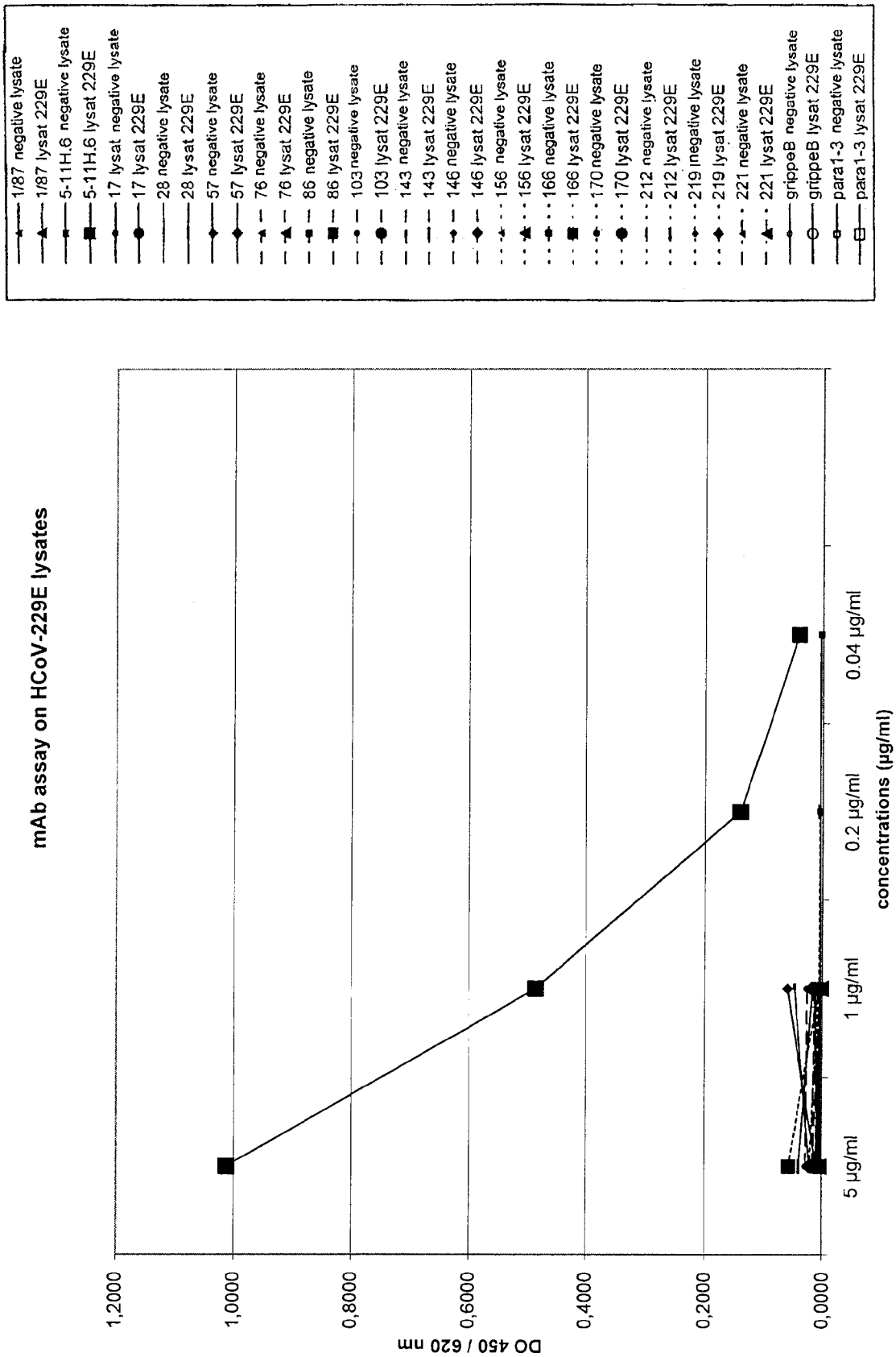

mAb assay on HCoV-229E lysates
DO 450 / 620 nm
1,2000
1,0000
0,8000
0,6000
0,4000
0,2000
0,0000
5 µg/ml
1 µg/ml
0.2 µg/ml
0.04 µg/ml
concentrations (µg/ml)
1/87 negative lysate
1/87 lysat 229E
5-11H.6 negative lysate
5-11H.6 lysat 229E
17 lysat negative lysate
17 lysat 229E
28 negative lysate
28 lysat 229E
57 negative lysate
57 lysat 229E
76 negative lysate
76 lysat 229E
86 negative lysate
86 lysat 229E
103 negative lysate
103 lysat 229E
143 negative lysate
143 lysat 229E
146 negative lysate
146 lysat 229E
156 negative lysate
156 lysat 229E
166 negative lysate
166 lysat 229E
170 negative lysate
170 lysat 229E
212 negative lysate
212 lysat 229E
219 negative lysate
219 lysat 229E
221 negative lysate
221 lysat 229E
grippeB negative lysate
grippeB lysat 229E
para1-3 negative lysate
para1-3 lysat 229E

FIGURE 19

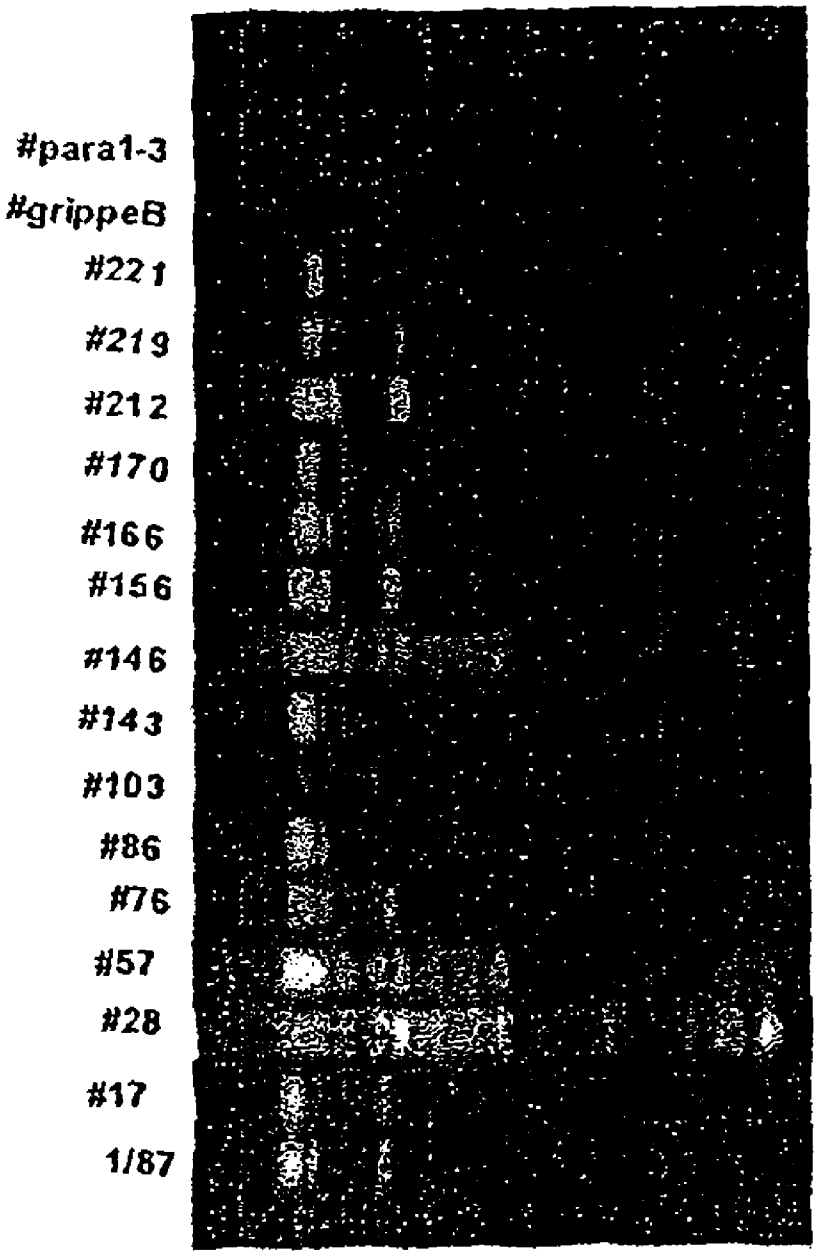

#para1-3
#grippeB
#221
#219
#212
#170
#166
#156
#146
#143
#103
#86
#76
#57
#28
#17
1/87

FIGURE 20

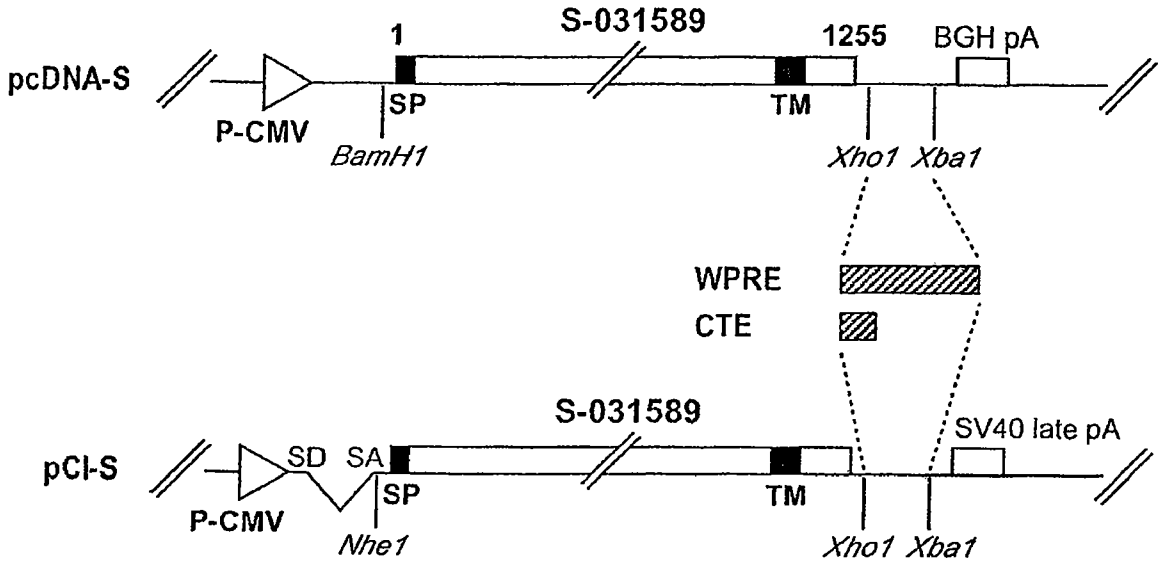

pcDNA-S
P-CMV
1
SP
BamH1
S-031589
1255
TM
BGH pA
Xho1
Xba1
WPRE
CTE
pCI-S
P-CMV
SD
SA
SP
Nhe1
S-031589
TM
SV40 late pA
Xho1
Xba1

FIGURE 21

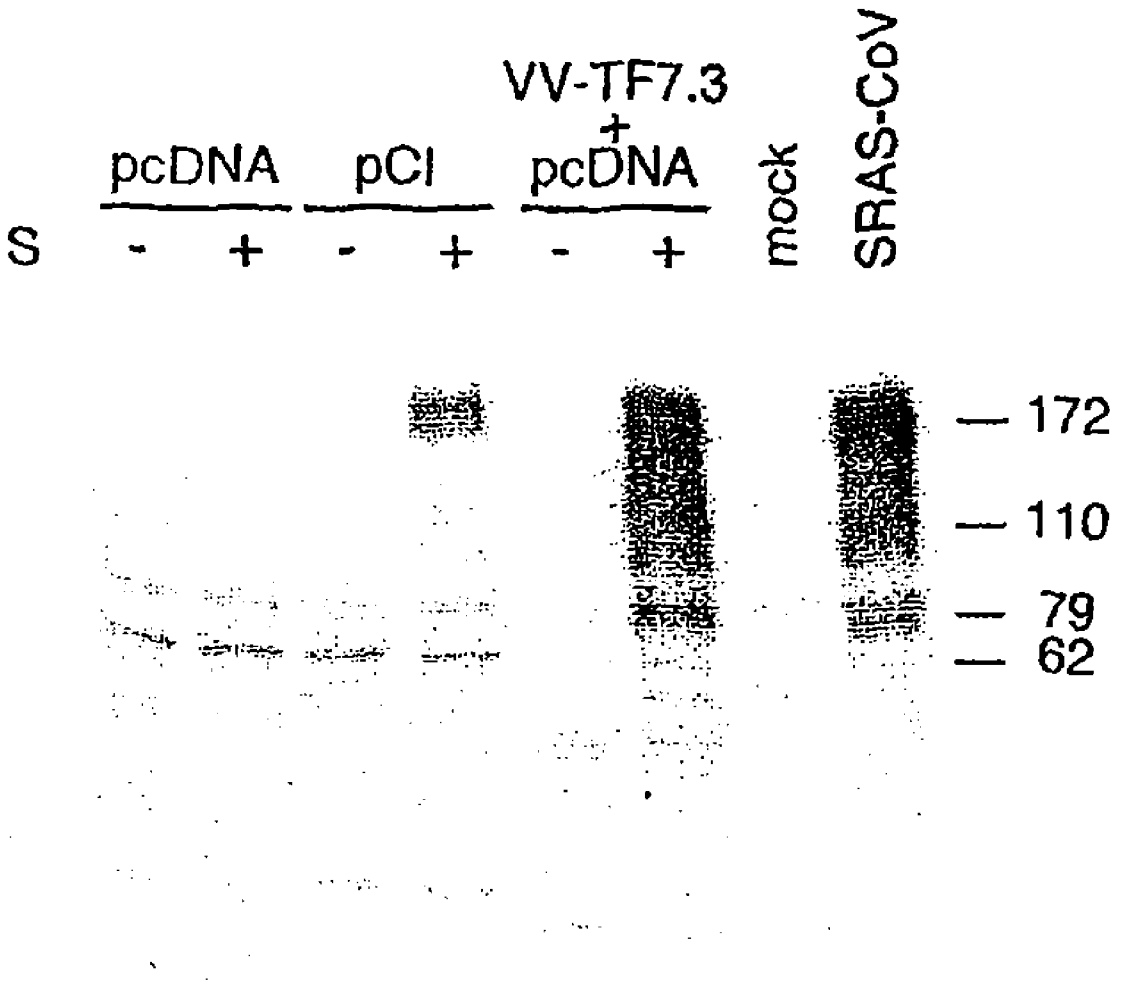
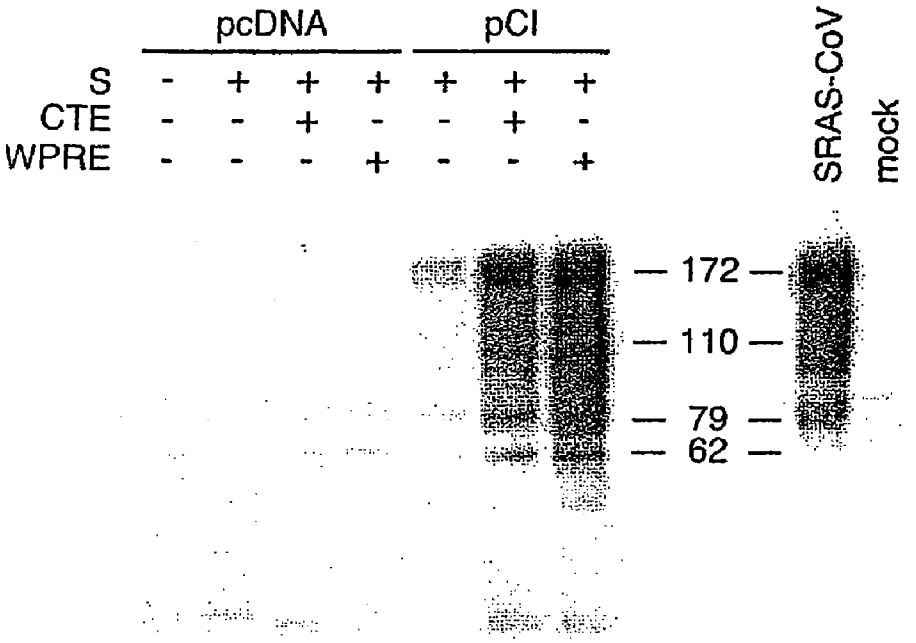


FIGURE 22

A.


pcDNA
pCI
S - + + + + + +
CTE - - + - - + -
WPRE - - - + - - +
SRAS-CoV
mock
172
110
79
62

B.

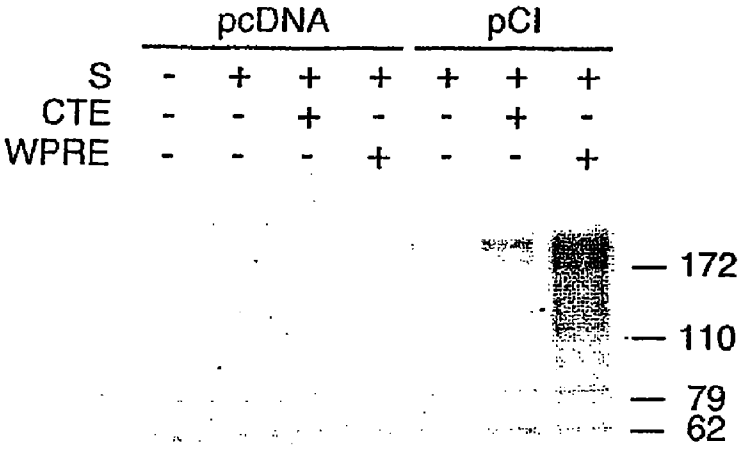

pcDNA
pCI
S - + + + + + +
CTE - - + - - + -
WPRE - - - + - - +
172
110
79
62

FIGURE 23

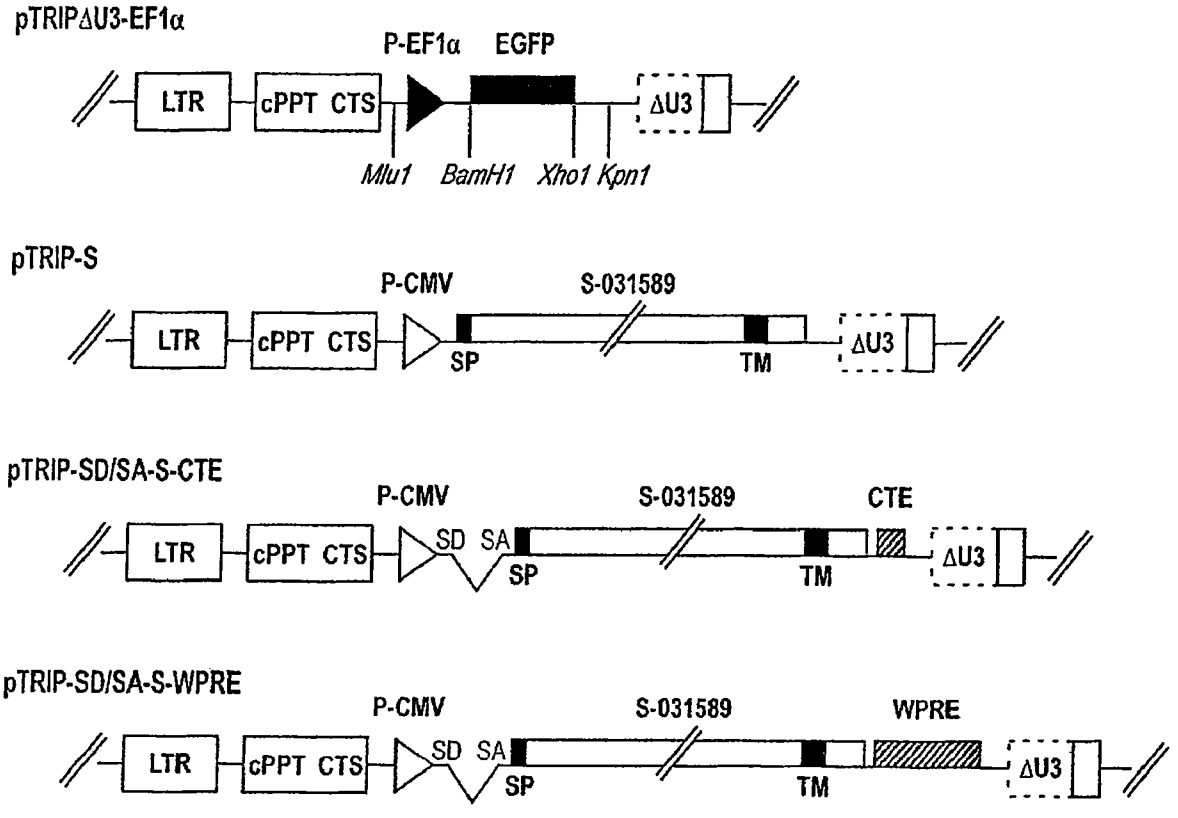

pTRIPΔU3-EF1α
P-EF1α
EGFP
LTR
cPPT CTS
ΔU3
Mlu1
BamH1
Xho1
Kpn1
pTRIP-S
P-CMV
S-031589
LTR
cPPT CTS
SP
TM
ΔU3
pTRIP-SD/SA-S-CTE
P-CMV
S-031589
CTE
LTR
cPPT CTS
SD
SA
SP
TM
ΔU3
pTRIP-SD/SA-S-WPRE
P-CMV
S-031589
WPRE
LTR
cPPT CTS
SD
SA
SP
TM
ΔU3

FIGURE 24

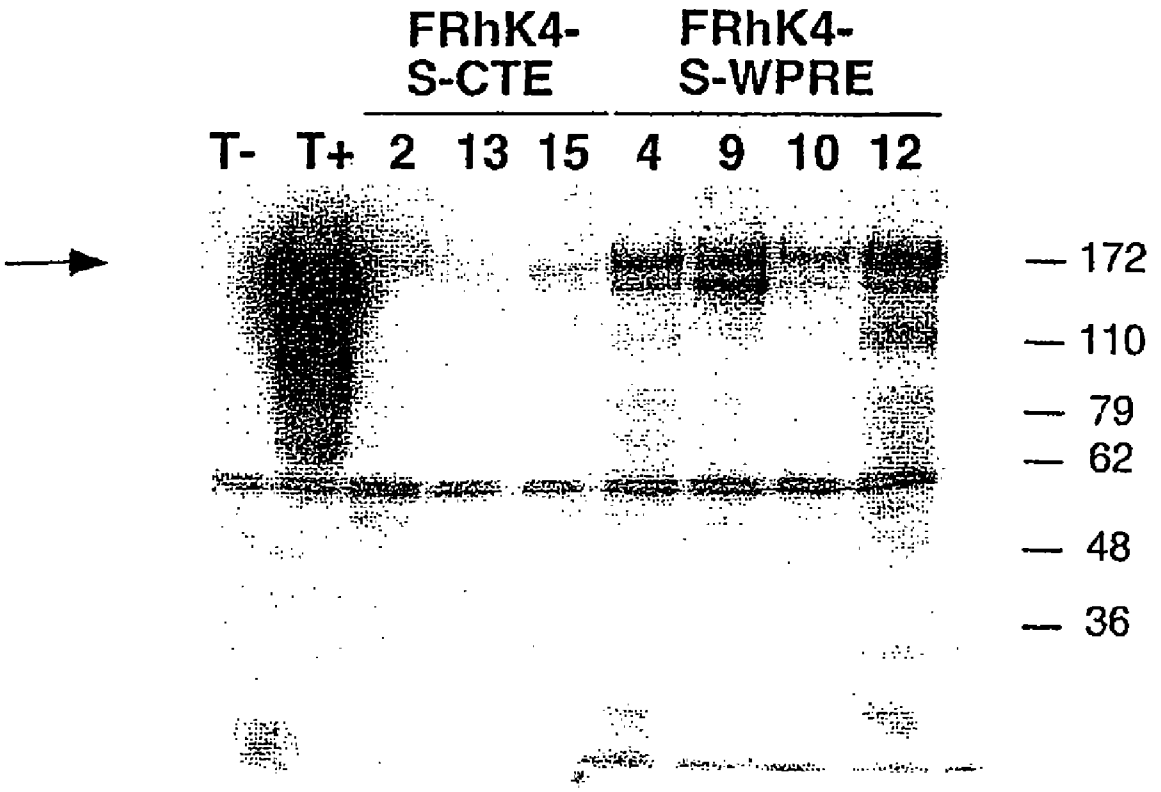


FIGURE 25

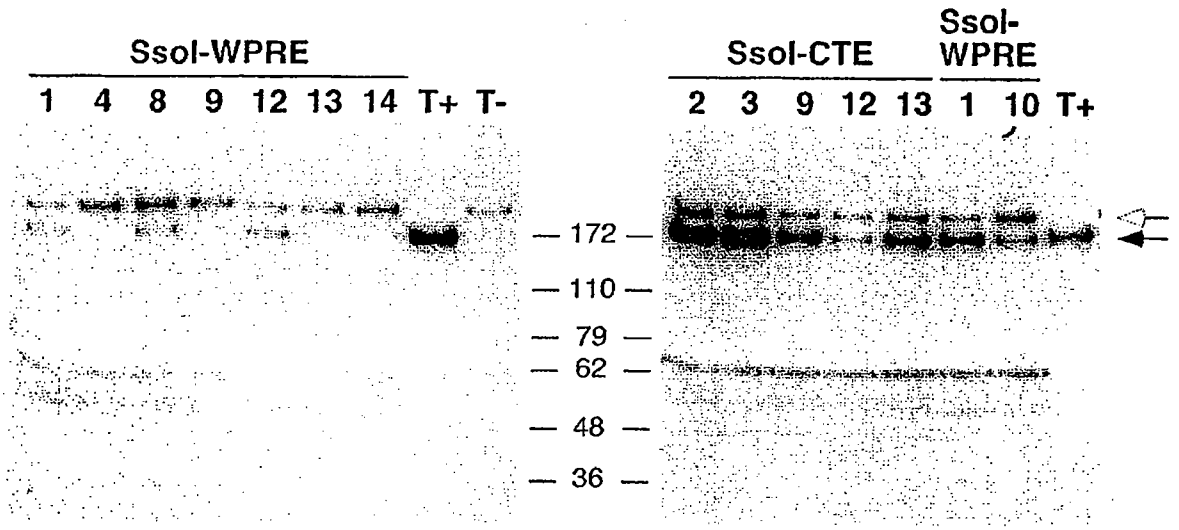
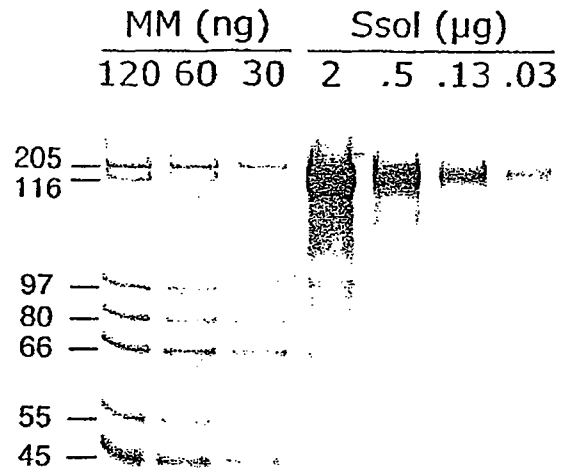
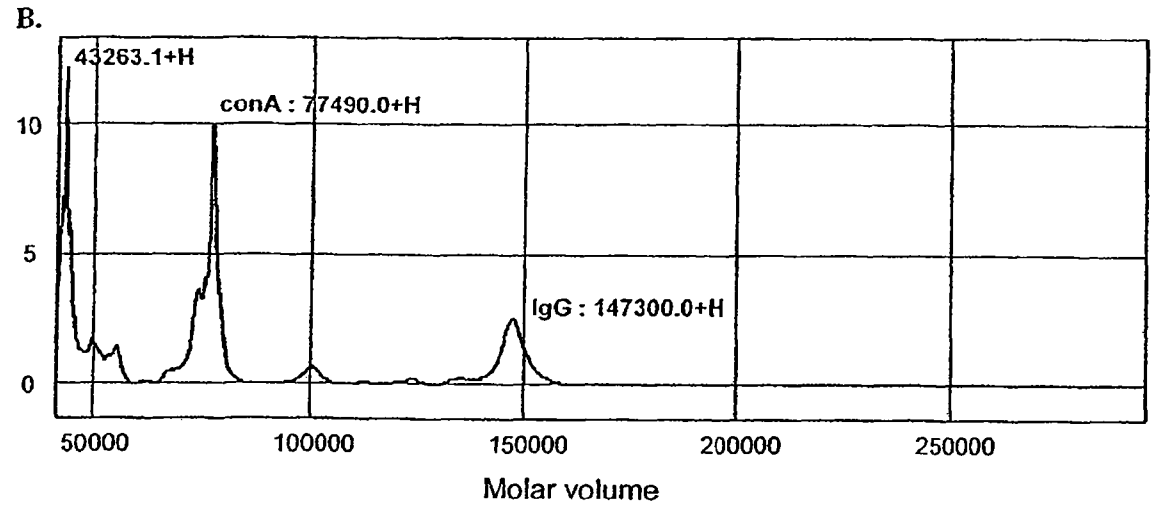

Ssol-WPRE
1 4 8 9 12 13 14 T+ T-
Ssol-CTE
Ssol-WPRE
2 3 9 12 13 1 10 T+
172
110
79
62
48
36

FIGURE 26

A.



B.



C.

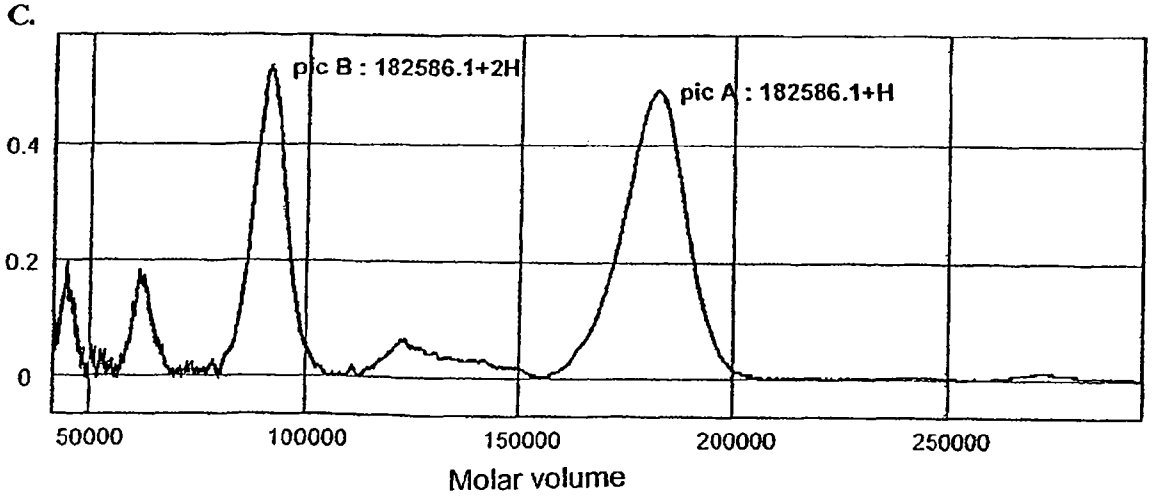


FIGURE 27 A-C

D.

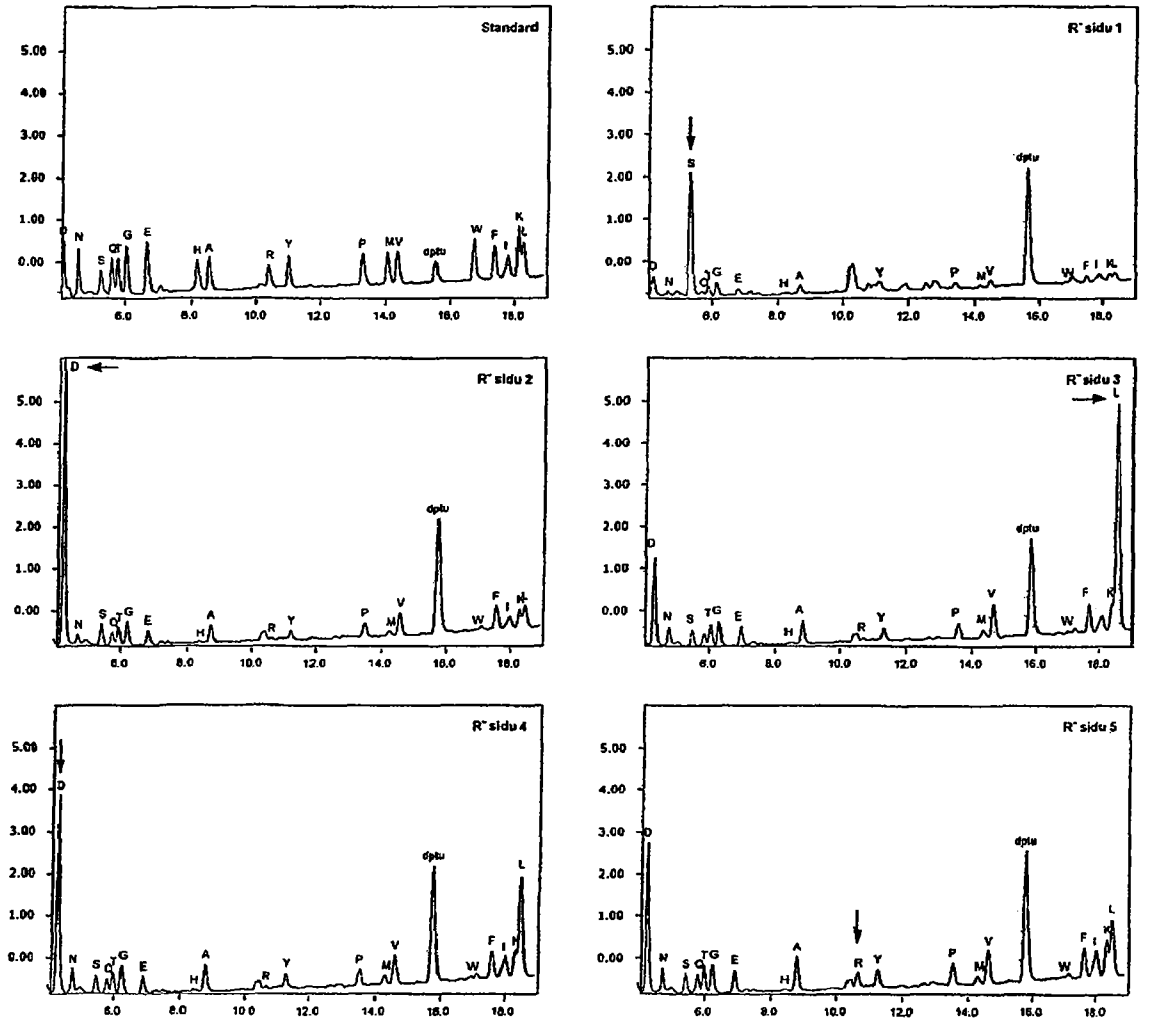
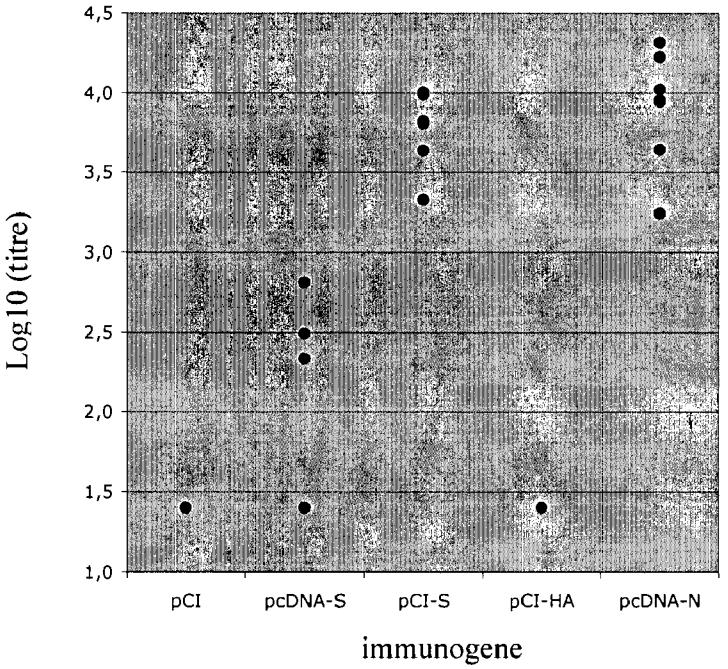


FIGURE 27 D

A.


4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
Log10 (titre)
pCI
pcDNA-S
pCI-S
pCI-HA
pcDNA-N
immunogene

B.

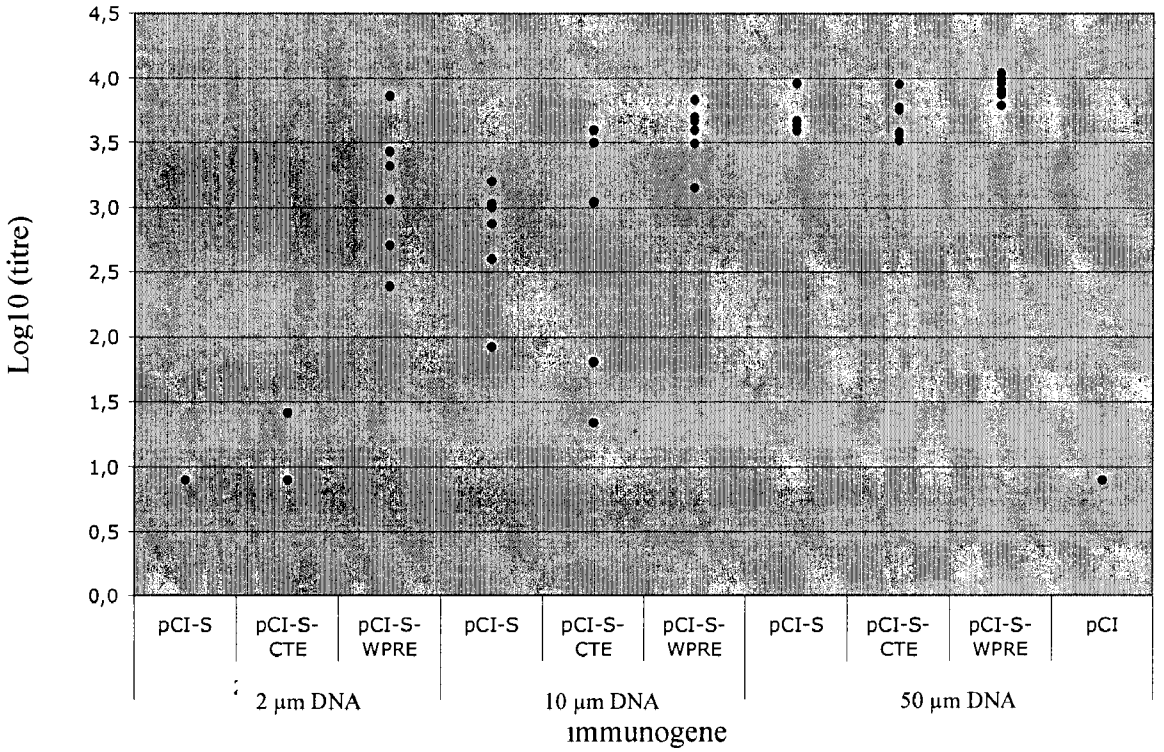
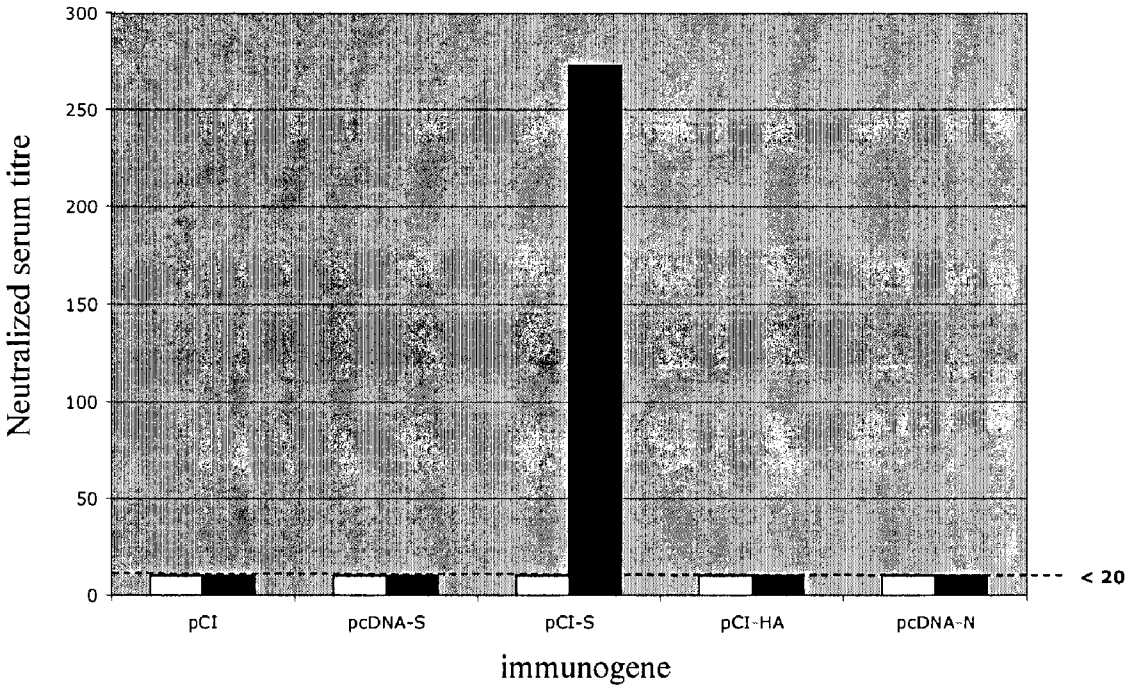

4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Log10 (titre)
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI
2 µm DNA
10 µm DNA
50 µm DNA
immunogene

FIGURE 28

A.


Neutralized serum titre
300
250
200
150
100
50
0
< 20
pCI
pcDNA-S
pCI-S
pCI-HA
pcDNA-N
immunogene

B.

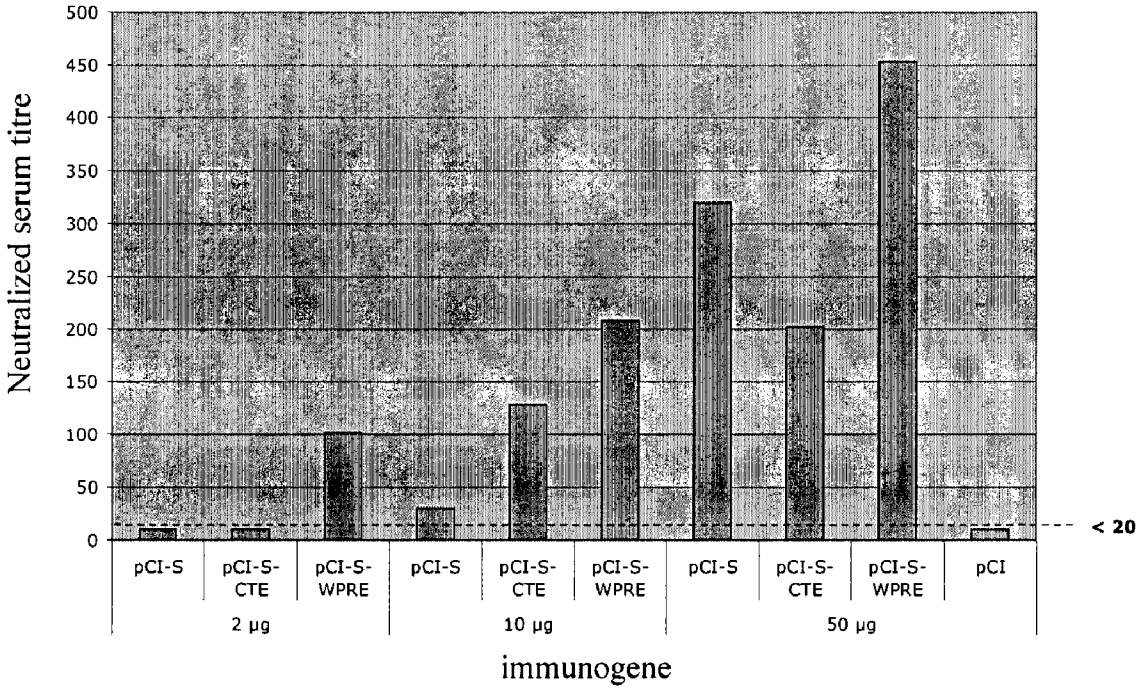

Neutralized serum titre
500
450
400
350
300
250
200
150
100
50
0
< 20
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI
2 µg
10 µg
50 µg
immunogene

FIGURE 29

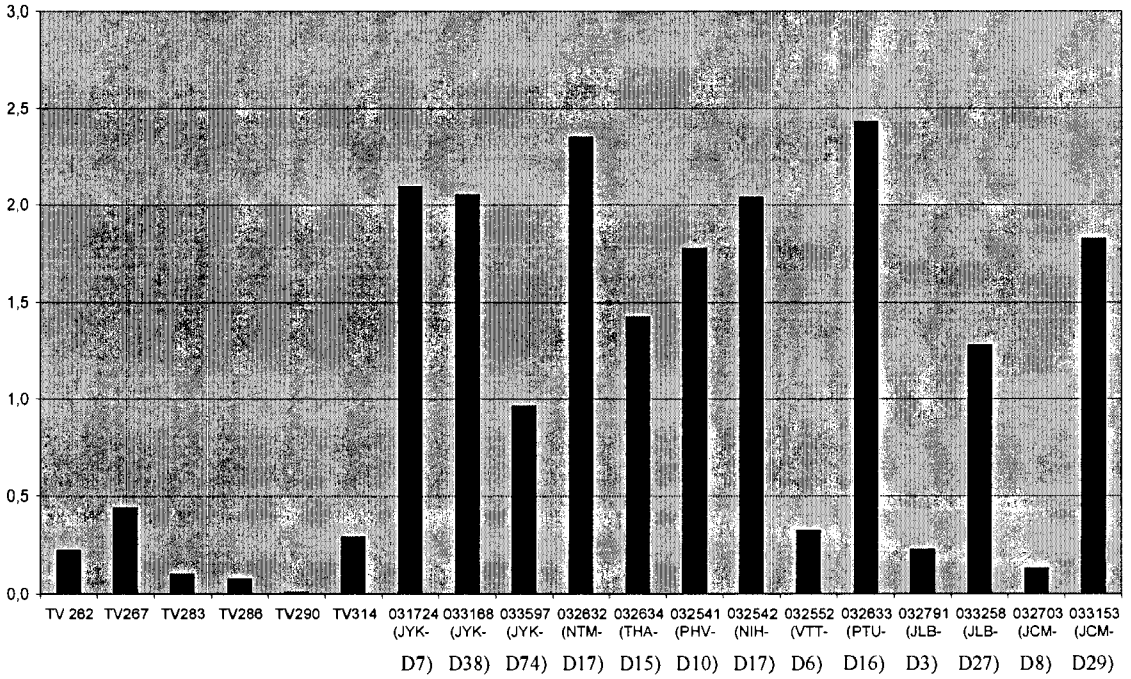

3,0
2,5
2,0
1,5
1,0
0,5
0,0
TV 262
TV267
TV283
TV286
TV290
TV314
031724 (JYK- D7)
033168 (JYK- D38)
033597 (JYK- D74)
032632 (NTM- D17)
032634 (THA- D15)
032541 (PHV- D10)
032542 (NIH- D17)
032552 (VTT- D6)
032633 (PTU- D16)
032791 (JLB- D3)
033258 (JLB- D27)
032703 (JCM- D8)
033153 (JCM- D29)
serums

FIGURE 30

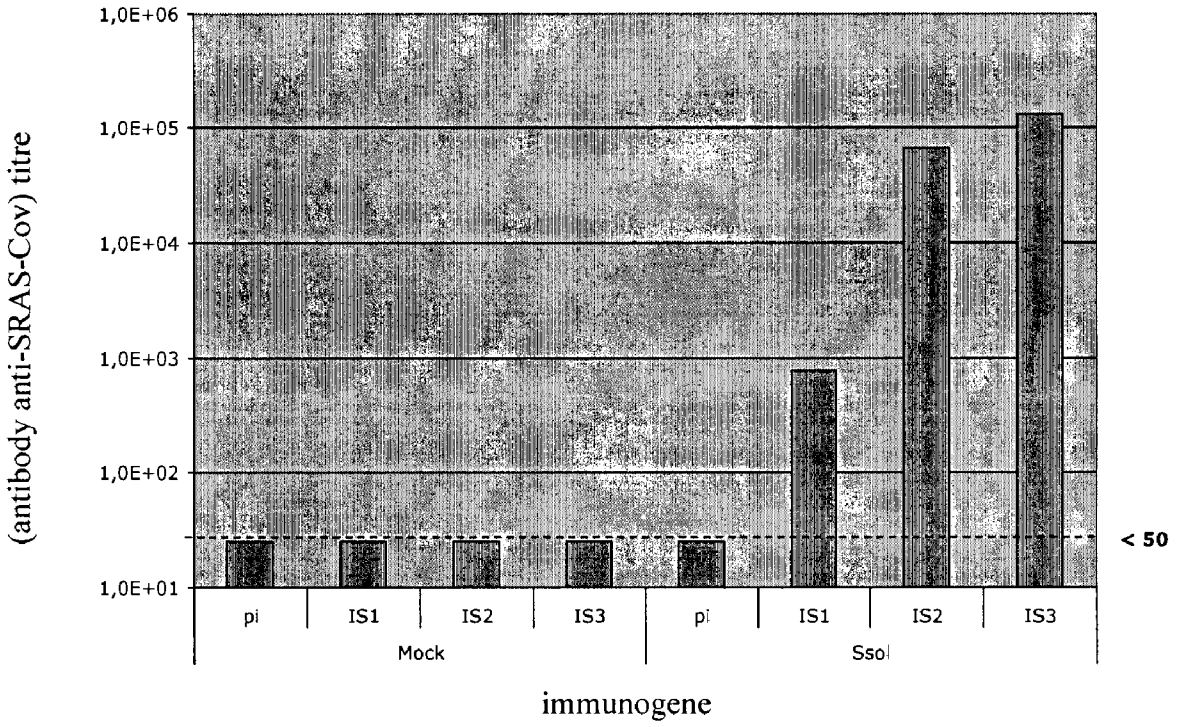

1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
(antibody anti-SRAS-Cov) titre
< 50
pi
IS1
IS2
IS3
pi
IS1
IS2
IS3
Mock
Ssol
immunogene

FIGURE 31

```
I-3059        1 CTCTTCTGGAAAAAGGTAGGCTTATCATTAGAGAAAACAACAGAGTTGTGGTTTCAAGTG
S-040530        ____________________________________________________________

I-3059       61 ATATTCTTGTTAACAACTAAACGAACATGTTTATTTTCTTATTATTTCTTACTCTCACTA
S-040530      1 _________________GG"T"C"C""""""C""C"""C"GC"G""C""G""C""G""C"

I-3059      121 GTGGTAGTGACCTTGACCGGTGCACCACTTTTGATGATGTTCAAGCTCCTAATTACACTC
S-040530     44 "C""C""C"""""G""""""""""""""""C""C""C""C""G""G""C""C""C""""""C"

I-3059      181 AACATACTT_CATCTATGAGGGGGGTTTACTATCCTGATGAAATTTTTAGATCAGACACT
S-040530    104 "G""C""CAG""G"_"""C""""C""G"""""C""C""C""G""C"""C"GAGC"""""C

I-3059      240 CTTTATTTAACTCAGGATTTATTTCTTCCATTTTATTCTAATGTTACAGGGTTTCATACT
S-040530    163 ""G""CC"G""C"""""CC"G""C""G""C""C""CAGC""C""G""C""C""C""C""C

I-3059      300 ATTAATCATACGTTTGGCAACCCTGTCATACCTTTTAAGGATGGTATTTATTTTGCTGCC
S-040530    223 ""C""C""C""C""C""""""""""C""G""C""C""C"""""C""C""C""C""C""C"""

I-3059      360 ACAGAGAAATCAAATGTTGTCCGTGGTTGGGTTTTTGGTTCTACCATGAACAACAAGTCA
S-040530    283 ""C"""""GAGC""C""G""G""G""C"""""G""C""CAGC""""""""""""""""AGC

I-3059      420 CAGTCGGTGATTATTATTAACAATTCTACTAATGTTGTTATACGAGCATGTAACTTTGAA
S-040530    343 """AGC"""""C""C""C"""""CAGC""C""C""G""G""C""G""C""C""""""C""G

I-3059      480 TTGTGTGACAACCCTTTCTTTGCTGTTTCTAAACCCATGGGTACACAGACACATACTATG
S-040530    403 C""""C""""""""""C"""""C""C""G""C""""""""""""""C""C""""""C""C""C"""

I-3059      540 ATATTCGATAATGCATTTAATTGCACTTTCGAGTACATATCTGATGCCTTTTCGCTTGAT
S-040530    463 ""C"""""C""C""C""C""C"""""C""""""""""""""CAGC""C"""""CAGC""G""C

I-3059      600 GTTTCAGAAAAGTCAGGTAATTTTAAACACTTACGAGAGTTTGTGTTTAAAAATAAAGAT
S-040530    523 ""GAGC""G"""AGC""C""C""C""G"""C"G""G"""""C""""""C""G""C""G""C

I-3059      660 GGGTTTCTCTATGTTTATAAGGGCTATCAACCTATAGATGTAGTTCGTGATCTACCTTCT
S-040530    583 ""C""C""G""C""G""C""""""""""C""G""C""C""C""G""GA"A""C""G""CAGC

I-3059      720 GGTTTTAACACTTTGAAACCTATTTTTAAGTTGCCTCTTGGTATTAACATTACAAATTTT
S-040530    643 ""C""C"""""CC""""G""C""C""C"""C""""C""G""C""C"""""C""C""C""C

I-3059      780 AGAGCCATTCTTACAGCCTTTTCACCTGCTCAAGACATTTGGGGCACGTCAGCTGCAGCC
S-040530    703 C"G"""""C""G""C""""""AGC"""""C""G"""""C"""""""""CAGC""C""C"""

I-3059      840 TATTTTGTTGGCTATTTAAAGCCAACTACATTTATGCTCAAGTATGATGAAAATGGTACA
S-040530    763 ""C""C""G"""""CC"G"""""T""C""C""C""""""G""""""C""C""G""C""C""C

I-3059      900 ATCACAGATGCTGTTGATTGTTCTCAAAATCCACTTGCTGAACTCAAATGCTCTGTTAAG
S-040530    823 """"""C""C""C""G""C""CAGC""G""C""C""G""C""G""G""G"""AGC""G"""

I-3059      960 AGCTTTGAGATTGACAAAGGAATTTACCAGACCTCTAATTTCAGGGTTGTTCCCTCAGGA
S-040530    883 """"""C""""""C"""""G""C""C""""""""""AGC""C"""""A""G""G""TAGC""C

I-3059     1020 GATGTTGTGAGATTCCCTAATATTACAAACTTGTGTCCTTTTGGAGAGGTTTTTAATGCT
S-040530    943 """"""G"""C"G"""""C"""""C""C"""C"""""C""C""C""C""A""G""C""C""C

I-3059     1080 ACTAAATTCCCTTCTGTCTATGCATGGGAGAGAAAAAAAATTTCTAATTGTGTTGCTGAT
S-040530   1003 ""C""G""""""CAGC""G""C""C""""""C"G""G""G""CAGC""C""C""G""C""C

I-3059     1140 TACTCTGTGCTCTACAACTCAACATTTTTTTCAACCTTTAAGTGCTATGGCGTTTCTGCC
S-040530   1063 """AGC""""""G""""""""""C""C""C""CAGC"""""C""""""""""C""""""GAGC"""

I-3059     1200 ACTAAGTTGAATGATCTTTGCTTCTCCAATGTCTATGCAGATTCTTTTGTAGTCAAGGGA
S-040530   1123 ""C"""C""""C""C""G"""""""AG"""C""G""C""C""CAGC""C""G""G"""""C

I-3059     1260 GATGATGTAAGACAAATAGCGCCAGGACAAACTGGTGTTATTGCTGATTATAATTATAAA
S-040530   1183 ""C""C""G"""""G""C""C""T""C""G""C""C""G""C""C""C""C""C""C""G
```

FIGURE 32.1

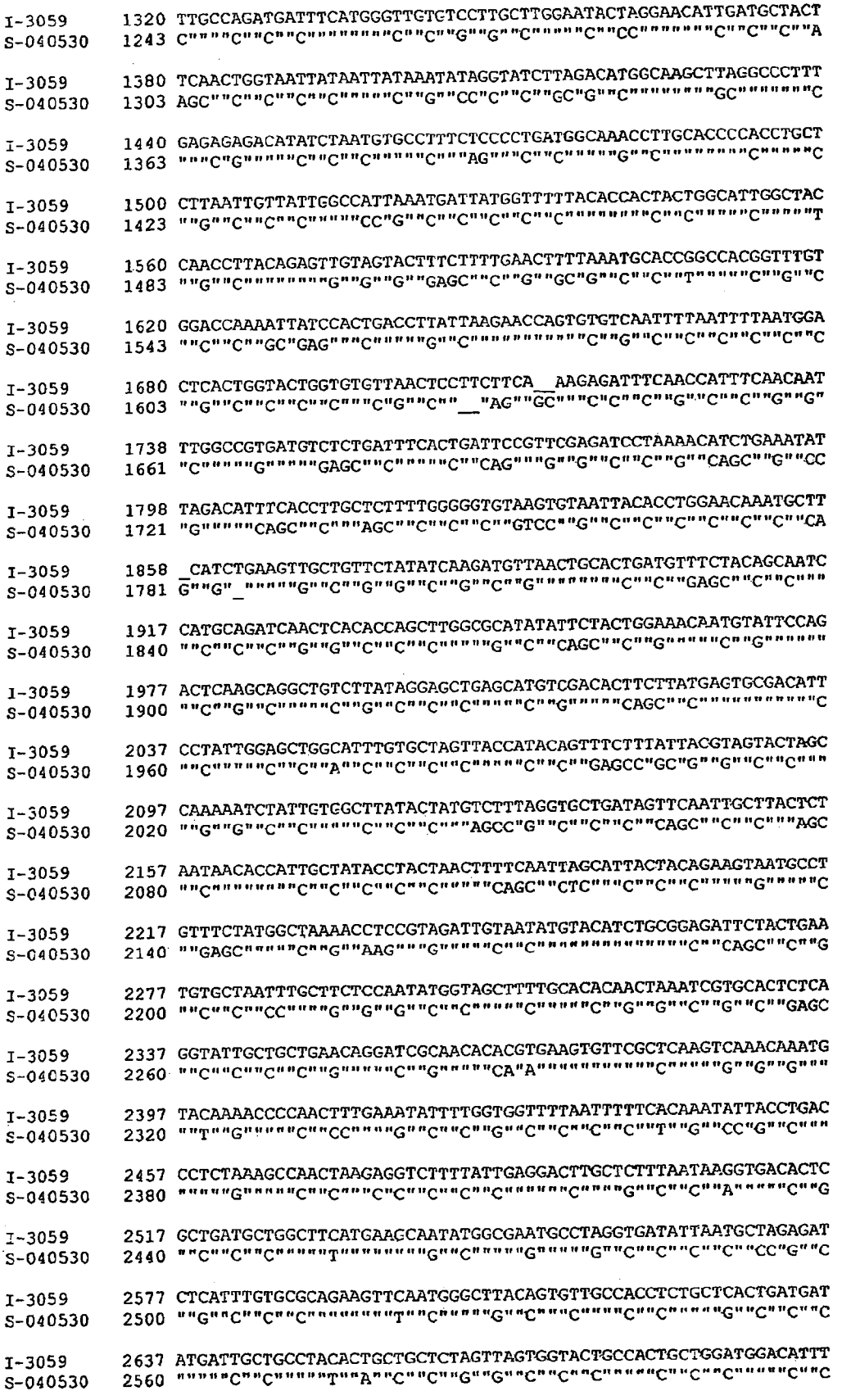

I-3059 1320 TTGCCAGATGATTTCATGGGTTGTGTCCTTGCTTGGAATACTAGGAACATTGATGCTACT
S-040530 1243
I-3059 1380 TCAACTGGTAATTATAATTATAAATATAGGTATCTTAGACATGGCAAGCTTAGGCCCTTT
S-040530 1303
I-3059 1440 GAGAGAGACATATCTAATGTGCCTTTCTCCCCTGATGGCAAACCTTGCACCCCACCTGCT
S-040530 1363
I-3059 1500 CTTAATTGTTATTGGCCATTAAATGATTATGGTTTTTACACCACTACTGGCATTGGCTAC
S-040530 1423
I-3059 1560 CAACCTTACAGAGTTGTAGTACTTTCTTTTGAACTTTTAAATGCACCGGCCACGGTTTGT
S-040530 1483
I-3059 1620 GGACCAAAATTATCCACTGACCTTATTAAGAACCAGTGTGTCAATTTTAATTTTAATGGA
S-040530 1543
I-3059 1680 CTCACTGGTACTGGTGTGTTAACTCCTTCTTCA__AAGAGATTTCAACCATTTCAACAAT
S-040530 1603
I-3059 1738 TTGGCCGTGATGTCTCTGATTTCACTGATTCCGTTCGAGATCCTAAAACATCTGAAATAT
S-040530 1661
I-3059 1798 TAGACATTTCACCTTGCTCTTTTGGGGGTGTAAGTGTAATTACACCTGGAACAAATGCTT
S-040530 1721
I-3059 1858 _CATCTGAAGTTGCTGTTCTATATCAAGATGTTAACTGCACTGATGTTTCTACAGCAATC
S-040530 1781
I-3059 1917 CATGCAGATCAACTCACACCAGCTTGGCGCATATATTCTACTGGAAACAATGTATTCCAG
S-040530 1840
I-3059 1977 ACTCAAGCAGGCTGTCTTATAGGAGCTGAGCATGTCGACACTTCTTATGAGTGCGACATT
S-040530 1900
I-3059 2037 CCTATTGGAGCTGGCATTTGTGCTAGTTACCATACAGTTTCTTTATTACGTAGTACTAGC
S-040530 1960
I-3059 2097 CAAAAATCTATTGTGGCTTATACTATGTCTTTAGGTGCTGATAGTTCAATTGCTTACTCT
S-040530 2020
I-3059 2157 AATAACACCATTGCTATACCTACTAACTTTTCAATTAGCATTACTACAGAAGTAATGCCT
S-040530 2080
I-3059 2217 GTTTCTATGGCTAAAACCTCCGTAGATTGTAATATGTACATCTGCGGAGATTCTACTGAA
S-040530 2140
I-3059 2277 TGTGCTAATTTGCTTCTCCAATATGGTAGCTTTTGCACACAACTAAATCGTGCACTCTCA
S-040530 2200
I-3059 2337 GGTATTGCTGCTGAACAGGATCGCAACACACGTGAAGTGTTCGCTCAAGTCAAACAAATG
S-040530 2260
I-3059 2397 TACAAAACCCCAACTTTGAAATATTTTGGTGGTTTTAATTTTTCACAAATATTACCTGAC
S-040530 2320
I-3059 2457 CCTCTAAAGCCAACTAAGAGGTCTTTTATTGAGGACTTGCTCTTTAATAAGGTGACACTC
S-040530 2380
I-3059 2517 GCTGATGCTGGCTTCATGAAGCAATATGGCGAATGCCTAGGTGATATTAATGCTAGAGAT
S-040530 2440
I-3059 2577 CTCATTTGTGCGCAGAAGTTCAATGGGCTTACAGTGTTGCCACCTCTGCTCACTGATGAT
S-040530 2500
I-3059 2637 ATGATTGCTGCCTACACTGCTGCTCTAGTTAGTGGTACTGCCACTGCTGGATGGACATTT
S-040530 2560

FIGURE 32.2

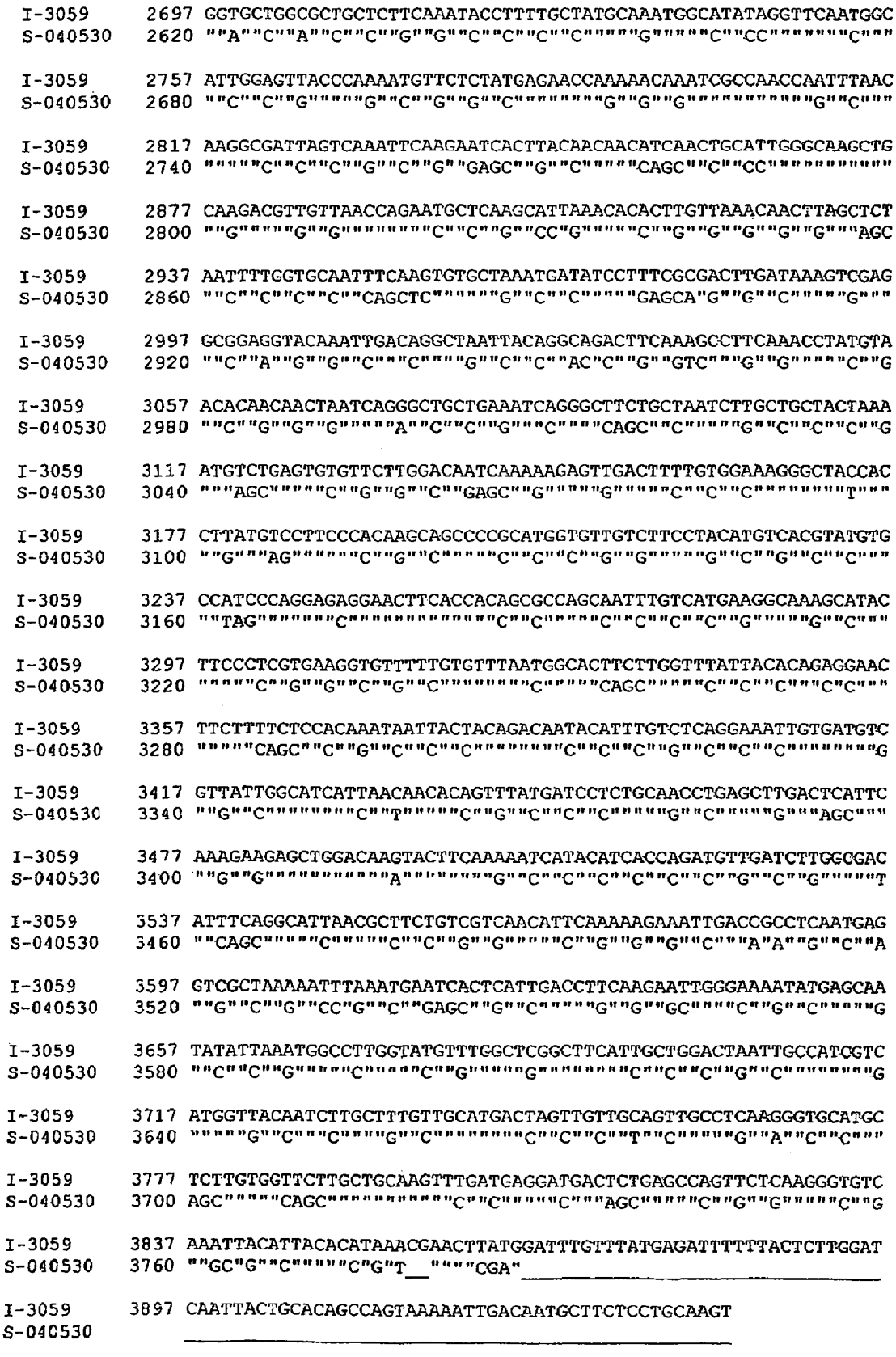

I-3059 2697 GGTGCTGGCGCTGCTCTTCAAATACCTTTTGCTATGCAAATGGCATATAGGTTCAATGGC
S-040530 2620
I-3059 2757 ATTGGAGTTACCCAAAATGTTCTCTATGAGAACCAAAAACAAATCGCCAACCAATTTAAC
S-040530 2680
I-3059 2817 AAGGCGATTAGTCAAATTCAAGAATCACTTACAACAACATCAACTGCATTGGGCAAGCTG
S-040530 2740
I-3059 2877 CAAGACGTTGTTAACCAGAATGCTCAAGCATTAAACACACTTGTTAAACAACTTAGCTCT
S-040530 2800
I-3059 2937 AATTTTGGTGCAATTTCAAGTGTGCTAAATGATATCCTTTCGCGACTTGATAAAGTCGAG
S-040530 2860
I-3059 2997 GCGGAGGTACAAATTGACAGGCTAATTACAGGCAGACTTCAAAGCCTTCAAACCTATGTA
S-040530 2920
I-3059 3057 ACACAACAACTAATCAGGGCTGCTGAAATCAGGGCTTCTGCTAATCTTGCTGCTACTAAA
S-040530 2980
I-3059 3117 ATGTCTGAGTGTGTTCTTGGACAATCAAAAAGAGTTGACTTTTGTGGAAAGGGCTACCAC
S-040530 3040
I-3059 3177 CTTATGTCCTTCCCACAAGCAGCCCCGCATGGTGTTGTCTTCCTACATGTCACGTATGTG
S-040530 3100
I-3059 3237 CCATCCCAGGAGAGGAACTTCACCACAGCGCCAGCAATTTGTCATGAAGGCAAAGCATAC
S-040530 3160
I-3059 3297 TTCCCTCGTGAAGGTGTTTTTGTGTTTAATGGCACTTCTTGGTTTATTACACAGAGGAAC
S-040530 3220
I-3059 3357 TTCTTTTCTCCACAAATAATTACTACAGACAATACATTTGTCTCAGGAAATTGTGATGTC
S-040530 3280
I-3059 3417 GTTATTGGCATCATTAACAACACAGTTTATGATCCTCTGCAACCTGAGCTTGACTCATTC
S-040530 3340
I-3059 3477 AAAGAAGAGCTGGACAAGTACTTCAAAAATCATACATCACCAGATGTTGATCTTGGCGAC
S-040530 3400
I-3059 3537 ATTTCAGGCATTAACGCTTCTGTCGTCAACATTCAAAAAGAAATTGACCGCCTCAATGAG
S-040530 3460
I-3059 3597 GTCGCTAAAAATTTAAATGAATCACTCATTGACCTTCAAGAATTGGGAAAATATGAGCAA
S-040530 3520
I-3059 3657 TATATTAAATGGCCTTGGTATGTTTGGCTCGGCTTCATTGCTGGACTAATTGCCATCGTC
S-040530 3580
I-3059 3717 ATGGTTACAATCTTGCTTTGTTGCATGACTAGTTGTTGCAGTTGCCTCAAGGGTGCATGC
S-040530 3640
I-3059 3777 TCTTGTGGTTCTTGCTGCAAGTTTGATGAGGATGACTCTGAGCCAGTTCTCAAGGGTGTC
S-040530 3700
I-3059 3837 AAATTACATTACACATAAACGAACTTATGGATTTGTTTATGAGATTTTTTACTCTTGGAT
S-040530 3760
I-3059 3897 CAATTACTGCACAGCCAGTAAAAATTGACAATGCTTCTCCTGCAAGT
S-040530

FIGURE 32.3

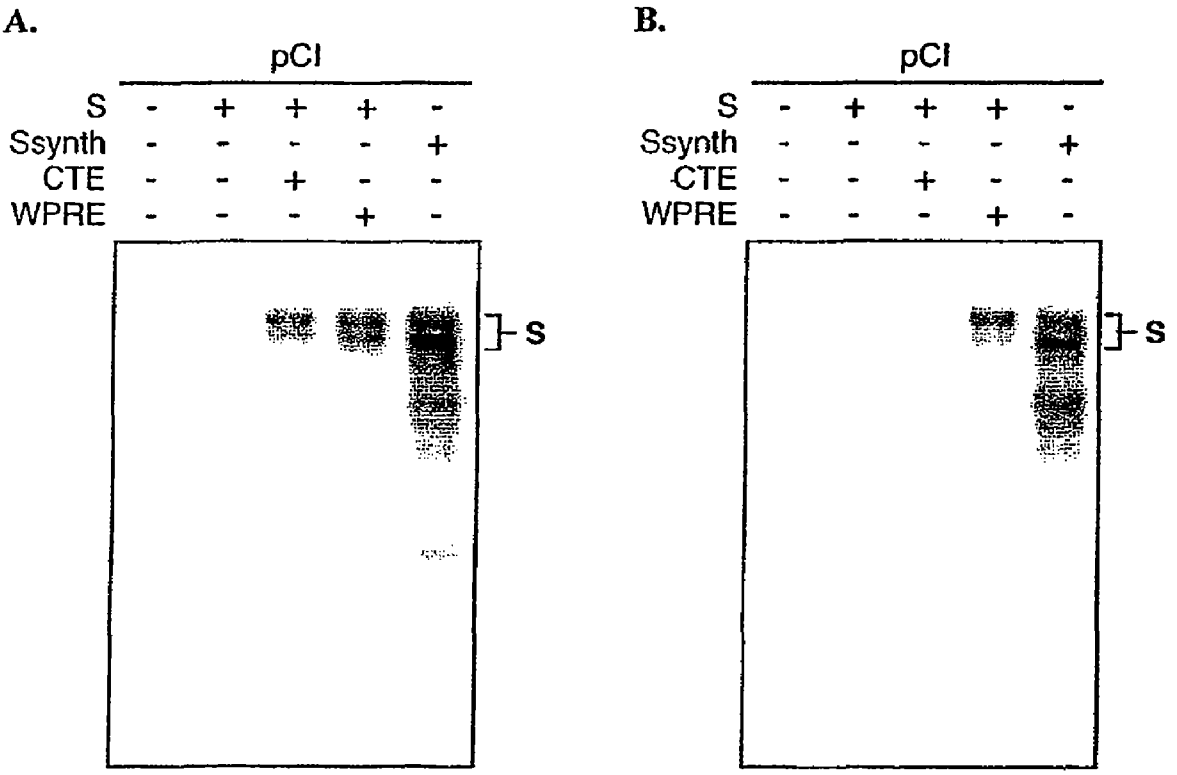
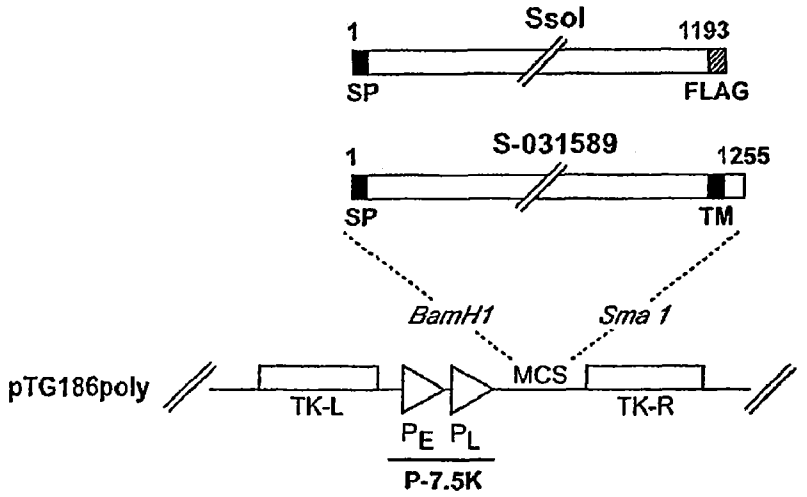
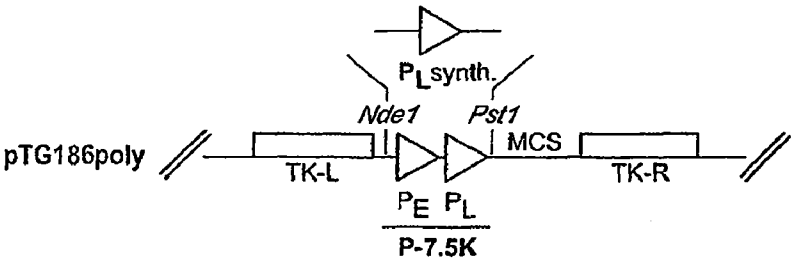


FIGURE 33

A.



B.



C.

CATATG AGC [T]$_{20}$GGCATATAAATA GACTC GGCGCGCC AT CTGCAG
Nde1 promoteur 480 *Asc1* *Pst1*

FIGURE 34 A-C

D.

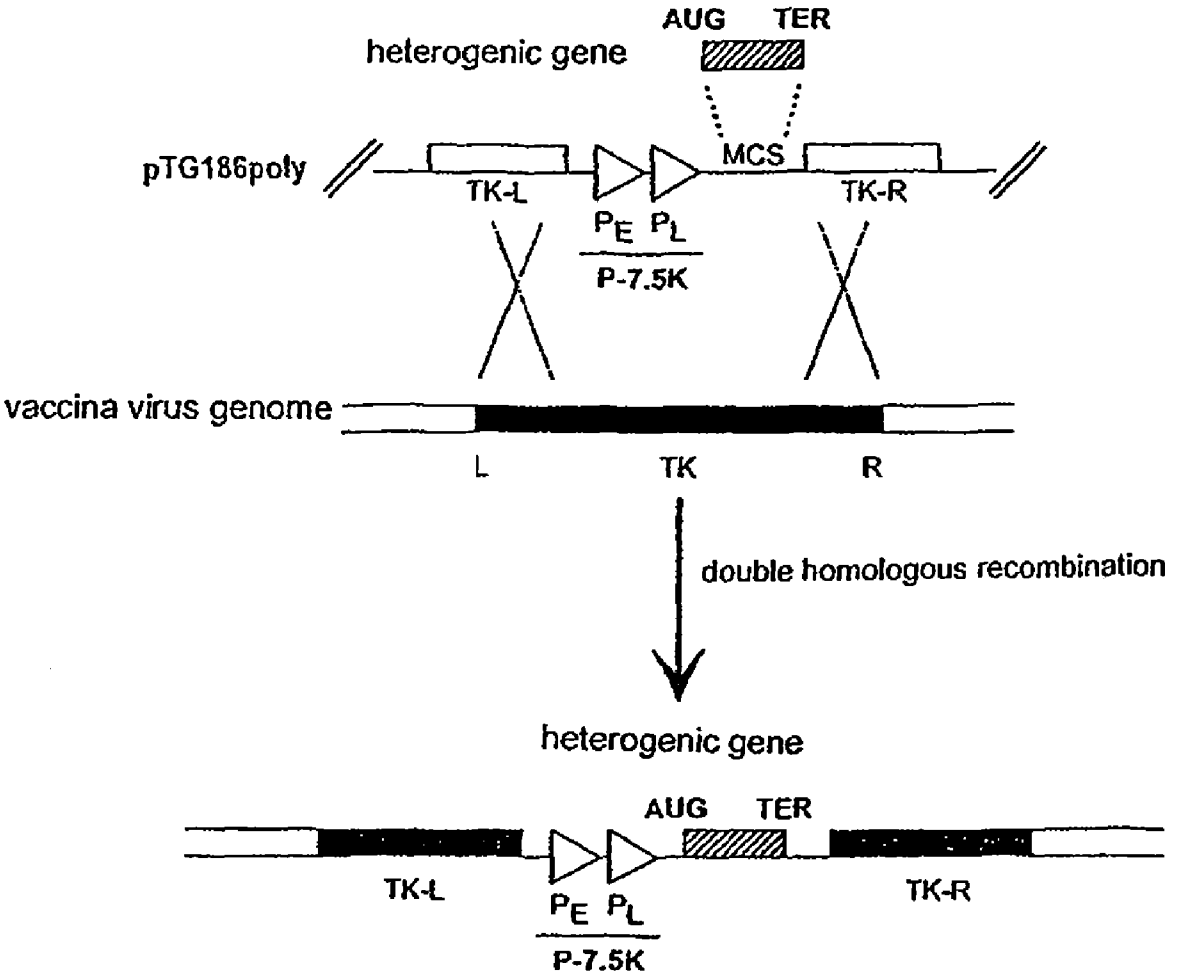

AUG
TER
heterogenic gene
MCS
pTG186poly
TK-L
TK-R
P_E P_L
P-7.5K
vaccina virus genome
L
TK
R
double homologous recombination
heterogenic gene
AUG
TER
TK-L
TK-R
P_E P_L
P-7.5K

FIGURE 34 D

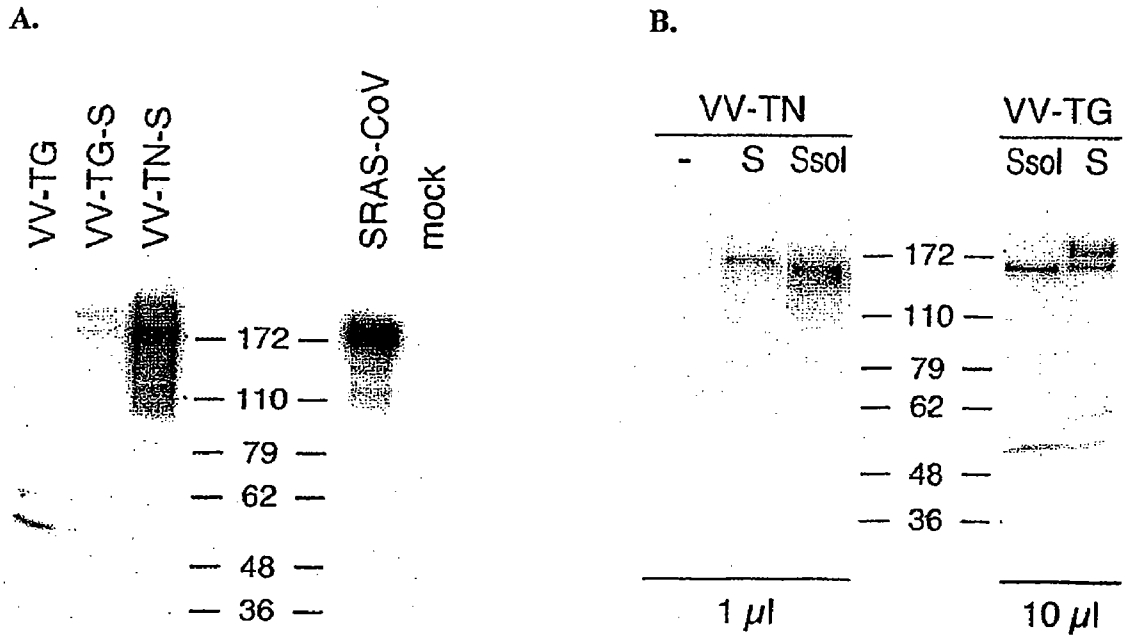
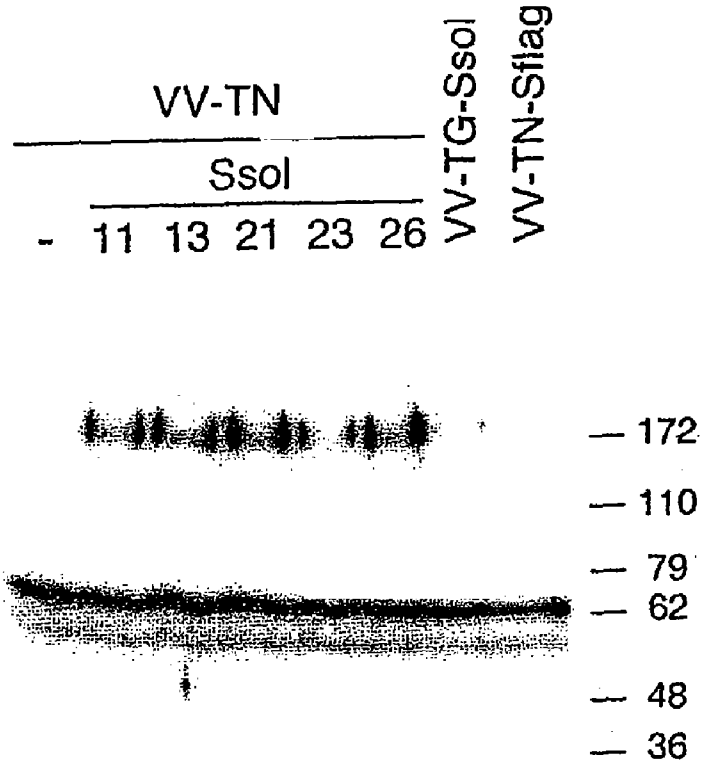

A.
VV-TG
VV-TG-S
VV-TN-S
SRAS-CoV
mock
172
110
79
62
48
36
B.
VV-TN
VV-TG
-
S
Ssol
Ssol
S
172
110
79
62
48
36
1 µl
10 µl

FIGURE 35

A.


VV-TN
Ssol
- 11 13 21 23 26
VV-TG-Ssol
VV-TN-Sflag
172
110
79
62
48
36

B.

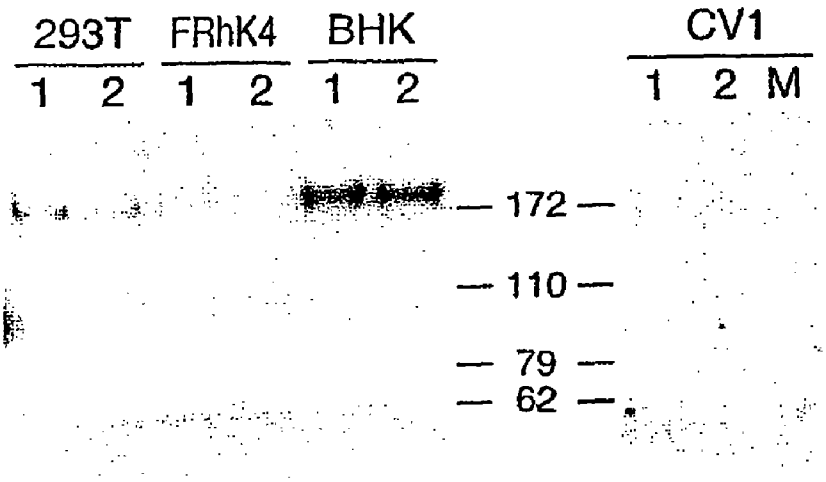

293T FRhK4 BHK
1 2 1 2 1 2
CV1
1 2 M
172
110
79
62

FIGURE 36

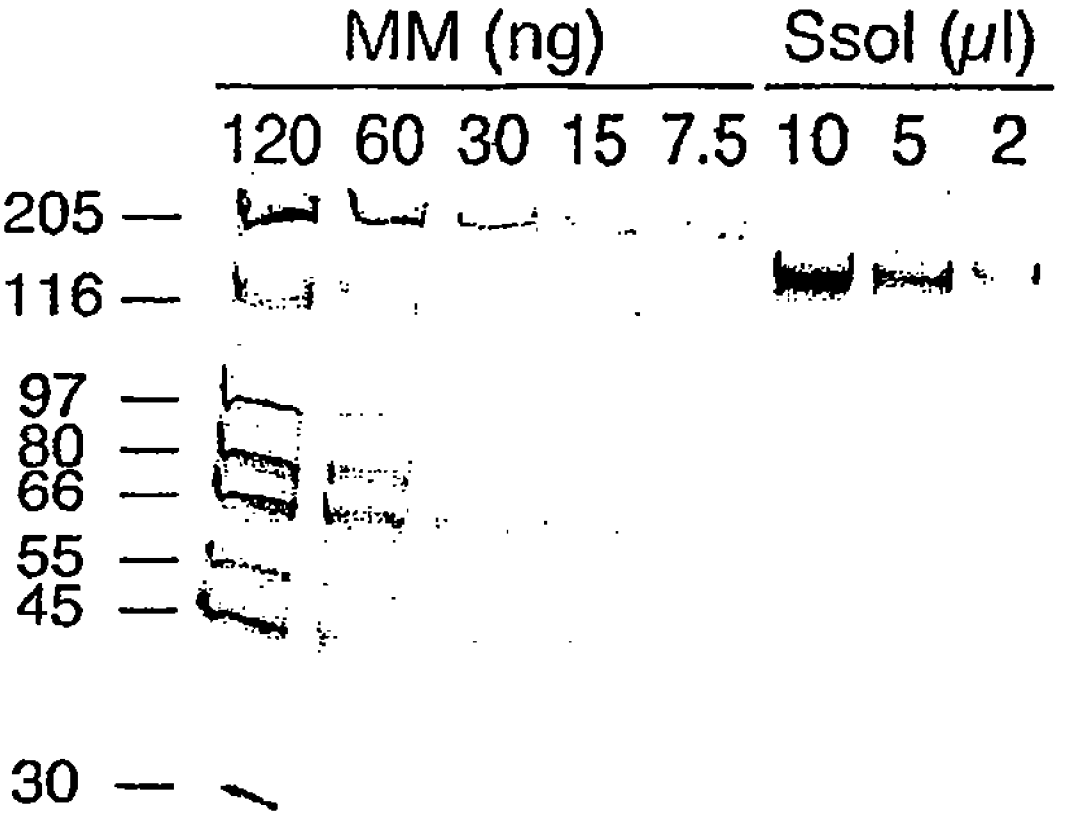


FIGURE 37

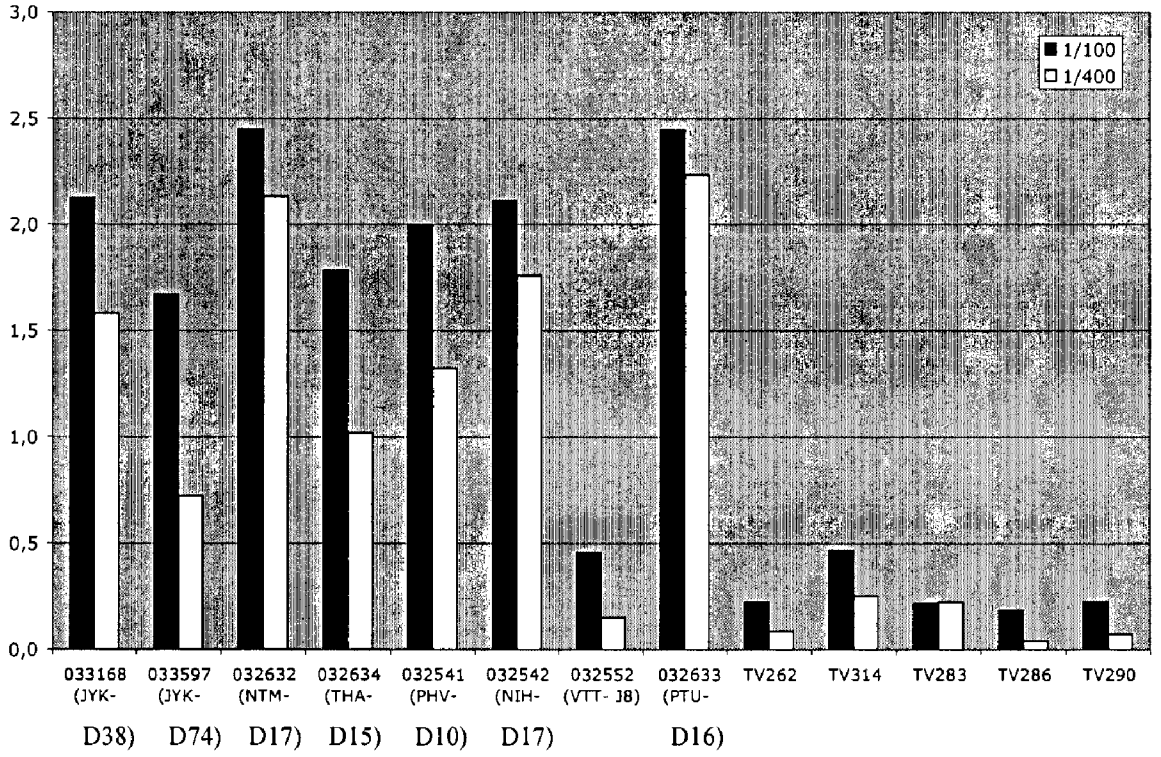
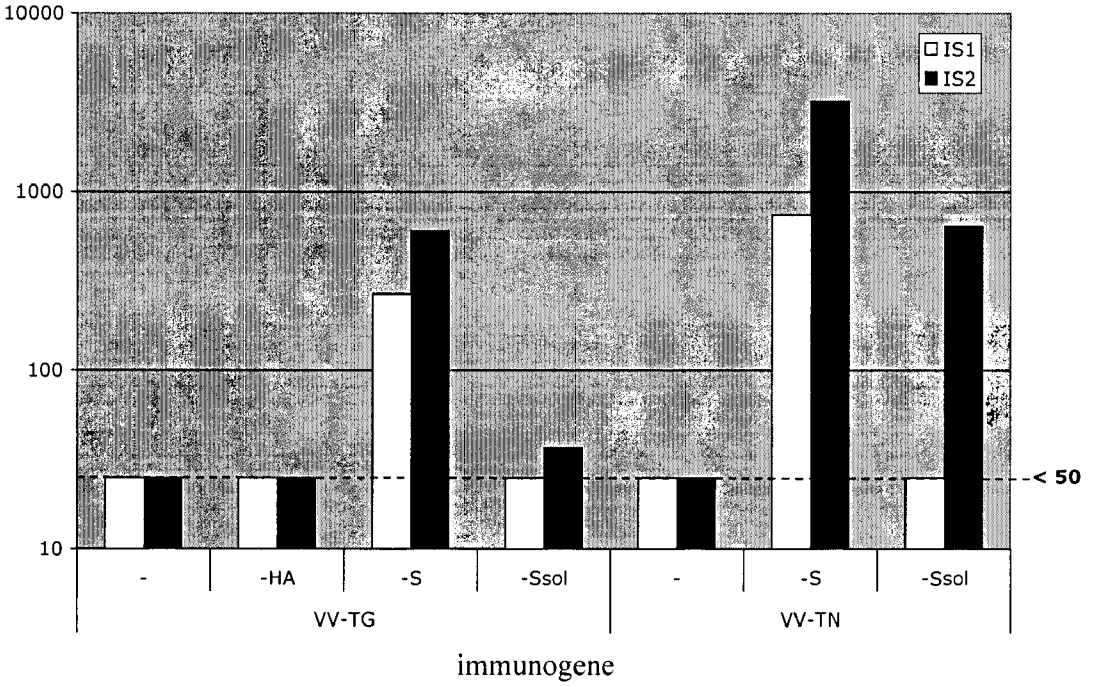

3,0
2,5
2,0
1,5
1,0
0,5
0,0
1/100
1/400
033168 (JYK- D38)
033597 (JYK- D74)
032632 (NTM- D17)
032634 (THA- D15)
032541 (PHV- D10)
032542 (NIH- D17)
032552 (VTT- J8)
032633 (PTU- D16)
TV262
TV314
TV283
TV286
TV290
serums

FIGURE 38

A.


10000
1000
100
10
IS1
IS2
< 50
-
-HA
-S
-Ssol
-
-S
-Ssol
VV-TG
VV-TN
immunogene

B.

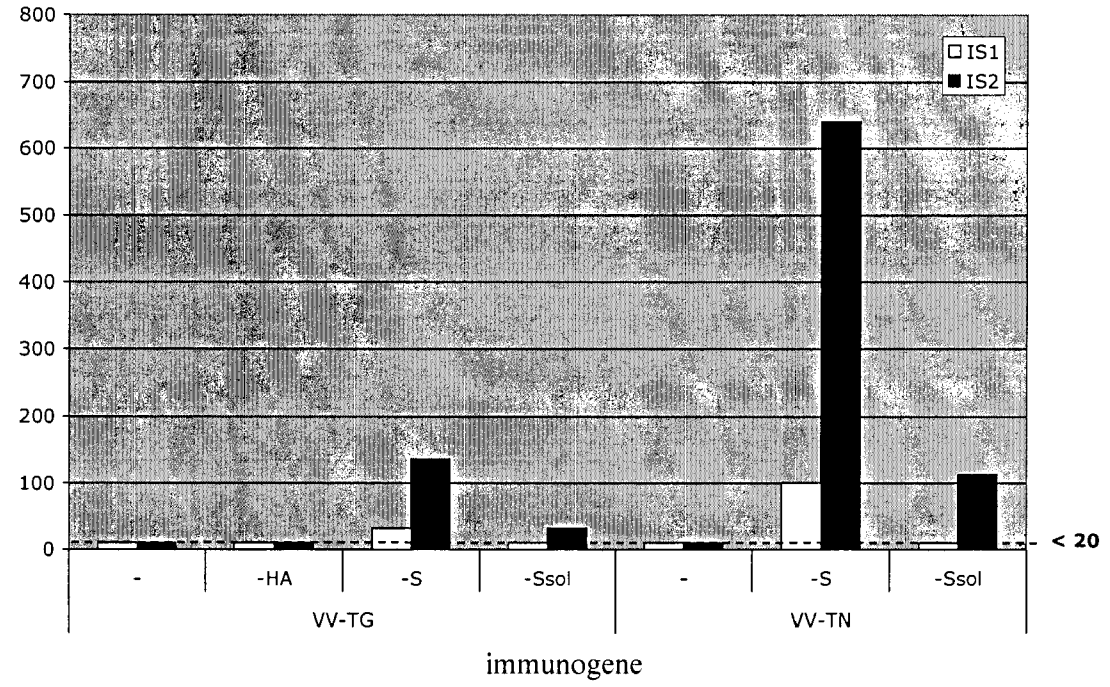

800
700
600
500
400
300
200
100
0
IS1
IS2
< 20
-
-HA
-S
-Ssol
-
-S
-Ssol
VV-TG
VV-TN
immunogene

FIGURE 39

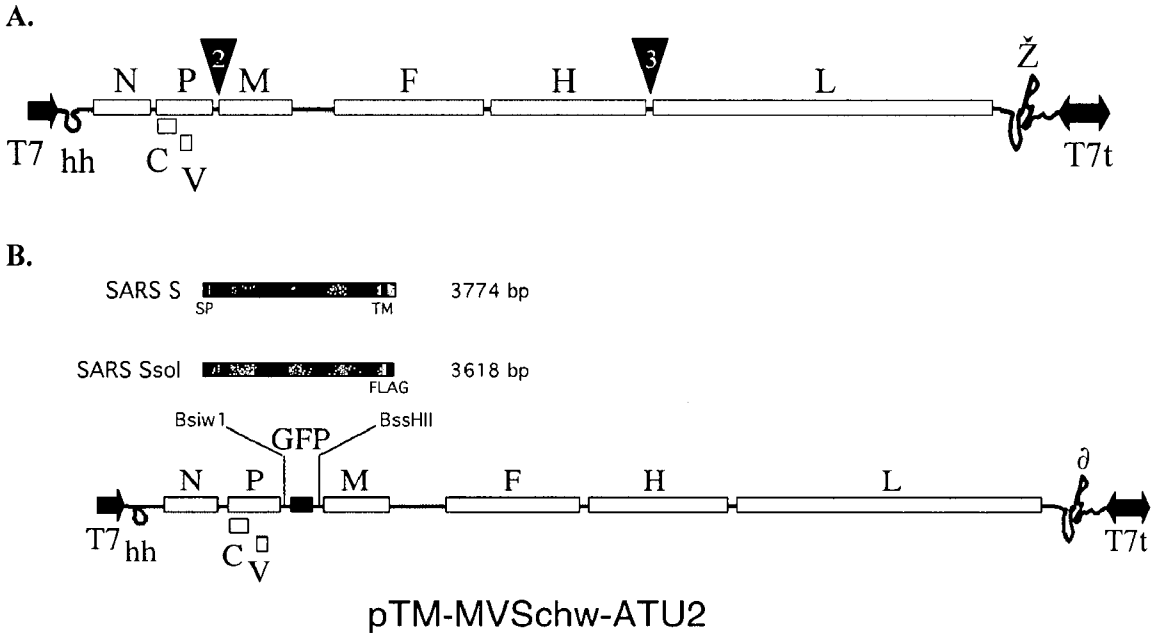


FIGURE 40

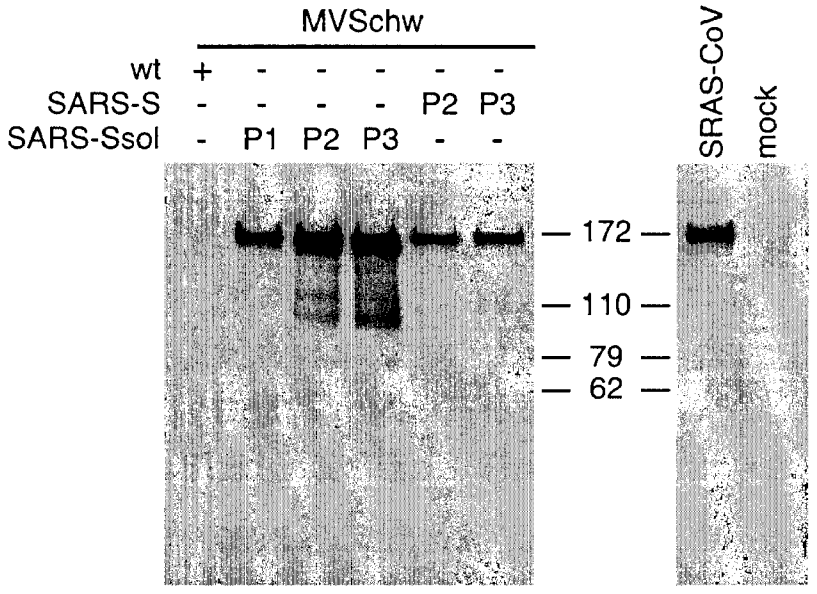
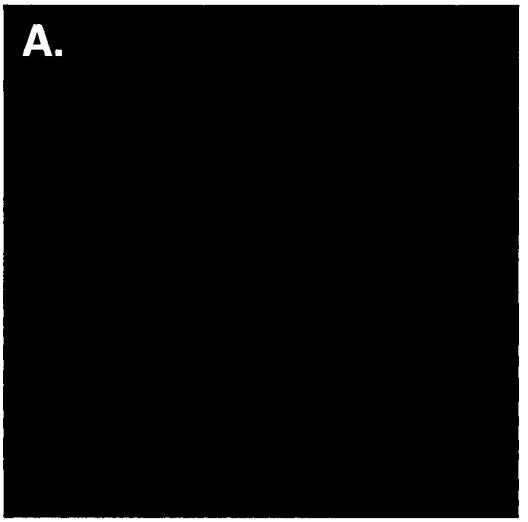
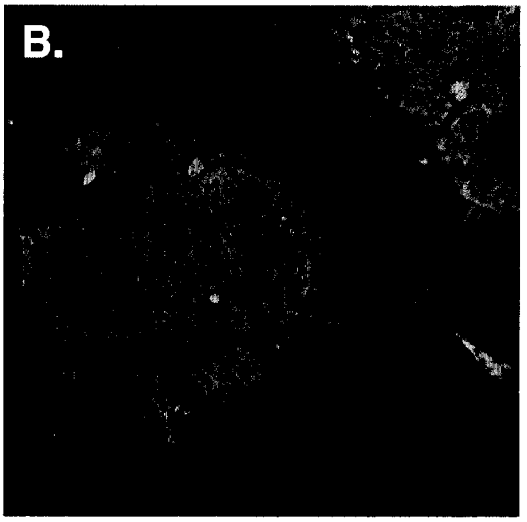


FIGURE 41





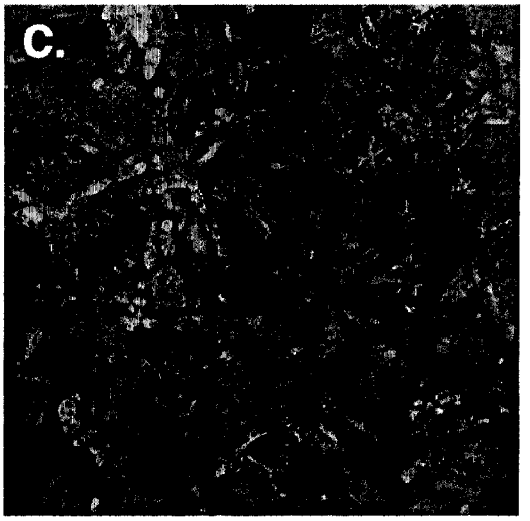


FIGURE 42

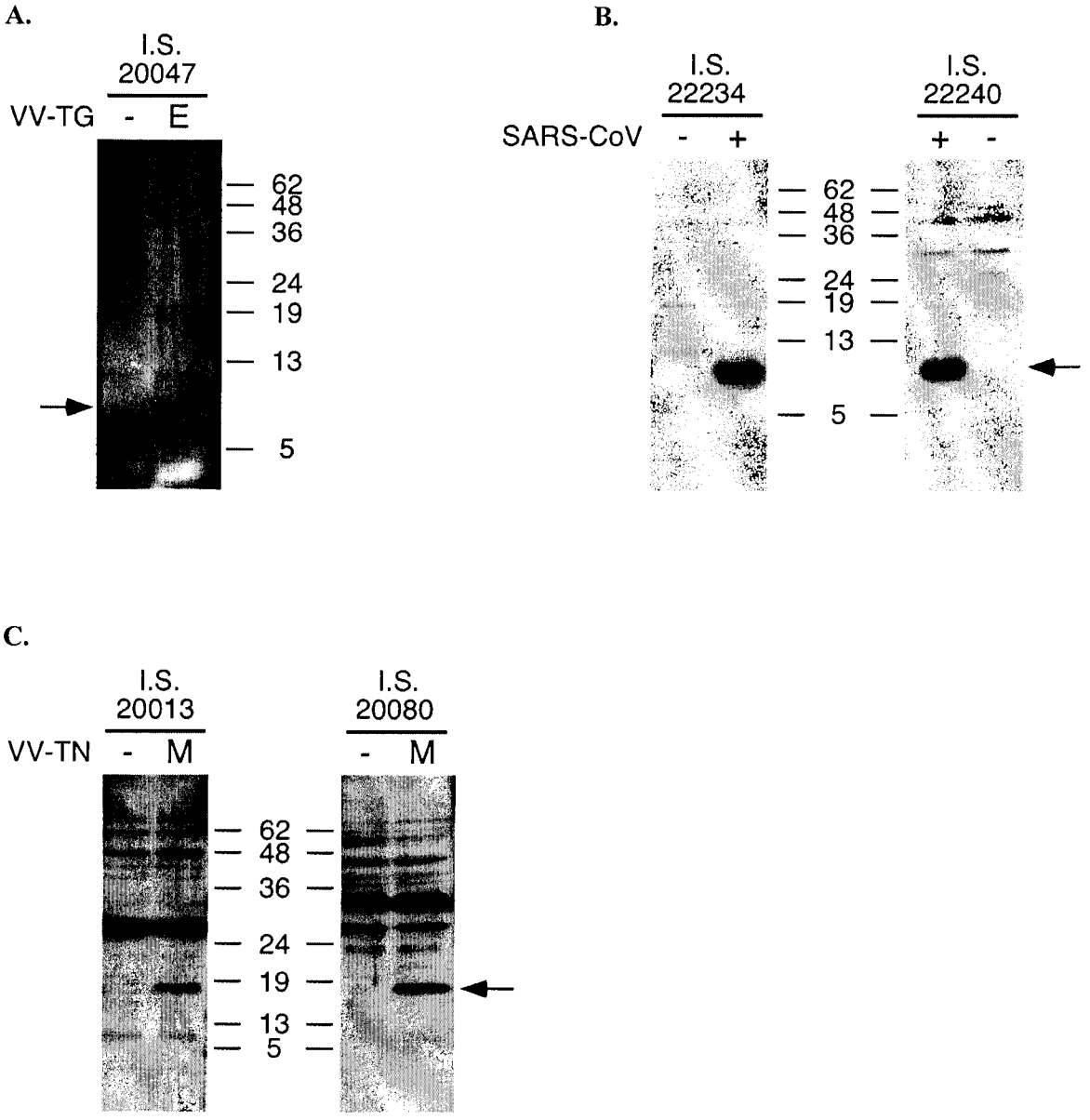

A.
I.S. 20047
VV-TG - E
62
48
36
24
19
13
5
B.
I.S. 22234
I.S. 22240
SARS-CoV - + + -
62
48
36
24
19
13
5
C.
I.S. 20013
I.S. 20080
VV-TN - M - M
62
48
36
24
19
13
5

FIGURE 43

STRAIN OF SARS-ASSOCIATED CORONAVIRUS AND APPLICATIONS THEREOF

This is a division of application Ser. No. 10/581,356, filed Feb. 8, 2007, now U.S. Pat. No. 7,736,850, which is a continuation of International Application No. PCT/FR2004/003106, filed Dec. 2, 2004, both of which are incorporated herein by reference.

The present invention relates to a novel strain of severe acute respiratory syndrome (SARS)-associated coronavirus derived from a sample recorded under No. 031589 and collected in Hanoi (Vietnam), to nucleic acid molecules derived from its genome, to the proteins and peptides encoded by said nucleic acid molecules and to their applications, in particular as diagnostic reagents and/or as vaccine.

Coronavirus is a virus containing single-stranded RNA, of positive polarity, of approximately 30 kilobases which replicates in the cytoplasm of the host cells; the 5' end of the genome has a capped structure and the 3' end contains a polyA tail. This virus is enveloped and comprises, at its surface, peplomeric structures called spicules.

The genome comprises the following open reading frames or ORFs, from its 5' end to its 3' end: ORF1a and ORF1b corresponding to the proteins of the transcription-replication complex, and ORF-S, ORF-E, ORF-M and ORF-N corresponding to the structural proteins S, E, M and N. It also comprises ORFs corresponding to proteins of unknown function encoded by: the region situated between ORF-S and ORF-E and overlapping the latter, the region situated between ORF-M and ORF-N, and the region included in ORF-N.

The S protein is a membrane glycoprotein (200-220 kDa) which exists in the form of spicules or spikes emerging from the surface of the viral envelope. It is responsible for the attachment of the virus to the receptors of the host cell and for inducing the fusion of the viral envelope with the cell membrane.

The small envelope protein (E), also called sM (small membrane), which is a nonglycosylated transmembrane protein of about 10 kDa, is the protein present in the smallest quantity in the virion. It plays a powerful role in the coronavirus budding process which occurs at the level of the intermediate compartment in the endoplasmic reticulum and the Golgi apparatus.

The M protein or matrix protein (25-30 kDa) is a more abundant membrane glycoprotein which is integrated into the viral particle by an M/E interaction, whereas the incorporation of S into the particles is directed by an S/M interaction. It appears to be important for the viral maturation of coronaviruses and for the determination of the site where the viral particles are assembled.

The N protein or nucleocapsid protein (45-50 kDa) which is the most conserved among the coronavirus structural proteins is necessary for encapsidating the genomic RNA and then for directing its incorporation into the virion. This protein is probably also involved in the replication of the RNA.

When the host cell is infected, the reading frame (ORF) situated in 5' of the viral genome is translated into a polyprotein which is cleaved by the viral proteases and then releases several nonstructural proteins such as the RNA-dependent RNA polymerase (Rep) and the ATPase helicase (Hel). These two proteins are involved in the replication of the viral genome and in the generation of transcripts which are used in the synthesis of the viral proteins. The mechanisms by which these subgenomic mRNAs are produced are not completely understood; however, recent facts indicate that the sequences for regulation of transcription at the 5' end of each gene

represent signals which regulate the discontinuous transcription of the subgenomic mRNAs.

The proteins of the viral membrane (S, E and M proteins) are inserted into the intermediate compartment, whereas the replicated RNA (+ strand) is assembled with the N (nucleocapsid) protein. This protein-RNA complex then combines with the M protein contained in the membranes of the endoplasmic reticulum and the viral particles form when the nucleocapsid complex buds into the endoplasmic reticulum. The virus then migrates across the Golgi complex and eventually leaves the cell, for example by exocytosis. The site of attachment of the virus to the host cell is at the level of the S protein.

Coronaviruses are responsible for 15 to 30% of colds in humans and for respiratory and digestive infections in animals, especially cats (FIPV: Feline infectious peritonitis virus), poultry (IBV: Avian infectious bronchitis virus), mice (MHV: Mouse hepatitis virus), pigs (TGEV: Transmissible gastroenteritis virus, PEDV: Porcine Epidemic diarrhea virus, PRCoV: Porcine Respiratory Coronavirus, HEV: Hemagglutinating encephalomyelitis Virus) and bovines (BCoV: Bovine coronavirus).

In general, each coronavirus affects only one species; in immunocompetent individuals, the infection induces optionally neutralizing antibodies and cell immunity, capable of destroying the infected cells.

An epidemy of atypical pneumonia, called severe acute respiratory syndrome (SARS) has spread in various countries (Vietnam, Hong Kong, Singapore, Thailand and Canada) during the first quarter of 2003, from an initial focus which appeared in China in the last quarter of 2002. The severity of this disease is such that its mortality rate is about 3 to 6%. The determination of the causative agent of this disease is underway by numerous laboratories worldwide.

In March 2003, a new coronavirus (SARS-CoV or SARS virus) was isolated, in association with cases of severe acute respiratory syndrome (T. G. KSIAZEK et al., The New England Journal of Medicine, 2003, 348, 1319-1330; C. DROSTEN et al., The New England Journal of Medicine, 2003, 348, 1967-1976; Peiris et al., Lancet, 2003, 361, 1319).

Genomic sequences of this new coronavirus have thus been obtained, in particular those of the Urbani isolate (Genbank accession No. AY274119.3 and A. MARRA et al., Science, May 1, 2003, 300, 1399-1404) and the Toronto isolate (Tor2, Genbank accession No. AY278741 and A. ROTA et al., Science, 2003, 300, 1394-1399).

The organization of the genome is comparable with that of other known coronaviruses, thus making it possible to confirm that SARS-CoV belongs to the Coronaviridae family; open reading frames ORF1a and 1b and open reading frames corresponding to the S, E, M and N proteins, and to proteins encoded by: the region situated between ORF-S and ORF-E (ORF3), the region situated between ORF-S and ORF-E and overlapping. ORF-E (ORF4), the region situated between ORF-M and ORF-N (ORF7 to ORF11) and the region corresponding to ORF-N (ORF13 and ORF14), have in particular been identified.

Seven differences have been identified between the sequences of the Tor2 and Urbani isolates; 3 correspond to silent mutations (c/t at position 16622 and a/g at position 19064 of ORF1b, t/c at position 24872 of ORF-S) and 4 modify the amino acid sequence of respectively: the proteins encoded by ORF1a (c/t at position 7919 corresponding to the A/V mutation), the S protein (g/t at position 23220 corresponding to the A/S mutation), the protein encoded by ORF3

(a/g at position 25298 corresponding to the R/G mutation) and the M protein (t/c at position 26857 corresponding to the S/P mutation).

In addition, phylogenetic analysis shows that SARS-CoV is distant from other coronaviruses and that it did not appear by mutation of human respiratory coronaviruses nor by recombination between known coronaviruses (for a review, see Holmes, J. C. I., 2003, 111, 1605-1609).

The determination and the taking into account of new variants are important for the development of reagents for the detection and diagnosis of SARS which are sufficiently sensitive and specific, and immunogenic compositions capable of protecting populations against epidemics of SARS.

The inventors have now identified another strain of SARS-associated coronavirus which is distinguishable from the Tor2 and Urbani isolates.

The subject of the present invention is therefore an isolated or purified strain of severe acute respiratory syndrome-associated human coronavirus, characterized in that its genome has, in the form of complementary DNA, a serine codon at position 23220-23222 of the gene for the S protein or a glycine codon at position 25298-25300 of the gene for ORF3, and an alanine codon at position 7918-7920 of ORF1a or a serine codon at position 26857-26859 of the gene for the M protein, said positions being indicated in terms of reference to the Genbank sequence AY274119.3.

According to an advantageous embodiment of said strain, the DNA equivalent of its genome has a sequence corresponding to the sequence SEQ ID No: 1; this coronavirus strain is derived from the sample collected from the bronchoaleveolar washings from a patient suffering from SARS, recorded under the No. 031589 and collected at the Hanoi (Vietnam) French hospital.

In accordance with the invention, said sequence SEQ ID No: 1 is that of the deoxyribonucleic acid corresponding to the ribonucleic acid molecule of the genome of the isolated coronavirus strain as defined above.

The sequence SEQ ID No: 1 is distinguishable from the Genbank sequence AY274119.3 (Tor2 isolate) in that it possesses the following mutations:

- g/t at position 23220; the alanine codon (gct) at position 577 of the amino acid sequence of the Tor2 S protein is replaced by a serine codon (tct),
- a/g at position 25298; the arginine codon (aga) at position 11 of the amino acid sequence of the protein encoded by the Tor2 ORF3 is replaced by a glycine codon (gga).

In addition, the sequence SEQ ID No: 1 is distinguishable from the Genbank sequence AY278741 (Urbani isolate) in that it possesses the following mutations:

- t/c at position 7919; the valine codon (gtt) in position 2552 of the amino acid sequence of the protein encoded by ORF1a is replaced by an alanine codon (gct),
- t/c at position 16622: this mutation does not modify the amino acid sequence of the proteins encoded by ORF1b (silent mutation),
- g/a at position 19064: this mutation does not modify the amino acid sequence of the proteins encoded by ORF1b (silent mutation),
- c/t at position 24872: this mutation does not modify the amino acid sequence of the S protein, and
- c/t at position 26857: the proline codon (ccc) at position 154 of the amino acid sequence of the M protein is replaced by a serine codon (tcc).

Unless otherwise stated, the positions of the nucleotide and peptide sequences are indicated with reference to the Genbank sequence AY274119.3.

The subject of the present invention is also an isolated or purified polynucleotide, characterized in that its sequence is that of the genome of the isolated coronavirus strain as defined above.

According to an advantageous embodiment of said polynucleotide, it has the sequence SEQ ID No: 1.

The subject of the present invention is also an isolated or purified polynucleotide, characterized in that its sequence hybridizes under high stringency conditions with the sequence of the polynucleotide as defined above.

The terms "isolated or purified" mean modified "by the hand of humans" from the natural state; in other words if an object exists in nature, it is said to be isolated or purified if it is modified or extracted from its natural environment or both. For example, a polynucleotide or a protein/peptide naturally present in a living organism is neither isolated nor purified; on the other hand, the same polynucleotide or protein/peptide separated from coexisting molecules in its natural environment, obtained by cloning, amplification and/or chemical synthesis is isolated for the purposes of the present invention. Furthermore, a polynucleotide or a protein/peptide which is introduced into an organism by transformation, genetic manipulation or by any other method, is "isolated" even if it is present in said organism. The term purified as used in the present invention means that the proteins/peptides according to the invention are essentially free of association with the other proteins or polypeptides, as is for example the product purified from the culture of recombinant host cells or the product purified from a nonrecombinant source.

For the purposes of the present invention, high stringency hybridization conditions are understood to mean temperature and ionic strength conditions chosen such that they make it possible to maintain the specific and selective hybridization between complementary polynucleotides.

By way of illustration, high stringency conditions for the purposes of defining the above polynucleotides are advantageously the following: the DNA-DNA or DNA-RNA hybridization is performed in two steps: (1) prehybridization at 42° C. for 3 hours in phosphate buffer (20 mM, pH 7.5) containing 5×SSC (1×SSC corresponds to a 0.15 M NaCl+0.015 M sodium citrate solution), 50% formamide, 7% sodium dodecyl sulfate (SDS), 10×Denhardt's, 5% dextran sulfate and 1% salmon sperm DNA; (2) hybridization for 20 hours at 42° C. followed by 2 washings of 20 minutes at 20° C. in 2×SSC+2% SDS, 1 washing of 20 minutes at 20° C. in 0.1×SSC+0.1% SDS. The final washing is performed in 0.1×SSC+0.1% SDS for 30 minutes at 60° C.

The subject of the present invention is also a representative fragment of the polynucleotide as defined above, characterized in that it is capable of being obtained either by the use of restriction enzymes whose recognition and cleavage sites are present in said polynucleotide as defined above, or by amplification with the aid of oligonucleotide primers specific for said polynucleotide as defined above, or by transcription in vitro, or by chemical synthesis.

According to an advantageous embodiment of said fragment, it is selected from the group consisting of: the cDNA corresponding to at least one open reading frame (ORF) chosen from: ORF1a, ORF1b, ORF-S, ORF-E, ORF-M, ORF-N, ORF3, ORF4, ORF7 to ORF11, ORF13 and ORF14 and the cDNA corresponding to the noncoding 5' or 3' ends of said polynucleotide.

According to an advantageous feature of this embodiment, said fragment has a sequence selected from the group consisting of:

- the sequences SEQ ID NO: 2 and 4 representing the cDNA corresponding to the ORF-S which encodes the S protein,
- the sequences SEQ ID NO: 13 and 15 representing the cDNA corresponding to the ORF-E which encodes the E protein,
- the sequences SEQ ID NO: 16 and 18 representing the cDNA corresponding to the ORF-M which encodes the M protein,
- the sequences SEQ ID NO: 36 and 38 representing the cDNA corresponding to the ORF-N which encodes the N protein,
- the sequences representing the cDNA corresponding respectively: to ORF1a and ORF1b (ORF1ab, SEQ ID NO: 31), to ORF3 and ORF4 (SEQ ID NO: 7, 8), to ORF7 to 11 (SEQ ID NO: 19, 20) to ORF13 (SEQ ID NO: 32) and to ORF14 (SEQ ID NO: 34), and
- the sequences representing the cDNAs corresponding respectively to the noncoding 5' (SEQ ID NO: 39 and 72) and 3' (SEQ ID NO: 40, 73) ends of said polynucleotide.

The subject of the present invention is also a cDNA fragment encoding the S protein, as defined above, characterized in that it has a sequence selected from the group consisting of the sequences SEQ ID NO: 5 and 6 (Sa and Sb fragments).

The subject of the present invention is also a cDNA fragment corresponding to ORF1a and ORF1b as defined above, characterized in that it has a sequence selected from the group consisting of the sequences SEQ ID NO: 41 to 54 (L0 to L12 fragments).

The subject of the present invention is also a polynucleotide fragment as defined above, characterized in that it has at least 15 consecutive bases or base pairs of the sequence of the genome of said strain including at least one of those situated in position 7979, 16622, 19064, 23220, 24872, 25298 and 26857. Preferably this is a fragment of 20 to 2500 bases or base pairs, preferably from 20 to 400.

According to an advantageous embodiment of said fragment, it includes at least one pair of bases or base pairs corresponding to the following positions: 7919 and 23220, 7919 and 25298, 16622 and 23220, 19064 and 23220, 16622 and 25298, 19064 and 25298, 23220 and 24872, 23220 and 26857, 24872 and 25298, 25298 and 26857.

The subject of the present invention is also primers of at least 18 bases capable of amplifying a fragment of the genome of a SARS-associated coronavirus or of the DNA equivalent thereof.

According to an embodiment of said primers, they are selected from the group consisting of:

- the pair of primers No. 1 corresponding respectively to positions 28507 to 28522 (sense primer, SEQ ID NO: 60) and 28774 to 28759 (antisense primer, SEQ ID NO: 61) of the sequence of the polynucleotide as defined above,
- the pair of primers No. 2 corresponding respectively to positions 28375 to 28390 (sense primer, SEQ ID NO: 62) and 28702 to 28687 (antisense primer, SEQ ID NO: 63) of the sequence of the polynucleotide as defined above, and
- the pair of primers consisting of the primers SEQ ID Nos: 55 and 56.

The subject of the present invention is also a probe capable of detecting the presence of the genome of a SARS-associated coronavirus or of a fragment thereof, characterized in that it is selected from the group consisting of: the fragments as defined above and the fragments corresponding to the following positions of the polynucleotide sequence as defined above: 28561 to 28586, 28588 to 28608, 28541 to 28563 and 28565 to 28589 (SEQ ID NO: 64 to 67).

The probes and primers according to the invention may be labeled directly or indirectly with a radioactive or nonradioactive compound by methods well known to persons skilled in the art so as to obtain a detectable and/or quantifiable signal. Among the radioactive isotopes used, there may be mentioned ^{32}P, ^{33}P, ^{35}S, ^{3}H or ^{125}I. The nonradioactive entities are selected from ligands such as biotin, avidin, streptavidin, digoxygenin, haptens, dyes, luminescent agents such as radioluminescent, chemoluminescent, bioluminescent, fluorescent and phosphorescent agents.

The invention encompasses the labeled probes and primers derived from the preceding sequences.

Such probes and primers are useful for the diagnosis of infection by a SARS-associated coronavirus.

The subject of the present invention is also a method for the detection of a SARS-associated coronavirus, from a biological sample, which method is characterized in that it comprises at least:

(a) the extraction of nucleic acids present in said biological sample,

(b) the amplification of a fragment of ORF-N by RT-PCR with the aid of a pair of primers as defined above, and

(c) the detection, by any appropriate means, of the amplification products obtained in (b).

The amplification products (amplicons) in (b) are 268 bp for the pair of primers No. 1 and 328 bp for the pair of primers No. 2.

According to an advantageous embodiment of said method, the step (b) of detection is carried out with the aid of at least one probe corresponding to positions 28561 to 28586, 28588 to 28608, 28541 to 28563 and 28565 to 28589 of the sequence of the polynucleotide as defined above.

Preferably, the SARS-associated coronavirus genome is detected and optionally quantified by PCR in real time with the aid of the pair of primers No. 2 and probes corresponding to positions 28541 to 28563 and 28565 to 28589 labeled with different compounds, in particular different fluorescent agents.

The real time RT-PCR which uses this pair of primers and this probe is very sensitive since it makes it possible to detect 10^2 copies of RNA and up to 10 copies, of RNA; it is in addition reliable and reproducible.

The invention encompasses the single-stranded, double-stranded and triple-stranded polydeoxyribonucleotides and polyribonucleotides corresponding to the sequence of the genome of the isolated strain of coronavirus and its fragments as defined above, and to their sense or antisense complementary sequences, in particular the RNAs and cDNAs corresponding to the sequence of the genome and of its fragments as defined above.

The present invention also encompasses the amplification fragments obtained with the aid of primers specific for the genome of the purified or isolated strain as defined above, in particular with the aid of primers or pairs of primers as defined above, the restriction fragments formed by or comprising the sequence of fragments as defined above, the fragments obtained by transcription in vitro from a vector containing the sequence SEQ ID NO: 1 or a fragment as defined above, and fragments obtained by chemical synthesis. Examples of restriction fragments are deduced from the restriction map of the sequence SEQ ID NO: 1 illustrated by FIG. **13**. In accordance with the invention, said fragments are either in the form of isolated fragments, or in the form of mixtures of fragments. The invention also encompasses fragments modified, in relation to the preceding ones, by removal

or addition of nucleotides in a proportion of about 15%, relative to the length of the above fragments and/or modified in terms of the nature of the nucleotides, as long as the modified nucleotide fragments retain a capacity for hybridization with the genomic or antigenomic RNA sequences of the isolate as defined above.

The nucleic acid molecules according to the invention are obtained by conventional methods, known per se, following standard protocols such as those described in *Current Protocols in Molecular Biology* (Frederick M. AUSUBEL, 2000, Wiley and son Inc., Library of Congress, USA). For example, they may be obtained by amplification of a nucleic sequence by PCR or RT-PCR or alternatively by total or partial chemical synthesis.

The subject of the present invention is also a DNA or RNA chip or filter, characterized in that it comprises at least one polynucleotide or one of its fragments as defined above.

The DNA or RNA chips or filters according to the invention are prepared by conventional methods, known per se, such as for example chemical or electrochemical grafting of oligonucleotides on a glass or nylon support.

The subject of the present invention is also a recombinant cloning and/or expression vector, in particular a plasmid, a virus, a viral vector or a phage comprising a nucleic acid fragment as defined above. Preferably, said recombinant vector is an expression vector in which said nucleic acid fragment is placed under the control of appropriate elements for regulating transcription and translation. In addition, said vector may comprise sequences (tags) fused in phase with the 5' and/or 3' end of said insert, which are useful for the immobilization and/or detection and/or purification of the protein expressed from said vector.

These vectors are constructed and introduced into host cells by conventional recombinant DNA and genetic engineering methods which are known per se. Numerous vectors into which a nucleic acid molecule of interest may be inserted in order to introduce it and to maintain it in a host cell are known per se; the choice of an appropriate vector depends on the use envisaged for this vector (for example replication of the sequence of interest, expression of this sequence, maintenance of the sequence in extrachromosomal form or alternatively integration into the chromosomal material of the host), and on the nature of the host cell.

In accordance with the invention, said plasmid is selected in particular from the following plasmids:

- the plasmid, called SARS-S, contained in the bacterial strain deposited under the No. I-3059, on Jun. 20, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence encoding the S protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, said sequence corresponding to the nucleotides at positions 21406 to 25348 (SEQ ID NO: 4), with reference to the Genbank sequence AY274119.3,
- the plasmid, called SARS-S1, contained in the bacterial strain deposited under the No. I-3020, on May 12, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains a 5' fragment of the cDNA sequence encoding the S protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said fragment corresponding to the nucleotides at positions 21406 to 23454 (SEQ ID NO: 5), with reference to the Genbank sequence AY274119.3 Tor2,
- the plasmid, called SARS-S2, contained in the bacterial strain deposited under the No. I-3019, on May 12, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains a 3' fragment of the cDNA sequence encoding the S protein of the SARS-CoV strain derived from the sample recorded under the number No. 031589, as defined above, said fragment corresponding to the nucleotides at positions 23322 to 25348 (SEQ ID NO: 6), with reference to the Genbank sequence accession No. AY274119.3,
- the plasmid, called SARS-SE, contained in the bacterial strain deposited under the No. I-3126, on Nov. 13, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA corresponding to the region situated between ORF-S and ORF-E and overlapping ORF-E of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said region corresponding to the nucleotides at positions 25110 to 26244 (SEQ ID NO: 8), with reference to the Genbank sequence accession No. AY274119.3,
- the plasmid, called SARS-E, contained in the bacterial strain deposited under the No. I-3046, on May 28, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence encoding the E protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 26082 to 26413 (SEQ ID NO: 15), with reference to the Genbank sequence accession No. AY274119.3,
- the plasmid, called SARS-M, contained in the bacterial strain deposited under the No. I-3047, on May 28, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence encoding the M protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above; said sequence corresponding to the nucleotides at positions 26330 to 27098 (SEQ ID NO: 18), with reference to the Genbank sequence accession No. AY274119.3,
- the plasmid, called SARS-MN, contained in the bacterial sequence deposited under the No. I-3125, on Nov. 13, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence corresponding to the region situated between ORF-M and ORF-N of the SARS-CoV strain derived from the sample recorded under the No. 031589 and collected in Hanoi, as defined above, said sequence corresponding to the nucleotides at positions 26977 to 28218 (SEQ ID NO: 20), with reference to the Genbank accession No. AY274119.3,
- the plasmid, called SARS-N, contained in the bacterial strain deposited under the No. I-3048, on Jun. 5, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA encoding the N protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 28054 to 29430 (SEQ ID NO: 38), with reference to the Genbank sequence accession No. AY274119.3; thus, this plasmid comprises an insert of sequence SEQ ID NO: 38 and is contained in a bacterial strain which was deposited under the No. I-3048, on Jun. 5, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15,

the plasmid, called SARS-5'NC, contained in the bacterial strain deposited under the No. I-3124, on Nov. 7, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA corresponding to the noncoding 5' end of the genome of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 1 to 204 (SEQ ID NO: 39), with reference to the Genbank sequence accession No. AY274119.3,

the plasmid called SARS-3'NC, contained in the bacterial strain deposited under the No. I-3123 on Nov. 7, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence corresponding to the noncoding 3' end of the genome of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to that situated between the nucleotide and position 28933 to 29727 (SEQ ID NO: 40), with reference to the Genbank sequence accession No. AY274119.3, ends with a series of nucleotides a,

the expression plasmid, called pIV2.3N, containing a cDNA fragment encoding a C-terminal fusion of the N protein (SEQ ID NO: 37) with a polyhistidine tag,

the expression plasmid, called pIV2.3S_C, containing a cDNA fragment encoding a C-terminal fusion of the fragment corresponding to positions 475 to 1193 of the amino acid sequence of the S protein (SEQ ID NO: 3) with a polyhistidine tag,

the expression plasmid, pIV2.3S_L, containing a cDNA fragment encoding a C-terminal fusion of the fragment corresponding to positions 14 to 1193 of the amino acid sequence of the S protein (SEQ ID NO: 3) with a polyhistidine tag,

the expression plasmid, called pIV2.4N, containing a cDNA fragment encoding a N-terminal fusion of the N protein (SEQ ID NO: 3) with a polyhistidine tag,

the expression plasmid, called pIV2.4S_C or pIV2.4S_1, containing an insert encoding a N-terminal fusion of the fragment corresponding to positions 475 to 1193 of the amino acid sequence of the S protein (SEQ ID NO: 3) with a polyhistidine tag, and

the expression plasmid, called pIV2.4S_L, containing a cDNA fragment encoding an N-terminal fusion of the fragment corresponding to positions 14 to 1193 of the amino acid sequence of the S protein (SEQ ID NO: 3) with a polyhistidine tag.

According to an advantageous feature of the expression plasmid as defined above, it is contained in a bacterial strain which was deposited under the No. I-3117, on Oct. 23, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15.

According to another advantageous feature of the expression plasmid as defined above, it is contained in a bacterial strain which was deposited under the No. I-3118, on Oct. 23, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15.

According to another feature of the expression plasmid as defined above, it is contained in a bacterial strain which was deposited at the CNCM, 25 rue du Docteur Roux, 75724 Paris Cedex 15 under the following numbers:

a) strain No. I-3118, deposited on Oct. 23, 2003,
b) strain No. I-3019, deposited on May 12, 2003,
c) strain No. I-3020, deposited on May 12, 2003,
d) strain No. I-3059, deposited on Jun. 20, 2003,
e) strain No. I-3323, deposited on Nov. 22, 2004,
f) strain No. I-3324, deposited on Nov. 22, 2004,
g) strain No. I-332, deposited on Dec. 1, 2004,
h) strain No. I-3327, deposited on Dec. 1, 2004,
i) strain No. I-3332, deposited on Dec. 1, 2004,
j) strain No. I-3333, deposited on Dec. 1, 2004,
k) strain No. I-3334, deposited on Dec. 1, 2004,
l) strain No. I-3335, deposited on Dec. 1, 2004,
m) strain No. I-3336, deposited on Dec. 1, 2004,
n) strain No. I-3337, deposited on Dec. 1, 2004,
o) strain No. I-3338, deposited on Dec. 2, 2004,
p) strain No. I-3339, deposited on Dec. 2, 2004,
q) strain No. I-3340, deposited on Dec. 2, 2004,
r) strain No. I-3341, deposited on Dec. 2, 2004.

The subject of the present invention is also a nucleic acid insert of viral origin, characterized in that it is contained in any of the strains as defined above in a)-r).

The subject of the present invention is also a nucleic acid containing a synthetic gene allowing optimized expression of the S protein in eukaryotic cells, characterized in that it possesses the sequence SEQ ID NO: 140.

The subject of the present invention is also an expression vector containing a nucleic acid containing a synthetic gene allowing optimized expression of the S protein, which vector is contained in the bacterial strain deposited at the CNCM, on Dec. 1, 2004, under the No. I-3333.

According to one embodiment of said expression vector, it is a viral vector, in the form of a viral particle or in the form of a recombinant genome.

According to an advantageous feature of this embodiment, this is a recombinant viral particle or a recombinant viral genome capable of being obtained by transfection of a plasmid according to paragraphs g), h) and k) to r) as defined above, in an appropriate cellular system, that is to say, for example, cells transfected with one or more other plasmids intended to transcomplement certain functions of the virus that are deleted in the vector and that are necessary for the formation of the viral particles.

The expression "S protein family" is understood here to mean the complete S protein, its ectodomaine and fragments of this ectodomaine which are preferably produced in a eukaryotic system.

The subject of the present invention is also a lentiviral vector encoding a polypeptide of the S protein family, as defined above.

The subject of the present invention is also a recombinant measles virus encoding a polypeptide of the S protein family, as defined above.

The subject of the present invention is also a recombinant vaccinia virus encoding a polypeptide of the S protein family, as defined above.

The subject of the present invention is also the use of a vector according to paragraphs e) to r) as defined above, or of a vector containing a synthetic gene for the S protein, as defined above, for the production, in a eukaryotic system, of the SARS-associated coronavirus S protein or of a fragment of this protein.

The subject of the present invention is also a method for producing the S protein in a eukaryotic system, comprising a step of transfecting eukaryotic cells in culture with a vector chosen from the vectors contained in the bacterial strains mentioned in paragraphs e) to r) above or a vector containing a synthetic gene allowing optimized expression of the S protein.

The subject of the present invention is also a cDNA library characterized in that it comprises fragments as defined above,

in particular amplification fragments or restriction fragments, cloned into a recombinant vector, in particular an expression vector (expression library).

The subject of the present invention is also cells, in particular prokaryotic cells, modified by a recombinant vector as defined above.

The subject of the present invention is also a genetically modified eukaryotic cell expressing a protein or a polypeptide as defined above. Quite obviously, the terms "genetically modified eukaryotic cell" do not denote a cell modified with a wild-type virus.

According to an advantageous embodiment of said cell, it is capable of being obtained by transfection with any of the vectors mentioned in paragraphs i) to l) above.

According to an advantageous feature of this embodiment, this is the cell FRhK4-Ssol-30, deposited at the CNCM on Nov. 22, 2004, under the No. I-3325.

The recombinant vectors as defined above and the cells transformed with said expression vectors are advantageously used for the production of the corresponding proteins and peptides. The expression libraries derived from said vectors, and the cells transformed with said expression libraries are advantageously used to identify the immunogenic epitopes (B and T epitopes) of the SARS-associated coronavirus proteins.

The subject of the present invention is also the purified or isolated proteins and peptides, characterized in that they are encoded by the polynucleotide or one of its fragments as defined above.

According to an advantageous embodiment of the invention, said protein is selected from the group consisting of:

- the S protein having the sequence SEQ ID NO: 3 or its ectodomaine
- the E protein having the sequence SEQ ID NO: 14
- the M protein having the sequence SEQ ID NO: 17
- the N protein having the sequence SEQ ID NO: 37
- the proteins encoded by the ORFs: ORF1a, ORF1b, ORF3, ORF4 and ORF7 to ORF11, ORF13 and ORF14 and having the respective sequence, SEQ ID NO: 74, 75, 10, 12, 22, 24, 26, 28, 30, 33 and 35.

The terms "ectodomaine of the S protein" and "soluble form of the S protein" will be used interchangeably below.

According to an advantageous embodiment of the invention, said polypeptide consists of the amino acids corresponding to positions 1 to 1193 of the amino acid sequence of the S protein.

According to another advantageous embodiment of the invention, said peptide is selected from the group consisting of:

a) the peptides corresponding to positions 14 to 1193 and 475 to 1193 of the amino acid sequence of the S protein,

b) the peptides corresponding to positions 2 to 14 (SEQ ID NO: 69) and 100 to 221 of the amino acid sequence of the M protein; these peptides correspond respectively to the ectodomaine and to the endodomaine of the M protein, and

c) the peptides corresponding to positions 1 to 12 (SEQ ID NO: 70) and 53 to 76 (SEQ ID NO: 71) of the amino acid sequence of the E protein; these peptides correspond respectively to the ectodomaine and to the C-terminal end of the E protein, and

d) the peptides of 5 to 50 consecutive amino acids, preferably of 10 to 30 amino acids, inclusive or partially or completely overlapping the sequence of the peptides as defined in a), b) or c).

The subject of the present invention is also a peptide, characterized in that it has a sequence of 7 to 50 amino acids including an amino acid residue selected from the group consisting of:

- the alanine situated at position 2552 of the amino acid sequence of the protein encoded by ORF1a,
- the serine situated at position 577 of the amino acid sequence of the S protein of the SARS-CoV strain as defined above,
- the glycine at position 11 of the amino acid sequence, of the protein encoded by ORF3 of the SARS-CoV strain as defined above,
- the serine at position 154 of the amino acid sequence of the M protein of the SARS-CoV strain as defined above.

The subject of the present invention is also an antibody or a polyclonal or monoclonal antibody fragment which can be obtained by immunization of an animal with a recombinant vector as defined above, a cDNA library as defined above or alternatively a protein or a peptide as defined above, characterized in that it binds to at least one of the proteins encoded by SARS-CoV as defined above.

The invention encompasses the polyclonal antibodies, the monoclonal antibodies, the chimeric antibodies such as the humanized antibodies, and fragments thereof (Fab, Fv, scFv).

A subject of the present invention is also a hybridoma producing a monoclonal antibody against the N protein, characterized in that it is chosen from the following hybridomas:

- the hybridoma producing the monoclonal antibody 87, deposited at the CNCM on Dec. 1, 2004 under the number I-3328,
- the hybridoma producing the monoclonal antibody 86, deposited at the CNCM on Dec. 1, 2004 under the number I-3329,
- the hybridoma producing the monoclonal antibody 57, deposited at the CNCM on Dec. 1, 2004 under the number I-3330, and
- the hybridoma producing the monoclonal antibody 156, deposited at the CNCM on Dec. 1, 2004 under the number I-3331.

The subject of the present invention is also a polyclonal or monoclonal antibody or antibody fragment directed against the N protein, characterized in that it is produced by a hybridoma as defined above.

For the purposes of the present invention, the expression chimeric antibody is understood to mean, in relation to an antibody of a particular animal species or of a particular class of antibody, an antibody comprising all or part of a heavy chain and/or of a light chain of an antibody of another animal species or of another class of antibody.

For the purposes of the present invention, the expression humanized antibody is understood to mean a human immunoglobulin in which the residues of the CDRs (Complementary Determining Regions) which form the antigen-binding site are replaced by those of a nonhuman monoclonal antibody possessing the desired specificity, affinity or activity. Compared with the nonhuman antibodies, the humanized antibodies are less immunogenic and possess a prolonged half-life in humans because they possess only a small proportion of nonhuman sequences given that practically all the residues of the FR (Framework) regions and of the constant (Fc) region of these antibodies are those of a consensus sequence of human immunoglobulins.

A subject of the present invention is also a protein chip or filter, characterized in that it comprises a protein, a peptide or alternatively an antibody as defined above.

The protein chips according to the invention are prepared by conventional methods known per se. Among the appropri-

ate supports on which proteins may be immobilized, there may be mentioned those made of plastic or glass, in particular in the form of microplates.

The subject of the present invention is also reagents derived from the isolated strain of SARS-associated coronavirus, derived from the sample recorded under the No. 031589, which are useful for the study and diagnosis of the infection caused by a SANS-associated coronavirus, said reagents are selected from the group consisting of:

(a) a pair of primers, a probe or a DNA chip as defined above,

(b) a recombinant vector or a modified cell as defined above,

(c) an isolated coronavirus strain or a polynucleotide as defined above,

(d) a protein or a peptide as defined above,

(e) an antibody or an antibody fragment as defined above, and

(f) a protein chip as defined above.

These various reagents are prepared and used according to conventional molecular biology and immunology techniques following standard protocols such as those described in *Current Protocols in Molecular Biology* (Frederick M. AUSUBEL, 2000, Wiley and Son Inc., Library of Congress, USA), in *Current Protocols in Immunology* (John E. Cologan, 2000, Wiley and Son Inc., Library of Congress, USA) and in *Antibodies: A Laboratory Manual* (E. Howell and D. Lane, Cold Spring Harbor Laboratory, 1988).

The nucleic acid fragments according to the invention are prepared and used according to conventional techniques as defined above. The peptides and proteins according to the invention are prepared by recombinant DNA techniques, known to persons skilled in the art, in particular with the aid of the recombinant vectors as defined above. Alternatively, the peptides according to the invention may be prepared by conventional techniques of solid or liquid phase synthesis, known to persons skilled in the art.

The polyclonal antibodies are prepared by immunizing an appropriate animal with a protein or a peptide as defined above, optionally coupled to KLH or to albumin and/or combined with an appropriate adjuvant such as (complete or incomplete) Freund's adjuvant or aluminum hydroxide; after obtaining a satisfactory antibody titer, the antibodies are harvested by collecting serum from the immunized animals and enriched with IgG by precipitation, according to conventional techniques, and then the IgGs specific for the SARS-CoV proteins are optionally purified by affinity chromatography on an appropriate column to which said peptide or said protein is attached, as defined above, so as to obtain a monospecific IgG preparation.

The monoclonal antibodies are produced from hybridomas obtained by fusion of B lymphocytes from an animal immunized with a protein or a peptide as defined above with myelomas, according to the Köhler and Milstein technique (Nature, 1975, 256, 495-497); the hybridomas are cultured in vitro, in particular in fermenters or produced in vivo, in the form of as cites; alternatively, said monoclonal antibodies are produced by genetic engineering as described in American patent U.S. Pat. No. 4,816,567.

The humanized antibodies are produced by general methods such as those described in International application WO 98/45332.

The antibody fragments are produced from the cloned V_H and V_L regions, from the mRNAs of hybridomas or splenic lymphocytes of an immunized mouse; for example, the Fv, scFv or Fab fragments are expressed at the surface of filamentous phages according to the Winter and Milstein technique (Nature, 1991, 349, 293-299); after several selection steps, the antibody fragments specific for the antigen are isolated and expressed in an appropriate expression system, by conventional techniques for cloning and expression of recombinant DNA.

The antibodies or fragments thereof as defined above are purified by conventional techniques known to persons skilled in the art, such as affinity chromatography.

The subject of the present invention is additionally the use of a product selected from the group consisting of: a pair of primers, a probe, a DNA chip, a recombinant vector, a modified cell, an isolated coronavirus strain, a polynucleotide, a protein or a peptide, an antibody or an antibody fragment and a protein chip as defined above, for the preparation of a reagent for the detection and optionally genotyping/serotyping of a SARS-associated coronavirus.

The proteins and peptides according to the invention, which are capable of being recognized and/or of inducing the production of antibodies specific for the SARS-associated coronavirus, are useful for the diagnosis of infection with such a coronavirus; the infection is detected, by an appropriate technique—in particular EIA, ELISA, RIA, immunofluorescence—, in a biological sample collected from an individual capable of being infected.

According to an advantageous feature of said use, said proteins are selected from the group consisting of the S, E, M and/or N proteins and the peptides as defined above.

The S, E, M and/or N proteins and the peptides derived from these proteins as defined above, for example the N protein, are used for the indirect diagnosis of a SARS-associated coronavirus infection (serological diagnosis; detection of an antibody specific for SARS-CoV), in particular by an immunoenzymatic method (ELISA).

The antibodies and antibody fragments according to the invention, in particular those directed against the S, E, M and/or N proteins and the derived peptides as defined above, are useful for the direct diagnosis of a SARS-associated coronavirus infection; the detection of the protein(s) of SARS-CoV is carried out by an appropriate technique, in particular EIA, ELISA, RIA, immunofluorescence, in a biological sample collected from an individual capable of being infected.

The subject of the present invention is also a method for the detection of a SARS-associated coronavirus, from a biological sample, which method is characterized in that it comprises at least:

(a) bringing said biological sample into contact with at least one antibody or one antibody fragment, one protein, one peptide or alternatively one protein or peptide chip or filter as defined above, and

(b) visualizing by any appropriate means antigen-antibody complexes formed in (a), for example by EIA, ELISA, RIA, or by immunofluorescence.

According to one advantageous embodiment of said process, step (a) comprises:

(a_1) bringing said biological sample into contact with at least a first antibody or an antibody fragment which is attached to an appropriate support, in particular a microplate,

(a_2) washing the solid phase, and

(a_3) adding at least a second antibody or an antibody fragment, different from the first, said antibody or antibody fragment being optionally appropriately labeled.

This method, which makes it possible to capture the viral particles present in the biological sample, is also called immunocapture method.

For example:

- step (a_1) is carried out with at least a first monoclonal or polyclonal antibody or a fragment thereof, directed against the S, M and/or E protein, and/or a peptide corresponding to the ectodomaine of one of these proteins (M2-14 or E1-12 peptides)
- step (a_3) is carried out with at least one antibody or an antibody fragment directed against another epitope of the same protein or preferably against another protein, preferably against an inner protein such as the N nucleoprotein or the endodomaine of the E or M protein, more preferably still these are antibodies or antibody fragments directed against the N protein which is very abundant in the viral particle; when an antibody or an antibody fragment directed against an inner protein (N) or against the endodomaine of the E or M proteins is used, said antibody is incubated in the presence of detergent, such as Tween 20 for example, at concentrations of the order of 0.1%.
- step (b) for visualizing the antigen-antibody complexes formed is carried out, either directly with the aid of a second antibody labeled for example with biotin or an appropriate enzyme such as peroxidase or alkaline phosphatase, or indirectly with the aid of an anti-immunoglobulin serum labeled as above. The complexes thus formed are visualized with the aid of an appropriate substrate.

According to a preferred embodiment of this aspect of the invention, the biological sample is mixed with the visualizing monoclonal antibody prior to its being brought into contact with the capture monoclonal antibodies. Where appropriate, the serum-visualizing antibody mixture is incubated for at least 10 minutes at room temperature before being applied to the plate.

The subject of the present invention is also an immunocapture test intended to detect an infection by the SARS-associated coronavirus by detecting the native nucleoprotein (N protein), in particular characterized in that the antibody used for the capture of the native viral nucleoprotein is a monoclonal antibody specific for the central region and/or for a conformational epitope.

According to one embodiment of said test, the antibody used for the capture of the N protein is the monoclonal antibody mAb87, produced by the hybridoma deposited at the CNCM on Dec. 1, 2004 under the number I-3328.

According to another embodiment of said immunocapture test, the antibody used for the capture of the N protein is the monoclonal antibody mAb86, produced by the hybridoma deposited at the CNCM on Dec. 1, 2004 under the number I-3329.

According to another embodiment of said immunocapture test, the monoclonal antibodies mAb86 and mAb87 are used for the capture of the N protein.

In the immunocapture tests according to the invention, it is possible to use, for visualizing the N protein, the monoclonal antibody mAb57, produced by the hybridoma deposited at the CNCM on Dec. 1, 2004 under the number I-3330, said antibody being conjugated with a visualizing molecule or particle.

In accordance with said immunocapture test, a combination of the antibodies mAb57 and mAb87, conjugated with a visualizing molecule or particle, is used for the visualization of the N protein.

A visualizing molecule may be a radioactive atom, a dye, a fluorescent molecule, a fluorophore, an enzyme; a visualizing particle may be for example: colloidal gold, a magnetic particle or a latex bead.

The subject of the present invention is also a reagent for detecting a SARS-associated coronavirus, characterized in that it is selected from the group consisting of:

(a) a pair of primers or a probe as defined above,
(b) a recombinant vector as defined above or a modified cell as defined above,
(c) an isolated coronavirus strain as defined above or a polynucleotide as defined above,
(d) an antibody or an antibody fragment as defined above,
(e) a combination of antibodies comprising the monoclonal antibodies mAb86 and/or mAb87, and the monoclonal antibody mAb57, as defined above,
(f) a chip or a filter as defined above.

The subject of the present invention is also a method for the detection of a SARS-associated coronavirus infection, from a biological sample, by indirect IgG ELISA using the N protein, which method is characterized in that the plates are sensitized with an N protein solution at a concentration of between 0.5 and 4 μg/ml, preferably to 2 μg/ml, in a 10 mM PBS buffer pH 7.2, phenol red at 0.25 ml/l.

The subject of the present invention is additionally a method for the detection of a SARS-associated coronavirus infection, from a biological sample, by double epitope ELSA, characterized in that the serum to be tested is mixed with the visualizing antigen, said mixture then being brought into contact with the antigen attached to a solid support.

According to one variant of the tests for detecting SARS-associated coronaviruses, these tests combine an ELSA using the N protein, and another ELSA using the S protein, as described below.

The subject of the present invention is also an immune complex formed of a polyclonal or monoclonal antibody or antibody fragment as defined above, and of a SARS-associated coronavirus protein or peptide.

The subject of the present invention is additionally a SARS-associated coronavirus detection kit, characterized in that it comprises at least one reagent selected from the group consisting of: a pair of primers, a probe, a DNA or RNA chip, a recombinant vector, a modified cell, an isolated coronavirus strain, a polynucleotide, a protein or a peptide, an antibody, and a protein chip as defined above.

The subject of the present invention is additionally an immunogenic composition, characterized in that it comprises at least one product selected from the group consisting of:

a) a protein or a peptide as defined above,
b) a polynucleotide of the DNA or RNA type or one of its representative fragments as defined above, having a sequence chosen from:
(i) the sequence SEQ ID NO: 1 or its RNA equivalent
(ii) the sequence hybridizing under high stringency conditions with the sequence SEQ ID NO: 1,
(iii) the sequence complementary to the sequence SEQ ID NO: 1 or to the sequence hybridizing under high stringency conditions with the sequence SEQ ID NO: 1,
(iv) the nucleotide sequence of a representative fragment of the polynucleotide as defined in (i), (ii) or (iii),
(v) the sequence as defined in (i), (ii), (iii) or (iv), modified, and
c) a recombinant expression vector comprising a polynucleotide as defined in b), and
d) a cDNA library as defined above,

said immunogenic composition being capable of inducing protective humoral or cellular immunity specific for the

SARS-associated coronavirus, in particular the production of an antibody directed against a specific epitope of the SARS-associated coronavirus.

The proteins and peptides as defined above, in particular the S, M, E and/or N proteins and the derived peptides, and the nucleic acid (DNA or RNA) molecules encoding said proteins or said peptides are good candidate vaccines and may be used in immunogenic compositions for the production of a vaccine against the SARS-associated coronavirus.

According to an advantageous embodiment of the compositions according to the invention, they additionally contain at least one pharmaceutically acceptable vehicle and optionally carrier substances and/or adjuvants.

The pharmaceutically acceptable vehicles, the carrier substances and the adjuvants are those conventionally used.

The adjuvants are advantageously chosen from the group consisting of oily emulsions, saponin, mineral substances, bacterial extracts, aluminum hydroxide and squalene.

The carrier substances are advantageously selected from the group consisting of unilamellar liposomes, multilamellar liposomes, micelles of saponin or solid microspheres of a saccharide or auriferous nature.

The compositions according to the invention are administered by the general route, in particular by the intramuscular or subcutaneous route or alternatively by the local, in particular nasal (aerosol) route.

The subject of the present invention is also the use of an isolated or purified protein or peptide having a sequence selected from the group consisting of the sequences SEQ ID NO: 3, 10, 12, 14, 17, 22, 24, 26, 28, 30, 33, 35, 37, 69, 70, 71, 74 and 75 to form an immune complex with an antibody specifically directed against an epitope of the SARS-associated coronavirus.

The subject of the present invention is also an immune complex consisting of an isolated or purified protein or peptide having a sequence selected from the group consisting of the sequences SEQ ID NO: 3, 10, 12, 14, 17, 22, 24, 26, 28, 30, 33, 35, 37, 69, 70, 71, 74 and 75, and of an, antibody specifically directed against an epitope of the SARS-associated coronavirus.

The subject of the present invention is also the use of an isolated or purified protein or peptide having a sequence selected from the group-consisting of the sequences SEQ ID NO: 3, 10, 12, 14, 17, 22, 24, 26, 28, 30, 33, 35, 37, 69, 70, 71, 74 and 75 to induce the production of an antibody capable of specifically recognizing an epitope of the SARS-associated coronavirus.

The subject of the present invention is also the use of an isolated or purified polynucleotide having a sequence selected from the group consisting of the sequences SEQ ID NO: 1, 2, 4, 7, 8, 13, 15, 16, 18, 19, 20, 31, 36 and 38 to induce the production of an antibody directed against the protein encoded by said polynucleotide and capable of specifically recognizing an epitope of the SARS-associated coronavirus.

The subject of the present invention is also monoclonal antibodies recognizing the native S protein of a SARS-associated coronavirus.

The subject of the present invention is also the use of a protein or a polypeptide of the S protein family, as defined above, or of an antibody recognizing the native S protein, as defined above, to detect an infection by a SARS-associated coronavirus, in a biological sample.

The subject of the present invention is also a method for detecting an infection by a SARS-associated coronavirus, in a biological sample, characterized in that the detection is carried out by ELISA using the recombinant S protein, expressed in a eukaryotic system.

According to an advantageous embodiment of said method, it is a double epitope ELISA method, and the serum to be tested is mixed with the visualizing antigen, said mixture then being brought into contact with the antigen attached to a solid support.

The subject of the present invention is also an immune complex consisting of a monoclonal antibody or antibody fragment recognizing the native S protein, and of a protein or a peptide of the SARS-associated coronavirus.

The subject of the present invention is also an immune complex consisting of a protein or a polypeptide of the S protein family, as defined above, and of an antibody specifically directed against an epitope of the SARS-associated coronavirus.

The subject of the present invention is additionally a SARS-associated coronavirus detection kit or box, characterized in that it comprises at least one reagent selected from the group consisting of: a protein or polypeptide of the S protein family, as defined above, a nucleic acid encoding a protein or peptide of the S protein family, as defined above, a cell expressing a protein or polypeptide of the S protein family, as defined above, or an antibody recognizing the native S protein of a SARS-associated coronavirus.

The subject of the present invention is an immunogenic and/or vaccine composition, characterized in that it comprises a polypeptide or a recombinant protein of the S protein family, as defined above, obtained in a eukaryotic expression system.

The subject of the present invention is also an immunogenic and/or vaccine composition, characterized in that it comprises a vector or recombinant virus, expressing a protein or a polypeptide of the S protein family, as defined above.

In addition to the preceding features, the invention further comprises other features, which will emerge from the description which follows, which refers to examples of use of the polynucleotide representing the genome of the SARS-CoV strain derived from the sample recorded under the number 031589, and derived cDNA fragments which are the subject of the present invention, and to Table I presenting the sequence listing:

TABLE I

Sequence listing

Identification number	Sequence	Position of the cDNA with reference to Genbank AY274119.3	Deposit number at the CNCM of the corresponding plasmid
SEQ ID NO: 1	genome of the strain derived from the sample 031589	—	—
SEQ ID NO: 2	ORF-S*	21406-25348	—
SEQ ID NO: 3	S protein	—	—
SEQ ID NO: 4	ORF-S**	21406-25348	I-3059
SEQ ID NO: 5	Sa fragment	21406-23454	I-3020
SEQ ID NO: 6	Sb fragment	23322-25348	I-3019
SEQ ID NO: 7	ORF-3 + ORF-4*	25110-26244	—
SEQ ID NO: 8	ORF-3 + ORF-4**	25110-26244	I-3126
SEQ ID NO: 9	ORF3	—	—
SEQ ID NO: 10	ORF-3 protein	—	—
SEQ ID NO: 11	ORF4	—	—
SEQ ID NO: 12	ORF-4 protein	—	—
SEQ ID NO: 13	ORF-E*	26082-26413	—
SEQ ID NO: 14	E protein	—	—
SEQ ID NO: 15	ORF-E**	26082-26413	I-3046
SEQ ID NO: 16	ORF-M*	26330-27098	—
SEQ ID NO: 17	M protein	—	—

TABLE I-continued

Sequence listing			
Identification number	Sequence	Position of the cDNA with reference to Genbank AY274119.3	Deposit number at the CNCM of the corresponding plasmid
SEQ ID NO: 18	ORF-M**	26330-27098	I-3047
SEQ ID NO: 19	ORF7 to 11*	26977-28218	—
SEQ ID NO: 20	ORF7 to 11**	26977-28218	I-3125
SEQ ID NO: 21	ORF7	—	—
SEQ ID NO: 22	ORF7 protein	—	—
SEQ ID NO: 23	ORF8	—	—
SEQ ID NO: 24	ORF8 protein	—	—
SEQ ID NO: 25	ORF9	—	—
SEQ ID NO: 26	ORF9 protein	—	—
SEQ ID NO: 27	ORF10	—	—
SEQ ID NO: 28	ORF10 protein	—	—
SEQ ID NO: 29	ORF11	—	—
SEQ ID NO: 30	ORF11 protein	—	—
SEQ ID NO: 31	OrF1ab	265-21485	—
SEQ ID NO: 32	ORF13	28130-28426	—
SEQ ID NO: 33	ORF13 protein	—	—
SEQ ID NO: 34	ORF14	—	—
SEQ ID NO: 35	ORF14 protein	28583-28795	—
SEQ ID NO: 36	ORF-N*	28054-29430	
SEQ ID NO: 37	N protein	—	—
SEQ ID NO: 38	ORF-N**	28054-29430	I-3048
SEQ ID NO: 39	noncoding 5'**	1-204	I-3124
SEQ ID NO: 40	noncoding 3'**	28933-29727	I-3123
SEQ ID NO: 41	ORF1ab Fragment L0	30-500	—
SEQ ID NO: 42	Fragment L1	211-2260	—
SEQ ID NO: 43	Fragment L2	2136-4187	—
SEQ ID NO: 44	Fragment L3	3892-5344	—
SEQ ID NO: 45	Fragment L4b	4932-6043	—
SEQ ID NO: 46	Fragment L4	5305-7318	—
SEQ ID NO: 47	Fragment L5	7275-9176	—
SEQ ID NO: 48	Fragment L6	9032-11086	—
SEQ ID NO: 49	Fragment L7	10298-12982	—
SEQ ID NO: 50	Fragment L8	12815-14854	—
SEQ ID NO: 51	Fragment L9	14745-16646	—
SEQ ID NO: 52	Fragment L10	16514-18590	—
SEQ ID NO: 53	Fragment L11	18500-20602	—
SEQ ID NO: 54	Fragment L12	20319-22224	—
SEQ ID NO: 55	Sense N primer	—	—
SEQ ID NO: 56	Antisense N primer	—	—
SEQ ID NO: 57	Sense S_C primer	—	—
SEQ ID NO: 58	Sense S_L primer	—	—
SEQ ID NO: 59	Antisense S_C and S_L primer	—	—
SEQ ID NO: 60	Sense primer series 1	28507-28522	—
SEQ ID NO: 61	Antisense primer series 1	28774-28759	
SEQ ID NO: 62	Sense primer series 2	28375-28390	—
SEQ ID NO: 63	Antisense primer series 2	28702-28687	—
SEQ ID NO: 64	Probe 1/series 1	28561-28586	—
SEQ ID NO: 65	Probe 2/series 1	28588-28608	—
SEQ ID NO: 66	Probe 1/series 2	28541-28563	—
SEQ ID NO: 67	Probe 2/series 2	28565-28589	—
SEQ ID NO: 68	Anchor primer 14T		
SEQ ID NO: 69	Peptide M2-14	—	—
SEQ ID NO: 70	Peptide E1-12	—	—
SEQ ID NO: 71	Peptide E53-76	—	—
SEQ ID NO: 72	Noncoding 5'*	1-204	—
SEQ ID NO: 73	Noncoding 3'*	28933-29727	—
SEQ ID NO: 74	ORF1a protein	—	—
SEQ ID NO: 75	ORF1b protein	—	—
SEQ ID NO: 76-139	Primers		
SEQ ID NO: 140	Pseudogene of S		
SEQ ID NO: 141-148	Primers		
SEQ ID NO: 149	Aa1-13 of S		
SEQ ID NO: 150	Polypeptide		
SEQ ID NO: 151-158	Primers		

*PCR amplification product (amplicon)

**Insert cloned into the plasmid deposited at the CNCM and to the appended drawings in which:

FIG. 1 illustrates Western-blot analysis of the expression in vitro of the recombinant proteins N, S_C and S_L from the expression vectors pIVEX. Lane 1: pIV2.3N. Lane 2: pIV2.3S_C. Lane 3: pIV2.3S_L. Lane 4: pIV2.4N. Lane 5: pIV2.4S_1 or pIV2.4S_C. Lane 6: pIV2.4S_L. The expression of the GFP protein expressed from the same vector is used as a control.

FIG. 2 illustrates the analysis, by polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE) and staining with Coomassie blue, of the expression in vivo of the N protein from the expression vectors pIVEX. The *E. coli* BL21(DE3)pDIA17 strain transformed with the recombinant vectors pIVEX is cultured at 30° C. in LB medium, in the presence or in the absence of inducer (IPTG 1 mM). Lane 1: pIV2.3N. Lane 2: pIV2.4N.

FIG. 3 illustrates the analysis, by polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE) and staining with Coomassie blue, of the expression in vivo of the S_L and S_C polypeptides from the expression vectors pIVEX. The *E. coli* BL21(DE3)pDIA17 strain transformed with the recombinant vectors pIVEX is cultured at 30° C. in LB medium, in the presence or in the absence of inducer (IPTG 1 mM). Lane 1: pIV2.3S_C. Lane 2: pIV2.3S_L. Lane 3: pIV2.4S_1. Lane 4: pIV2.4S_L.

FIG. 4 illustrates the antigenic activity of the recombinant N, S_L and S_C proteins produced in the *E. coli* BL21(DE3)pDIA17 strain transformed with the recombinant vectors pIVEX. A: electrophoresis (SDS-PAGE) of the bacterial lysates. B and C: Western-blot with the sera, obtained from the same patient infected with SARS-CoV, collected 8 days (B: serum M12) and 29 days (C: serum M13) respectively after the onset of the SARS symptoms. Lane 1: pIV2.3N. Lane 2: pIV2.4N. Lane 3: pIV2.3S_C. Lane 4: pIV2.4S_1. Lane 5: pIV2.3S_L. Lane 6: pIV2.4S_L.

FIG. 5 illustrates the purification on an Ni-NTA agarose column of the recombinant N protein produced in the *E. coli* BL21(DE3)pDIA17 strain from the vector pIV2.3N. Lane 1: total bacterial extract. Lane 2: soluble extract. Lane 3: insoluble extract. Lane 4: extract deposited on the Ni-NTA column. Lane 5: unbound proteins. Lane 6: fractions of peak 1. Lane 7: fractions of peak 2.

FIG. 6 illustrates the purification of the recombinant S_C protein from the inclusion bodies produced in the *E. coli* BL21(DE3)pDIA17 strain transformed with pIV2.4S_1. A. Treatment with Triton X-100 (2%): Lane 1: total bacterial extract. Lane 2: soluble extract. Lane 3: insoluble extract. Lane 4: supernatant after treatment with Triton X-100 (2%). Lanes 5 and 6: pellet after treatment with Triton X-100 (2%). B: Treatment with 4 M, 5 M, 6 M and 7 M urea of the soluble and insoluble extracts.

FIG. 7 represents the immunoblot produced with the aid of a lysate of cells infected with SARS-CoV and a serum from a patient suffering from a typical pneumopathy.

FIG. 8 represents immunoblots produced with the aid of a lysate of cells infected with SARS-CoV and rabbit immunosera specific for the nucleoprotein N (A) and for the spicule protein S (B). I.S.: immune serum. p.i.: preimmune serum. The anti-N immune serum was used at 1/50 000 and the anti-S immune serum at 1/10 000.

FIG. 9 illustrates the ELISA reactivity of the rabbit monospecific polyclonal sera directed against the N protein or the short fragment of the S protein (S_C), toward the corresponding recombinant proteins used for immunization. A: rabbits P13097, P13081 and P13031 immunized with the purified recombinant N protein. B: rabbits P11135, P13042 and P14001 immunized with a preparation of inclusion bodies corresponding to the short fragment of the S protein (S_C). I.S.: immune serum. p.i.: preimmune serum.

FIG. 10 illustrates the ELISA reactivity of the purified recombinant N protein, toward sera from patients suffering from a typical pneumonia caused by SARS-CoV. FIG. 10a: ELISA plates prepared with the N protein at the concentration of 4 μg/ml and 2 μg/ml. FIG. 10B: ELISA plate prepared with the N protein at the concentration of 1 μg/ml. The sera designated A, B, D, E, F, G, H correspond to those of Table IV.

FIG. 11 illustrates the amplification by RT-PCR of decreasing quantities of synthetic RNA of the SARS-CoV N gene (10^7 to 1 copy), with the aid of pairs of primers No. 1 (N/+/28507, N/−/28774) (A) and No. 2 (N/+/28375, N/−/28702) (B). T: amplification performed in the absence of RNA. MW: DNA marker.

FIG. 12 illustrates the amplification by RT-PCR in real time of synthetic RNA for the SARS-CoV N gene: decreasing quantities of synthetic RNA as replica (repli.; lanes 16 to 29) and of viral RNA diluted $1/20 \times 10^{-4}$ (lane 32) were amplified by RT-PCR in real time with the aid of the kit "Light Cycler RNA Amplification Kit Hybridization Probes" and pairs of primers and probes of the No. 2 series, under the conditions described in Example 8.

FIG. 13 (FIG. 13.1 to 13.7) represents the restriction map of the sequence SEQ ID NO: 1 corresponding to the DNA equivalent of the genome of the SARS-CoV strain derived from the sample recorded under the number 031589.

FIG. 14 shows the result of the SARS serology test by indirect N ELISA (1^{st} series of sera tested).

FIG. 15 shows the result of the SARS serology test by indirect N ELISA (2^{nd} series of sera tested).

FIG. 16 presents the result of the SARS serology test by double epitope N ELISA (1^{st} series of sera tested).

FIG. 17 shows the result of the SARS serology test by double epitope N ELISA (2^{nd} series of sera tested).

FIG. 18 illustrates the test of reactivity of the anti-N monoclonal antibodies by ELISA on the native nucleoprotein N of SARS-CoV. The antibodies were tested in the form of hybridoma culture supernatants by indirect ELISA using an irradiated lysate of VeroE6 cells infected with SARS-CoV as antigen (SARS lysate curves). A negative control for reactivity is performed for each antibody on a lysate of uninfected VeroE6 cells (negative lysate curves). Several monoclonal antibodies of known specificity were used as negative control antibodies: para1-3 directed against the antigens of the parainfluenza viruses type 1-3 (Bio-Rad) and influenza B directed against the antigens of the influenza virus type B (Bio-Rad).

TABLE I-continued

Sequence listing

Identification number	Sequence	Position of the cDNA with reference to Genbank AY274119.3	Deposit number at the CNCM of the corresponding plasmid

FIG. 19 illustrates the test of reactivity of the anti-N of SARS-CoV monoclonal antibodies by ELISA on the native antigens of the human coronavirus 229E (HCoV-229E). The antibodies were tested in the form of hybridoma culture supernatants by an indirect ELISA test using a lysate of MRC-5 cells infected with the human coronavirus 229E as antigen (229E lysate curves). A negative control for immunoreactivity was performed for each antibody on a lysate of noninfected MRC-5 cells (negative lysate curves). The monoclonal antibody 5-11H.6 directed against the S protein of the human coronavirus 229E (Sizun et al. 1998, J. Virol. Met. 72: 145-152) is used as positive control antibody. The antibodies para1-3 directed against the antigens of the parainfluenza virus type 1-3 (Bio-Rad) and influenza B directed against the antigens of the influenza virus type B (Bio-Rad) were added to the panel of monoclonal antibodies tested.

FIG. 20 shows a test of reactivity of the anti-N of SARS-CoV monoclonal antibodies by Western blotting on the denatured native nucleoprotein N of SARS-CoV. A lysate of VeroE6 cells infected with SARS-CoV was prepared in the loading buffer according to Laemmli and caused to migrate in a 12% SDS polyacrylamide gel and then the proteins were transferred onto PVDF membrane. The anti-N monoclonal antibodies tested were used for the immunoassay at the concentration of 0.05 μg/ml. The visualization is carried out with anti-mouse IgG(H + L) antibodies coupled to peroxidase (NA93IV, Amersham) and the ECL+ system. Two monoclonal antibodies were used as negative controls for reactivity: influenza B directed against the antigens of the influenza virus type B (Bio-Rad) and para1-3 directed against the antigens of the parainfluenza virus type 1-3 (Bio-Rad).

FIG. 21 presents the plasmids for expression in mammalian cells of the SARS-CoV S protein. The cDNA for the SARS-CoV S was inserted between the BamH1 and Xho1 sites of the expression plasmid pcDNA3.1(+) (Clontech) in order to obtain the plasmid pcDNA-S and between the Nhe1 and Xho1 sites of the expression plasmid pCI (Promega) in order to obtain the plasmid pCI-S. The WPRE and CTE sequences were inserted between each of the two plasmids pcDNA-S and pCI-S between the Xho1 and Xba1 sites in order to obtain the plasmids pcDNA-S-CTE, pcDNA-S-WPRE, pCI-S-CTE and pCI-S-WPRE, respectively.

SP: signal peptide predicted (aa 1-13) with the software signalP v2.0 (Nielsen et al., 1997, Protein Engineering, 10: 1-6)

TM: transmembrane region predicted (aa 1196-1218) with the software TMHMM v2.0 (Sonnhammer et al., 1998, Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, pp. 175-182, AAAI Press). It should be noted that the amino acids W1194 and P1195 are possibly part of the transmembrane region with the respective probabilities of 0.13 and 0.42

P-CMV: cytomegalovirus immediate/early promoter.

BGH pA: polyadenylation signal of the bovine growth hormone gene

SV40 late pA: SV40 virus late polyadenylation signal

SD/SA: splice donor and acceptor sites

WPRE: sequences of the "Woodchuck Hepatitis Virus posttranscriptional regulatory element" of the woodchuck hepatitis virus

CTE: sequences of the "constitutive transport element" of the Mason-Pfizer simian retrovirus

FIG. 22 illustrates the expression of the S protein after transfection of VeroE6 cells. Cellular extracts were prepared 48 hours after transfection of VeroE6 cells with the plasmids pcDNA, pcDNA-S, pCI and pCI-S. Cellular extracts were also prepared 18 hours after infection with the recombinant vaccinia virus VV-TF7.3 and transfection with the plasmids pcDNA or pcDNA-S. As a control, extracts of VeroE6 cells were prepared 8 hours after infection with SARS-CoV at a multiplicity of infection of 3. They were separated on an 8% SDS acrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H + L) polyclonal antibody coupled to peroxidase (NA934V, Amersham). A molecular mass ladder (kDa) is presented in the figure.

SARS-CoV: extract of VeroE6 cells infected with SARS-CoV

Mock: control extract of noninfected cells

FIG. 23 illustrates the effect of the CTE and WPRE sequences on the expression of the S protein after transfection of VeroE6 and 293T cells. Cellular extracts were prepared 48 hours after transfection of VeroE6 cells (A) or 293T cells (B) with the plasmids pcDNA, pcDNA-S, pcDNA-S-CTE, pcDNA-S-WPRE, pCI-S, pCI-S-CTE and pCI-S-WPRE separated on 8% SDS polyacrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H + L) polyclonal antibody coupled to peroxidase (NA934V, Amersham). A molecular mass ladder (kDa) is presented in the figure.

SARS-CoV: extract of VeroE6 cells prepared 8 hours after infection with SARS-CoV at a multiplicity of infection of 3.

Mock: control extract of noninfected VeroE6 cells

FIG. 24 presents defective lentiviral vectors with central DNA flap for the expression of SARS-CoV S. The cDNA for the SARS-CoV S protein was cloned in the form of a BamH1-Xho1 fragment into the plasmid pTRIPΔU3-CMV containing a defective lentiviral vector TRIP with central DNA flap (Sirven et al., 2001, Mol. Ther., 3: 438-448) in order to obtain the plasmid pTRIP-S. The optimum expression cassettes consisting of the CMV virus immediate/early promoter, a splice signal, cDNA for S and either of the posttranscriptional signals CTE or WPRE were substituted for the cassette EF1α-EGFP of the defective lentiviral expression vector with central DNA flap TRIPΔU3-EF1α (Sirven et al., 2001, Mol. Ther., 3: 438-448) in order to obtain the plasmids pTRIP-SD/SA-S-CTE and pTRIP-SD/SA-S-WPRE.

SP: signal peptide

TM: transmembrane region

P-CMV: cytomegalovirus immediate/early promoter

P-EF1α: EF1α gene promoter

SD/SA: splice donor and acceptor sites

WPRE: sequences of the "Woodchuck Hepatitis Virus posttranscriptional regulatory element" of the woodchuck hepatitis virus

CTE: sequences of the "constitutive transport element" of the Mason-Pfizer simian retrovirus

LTR: long terminal repeat ΔU3: LTR deleted for the "promoter/enhancer" sequences

cPPT: "polypurine tract cis-active sequence"

CTS: "central termination sequence"

TABLE I-continued

Sequence listing

Identification number	Sequence	Position of the cDNA with reference to Genbank AY274119.3	Deposit number at the CNCM of the corresponding plasmid

FIG. 25 shows the Western-blot analysis of the expression of the SARS-CoV S by cell lines transduced with the lentiviral vectors TRIP-SD/SA-S-WPRE and TRIP-SD/SA-S-CTE. Cellular extracts were prepared from established lines FrhK4-S-CTE and FrhK4-S-WPRE after transduction with the lentiviral vectors TRIP-SD/SA-S-CTE and TRIP-SD/SA-S-WPRE respectively. They were separated on an 8% SDS acrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H + L) conjugate coupled to peroxidase. A molecular mass ladder (kDa) is presented in the figure.

T−: control extract of FrhK-4 cells

T+: extract of FrhK-4 cells prepared 24 hours after infection with SARS-CoV at a multiplicity of infection of 3.

FIG. 26 relates to the analysis of the expression of SsoI polypeptide by cell lines transduced with the lentiviral vectors TRIP-SD/SA-SsoI-WPRE and TRIP-SD/SA-SsoI-CTE. The secretion of the SsoI polypeptide was determined in the supernatant of a series of cell clones isolated after transduction of FrhK-4 cells with the lentiviral vectors TRIP-SD/SA-SsoI-WPRE and TRIP-SD/SA-SsoI-CTE. 5 μl of supernatant, diluted 1/2 in loading buffer according to Laemmli, were analyzed by Western blotting, visualized with an anti-FLAG monoclonal antibody (M2, Sigma) and an anti-mouse IgG(H + L) conjugate coupled to peroxidase.

T−: supernatant of the parental FRhK-4 line.

T+: supernatant of BHK cells infected with a recombinant vaccinia virus expressing the SsoI polypeptide. The solid arrow indicates the SsoI polypeptide, while the empty arrow indicates a cross reaction with a protein of cellular origin.

FIG. 27 shows the results relating to the analysis of the purified SsoI polypeptide

A. 8, 2, 0.5 and 0.125 μg of recombinant SsoI polypeptide purified by anti-FLAG affinity chromatography and gel filtration (G75) were separated on 8% SDS polyacrylamide gel. The SsoI polypeptide and variable quantities of molecular mass markers (MM) were visualized by staining with silver nitrate (Gelcode SilverSNAP stain kit II, Pierce).

B. Standard markers for analysis by SELDI-TOF mass spectrometry

IgG: bovine IgG of MM 147300

ConA: conalbumin of MM 77490

HRP: horseradish peroxidase analyzed as a control and of MM 43240

C. Analysis by mass spectrometry (SELDI-TOF) of the recombinant SsoI polypeptide.

The peaks A and B correspond to the single and double charged SsoI polypeptide.

D. Sequencing of the N-terminal end of the recombinant SsoI polypeptide. 5 Edman degradation cycles in liquid phase were carried out on an ABI494 sequencer (Applied Biosystems).

FIG. 28 illustrates the influence of a splicing signal and of the CTE and WPRE sequences on the efficacy of the gene immunization with the aid of plasmid DNA encoding the SARS-CoV S

A. Groups of 7 BALB/c mice were immunized twice at 4 weeks' interval with the aid of 50 μg of plasmid DNA of pCI, pcDNA-S, pCI-S, pcDNA-N and pCI-HA.

B. Groups of 6 BALB/c mice were immunized twice at 4 weeks' interval with the aid of 2 μg, 10 μg or 50 μg of plasmid DNA of pCI, pCI-S, pCI-S-CTE and pCI-S-WPRE.

The immune sera collected 3 weeks after the second immunization were analyzed by indirect ELISA using a lysate of VeroE6 cells infected with SARS-CoV as antigen. The anti-SARS-CoV antibody titers are calculated as the reciprocal of the dilution producing a specific OD of 0.5 after visualization with an anti-mouse IgG polyclonal antibody coupled to peroxidase (NA931V, Amersham) and TMB (KPL).

FIG. 29 shows the seroneutralization of the

infectivity of SARS-CoV with the antibodies induced in

mice after gene immunization with the aid of plasmid

DNA encoding SARS-CoV S. Pools of immune sera collected

3 weeks after the second immunization were prepared for

each of the groups of experiments described in

FIG. 28 and evaluated for their capacity to seroneutralize the infectivity of 100 TCID50 of SARS-CoV on FRhK-4 cells. 4 points are produced for each of the 2-fold dilutions tested from 1/20. The seroneutralizing titer is calculated according to the Reed and Munsch method as the reciprocal of the dilution neutralizing the infectivity of 2 wells out of 4.

A. Groups by BALB/c mice immunized twice at 4 weeks' interval with the aid of 50 μg of plasmid DNA of pCI, pcDNA-S, pCI-S, pcDNA-N and pCI-HA. □: preimmune serum. ■: immune serum.

B. Groups of BALB/c mice immunized twice at 4 weeks' interval with the aid of 2 μg, 10 μg or 50 μg of plasmid DNA of pCI, pCI-S, pCI-S-CTE and pCI-S-WPRE.

FIG. 30 illustrates the immunoreactivity of the recombinant SsoI polypeptide toward sera from patients suffering from SARS. The reactivity of sera from patients was analyzed by indirect ELISA test against solid phases prepared with the aid of the purified recombinant SsoI polypeptide. The antibodies from patients reacting with the solid phase at a dilution of 1/400 are visualized with a human anti-IgG(H + L) polyclonal antibody coupled to peroxidase (Amersham NA933V) and TMB plus. H202 (KPL). The sera of probable SARS cases are identified by a National Reference Center for Influenza Viruses serial number and by the initials of the patient and the number of days elapsed since the onset of symptoms, where appropriate. The TV sera are control sera from subjects which were collected in France before the SARS epidemic which occurred in 2003.

FIG. 31 shows the induction of antibodies directed against SARS-CoV after immunization with the recombinant SsoI polypeptide. Two groups of 6 mice were immunized at 3 weeks' interval with 10 μg of recombinant SsoI polypeptide (SsoI group) adjuvanted with aluminum hydroxide or, as a control, of adjuvant alone (mock group). Three successive immunizations were performed and the immune sera were collected 3 weeks after each of the three immunizations (IS1, IS2, IS3). The immune sera were analyzed per pool for each of the 2 groups by indirect ELISA using a lysate of VeroE6 cells infected with SARS-CoV as antigen. The anti-SARS-CoV antibody titers are calculated as the reciprocal of the dilution producing a specific OD of 0.5 after visualization with an anti-mouse IgG polyclonal antibody coupled to peroxidase (Amersham) and TMB (KPL).

FIG. 32 presents the nucleotide alignment of the sequences of the synthetic gene 040530 with the sequence of the wild-type gene of the SARS-CoV isolate 031589. I-3059 corresponds to nucleotides 21406-25348 of the SARS-CoV isolate 031589 deposited at the C.N.C.M. under the number I-3059 (SEQ ID NO: 4, plasmid pSARS-S) S-040530 is the sequence of the synthetic gene 040530.

TABLE I-continued

Sequence listing			
Identification number	Sequence	Position of the cDNA with reference to Genbank AY274119.3	Deposit number at the CNCM of the corresponding plasmid

FIG. 33 illustrates the use of a synthetic gene for the expression of the SARS-CoV S. Cellular extracts prepared 48 hours after transfection of VeroE6 cells (A) or 293T cells (B) with the plasmids pCI, pCI-S, pCI-S-CTE, pCI-S-WPRE and pCI-Ssynth were separated on 8% SDS acrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H + L) polyclonal antibody coupled to peroxidase (NA934V, Amersham). The Western blot is visualized by luminescence (ECL+, Amersham) and acquisition on a digital imaging device (FluorS, BioRad). The levels of expression of the S protein were measured by quantifying the 2 predominant bands identified on the image.

FIG. 34 presents a diagram for the construction of recombinant vaccinia viruses VV-TG-S, VV-TG-Ssol, VV-TN-S and VV-TN-Ssol

A. The cDNAs for the S protein and the Ssol polypeptide of SARS-CoV were inserted between the BamH1 and Sma1 sites of the transfer plasmid pTG186 in order to obtain the plasmids pTG-S and pTG-Ssol.

B. The sequences of the synthetic promoter 480 were then substituted for those of the 7.5 promoter by exchange of the Nde1-Pst1 fragments of the plasmids pTG186poly, pTG-S and pTG-Ssol in order to obtain the transfer plasmids pTN480, pTN-S and pTN-Ssol.

C. Sequence of the synthetic promoter 480 as contained between the Nde1 and Pst1 sites of the transfer plasmids of the pTN series. An Asc1 site was inserted in order to facilitate subsequent handling. The restriction sites and the promoter sequence are underlined.

D. The recombinant vaccinia viruses are obtained by double homologous recombination in vivo between the TK cassette of the transfer plasmids of the pTG and pTN series and the TK gene of the Copenhagen strain of the vaccinia virus.

SP: signal peptide predicted (aa 1-13) with the software signalP v2.0 (Nielsen et al., 1997, Protein Engineering, 10: 1-6)

TM: transmembrane region predicted (aa 1196-1218) with the software TMHMM v2.0 (Sonnhammer et al., 1998, Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, pp. 175-182, AAAI Press). It should be noted that the amino acids W1194 and P1195 possibly form part of the transmembrane region with respective probabilities of 0.13 and 0.42.

TK-L, TK-R: left- and right-hand parts of the vaccinia virus thymidine kinase gene

MCS: multiple cloning site

PE: early promoter

PL: late promoter

PL synth: synthetic late promoter 480

FIG. 35 illustrates the expression of the S protein by recombinant vaccinia viruses, analyzed by Western blotting. Cellular extracts were prepared 18 hours after infection of CV1 cells with the recombinant vaccinia viruses VV-TG, VV-TG-S and VV-TN-S at an M.O.I. of 2 (A). As a control, extracts of VeroE6 cells were prepared 8 hours after infection with SARS-CoV at a multiplicity of infection of 2. Cellular extracts were also prepared 18 hours after infection of CV1 cells with the recombinant vaccinia viruses VV-TG-S, VV-TG-Ssol, VV-TN, VV-TN-S and VV-TN-Ssol (B). They were separated on 8% SDS acrylamide gels and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H + L) polyclonal antibody coupled to peroxidase (NA934V, Amersham). "1 µl" and "10 µl" indicates the quantities of cellular extracts deposited on the gel. A molecular mass ladder (kDa) is presented in the figure.

SARS-CoV: extract of VeroE6 cells infected with SARS-CoV

Mock: control extract of noninfected cells

FIG. 36 shows the result of a Western-blot analysis of the secretion of the Ssol polypeptide by the recombinant vaccinia viruses.

A. Supernatants of CV1 cells infected with the recombinant vaccinia virus VV-TN, various clones of the VV-TN-Ssol virus and with the viruses VV-TG-Ssol or VV-TN-Sflag were harvested 18 hours after infection of CV1 cells at an M.O.I. of 2.

B. Supernatants of 293T, FRhK-4, BHK-21 and CV1 cells infected in duplicate (1.2) with the recombinant vaccinia virus VV-TN-Ssol at an M.O.I. of 2 were harvested 18 hours after infection. The supernatant of CV1 cells infected with the virus VV-TN was also harvested as a control (M).

All the supernatants were separated on 8% SDS acrylamide gel according to Laemmli and analyzed by Western blotting with the aid of an anti-FLAG mouse monoclonal antibody and an anti-mouse IgG(H + L) polyclonal antibody coupled to peroxidase (NA931V, Amersham) (A) or with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H + L) polyclonal antibody coupled to peroxidase (NA934V, Amersham) (B).

A molecular mass ladder (kDa) is presented in the figure.

FIG. 37 shows the analysis of the Ssol polypeptide, purified on SDS polyacrylamide gel 10, 5 and 2 µl of recombinant Ssol polypeptide purified by anti-FLAG affinity chromatography were separated on 4 to 15% gradient SDS polyacrylamide gel. The Ssol polypeptide and variable quantities of molecular mass markers (MM) were visualized by staining with silver nitrate (Gelcode SilverSNAP stain kit II, Pierce).

FIG. 38 illustrates the immunoreactivity of the recombinant Ssol polypeptide produced by the recombinant vaccinia virus VV-TN-Ssol toward sera of patients suffering from SARS. The reactivity of sera from patients was analyzed by indirect ELISA test against solid phases prepared with the aid of the purified recombinant Ssol polypeptide. The antibodies from patients reacting with the solid phase at a dilution of 1/100 and 1/400 are visualized with a human anti-IgG(H + L) polyclonal antibody coupled to peroxidase (Amersham NA933V) and TMB plus H202 (KPL). The sera of probable SARS cases are identified by a National Reference Center for Influenza Virus serial number and by the initials of the patient and the number of days elapsed since the onset of symptoms, where appropriate. The TV sera are control sera from subjects which were collected in France before the SARS epidemic which occurred in 2003.

FIG. 39 shows the anti-SARS-CoV antibody response in mice after immunization with the recombinant vaccinia viruses. Groups of 7 BALB/c mice were immunized by the i.v. route twice at 4 weeks' interval with 106 pfu of recombinant vaccinia viruses VV-TG, VV-TG-HA, VV-TG-S, VV-TG-Ssol, VV-TN, VV-TN-S, VV-TN-Ssol.

A. Pools of immune sera collected 3 weeks after each of the two immunizations were prepared for each of the groups and were analyzed by indirect ELISA using a lysate of VeroE6 cells infected with SARS-CoV as antigen. The anti-SARS-CoV antibody titers are calculated as the reciprocal of the dilution producing a specific OD of 0.5 after visualization with an anti-mouse IgG polyclonal antibody coupled to peroxidase (NA931V, Amersham) and TMB (KPL).

TABLE I-continued

Sequence listing			
Identification number	Sequence	Position of the cDNA with reference to Genbank AY274119.3	Deposit number at the CNCM of the corresponding plasmid

B. The pools of immune sera were evaluated for their capacity to seroneutralize the infectivity of 100 TCID50 of SARS-CoV on FRhK-4 cells. 4 points are produced for each of the 2-fold dilutions tested from 1/20. The seroneutralizing titer is calculated according to the Reed and Munsch method as the reciprocal of the dilution neutralizing the infectivity of 2 wells out of 4.

FIG. 40 describes the construction of the recombinant viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol.

A. The measles vector is a complete genome of the Schwarz vaccine strain of the measles virus (MV) into which an additional transcription unit has been introduced (Combredet, 2003, Journal of Virology, 77: 11546-11554). The expression of the additional open reading frames (ORF) is controlled by cis-acting elements necessary for the transcription, for the formation of the cap and for the polyadenylation of the transgene which were copied from the elements present at the N/P junction. 2 different vectors allow the insertion between the P (phosphoprotein) and M (matrix) genes on the one hand and the H (hemagglutinin) and L (polymerase) genes on the other hand.

B. The recombinant genomes MVSchw2-SARS-S and MVSchw2-SARS-Ssol of the measles virus were constructed by inserting the ORFs of the S protein and of the Ssol polypeptide into an additional transcription unit located between the P and M genes of the vector.

The various genes of the measles virus (MV) are indicated: N (nucleoprotein), PVC (V/C phosphoprotein and protein), M (matrix), F (fusion), H (hemagglutinin), L (polymerase). T7 = T7 RNA polymerase promoter, hh = hammerhead ribozyme, T7t = T7 phage RNA polymerase terminator sequence, δ = ribozyme of the hepatitis δ virus, (2), (3) = additional transcription units (ATU).

Size of the MV genome: 15 894 nt.

SP: signal peptide

TM: transmembrane region

FLAG: FLAG tag

FIG. 41 illustrates the expression of the S protein by the recombinant measles viruses, analyzed by Western blotting.

Cytoplasmic extracts were prepared after infection of Vero cells by different passages of the viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol and the wild-type virus MWSchw as control. Cellular extracts in loading buffer according to Laemmli were also prepared 8 hours after infection of VeroE6 cells with SARS-CoV at a multiplicity of infection of 3. They were separated on 8% SDS acrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H + L) polyclonal antibody coupled to peroxidase (NA934V, Amersham).

A molecular mass ladder (kDa) is presented in the figure.

Pn: nth passage of the virus after coculture of 293-3-46 and Vero cells

SARS-CoV: extract of VeroE6 cells infected with SARS-CoV

Mock: control extract of noninfected VeroE6 cells

FIG. 42 shows the expression of the S protein by the recombinant measles viruses, analyzed by immunofluorescence

Vero cells in monolayers on glass slides were infected with the wild-type virus MWSchw (A) or the viruses MVSchw2-SARS-S (B) and MVSchw2-SARS-Ssol (C). When the syncytia have reached 30 to 40% confluence (A., B.) or 90-100% (C), the cells were fixed, permeabilized and labeled with anti-SARS-CoV rabbit polyclonal antibodies and an anti-rabbit IgG(H + L) conjugate coupled to FITC (Jackson).

FIG. 43 illustrates the Western-blot analysis of the immunoreactivity of rabbit sera directed against the peptides E1-12, E53-76 and M2-14. The rabbit 20047 was immunized with the peptide E1-12 coupled to KLH. The rabbits 22234 and 22240 were immunized with the peptide E53-76 coupled to KLH. The rabbits 20013 and 20080 were immunized with the peptide M2-14 coupled to KLH. The immune sera were analyzed by Western blotting with the aid of extracts of cells infected with SARS-CoV (B) or with the aid of extracts of cells infected with a recombinant vaccinia virus expressing the protein E (A) or M (C) of the SARS-CoV 031589 isolate. The immunoblots were visualized with the aid of an anti-rabbit IgG(H + L) conjugate coupled to peroxidase (NA934V, Amersham).

The position of the E and M proteins is indicated by an arrow.

A molecular mass ladder (kDa) is presented in the figure.

It should be understood, however, that these examples are given solely by way of illustration of the subject of the invention, and do not constitute in any manner a limitation thereto.

EXAMPLE 1

Cloning and Sequencing of the Genome of the SARS-CoV Strain Derived from the Sample Recorded Under the Number 031589

The RNA of the SARS-CoV strain was extracted from the sample of bronchoalveolar washing recorded under the number 031589, performed on a patient at the Hanoi (Vietnam) French hospital suffering from SARS.

The isolated RNA was used as template to amplify the cDNAs corresponding to the various open reading frames of the genome (ORF1a, ORF1b, ORF-S, ORF-E, ORF-M, ORF-N (including ORF-13 and ORF-14), ORF3, ORF4, ORF7 to ORF11), and at the noncoding 5' and 3' ends. The sequences of the primers and of the probes used for the amplification/detection were defined based on the available SARS-CoV nucleotide sequence.

In the text which follows, the primers and the probes are identified by: the letter S, followed by a letter which indicates the corresponding region of the genome (L for the 5' end including ORF1a and ORF1b; S, N and N for ORF-S, ORF-M, ORF-N, SE and MN for the corresponding intergene regions), and then optionally by Fn, Rn, with n between 1 and 6 corresponding to the primers used for the nested PCR (F1+R1 pair for the first amplification, F2+R2 pair for the second amplication, and the like), and then by /+/ or /−/ corresponding to a sense or antisense primer and finally by the positions of the primers with reference to the Genbank sequence AY27411.3; for the sense and antisense S and N primers and the other sense primers only, when a single position is indicated, it corresponds to that of the 5' end of a probe or of a primer of about 20 bases; for the antisense primers other than the S and N primers, when a single position is indicated, it corresponds to that of the 3' end of a probe or of a primer of about 20 bases.

The amplification products thus generated were sequenced with the aid of specific primers in order to determine the complete sequence of the genome of the SARS-CoV strain derived from the sample recorded under the number 031589. These amplification products, with the exception of those corresponding to ORF1a and ORF1b, were then cloned into expression vectors in order to produce the corresponding viral proteins and the antibodies directed against these proteins, in particular by DNA-based immunization.

1. Extraction of the RNAs

The RNAs were extracted with the aid of the QIamp viral RNA extraction mini kit (QIAGEN) according to the manufacturer's recommendations. More specifically: 140 μl of the sample and 560 μl of AVL buffer were vigorously mixed for 15 seconds, incubated for 10 minutes at room temperature and then briefly centrifuged at maximum speed. 560 μl of 100% ethanol were added to the supernatant and the mixture thus obtained was very vigorously stirred for 15 sec. 630 μl of the mixture were then deposited on the column.

The column was placed on a 2 ml tube, centrifuged for 1 min at 8000 rpm, and then the remainder of the preceding mixture was deposited on the same column, centrifuged again, for 1 min at 8000 rpm, and the column was transferred over a clean 2 ml tube. Next, 500 μl of AW1 buffer were added to the column, and then the column was centrifuged for 1 min at 8000 rpm and the eluate was discarded. 500 μl of AW2 buffer were added to the column which was then centrifuged for 3 min at 14 000 rpm and transferred onto a 1.5 ml tube. Finally, 60 μl of AVE buffer were added to the column which was incubated for 1 to 2 min at room temperature and then centrifuged for 1 min at 8000 rpm. The eluate corresponding to the purified RNA was recovered and frozen at −20° C.

2. Amplification, Sequencing and Cloning of the cDNAs

2.1) cDNA Encoding the S Protein

The RNAs extracted from the sample were subjected to reverse transcription with the aid of random sequence hexameric oligonucleotides (pdN6), so as to produce cDNA fragments.

The sequence encoding the SARS-CoV S glycoprotein was amplified in the form of two overlapping DNA fragments: 5' fragment (SARS-Sa, SEQ ID NO: 5) and 3' fragment (SARS-Sb, SEQ ID NO: 6), by carrying out two successive amplifications with the aid of nested primers. The amplicons thus obtained were sequenced, cloned into the PCR plasmid vector 2.1-TOPO™ (INVITROGEN), and then the sequence of the cloned cDNAs was determined.

a) Cloning and Sequencing of the Sa and Sb Fragments

a.1) Synthesis of the cDNA

The reaction mixture containing: RNA (5 μl), H_2O for injection (3.5 μl), 5× reverse transcriptase buffer (4 μl), 5 mM dNTP (2 μl), pdN6 100 μg/ml (4 μl), RNasin 40 IU/μl (0.5 μl) and reverse transcriptase AMV-RT, 10 IU/μl, PROMEGA (1 μl) was incubated in a thermocycler under the following conditions: 45 min at 42° C., 15 min at 55° C., 5 min at 95° C., and then the cDNA obtained was kept at +4° C.

a.2) First PCR Amplification

The 5' and 3' ends of the S gene were respectively amplified with the pairs of primers S/F1/+/21350-21372 and S/R1/−/23518-23498, S/F3/+/23258-23277 and S/R3/−/25382-25363. The 50 μl reaction mixture containing: cDNA (2 μl), 50 μM primers (0.5 μl), 10× buffer (5 μl), 5 mM dNTP (2 μl), Taq Expand High Fidelity, Roche (0.75 μl) and H_2O (39, 75 μl) was amplified in a thermocycler, under the following conditions: an initial step of denaturation at 94° C. for 2 min was followed by 40 cycles comprising: a step of denaturation at 94° C. for 30 sec, a step of annealing at 55° C. for 30 sec and then a step of extension at 72° C. for 2 min 30 sec, with 10 sec of additional extension at each cycle, and then a final step of extension at 72° C. for 5 min.

a.3) Second PCR Amplification

The products of the first PCR amplification (5' and 3' amplicons) were subjected to a second PCR amplification step (nested PCR) under conditions identical to those of the first amplification, with the pairs of primers S/F2/+/21406-21426 and S/R2/−/23454-23435 and S/F4/+/23322-23341 and S/R4/−/25348-25329, respectively for the 5' amplicon and the 3' amplicon.

a.4) Cloning and Sequencing of the Sa and Sb Fragments

The Sa (5' end) and Sb (3' end) amplicons thus obtained were purified with the aid of the QIAquick PCR purification kit (QIAGEN), following the manufacturer's instructions, and then they were cloned into the vector PCR2.1-TOPO (Invitrogen kit), to give the plasmids called SARS-S1 and SARS-S2.

The DNA of the Sa and Sb clones was isolated and then the corresponding insert was sequenced with the aid of the Big Dye kit, Applied Biosystem® and universal primers M13 forward and M13 reverse, and primers: S/S/+/21867, S/S/+/22353, S/S/+/22811, S/S/+/23754, S/S/+/24207, S/S/+/24699, S/S/+/24348, S/S/−/24209, S/S/−/23630, S/S−/23038, S/S/−/22454, S/S/−/21815, S/S/−/24784, S/S/+/21556, S/S/+/23130 and S/S/+/24465 following the manufacturer's instructions; the sequences of the Sa and Sb fragments thus obtained correspond to the sequences SEQ ID NO: 5 and SEQ ID NO: 6 in the sequence listing appended as an annex.

The plasmid, called SARS-S1, was deposited under the No. I-3020, on May 12, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains a 5' fragment of the sequence of the S gene of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said fragment called Sa corresponding to the nucleotides at positions 21406 to 23454 (SEQ ID NO: 5), with reference to the Genbank sequence AY274119.3 Tor2.

The plasmid, called TOP10F'-SARS-S2, was deposited under the No. I-3019, on May 12, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains a 3' fragment of the sequence of the S gene of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said fragment called Sb corresponding to the nucleotides at positions 23322 to 25348 (SEQ ID NO: 6), with reference to the Genbank sequence accession No. AY274119.3.

b) Cloning and Sequencing of the Complete cDNA (SARS-S Clone of 4 kb)

The complete S cDNA was obtained from the abovementioned clones SARS-S1 and SARS-S2, in the following manner:

1) A PCR amplification reaction was carried out on a SARS-S2 clone in the presence of the abovementioned primer S/R4/−/25348-25329 and of the primer S/S/+/24696-24715: an amplicon of 633 bp was obtained,

2) Another PCR amplification reaction was carried out on another SARS-S2 clone, in the presence of the primers S/F4/+/23322-23341 mentioned above and S/S/−/24803-24784: an amplicon of 1481 bp was obtained.

The amplification reaction was carried out under the conditions as defined above for the amplification of the Sa and Sb fragments, with the exception that 30 amplification cycles comprising a step of denaturation at 94° C. for 20 sec and a step of extension at 72° C. for 2 min 30 sec were carried out.

3) The 2 amplicons (633 bp and 1481 bp) were purified under the conditions as defined above for the Sa and Sb fragments.

4) Another PCR amplification reaction with the aid of the abovementioned primers S/F4/+/23322-23341 and S/R4/−/25348-25329 was carried out on the purified amplicons obtained in 3). The amplification reaction was carried out under the conditions as defined above for the amplification of the Sa and Sb fragments, except that 30 amplification cycles were performed.

The 2026 bp amplicon thus obtained was purified, cloned into the vector PCR2.1-TOPO and then sequenced as above, with the aid of the primers as defined above for the Sa and Sb fragments. The clone thus obtained was called clone 3'.

5) The clone SARS-S1 obtained above and the clone 3' were digested with EcoR I, the bands of about 2 kb thus obtained were gel purified and then amplified by PCR with the abovementioned primers S/F2/+/21406-21426 and S/R4/−/25348-25329. The amplification reaction was carried out under the conditions as defined above for the amplification of the Sa and Sb fragments, except that 30 amplification cycles were performed. The amplicon of about 4 kb was purified and sequenced. It was then cloned into the vector PCR2.1-TOPO in order to give the plasmid, called SARS-S, and the insert obtained in this plasmid was sequenced as above, with the aid of the primers as defined above for the Sa and Sb fragments. The cDNA sequences of the insert and of the amplicon encoding the S protein correspond respectively to the sequences SEQ ID NO: 4 and SEQ ID NO: 2 in the sequence listing appended as an annex, they encode the S protein (SEQ ID NO: 3).

The sequence of the amplicon corresponding to the cDNA encoding the S protein of the SARS-CoV strain derived from the sample No. 031589 has the following two mutations compared with the corresponding sequences of respectively the Tor2 and Urbani isolates, the positions of the mutations being indicated with reference to the complete sequence of the genome of the Tor2 isolate (Genbank AY274119.3):

- g/t in position 23220; the alanine codon (gct) in position 577 of the amino acid sequence of the S protein of Tor2 is replaced with a serine codon (tct),
- c/t in position 24872: this mutation does not modify the amino acid sequence of the S protein, and

the plasmid, called SARS-S, was deposited under the No. I-3059, on Jun. 20, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence encoding the S protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, said sequence corresponding to the nucleotides at positions 21406 to 25348 (SEQ ID NO: 4), with reference to the Genbank sequence AY274119.3.

2.2) cDNA Encoding the M and E Proteins

The RNAs derived from the sample 031589, extracted as above, were subjected to a reverse transcription, combined, during the same step (Titan One Step RT-PCR® kit, Roche), with a PCR amplification reaction, with the aid of the pairs of primers:

- S/E/F1/+/26051-26070 and S/E/R1/−/26455-26436 in order to amplify ORF-E, and
- S/M/F1/+/26225-26244 and S/M/R1/−/27148-27129 in order to amplify ORF-M.

A first reaction mixture containing: 8.6 μl of H_2O for injection, 1 μl of dNTP (5 mM), 0.2 μl of each of the primers (50 μM), 1.25 μl of DTT (100 mM) and 0.25 μl of RNAsin (40 IU/μl) was combined with a second reaction mixture containing: 1 μl of RNA, 7 μl of H_2O for injection, 5 μl of 5×RT-PCR buffer and 0.5 μl of enzyme mixture and the combined mixtures were incubated in a thermocycler under the following conditions: 30 min at 42° C., 10 min at 55° C., 2 min at 94° C. followed by 40 cycles comprising a step of denaturation at 94° C. for 10 sec, a step of annealing at 55° C. for 30 sec and a step of extension at 68° C. for 45 sec, with 3 sec increment per cycle and finally a step of terminal extension at 68° C. for 7 min.

The amplification products thus obtained (M and E amplicons) were subjected to a second PCR amplification (nested PCR) using the Expand High-Fi® kit, Roche), with the aid of the pairs of primers:

- S/E/F2/+/26082-26101 and S/E/R2/−/26413-26394 for the amplicon E, and
- S/M/F2/+/26330-26350 and S/M/R2/−/27098-27078 for the amplicon M.

The reaction mixture containing: 2 μl of the product of the first PCR, 39.25 μl of H_2O for injection, 5 μl of 10× buffer containing $MgCl_2$, 2 μl of dNTP (5 mM), 0.5 μl of each of the primers (50 μM) and 0.75 μl of enzyme mixture was incubated in a thermocycler under the following conditions: a step of denaturation at 94° C. for 2 min was followed by 30 cycles comprising a step of denaturation at 94° C. for 15 sec, a step of annealing at 60° C. for 30 sec and a step of extension at 72° C. for 45 sec, with 3 sec increment per cycle, and finally a step of terminal extension at 72° C. for 7 min. The amplification products obtained corresponding to the cDNAs encoding the E and M proteins were sequenced as above, with the aid of the primers: S/E/F2/+/26082 and S/E/R2/−/126394, S/M/F2/+/26330, S/M/R2/−/27078 cited above and the primers S/M/+/26636-26655 and S/M/−/26567-26548. They were then cloned, as above, in order to give the plasmids called SARS-E and SARS-M. The DNA of these clones was then isolated and sequenced with the aid of the universal primers M13 forward and M13 reverse and the primers S/M/+/26636 and S/M/−/26548 mentioned above.

The sequence of the amplicon representing the cDNA encoding the E protein (SEQ ID NO: 13) of the SARS-CoV strain derived from the sample No. 031589 does not contain differences in relation to the corresponding sequences of the isolates AY274119.3-Tor2 and AY278741-Urbani. The sequence of the E protein of the SARS-CoV 031589 strain corresponds to the sequence SEQ ID NO: 14 in the sequence listing appended as an annex.

The plasmid, called SARS-E, was deposited under the No. I-3046, on May 28, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence encoding the E protein of the SARS-CoV strain derived from the

sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 26082 to 26413 (SEQ ID NO: 15), with reference to the Genbank sequence accession No. AY274119.3.

The sequence of the amplicon representing the cDNA encoding M (SEQ ID NO: 16) from the SARS-CoV strain derived from the sample No. 031589 does not contain differences in relation to the corresponding sequence of the isolate AY274119.3-Tor2. By contrast, at position 26857, the isolate AY278741-Urbani contains a c and the sequence of the SARS-CoV strain derived from the sample recorded under the No. 031589 contains a t. This mutation results in a modification of the amino acid sequence of the corresponding protein: at position 154, a proline (AY278741-Urbani) is changed to serine in the SARS-CoV strain derived from the sample recorded under the No. 031589. The sequence of the M protein of the SARS-CoV strain derived from the sample recorded under the No. 031589 corresponds to the sequence SEQ ID NO: 17 in the sequence listing appended as an annex.

The plasmid, called SARS-M, was deposited under the No. I-3047, on May 28, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence encoding the M protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above; said sequence corresponding to the nucleotides at positions 26330 to 27098 (SEQ ID NO: 18), with reference to the Genbank sequence accession No. AY274119.3.

2.3) cDNA Corresponding to ORF3, ORF4, ORF7 to ORF11

The same amplification, cloning and sequencing strategy was used to obtain the cDNA fragments corresponding respectively to the following ORFs: ORF3, ORF4, ORF7, ORF8, ORF9, ORF10 and ORF11. The pairs of primers used for the first amplification are:

ORF3 and ORF4: S/SE/F1/+/25069-25088 and S/SE/R1/−/26300-26281

ORF7 to ORF11: S/MN/F1/+/26898-26917 and S/MN/R1/−/28287-28266

The pairs of primers used for the second amplification are:

ORF3 and ORF4: S/SE/F2/+/25110-25129 and S/SE/R2/−/26244-26225

ORF7 to ORF11: S/NN/F2/+/26977-26996 and S/MN/R2/−/28218-28199

The conditions for the first amplification (RT-PCR) are the following: 45 min at 42° C., 10 min at 55° C., 2 min at 94° C. followed by 40 cycles comprising a step of denaturation at 94° C. for 15 sec, a step of annealing at 58° C. for 30 sec and a step of extension at 68° C. for 1 min, with 5 sec increment per cycle and finally a step of terminal extension at 68° C. for 7 min.

The conditions for the nested PCR are the following: a step of denaturation at 94° C. for 2 min was followed by 40 cycles comprising a step of denaturation at 94° C. for 20 sec, a step of annealing at 58° C. for 30 sec and a step of extension at 72° C. for 50 sec, with 4 sec increment per cycle and finally a step of terminal extension at 72° C. for 7 min.

The amplification products obtained corresponding to the cDNAs containing respectively ORF3 and 4 and ORF7 to 11 were sequenced with the aid of the primers: S/SE/+/25363, S/SE/+/25835, S/SE/−/25494, S/SE/−/25875, S/MN/+/27839, S/MN/+/27409, S/MN/−/27836, S/MN/−/27799 and cloned as above for the other ORFs, to give the plasmids called SARS-SE and SARS-MN. The DNA of these clones was isolated and sequenced with the aid of these same primers and of the universal primers M13 sense and M13 antisense.

The sequence of the amplicon representing the cDNA of the region containing OFR3 and ORF4 (SEQ ID NO: 7) of the SARS-CoV strain derived from the sample No. 031589 contains a nucleotide difference in relation to the corresponding sequence of the isolate AY274119-Tor2. This mutation at position 25298 results in a modification of the amino acid sequence of the corresponding protein (ORF3): at position 11, an arginine (AY274119-Tor2) is changed to glycine in the SARS-CoV strain derived from the sample No. 031589. By contrast, no mutation was identified in relation to the corresponding sequence of the isolate AY278741-Urbani. The sequences of ORF3 and 4 of the SARS-COV strain derived from the sample No. 031589 correspond respectively to the sequences SEQ ID NO: 10 and 12 in the sequence listing appended as an annex.

The plasmid, called SARS-SE, was deposited under the No. I-3126, on Nov. 13, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA corresponding to the region situated between ORF-S and ORF-E and overlapping ORF-E of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said region corresponding to the nucleotides at positions 25110 to 26244 (SEQ ID NO: 8), with reference to the Genbank sequence accession No. AY274119.3.

The sequence of the amplicon representing the cDNA corresponding to the region containing ORF7 to ORF11 (SEQ ID NO: 19) of the SARS-CoV strain derived from the sample No. 031589 does not contain differences in relation to the corresponding sequences of the isolates AY274119-Tor2 and AY278741-Urbani. The sequences of ORF7 to 11 of the SARS-CoV strain derived from the sample No. 031589 correspond respectively to the sequences SEQ ID NO: 22, 24, 26, 28 and 30 in the sequence listing appended as an annex.

The plasmid, called SARS-MN, was deposited under the No. I-3125, on Nov. 13, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence corresponding to the region situated between ORF-M and ORF-N of the SARS-CoV strain derived from the sample recorded under the No. 031589 and collected in Hanoi, as defined above, said sequence corresponding to the nucleotides at positions 26977 to 28218 (SEQ ID NO: 20), with reference to the Genbank sequence accession No. AY274119.3.

The sequence of the amplicon representing the cDNA corresponding to the region containing ORF7 to ORF11 (SEQ ID NO: 19) of the SARS-CoV strain derived from the sample No. 031589 does not contain differences in relation to the corresponding sequences of the isolates AY274119-Tor2 and AY278741-Urbani. The sequences of ORF7 to 11 of the SARS-CoV strain derived from the sample No. 031589 correspond respectively to the sequences SEQ ID NO: 22, 24, 26, 28 and 30 in the sequence listing appended as an annex.

2.4) cDNA Encoding the N Protein and Including ORF13 and ORF14

The cDNA was synthesized and amplified as described above for the fragments Sa and Sb. More specifically, the reaction mixture containing: 5 μl of RNA, 5 μl of H_2O for injection, 4 μl of 5× reverse transcriptase buffer, 2 μl of dNTP (5 mM), 2 μl of oligo 20 T (5 μM), 0.5 μl of RNasin (40 IU/μl) and 1.5 μl of AMV-RT (10 IU/μl Promega) was incubated in a thermocycler under the following conditions: 45 min at 42° C., 15 min at 55° C., 5 min at 95° C., and it was then kept at +4° C.

A first PCR amplification was performed with the pair of primers S/N/F3/+/28023 and S/N/R3/−/29460.

The reaction mixture as above for the amplification of the S1 and S2 fragments was incubated in a thermo-cycler, under the following conditions: an initial step of denaturation at 94° C. for 2 min was followed by 40 cycles comprising a step of denaturation at 94° C. for 20 sec, a step of annealing at 55° C. for 30 sec and then a step of extension at 72° C. for 1 min 30 sec with 10 sec of additional extension at each cycle, and then a final step of extension at 72° C. for 5 min.

The amplicon obtained at the first PCR amplification was subjected to a second PCR amplification step (nested PCR) with the pairs of primer S/N/F4/+/28054 and S/N/R4/−/29430 under conditions identical to those of the first amplification.

The amplification product obtained, corresponding to the cDNA encoding the N protein of the SARS-CoV strain derived from the sample No. 031589, was sequenced with the aid of the primers: S/N/F4/+/28054, S/N/R4/−/29430, S/N/+/28468, S/N/+/28918 and S/N/−/28607 and cloned as above for the other ORFs, to give the plasmid called SARS-N. The DNA of these clones was isolated and sequenced with the aid of the universal primers M13 sense and M13 antisense, and the primers S/N/+/28468, S/N/+/28918 and S/N/−/28607.

The sequence of the amplicon representing the cDNA corresponding to ORF-N and including ORF13 and ORF14 (SEQ ID NO: 36) of the SARS-CoV strain derived from the sample No. 031589 does not contain differences in relation to the corresponding sequences of the isolates AY274119.3-Tor2 and AY278741-Urbani. The sequence of the N protein of the SARS-CoV strain derived from the sample No. 031589 corresponds to the sequence SEQ ID NO: 37 in the sequence listing appended as an annex.

The sequences of ORF13 and 14 of the SARS-CoV strain derived from the sample No. 031589 correspond respectively to the sequences SEQ ID NO: 32 and 34 in the sequence listing appended as an annex.

The plasmid, called SARS-N, was deposited under the No. I-3048, on Jun. 5, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA encoding the N protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 28054 to 29430 (SEQ ID NO: 38), with reference to the Genbank sequence accession No. AY274119.3.

2.5) Noncoding 5' and 3' Ends

a) Noncoding end (5'NC)

a_1) Synthesis of the cDNA

The RNAs derived from the sample 031589, extracted as above, were subjected to reverse transcription under the following conditions:

The RNA (15 μl) and the primer S/L/−/443 (3 μl at the concentration of 5 μm) were incubated for 10 min at 75° C.

Next, the 5× reverse transcriptase buffer (6 μl, INVITROGEN), 10 Mm dNTP (1 μl), 0.1 M DTT (3 μl) were added and the mixture was incubated at, 50° C. for 3 min.

Finally, the reverse transcriptase (3 μl of Superscript®, INVITROGEN) was added to the preceding mixture which was incubated at 50° C. for 1 h 30 min and then at 90° C. for 2 min.

The cDNA thus obtained was purified with the aid of the QIAquick PCR purification kit (QIAGEN), according to the manufacturer's recommendations.

b_1) Terminal Transferase Reaction (TdT)

The cDNA (10 μl) is incubated for 2 min at 100° C., stored in ice, and the following are then added: H_2O (2.5 μl), 5× TdT buffer (4 μl, AMERSHAM), 5 mM dATP (2 μl) and TdT (1.5 μl, AMERSHAM). The mixture thus obtained is incubated for 45 min at 37° C. and then for 2 min at 65° C.

The product obtained is amplified by a first PCR reaction with the aid of the primers: S/L/−225-206 and anchor 14T: 5'-AGATGAATTCGGTACCTTTTTTTTTTTTTT-3' (SEQ ID NO: 68). The amplification conditions are the following: an initial step of denaturation at 94° C. for 2 min is followed by 10 cycles comprising a step of denaturation at 94° C. for 10 sec, a step of annealing at 45° C. for 30 sec and then a step of extension at 72° C. for 30 sec and then by 30 cycles comprising a step of denaturation at 94° C. for 10 sec, a step of annealing at 50° C. for 30 sec and then a step of extension at 72° C. for 30 sec, and then a final step of extension at 72° C. for 5 min.

The product of the first PCR amplification was subjected to a second amplification step with the aid of the primers: S/L/−/204-185 and anchor 14 T mentioned above under conditions identical to those of the first amplification. The amplicon thus obtained was purified, sequenced with the aid of the primer S/L/−/182-163 and it was then cloned as above for the different ORFs, to give the plasmid called SARS-5'NC. The DNA of this clone was isolated and sequenced with the aid of the universal primers M13 sense and M13 antisense and the primer S/L/−/182-163 mentioned above.

The amplicon representing the cDNA corresponding to the 5'NC end of the SARS-CoV strain derived from the sample recorded under the No. 031589 corresponds to the sequence SEQ ID NO: 72 in the sequence listing appended as an annex; this sequence does not contain differences in relation to the corresponding sequences of the isolates AY274119.3-Tor2 and AY278741-Urbani.

The plasmid, called SARS-5'NC, was deposited under the No. I-3124, on Nov. 7, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA corresponding to the noncoding 5' end of the genome of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 1 to 204 (SEQ ID NO: 39), with reference to the Genbank sequence accession No. AY274119.3.

b) Noncoding 3' End (3'NC)

a_1) Synthesis of the cDNA

The RNAs derived from the sample 031589, extracted as above, were subjected to reverse transcription, according to the following protocol: the reaction mixture containing: RNA (5 μl), H_2O (5 μl), 5× reverse transcriptase buffer (4 μl), 5 mM dNTP (2 μl), 5 μM Oligo 20 T (2 μl), 40 U/μl RNasin (0.5 μl) and 10 IU/μl RT-AMV (1.5 μl, PROMEGA) was incubated in a thermo-cycler, under the following conditions: 45 min at 42° C., 15 min at 55° C., 5 min at 95° C., and it was then kept at +4° C.

The cDNA obtained was amplified by a first PCR reaction with the aid of the primers S/N/+/28468-28487 and anchor 14 T mentioned above. The amplification conditions are the following: an initial step of denaturation at 94° C. for 2 min is followed by 10 cycles comprising a step of denaturation at 94° C. for 20 sec, a step of annealing at 45° C. for 30 sec and then a step of extension at 72° C. for 50 sec and then 30 cycles comprising a step of denaturation at 94° C. for 20 sec, a step of annealing at 50° C. for 30 sec and then a step of extension at 72° C. for 50 sec, and then a final step of extension at 72° C. for 5 min.

The product of the first PCR amplification was subjected to a second amplification step with the aid of the primers S/N/+/28933-28952 and anchor 14 T mentioned above, under conditions identical to those of the first amplification. The amplicon thus obtained was purified, sequenced with the aid of the primer S/N/+/29257-29278 and cloned as above for the different ORFs, to give the plasmid called SARS-3'NC. The DNA of this clone was isolated and sequenced with the aid of the universal primers M13 sense and M13 antisense and the primer S/N/+/29257-29278 mentioned above.

031589 was performed by carrying out RT-PCR reactions followed by nested PCRs according to the same principles as those described above for the other ORFs. The amplified fragments overlap over several tenths of bases, thus allowing computer reconstruction of the complete sequence of this part of the genome. On average, the amplified fragments are of two kilobases.

14 overlapping fragments, called L0 to L12, were thus amplified with the aid of the following primers:

TABLE II

Primers used for the amplification of the 5' region (ORF1a and ORF1b)

REGION AMPLIFIED AND SEQUENCED (does not include the primers)	RT-PCR sense primer	RT-PCR antisense primer	Nested PCR sense primer	Nested PCR antisense primer
L0 50-480	S/L0/F1/+30	S/L0/R1/−481		
L1 231-2240	S/L1/F1/+147	S/L1/R1/−2336	S/L1/F2/+211	S/L1/R2/−2241
L2 2156-4167	S/L2/F1/+2033	S/L2/R1/−4192	S/L2/F2/+2136	S/L2/R2/−4168
L3 3913-5324	S/L3bis/F1/+3850	S/L3bis/R1/−5365	S/L3bis/F2/+3892	S/L3bis/R2/−5325
L4b 4952-6023	S/L4b/F1/+4878	S/L4b/R1/−6061	S/L4b/F2/+4932	S/L4b/R2/−6024
L4 5325-7318	S/L4/F1/+5272	S/L4/R1/−7392	S/L4/F2/+5305	S/L4/R2/−7323
L5 7296-9156	S/L5/F1/+7111	S/L5/R1/−9253	S/L5/F2/+7275	S/L5/R2/−9157
L6 9053-11066	S/L6/F1/+8975	S/L6/R1/−11151	S/L6/F2/+9032	S/L6/R2/−11067
L7 10928-12962	S/L7/F1/+10883	S/L7/R1/−13050	S/L7/F2/+10928	S/L7/R2/−12963
L8 12835-14834	S/L8/F1/+12690	S/L8/R1/−14857	S/L8/F2/+12815	S/L8/R2/−14835
L9 14765-16624	S/L9/F1/+14688	S/L9/R1/−16678	S/L9/F2/+14745	S/L9/R2/−16625
L10 16534-18570	S/L10/F1/+16451	S/L10/R1/−18594	S/L10/F2/+16514	S/L10/R2/−18571
L11 18521-20582	S/L11/F1/+18441	S/L11/R1/−20612	S/L11/F2/+18500	S/L11/R2/−20583
L12 20338-22205.	S/L12/F1/+20279	S/L12/R1/−22229	S/L12/F2/+20319	S/L12/R2/−22206

The amplicon representing the cDNA corresponding to the 3'NC end of the SARS-CoV strain derived from the sample recorded under the No. 031589 corresponds to the sequence SEQ ID NO: 73 in the sequence listing appended as an annex; this sequence does not contain differences in relation to the corresponding sequences of the isolates AY274119.3-Tor2 and AY278741-Urbani.

The plasmid called SARS-3'NC was deposited under the No. I-3123 on Nov. 7, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence corresponding to the noncoding 3' end of the genome of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to that situated between the nucleotide at positions 28933 to 29727 (SEQ ID NO: 40), with reference to the Genbank sequence accession No. AY274119.3, ends with a series of nucleotides a.

2.6) ORF1a and ORF1b

The amplification of the 5' region containing ORF1a and ORF1b of the SARS-CoV genome derived from the sample

All the fragments were amplified under the following conditions, except fragment L0 which was amplified as described above for ORF-M:

RT-PCR: 30 min at 42° C., 15 min at 55° C., 2 min at 94° C., and then the cDNA obtained is amplified under the following conditions: 40 cycles comprising: a step of denaturation at 94° C. for 15 sec, a step of annealing at 58° C. for 30 sec and then a step of extension at 68° C. for 1 min 30 sec, with 5 sec additional extension at each cycle, and then a final step of extension at 68° C. for 7 min.

Nested PCR: An initial step of denaturation at 94° C. for 2 min is followed by 35 cycles comprising: a step of denaturation at 94° C. for 15 sec, a step of annealing at 60° C. for 30 sec and then a step of extension at 72° C. for 1 min 30 sec, with 5 sec of additional extension at each cycle, and then a final step of extension at 72° C. for 7 min.

The amplification products were sequenced with the aid of the primers defined in table III below:

TABLE III

Primers used for the sequencing of the 5' region (ORF1a and ORF1b)

Names	Sequences (SEQ ID NO: 76 to 139)
S/L3/+/4932	5'-CCACACACAGCTTGTGGATA-3'
S/L4/+/6401	5'-CCGAAGTTGTAGGCAATGTC-3'
S/L4/+/6964	5'-TTTGGTGCTCCTTCTTATTG -3'
S/L4/-/6817	5'-CCGGCATCCAAACATAATTT-3'
S/L5/-/7633	5'-TGGTCAGTAGGGTTGATTGG-3'
S/L5/-/8127	5'-CATCCTTTGTGTCAACATCG-3'
S/L5/-/8633	5'-GTCACGAGTGACACCATCCT-3'
S/L5/+/7839	5'-ATGCGACGAGTCTGCTTCTA-3'
S/L5/+/8785	5'-TTCATAGTGCCTGGCTTACC-3'
S/L5/+/8255	5'-ATCTTGGCGCATGTATTGAC-3'
S/L6/-/9422	5'-TGCATTAGCAGCAACAACAT-3'
S/L6/-/9966	5'-TCTGCAGAACAGCAGAAGTG-3'
S/L6/-/10542	5'-CCTGTGCAGTTTGTCTGTCA-3'
S/L6/+/10677	5'-CCTTGTGGCAATGAAGTACA-3'
S/L6/+/10106	5'-ATGTCATTTGCACAGCAGAA-3'
S/L6/+/9571	5'-CTTCAATGGTTTGCCATGTT-3'
S/L7/-/11271	5'-TGCGAGCTGTCATGAGAATA-3'
S/L7/-/11801	5'-AACCGAGAGCAGTACCACAG-3'
S/L7/-/12383	5'-TTTGGCTGCTGTAGTCAATG-3'
S/L7/+/12640	5'-CTACGACAGATGTCCTGTGC-3'
S/L7/+/12088	5'-GAGCAGGCTGTAGCTAATGG-3'
S/L7/+/11551	5'-TTAGGCTATTGTTGCTGCTG-3'
S/L8/-/13160	5'-CAGACAACATGAAGCACCAC-3'
S/L8/-/13704	5'-CGCTGACGTGATATATGTGG-3'
S/L8/-14284	5'-TGCACAATGAAGGATACACC-3'
S/L8/+/14453	5'-ACATAGCTCGCGTCTCAGTT-3'
S/L8/+/13968	5'-GGCATTGTAGGCGTACTGAC-3'
S/L8/+/13401	5'-GTTTGCGGTGTAAGTGCAG-3'
S/L9/-15099	5'-TAGTGGCGGCTATTGACTTC-3'
S/L9/-15677	5'-CTAAACCTTGAGCCGCATAG-3'
S/L9/-16247	5'-CATGGTCATAGCAGCACTTG-3'
S/L9/+16323	5'-CCAGGTTGTGATGTCACTGAT-3'
S/L9/+15858	5'-CCTTACCCAGATCCATCAAG-3'
S/L9/+15288	5'-CGCAAACATAACACTTGCTG-3'
S/L10/-16914	5'-AGTGTTGGGTACAAGCCAGT-3'
S/L10/-17466	5'-GTTCCAAGGAACATGTCTGG-3'
S/L10/-18022	5'-AGGTGCCTGTGTAGGATGAA-3'
S/L10/+18245	5'-GGGCTGTCATGCAACTAGAG-3'

TABLE III-continued

Primers used for the sequencing of the 5' region (ORF1a and ORF1b)

Names	Sequences (SEQ ID NO: 76 to 139)
S/L10/+17663	5'-TCTTACACGCAATCCTGCTT-3'
S/L10/+17061	5'-TACCCATCTGCTCGCATAGT-3'
S/L11/-/18877	5'-GCAAGCAGAATTAACCCTCA-3'
S/L11/-19396	5'-AGCACCACCTAAATTGCATC-3'
S/L11/-20002	5'-TGGTCCCTTTGAAGGTGTTA-3'
S/L11/+20245	5'-TCGAACACATCGTTTATGGA-3'
S/L11/+/19611	5'-GAAGCACCTGTTTCCATCAT-3'
S/L11/+/19021	5'-ACGATGCTCAGCCATGTAGT-3'
SARS/L1/F3/+800	5'-GAGGTGCAGTCACTCGCTAT-3'
SARS/L1/F4/+1391	5'-CAGAGATTGGACCTGAGCAT-3'
SARS/L1/F5/+1925	5'-CAGCAAACCACTCAATTCCT-3'
SARS/L1/R3/-1674	5'-AAATGATGGCAACCTCTTCA-3'
SARS/L1/R4/-1107	5'-CACGTGGTTGAATGACTTTG-3'
SARS/L1/R5/-520	5'-ATTTCTGCAACCAGCTCAAC-3'
SARS/L2/F3/+2664	5'-CGCATTGTCTCCTGGTTTAC-3'
SARS/L2/F4/+3232	5'-GAGATTGAGCCAGAACCAGA-3'
SARS/L2/F5/+3746	5'-ATGAGCAGGTTGTCATGGAT-3'
SARS/L2/R3/-3579	5'-CTGCCTTAAGAAGCTGGATG-3'
SARS/L2/R4/-2991	5'-TTTCTTCACCAGCATCATCA-3'
SARS/L2/R5/-2529	5'-CACCGTTCTTGAGAACAACC-3'
SARS/L3/F3/+4708	5'-TCTTTGGCTGGCTCTTACAG-3'
SARS/L3/F4/+5305	5'-GCTGGTGATGCTGCTAACTT-3'
SARS/L3/F5/+5822	5'-CCATCAAGCCTGTGTCGTAT-3'
SARS/L3/R3/-5610	5'-CAGGTGGTGCAGACATCATA-3'
SARS/L3/R4/-4988	5'-AACATCAGCACCATCCAAGT-3'
SARS/L3/R5/-4437	5'-ATCGGACACCATAGTCAACG-3'

The sequences of the fragments L0 to L12 of the SARS-CoV strain derived from the sample recorded under the No. 031589 correspond respectively to the sequences SEQ ID NO: 41 to SEQ ID NO: 54 in the sequence listing appended as an annex. Among these sequences, only that corresponding to the fragments L5 contains a nucleotide difference in relation to the corresponding sequence of the isolate AY278741-Urbani. This t/c mutation at position 7919 results in a modification of the amino acid sequence of the corresponding protein, encoded by ORF1a: at position 2552, a valine (gtt codon; AY278741) is changed to alanine (gct codon) in the SARS-CoV strain 031589. By contrast, no mutation was identified in relation to the corresponding sequence of the isolate AY274119.3-Urbani. The other fragments do not exhibit differences in relation to the corresponding sequences of the isolates Tor2 and Urbani.

EXAMPLE 2

Production and Purification of the Recombinant N and S Proteins of the SARS-CoV Strain Derived from the Sample Recorded Under the Number 031589

The entire N protein and two polypeptide fragments of the S protein of the SARS-CoV strain derived from the sample recorded under the number 031589 were produced in *E. coil*, in the form of fusion proteins comprising an N- or C-terminal polyhistidine tag. In the two S polypeptides, the N- and C-terminal, hydrophobic sequences of the S protein (signal peptide: positions 1 to 13 and transmembrane helix: positions 1196 to 1218) were deleted whereas the β helix (positions 565 to 687) and the two motifs of the coiled-coil type (positions 895 to 980 and 1155 to 1186) of the S protein were preserved. These two polypeptides consist of: a long fragment (S_L) corresponding to positions 14 to 1193 of the amino acid sequence of the S protein and a short fragment (S_C) corresponding to positions 475 to 1193 of the amino acid sequence of the S protein.

1) Cloning of the cDNAs N, S_L and S_C into the Expression Vectors pIVEX2.3 and pIVEX2.4

The cDNAs corresponding to the N protein and to the S_L and S_C fragments were amplified by PCR under standard conditions, with the aid of the DNA polymerase Platinium Pfx® (INVITROGEN). The plasmids SRAS-N and SRAS-S were used as template and the following oligo-nucleotides as primers:

5'-CCCATATGTCTGATAATGGACCCCAATCAAAC-3' (Nsense, SEQ ID NO: 55)

5'-CCCCCGGGTGCCTGAGTTGAATCAGCAGAAGC-3' (N antisense, SEQ ID NO: 56)

5'-CCCATATGAGTGACCTTGACCGGTGCACCAC-3' (S_C sense, SEQ ID NO: 57)

5'-CCCATATGAAACCTTGCACCCCACCTGCTC-3' (S_L sense, SEQ ID NO: 58)

5'-CCCCCGGGTTTAATATATTGCTCATATTTTCCC-3' (S_C and S_L antisense, SEQ ID NO: 29).

The sense primers introduce an NdeI site (underlined) while the antisense primers introduce an XmaI or SmaI site (underlined). The 3 amplification products were column purified (QIAquick PCR Purification kit, QIAGEN) and cloned into an appropriate vector. The plasmid DNA purified from the 3 constructs (QIAFilter Midi Plasmid kit, QIAGEN) was verified by sequencing and digested with the enzymes NdeI and XmaI. The 3 fragments corresponding to the cDNAs N, S_L and S_C were purified on agarose gel and then inserted into the plasmids pIVEX2.3MCS (C-terminal polyhistidine tag) and pIVEX2.4d (N-terminal polyhistidine tag) digested beforehand with the same enzymes. After verification of the constructs, the 6 expression vectors thus obtained (pIV2.3N, pIV2.3S_C, pIV2.3S_L, pIV2.4N, pIV2.4S_C also called pIV2.4S_1, pIV2.4S_L) were then used, on the one hand to test the expression of the proteins in vitro, and on the other hand to transform the bacterial strain BL21(DE3)pDIA17 (NOVAGEN). These constructs encode proteins whose expected molecular mass is the following: pIV2.3N (47174 Da), pIV2.3S_C (82897 Da), pIV2.3S_L (132056 Da), pIV2.4N (48996 Da), pIV2.4S_1 (81076 Da) and pIV2.4S_L (133877 Da). Bacteria transformed with pIV2.3N were deposited at the CNCM on Oct. 23, 2003, under the number I-3117, and bacteria transformed with pIV2.4S_1 were deposited at the CNCM on Oct. 23, 2003, under the number I-3118.

2) Analysis of the Expression of the Recombinant Proteins In Vitro and In Vivo

The expression of recombinant proteins from the 6 recombinant vectors was tested, in a first instance, in a system in vitro (RTS100, Roche). The proteins produced in vitro, after incubation of the recombinant vectors pIVEX for 4 h at 30° C., in the RTS100 system, were analyzed by Western blotting with the aid of an anti-$(his)_6$ antibody coupled to peroxidase. The result of expression in vitro (FIG. **1**) shows that only the N protein is expressed in large quantities, regardless of the position, N- or C-terminal, of the polyhistidine tag. In a second step, the expression of the N and S proteins was tested in vivo at 30° C. in LB medium in the presence or in the absence of inducer (1 mM IPTG). The N protein is very well produced in this bacterial system (FIG. **2**) and is found mainly in a soluble fraction after lysis of the bacteria. By contrast, the long version of S (S_L) is very weakly produced and is completely insoluble (FIG. **3**). The short version (S_C) also exhibits a very weak solubility, but an expression level that is much higher than that of the long version. Moreover, the construct S_C fused with a polyhistidine tag at the C-terminal position has a smaller size than that expected. An immunodetection experiment with an anti-polyhistidine antibody has shown that this construct was incomplete. In conclusion, the two constructs, pIV2.3N and pIV2.4S_1, which express respectively the entire N protein fused with the C-terminal polyhistidine tag and the short S protein fused with the N-terminal polyhistidine tag, were selected in order to produce the two proteins in a large quantity so as to purify them. The plasmids pIV2.3N and pIV2.4S_1 were deposited respectively under the No. I-3117 and I-3118 at the CNCM, 25 rue du Docteur Roux, 75724 PARIS 15, on Oct. 23, 2003.

3) Analysis of the Antigenic Activity of the Recombinant Proteins

The antigenic activity of the N, S_L and S_C proteins was tested by Western blotting with the aid of two serum samples, obtained from the same patient infected with SARS-CoV, collected 8 days (M12) and 29 days (M13) after the onset of the SARS symptoms. The experimental protocol is as described in example 3. The results illustrated by FIG. **4** show (i) the seroconversion of the patient, and (ii) that the N protein possesses a higher antigenic reactivity than the short S protein.

4) Purification of the N protein from pIV2.3N

Several experiments for purifying the N protein, produced from the vector pIV2.3N, were carried out according to the following protocol. The bacteria BL21(DE3)pDIA17, transformed with the expression vector pIV2.3N, were cultured at 30° C. in 1 liter of culture medium containing 0.1 mg/ml of ampicillin, and induced with 1 mM IPTG when the cell density equivalent to A_{600}=0.8 is reached (about 3 hours). After 2 hours of culture in the presence of inducer, the cells were recovered by centrifugation (10 min at 5000 rpm), resuspended in the lysis buffer (50 mM NaH_2PO_4, 0.3 M NaCl, 20 mM imidazole, pH 8, containing the mixture of protease inhibitors Complete®, Roche), and lysed with the French press (12 000 psi). After centrifugation of the bacterial lysate (15 min at 12 000 rpm), the supernatant (50 ml) was deposited at a flow rate of 1 ml/min on a metal chelation column (15 ml) (Ni-NTA superflow, Qiagen), equilibrated with the lysis buffer. After washing the column with 200 ml of lysis buffer,

the N protein was eluted with an imidazole gradient (20→250 mM) in 10 column volumes. The fractions containing the N protein were assembled and analyzed by polyacrylamide gel electrophoresis under denaturing conditions followed by staining with Coomassie blue. The results illustrated by FIG. **5** show that the protocol used makes it possible to purify the N protein with a very satisfactory homogeneity (95%) and a mean yield of 15 mg of protein per liter of culture.

5) Purification of the S_c Protein from pIV2.4S_c (pIV2.4S_1)

The protocol followed for purifying the short S protein is very different from that described above because the protein is highly aggregated in the bacterial system (inclusion bodies). The bacteria BL21(DE3)pDIA17, transformed with the expression vector pIV2.4S_1, were cultured at 30° C. in 1 liter of culture medium containing 0.1 mg/ml of ampicillin, and induced with 1 mM IPTG when the cell density equivalent to A_{600}=0.8 is reached (about 3 hours). After 2 hours of culture in the presence of inducer, the cells were recovered by centrifugation (10 min at 5000 rpm), resuspended in the lysis buffer (0.1 M Tris-HCl, 1 mM EDTA, pH 7.5), and lysed with the French press (1200 psi). After centrifugation of the bacterial lysate (15 min at 12 000 rpm), the pellet was resuspended in 25 ml of lysis buffer containing 2% Triton X100 and 10 mM β-mercaptoethanol, and then centrifuged for 20 min at 12 000 rpm. The pellet was resuspended in 10 mM Tris-HCl buffer containing 7 M urea, and gently stirred for 30 min at room temperature. This final washing of the inclusion bodies with 7 M urea is necessary in order to remove most of the *E. coli* membrane proteins which co-sediment with the aggregated S_c protein. After a final centrifugation for 20 min at 12 000 rpm, the final pellet is resuspended in the 10 mM Tris-HCl buffer. The electrophoretic analysis of this preparation (FIG. **6**) shows that the short S protein may be purified with a satisfactory homogeneity (about 90%) from the inclusion bodies (insoluble extract).

EXAMPLE 3

Immunodominance of the N Protein

The reactivity of the antibodies present in the serum of patients suffering from atypical pneumopathy caused by the SARS-associated coronavirus (SARS-CoV), toward the various proteins of this virus, was analyzed by Western blotting under the conditions described below.

1) Materials

a) Lysate of Cells Infected with SARS-CoV

Vero E6 cells (2×10^6) were infected with SARS-CoV (isolate recorded under the number FFM/MA104) at a multiplicity of infection (M.O.I.) of 10^{-1} or 10^{-2} and then incubated in DMEM medium containing 2% FCS, at 35° C. in an atmosphere containing 5% CO_2. 48 hours later, the cellular lawn was washed with PBS and then lysed with 500 μl of loading buffer prepared according to Laemmli and containing β-mercaptoethanol. The samples were then boiled for 10 minutes and then sonicated for 3 times 20 seconds.

b) Antibodies

b_1) Serum from a Patient Suffering from Atypical Pneumopathy

The serum designated by a reference at the National Reference Center for Influenza Viruses (Northern region) under the No. 20033168 is that from a French patient suffering from atypical pneumopathy caused by SARS-CoV collected on day 38 after the onset of the symptoms; the diagnosis of SARS-CoV infection was performed by nested RT-PCR and quantitative PCR.

b_2) Monospecific Rabbit Polyclonal sera Directed Against the N Protein or the S Protein

The sera are those produced from the recombinant N and S_c proteins (example 2), according to the immunization protocol described in example 4; they are the rabbit P13097 serum (anti-N serum) and the rabbit P11135 serum (anti-S serum).

2) Method

20 μl of lysate of cells infected with SARS-CoV at M.O.I. values of 10^{-1} and 10^{-2} and, as a control, 20 μl of a lysate of noninfected cells (mock) were separated on 10% SDS polyacrylamide gel and then transferred onto a nitrocellulose membrane. After blocking in a solution of PBS/5% milk/0.1% Tween and washing in PBS/0.1% Tween, this membrane was hybridized overnight at 4° C. with: (1) the immune serum No. 20033168 diluted 1/300, 1/1000 and 1/3000 in the buffer PBS/1% BSA/0.1% Tween, (ii) the rabbit P13097 serum (anti-N serum) diluted 1/50 000 in the same buffer and (iii) the rabbit P11135 serum (anti-S serum) diluted 1/10 000 in the same buffer. After washing in PBS/Tween, a secondary hybridization was performed with the aid of either sheep polyclonal antibodies directed against the heavy and light chains of human G immunoglobulins and coupled with peroxidase (NA933V, Amersham), or of donkey polyclonal antibodies directed against the heavy and light chains of the rabbit G immunoglobulins and coupled with peroxidase (NA934V, Amersham). The bound antibodies were visualized with the aid of the ECL+ kit (Amersham) and of Hyperfilm MP autoradiography films (Amersham). A molecular mass ladder (kDa) is presented in the figure.

3) Results

FIG. **7** shows that three polypeptides of apparent molecular mass 35, 55 and 200 kDa are specifically detected in the extracts of cells infected with SARS-CoV.

In order to identify these polypeptides, two other immunoblots (FIG. **8**) were prepared on the same samples and under the same conditions with rabbit polyclonal antibodies specific for the nucleoprotein N (rabbit P13097, FIG. **8**A) and for the spicule protein S (rabbit P11135, FIG. **8**B). This experiment shows that the 200 kDa polypeptide corresponds to the SARS-CoV spicule glycoprotein S, that the 55 kDa polypeptide corresponds to the nucleoprotein N while the 35 kDa polypeptide probably represents a truncated or degraded form of N.

The data presented in FIG. **7** therefore show that the serum 20033168 strongly reacts with N and a lot more weakly with the SARS-CoV S since the 35 and 55 kDa polypeptides are visualized in the form of intense bands for 1/300, 1/1000 and 1/3000 dilutions of the immunoserum whereas the 200 kDa polypeptide is only weakly visualized for a dilution of 1/300. It is also possible to note that no other SARS-CoV polypeptide is detected for dilutions greater than 1/300 of the serum 20033168.

This experiment indicates that the antibody response specific for the SARS-CoV N dominates the antibody responses specific for the other SARS-CoV polypeptides and in particular the antibody response directed against the S glycoprotein. It indicates an immunodominance of the nucleoprotein N during human infections with SARS-CoV.

EXAMPLE 4

Preparation of Monospecific Polyclonal Antibodies Directed Against the SRAS-Associated Coronavirus (SARS-CoV) N and S Proteins

1) Materials and Method

Three rabbits (P13097, P13081, P13031) were immunized with the purified recombinant polypeptide corresponding to the entire nucleoprotein (N), prepared according to the protocol described in example 2. After a first injection of 0.35 mg per rabbit of protein emulsified in complete Freund's adjuvant (intradermal route), the animals received 3 booster injections at 3 and then 4 weeks' interval, of 0.35 mg of recombinant protein emulsified in incomplete Freund's adjuvant.

Three rabbits (P11135, P13042, P14001) were immunized with the recombinant polypeptide corresponding to the short fragment of the S protein (S_c) produced as described in example 2. As this polypeptide is found mainly in the form of inclusion bodies in the bacterial cytoplasm, the animals received 4 intradermal injections at 3-4 weeks' interval of a preparation of inclusion bodies corresponding to 0.5 mg of recombinant protein emulsified in incomplete Freund's adjuvant. The first 3 injections were made with a preparation of inclusion bodies prepared according to the protocol described in example 2, while the fourth injection was made with a preparation of inclusion bodies which were prepared according to the protocol described in example 2 and then purified on sucrose gradient and washed in 2% Triton X100.

For each rabbit, a preimmune (p.i.) serum was prepared before the first immunization and an immune serum (I.S.) 5 weeks after the fourth immunization.

In a first instance, the reactivity of the sera was analyzed by ELISA test on preparations of recombinant proteins similar to those used for the immunizations; the ELISA tests were carried out according to the protocol and with the reagents as described in example 6.

In a second instance, the reactivity of the sera was analyzed by preparing an immunoblot (Western blot) of a lysate of cells infected with SARS-CoV, according to the protocol as described in example 3.

2) Results

The ELISA tests (FIG. **9**) demonstrate that the preparations of recombinant N protein and of inclusion bodies of the short fragment of the S protein (S_c) are immunogenic in animals and that the titer of the immune sera is high (more than 1/25 000).

The immunoblot (FIG. **8**) shows that the rabbit P13097 immune serum recognizes two polypeptides present in the lysates of cells infected with SARS-CoV: a polypeptide whose apparent molecular mass (50-55 kDa based on experiments) is compatible with that of the nucleo-protein N (422 residues, predicted molecular mass of 46 kDa) and a polypeptide of 35 kDa, which probably represents a truncated or degraded form of N.

This experiment also shows that the rabbit P11135 serum mainly recognizes a polypeptide whose apparent molecular mass (180-220 kDa based on experiments) is compatible with a glycosylated form of S (1255 residues, nonglycosylated polypeptide chain of 139 kDa), as well as lighter polypeptides, which probably represent truncated and/or nonglycosylated forms of S.

In conclusion, all these experiments demonstrate that the recombinant polypeptides expressed in *E. coli* and corresponding to the SARS-CoV N and S proteins make it possible to induce, in animals, polyclonal antibodies capable of recognizing the native forms of these proteins.

EXAMPLE 5

Preparation of Monospecific Polyclonal Antibodies Directed Against the SARS-Associated Coronavirus (SARS-CoV) M and E Proteins

1) Analysis of the Structure of the M and E Proteins

a) E Protein

The structure of the SARS-CoV E protein (76 amino acids) was analyzed in silico, with the aid of various software packages such as signalP v1.1, NetNGlyc 1.0, THMM 1.0 and 2.0 (Krogh et al., 2001, J. Mol. Biol., 305(3):567-580) or alternatively TOPPRED (von Heijne, 1992, J. Mol. Biol. 225, 487-494). The analysis shows that this nonglycosylated polypeptide is a type 1 membrane protein, containing a single transmembrane helix (aa 12-34 according to THMM), and in which the majority of the hydrophilic domain (42 residues) is located at the C-terminal end and probably inside the viral particle (endodomain). It is possible to note an inversion in the topology predicted by versions 1.0 (N-ter is external) and 2.0 (N-ter is internal) of the THMM software, but that other algorithms, in particular TOPPRED and THUMBUP (Zhou et Zhou, 2003, Protein Science 12:1547-1555) confirm an external location of the N-terminal end of E.

b) M Protein

A similar analysis carried out on the SARS-CoV M protein (221 amino acids) shows that this polypeptide does not possess a signal peptide (according to the software signalP v1.1) but three transmembrane domains (residues 15-37, 50-72, 77-99 according to THMM2.0) and a large hydrophilic domain (aa 100-221) located inside the viral particle (endodomain). It is probably glycosylated on the asparagine at position 4 (according to NetNGlyc 1.0).

Thus, in agreement with the experimental data known for the other coronaviruses, it is remarkable that the two M and E proteins exhibit endodomains corresponding to the majority of the polypeptides and of the ectodomains that are very small in size.

- The ectodomain of E probably corresponds to residues 1 to 11 or 1 to 12 of the protein: MYSFVSEETGT(L), SEQ ID NO: 70. Indeed, the probability associated with the transmembrane location of residue 12 is intermediate (0.56 according to THMM 2.0).
- The ectodomain of M probably corresponds to residues 2 to 14 of the protein: ADNGTITVEELKQ, SEQ ID NO: 69. Indeed, the N-terminal methionine of M is very probably cleaved from the mature polypeptide because the residue at position 2 is an alanine (Varshaysky, 1996, 93:12142-12149).

Moreover, the analysis of the hydrophobicity (Kyte & Doolittle Hopp & Woods) of the E protein demonstrates that the C-terminal end of the endodomain of E is hydrophilic and therefore probably exposed at the surface of this domain. Thus, a synthetic peptide corresponding to this end is a good immunogenic candidate for inducing, in animals, antibodies directed against the endodomain of E. Consequently, a peptide corresponding to 24 C-terminal residues of E was synthesized.

2) Preparation of Antibodies Directed Against the Ectodomain of the M and E Proteins and the Endodomain of the E Protein

The peptides M2-14 (ADNGTITVEELKQ, SEQ. ID NO: 69), E1-12 (MYSFVSEETGTL, SEQ ID NO: 70) and E53-76 (KPTVYVYSRV KNLNSSEGVP DLLV, SEQ ID NO: 71) were synthesized by Neosystem. They were coupled with KLH (Keyhole Limpet Hemocyanin) with the aid of MBS (m-maleimido-benzoyl-N-hydroxysuccinimide ester) via a

cysteine added during the synthesis either at the N-terminus of the peptide (case for E53-76) or at the C-terminus (case of M2-14 and E1-12).

Two rabbits were immunized with each of the conjugates, according to the following immunization protocol: after a first injection of 0.5 mg of peptide coupled with KLH and emulsified in complete Freund's adjuvant (intradermal route), the animals receive 2 to 4 booster injections at 3 or 4 weeks' interval of 0.25 mg of peptide coupled to KLH and emulsified in incomplete Freund's adjuvant.

For each rabbit, a preimmune (p.i.) serum was prepared before the first immunization and an immune serum (I.S.) is prepared 3 to 5 weeks after the booster injections.

The reactivity of the sera was analyzed by Western blotting with the aid of extracts of cells infected with SARS-CoV (FIG. **43**B) or with the aid of extracts of cells infected with a recombinant vaccinia virus expressing the protein E (VV-TG-E, FIG. **43**A) or M (VV-TN-M, FIG. **43**C) of the SARS-CoV 031589 isolate.

The immune sera of the rabbits 22234 and 22240, immunized with the conjugate KLH-E53-76, recognize a polypeptide of about 9 to 10 kD, which is present in the extracts of cells infected with SARS-CoV but absent from the extracts, of noninfected cells (FIG. **43**B). The apparent mass of this polypeptide is compatible with the predicted mass of the E protein, which is 8.4 kD. Similarly, the immune serum of the rabbit 20047, immunized with the conjugate KLH-E1-12, recognizes a polypeptide present in the extracts of cells infected with the VV-TG-E virus, whose apparent molar mass is compatible with that of the E protein (FIG. **43**A).

The immune serum of the rabbits 20013 and 20080, immunized with the conjugate KLH-M2-14, recognizes a polypeptide present in the extracts of cells infected with the VV-TN-M virus (FIG. **43**C), whose apparent molar mass (about 18 kD) is compatible with that of the glycoprotein M, which is 25.1 kD and has a high iso-electric point (9.1 for the naked polypeptide).

These results demonstrate that the peptides E1-12 and E53-76, on the one hand, and the peptide M2-14, on the other hand, make it possible to induce, in animals, polyclonal antibodies capable of recognizing the native forms of the SARS-CoV E and M proteins, respectively.

EXAMPLE 6

Analysis of the ELISA Reactivity of the Recombinant N Protein Toward Sera from Patients Suffering from SARS

1) Materials

The antigen used to prepare the solid phases is the purified recombinant nucleoprotein N prepared according to the protocol described in example 2.

The sera to be tested (table IV) were chosen on the basis of the results of analysis of their reactivity by immunofluorescence (IF-SARS titer), toward cells infected with SARS-CoV.

TABLE IV

Sera tested by ELISA

Reference	Serum No.	Type of serum	Date of the serum***	IF-SARS titer
3050	A	Control	na*	nt**
3048	B	Control	na	nt
033168	D	Patient 1-SARS	Apr. 27, 2003 (D38)	320
033397	E	Patient-1 SARS	May 11, 2005 (D52)	320
032632	F	Patient-2 SARS	Mar. 21, 2003 (D17)	2500
032791	G	Patient-3 SARS	Apr. 04, 2003 (D3)	<40
033258	H	Patient-3 SARS	Apr. 28, 2003 (D27)	160

*na: not applicable.

**nt: not tested.

***the dates indicated correspond to the number of days after the onset of the SARS symptoms.

2) Method

The N protein (100 μl) diluted at various concentrations in 0.1 M carbonate buffer, pH 9.6 (1, 2 or 4 μg/ml) is distributed into the wells of ELISA plates, and then the plates are incubated overnight at laboratory temperature. The plates are washed with PBS-Tween buffer saturated with PBS-skimmed milk-sucrose (5%) buffer. The test sera (100 μl), diluted beforehand (1/50, 1/100, 1/200, 1/400, 1/800, 1/1600 and 1/3200) are added and then the plates are incubated for 1 h at 37° C. After 3 washings, the peroxidase-labeled anti-human IgG conjugate (reference 209-035-098, JACKSON) diluted 1/18 000 is added and then the plates are incubated for 1 h at 37° C. After 4 washings, the chromogen (TMB) and the substrate (H_2O_2) are added and the plates are incubated for 30 min at room temperature, protected from light. The reaction is then stopped and then the absorbance at 450 nm is measured with the aid of an automated reader.

3) Results

The ELISA tests (FIG. **10**) demonstrate that the recombinant N protein preparation is specifically recognized by the antibodies of sera from patients suffering from SARS collected in the late phase of the infection (≧17 days after the onset of the symptoms) whereas it is not significantly recognized by the antibodies of a patient's serum collected in the early phase of the infection (3 days after the onset of the symptoms) or by control sera from subjects not suffering from SARS.

EXAMPLE 7

ELISA Tests Prepared for a Very Specific and Sensitive Detection of a SARS-Associated Coronavirus Infection, from Sera of Patients

1) Indirect ELISA IgG Test

a) Reagents

Preparation of the Plates

The plates are sensitized with a solution of N protein at 2 μg/ml in a 10 mM PBS buffer, pH 7.2, phenol red at 0.25 ml/l. 100 μl of solution are deposited in the wells and left to incubate at room temperature overnight. Saturation is obtained by prewashing in 10 mM PBS/0.1% Tween buffer, followed by washing with a saturation solution PBS, 25% milk/sucrose.

Diluent Sera

Buffer 0.48 g/l TRIS, 10 mM PBS, 3.7 g/l EDTA, 15% v/v milk, pH 6.7

Diluent Conjugate

Citrate buffer (15 g/l), 0.5% Tween, 25% bovine serum, 12% NaCl, 6% v/v skimmed milk pH 6.5

Conjugate

50× anti-human IgG conjugate, marketed by Bio-Rad: Platelia *H. pylori* kit ref 72778

Other Solutions:

Washing solution R2, solutions for visualizing with TMB R8 diluent, R9 chromogen, R10 stopping solution: reagents marketed by Bio-Rad (e.g.: Platelia *pylori* kit, ref 72778)

b) Procedure

Dilute the sera 1/200 in the sample diluent

Distribute 100 µl/well

Incubation 1 h at 37° C.

3 washings in 10× WASHING solution R2 diluted beforehand 10-fold in demineralized water (i.e., 1× washing solution)

Distribute 100 µl of conjugate (50× conjugate to be diluted immediately before use in the diluent conjugate provided)

Incubation 1 h at 37° C.

4 washings in 1× washing solution

Distribute 200 µl/well of visualization solution (to be diluted immediately before use e.g.: 1 ml of R9 in 10 ml of R8)

Incubation for 30 min at room temperature in the dark

Stop the reaction with 100 µl/well of R10

READING at 450/620 nm

The results can be interpreted by taking a THRESHOLD serum giving a response above which the sera tested would be considered as positive. This serum is chosen and diluted so as to give a significantly higher signal than the background noise.

2) Double Epitope Elisa Test

Reagents

Preparation of the Plates

The plates are sensitized with a solution of N protein at 1 µg/ml in a 10 mM PBS buffer, pH 7.2, phenol red at 0.25 ml/l. 100 µl of solution are deposited in the wells and left to incubate at room temperature overnight. Saturation is obtained by prewashing in 10 mM PBS/0.1% Tween buffer, followed by washing with a saturation solution 10 mM PBS, 25% (V/V) milk.

Diluent sera and Conjugate

Buffer 50 mM TRIS saline, pH 8, 2% milk

Conjugate

This is the purified recombinant N protein coupled with peroxidase according to the Nakane protocol (Nakane P. K. and Kawaoi A.; (1974): *Peroxydase-labeled antibody, a new method of conjugation. The Journal of Histochemistry and Cytochemistry* Vol. 22, N) 23, pp. 1084-1091), in respective molar ratios 1/2. This ProtN POD conjugate is used at a concentration of 2 µg/ml in serum/conjugate diluent.

Other Solutions:

Washing solution R2, solutions for visualization with TMB R8, diluent, R9 chromogen, R10 stopping solution: reagents marketed by Bio-Rad (e.g. Platelia pylori kit, ref 72778).

b) Procedure

1st step in "predilution" plate

Dilute each serum 1/5 in the predilution plate (48 µl of diluent+12 µl of serum).

After having diluted all the sera, distribute 60 µl of conjugate.

Where appropriate, the serum+conjugate mix is left to incubate.

2nd step in "reaction" plate

Transfer 100 µl of mixture/well into the reaction plate

Incubation 1 h 37° C.

5 washings in 10× WASHING solution R2 diluted 10-fold beforehand in demineralized water (→1× washing solution)

Distribute 200 µl/well of visualization solution (to be diluted immediately before use e.g.: 1 ml of R9 in 10 ml of R8)

Incubation 30 min at room temperature and protected from light

Stop the reaction with 100 µl/well of R10

READING at 450/620 nm

Likewise as for the indirect ELISA test, the results can be interpreted using a "threshold value" serum. Any serum having a response greater than the threshold value serum will be considered as positive.

2) Results

The sera of patients classified as probable cases of SARS from the French hospital of Hanoi, Vietnam or in relation with the French hospital of Hanoi (JYK) were analyzed using the indirect IgG-N test and the double epitope N test.

The results of the indirect IgG-N test (FIGS. **14** and **15**) and double epitope N test (FIGS. **16** and **17**) show an excellent correlation between them and with an indirect ELISA test comparing the reactivity of the sera toward a lysate of VeroE6 cells infected or not infected with SARS-CoV (ELISA-SARS-CoV lysate; see table V below). All the sera collected 12 days or more after the onset of the symptoms were found to be positive, including in patients for whom it had not been possible to document the SARS-CoV virus infection by analyzing respiratory samples by RT-PCR, probably because of a sample being collected too late during the infection (≧D12). In the case of the patient TTH for whom a nasal sample collected on D7 was found to be negative by RT-PCR, the quality of the sample may be in question.

Some sera were found to be negative whereas the presence of SARS-CoV was detected by RT-PCR. They are in all cases early sera collected less than 10 days after the onset of the symptoms (e.g.: serum #032637). In the case of a patient PTTH (serum #032673), only a suspicion of SARS was raised at the time the samples were collected.

In conclusion, the indirect IgG-N and N-double epitope serological tests make it possible to document the SARS-CoV infection in all the patients for the sera collected 12 days or more after the infection.

TABLE V

Results of the ELISA tests

Sample Num	Patient	Day	PCR-SARS (1)	ELISA SARS-CoV lysate (2)	IgG-N (2nd series)	2Xepitope (2nd series)
033168	JYK	38	POS	+++	>5000	NT
033597	J K	74	POS	NT	≈5000	NT
032552	VTT	8	NEG-D3&D8&D12	NEG	<200	<5
032544	CTP	16	NEG D16&D20	++	>5000	>>20
032546	CJF	15	NEG D15&D19	++	>5000	>>20

TABLE V-continued

Results of the ELISA tests

Sample Num	Patient	Day	PCR-SARS (1)	ELISA SARS-CoV lysate (2)	IgG-N (2nd series)	2Xepitope (2nd series)
032548	PTL	17	NEG D17&D21	++	>5000	>>20
032550	NTH	17	NEG- D17&D21	++	>5000	>>20
032553	VTT	8	NEG- D3&D8&D12	NEG	<200	<5
032554	NTBV	4	POS	NEG	<200	<5
032555	NTBV	4	POS	NEG	<200	
032564	NTP	15	POS	++	>5000	>>20
032629	NVH	4	POS	NEG	<200	<5
032631	BTTX	9	POS	NEG	<200	<5
032635	NHH	4	POS	NEG	<200	<5
032637	NHB	10	POS	NEG	<200	<5
032642	BTTX	9	POS	NEG	<200	<5
032643	LTDH	1	POS	NEG	<200	<5
032644	NTBV	4	POS	NEG	<200	<5
032646	TTH	12	NEG D7&D12&D16	++	>5000	>>20
032647	DTH	17	NEG D17&D21	++	>5000	>>20
032648	NNT	15	NEG D15&D19	++	>5000	>>20
032649	PTH	17	NEG D17&D21	++	>5000	>>20
032672	LVV	16	NEG D16&D20	+	>5000	>>20
032673	PTTH	NA	NEG	NEG	<200	<5
032674	PNB	17	NEG D17&D21	++	>5000	>>20
032682	VTH	12	NEG D12&D16	++	>5000	>>20
032683	DTV	17	NEG D17&D21	+	>1000	>>20

Remarks:
(1): The RT-PCR analyses were carried out by nested RT-PCR BNI, LC Artus and LC-N on nasal or pharyngeal swabs; POS means that at least one sample was found to be positive in this patient.
(2): The reactivity of the sera in the ELISA test using a lysate of cells infected with SARS-CoV was classified as very highly reactive (+++), highly reactive (++), reactive (+) and negative according to the OD value obtained at the dilutions tested.

EXAMPLE 8

Detection of SARS-Associated Coronavirus (SARS-CoV) by RT-PCR

1) Real Time Development of RT-PCR Conditions with the Aid of Primers Specific for the Gene for the Nucleocapsid Protein—"Light Cycler N" Test

a) Design of the Primers and Probes

The primers and probes were designed from the sequence of the genome of the SARS-CoV strain derived from the sample recorded under the number 031589, with the aid of the programme "Light Cycler Probe Design (Roche)". Thus, the following two series of primers and probes were selected:

series 1 (SEQ ID NO: 60, 61, 64, 65):

```
sense primer: N/+/28507:
5'-GGC ATC GTA TGG GTT G-3' [28507-28522]

antisense primer: N/-/28774:
5'-CAG TTT CAC CAC CTC C-3' [28774-28759]

probe 1:
5'-GGC ACC CGC AAT CCT AAT AAC AAT GC-fluorescein

3' [28561-28586]
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-continued

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probe 2:
5' Red705-GCC ACC GTG CTA CAA CTT CCT-phosphate

[28588-28608]
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series 2 (SEQ ID NO: 62, 63, 66, 67)

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sense primer: N/+/28375:
5'-GGC TAC TAC CGA AGA G-3' [28375-28390]

antisense primer: N/-/28702:
5'-AAT TAC CGC GAC TAC G-3' [28702-28687]

probe 1: SARS/N/FL:
5'-ATA CAC CCA AAG ACC ACA TTG GC-fluorescein 3'

[28541-28563]

probe 2: SARS/N/LC705:
5' Red705-CCC GCA ATC CTA ATA ACA ATG CTG C-

phosphate 3' [28565-28589]
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b) Analysis of the Efficacy of the Two Primer Pairs

In order to test the respective efficacy of the two pairs of primers, an RT-PCR amplification was carried out on a synthetic RNA corresponding to nucleotides 28054-29430 of the

genome of the SARS-CoV strain derived from the sample recorded under the number 031589 and containing the sequence of the N gene.

More specifically:

This synthetic RNA was prepared by in vitro transcription with the aid of the T7 phage RNA polymerase, of a DNA template obtained by linearization of the plasmid SRAS-N with the enzyme Bam H1. After eliminating the DNA template by digestion with the aid of DNAse 1, the synthetic RNAs are purified by a phenol-chloroform extraction, followed by two successive precipitations in ammonium acetate and isopropanol. They are then quantified by measuring the absorbance at 260 nm and their quality is checked by the ratio of the absorbances at 260 and 280 nm and by agarose gel electrophoresis. Thus, the concentration of the synthetic RNA preparation used for these studies is 1.6 mg/ml, which corresponds to 2.1×10^{15} copies/ml of RNA.

Decreasing quantities of synthetic RNA were amplified by RT-PCR with the aid of the "Superscript™ One-Step RT-PCR with Platinum® Taq" kit and the pairs of primers No. 1 (N/+/28507, N/−/28774) (FIG. 1A) and No. 2 (N/+/28375, N/−/28702) (FIG. 1B), according to the supplier's instructions. The amplification conditions used are the following: the cDNA was synthesized by incubation for 30 min at 45° C., 15 min at 55° C. and then 2 min at 94° C. and it was then amplified by 5 cycles comprising: a step of denaturation at 94° C. for 15 sec, a step of annealing at 45° C. for 30 sec and, then a step of extension at 72° C. for 30 sec, followed by 35 cycles comprising: a step of denaturation at 94° C. for 15 sec, a step of annealing at 55° C. for 30 sec and then a step of extension at 72° C. for 30 sec, with 2 sec of additional extension at each cycle, and a final step of extension at 72° C. for 5 min. The amplification products obtained were then kept at 10° C.

The results presented in FIG. **11** show that the pair of primers No. 2 (N/+/28375, N/−/28702) makes it possible to detect up to 10 copies of RNA (band of weak intensity) or 10^2 copies (band of good intensity) against 10^4 copies for the pair of primers No. 1 (N/+/28507, N/−/28774). The amplicons are respectively 268 bp (pair 1) and 328 bp (pair 2).

c) Development of Real Time RT-PCR

A real time RT-PCR was developed with the aid of the pair of primers No. 2 and of the pair of probes consisting of SRAS/N/FL and SRAS/N/LC705 (FIG. **2**).

The amplification was carried out on a LightCycler™ (Roche) with the aid of the "Light Cycler RNA Amplification Kit Hybridization Probes" kit (reference 2 015 145, Roche) under the following optimized conditions. A reaction mixture containing: H_2O (6.8 µl), 25 mM $MgCl_2$ (0.8 µl, 4 µM Mg2+ final), 5× reaction mixture (4 µl), 3 µm probe SRAS/N/FL (0.5 µl, 0.075 µM final), 3 µm probe SRAS/N/LC705 (0.5 µl, 0.075 µM final), 10 µM primer N/+/28375 (1 µl, 0.5 µM final), 10 µM primer N/−/28702 (1 µl, 0.5 µM final), enzyme mixture (0.4 µl) and sample (viral RNA, 5 µl) was amplified according to the following program:

Reverse transcription: 50° C. 10:00 min analysis mode: none

Denaturation: 95° C. 30 sec×1 analysis mode: none

Amplification: 95° C. 2 sec

50° C. 15 sec analysis mode: quantification*{×45

72° C. 13 sec thermal ramp 2.0° C./sec}

* The fluorescence is measured at the end of the annealing and at each cycle (in SINGLE mode).

Annealing: 40° C. 30 sec×1 analysis mode: none

The results presented in FIG. **12** show that this real time RT-PCR is very sensitive since it makes it possible to detect 10^2 copies of synthetic RNA in 100% of the 5 samples analyzed (29/29 samples in 8 experiments) and up to 10 copies of RNA in 100% of the 5 samples analyzed (40/45 samples in 8 experiments). It also shows that this RT-PCR makes it possible to detect the presence of the SARS-CoV genome in a sample and to quantify the number of genomes present. By way of example, the viral RNA of a SARS-CoV stock cultured on Vero E6 cells was extracted with the aid of the "Qiamp viral RNA extraction" kit (Qiagen), diluted to 0.05×10^{-14} and analyzed by real time RT-PCR according to the protocol described above; the analysis presented in FIG. **12** shows that this virus stock contains 6.5×10^9 genome-equivalents/ml (geq/ml), which is entirely similar to the 1.0×10^{10} geq/ml value measured with the aid of the "RealArt™ HPA-Coronavirus LC RT PCR Reagents" kit marketed by Artus.

2) Development of Nested RT-PCR Conditions Targeting the Gene for RNA Polymerase—"CDC (Centers for Disease Control and Prevention)/IP Nested RT-PCR" Test

a) Extraction of the Viral RNA

Clinical sample: QIAmp viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions, or an equivalent technique. The RNA is eluted in a volume of 60 µl.

b) "SNE/SAR" Nested RT-PCR

First step: "SNE" coupled RT-PCR

The Invitrogen "Superscript™ One-Step RT-PCR with Platinum® Taq" kit was used, but the "Titan" kit from Roche Boehringer can be used in its place with similar results.

Oligonucleotides:

```
SNE-S1    5' GGT TGG GAT TAT CCA AAA TGT GA 3'

SNE-AS1   5' GCA TCA TCA GAA AGA ATC ATC ATG 3'
```

→Expected size: 440 bp

1. Prepare a mix:

H2O	6.5 µl
Reaction mix 2X	12.5 µl
Oligo SNE-S1 50 µM	0.2 µl
Oligo SNE-AS1 50 µM	0.2 µl
RNAsin 40 U/µl	0.12 µl
RT/Platinum Taq mix	0.5 µl

2. To 20 µl of the mix, add 5 µl of RNA and carry out the amplification on a thermocycler (ABI 9600 conditions):

2.1	45° C.	30 min.	
	55° C.	15 min.	
	94° C.	2 min.	
2.2.	94° C.	15 sec.	} × 5 cycles
	45° C.	30 sec.	
	72° C.	30 sec.	
2.3.	94° C.	15 sec.	} × 35 cycles
	55° C.	30 sec.	
	72° C.	30 sec. + 2 sec./cycle	
2.4.	72° C.	5 min.	
2.5	10° C.	∞	

Storage at +4° C.

The RNAsin (N2511/N2515) from Promega was used as RNase inhibitors.

Synthetic RNAs served as positive control. As the control, 10^3, 10^2 and 10 copies of synthetic RNA R_{SNE} were amplified in each experiment.

Second step: "SAR" nested PCR
Oligonucleotides:

SAR1-S 5' CCT CTC TTG TTC TTG CTC GCA 3'

SAR1-AS 5' TAT AGT GAG CCG CCA CAC ATG 3'

→Expected size: 121 bp
1. Prepare a mix:

H2O	35.8 µl
Taq buffer 10X	5 µl
$MgCl_2$ 25 mM	4 µl
Mix dNTPs 5 mM	2 µl
Oligo SAR1-S 50 µM	0.5 µl
Oligo SAR1-AS 50 µM	0.5 µl
Taq DNA pol 5 U/µl	0.25 µl

AmpliTaq DNA Pot from Applied Biosystems was used (10× buffer without $MgCl_2$, ref 27216601).

2. To 48 µl of the mix, add 2 µl of the product from the first PCR and carry out the amplification (ABI 9600 conditions):

2.1.	94° C.	2 min.	
2.2.	94° C.	30 sec.	} × 5 cycles
	45° C.	45 sec.	
	72° C.	30 sec.	
2.3.	94° C.	30 sec.	} × 35 cycles
	55° C.	30 sec.	
	72° C.	30 sec. + 1 sec./cycle	
2.4.	72° C.	5 min.	
2.5	10° C.	∞	

3. Analyze 10 µl of the reaction product on "low-melting" gel (Seakem GTG type) containing 3% agarose.

The sensitivity of the nested test is routinely, under the conditions described, 10 copies of RNA.

4. The fragments can then be purified on QIAquick PCR kit (QIAGEN) and sequenced with the oligos SAR1-S and SAR1-AS.

3) Detection of the SARS-CoV RNA by PCR from Respiratory Samples

a) First Comparative Study

A comparative study was carried out on a series of respiratory samples received by the National Reference Center for the Influenza Virus (Northern region) and likely to contain SARS-CoV. To do this, the RNA was extracted from the samples with the aid of the "Qiamp viral RNA extraction" kit (Qiagen) and analyzed by real time RT-PCR, on the one hand with the aid of the pairs of primers and probes of the No. 2 series under the conditions described above on the one hand, and on the other hand with the aid of the kit "LightCycler SARS-CoV quantification kit" marketed by Roche (reference 03 604 438). The results are summarized in table VI below. They show that 18 of the 26 samples are negative and 5 of the 26 samples are positive for the two kits, while one sample is positive for the Roche kit alone and two for the "series 2" N reagents alone. Additionally, for 3 samples (20032701, 20032712, 20032714) the quantities of RNA detected are markedly higher with the reagents (probes and primers) of the No. 2 series. These results indicate that the "series 2" N primers and probes are more sensitive for the detection of the SARS-CoV genome in biological samples than those of the kit currently available.

TABLE VI

Real time RT-PCR analysis of the RNAs extracted from a series of samples from 5 patients with the aid of the pairs of primers and probes of the No. 2 series ("series 2" N) or of the kit "Lightcycler SARS-CoV quantification kit" (Roche). The type of sample is indicated as well as the number of copies of viral genome measured in each of the two tests. NEG: negative RT-PCR.

Sample No.	Patient	Type of sample	ROCHE KIT	"Series 2" N
20033082	K	nasal	NEG	NEG
20033083	K	pharyngeal	NEG	NEG
20033086	K	nasal	NEG	NEG
20033087	K	pharyngeal	NEG	NEG
20032802	M	nasal	NEG	NEG
20032803	M	expectoration	NEG	NEG
20032806	M	nasal or pharyngeal	NEG	NEG
20031746ARN2	C	pharyngeal	NEG	NEG
20032711	C	nasal or pharyngeal	39	NEG
20032910	B	nasal	NEG	NEG
20032911	B	pharyngeal	NEG	NEG
20033356	V	expectoration	NEG	NEG
20033357	V	expectoration	NEG	NEG
20031725	K	endotracheal asp.	NEG	150
20032657	K	endotracheal asp.	NEG	NEG
20032698	K	endotracheal asp.	NEG	NEG
20032720	K	endotracheal asp.	3	5
20033074	K	stools	115	257
20032701	M	pharyngeal	443	1676
20032702	M	expectoration	NEG	249
20031747ARN2	C	pharyngeal	NEG	NEG
20032712	C	unknown	634	6914
20032714	C	pharyngeal	17	223
20032800	B	nasal	NEG	NEG
20033353	V	nasal	NEG	NEG
20033384	V	nasal	NEG	NEG

b) Second Comparative Study

The performance of various nested RT-PCR and real time RT-PCR methods were then compared for 121 respiratory samples from possible cases of SARS at the French hospital in Hanoi, Vietnam, taken between the 4th and the 17th day after the onset of the symptoms. Among these samples, 14 were found to be positive during a first test using the nested RT-PCR method targeting ORF1b (encoding replicase) as described initially by Bernhard Nocht Institute (BNI nested RT-PCR). Information relating to this test is available on the internet, at the address www.15.bni-hamburq.de/bni2/neu2/getfile.acgi?area_engl=diagnostics&pid=4112.

The various tests compared in this study are:

- the quantitative RT-PCR method according to the invention, with the "series 2" N primers and probes described above (LightCycler N column),
- the nested RT-PCR test targeting the RNA polymerase gene described above, developed by the CDC, BNI and Institut Pasteur (CDC/IP nested RT-PCR),
- the ARTUS kit with the reference "HPA Corona LC RT-PCR Kit #5601-02", which is a real time RT-PCR test targeting the ORF1b gene,
- the BNI nested RT-PCR test, also targeting the RNA polymerase gene mentioned above.

The inventors observed:

1) an inter-test variability for the same technique, linked to the degradation of the RNA preparation during repeated thawing, in particular for the samples containing the lowest quantities of RNA,

2) a reduced sensitivity of the CDC/IP nested RT-PCR compared with the BNI nested RT-PCR, and

3) a comparable sensitivity of the quantitative RT-PCR test according to the invention (LightCycler N) compared with the Artus LightCycler (LC) test.

These results, which are presented in table VII below, show that the quantitative RT-PCR test according to the invention constitutes an excellent addition—or an alternative—to the tests currently available. Indeed, the SARS-linked coronavirus is an emergent virus which is capable of changing rapidly. In particular, the gene for the RNA polymerase of the SARS-linked coronavirus, which is targeted in most of the tests currently available, can recombine with that of other coronaviruses not linked to SARS. The use of a test targeting this gene exclusively could then lead to the production of false-negatives.

The quantitative RT-PCR test according to the invention does not target the same genomic region as the ARTUS kit since it targets the gene encoding the N protein. By carrying out a diagnostic test targeting two different genes of the SARS-linked coronavirus, it can therefore be hoped to avoid false-negative type results which could be due to the genetic evolution of the virus.

Furthermore, it appears particularly advantageous to target the gene for the nucleocapsid protein because it is very stable because of the high selection pressure linked to the high structural constraints regarding this protein.

TABLE VII

Comparison of various methods of analysis by gene amplification, from 121 samples of probable cases of SARS at the French hospital in Hanoi, Vietnam (epidemic 2003)

NRC No.	Sample type (1)	Sample collection day	Patient	CDC/IP nested RT-PCR	BNI nested RT-PCR	Artus Light Cycler kit	Light Cycler N (IP)
107 samples	N and P			Negative	Negative	Negative	Negative
032529	P	10	NHB	Negative	Positive	Negative	Negative
032530	N	10	NHB	Positive	Positive	3.10E+01	4.20E+01
032531	P	7	LP	Positive	Positive	7.70E+00	3.10E+00
032534	N	15	BND	Positive	Positive	1.60E+00	Negative
032600	P	4	NHH	Negative	Positive	Negative	1.30E+02
032612	P	17	NTS	Negative	Positive	Negitive	Negative
032688	P	9	BTX	Positive	Positive	Negative	Negative
032689	N	4	NVH	Positive	Positive	1.20E+01	2.30E+02
032690	P	4	NVH	Negative	Positive	1.60E+00	Negative
032727	P	8	NVH	Positive	Positive	2.30E+02	4.00E+02
032728	N	8	NVH	Positive	Positive	1.10E+03	1.60E+04
032729	P	14	NHB	Positive	Positive	5.90E+00	3.40E+01
032730	N	14	NHB	Positive	Positive	1.30E+02	4.80E+02
032741	P	8	NHH	Positive	Positive	2.10E+02	1.30E+02
positives				10	14	10	9
fraction detected from the 14 positives				71.4%	100.0%	71.4%	64.3%

(1) P = pharyngeal swab
N = nasal swab

EXAMPLE 9

Production and Characterization of Monoclonal Antibodies Directed Against the N Protein

Balb C mice were immunized with the purified recombinant N protein and their spleen cells fused with an appropriate murine myeloma according to the Köhler and Milstein techniques.

Nineteen anti-N antibody secreting hybridomas were preselected and their immunoreactivities determined. These antibodies do indeed recognize the recombinant N protein (in ELISA) with variable intensities, and the natural viral N protein in ELISA and/or in Western blotting. FIGS. **18** to **20** show the results of these tests for 15 of these 19 monoclonal antibodies.

The highly reactive clones 12, 17, 28, 57, 72, 76, 86, 87, 98, 103, 146, 156, 166, 170, 199, 212, 218, 219 and 222 were subcloned. Specificity studies were carried out with the appropriate tools in order to determine the epitopes recognized and verify the absence of reactivity toward other human coronaviruses and certain respiratory viruses.

Epitope mapping studies (performed on spot membrane with the aid of overlapping peptides of 15 aa) and additional studies performed on the natural N protein in Western blotting revealed the existence of 4 groups of monoclonal antibodies:

1. Monoclonal antibodies specific for a major linear epitope at the N-ter position (75-81, sequence: INTNSVP).

The representative of this group is antibody 156. The hybridoma producing this antibody was deposited at the Collection Nationale de Cultures de Microorganismes (CNCM) of the Institut Pasteur (Paris, France) on Dec. 1, 2004, under the number I-3331. This same epitope is also recognized by a rabbit serum (anti-N polyclonal) obtained by conventional immunization with the aid of this same N protein.

2. Monoclonal antibodies specific for a major linear epitope located in a central position (position 217-224, sequence: ETALALL); the representatives of this group are the monoclonal antibodies 87 and 166. The hybridoma producing antibody 87 was deposited at the CNCM on Dec. 1, 2004, under the number I-3328.

3. Monoclonal antibodies specific for a major linear epitope located at the C-terminal position (position 403-408, sequence: DFFRQL), the representatives of this group are the antibodies 28, 57 and 143. The hybridoma producing antibody 57 was deposited at the CNCM on Dec. 1, 2004, under the number I-3330.

4. Monoclonal antibodies specific for a discontinuous conformational epitope. This group of antibodies does not rec-

ognize any of the peptides spanning the sequence of the N protein, but react strongly on the non-denatured natural protein. The representative of this final group is the antibody 86. The hybridoma producing this antibody was deposited at the CNCM on Dec. 1, 2004, under the number I-3329.

Table VIII below summarizes the epitope mapping results obtained:

TABLE VIII

Epitope mapping of the monoclonal antibodies

Antibody	Epitope	Position	Region
28	DFSRQL *Q*	403 . . . 408	C - Ter.
143	DFSRQL *Q*		
76	DFSRQL *Q*		
57	DFSRQL *Q*		
	FFGMS RI	315 . . . 319	
146	LPQRQ	383 . . . 387	
166	ETALALL*LL*	217 . . . 224	central
87	ETALALL	217 . . . 224	
156	*INTNSGP*	75 . . . 81	N-Ter.
86	Conformational		
212	Conformational		
1170	Conformational		

In addition, as illustrated in particular in FIGS. **18** and **19**, these antibodies exhibit no reactivity in ELISA and/or in WB toward the N protein of the human corona-virus 229 E.

EXAMPLE 10

Combinations of the Monoclonal Antibodies for the Development of a Sensitive Immunocapture Test Specific for the Viral N Antigen in the Serum or Biological Fluids of Patients Infected with the SARS-CoV Virus

The antibodies listed below were selected because of their very specific properties for an additional capture and detection study of the viral N protein, in the serum of the subjects or patients.

These antibodies were produced in ascites on mice, purified by affinity chromatography and used alone or in combination, as capture antibodies and as signal antibodies.

List of the antibodies selected:

Ab anti-C-ter region (No. 28, 57, 143)

Ab anti-central region (No. 87, 166)

Ab anti-N-ter region (No. 156)

Ab anti-discontinuous conformational epitope (86)

1) Preparation of the Reagents:

a) Immunocapture ELISA Plates

The plates are sensitized with the antibody solutions at 5 μg/ml in 0.1 M carbonate buffer, pH 9.6. The (monovalent or plurivalent) solutions are deposited in a volume of 100 μl in the wells and incubated overnight at room temperature. These plates are then washed with PBS buffer (10 mM pH 7.4 supplemented with 0.1% Tween 20) and then saturated with a PBS solution supplemented with 0.3% BSA and 5% sucrose). The plates are then dried and then packaged in a bag in the presence of a desiccant. They are ready to use.

b) Conjugates

The purified antibodies were coupled with peroxidase according to the Nakane protocol (Nakane et al.—1974, J. of Histo and cytochemistry, vol. 22, pp. 1084-1091) in a ratio of one molecule of IgG per 3 molecules of peroxidase. These conjugates were purified by exclusion chromatography and stored concentrated (concentration between 1 and 2 mg/ml) in the presence of 50% glycerol and at −20° C. They are diluted for their use in the assays at the final concentration of 1 or 2 μg/ml in PBS buffer (pH 7.4) supplemented with 1% BSA.

c) Other Reagents

Human sera negative for all the serum markers for the HIV, HBV, HCV and THLV viruses

Pool of negative human sera supplemented with 0.5% Triton X 100

Inactivated viral Ag: viral culture supernatant inactivated by irradiation and inactivation verified after placing in culture on sensitive cells—titer of the suspension before inactivation about 10^7 infectious particles per ml or alternatively about 5×10^9 physical viral particles per ml of antigen

The Ag samples diluted in negative human serum: these samples were prepared by diluting 1:100 and then by 5-fold serial dilution.

These noninfectious samples mimic human samples thought to contain low to very low concentrations of viral nucleoprotein N. Such samples are not available for routine work.

Washing solution R2, solution for visualization TMB R8, chromogen R9 and stop solution R10, are the generic reagents marketed by Bio-Rad in its ELISA kits (e.g.: Platelia *pylori* kit ref. 72778).

2) Procedure

The samples of human sera overloaded with inactivated viral Ag are distributed in an amount of 100 μl per well, directly in the ready-to-use sensitized plates, and then incubated for 1 hour at 37° C. (Bio-Rad IPS incubation).

The material not bound to the solid phase is removed by 3 washings (washing with dilute R2 solution, automatic LP 35 washer).

The appropriate conjugates, diluted to the final concentration of 1 or 2 μg/ml, are distributed in an amount of 100 μl per well and the plates are again incubated for one hour at 37° C. (IPS incubation).

The excess conjugate is removed by 4 successive washings (dilute R2 solution—LP 35 washer).

The presence of conjugate attached to the plates is visualized after adding 100 μl of visualization solution prepared before use (1 ml of R9 and 10 ml of R8) and after incubation for 30 minutes, at room temperature and protected from light.

The enzymatic reaction is finally blocked by adding 100 μl of R10 reagent (1 N H_2SO_4) to all the wells.

The reading is carried out with the aid of an appropriate microplate reader at double wavelength (450/620 nm).

The results can be interpreted by using, as provisional threshold value, the mean of at least two negative controls multiplied by a factor of 2 or alternatively the mean of 100 negative sera supplemented with an increment corresponding to 6 SD (standard deviation calculated on the 100 individual measurements).

3) Results

Various capture antibody and signal antibody combinations were tested based on the properties of the antibodies

selected, and avoiding the combinations of antibodies specific for the same epitopes in solid phase and as conjugates.

The best results were obtained with the 4 combinations listed below. These results are reproduced in table IX below.

1. Combination F/28

Solid phase (Ab 166+87 central region): conjugate antibody 28 (C-ter)

2. Combination G/28

Solid phase (Ab 86—conformational epitope): conjugate antibody 28 (C-ter)

3. Combination H/28

Solid phase (Ab 86, 166 and 87 central region and conformational epitope): conjugate antibody 28 C-ter)

4. Combination H/28+87

Solid phase (Ab 86, 166 and 87 central region and conformational epitope): mixed conjugate antibodies 28 (C-ter) and 87 (central)

5. Combination G/87

Solid phase (Ab 86—conformational epitope): conjugate antibody 87 (central region)

The first 4 combinations exhibit equivalent and reproduced performance levels, greater than the other combinations used such as for example the combination G/87). Of course, in these combinations, a monoclonal antibody may be replaced with another antibody recognizing the same epitope. Thus, the following variants may be mentioned:

6. Variant of the combination F/28

Solid phase (Ab 87 only): conjugate antibody 57 (C-ter)

7. Variant of the combination G/28

Solid phase (Ab 86—conformational epitope): conjugate antibody 57 (C-ter)

8. Variant of the combination H/28

Solid phase (Ab 86 and 87 central region and conformational epitope): conjugate antibody 57 (C-ter)

9. Variant of the combination H/28+87

Solid phase (Ab 86 and 87 central region and conformational epitope): mixed conjugate antibodies 57 (C-ter) and 87 (central)

TABLE IX

Test of immunoreactivity of the anti-SARS-CoV nucleoprotein Abs: optical densities measured with each combination of antibodies according to the dilutions of the inactivated viral antigen.

No.	Dilution	F/28	G/28	G/87	H/28	H/28 + 87
0	1/100	5	5	3.495	3.900	5
1	1/500	3.795	3.814	1.379	3.702	3.804
2	1/2 500	2.815	2.950	0.275	3.268	2.680
3	1/12 500	0.987	1.038	0.135	1.374	0.865
4	1/62 500	0.404	0.348	0.125	0.480	0.328
5	1/312 500	0.285	0.211	0.123	0.240	0.215
6	Control	0.210	0.200	0.098	0.186	0.156
7	Control	0.269	0.153	0.104	0.193	0.202

The detection limit for these 4 experimental trials corresponds to the antigen dilution in negative serum 1:62 500. A rapid extrapolation suggests the detection of less than 10^3 infectious particles per ml of sera.

From this study, it is evident that the most appropriate antibodies for the capture of the native viral nucleoprotein are the antibodies specific for the central region and/or for a conformational epitope, both being antibodies also selected for their high affinity for the native antigen.

Having determined the best antibodies for the composition of the solid phase, the antibodies to be selected as a priority for the detection of the antigens attached to the solid phase are the complementary antibodies specific for a dominant epitope in the C-ter region. The use of any other complementary antibody specific for epitopes located in the N-ter region of the protein leads to average or poor results.

EXAMPLE 11

Eukaryotic Expression Systems for the SARS-Associated Coronavirus (SARS-CoV) Spicule (S) Protein

1) Optimization of the Conditions for Expression of the SARS-CoV S in Mammalian Cells

The conditions for transient expression of the SARS-CoV spicule (S) protein were optimized in mammalian cells (293T, VeroE6).

For that, a DNA fragment containing the cDNA for SARS-CoV S was amplified by PCR with the aid of the oligonucleotides 5'-ATAGGATCCA CCATGTTTAT TTTCTTATTA TTTCTTACTC TCACT-3' and 5'-ATACTCGAGTT ATGTGTAATG TAATTTGACA CCCTTG-3' from the plasmid pSARS-S (C.N.C.M. No. I-3059) and then inserted between the BamH1 and Xho1 sites of the plasmid pTRIPΔU3-CMV containing a lentiviral vector TRIP (Sirven, 2001, Mol. Ther., 3, 438-448) in order to obtain the plasmid pTRIP-S. The BamH1 and Xho1 fragment containing the cDNA for S was then subcloned between BamH1 and Xho1 of the eukaryotic expression plasmid pcDNA3.1(+) (Clontech) in order to obtain the plasmid pcDNA-S. The Nhe1 and Xho1 fragment containing the cDNA for S was then subcloned between the corresponding sites of the expression plasmid pCI (Promega) in order to obtain the plasmid pCI-S. The WPRE sequences of the woodchuck hepatitis virus ("Woodchuck Hepatitis Virus posttranscriptional regulatory element") and the CTE sequences ("constitutive transport element") of the simian retro-virus from Mason-Pfizer were inserted into each of the two plasmids pcDNA-S and pCI-S between the Xho1 and Xba1 sites in order to obtain respectively the plasmids pcDNA-S-CTE, pcDNA-S-WPRE, pCI-S-CTE and pCI-S-WPRE (FIG. **21**). The plasmid pCI-S-WPRE was deposited at the CNCM, on Nov. 22, 2004, under the number I-3323. All the inserts were sequenced with the aid of a BigDye Terminator v1.1 kit (Applied Biosystems) and an automated sequencer ABI377.

The capacity of the plasmid constructs to direct the expression of SARS-CoV S in mammalian cells was assessed after transfection of VeroE6 cells (FIG. **22**). In this experiment, monolayers of 5×10^5 VeroE6 cells in 35 mm Petri dishes were transfected with 2 μg of plasmids pcDNA as control), pcDNA-S, pCI and pCI-S and 6 μl of Fugene6 reagent according to the manufacturer's instructions (Roche). After 48 hours of incubation at 37° C. and under 5% CO_2, cellular extracts were prepared in loading buffer according to Laemmli, separated on 8% SDS polyacrylamide gel, and then transferred onto a PVDF membrane (BioRad). The detection of this immunoblot (Western blot) was carried out with the aid of an anti-S rabbit polyclonal serum (immune serum from the rabbit P11135; cf. example 4 above) and donkey polyclonal antibodies directed against rabbit IgGs and coupled with peroxidase (NA934V, Amersham). The bound antibodies were visualized by luminescence with the aid of the ECL+ kit (Amersham) and autoradiography films Hyperfilm MP (Amersham).

This experiment (FIG. **22**) shows that the plasmid pcDNA-S does not make it possible to direct the expression of SARS-CoV S at detectable levels whereas the plasmid pCI-S allows a weak expression, close to the limit of detection, which may be detected when the film is overexposed. Similar

results were obtained when the expression of S was sought by immunofluorescence (data not shown). This impossibility to detect effective expression of S cannot be attributed to the detection techniques used since the S protein can be detected at the expected size (180 kDa) in an extract of cells infected with SARS-CoV or in an extract of VeroE6 cells infected with the recombinant vaccinia virus VV-TF7.3 and transfected with the plasmid pcDNA-S. In this latter experiment, the virus VV-TF7.3 expresses the RNA polymerase of the T7 phage and allows the cytoplasmic transcription of an uncapped RNA capable of being efficiently translated. This experiment suggests that the expression defects described above are due to an intrinsic inability of the cDNA for S to be efficiently expressed when the step for transcription to messenger RNA is carried out at the nuclear level.

In a second experiment, the effect of the CTE and WPRE signals on the expression of S was assessed after transfection of VeroE6 (FIG. **23**A) and 293T (FIG. **23**B) cells and according to a protocol similar to that described above. Whereas the expression of S cannot be detected after transfection of the plasmids pcDNA-S-CTE and pcDNA-S-WPRE derived from pcDNA-S, the insertion of the WPRE and CTE signals greatly improves the expression of S in the context of the expression plasmid pCI-S.

To specify this result, a second series of experiments were carried out where the immunoblot is quantitatively visualized by luminescence and acquisition on a digital imaging device (FluorS, BioRad). The analysis of the results obtained with the QuantityOne v4.2.3 software (BioRad) shows that the WPRE and CTE sequences increase respectively the expression of S by a factor of 20 to 42 and 10 to 26 in Vero E6 cells (table X). In 293T cells (table X), the effect of the CTE sequence is more moderate (4 to 5 times) whereas that of the WPRE sequence remains high (13 to 22 times).

TABLE X

Quantitative analysis of the effect of the CTE and WPRE signals on the expression of SARS-CoV S:

Plasmid	cell	exp. 1	exp. 2
PCI	VeroE6	0.0	0.0
pCI-S	VeroE6	1.0 ± 0.1	1.0
pCI-S-CTE	VeroE6	9.8 ± 0.9	26.4
pCI-S-WPRE	VeroE6	20.1 ± 2.0	42.3
PCI	293T	0.0	0.0
PCI-S	293T	1.0	1.0
PCI-S-CTE	293T	4.6	4.0
PCI-S-WPRE	293T	27.6	12.8

Cellular extracts were prepared 48 hours after transfection of VeroE6 or 293T cells with the plasmid pCI, pCI-S, pCI-S-CTE and pCI-S-WPRE and analyzed by Western blotting as described in the legend to FIG. 22. The Western blot is visualized by luminescence (ECL+, Amersham) and acquisition on a digital imaging device (FluorS, BioRad). The expression levels are indicated according to an arbitrary scale where the value of 1 represents the level measured after transfection of the plasmid pCI-S. Two independent experiments were carried out for each of the two cell types. In experiment 1 on VeroE6 cells, the transfections were carried out in duplicate and the results are indicated in the form of the mean and standard deviation values for the expression levels measured.

In summary, all these results show that the expression, in mammalian cells, of the cDNA for the SARS-CoV S under the control of the RNA polymerase II promoter sequences requires, to be efficient, the expression of a splice signal and of either of the sequences WPRE and CTE.

2) Production of Stable Lines Allowing the Expression of SARS-CoV S

The cDNA for the SARS-CoV S protein was cloned in the form of a BamH1-Xho1 fragment into the plasmid pTRIPΔU3-CMV containing a defective lentiviral vector TRIP with central DNA flap (Sirven et al., 2001, Mol. Ther., 3: 438-448) in order to obtain the plasmid pTRIP-S (FIG. **24**). Transient cotransfection according to Zennou et al. (2000, Cell, 101: 173-185) of this plasmid, of an encapsidation plasmid (p8.2) and of a plasmid for expression of the VSV envelope glycoprotein. G (pHCMV-G) in 293T cells allowed the preparation of retroviral pseudoparticles containing the vector TRIP-S and pseudotyped with the envelope protein G. These pseudotyped TRIP-S vectors were used to translate 293T and FRhK-4 cells: no expression of the S protein could be detected by Western blotting and immunofluorescence in the transduced cells (data not presented).

The optimum expression cassettes consisting of the CMV virus immediate/early promoter, a splice signal, cDNA for S and either of the posttranscriptional signals WPRE or CTE described above were then substituted for the EF1α-EGFP cassette of the defective lentiviral expression vector with central DNA flap TRIPΔU3-EF1α (Sirven et al., 2001, Mol. Ther., 3: 438-448) (FIG. **25**). These substitutions were carried out by a series of successive subclonings of the S expression cassettes which were excised from the plasmids pCT-S-CTE (BglII-Apa1) or respectively pCI-S-WPRE (BglII-Sal1) and then inserted between the Mlu1 and Kpn1 sites or respectively Mlu1 or Xho1 sites of the plasmid TRIPΔU3-EF1α in order to obtain the plasmids pTRIP-SD/SA-S-CTE and pTRIP-SD/SA-S-WPRE, deposited at the CNCM, on Dec. 1, 2004, under the numbers I-3336 and I-3334, respectively. Pseudotyped vectors were produced according to Zennou et al. (2000, Cell, 101: 173-185) and used to transduce 293T cells (10 000 cells) and FRhK-4 cells (15 000 cells) according to a series of 5 successive transduction cycles with a quantity of vectors corresponding to 25 ng (TRIP-SD/SA-S-CTE) or 22 ng TRIP-SD/SA-S-WPRE) of p24 per cycle.

The transduced cells were cloned by limiting dilution and a series of clones were qualitatively analyzed for the expression of SARS-CoV S by immunofluorescence (data not shown), and then quantitatively by Western blotting (FIG. **25**) with the aid of an anti-S rabbit polyclonal serum. The results presented in FIG. **25** show that clones 2 and 15 of FrhK4-s-CTE cells transduced with TRIP-SD/SA-S-CTE and clones 4, 9 and 12 of FRhK4-S-WPRE cells transduced with TRIP-SD/SA-S-WPRE allow the expression of the SARS-CoV S at respectively low, or moderate levels if they are compared to those which can be observed during infection with SARS-CoV.

In summary, the vectors TRIP-SD/SA-S-CTE and TRIP-SD/SA-S-WPRE allow the production of stable clones of FRhK-4 cells and similarly 293T cells expressing SARS-CoV S, whereas the assays carried out with the "parent" vector TRIP-S remained unsuccessful, which demonstrates the need for a splice signal and for either of the sequences CTE and WPRE for the production of stable cell clones expressing the S protein.

In addition, these modifications of the vector TRIP (insertion of a splice signal and of a post-transcriptional signal like CTE and WPRE) could prove advantageous for improving the expression of other cDNAs than that for S.

3) Production of Stable Lines Allowing the Expression of a Soluble Form of SARS-CoV S. Purification of this Recombinant Antigen.

A cDNA encoding a soluble form of the S protein (Ssol) was obtained by fusing the sequences encoding the ectodomain of the protein (amino acids 1 to 1193) with those of a tag (FLAG:DYKDDDDK) via a BspE1 linker encoding the SG dipeptide. Practically, in order to obtain the plasmid pcDNA-Ssol, a DNA fragment encoding the ectodomain of SARS-CoV S was amplified by PCR with the aid of the oligonucleotides 5'-ATAGGATCCA CCATGTTTAT TTTCTTATTA TTTCTTACTC TCACT-3' and 5'-ACCTC-

CGGAT TTAATATATT GCTCATATTT TCCCAA-3' from the plasmid pcDNA-S, and then inserted between the unique BamH1 and BspE1 sites of a modified eukaryotic expression plasmid pcDNA3.1(+) (Clontech) containing the tag sequence FLAG between its BamH1 and Xho1 sites:

```
// GGATCC ...nnn... TCC GGA GAT TAT AAA GAT GAC
    BamH1            S   G   D   Y   K   D   D

GAC GAT AAA TAA CTCGAG //
 D   D   K  ter Xho1
```

The Nhe1-Xho1 and BamH1-Xho1 fragments, containing the cDNA for S, were then excised from the plasmid pcDNA-Ssol, and subcloned between the corresponding sites of the plasmid pTRIP-SD/SA-S-CTE and of the plasmid pTRIP-SD-SA-S-WPRE, respectively, in order to obtain the plasmids pTRIP-SD/SA-Ssol-CTE and pTRIP-SD/SA-Ssol-WPRE, deposited at the CNCM, on Dec. 1, 2004, under the numbers I-3337 and I-3335, respectively.

Pseudotyped vectors were produced according to Zennou et al. (2000, Cell, 101:173-185) and used to transduce FRhK-4 cells (15 000 cells) according to a series of 5 successive transduction cycles (15 000 cells) with a quantity of vector corresponding to 24 ng (TRIP-SD/SA-Ssol-CTE) or 40 ng (TRIP-SD/SA-Ssol-WPRE) of p24 per cycle. The transduced cells were cloned by limiting dilution and a series of 16 clones transduced with TRIP-SD/SA-Ssol-CTE and of 15 clones with TRIP-SD/SA-Ssol-WPRE were analyzed for the expression of the Ssol polypeptide by Western blotting visualized with an anti-FLAG monoclonal antibody (FIG. **26** and data not presented), and by capture ELISA specific for the Ssol polypeptide which was developed for this purpose (table XI and data not presented). Part of the process for selecting the best secretory clones is shown in FIG. **26**. Capture ELISA is based on the use of solid phases coated with polyclonal antibodies of rabbits immunized with purified and inactivated SARS-CoV. These solid phases allow the capture of the Ssol polypeptide secreted into the cellular supernatants, whose presence is then visualized with a series of steps successively involving the attachment of an anti-FLAG monoclonal antibody (M2, SIGMA), of anti-mouse IgG(H+L) biotinylated rabbit polyclonal antibodies (Jackson) and of a streptavidin-peroxidase conjugate (Amersham) and then the addition of chromogen and substrate (TMB+H_2O_2, KPL).

TABLE XI

Analysis of the expression of the Ssol polypeptide by cell lines transduced with the lentiviral vectors TRIP-SD/SA-Ssol-WPRE and TRIP-SD/SA-Ssol-CTE.

Vector	Clone	OD (450 nm)
Control	—	0.031
TRIP-SD/SA-Ssol-CTE	CTE2	0.547
	CTE3	0.668
	CTE9	0.171
	CTE12	0.208
	CTE13	0.133
TRIP-SD/SA-Ssol-WPRE	WPRE1	0.061
	WPRE10	0.134

The secretion of the Ssol polypeptide was assessed in the supernatant of a series of cell clones isolated after transduction of FRhK-4 cells with the lentiviral vectors TRIP-SD/SA-Ssol-WPRE and TRIP-SD/SA-Ssol-CTE. The supernatants diluted 1/50 were analyzed by a capture ELISA test specific for SARS-CoV S.

The cell line secreting the highest quantities of Ssol polypeptide in the culture supernatant is the FRhK4-Ssol-CTE3 line. It was subjected to a second series of 5 cycles of transduction with the vector TRIP-SD/SA-Ssol-CTE under conditions similar to those described above and then cloned. The subclone secreting the highest quantities of Ssol was selected by a combination of Western blot and capture ELISA analysis: it is the subclone FRhK4-Ssol-30, which was deposited at the CNCM, on Nov. 22, 2004, under the name I-3325.

The FRhK4-Ssol-30 line allows the quantitative production and purification of the recombinant Ssol polypeptide. In a typical experiment where the experimental conditions for growth, production and purification were optimized, the cells of the FRhK4-Ssol-30 line are inoculated in standard culture medium (pyruvate-free DMEM containing 4.5 g/l of glucose and supplemented with 5% FCS, 100 U/ml of penicillin and 100 μg/ml of streptomycin) in the form of a subconfluent monolayer (1 million cells per each 100 cm^2 in 20 ml of medium). At confluence, the standard medium is replaced with the secretion medium where the quantity of FCS is reduced to 0.5% and the quantity of medium reduced to 16 ml per each 100 cm^2. The culture supernatant is removed after 4 to 5 days of incubation at 35° C. and under 5% CO_2. The recombinant polypeptide Ssol is purified from the supernatant by the succession of steps of filtration on 0.1 μm polyethersulfone (PES) membrane, concentration by ultrafiltration on a PES membrane with a 50 kD cut-off, affinity chromatography on anti-FLAG matrix with elution with a solution of FLAG peptide (DYKDDDDK) at 100 μg/ml in TBS (50 mM tris, pH 7.4, 150 mM NaCl) and then gel filtration chromatography in TBS on sephadex G-75 beads (Pharmacia). The concentration of the purified recombinant Ssol polypeptide was determined by micro-BCA test (Pierce) and then its biochemical characteristics analyzed.

Analysis by 8% SDS acrylamide gel stained with silver nitrate demonstrates a predominant polypeptide whose molecular mass is about 180 kD and whose degree of purity may be evaluated at 98% (FIG. **27**A). Two main peaks are detected by SELDI-TOF mass spectrometry (Cyphergen): they correspond to single and double charged forms of a predominant polypeptide whose molecular mass is thus determined at 182.6±3.7 kD (FIGS. **27**B and C). After transfer onto Prosorb membrane and rinsing in 0.1% TFA, the N-terminal end of the Ssol polypeptide was sequenced in liquid phase by Edman degradation on 5 residues (ABI494, Applied Biosystems) and determined as being SDLDR (FIG. **27**D). This demonstrates that the signal peptide located at the N-terminal end of the SARS-CoV S protein, composed of aa 1 to 13 (MFIFLLFLTLTSG) according to an analysis carried out with the software signalP v2.0 (Nielsen et al., 1997, Protein Engineering, 10:1-6), is cleaved from the mature Ssol polypeptide. The recombinant Ssol polypeptide therefore consists of amino acids 14 to 1193 of the SARS-CoV S protein fused at the C-terminals with a sequence SG DYKDDDDK containing the sequence of the FLAG tag (underlined). The difference between the theoretical molar mass of the naked Ssol polypeptide (132.0 kD) and the real molar mass of the mature polypeptide (182.6 kD) suggests that the Ssol polypeptide is glycosylated.

A preparation of purified Ssol polypeptide, whose protein concentration was determined by micro-BCA test, makes it possible to prepare a calibration series in order to measure, with the aid of the capture ELISA test described above, the concentrations of Ssol present in the culture supernatants and to review the characteristics of the secretory lines. According to this test, the FRhK4-Ssol-CT3 line secretes 4 to 6 μg/ml of polypeptide Ssol while the FRhK4-Ssol-30 line secretes 9 to 13 μg/ml of Ssol after 4 to 5 days of culture at confluence. In addition, the purification scheme presented above makes it

possible routinely to purify from 1 to 2 mg of Ssol polypeptide per liter of culture supernatant.

EXAMPLE 12

Gene Immunization Involving the SARS-Associated Corona Virus (SARS-CoV) Spicule (S) Protein

The effect of a splice signal and of the posttranscriptional signals WPRE and CTE was analyzed after gene immunization of BALB/c mice (FIG. **28**).

For that, BALB/c mice were immunized at intervals of 4 weeks by injecting into the tibialis anterior a saline solution of 50 μg of plasmid DNA of pcDNA-S and pCI-S and, as a control, 50 μg of plasmid DNA of pcDNA-N (directing the expression of SARS-CoV N) or of pCI-HA (directing the expression of the HA of the influenza virus A/PR/8/34) and the immune sera collected 3 weeks after the 2^{nd} injection. The presence of antibodies directed against the SARS-CoV S was assessed by indirect ELISA using as antigen a lysate of VeroE6 cells infected with SARS-CoV and, as a control, a lysate of noninfected VeroE6 cells. The anti-SARS-CoV antibody titers (TI) are calculated as the reciprocal of the dilution producing a specific OD of 0.5 (difference between OD measured on a lysate of infected cells and OD measured on a lysate of noninfected cells) after visualization with an anti-mouse IgG polyclonal antibody coupled with peroxidase (NA931V, Amersham) and TMB supplemented with H_2O_2 (KPL) (FIG. **28**A).

Under these conditions, the expression plasmid pcDNA-S only allows the induction of low antibody titers directed against SARS-CoV S in 3 mice out of 6 ($LOG_{10}(TI)$= 1.9±0.6) whereas the plasmid pcDNA-N allows the induction of anti-N antibodies at high titers ($LOG_{10}(TI)$=3.9±0.3) in all the animals, and the control plasmids (pCI, pCI-HA) do not result in any detectable antibody ($LOG_{10}(TI)$<1.7). The plasmid pCI-S equipped with a splice signal allows the induction of antibodies at high titers ($LOG_{10}(TI)$=3.7±0.2), which are approximately 60 times higher than those observed after injection of the plasmid pcDNA-S ($p<10^{-5}$).

The efficiency of the posttranscriptional signals was studied by carrying out a dose-response study of the anti-S antibody titers induced in the BALB/c mouse as a function of the quantity of plasmid DNA used as immunogen (2 μg, 10 μg and 50 μg). This study (FIG. **28**B) demonstrates that the posttranscriptional signal WPRE greatly improves the efficiency of gene immunization when small doses of DNA are used ($p<10^{-5}$ for a dose of 2 μg of DNA and $p<10^{-2}$ for a dose of 10 μg), whereas the effect of the CTE signal remains marginal (p=0.34 for a dose of 2 μg of DNA).

Finally, the antibodies induced in mice after gene immunization neutralize the infectivity of SARS-CoV in vitro (FIGS. **29**A and **29**B) at titers which are consistent with the titers measured by ELISA.

In summary, the use of a splice signal and of the posttranscriptional signal WPRE of the woodchuck hepatitis virus considerably improves the induction of neutralizing antibodies directed against SARS-CoV after gene immunization with the aid of plasmid DNA directing the expression of the cDNA for SARS-CoV S.

EXAMPLE 13

Diagnostic Applications of the S Protein

The ELISA reactivity of the recombinant Ssol polypeptide was analyzed with respect to sera from patients suffering from SARS.

The sera from probable cases of SARS tested were chosen on the basis of the results (positive or negative) of analysis of their specific reactivity toward the native antigens of SARS-CoV by immunofluorescence test on VeroE6 cells infected with SARS-CoV and/or by indirect ELISA test using as antigen a lysate of VeroE6 cells infected with SARS-CoV. The sera of these patients are identified by a serial number of the National Reference Center for Influenza Viruses and by the initials of the patient and the number of days elapsed since the onset of the symptoms. All the sera of probable cases (cf. Table XII) recognize the native antigens of SARS-CoV, with the exception of the serum 032552 of the patient VTT for whom infection with SARS-CoV could not be confirmed by RT-PCR performed on respiratory samples of days 3, 8 and 12. A panel of control sera was used as control (TV sera): they are sera collected in France before the SARS epidemic that occurred in 2003.

TABLE XII

Sera of probable cases of SARS

Serum	Patient	Sample collection day
031724	JYK	7
033168	JYK	38
033597	JYK	74
032632	NTM	17
032634	THA	15
032541	PHV	10
032542	NIH	17
032552	VTT	8
032633	PTU	16
032791	JLB	3
033258	JLB	27
032703	JCM	8
033153	JCM	29

Solid phases sensitized with the recombinant Ssol polypeptide were prepared by adsorption of a solution of purified Ssol polypeptide at 2 μg/ml in PBS in the wells of an ELISA plate, and then the plates are incubated overnight at 4° C. and washed with PBS-Tween buffer (PBS, 0.1% Tween 20). After saturating the ELISA plates with a solution of PBS-10% skimmed milk (weight/volume) and washing in PBS-Tween, the sera to be tested (100 μl) are diluted 1/400 in PBS skimmed milk-Tween buffer (PBS, 3% skimmed milk, 0.1% Tween) and then added to the wells of the sensitized ELISA plate. The plates are incubated for 1 h at 37° C. After 3 washings with PBS-Tween buffer, the anti-human IgG conjugate labeled with peroxidase (ref. NA933V, Amersham) diluted 1/4000 in PBS-skimmed milk-Tween buffer is added, and then the plates are incubated for 1 hour at 37° C. After 6 washings with PBS-Tween buffer, the chromogen (TMB) and the substrate (H_2O_2) are added and the plates are incubated for 10 minutes protected from light. The reaction is stopped by adding a 1 N H_3PO_4 solution, and then the absorbance is measured at 450 nm with a reference at 620 nm.

The ELISA tests (FIG. **30**) demonstrate that the recombinant Ssol polypeptide is specifically recognized by the serum antibodies of patients suffering from SARS collected at the medium or late phase of infection (≧10 days after the onset of the symptoms) whereas it is not significantly recognized by the serum antibodies of 2 patients (JLB and JCM) collected in the early phase of infection (3 to 8 days after the onset of the symptoms) or by control sera of subjects not suffering from SARS. The serum antibodies of patients JLB and JCM show a seroconversion between days 3 and 27 for the first and 8 and

29 for the second after the onset of the symptoms, which confirms the specificity of the reactivity of these sera toward the Ssol polypeptide.

In conclusion, these results demonstrate that the recombinant. Ssol polypeptide may be used as an antigen for the development of an ELISA test for serological diagnosis of infection with SARS-CoV.

EXAMPLE 14

Vaccine Applications of the Recombinant Soluble S Protein

The immunogenicity of the recombinant Ssol polypeptide was studied in mice.

For that, a group of 6 mice was immunized at 3 weeks' interval with 10 μg of recombinant Ssol polypeptide adjuvanted with 1 mg of aluminum hydroxide (Alu-gel-S, Serva) diluted in PBS. Three successive immunizations were performed and the immune sera were collected 3 weeks after each of the immunizations (IS1, IS2, IS3). As a control, a group of mice (mock group) received aluminum hydroxide alone according to the same protocol.

The immune sera were analyzed per pool for each of the 2 groups by indirect ELISA using a lysate of VeroE6 cells infected with SARS-CoV as antigen and as a control a lysate of noninfected VeroE6 cells. The anti-SARS-CoV antibody titers are calculated as the reciprocal of the dilution producing a specific OD of 0.5 after visualization with an anti-mouse IgG(H+L) polyclonal antibody coupled with peroxidase (NA931V, Amersham) and TMB supplemented with H_2O_2 (KPL). This analysis (FIG. **31**) shows that the immunization with the Ssol polypeptide induces in mice, from the first immunization, antibodies directed against the native form of the SARS-CoV spicule protein present in the lysate of infected VeroE6 cells. After 2 then 3 immunizations, the anti-S antibody titers become very high.

The immune sera were analyzed per pool for each of the two groups for their capacity to seroneutralize the infectivity of SARS-CoV. 4 points of seroneutralization on FRhK-4 cells (100 TCID50 of SARS-CoV) are produced for each of the 2-fold dilutions tested from 1/20. The seroneutralizing titer is calculated according to the Reed and Munsch method as the reciprocal of the dilution neutralizing the infectivity of 2 wells out of 4. This analysis shows that the antibodies induced in mice by the Ssol polypeptide are neutralizing: the titers observed are very high after 2 and then 3 immunizations (greater than 2560 and 5120 respectively, table XIII).

TABLE XIII

Induction of antibodies directed against SARS-CoV after immunization with the recombinant Ssol polypeptide.

Group	Sera	Neutralizing Ab
Mock	pi	<20
	IS1	<20
	IS2	<20
	IS3	<20
Ssol	pi	<20
	IS1	57
	IS2	>2560
	IS3	>5120

The immune sera were analyzed per pool for each of the two groups for their capacity to seroneutralize the infectivity of 100 TCID50 of SARS-CoV on FRhK-4 cells. 4 points are produced for each of the 2-fold dilutions tested from 1/20. The seroneutralizing titer is calculated according to the Reed and Munsch method as the reciprocal of the dilution neutralizing the infectivity of 2 wells out of 4.

The neutralizing titers observed in mice immunized with the Ssol polypeptide reach levels far greater than the titers observed by Yang et al. in mice (2004, Nature, 428:561-564) and those observed by Buchholz in the hamster (2004, PNAS 101:9804-9809) which protect respectively mice and hamsters from infection with SARS-CoV. It is therefore probable that the neutralizing antibodies induced in mice after immunization with the Ssol polypeptide protect these animals against infection with SARS-CoV.

EXAMPLE 15

Optimized Synthetic Gene for the Expression in Mammalian Cells of the SARS-Associated Coronavirus (SARS-CoV) Spicule (S) Protein

1) Design of the Synthetic Gene

A synthetic gene encoding the SARS-CoV spicule protein was designed from the gene of the isolate 031589 (plasmid pSARS-S, C.N.C.M. No. I-3059) so as to allow high levels of expression in mammalian cells and in particular in cells of human origin.

For that:

- the use of codons of the wild-type gene of the isolate 031589 was modified so as to become close to the bias observed in humans and to improve the efficiency of translation of the corresponding mRNA
- the overall GC content of the gene was increased so as to extend the half-life of the corresponding mRNA
- the optionally cryptic motifs capable of interfering with an efficient expression of the gene were deleted (splice donor and acceptor sites, polyadenylation signals, sequences very rich (>80%) or very low (<30%) in GC, repeat sequences, sequences involved in the formation of secondary RNA structures, TATA boxes)
- a second STOP codon was added to allow efficient termination of translation.

In addition, CpG motifs were introduced into the gene so as to increase its immunogenicity as DNA vaccine. In order to facilitate the manipulation of the synthetic gene, two BamH1 and Xho1 restriction sites were placed on either side of the open reading frame of the S protein, and the BamH1, Xho1, Nhe1, Kpn1, BspE1 and Sal1 restriction sites were avoided in the synthetic gene.

The sequence of the synthetic gene designed (gene 040530) is given in SEQ ID No: 140.

An alignment of the synthetic gene 040530 with the sequence of the wild-type gene of the isolate 031589 of SARS-CoV deposited at the C.N.C.M. under the number I-3059 (SEQ ID No: 4, plasmid pSRAS-S) is presented in FIG. **32**.

2) Plasmid Constructs

The synthetic gene SEQ ID No: 140 was assembled from synthetic oligonucleotides and cloned between the Kpn1 and Sac1 sites of the plasmid pUC-Kana in order to give the plasmid 040530pUC-Kana. The nucleotide sequence of the insert of the plasmid 040530pUC-Kana was verified by automated sequencing (Applied).

A Kpn1-Xho1 fragment containing the synthetic gene 040530 was excised from the plasmid 040530pUC-Kana and subcloned between the Nhe1 and Xho1 sites of the expression plasmic pCI (Promega) in order to obtain the plasmid pCI-SSYNTH, deposited at the CNCM on Dec. 1, 2004, under the number I-3333.

A synthetic gene encoding the soluble form of the S protein was then obtained by fusing the synthetic sequences encoding the ectodomain of the S protein (amino acids 1 to 1193) with

those of the tag (FLAG:DYKDDDDK) via a linker BspE1 encoding the dipeptide SG. Practically, a DNA fragment encoding the ectodomain of the SARS-CoV S was amplified by PCR with the aid of the oligonucleotides 5'-ACTA GCTAGC GGATCCACCATGTTCATCTT CCTG-3' and 5'-AGTATCCGGAC TTG ATGTACT GCTCGTACTTGC-3' from the plasmid 040530pUC-Kana, digested with Nhe1 and BspE1 and then inserted between the unique Nhe1 and BspE1 sites of the plasmid pCI-Ssol, to give the plasmid pCI-SCUBE, deposited at the CNCM on Dec. 1, 2004, under the number I-3332. The plasmids pCI-Ssol, pCI-Ssol-CTE, and pCI-Ssol-WPRE (deposited at the CNCM, on Nov. 22, 2004, under the number I-3324) had been previously obtained by subcloning the Kpn1-Xho1 fragment excised from the plasmid pcDNA-Ssol (see technical note of DI 2004-106) between the Nhe1 and Xho1 sites of the plasmids pCI, pCI-S-CTE and pCI-S-WPRE respectively.)

The plasmids pCI-Scube and pCI-Ssol encode the same recombinant Ssol polypeptide.

3) Results

The capacity of the synthetic gene encoding the S protein to efficiently direct the expression of the SARS-CoV S in mammalian cells was compared with that of the wild-type gene after transient transfection of primate cells (VeroE6) and of human cells (293T).

In the experiment presented in FIG. **33** and in table XIV, monolayers of 5×10^5 VeroE6 cells or 7×10^5 293T cells in 35 mm Petri dishes were transfected with 2 μg of plasmids pCI (as control), pCI-S, pCI-S-CTE, pCI-S-WPRE and pCI-S-Ssynth and 6 μl of Fugene6 reagent according to the manufacturer's instructions (Roche). After 48 hours of incubation at 37° C. and under 5% CO_2, cell extracts were prepared in loading buffer according to Laemmli, separated on 8% SDS polyacrylamide gel and then transferred onto a PVDF membrane (BioRad). The detection of this immunoblot (Western blot) was carried out with the aid of an anti-S rabbit polyclonal serum (immune serum of the rabbit P11135: cf example 4 above) and of donkey polyclonal antibodies directed against rabbit IgGs and coupled with peroxidase (NA934V, Amersham). The immunoblot was quantitatively visualized by luminescence with the aid of the ECL+ kit (Amersham) and acquisition on a digital imaging device (FluorS, BioRad).

The analysis of the results obtained with the software QuantityOne v4.2.3 (BioRad) shows that in this experiment, the plasmid pCI-Synth allows the transient expression of the S protein at high levels in the VeroE6 and 293T cells, whereas the plasmid pCI-S does not make it possible to induce expression at sufficient levels to be detected. The expression. Levels observed are of the order of twice as high as those observed with the plasmid pCI-S-WPRE.

TABLE XIV

Use of a synthetic gene for the expression of the SARS-CoV S.

Plasmid	VeroE6	293T
pCI	0.0	0.0
pCI-S	≤0.1	≤0.1
pCI-S-CTE	0.5	≤0.1
pCI-S-WPRE	1.0	1.0
pCI-Ssynth	1.8	1.9

Cell extracts prepared 48 hours after transfection of VeroE6 or 293T cells with the plasmids pCI, pCI-S, pCI-S-CTE, pCI-S-WPRE and pCI-S-Ssynth were separated on 8% SDS acrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H + L) polyclonal antibody coupled with peroxidase (NA934V, Amersham). The Western blot is visualized by luminescence (ECL+, Amersham) and acquisition on a digital imaging device (FluorS, BioRad). The expression levels of the S protein were measured by quantifying the two predominant bands identified on the image (see FIG. 33) and are indicated according to an arbitrary scale where the value 1 represents the level measured after transfection of the plasmid pCI-S-WPRE.

In a second instance, the capacity of the synthetic gene Scube to efficiently direct the synthesis and the secretion of the Ssol polypeptide by mammalian cells was compared with that of the wild-type gene after transient transfection of hamster cells (BHK-21) and of human cells (293T).

In the experiment presented in table XV, monolayers of 6×10^5 BHK-21 cells and 7×10^5 293T cells in 35 mm Petri dishes were transfected with 2 μg of plasmids pCI (as control), pCI-Ssol, pCI-Ssol-CTE, pCI-Ssol-WPRE and pCI-Scube and 6 μl of Fugene6 reagent according to the manufacturer's instructions (Roche). After 48 hours of incubation at 37° C. and under 5% CO_2, the cellular supernatants were collected and quantitatively analyzed for the secretion of the Ssol polypeptide by a capture ELISA test specific for the Ssol polypeptide.

Analysis of the results shows that, in this experiment, the plasmid pCI-Scube allows the expression of the Ssol polypeptide at levels 8 times (BHK-21 cells) to 20 times (293T cells) higher than the plasmid pCI-Ssol. The levels of expression observed are of the order of twice (293T cells) to 5 times (BHK-21 cells) as high as those observed with the plasmid pCI-Ssol-WPRE.

TABLE XV

Use of a synthetic gene for the expression of the Ssol polypeptide.

Plasmid	BHK	293T
pci	<20	<20
pCI-Ssol	<20	56 ± 10
pCI-Ssol-CTE	<20	63 ± 8
pCI-Ssol-WPRE	28 ± 1	531 ± 15
pCI-Scube	152 ± 6	1140 ± 20

The supernatants were harvested 48 hours after transfection of BHK or 293T cells with the plasmids pCI, pCI-Ssol, pCI-Ssol-CTE, pCI-Ssol-WPRE and pCI-Scube and quantitatively analyzed for the secretion of the Ssol polypeptide by an ELISA test specific for the Ssol polypeptide. The transfections were carried out in duplicate and the results are presented in the form of means and standard deviations of the concentrations of Ssol polypeptide (ng/ml) measured in the supernatants.

In summary, these results show that the expression, in mammalian cells, of the synthetic gene 040530 encoding SARS-CoV S under the control of RNA polymerase II promoter sequences is much more efficient than that of the wild-type gene of the 031589 isolate. This expression is even more efficient than that directed by the wild-type gene in the presence of the WPRE sequences of the woodchuck hepatitis virus.

4) Applications

The use of the synthetic gene 040530 encoding SARS-CoV S or its Scube variant encoding the polypeptide Ssol is capable of advantageously replacing the wild-type gene in numerous applications where the expression of S is necessary at high levels. In particular in order to:

- improve the efficiency of gene immunization with plasmids of the pCI-Ssynth or even pCI-Ssynth-CTE or pCI-Ssynth-WPRE type
- establish novel cell lines expressing higher quantities of the S protein or of the Ssol polypeptide with the aid of recombinant lentiviral vectors carrying the Ssynth gene or the Scube gene respectively
- improve the immunogenicity of the recombinant lentiviral vectors allowing the expression of the S protein or of the Ssol polypeptide
- improve the immunogenicity of live vectors allowing the expression of the S protein or of the Ssol polypeptide like recombinant vaccinia viruses or recombinant measles viruses (see examples 16 and 17 below)

EXAMPLE 16

Expression of the SARS-Associated Coronavirus (SARS-CoV) Spicule (S) Protein with the Aid of Recombinant Vaccinia Viruses

Vaccine Application

Application to the Production of a Soluble Form of the Spicule (S) Protein and Design of a Serological Test for SARS

1) Introduction

The aim of this example is to evaluate the capacity of recombinant vaccinia viruses (VV) expressing various SARS-associated coronavirus (SARS-CoV) antigens to constitute novel vaccine candidates against SARS and a means of producing recombinant antigens in mammalian cells.

For that, the inventors focused on the SARS-CoV spicule (S) protein which makes it possible to induce, after gene immunization in animals, antibodies neutralizing the infectivity of SARS-CoV, and a soluble and secreted form of this protein, the Ssol polypeptide, which is composed of the ectodomain (aa 1-1193) of S fused at its C-ter end with a tag FLAG (DYKDDDDK) via a BspE1 linker encoding the SG dipeptide. This Ssol polypeptide exhibits an antigenicity similar to that of the S protein and allows, after injection into mice in the form of a purified protein adjuvanted with aluminum hydroxide, the induction of high neutralizing antibody titers against SARS-CoV.

The various forms of the S gene were placed under the control of the promoter of the 7.5K gene and then introduced into the thymidine kinase (TK) locus of the Copenhagen strain of the vaccinia virus by double homologous recombination in vivo. In order to improve the immunogenicity of the recombinant vaccinia viruses, a synthetic late promoter was chosen in place of the 7.5K promoter, in order to increase the production of S and Ssol during the late phases of the viral cycle.

After having isolated the recombinant vaccinia viruses and verified their capacity to express the SARS-CoV S antigen, their capacity to induce in mice an immune response against SARS was tested. After having purified the Ssol antigen from the supernatant of infected cells, an ELISA test for serodiagnosis of SARS was designed, and its efficiency was evaluated with the aid of sera from probable cases of SARS.

2) Construction of the Recombinant Viruses

Recombinant vaccinia viruses directing the expression of the S glycoprotein of the 031589 isolate of SARS-CoV and of a soluble and secreted form of this protein, the Ssol polypeptide, under the control of the 7.5K promoter were obtained. With the aim of increasing the levels of expression of S and Ssol, recombinant viruses in which the cDNAs for S and for Ssol are placed under the control of a late synthetic promoter were also obtained.

The plasmid pTG186poly is a transfer plasmid for the construction of recombinant vaccinia viruses (Kieny, 1986, Biotechnology, 4:790-795). As such, it contains the VV thymidine kinase gene into which the promoter of the 7.5K gene has been inserted followed by a multiple cloning site allowing the insertion of heterologous genes (FIG. **34**A). The promoter of the 7.5K gene in fact contains a tandem of two promoter sequences that are respectively active during the early (P_E) and late (P_L) phases of the vaccinia virus replication cycle. The BamH1-Xho1 fragments were excised from the plasmids pTRIP-S and pcDNA-Ssol respectively and inserted between the BamH1 and Sma1 sites of the plasmid pTG186poly in order to give the plasmids pTG-S and pTG-Ssol (FIG. **34**A). The plasmids pTG-S and pTG-Ssol were deposited at the CNCM, on Dec. 2, 2004, under the numbers I-3338 and I-3339, respectively.

The plasmids pTN480, pTN-S and pTN-Ssol were obtained from the plasmids pTG186poly, pTG-S and pTG-Ssol respectively, by substituting the Nde1-Pst1 fragment containing the 7.5K promoter by a DNA fragment containing the synthetic late promoter 480, which was obtained by hybridization of the oligonucleotides 5'-TATGAGCTTT TTTTTTTTTT TTTTTTTGGC ATATAAATAG ACTCG-GCGCG CCATCTGCA-3' and 5'-GATGGCGCGC-CGAGTCTATT TATATGCCAA AAAAAAAAAA AAAAAAAAGC TCA-3' (FIG. **34**B). The insert was sequenced with the aid of a BigDye Terminator v1.1 kit (Applied Biosystems) and an automated sequencer ABI377. The sequence of the late synthetic promoter 480 as cloned into the transfer plasmids of the pTN series is indicated in FIG. **34**C. The plasmids pTN-S and pTN-Ssol were deposited at the CNCM, on Dec. 2, 2004, under the numbers I-3340 and I-3341, respectively.

The recombinant vaccinia viruses were obtained, by double homologous recombination in vivo between the TK cassette of the transfer plasmids of the series pTG and pTN and the TK gene of the Copenhagen strain of the vaccinia virus according to a procedure described by Kieny et al. (1984, Nature, 312:163-166). Briefly, CV-1 cells are transfected with the aid of DOTAP (Roche) with genomic DNA of the Copenhagen strain of the vaccinia virus and each of the transfer plasmids of the pTG and pTN series described above, and then superinfected with the helper vaccinia virus VV-ts7 for 24 hours at 33° C. The helper virus is counter-selected by incubation at 40° C. for 2 days and then the recombinant viruses (TK− phenotype) selected by two cloning cycles under agar medium on 143Btk− cells in the presence of BuDr (25 μg/ml). The 6 viruses VV-TG, VV-TG-S, VV-TG-Ssol, VV-TN, VV-TN-S, and VV-TN-Ssol are respectively obtained with the aid of the transfer plasmids pTG186poly, pTG-S, pTG-Ssol, pTN480, pTN-Ssol. The viruses VV-TG and VV-TN do not express any heterologous gene and were used as TK− control in the experiments. The preparations of recombinant viruses were performed on monolayers of CV-1 or BHK-21 cells and the titer in plaque forming units (p.f.u) determined on CV-1 cells according to Earl and Moss (1998, Current Protocols in Molecular Biology, 16.16.1-16.16.13).

3) Characterization of the Recombinant Viruses

The expression of the transgenes encoding the S protein and the Ssol polypeptide was assessed by Western blotting.

Monolayers of CV-1 cells were infected at a multiplicity of 2 with various recombinant vaccinia viruses VV-TG, VV-TG-S, VV-TG-Ssol, VV-TN, VV-TN-S and VV-TN-Ssol. After 18 hours of incubation at 37° C. and under 5% CO2, cellular

extracts were prepared in loading buffer according to Laemmli, separated on 8% SDS polyacrylamide gel and then transferred onto a PVDF membrane (BioRad). The detection of this immunoblot (Western blot) was performed with the aid of an anti-S rabbit polyclonal serum (immune serum from the rabbit P11135: cf. example 4) and donkey polyclonal antibodies directed against rabbit IgGs and coupled with peroxidase (NA934V, Amersham). The bound antibodies were visualized by luminescence with the aid of the ECL+ kit (Amersham) and autoradiography films Hyperfilm MP (Amersham).

As shown in FIG. **35**A, the recombinant virus VV-TN-S directs the expression of the S protein at levels which are comparable to those which can be observed 8 h after infection with SARS-CoV but which are much higher than those which can be observed after infection with VV-TG-S. In a second experiment (FIG. **35**B), the analysis of variable quantities of cellular extracts shows that the levels of expression observed after infection with viruses of the TN series (VV-TN-S and VV-TN-Ssol) are about 10 times as high as those observed with the viruses of the TG series (VV-TG-S and VV-TG-Ssol, respectively). In addition, the Ssol polypeptide is secreted into the supernatant of CV-1 cells infected with the VV-TN-Ssol virus more efficiently than in the supernatant of cells infected with VV-TG-Ssol (FIG. **36**A). In this experiment, the VV-TN-Sflag virus was used as a control because it expresses the membrane form of the S protein fused at its C-ter end with the FLAG tag. The Sflag protein is not detected in the supernatant of cells infected with VV-TN-Sflag, demonstrating that the Ssol polypeptide is indeed actively secreted after infection with VV-TN-Ssol.

These results demonstrate that the recombinant vaccinia viruses are indeed carriers of the transgenes and allow the expression of the SRAS glycoprotein in its membrane form (S) or in a soluble or secreted form (Ssol). The vaccinia viruses carrying the synthetic promoter 480 allow the expression of S and the secretion of Ssol at levels much higher than the viruses carrying the promoter of the 7.5K gene.

4) Application to the Production of a Soluble Form of SARS-CoV S. Purification of this Recombinant Antigen and Diagnostic Applications

The BHK-21 line is the cell line which secretes the highest quantities of Ssol polypeptide after infection with the VV-TN-Ssol virus among the lines tested (BHK-21, CV1, 293T and FrhK-4, FIG. **36**B); it allows the quantitative production and purification of the recombinant Ssol polypeptide. In a typical experiment where the experimental conditions for infection, production and purification were optimized, the BHK-21 cells are inoculated in standard culture medium (pyruvate-free DMEM containing 4.5 g/l of glucose and supplemented with 5% TPB, 5% FCS, 100 U/ml of penicillin and 100 μg/ml of streptomycin) in the form of a subconfluent monolayer (10 million cells for each 100 cm^2 in 25 ml of medium). After 24 h of incubation at 37° C. under 5% CO_2, the cells are infected at an M.O.I. of 0.03 and the standard medium replaced with the secretion medium where the quantity of FCS is reduced to 0.5% and the TPB eliminated. The culture supernatant is removed after 2.5 days of incubation at 35° C. and under 5% CO_2 and the vaccinia virus inactivated by addition of Triton X-100 (0.1%). After filtration on 0.1 μm polyethersulfone (PES) membrane, the recombinant Ssol polypeptide is purified by affinity chromatography on an anti-FLAG matrix with elution with a solution of FLAG peptide (DYKDDDDK) at 100 μg/ml in TBS (50 mM Tris, pH 7.4, 150 mM NaCl).

The analysis by 8% SDS acrylamide gel stained with silver nitrate identified a predominant polypeptide whose molecular mass is about 180 kD and whose degree of purity is greater than 90% (FIG. **37**). The concentration of the purified Ssol recombinant polypeptide was determined by comparison with molecular mass markers and estimated at 24 ng/μl.

This purified Ssol polypeptide preparation makes it possible to produce a calibration series in order to measure, with the aid of a capture ELISA test, the Ssol concentrations present in the culture supernatants. According to this test, the BHK-21 line secretes about 1 μg/ml of Ssol polypeptide under the production conditions described above. In addition, the purification scheme presented makes it possible to purify of the order of 160 μg of Ssol polypeptide per liter of culture supernatant.

The ELISA reactivity of the recombinant Ssol polypeptide was analyzed toward sera from patients suffering from SARS.

The sera of probable cases of SARS tested were chosen on the basis of the results (positive or negative) of analysis of their specific reactivity toward the native antigens of SARS-CoV by immunofluorescence test on VeroE6 cells infected with SARS-CoV and/or by indirect ELISA test using, as antigen, a lysate of VeroE6 cells infected with SARS-CoV. The sera of these patients are identified by a serial number of the National Reference Center for Influenza Viruses and by the patient's initials and the number of days elapsed since the onset of the symptoms. All the sera of probable cases (cf. table XVI) recognize the native antigens of SARS-CoV with the exception of the serum 032552 of the patient VTT, for which infection with SARS-CoV could not be confirmed by RT-PCR performed on respiratory samples of days 3, 8 and 12. A panel of control sera was used as control (TV sera): they are sera collected in France before the SARS epidemic which occurred in 2003.

TABLE XVI

Sera of probable cases of SARS

Serum	Patient	Sample collection day
033168	JYK	38
033597	JYK	74
032632	NTM	17
032634	THA	15
032541	PHV	10
032542	NIH	17
032552	VTT	8
032633	PTU	16

Solid phases sensitized with the recombinant Ssol polypeptide were prepared by adsorption of a solution of purified Ssol polypeptide at 4 μg/ml in PBS in the wells of an ELISA plate. The plates are incubated overnight at 4° C. and then washed with PES-Tween buffer (PBS, 0.1% Tween 20). After washing with PBS-Tween, the sera to be tested (100 μl) are diluted 1/100 and 1/400 in PBS-skimmed milk-Tween buffer (PBS, 3% skimmed milk, 0.1% Tween) and then added to the wells of the sensitized ELISA plate. The plates are then incubated for 1 h at 37° C. After 3 washings with PBS-Tween buffer, the anti-human IgG conjugate labeled with peroxidase (ref. NA933V, Amersham) diluted 1/4000 in PBS-skimmed milk-Tween buffer is added and then the plates are incubated for one hour at 37° C. After 6 washings with PBS-Tween buffer, the chromogen (TMB) and the substrate (H_2O_2) are added and the plates are incubated for 10 minutes protected from light. The reaction is stopped by adding a 1M solution of H_3PO_4 and then the absorbance is measured at 450 nm with a reference at 620 nm.

The ELISA tests (FIG. **38**) demonstrate that the recombinant Ssol polypeptide is specifically recognized by the serum

antibodies of patients suffering from SARS, collected at the middle or late phase of infection (≧10 days after the onset of the symptoms), whereas it is not significantly recognized by the serum antibodies of the control sera of subjects not suffering from SARS.

In conclusion, these results demonstrate that the recombinant Ssol polypeptide can be purified from the supernatant of mammalian cells infected with the recombinant vaccinia virus VV-TN-Ssol and can be used as antigen for developing an ELISA test for serological diagnosis of infection with SARS-CoV.

5. Vaccine Applications

The immunogenicity of the recombinant vaccinia viruses was studied in mice.

For that, groups of 7 BALB/c mice were immunized by the i.v. route twice at 4 weeks' interval with 10^6 p.f.u. of recombinant vaccinia viruses VV-TG, VV-TG-S, VV-TG-Ssol, VV-TN, VV-TN-S and VV-TN-Ssol and, as a control, VV-TG-HA which directs the expression of hemagglutinin of the A/PR/8/34 strain of the influenza virus. The immune sera were collected 3 weeks after each of the immunizations (IS1, IS2).

The immune sera were analyzed per pool for each of the groups by indirect ELISA using a lysate of VeroE6 cells infected with SARS-CoV as antigen and, as control, a lysate of noninfected VeroE6 cells. The anti-SARS-CoV antibody titers (TI) are calculated as the reciprocal of the dilution producing a specific OD of 0.5 after visualization with an anti-mouse IgG(H+L) polyclonal antibody coupled with peroxidase (NA931V, Amersham) and TMB supplemented with H_2O_2 (KPL). This analysis (FIG. **39**A) shows that immunization with the virus VV-TG-S and VV-TN-S induces in mice, from the first immunization, antibodies directed against the native form of the SARS-CoV spicule protein present in the lysate of infected VeroE6 cells. The responses induced by the VV-TN-S virus are higher than those induced by the VV-TG-S virus after the first (TI=740 and TI=270 respectively) and the second (TI=3230 and TI=600 respectively) immunization. The VV-TN-Ssol virus induces high anti-SARS-CoV antibody titers after two immunizations (TI=640), whereas the virus VV-TG-Ssol induces a response at the detection limit (TI=40).

The immune sera were analyzed per pool for each of the groups for their capacity to seroneutralize the infectivity of SARS-CoV. 4 seroneutralization points on FRhK-4 cells (100 TCID50 of SARS-CoV) are produced for each of the 2-fold dilutions tested from 1/20. The seroneutralizing titer is calculated according to the Reed, and Munsch method as the reciprocal of the dilution neutralizing the infectivity of 2 wells out of 4. This analysis shows that the antibodies induced in mice by the vaccinia viruses expressing the S protein or the Ssol polypeptide are neutralizing and that the viruses with synthetic promoters are more efficient immunogens than the viruses carrying the 7.5K promoter: the highest titers (640) are observed after 2 immunizations with the virus VV-TN-S (FIG. **39**B).

The protective power of the neutralizing antibodies induced in mice after immunization with the recombinant vaccinia viruses is evaluated with the aid of a challenge infection with SARS-CoV.

6) Other Applications

Third generation recombinant vaccinia viruses are constructed by substituting the wild-type sequences of the S and Ssol genes by synthetic genes optimized for the expression in mammalian cells, described above. These recombinant vaccinia viruses are capable of expressing larger quantities of S and Ssol antigens and therefore of exhibiting increased immunogenicity.

The recombinant vaccinia virus VV-TN-Ssol can be used for the quantitative production and purification of the Ssol antigen for diagnostic (serology by ELISA) and vaccine (subunit vaccine) applications.

EXAMPLE 17

Recombinant Measles Virus Expressing the SARS-Associated Coronavirus (SARS-CoV) Spicule (S) Protein. Vaccine Applications

1) Introduction

The measles vaccine (MV) induces a lasting protective immunity in humans after a single injection (Hilleman, 2002, Vaccine, 20: 651-665). The protection conferred is very robust and is based on the induction of an antibody response and of a CD4 and CD8 cell response. The MV genome is very stable and no reversion of the vaccine strains to virulence has ever been observed. The measles virus belongs to the genus *Morbillivirus* of the Paramyxoviridae family; it is an enveloped virus whose genome is a 16 kb single-stranded RNA of negative polarity (FIG. **40**A) and whose exclusively cytoplasmic replication cycle excludes any possibility of integration into the genome of the host. The measles vaccine is thus one of the most effective and one of the safest live vaccines used in the human population. Frédéric Tangy's team recently developed an expression vector on the basis of the Schwarz strain of the measles virus, which is the safest attenuated strain and the most widely used in humans as vaccine against measles. This vaccine strain may be isolated from an infectious molecular clone while preserving its immunogenicity in primates and in mice that are sensitive to the infection. It constitutes, after insertion of additional transcription units, a vector for the expression of heterologous sequences (Combredet, 2003, J. Virol. 77: 11546-11554). In addition, a recombinant MV Schwarz expressing the envelope glycoprotein of the West Nile virus (WNV) induces an effective and lasting antibody response which protects mice from a lethal challenge infection with WNV (Despres et al., 2004, J. Infect. Dis., in press). All these characteristics make the attenuated Schwarz strain of the measles virus an extremely promising candidate vector for the construction of novel recombinant live vaccines.

The aim of this example is to evaluate the capacity of recombinant measles viruses (MV) expressing various SARS-associated coronavirus (SARS-CoV) antigens to constitute novel candidate vaccines against SARS.

The inventors focused on the SARS-CoV spicule (S) protein, which makes it possible to induce, after gene immunization in animals, antibodies neutralizing the infectivity of SARS-CoV, and on a soluble and secreted form of this protein, the Ssol polypeptide, which is composed of the ectodomain (aa 1-1193) of S fused at its C-ter end with a FLAG tag (DYKDDDDK) via a BspE1 linker encoding the SG dipeptide. This Ssol polypeptide exhibits a similar antigenicity to that of the S protein and allows, after injection into mice in the form of a purified protein adjuvanted with aluminum hydroxide, the induction of high neutralizing antibody titers against SARS-CoV.

The various forms of the S gene were introduced in the form of an additional transcription unit between the P (phosphoprotein) and M (matrix) genes into the cDNA of the Schwarz strain of MV previously described (Combredet, 2003, J. Virol. 77: 11546-11554; EP application No. 02291551.6 of Jun. 20, 2002, and EP application No. 02291550.8 of Jun. 20, 2002). After having isolated the recombinant viruses MVSchw2-SARS-S and MVSchw2-

SARS-Ssol and checked their capacity to express the SARS-CoV S antigen, their capacity to induce a protective immune response against SARS in mice and then in monkeys was tested.

2) Construction of the Recombinant Viruses

The plasmid pTM-MVSchw-ATU2 (FIG. **40**B) contains an infectious cDNA corresponding to the antigenome of the Schwarz vaccine strain of the measles virus (MV) into which an additional transcription unit (ATU) has been introduced between the P (phosphoprotein) and M (matrix) genes (Combredet, 2003, Journal of Virology, 77: 11546-11554). Recombinant genomes MVSchw2-SARS-S and MVSchw2-SARS-Ssol of the measles virus were constructed by inserting ORFs of the S protein and of the Ssol polypeptide into the additional transcription unit of the MVSchw-ATU2 vector.

For that, a DNA fragment containing the SARS-CoV S cDNA was amplified by PCR with the aid of the oligo-nucleotides 5'-ATACGTACGA CCATGTTTAT TTTCTTATTA TTTCTTACTC TCACT-3' and 5'-ATAGCGCGCT CATTATGTGT AATGTAATTT GACACCCTTG-3' using the plasmid pcDNA-S as template and then inserted into the plasmid pCR®2.1-TOPO (Invitrogen) in order to obtain the plasmid pTOPO-S-MV. The two oligonucleotides used contain restriction sites BsiW1 and BssHII, so as to allow subsequent insertion into the measles vector, and were designed so as to generate a sequence of 3774 nt including the codons for initiation and termination, so as to observe the rule of 6 which stipulates that the length of the genome of a measles virus must be divisible by 6 (Calain & Roux, 1993, J. Virol., 67: 4822-4830; Schneider et al., 1997, Virology, 227: 314-322). The insert was sequenced with the aid of a BigDye Terminator v1.1 kit (Applied. Biosystems) and an automated sequencer ABI377.

To express a soluble and secreted form of SARS-CoV S, a plasmid containing the cDNA of the Ssol polypeptide corresponding to the ectodomain (aa 1-1193) of SARS-CoV S fused at its C-ter end with the sequence of a FLAG tag (DYKDDDDK) via a BspE1 linker encoding the SG dipeptide was then obtained. For that, a DNA fragment was amplified with the aid of the oligonucleotides 5'-CCATTTCAAC AATTTGGCCG-3' and 5'-ATAGGATCCGCGCGCTCATT ATTTATCGTC GTCATCTTTA TAATC-3' from the plasmid pCDNA-Ssol and then inserted into the plasmid pTOPO-S-MV between the Sal1 and BamH1 sites in order to obtain the plasmid pTOPO-S-MV-SF. The sequence generated is 3618 nt long between the BsiW1 and BssHII sites and observes the rule of 6. The insert was sequenced as indicated above.

The BsiW1-BssHII fragments containing the cDNAs for the S protein and the Ssol polypeptide were then excised by digestion of the plasmids pTOPO-S-MV and pTOPO-S-MV-SF and then subcloned between the corresponding sites of the plasmid pTM-MVSchw-ATU2 in order to give the plasmids pTM-MVSchw2-SARS-S and pTM-MVSchw2-SARS-Ssol (FIG. **40**B). These two plasmids were deposited at the C.N.C.M. on Dec. 1, 2004, under the numbers I-3326 and I-3327, respectively.

The recombinant measles viruses corresponding to the plasmids pTM-MVSchw2-SARS-S and pTM-MVSchw2-SARS-Ssol were obtained by reverse genetics according to the system based on the use of a helper cell line, described by Radecke et al. (1995, Embo J., 14: 5773-5784) and modified by Parks et al. (1999, J. Virol., 73: 3560-3566). Briefly, the helper cells 293-3-46 are transfected according to the calcium phosphate method with 5 pg of the plasmids pTM-MVSchw2-SARS-S or pTM-MVSchw2-SARS-Ssol and 0.02 μg of the plasmid pEMC-La directing the expression of the MV L polymerase (gift from M. A. Billeter). After incubating, overnight at 37° C., a heat shock is produced for 2 hours at 43° C. and the transfected cells are transferred onto a monolayer of Vero cells. For each of the two plasmids, syncytia appeared after 2 to 3 days of coculture and were transferred successively onto monolayers of Vero cells at 70% confluence in 35 mm Petri dishes and then in, 25 and 75 cm^2 flasks. When the syncytia have reached 80-90% confluence, the cells are recovered with the aid of a scraper and then frozen and thawed once. After low-speed centrifugation, the supernatant containing the virus is stored in aliquots at −80° C. The titers of the recombinant viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol were determined by limiting dilution on Vero cells and the titer as dose infecting 50% of the wells ($TCID_{50}$) calculated according to the Kärber method.

3) Characterization of the Recombinant Viruses

The expression of the transgenes encoding the S protein and the Ssol polypeptide was assessed by Western blotting and immunofluorescence.

Monolayers of Vero cells in T-25 flasks were infected at a multiplicity of 0.05 by various passages of the two viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol and the wild-type virus MWSchw as a control. When the syncytia had reached 80 to 90% confluence, cytoplasmic extracts were prepared in an extraction buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.2, 1% Triton X-100, 0.1% SDS, 1% DOC) and then diluted in loading buffer according to Laemmli, separated on 8% SDS polyacrylamide gel and transferred onto a PVDF membrane (BioRad). The detection of this immunoblot (Western blot) was carried out with the aid of an anti-S rabbit polyclonal serum (immune serum of the rabbit P11135: cf. example 4 above) and donkey polyclonal antibodies directed against rabbit IgGs and coupled with peroxidase (NA934V, Amersham). The bound antibodies were visualized by luminescence with the aid of the ECL+ kit (Amersham) and Hyperfilm MP autoradiography films (Amersham).

Vero cells in monolayers on glass slides were infected with the two viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol and the wild-type virus MWSchw as a control at multiplicities of infection of 0.05. When the syncytia had reached 90 to 100% (MVSchw2-SARS-Ssol virus) or 30 to 40% (MVSchw2-SARS-S, MWSchw) confluence, the cells were fixed in a 4% PBS-PFA solution, permeabilized with a PBS solution containing 0.2% Triton and then labeled with rabbit polyclonal antibodies hyperimmunized with purified and inactivated SARS-CoV virions and with an anti-rabbit IgG (H+L) goat antibody conjugate coupled with FITC (Jackson).

As shown in FIGS. **41** and **42**, the recombinant viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol direct the expression of the S protein and the Ssol polypeptide respectively at levels comparable to those which can be observed 8 h after infection with SARS-CoV. The expression of these polypeptides is stable after 3 passages of the recombinant viruses in cell culture. These results demonstrate that the recombinant measles viruses are indeed carriers of the transgenes and allow the expression of the SARS glycoprotein in its membrane form (S) or in a soluble form (Ssol). The Ssol polypeptide is expected to be secreted by cells infected with the MVSchw2-SARS-Ssol virus as is the case when this same polypeptide is expressed in mammalian cells after transient transfection of the corresponding sequences (cf. example 11 above).

4) Applications

Having shown that the viruses. MVSchw2-SARS-S and MVSchw2-SARS-Ssol allow the expression of the SARS-CoV S, their capacity to induce a protective immune response against SARS-CoV in $CD46^{+/-}$ IFN-$\alpha\beta R^{-/-}$ mice, which is

sensitive to infection by MV, is evaluated. The antibody response of the immunized mice is evaluated by ELISA test against the native antigens of SARS-CoV and for their capacity to neutralize the infectivity of SARS-CoV in vitro, using the methodologies described above. The protective power of the response will be evaluated by measuring the reduction in the pulmonary viral load 2 days after a nonlethal challenge infection with SARS-CoV.

Second generation recombinant measles viruses are constructed by substituting the wild-type sequences of the S and Sol genes by synthetic genes optimized for expression in mammalian cells, described in example 15 above. These recombinant measles viruses are capable of expressing larger quantities of the S and Ssol antigens and therefore of exhibiting increased immunogenicity.

Alternatively, the wild-type or synthetic genes encoding the S protein or the Ssol polypeptide may be inserted into the measles vector MVSchw-ATU3 in the form of an additional transcription unit located between the H and L genes, and then the recombinant viruses produced and characterized in a similar manner. This insertion is capable of generating recombinant viruses possessing different characteristics (multiplication of the virus, level of expression of the transgene) and possibly an improved immunogenicity compared with those obtained after insertion of the transgenes between the P and N genes.

The recombinant measles virus MVSchw2-SARS-Ssol may be used for the quantitative production and the purification of the Ssol antigen for diagnostic and vaccine applications.

EXAMPLE 18

Other Applications Linked to the S Protein

a) The lentiviral vectors allowing the expression of S or Ssol (or even of fragments of S) can constitute a recombinant vaccine against SARS-CoV, to be used in human or veterinary prophylaxis. In order to demonstrate the feasibility of such a vaccine, the immunogenicity of the recombinant lentiviral vectors TRIP-SD/SA-S-WPRE and TRIP-SD/SA-Ssol-WPRE is studied in mice.

b) Monoclonal antibodies are produced with the aid of the recombinant Ssol polypeptide. According to the results presented in example 14 above, these antibodies or at least the majority of them will recognize the native form of the SARS-CoV S and will be capable of diagnostic and/or prophylactic applications.

c) A serological test for SARS is developed with the Ssol polypeptide used as antigen and the double epitope methodology.

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atctcacata gcaatcttta atcaatgtgt aacattaggg aggacttgaa agagccacca 29580

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Met Phe Ile Phe Leu Leu Phe Leu
1 5

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Thr Leu Thr Ser Gly Ser Asp Leu Asp Arg Cys Thr Thr Phe Asp Asp
10 15 20

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Val Gln Ala Pro Asn Tyr Thr Gln His Thr Ser Ser Met Arg Gly Val
25 30 35 40

tac tat cct gat gaa att ttt aga tca gac act ctt tat tta act cag 256
Tyr Tyr Pro Asp Glu Ile Phe Arg Ser Asp Thr Leu Tyr Leu Thr Gln
45 50 55

gat tta ttt ctt cca ttt tat tct aat gtt aca ggg ttt cat act att 304
Asp Leu Phe Leu Pro Phe Tyr Ser Asn Val Thr Gly Phe His Thr Ile
60 65 70

aat cat acg ttt ggc aac cct gtc ata cct ttt aag gat ggt att tat 352
Asn His Thr Phe Gly Asn Pro Val Ile Pro Phe Lys Asp Gly Ile Tyr
75 80 85

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Phe Ala Ala Thr Glu Lys Ser Asn Val Val Arg Gly Trp Val Phe Gly
90 95 100

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Ser Thr Met Asn Asn Lys Ser Gln Ser Val Ile Ile Ile Asn Asn Ser
105 110 115 120

act aat gtt gtt ata cga gca tgt aac ttt gaa ttg tgt gac aac cct 496
Thr Asn Val Val Ile Arg Ala Cys Asn Phe Glu Leu Cys Asp Asn Pro
125 130 135

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Phe Phe Ala Val Ser Lys Pro Met Gly Thr Gln Thr His Thr Met Ile
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Phe Asp Asn Ala Phe Asn Cys Thr Phe Glu Tyr Ile Ser Asp Ala Phe
155 160 165

tcg ctt gat gtt tca gaa aag tca ggt aat ttt aaa cac tta cga gag 640
Ser Leu Asp Val Ser Glu Lys Ser Gly Asn Phe Lys His Leu Arg Glu
170 175 180

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Phe Val Phe Lys Asn Lys Asp Gly Phe Leu Tyr Val Tyr Lys Gly Tyr
185 190 195 200

caa cct ata gat gta gtt cgt gat cta cct tct ggt ttt aac act ttg 736
Gln Pro Ile Asp Val Val Arg Asp Leu Pro Ser Gly Phe Asn Thr Leu
205 210 215

aaa cct att ttt aag ttg cct ctt ggt att aac att aca aat ttt aga 784
Lys Pro Ile Phe Lys Leu Pro Leu Gly Ile Asn Ile Thr Asn Phe Arg
220 225 230

gcc att ctt aca gcc ttt tca cct gct caa gac att tgg ggc acg tca 832
Ala Ile Leu Thr Ala Phe Ser Pro Ala Gln Asp Ile Trp Gly Thr Ser
235 240 245

gct gca gcc tat ttt gtt ggc tat tta aag cca act aca ttt atg ctc 880
Ala Ala Ala Tyr Phe Val Gly Tyr Leu Lys Pro Thr Thr Phe Met Leu
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aag tat gat gaa aat ggt aca atc aca gat gct gtt gat tgt tct caa 928

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Lys Tyr Asp Glu Asn Gly Thr Ile Thr Asp Ala Val Asp Cys Ser Gln
265                 270                 275                 280

aat cca ctt gct gaa ctc aaa tgc tct gtt aag agc ttt gag att gac      976
Asn Pro Leu Ala Glu Leu Lys Cys Ser Val Lys Ser Phe Glu Ile Asp
                285                 290                 295

aaa gga att tac cag acc tct aat ttc agg gtt gtt ccc tca gga gat     1024
Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val Val Pro Ser Gly Asp
            300                 305                 310

gtt gtg aga ttc cct aat att aca aac ttg tgt cct ttt gga gag gtt     1072
Val Val Arg Phe Pro Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val
        315                 320                 325

ttt aat gct act aaa ttc cct tct gtc tat gca tgg gag aga aaa aaa     1120
Phe Asn Ala Thr Lys Phe Pro Ser Val Tyr Ala Trp Glu Arg Lys Lys
    330                 335                 340

att tct aat tgt gtt gct gat tac tct gtg ctc tac aac tca aca ttt     1168
Ile Ser Asn Cys Val Ala Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe
345                 350                 355                 360

ttt tca acc ttt aag tgc tat ggc gtt tct gcc act aag ttg aat gat     1216
Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp
                365                 370                 375

ctt tgc ttc tcc aat gtc tat gca gat tct ttt gta gtc aag gga gat     1264
Leu Cys Phe Ser Asn Val Tyr Ala Asp Ser Phe Val Val Lys Gly Asp
            380                 385                 390

gat gta aga caa ata gcg cca gga caa act ggt gtt att gct gat tat     1312
Asp Val Arg Gln Ile Ala Pro Gly Gln Thr Gly Val Ile Ala Asp Tyr
        395                 400                 405

aat tat aaa ttg cca gat gat ttc atg ggt tgt gtc ctt gct tgg aat     1360
Asn Tyr Lys Leu Pro Asp Asp Phe Met Gly Cys Val Leu Ala Trp Asn
    410                 415                 420

act agg aac att gat gct act tca act ggt aat tat aat tat aaa tat     1408
Thr Arg Asn Ile Asp Ala Thr Ser Thr Gly Asn Tyr Asn Tyr Lys Tyr
425                 430                 435                 440

agg tat ctt aga cat ggc aag ctt agg ccc ttt gag aga gac ata tct     1456
Arg Tyr Leu Arg His Gly Lys Leu Arg Pro Phe Glu Arg Asp Ile Ser
                445                 450                 455

aat gtg cct ttc tcc cct gat ggc aaa cct tgc acc cca cct gct ctt     1504
Asn Val Pro Phe Ser Pro Asp Gly Lys Pro Cys Thr Pro Pro Ala Leu
            460                 465                 470

aat tgt tat tgg cca tta aat gat tat ggt ttt tac acc act act ggc     1552
Asn Cys Tyr Trp Pro Leu Asn Asp Tyr Gly Phe Tyr Thr Thr Thr Gly
        475                 480                 485

att ggc tac caa cct tac aga gtt gta gta ctt tct ttt gaa ctt tta     1600
Ile Gly Tyr Gln Pro Tyr Arg Val Val Val Leu Ser Phe Glu Leu Leu
    490                 495                 500

aat gca ccg gcc acg gtt tgt gga cca aaa tta tcc act gac ctt att     1648
Asn Ala Pro Ala Thr Val Cys Gly Pro Lys Leu Ser Thr Asp Leu Ile
505                 510                 515                 520

aag aac cag tgt gtc aat ttt aat ttt aat gga ctc act ggt act ggt     1696
Lys Asn Gln Cys Val Asn Phe Asn Phe Asn Gly Leu Thr Gly Thr Gly
                525                 530                 535

gtg tta act cct tct tca aag aga ttt caa cca ttt caa caa ttt ggc     1744
Val Leu Thr Pro Ser Ser Lys Arg Phe Gln Pro Phe Gln Gln Phe Gly
            540                 545                 550

cgt gat gtt tct gat ttc act gat tcc gtt cga gat cct aaa aca tct     1792
Arg Asp Val Ser Asp Phe Thr Asp Ser Val Arg Asp Pro Lys Thr Ser
        555                 560                 565

gaa ata tta gac att tca cct tgc tct ttt ggg ggt gta agt gta att     1840
Glu Ile Leu Asp Ile Ser Pro Cys Ser Phe Gly Gly Val Ser Val Ile
    570                 575                 580

aca cct gga aca aat gct tca tct gaa gtt gct gtt cta tat caa gat     1888
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Thr Pro Gly Thr Asn Ala Ser Ser Glu Val Ala Val Leu Tyr Gln Asp
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gtt aac tgc act gat gtt tct aca gca att cat gca gat caa ctc aca     1936
Val Asn Cys Thr Asp Val Ser Thr Ala Ile His Ala Asp Gln Leu Thr
                605                 610                 615

cca gct tgg cgc ata tat tct act gga aac aat gta ttc cag act caa     1984
Pro Ala Trp Arg Ile Tyr Ser Thr Gly Asn Asn Val Phe Gln Thr Gln
            620                 625                 630

gca ggc tgt ctt ata gga gct gag cat gtc gac act tct tat gag tgc     2032
Ala Gly Cys Leu Ile Gly Ala Glu His Val Asp Thr Ser Tyr Glu Cys
        635                 640                 645

gac att cct att gga gct ggc att tgt gct agt tac cat aca gtt tct     2080
Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala Ser Tyr His Thr Val Ser
    650                 655                 660

tta tta cgt agt act agc caa aaa tct att gtg gct tat act atg tct     2128
Leu Leu Arg Ser Thr Ser Gln Lys Ser Ile Val Ala Tyr Thr Met Ser
665                 670                 675                 680

tta ggt gct gat agt tca att gct tac tct aat aac acc att gct ata     2176
Leu Gly Ala Asp Ser Ser Ile Ala Tyr Ser Asn Asn Thr Ile Ala Ile
                685                 690                 695

cct act aac ttt tca att agc att act aca gaa gta atg cct gtt tct     2224
Pro Thr Asn Phe Ser Ile Ser Ile Thr Thr Glu Val Met Pro Val Ser
            700                 705                 710

atg gct aaa acc tcc gta gat tgt aat atg tac atc tgc gga gat tct     2272
Met Ala Lys Thr Ser Val Asp Cys Asn Met Tyr Ile Cys Gly Asp Ser
        715                 720                 725

act gaa tgt gct aat ttg ctt ctc caa tat ggt agc ttt tgc aca caa     2320
Thr Glu Cys Ala Asn Leu Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln
    730                 735                 740

cta aat cgt gca ctc tca ggt att gct gct gaa cag gat cgc aac aca     2368
Leu Asn Arg Ala Leu Ser Gly Ile Ala Ala Glu Gln Asp Arg Asn Thr
745                 750                 755                 760

cgt gaa gtg ttc gct caa gtc aaa caa atg tac aaa acc cca act ttg     2416
Arg Glu Val Phe Ala Gln Val Lys Gln Met Tyr Lys Thr Pro Thr Leu
                765                 770                 775

aaa tat ttt ggt ggt ttt aat ttt tca caa ata tta cct gac cct cta     2464
Lys Tyr Phe Gly Gly Phe Asn Phe Ser Gln Ile Leu Pro Asp Pro Leu
            780                 785                 790

aag cca act aag agg tct ttt att gag gac ttg ctc ttt aat aag gtg     2512
Lys Pro Thr Lys Arg Ser Phe Ile Glu Asp Leu Leu Phe Asn Lys Val
        795                 800                 805

aca ctc gct gat gct ggc ttc atg aag caa tat ggc gaa tgc cta ggt     2560
Thr Leu Ala Asp Ala Gly Phe Met Lys Gln Tyr Gly Glu Cys Leu Gly
    810                 815                 820

gat att aat gct aga gat ctc att tgt gcg cag aag ttc aat gga ctt     2608
Asp Ile Asn Ala Arg Asp Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu
825                 830                 835                 840

aca gtg ttg cca cct ctg ctc act gat gat atg att gct gcc tac act     2656
Thr Val Leu Pro Pro Leu Leu Thr Asp Asp Met Ile Ala Ala Tyr Thr
                845                 850                 855

gct gct cta gtt agt ggt act gcc act gct gga tgg aca ttt ggt gct     2704
Ala Ala Leu Val Ser Gly Thr Ala Thr Ala Gly Trp Thr Phe Gly Ala
            860                 865                 870

ggc gct gct ctt caa ata cct ttt gct atg caa atg gca tat agg ttc     2752
Gly Ala Ala Leu Gln Ile Pro Phe Ala Met Gln Met Ala Tyr Arg Phe
        875                 880                 885

aat ggc att gga gtt acc caa aat gtt ctc tat gag aac caa aaa caa     2800
Asn Gly Ile Gly Val Thr Gln Asn Val Leu Tyr Glu Asn Gln Lys Gln
    890                 895                 900

atc gcc aac caa ttt aac aag gcg att agt caa att caa gaa tca ctt     2848
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Ile Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu
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aca aca aca tca act gca ttg ggc aag ctg caa gac gtt gtt aac cag     2896
Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln
                925                 930                 935

aat gct caa gca tta aac aca ctt gtt aaa caa ctt agc tct aat ttt     2944
Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe
            940                 945                 950

ggt gca att tca agt gtg cta aat gat atc ctt tcg cga ctt gat aaa     2992
Gly Ala Ile Ser Ser Val Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys
        955                 960                 965

gtc gag gcg gag gta caa att gac agg tta att aca ggc aga ctt caa     3040
Val Glu Ala Glu Val Gln Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln
    970                 975                 980

agc ctt caa acc tat gta aca caa caa cta atc agg gct gct gaa atc     3088
Ser Leu Gln Thr Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile
985                 990                 995                 1000

agg gct tct gct aat  ctt gct gct act aaa  atg tct gag tgt gtt       3133
Arg Ala Ser Ala Asn  Leu Ala Ala Thr Lys  Met Ser Glu Cys Val
                1005                1010                1015

ctt gga caa tca aaa  aga gtt gac ttt tgt  gga aag ggc tac cac       3178
Leu Gly Gln Ser Lys  Arg Val Asp Phe Cys  Gly Lys Gly Tyr His
                1020                1025                1030

ctt atg tcc ttc cca  caa gca gcc ccg cat  ggt gtt gtc ttc cta       3223
Leu Met Ser Phe Pro  Gln Ala Ala Pro His  Gly Val Val Phe Leu
                1035                1040                1045

cat gtc acg tat gtg  cca tcc cag gag agg  aac ttc acc aca gcg       3268
His Val Thr Tyr Val  Pro Ser Gln Glu Arg  Asn Phe Thr Thr Ala
                1050                1055                1060

cca gca att tgt cat  gaa ggc aaa gca tac  ttc cct cgt gaa ggt       3313
Pro Ala Ile Cys His  Glu Gly Lys Ala Tyr  Phe Pro Arg Glu Gly
                1065                1070                1075

gtt ttt gtg ttt aat  ggc act tct tgg ttt  att aca cag agg aac       3358
Val Phe Val Phe Asn  Gly Thr Ser Trp Phe  Ile Thr Gln Arg Asn
                1080                1085                1090

ttc ttt tct cca caa  ata att act aca gac  aat aca ttt gtc tca       3403
Phe Phe Ser Pro Gln  Ile Ile Thr Thr Asp  Asn Thr Phe Val Ser
                1095                1100                1105

gga aat tgt gat gtc  gtt att ggc atc att  aac aac aca gtt tat       3448
Gly Asn Cys Asp Val  Val Ile Gly Ile Ile  Asn Asn Thr Val Tyr
                1110                1115                1120

gat cct ctg caa cct  gag ctt gac tca ttc  aaa gaa gag ctg gac       3493
Asp Pro Leu Gln Pro  Glu Leu Asp Ser Phe  Lys Glu Glu Leu Asp
                1125                1130                1135

aag tac ttc aaa aat  cat aca tca cca gat  gtt gat ctt ggc gac       3538
Lys Tyr Phe Lys Asn  His Thr Ser Pro Asp  Val Asp Leu Gly Asp
                1140                1145                1150

att tca ggc att aac  gct tct gtc gtc aac  att caa aaa gaa att       3583
Ile Ser Gly Ile Asn  Ala Ser Val Val Asn  Ile Gln Lys Glu Ile
                1155                1160                1165

gac cgc ctc aat gag  gtc gct aaa aat tta  aat gaa tca ctc att       3628
Asp Arg Leu Asn Glu  Val Ala Lys Asn Leu  Asn Glu Ser Leu Ile
                1170                1175                1180

gac ctt caa gaa ttg  gga aaa tat gag caa  tat att aaa tgg cct       3673
Asp Leu Gln Glu Leu  Gly Lys Tyr Glu Gln  Tyr Ile Lys Trp Pro
                1185                1190                1195

tgg tat gtt tgg ctc  ggc ttc att gct gga  cta att gcc atc gtc       3718
Trp Tyr Val Trp Leu  Gly Phe Ile Ala Gly  Leu Ile Ala Ile Val
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Leu Lys Gly Ala Cys  Ser Cys Gly Ser Cys  Cys Lys Phe Asp Glu
                1230                1235                1240

gat gac tct gag cca  gtt ctc aag ggt gtc  aaa tta cat tac aca       3853
Asp Asp Ser Glu Pro  Val Leu Lys Gly Val  Lys Leu His Tyr Thr
                1245                1250                1255

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<211> LENGTH: 1255
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            20                  25                  30

His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
        35                  40                  45

Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
    50                  55                  60

Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
65                  70                  75                  80

Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
                85                  90                  95

Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
            100                 105                 110

Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
        115                 120                 125

Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
    130                 135                 140

Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
145                 150                 155                 160

Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
                165                 170                 175

Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
            180                 185                 190

Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
        195                 200                 205

Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
    210                 215                 220

Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
225                 230                 235                 240

Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
                245                 250                 255

Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
            260                 265                 270

Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
        275                 280                 285

Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
    290                 295                 300
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Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
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Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
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Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
            340                 345                 350

Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
        355                 360                 365

Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
    370                 375                 380

Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
385                 390                 395                 400

Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
                405                 410                 415

Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
            420                 425                 430

Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
        435                 440                 445

Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
    450                 455                 460

Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
465                 470                 475                 480

Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
                485                 490                 495

Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
            500                 505                 510

Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
        515                 520                 525

Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
    530                 535                 540

Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
545                 550                 555                 560

Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
                565                 570                 575

Ser Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
            580                 585                 590

Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
        595                 600                 605

Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
    610                 615                 620

Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
625                 630                 635                 640

His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
                645                 650                 655

Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
            660                 665                 670

Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
        675                 680                 685

Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
    690                 695                 700

Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
705                 710                 715                 720

Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
                725                 730                 735
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Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
            740                 745                 750

Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
        755                 760                 765

Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe
    770                 775                 780

Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
785                 790                 795                 800

Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
                805                 810                 815

Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
            820                 825                 830

Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
        835                 840                 845

Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
    850                 855                 860

Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe
865                 870                 875                 880

Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn
                885                 890                 895

Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala
            900                 905                 910

Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly
        915                 920                 925

Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu
    930                 935                 940

Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn
945                 950                 955                 960

Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp
                965                 970                 975

Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln
            980                 985                 990

Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu Ala Ala
        995                 1000                1005

Thr Lys  Met Ser Glu Cys Val  Leu Gly Gln Ser Lys  Arg Val Asp
    1010                1015                1020

Phe Cys  Gly Lys Gly Tyr His  Leu Met Ser Phe Pro  Gln Ala Ala
    1025                1030                1035

Pro His  Gly Val Val Phe Leu  His Val Thr Tyr Val  Pro Ser Gln
    1040                1045                1050

Glu Arg  Asn Phe Thr Thr Ala  Pro Ala Ile Cys His  Glu Gly Lys
    1055                1060                1065

Ala Tyr  Phe Pro Arg Glu Gly  Val Phe Val Phe Asn  Gly Thr Ser
    1070                1075                1080

Trp Phe  Ile Thr Gln Arg Asn  Phe Phe Ser Pro Gln  Ile Ile Thr
    1085                1090                1095

Thr Asp  Asn Thr Phe Val Ser  Gly Asn Cys Asp Val  Val Ile Gly
    1100                1105                1110

Ile Ile  Asn Asn Thr Val Tyr  Asp Pro Leu Gln Pro  Glu Leu Asp
    1115                1120                1125

Ser Phe  Lys Glu Glu Leu Asp  Lys Tyr Phe Lys Asn  His Thr Ser
    1130                1135                1140

Pro Asp  Val Asp Leu Gly Asp  Ile Ser Gly Ile Asn  Ala Ser Val
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    1145                1150                1155

Val Asn  Ile Gln Lys Glu Ile  Asp Arg Leu Asn Glu  Val Ala Lys
    1160                1165                1170

Asn Leu  Asn Glu Ser Leu Ile  Asp Leu Gln Glu Leu  Gly Lys Tyr
    1175                1180                1185

Glu Gln  Tyr Ile Lys Trp Pro  Trp Tyr Val Trp Leu  Gly Phe Ile
    1190                1195                1200

Ala Gly  Leu Ile Ala Ile Val  Met Val Thr Ile Leu  Leu Cys Cys
    1205                1210                1215

Met Thr  Ser Cys Cys Ser Cys  Leu Lys Gly Ala Cys  Ser Cys Gly
    1220                1225                1230

Ser Cys  Cys Lys Phe Asp Glu  Asp Asp Ser Glu Pro  Val Leu Lys
    1235                1240                1245

Gly Val  Lys Leu His Tyr Thr
    1250                1255


<210> SEQ ID NO 4
<211> LENGTH: 3943
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 4

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gtggtagtga ccttgaccgg tgcaccactt ttgatgatgt tcaagctcct aattacactc    180

aacatacttc atctatgagg ggggtttact atcctgatga aatttttaga tcagacactc    240

tttatttaac tcaggattta tttcttccat tttattctaa tgttacaggg tttcatacta    300

ttaatcatac gtttggcaac cctgtcatac cttttaagga tggtatttat tttgctgcca    360

cagagaaatc aaatgttgtc cgtggttggg tttttggttc taccatgaac aacaagtcac    420

agtcggtgat tattattaac aattctacta atgttgttat acgagcatgt aactttgaat    480

tgtgtgacaa ccctttcttt gctgtttcta aacccatggg tacacagaca catactatga    540

tattcgataa tgcatttaat tgcactttcg agtacatatc tgatgccttt tcgcttgatg    600

tttcagaaaa gtcaggtaat tttaaacact tacgagagtt tgtgtttaaa aataaagatg    660

ggtttctcta tgtttataag ggctatcaac ctatagatgt agttcgtgat ctaccttctg    720

gttttaacac tttgaaacct atttttaagt tgcctcttgg tattaacatt acaaatttta    780

gagccattct tacagccttt tcacctgctc aagacatttg gggcacgtca gctgcagcct    840

attttgttgg ctatttaaag ccaactacat ttatgctcaa gtatgatgaa aatggtacaa    900

tcacagatgc tgttgattgt tctcaaaatc cacttgctga actcaaatgc tctgttaaga    960

gctttgagat tgacaaagga atttaccaga cctctaattt cagggttgtt ccctcaggag   1020

atgttgtgag attccctaat attacaaact tgtgtccttt tggagaggtt tttaatgcta   1080

ctaaattccc ttctgtctat gcatgggaga gaaaaaaaat ttctaattgt gttgctgatt   1140

actctgtgct ctacaactca acatttttttt caacctttaa gtgctatggc gtttctgcca   1200

ctaagttgaa tgatctttgc ttctccaatg tctatgcaga ttcttttgta gtcaagggag   1260

atgatgtaag acaaatagcg ccaggacaaa ctggtgttat tgctgattat aattataaat   1320

tgccagatga tttcatgggt tgtgtccttg cttggaatac taggaacatt gatgctactt   1380

caactggtaa ttataattat aaatataggt atcttagaca tggcaagctt aggccctttg   1440

agagagacat atctaatgtg cctttctccc ctgatggcaa accttgcacc ccacctgctc   1500
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ttaattgtta ttggccatta aatgattatg gtttttacac cactactggc attggctacc 1560

aaccttacag agttgtagta ctttcttttg aacttttaaa tgcaccggcc acggtttgtg 1620

gaccaaaatt atccactgac cttattaaga accagtgtgt caattttaat tttaatggac 1680

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gccgtgatgt ctctgatttc actgattccg ttcgagatcc taaaacatct gaaatattag 1800

acatttcacc ttgctctttt gggggtgtaa gtgtaattac acctggaaca aatgcttcat 1860

ctgaagttgc tgttctatat caagatgtta actgcactga tgtttctaca gcaatccatg 1920

cagatcaact cacaccagct tggcgcatat attctactgg aaacaatgta ttccagactc 1980

aagcaggctg tcttatagga gctgagcatg tcgacacttc ttatgagtgc gacattccta 2040

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aatctattgt ggcttatact atgtctttag gtgctgatag ttcaattgct tactctaata 2160

acaccattgc tatacctact aacttttcaa ttagcattac tacagaagta atgcctgttt 2220

ctatggctaa aacctccgta gattgtaata tgtacatctg cggagattct actgaatgtg 2280

ctaatttgct tctccaatat ggtagctttt gcacacaact aaatcgtgca ctctcaggta 2340

ttgctgctga acaggatcgc aacacacgtg aagtgttcgc tcaagtcaaa caaatgtaca 2400

aaaccccaac tttgaaatat tttggtggtt ttaatttttc acaaatatta cctgaccctc 2460

taaagccaac taagaggtct tttattgagg acttgctctt taataaggtg acactcgctg 2520

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ctggcgctgc tcttcaaata ccttttgcta tgcaaatggc atataggttc aatggcattg 2760

gagttaccca aaatgttctc tatgagaacc aaaaacaaat cgccaaccaa tttaacaagg 2820

cgattagtca aattcaagaa tcacttacaa caacatcaac tgcattgggc aagctgcaag 2880

acgttgttaa ccagaatgct caagcattaa acacacttgt taaacaactt agctctaatt 2940

ttggtgcaat ttcaagtgtg ctaaatgata tcctttcgcg acttgataaa gtcgaggcgg 3000

aggtacaaat tgacaggcta attacaggca gacttcaaag ccttcaaacc tatgtaacac 3060

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ttggcatcat taacaacaca gtttatgatc ctctgcaacc tgagcttgac tcattcaaag 3480

aagagctgga caagtacttc aaaaatcata catcaccaga tgttgatctt ggcgacattt 3540

caggcattaa cgcttctgtc gtcaacattc aaaaagaaat tgaccgcctc aatgaggtcg 3600

ctaaaaattt aaatgaatca ctcattgacc ttcaagaatt gggaaaatat gagcaatata 3660

ttaaatggcc ttggtatgtt tggctcggct tcattgctgg actaattgcc atcgtcatgg 3720

ttacaatctt gctttgttgc atgactagtt gttgcagttg cctcaagggt gcatgctctt 3780

gtggttcttg ctgcaagttt gatgaggatg actctgagcc agttctcaag ggtgtcaaat 3840

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<210> SEQ ID NO 5
<211> LENGTH: 2049
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 5

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gtggtagtga ccttgaccgg tgcaccactt ttgatgatgt tcaagctcct aattacactc 180

aacatacttc atctatgagg ggggtttact atcctgatga aatttttaga tcagacactc 240

tttatttaac tcaggattta tttcttccat tttattctaa tgttacaggg tttcatacta 300

ttaatcatac gtttggcaac cctgtcatac cttttaagga tggtatttat tttgctgcca 360

cagagaaatc aaatgttgtc cgtggttggg tttttggttc taccatgaac aacaagtcac 420

agtcggtgat tattattaac aattctacta atgttgttat acgagcatgt aactttgaat 480

tgtgtgacaa ccctttcttt gctgtttcta aacccatggg tacacagaca catactatga 540

tattcgataa tgcatttaat tgcactttcg agtacatatc tgatgccttt tcgcttgatg 600

tttcagaaaa gtcaggtaat tttaaacact tacgagagtt tgtgtttaaa aataaagatg 660

ggtttctcta tgtttataag ggctatcaac ctatagatgt agttcgtgat ctaccttctg 720

gttttaacac tttgaaacct atttttaagt tgcctcttgg tattaacatt acaaatttta 780

gagccattct tacagccttt tcacctgctc aagacatttg gggcacgtca gctgcagcct 840

attttgttgg ctatttaaag ccaactacat ttatgctcaa gtatgatgaa aatggtacaa 900

tcacagatgc tgttgattgt tctcaaaatc cacttgctga actcaaatgc tctgttaaga 960

gctttgagat tgacaaagga atttaccaga cctctaattt cagggttgtt ccctcaggag 1020

atgttgtgag attccctaat attacaaact tgtgtccttt tggagaggtt tttaatgcta 1080

ctaaattccc ttctgtctat gcatgggaga gaaaaaaaat ttctaattgt gttgctgatt 1140

actctgtgct ctacaactca acatttttttt caacctttaa gtgctatggc gtttctgcca 1200

ctaagttgaa tgatctttgc ttctccaatg tctatgcaga ttcttttgta gtcaagggag 1260

atgatgtaag acaaatagcg ccaggacaaa ctggtgttat tgctgattat aattataaat 1320

tgccagatga tttcatgggt tgtgtccttg cttggaatac taggaacatt gatgctactt 1380

caactggtaa ttataattat aaatataggt atcttagaca tggcaagctt aggccctttg 1440

agagagacat atctaatgtg cctttctccc ctgatggcaa accttgcacc ccacctgctc 1500

ttaattgtta ttggccatta aatgattatg gtttttacac cactactggc attggctacc 1560

aaccttacag agttgtagta ctttcttttg aacttttaaa tgcaccggcc acggtttgtg 1620

gaccaaaatt atccactgac cttattaaga accagtgtgt caattttaat tttaatggac 1680

tcactggtac tggtgtgtta actccttctt caaagagatt tcaaccattt caacaatttg 1740

gccgtgatgt ctctgatttc actgattccg ttcgagatcc taaaacatct gaaatattag 1800

acatttcacc ttgctctttt gggggtgtaa gtgtaattac acctggaaca aatgcttcat 1860

ctgaagttgc tgttctatat caagatgtta actgcactga tgtttctaca gcaatccatg 1920

cagatcaact cacaccagct tggcgcatat attctactgg aaacaatgta ttccagactc 1980

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ttggagctg 2049

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<210> SEQ ID NO 6
<211> LENGTH: 2027
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 6

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actcaagcag gctgtcttat aggagctgag catgtcgaca cttcttatga gtgcgacatt 120

cctattggag ctggcatttg tgctagttac catacagttt ctttattacg tagtactagc 180

caaaaatcta ttgtggctta tactatgtct ttaggtgctg atagttcaat tgcttactct 240

aataacacca ttgctatacc tactaacttt tcaattagca ttactacaga agtaatgcct 300

gtttctatgg ctaaaacctc cgtagattgt aatatgtaca tctgcggaga ttctactgaa 360

tgtgctaatt tgcttctcca atatggtagc ttttgcacac aactaaatcg tgcactctca 420

ggtattgctg ctgaacagga tcgcaacaca cgtgaagtgt tcgctcaagt caaacaaatg 480

tacaaaaccc caactttgaa atattttggt ggttttaatt tttcacaaat attacctgac 540

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gctgatgctg gcttcatgaa gcaatatggc gaatgcctag gtgatattaa tgctagagat 660

ctcatttgtg cgcagaagtt caatgggctt acagtgttgc cacctctgct cactgatgat 720

atgattgctg cctacactgc tgctctagtt agtggtactg ccactgctgg atggacattt 780

ggtgctggcg ctgctcttca aatacctttt gctatgcaaa tggcatatag gttcaatggc 840

attggagtta cccaaaatgt tctctatgag aaccaaaaac aaatcgccaa ccaatttaac 900

aaggcgatta gtcaaattca agaatcactt acaacaacat caactgcatt gggcaagctg 960

caagacgttg ttaaccagaa tgctcaagca ttaaacacac ttgttaaaca acttagctct 1020

aattttggtg caatttcaag tgtgctaaat gatatccttt cgcgacttga taaagtcgag 1080

gcggaggtac aaattgacag gttaattaca ggcagacttc aaagccttca aacctatgta 1140

acacaacaac taatcagggc tgctgaaatc agggcttctg ctaatcttgc tgctactaaa 1200

atgtctgagt gtgttcttgg acaatcaaaa agagttgact tttgtggaaa gggctaccac 1260

cttatgtcct tcccacaagc agccccgcat ggtgttgtct tcctacatgt cacgtatgtg 1320

ccatcccagg agaggaactt caccacagcg ccagcaattt gtcatgaagg caaagcatac 1380

ttccctcgtg aaggtgtttt tgtgtttaat ggcacttctt ggtttattac acagaggaac 1440

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gttattggcg tcattaacaa cacagtttat gatcctctgc aacctgagct tgactcattc 1560

aaagaagagc tggacaagta cttcaaaaat catacatcac cagatgttga tcttggcgac 1620

atttcaggca ttaacgcttc tgtcgtcaac attcaaaaag aaattgaccg cctcaatgag 1680

gtcgctaaaa atttaaatga atcactcatt gaccttcaag aattgggaaa atatgagcaa 1740

tatattaaat ggccttggta tgtttggctc ggcttcattg ctggactaat tgccatcgtc 1800

atggttacaa tcttgctttg ttgcatgact agttgttgca gttgcctcaa gggtgcatgc 1860

tcttgtggtt cttgctgcaa gtttgatgag gatgactctg agccagttct caagggtgtc 1920

aaattacatt acacataaac gaacttatgg atttgtttat gagatttttt actcttggat 1980

caattactgc acagccagta aaaattgaca atgcttctcc tgcaagt 2027

<210> SEQ ID NO 7
<211> LENGTH: 1096

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<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 7

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acacataaac gaacttatgg atttgtttat gagatttttt actcttggat caattactgc 180

acagccagta aaaattgaca atgcttctcc tgcaagtact gttcatgcta cagcaacgat 240

accgctacaa gcctcactcc ctttcggatg gcttgttatt ggcgttgcat ttcttgctgt 300

ttttcagagc gctaccaaaa taattgcgct caataaaaga tggcagctag ccctttataa 360

gggcttccag ttcatttgca atttactgct gctatttgtt accatctatt cacatctttt 420

gcttgtcgct gcaggtatgg aggcgcaatt tttgtacctc tatgccttga tatattttct 480

acaatgcatc aacgcatgta gaattattat gagatgttgg ctttgttgga agtgcaaatc 540

caagaaccca ttactttatg atgccaacta ctttgtttgc tggcacacac ataactatga 600

ctactgtata ccatataaca gtgtcacaga tacaattgtc gttactgaag gtgacggcat 660

ttcaacacca aaactcaaag aagactacca aattggtggt tattctgagg ataggcactc 720

aggtgttaaa gactatgtcg ttgtacatgg ctatttcacc gaagtttact accagcttga 780

gtctacacaa attactacag acactggtat tgaaaatgct acattcttca tctttaacaa 840

gcttgttaaa gacccaccga atgtgcaaat acacacaatc gacggctctt caggagttgc 900

taatccagca atggatccaa tttatgatga gccgacgacg actactagcg tgcctttgta 960

agcacaagaa agtgagtacg aacttatgta ctcattcgtt tcggaagaaa caggtacgtt 1020

aatagttaat agcgtacttc tttttcttgc tttcgtggta ttcttgctag tcacactagc 1080

catccttact gcgctt 1096

<210> SEQ ID NO 8
<211> LENGTH: 1135
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 8

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ctcaagggtg tcaaattaca ttacacataa acgaacttat ggatttgttt atgagatttt 180

ttactcttgg atcaattact gcacagccag taaaaattga caatgcttct cctgcaagta 240

ctgttcatgc tacagcaacg ataccgctac aagcctcact ccctttcgga tggcttgtta 300

ttggcgttgc atttcttgct gtttttcaga gcgctaccaa aataattgcg ctcaataaaa 360

gatggcagct agcccctttat aagggcttcc agttcatttg caatttactg ctgctatttg 420

ttaccatcta ttcacatctt ttgcttgtcg ctgcaggtat ggaggcgcaa tttttgtacc 480

tctatgcctt gatatatttt ctacaatgca tcaacgcatg tagaattatt atgagatgtt 540

ggctttgttg gaagtgcaaa tccaagaacc cattacttta tgatgccaac tactttgttt 600

gctggcacac acataactat gactactgta taccatataa cagtgtcaca gatacaattg 660

tcgttactga aggtgacggc atttcaacac caaaactcaa agaagactac caaattggtg 720

gttattctga ggataggcac tcaggtgtta aagactatgt cgttgtacat ggctatttca 780

ccgaagttta ctaccagctt gagtctacac aaattactac agacactggt attgaaaatg 840

ctacattctt catctttaac aagcttgtta aagacccacc gaatgtgcaa atacacacaa 900

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tcgacggctc ttcaggagtt gctaatccag caatggatcc aatttatgat gagccgacga    960

cgactactag cgtgcctttg taagcacaag aaagtgagta cgaacttatg tactcattcg   1020

tttcggaaga aacaggtacg ttaatagtta atagcgtact tctttttctt gctttcgtgg   1080

tattcttgct agtcacacta gccatcctta ctgcgcttcg attgtgtgcg tactg        1135


<210> SEQ ID NO 9
<211> LENGTH: 1096
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (137)..(958)
<223> OTHER INFORMATION:

<400> SEQUENCE: 9

tcttgctttg ttgcatgact agttgttgca gttgcctcaa gggtgcatgc tcttgtggtt     60

cttgctgcaa gtttgatgag gatgactctg agccagttct caagggtgtc aaattacatt    120

acacataaac gaactt atg gat ttg ttt atg aga ttt ttt act ctt gga tca    172
                 Met Asp Leu Phe Met Arg Phe Phe Thr Leu Gly Ser
                 1               5                  10

att act gca cag cca gta aaa att gac aat gct tct cct gca agt act      220
Ile Thr Ala Gln Pro Val Lys Ile Asp Asn Ala Ser Pro Ala Ser Thr
        15                  20                  25

gtt cat gct aca gca acg ata ccg cta caa gcc tca ctc cct ttc gga      268
Val His Ala Thr Ala Thr Ile Pro Leu Gln Ala Ser Leu Pro Phe Gly
    30                  35                  40

tgg ctt gtt att ggc gtt gca ttt ctt gct gtt ttt cag agc gct acc      316
Trp Leu Val Ile Gly Val Ala Phe Leu Ala Val Phe Gln Ser Ala Thr
45                  50                  55                  60

aaa ata att gcg ctc aat aaa aga tgg cag cta gcc ctt tat aag ggc      364
Lys Ile Ile Ala Leu Asn Lys Arg Trp Gln Leu Ala Leu Tyr Lys Gly
                65                  70                  75

ttc cag ttc att tgc aat tta ctg ctg cta ttt gtt acc atc tat tca      412
Phe Gln Phe Ile Cys Asn Leu Leu Leu Leu Phe Val Thr Ile Tyr Ser
            80                  85                  90

cat ctt ttg ctt gtc gct gca ggt atg gag gcg caa ttt ttg tac ctc      460
His Leu Leu Leu Val Ala Ala Gly Met Glu Ala Gln Phe Leu Tyr Leu
        95                  100                 105

tat gcc ttg ata tat ttt cta caa tgc atc aac gca tgt aga att att      508
Tyr Ala Leu Ile Tyr Phe Leu Gln Cys Ile Asn Ala Cys Arg Ile Ile
    110                 115                 120

atg aga tgt tgg ctt tgt tgg aag tgc aaa tcc aag aac cca tta ctt      556
Met Arg Cys Trp Leu Cys Trp Lys Cys Lys Ser Lys Asn Pro Leu Leu
125                 130                 135                 140

tat gat gcc aac tac ttt gtt tgc tgg cac aca cat aac tat gac tac      604
Tyr Asp Ala Asn Tyr Phe Val Cys Trp His Thr His Asn Tyr Asp Tyr
                145                 150                 155

tgt ata cca tat aac agt gtc aca gat aca att gtc gtt act gaa ggt      652
Cys Ile Pro Tyr Asn Ser Val Thr Asp Thr Ile Val Val Thr Glu Gly
            160                 165                 170

gac ggc att tca aca cca aaa ctc aaa gaa gac tac caa att ggt ggt      700
Asp Gly Ile Ser Thr Pro Lys Leu Lys Glu Asp Tyr Gln Ile Gly Gly
        175                 180                 185

tat tct gag gat agg cac tca ggt gtt aaa gac tat gtc gtt gta cat      748
Tyr Ser Glu Asp Arg His Ser Gly Val Lys Asp Tyr Val Val Val His
    190                 195                 200

ggc tat ttc acc gaa gtt tac tac cag ctt gag tct aca caa att act      796
Gly Tyr Phe Thr Glu Val Tyr Tyr Gln Leu Glu Ser Thr Gln Ile Thr
205                 210                 215                 220
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aca gac act ggt att gaa aat gct aca ttc ttc atc ttt aac aag ctt      844
Thr Asp Thr Gly Ile Glu Asn Ala Thr Phe Phe Ile Phe Asn Lys Leu
                225                 230                 235

gtt aaa gac cca ccg aat gtg caa ata cac aca atc gac ggc tct tca      892
Val Lys Asp Pro Pro Asn Val Gln Ile His Thr Ile Asp Gly Ser Ser
            240                 245                 250

gga gtt gct aat cca gca atg gat cca att tat gat gag ccg acg acg      940
Gly Val Ala Asn Pro Ala Met Asp Pro Ile Tyr Asp Glu Pro Thr Thr
        255                 260                 265

act act agc gtg cct ttg taagcacaag aaagtgagta cgaacttatg             988
Thr Thr Ser Val Pro Leu
    270

tactcattcg tttcggaaga aacaggtacg ttaatagtta atagcgtact tctttttctt   1048

gctttcgtgg tattcttgct agtcacacta gccatcctta ctgcgctt               1096


<210> SEQ ID NO 10
<211> LENGTH: 274
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 10

Met Asp Leu Phe Met Arg Phe Phe Thr Leu Gly Ser Ile Thr Ala Gln
1               5                   10                  15

Pro Val Lys Ile Asp Asn Ala Ser Pro Ala Ser Thr Val His Ala Thr
            20                  25                  30

Ala Thr Ile Pro Leu Gln Ala Ser Leu Pro Phe Gly Trp Leu Val Ile
        35                  40                  45

Gly Val Ala Phe Leu Ala Val Phe Gln Ser Ala Thr Lys Ile Ile Ala
    50                  55                  60

Leu Asn Lys Arg Trp Gln Leu Ala Leu Tyr Lys Gly Phe Gln Phe Ile
65                  70                  75                  80

Cys Asn Leu Leu Leu Leu Phe Val Thr Ile Tyr Ser His Leu Leu Leu
                85                  90                  95

Val Ala Ala Gly Met Glu Ala Gln Phe Leu Tyr Leu Tyr Ala Leu Ile
            100                 105                 110

Tyr Phe Leu Gln Cys Ile Asn Ala Cys Arg Ile Ile Met Arg Cys Trp
        115                 120                 125

Leu Cys Trp Lys Cys Lys Ser Lys Asn Pro Leu Leu Tyr Asp Ala Asn
    130                 135                 140

Tyr Phe Val Cys Trp His Thr His Asn Tyr Asp Tyr Cys Ile Pro Tyr
145                 150                 155                 160

Asn Ser Val Thr Asp Thr Ile Val Val Thr Glu Gly Asp Gly Ile Ser
                165                 170                 175

Thr Pro Lys Leu Lys Glu Asp Tyr Gln Ile Gly Gly Tyr Ser Glu Asp
            180                 185                 190

Arg His Ser Gly Val Lys Asp Tyr Val Val Val His Gly Tyr Phe Thr
        195                 200                 205

Glu Val Tyr Tyr Gln Leu Glu Ser Thr Gln Ile Thr Thr Asp Thr Gly
    210                 215                 220

Ile Glu Asn Ala Thr Phe Phe Ile Phe Asn Lys Leu Val Lys Asp Pro
225                 230                 235                 240

Pro Asn Val Gln Ile His Thr Ile Asp Gly Ser Ser Gly Val Ala Asn
                245                 250                 255

Pro Ala Met Asp Pro Ile Tyr Asp Glu Pro Thr Thr Thr Thr Ser Val
            260                 265                 270
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Pro Leu

<210> SEQ ID NO 11
<211> LENGTH: 1096
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (558)..(1019)
<223> OTHER INFORMATION:

<400> SEQUENCE: 11

tcttgctttg ttgcatgact agttgttgca gttgcctcaa gggtgcatgc tcttgtggtt 60

cttgctgcaa gtttgatgag gatgactctg agccagttct caagggtgtc aaattacatt 120

acacataaac gaacttatgg atttgtttat gagatttttt actcttggat caattactgc 180

acagccagta aaaattgaca atgcttctcc tgcaagtact gttcatgcta cagcaacgat 240

accgctacaa gcctcactcc ctttcggatg gcttgttatt ggcgttgcat ttcttgctgt 300

ttttcagagc gctaccaaaa taattgcgct caataaaaga tggcagctag ccctttataa 360

gggcttccag ttcatttgca atttactgct gctatttgtt accatctatt cacatctttt 420

gcttgtcgct gcaggtatgg aggcgcaatt tttgtacctc tatgccttga tatattttct 480

acaatgcatc aacgcatgta gaattattat gagatgttgg ctttgttgga agtgcaaatc 540

caagaaccca ttacttt atg atg cca act act ttg ttt gct ggc aca cac 590
Met Met Pro Thr Thr Leu Phe Ala Gly Thr His
1 5 10

ata act atg act act gta tac cat ata aca gtg tca cag ata caa ttg 638
Ile Thr Met Thr Thr Val Tyr His Ile Thr Val Ser Gln Ile Gln Leu
15 20 25

tcg tta ctg aag gtg acg gca ttt caa cac caa aac tca aag aag act 686
Ser Leu Leu Lys Val Thr Ala Phe Gln His Gln Asn Ser Lys Lys Thr
30 35 40

acc aaa ttg gtg gtt att ctg agg ata ggc act cag gtg tta aag act 734
Thr Lys Leu Val Val Ile Leu Arg Ile Gly Thr Gln Val Leu Lys Thr
45 50 55

atg tcg ttg tac atg gct att tca ccg aag ttt act acc agc ttg agt 782
Met Ser Leu Tyr Met Ala Ile Ser Pro Lys Phe Thr Thr Ser Leu Ser
60 65 70 75

cta cac aaa tta cta cag aca ctg gta ttg aaa atg cta cat tct tca 830
Leu His Lys Leu Leu Gln Thr Leu Val Leu Lys Met Leu His Ser Ser
80 85 90

tct tta aca agc ttg tta aag acc cac cga atg tgc aaa tac aca caa 878
Ser Leu Thr Ser Leu Leu Lys Thr His Arg Met Cys Lys Tyr Thr Gln
95 100 105

tcg acg gct ctt cag gag ttg cta atc cag caa tgg atc caa ttt atg 926
Ser Thr Ala Leu Gln Glu Leu Leu Ile Gln Gln Trp Ile Gln Phe Met
110 115 120

atg agc cga cga cga cta cta gcg tgc ctt tgt aag cac aag aaa gtg 974
Met Ser Arg Arg Arg Leu Leu Ala Cys Leu Cys Lys His Lys Lys Val
125 130 135

agt acg aac tta tgt act cat tcg ttt cgg aag aaa cag gta cgt 1019
Ser Thr Asn Leu Cys Thr His Ser Phe Arg Lys Lys Gln Val Arg
140 145 150

taatagttaa tagcgtactt ctttttcttg ctttcgtggt attcttgcta gtcacactag 1079

ccatccttac tgcgctt 1096

<210> SEQ ID NO 12
<211> LENGTH: 154
<212> TYPE: PRT

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<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 12

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Met Met Pro Thr Thr Leu Phe Ala Gly Thr His Ile Thr Met Thr Thr
1               5                   10                  15

Val Tyr His Ile Thr Val Ser Gln Ile Gln Leu Ser Leu Leu Lys Val
            20                  25                  30

Thr Ala Phe Gln His Gln Asn Ser Lys Lys Thr Thr Lys Leu Val Val
        35                  40                  45

Ile Leu Arg Ile Gly Thr Gln Val Leu Lys Thr Met Ser Leu Tyr Met
    50                  55                  60

Ala Ile Ser Pro Lys Phe Thr Thr Ser Leu Ser Leu His Lys Leu Leu
65                  70                  75                  80

Gln Thr Leu Val Leu Lys Met Leu His Ser Ser Ser Leu Thr Ser Leu
                85                  90                  95

Leu Lys Thr His Arg Met Cys Lys Tyr Thr Gln Ser Thr Ala Leu Gln
            100                 105                 110

Glu Leu Leu Ile Gln Gln Trp Ile Gln Phe Met Met Ser Arg Arg Arg
        115                 120                 125

Leu Leu Ala Cys Leu Cys Lys His Lys Lys Val Ser Thr Asn Leu Cys
    130                 135                 140

Thr His Ser Phe Arg Lys Lys Gln Val Arg
145                 150
```

<210> SEQ ID NO 13
<211> LENGTH: 332
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (36)..(263)
<223> OTHER INFORMATION:

<400> SEQUENCE: 13

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tgcctttgta agcacaagaa agtgagtacg aacttatg tac tca ttc gtt tcg        53
                                       Met Tyr Ser Phe Val Ser
                                       1               5

gaa gaa aca ggt acg tta ata gtt aat agc gta ctt ctt ttt ctt gct      101
Glu Glu Thr Gly Thr Leu Ile Val Asn Ser Val Leu Leu Phe Leu Ala
            10                  15                  20

ttc gtg gta ttc ttg cta gtc aca cta gcc atc ctt act gcg ctt cga      149
Phe Val Val Phe Leu Leu Val Thr Leu Ala Ile Leu Thr Ala Leu Arg
        25                  30                  35

ttg tgt gcg tac tgc tgc aat att gtt aac gtg agt tta gta aaa cca      197
Leu Cys Ala Tyr Cys Cys Asn Ile Val Asn Val Ser Leu Val Lys Pro
    40                  45                  50

acg gtt tac gtc tac tcg cgt gtt aaa aat ctg aac tct tct gaa gga      245
Thr Val Tyr Val Tyr Ser Arg Val Lys Asn Leu Asn Ser Ser Glu Gly
55                  60                  65                  70

gtt cct gat ctt ctg gtc taaacgaact aactattatt attattctgt            293
Val Pro Asp Leu Leu Val
                75

ttggaacttt aacattgctt atcatggcag acaacggta                          332
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<210> SEQ ID NO 14
<211> LENGTH: 76
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 14

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Met Tyr Ser Phe Val Ser Glu Glu Thr Gly Thr Leu Ile Val Asn Ser
1               5                   10                  15

Val Leu Leu Phe Leu Ala Phe Val Val Phe Leu Leu Val Thr Leu Ala
            20                  25                  30

Ile Leu Thr Ala Leu Arg Leu Cys Ala Tyr Cys Cys Asn Ile Val Asn
        35                  40                  45

Val Ser Leu Val Lys Pro Thr Val Tyr Val Tyr Ser Arg Val Lys Asn
    50                  55                  60

Leu Asn Ser Ser Glu Gly Val Pro Asp Leu Leu Val
65                  70                  75


<210> SEQ ID NO 15
<211> LENGTH: 332
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 15

tgcctttgta agcacaagaa agtgagtacg aacttatgta ctcattcgtt tcggaagaaa     60

caggtacgtt aatagttaat agcgtacttc tttttcttgc tttcgtggta ttcttgctag    120

tcacactagc catccttact gcgcttcgat tgtgtgcgta ctgctgcaat attgttaacg    180

tgagtttagt aaaaccaacg gtttacgtct actcgcgtgt taaaaatctg aactcttctg    240

aaggagttcc tgatcttctg gtctaaacga actaactatt attattattc tgtttggaac    300

tttaacattg cttatcatgg cagacaacgg ta                                  332


<210> SEQ ID NO 16
<211> LENGTH: 708
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (41)..(703)
<223> OTHER INFORMATION:

<400> SEQUENCE: 16

tattattatt attctgtttg gaactttaac attgcttatc atg gca gac aac ggt       55
                                            Met Ala Asp Asn Gly
                                            1               5

act att acc gtt gag gag ctt aaa caa ctc ctg gaa caa tgg aac cta      103
Thr Ile Thr Val Glu Glu Leu Lys Gln Leu Leu Glu Gln Trp Asn Leu
                10                  15                  20

gta ata ggt ttc cta ttc cta gcc tgg att atg tta cta caa ttt gcc      151
Val Ile Gly Phe Leu Phe Leu Ala Trp Ile Met Leu Leu Gln Phe Ala
            25                  30                  35

tat tct aat cgg aac agg ttt ttg tac ata ata aag ctt gtt ttc ctc      199
Tyr Ser Asn Arg Asn Arg Phe Leu Tyr Ile Ile Lys Leu Val Phe Leu
        40                  45                  50

tgg ctc ttg tgg cca gta aca ctt gct tgt ttt gtg ctt gct gct gtc      247
Trp Leu Leu Trp Pro Val Thr Leu Ala Cys Phe Val Leu Ala Ala Val
    55                  60                  65

tac aga att aat tgg gtg act ggc ggg att gcg att gca atg gct tgt      295
Tyr Arg Ile Asn Trp Val Thr Gly Gly Ile Ala Ile Ala Met Ala Cys
70                  75                  80                  85

att gta ggc ttg atg tgg ctt agc tac ttc gtt gct tcc ttc agg ctg      343
Ile Val Gly Leu Met Trp Leu Ser Tyr Phe Val Ala Ser Phe Arg Leu
                90                  95                  100

ttt gct cgt acc cgc tca atg tgg tca ttc aac cca gaa aca aac att      391
Phe Ala Arg Thr Arg Ser Met Trp Ser Phe Asn Pro Glu Thr Asn Ile
            105                 110                 115

ctt ctc aat gtg cct ctc cgg ggg aca att gtg acc aga ccg ctc atg      439
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Leu Leu Asn Val Pro Leu Arg Gly Thr Ile Val Thr Arg Pro Leu Met
        120                 125                 130

gaa agt gaa ctt gtc att ggt gct gtg atc att cgt ggt cac ttg cga      487
Glu Ser Glu Leu Val Ile Gly Ala Val Ile Ile Arg Gly His Leu Arg
    135                 140                 145

atg gcc gga cac tcc cta ggg cgc tgt gac att aag gac ctg cca aaa      535
Met Ala Gly His Ser Leu Gly Arg Cys Asp Ile Lys Asp Leu Pro Lys
150                 155                 160                 165

gag atc act gtg gct aca tca cga acg ctt tct tat tac aaa tta gga      583
Glu Ile Thr Val Ala Thr Ser Arg Thr Leu Ser Tyr Tyr Lys Leu Gly
                170                 175                 180

gcg tcg cag cgt gta ggc act gat tca ggt ttt gct gca tac aac cgc      631
Ala Ser Gln Arg Val Gly Thr Asp Ser Gly Phe Ala Ala Tyr Asn Arg
            185                 190                 195

tac cgt att gga aac tat aaa tta aat aca gac cac gcc ggt agc aac      679
Tyr Arg Ile Gly Asn Tyr Lys Leu Asn Thr Asp His Ala Gly Ser Asn
        200                 205                 210

gac aat att gct ttg cta gta cag taagt                               708
Asp Asn Ile Ala Leu Leu Val Gln
    215                 220


<210> SEQ ID NO 17
<211> LENGTH: 221
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 17

Met Ala Asp Asn Gly Thr Ile Thr Val Glu Glu Leu Lys Gln Leu Leu
1               5                   10                  15

Glu Gln Trp Asn Leu Val Ile Gly Phe Leu Phe Leu Ala Trp Ile Met
            20                  25                  30

Leu Leu Gln Phe Ala Tyr Ser Asn Arg Asn Arg Phe Leu Tyr Ile Ile
        35                  40                  45

Lys Leu Val Phe Leu Trp Leu Leu Trp Pro Val Thr Leu Ala Cys Phe
    50                  55                  60

Val Leu Ala Ala Val Tyr Arg Ile Asn Trp Val Thr Gly Gly Ile Ala
65                  70                  75                  80

Ile Ala Met Ala Cys Ile Val Gly Leu Met Trp Leu Ser Tyr Phe Val
                85                  90                  95

Ala Ser Phe Arg Leu Phe Ala Arg Thr Arg Ser Met Trp Ser Phe Asn
            100                 105                 110

Pro Glu Thr Asn Ile Leu Leu Asn Val Pro Leu Arg Gly Thr Ile Val
        115                 120                 125

Thr Arg Pro Leu Met Glu Ser Glu Leu Val Ile Gly Ala Val Ile Ile
    130                 135                 140

Arg Gly His Leu Arg Met Ala Gly His Ser Leu Gly Arg Cys Asp Ile
145                 150                 155                 160

Lys Asp Leu Pro Lys Glu Ile Thr Val Ala Thr Ser Arg Thr Leu Ser
                165                 170                 175

Tyr Tyr Lys Leu Gly Ala Ser Gln Arg Val Gly Thr Asp Ser Gly Phe
            180                 185                 190

Ala Ala Tyr Asn Arg Tyr Arg Ile Gly Asn Tyr Lys Leu Asn Thr Asp
        195                 200                 205

His Ala Gly Ser Asn Asp Asn Ile Ala Leu Leu Val Gln
    210                 215                 220


<210> SEQ ID NO 18
<211> LENGTH: 769
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<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 18

cctgatcttc tggtctaaac gaactaacta ttattattat tctgtttgga actttaacat 60

tgcttatcat ggcagacaac ggtactatta ccgttgagga gcttaaacaa ctcctggaac 120

aatggaacct agtaataggt ttcctattcc tagcctggat tatgttacta caatttgcct 180

attctaatcg gaacaggttt ttgtacataa taaagcttgt tttcctctgg ctcttgtggc 240

cagtaacact tgcttgtttt gtgcttgctg ctgtctacag aattaattgg gtgactggcg 300

ggattgcgat tgcaatggct tgtattgtag gcttgatgtg gcttagctac ttcgttgctt 360

ccttcaggct gtttgctcgt acccgctcaa tgtggtcatt caacccagaa acaaacattc 420

ttctcaatgt gcctctccgg gggacaattg tgaccagacc gctcatggaa agtgaacttg 480

tcattggtgc tgtgatcatt cgtggtcact tgcgaatggc cggacactcc ctagggcgct 540

gtgacattaa ggacctgcca aaagagatca ctgtggctac atcacgaacg ctttcttatt 600

acaaattagg agcgtcgcag cgtgtaggca ctgattcagg ttttgctgca tacaaccgct 660

accgtattgg aaactataaa ttaaatacag accacgccgg tagcaacgac aatattgctt 720

tgctagtaca gtaagtgaca acagatgttt catcttgttg acttccagg 769

<210> SEQ ID NO 19
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 19

taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct 60

ttgctagtac agtaagtgac aacagatgtt tcatcttgtt gacttccagg ttacaatagc 120

agagatattg attatcatta tgaggacttt caggattgct atttggaatc ttgacgttat 180

aataagttca atagtgagac aattatttaa gcctctaact aagaagaatt attcggagtt 240

agatgatgaa gaacctatgg agttagatta tccataaaac gaacatgaaa attattctct 300

tcctgacatt gattgtattt acatcttgcg agctatatca ctatcaggag tgtgttagag 360

gtacgactgt actactaaaa gaaccttgcc catcaggaac atacgagggc aattcaccat 420

ttcaccctct tgctgacaat aaatttgcac taacttgcac tagcacacac tttgcttttg 480

cttgtgctga cggtactcga catacctatc agctgcgtgc aagatcagtt tcaccaaaac 540

ttttcatcag acaagaggag gttcaacaag agctctactc gccacttttt ctcattgttg 600

ctgctctagt atttttaata ctttgcttca ccattaagag aaagacagaa tgaatgagct 660

cactttaatt gacttctatt tgtgcttttt agcctttctg ctattccttg ttttaataat 720

gcttattata ttttggtttt cactcgaaat ccaggatcta gaagaacctt gtaccaaagt 780

ctaaacgaac atgaaacttc tcattgtttt gacttgtatt tctctatgca gttgcatatg 840

cactgtagta cagcgctgtg catctaataa acctcatgtg cttgaagatc cttgtaaggt 900

acaacactag gggtaatact tatagcactg cttggctttg tgctctagga aaggttttac 960

cttttcatag atggcacact atggttcaaa catgcacacc taatgttact atcaactgtc 1020

aagatccagc tggtggtgcg cttatagcta ggtgttggta ccttcatgaa ggtcaccaaa 1080

ctgctgcatt tagagacgta cttgttgttt taaataaacg aacaaattaa aatgtctgat 1140

aatggacccc aatcaaacca acgtagtgcc ccccgcatta catttggtgg acccacagat 1200

tcaactgaca ataaccagaa tggaggacgc a 1231

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<210> SEQ ID NO 20
<211> LENGTH: 1242
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 20

gcatacaacc gctaccgtat tggaaactat aaattaaata cagaccacgc cggtagcaac 60

gacaatattg ctttgctagt acagtaagtg acaacagatg tttcatcttg ttgacttcca 120

ggttacaata gcagagatat tgattatcat tatgaggact ttcaggattg ctatttggaa 180

tcttgacgtt ataataagtt caatagtgag acagttattt aagcctctaa ctaagaagaa 240

ttattcggag ttagatgatg aagaacctat ggagttagat tatccataaa acgaacatga 300

aaattattct cttcctgaca ttgattgtat ttacatcttg cgagctatat cactatcagg 360

agtgtgttag aggtacgact gtactactaa aagaaccttg cccatcagga acatacgagg 420

gcaattcacc atttcaccct cttgctgaca ataaatttgc actaacttgc actagcacac 480

actttgcttt tgcttgtgct gacggtactc gacataccta tcagctgcgt gcaagatcag 540

tttcaccaaa acttttcatc agacaagagg aggttcaaca agagctctac tcgccacttt 600

ttctcattgt tgctgctcta gtatttttaa tactttgctt caccattaag agaaagacag 660

aatgaatgag ctcactttaa ttgacttcta tttgtgcttt ttagcctttc tgctattcct 720

tgttttaata atgcttatta tattttggtt ttcactcgaa atccaggatc tagaagaacc 780

ttgtaccaaa gtctaaacga acatgaaact tctcattgtt ttgacttgta tttctctatg 840

cagttgcata tgcactgtag tacagcgctg tgcatctaat aaacctcatg tgcttgaaga 900

tccttgtaag gtacaacact aggggtaata cttatagcac tgcttggctt tgtgctctag 960

gaaaggtttt acctttttcat agatggcaca ctatggttca aacatgcaca cctaatgtta 1020

ctatcaactg tcaagatcca gctggtggtg cgcttatagc taggtgttgg taccttcatg 1080

aaggtcacca aactgctgca tttagagacg tacttgttgt tttaaataaa cgaacgaatt 1140

aaaatgtctg ataatggacc ccaatcaaac caacgtagtg ccccccgcat tacatttggt 1200

ggacccacag attcaactga caataaccag aatggaggac gc 1242

<210> SEQ ID NO 21
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (86)..(274)
<223> OTHER INFORMATION:

<400> SEQUENCE: 21

taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct 60

ttgctagtac agtaagtgac aacag atg ttt cat ctt gtt gac ttc cag gtt 112
Met Phe His Leu Val Asp Phe Gln Val
1 5

aca ata gca gag ata ttg att atc att atg agg act ttc agg att gct 160
Thr Ile Ala Glu Ile Leu Ile Ile Ile Met Arg Thr Phe Arg Ile Ala
10 15 20 25

att tgg aat ctt gac gtt ata ata agt tca ata gtg aga caa tta ttt 208
Ile Trp Asn Leu Asp Val Ile Ile Ser Ser Ile Val Arg Gln Leu Phe
30 35 40

aag cct cta act aag aag aat tat tcg gag tta gat gat gaa gaa cct 256
Lys Pro Leu Thr Lys Lys Asn Tyr Ser Glu Leu Asp Asp Glu Glu Pro
45 50 55

-continued

```
atg gag tta gat tat cca taaaacgaac atgaaaatta ttctcttcct            304
Met Glu Leu Asp Tyr Pro
        60

gacattgatt gtatttacat cttgcgagct atatcactat caggagtgtg ttagaggtac    364

gactgtacta ctaaaagaac cttgcccatc aggaacatac gagggcaatt caccatttca    424

ccctcttgct gacaataaat ttgcactaac ttgcactagc acacactttg cttttgcttg    484

tgctgacggt actcgacata cctatcagct gcgtgcaaga tcagtttcac caaaactttt    544

catcagacaa gaggaggttc aacaagagct ctactcgcca ctttttctca ttgttgctgc    604

tctagtattt ttaatacttt gcttcaccat taagagaaag acagaatgaa tgagctcact    664

ttaattgact tctatttgtg ctttttagcc tttctgctat tccttgtttt aataatgctt    724

attatatttt ggttttcact cgaaatccag gatctagaag aaccttgtac caaagtctaa    784

acgaacatga aacttctcat tgttttgact tgtatttctc tatgcagttg catatgcact    844

gtagtacagc gctgtgcatc taataaacct catgtgcttg aagatccttg taaggtacaa    904

cactaggggt aatacttata gcactgcttg gctttgtgct ctaggaaagg ttttaccttt    964

tcatagatgg cacactatgg ttcaaacatg cacacctaat gttactatca actgtcaaga   1024

tccagctggt ggtgcgctta tagctaggtg ttggtacctt catgaaggtc accaaactgc   1084

tgcatttaga gacgtacttg ttgttttaaa taaacgaaca aattaaaatg tctgataatg   1144

gaccccaatc aaaccaacgt agtgcccccc gcattacatt tggtggaccc acagattcaa   1204

ctgacaataa ccagaatgga ggacgca                                       1231


<210> SEQ ID NO 22
<211> LENGTH: 63
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 22

Met Phe His Leu Val Asp Phe Gln Val Thr Ile Ala Glu Ile Leu Ile
1               5                   10                  15

Ile Ile Met Arg Thr Phe Arg Ile Ala Ile Trp Asn Leu Asp Val Ile
            20                  25                  30

Ile Ser Ser Ile Val Arg Gln Leu Phe Lys Pro Leu Thr Lys Lys Asn
        35                  40                  45

Tyr Ser Glu Leu Asp Asp Glu Glu Pro Met Glu Leu Asp Tyr Pro
    50                  55                  60


<210> SEQ ID NO 23
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (285)..(650)
<223> OTHER INFORMATION:

<400> SEQUENCE: 23

taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct     60

ttgctagtac agtaagtgac aacagatgtt tcatcttgtt gacttccagg ttacaatagc    120

agagatattg attatcatta tgaggacttt caggattgct atttggaatc ttgacgttat    180

aataagttca atagtgagac aattatttaa gcctctaact aagaagaatt attcggagtt    240

agatgatgaa gaacctatgg agttagatta tccataaaac gaac atg aaa att att     296
                                                Met Lys Ile Ile
                                                1
```

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```
ctc ttc ctg aca ttg att gta ttt aca tct tgc gag cta tat cac tat      344
Leu Phe Leu Thr Leu Ile Val Phe Thr Ser Cys Glu Leu Tyr His Tyr
5                   10                  15                  20

cag gag tgt gtt aga ggt acg act gta cta cta aaa gaa cct tgc cca      392
Gln Glu Cys Val Arg Gly Thr Thr Val Leu Leu Lys Glu Pro Cys Pro
                25                  30                  35

tca gga aca tac gag ggc aat tca cca ttt cac cct ctt gct gac aat      440
Ser Gly Thr Tyr Glu Gly Asn Ser Pro Phe His Pro Leu Ala Asp Asn
            40                  45                  50

aaa ttt gca cta act tgc act agc aca cac ttt gct ttt gct tgt gct      488
Lys Phe Ala Leu Thr Cys Thr Ser Thr His Phe Ala Phe Ala Cys Ala
        55                  60                  65

gac ggt act cga cat acc tat cag ctg cgt gca aga tca gtt tca cca      536
Asp Gly Thr Arg His Thr Tyr Gln Leu Arg Ala Arg Ser Val Ser Pro
    70                  75                  80

aaa ctt ttc atc aga caa gag gag gtt caa caa gag ctc tac tcg cca      584
Lys Leu Phe Ile Arg Gln Glu Glu Val Gln Gln Glu Leu Tyr Ser Pro
85                  90                  95                  100

ctt ttt ctc att gtt gct gct cta gta ttt tta ata ctt tgc ttc acc      632
Leu Phe Leu Ile Val Ala Ala Leu Val Phe Leu Ile Leu Cys Phe Thr
                105                 110                 115

att aag aga aag aca gaa tgaatgagct cactttaatt gacttctatt             680
Ile Lys Arg Lys Thr Glu
            120

tgtgcttttt agcctttctg ctattccttg ttttaataat gcttattata ttttggtttt    740

cactcgaaat ccaggatcta gaagaacctt gtaccaaagt ctaaacgaac atgaaacttc    800

tcattgtttt gacttgtatt tctctatgca gttgcatatg cactgtagta cagcgctgtg    860

catctaataa acctcatgtg cttgaagatc cttgtaaggt acaacactag gggtaatact    920

tatagcactg cttggctttg tgctctagga aaggttttac cttttcatag atggcacact    980

atggttcaaa catgcacacc taatgttact atcaactgtc aagatccagc tggtggtgcg   1040

cttatagcta ggtgttggta ccttcatgaa ggtcaccaaa ctgctgcatt tagagacgta   1100

cttgttgttt taaataaacg aacaaattaa aatgtctgat aatggacccc aatcaaacca   1160

acgtagtgcc ccccgcatta catttggtgg acccacagat tcaactgaca ataaccagaa   1220

tggaggacgc a                                                        1231


<210> SEQ ID NO 24
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 24

Met Lys Ile Ile Leu Phe Leu Thr Leu Ile Val Phe Thr Ser Cys Glu
1               5                   10                  15

Leu Tyr His Tyr Gln Glu Cys Val Arg Gly Thr Thr Val Leu Leu Lys
            20                  25                  30

Glu Pro Cys Pro Ser Gly Thr Tyr Glu Gly Asn Ser Pro Phe His Pro
        35                  40                  45

Leu Ala Asp Asn Lys Phe Ala Leu Thr Cys Thr Ser Thr His Phe Ala
    50                  55                  60

Phe Ala Cys Ala Asp Gly Thr Arg His Thr Tyr Gln Leu Arg Ala Arg
65                  70                  75                  80

Ser Val Ser Pro Lys Leu Phe Ile Arg Gln Glu Glu Val Gln Gln Glu
                85                  90                  95

Leu Tyr Ser Pro Leu Phe Leu Ile Val Ala Ala Leu Val Phe Leu Ile
```

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            100                 105                 110

Leu Cys Phe Thr Ile Lys Arg Lys Thr Glu
        115                 120


<210> SEQ ID NO 25
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (650)..(781)
<223> OTHER INFORMATION:

<400> SEQUENCE: 25

taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct     60

ttgctagtac agtaagtgac aacagatgtt tcatcttgtt gacttccagg ttacaatagc    120

agagatattg attatcatta tgaggacttt caggattgct atttggaatc ttgacgttat    180

aataagttca atagtgagac aattatttaa gcctctaact aagaagaatt attcggagtt    240

agatgatgaa gaacctatgg agttagatta tccataaaac gaacatgaaa attattctct    300

tcctgacatt gattgtattt acatcttgcg agctatatca ctatcaggag tgtgttagag    360

gtacgactgt actactaaaa gaaccttgcc catcaggaac atacgagggc aattcaccat    420

ttcaccctct tgctgacaat aaatttgcac taacttgcac tagcacacac tttgcttttg    480

cttgtgctga cggtactcga catacctatc agctgcgtgc aagatcagtt tcaccaaaac    540

ttttcatcag acaagaggag gttcaacaag agctctactc gccacttttt ctcattgttg    600

ctgctctagt atttttaata ctttgcttca ccattaagag aaagacaga atg aat gag    658
                                                    Met Asn Glu
                                                    1

ctc act tta att gac ttc tat ttg tgc ttt tta gcc ttt ctg cta ttc      706
Leu Thr Leu Ile Asp Phe Tyr Leu Cys Phe Leu Ala Phe Leu Leu Phe
    5                   10                  15

ctt gtt tta ata atg ctt att ata ttt tgg ttt tca ctc gaa atc cag      754
Leu Val Leu Ile Met Leu Ile Ile Phe Trp Phe Ser Leu Glu Ile Gln
20                  25                  30                  35

gat cta gaa gaa cct tgt acc aaa gtc taaacgaaca tgaaacttct            801
Asp Leu Glu Glu Pro Cys Thr Lys Val
                40

cattgttttg acttgtattt ctctatgcag ttgcatatgc actgtagtac agcgctgtgc    861

atctaataaa cctcatgtgc ttgaagatcc ttgtaaggta caacactagg ggtaatactt    921

atagcactgc ttggctttgt gctctaggaa aggttttacc ttttcataga tggcacacta    981

tggttcaaac atgcacacct aatgttacta tcaactgtca agatccagct ggtggtgcgc   1041

ttatagctag gtgttggtac cttcatgaag gtcaccaaac tgctgcattt agagacgtac   1101

ttgttgtttt aaataaacga acaaattaaa atgtctgata atggacccca atcaaaccaa   1161

cgtagtgccc cccgcattac atttggtgga cccacagatt caactgacaa taaccagaat   1221

ggaggacgca                                                          1231


<210> SEQ ID NO 26
<211> LENGTH: 44
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 26

Met Asn Glu Leu Thr Leu Ile Asp Phe Tyr Leu Cys Phe Leu Ala Phe
1               5                   10                  15
```

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```
Leu Leu Phe Leu Val Leu Ile Met Leu Ile Ile Phe Trp Phe Ser Leu
            20                  25                  30

Glu Ile Gln Asp Leu Glu Glu Pro Cys Thr Lys Val
        35                  40


<210> SEQ ID NO 27
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (791)..(907)
<223> OTHER INFORMATION:

<400> SEQUENCE: 27

taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct     60

ttgctagtac agtaagtgac aacagatgtt tcatcttgtt gacttccagg ttacaatagc    120

agagatattg attatcatta tgaggacttt caggattgct atttggaatc ttgacgttat    180

aataagttca atagtgagac aattatttaa gcctctaact aagaagaatt attcggagtt    240

agatgatgaa gaacctatgg agttagatta tccataaaac gaacatgaaa attattctct    300

tcctgacatt gattgtattt acatcttgcg agctatatca ctatcaggag tgtgttagag    360

gtacgactgt actactaaaa gaaccttgcc catcaggaac atacgagggc aattcaccat    420

ttcaccctct tgctgacaat aaatttgcac taacttgcac tagcacacac tttgcttttg    480

cttgtgctga cggtactcga catacctatc agctgcgtgc aagatcagtt tcaccaaaac    540

ttttcatcag acaagaggag gttcaacaag agctctactc gccacttttt ctcattgttg    600

ctgctctagt atttttaata ctttgcttca ccattaagag aaagacagaa tgaatgagct    660

cactttaatt gacttctatt tgtgcttttt agcctttctg ctattccttg ttttaataat    720

gcttattata ttttggtttt cactcgaaat ccaggatcta gaagaacctt gtaccaaagt    780

ctaaacgaac atg aaa ctt ctc att gtt ttg act tgt att tct cta tgc       829
          Met Lys Leu Leu Ile Val Leu Thr Cys Ile Ser Leu Cys
          1               5                   10

agt tgc ata tgc act gta gta cag cgc tgt gca tct aat aaa cct cat      877
Ser Cys Ile Cys Thr Val Val Gln Arg Cys Ala Ser Asn Lys Pro His
    15                  20                  25

gtg ctt gaa gat cct tgt aag gta caa cac taggggtaat acttatagca        927
Val Leu Glu Asp Pro Cys Lys Val Gln His
30                  35

ctgcttggct ttgtgctcta ggaaaggttt tacctttca tagatggcac actatggttc    987

aaacatgcac acctaatgtt actatcaact gtcaagatcc agctggtggt gcgcttatag   1047

ctaggtgttg gtaccttcat gaaggtcacc aaactgctgc atttagagac gtacttgttg   1107

ttttaaataa acgaacaaat taaaatgtct gataatggac cccaatcaaa ccaacgtagt   1167

gccccccgca ttacatttgg tggacccaca gattcaactg acaataacca gaatggagga   1227

cgca                                                                1231


<210> SEQ ID NO 28
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 28

Met Lys Leu Leu Ile Val Leu Thr Cys Ile Ser Leu Cys Ser Cys Ile
1               5                   10                  15

Cys Thr Val Val Gln Arg Cys Ala Ser Asn Lys Pro His Val Leu Glu
```

-continued

```
           20                  25                  30

Asp Pro Cys Lys Val Gln His
        35
```

<210> SEQ ID NO 29
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (876)..(1127)
<223> OTHER INFORMATION:

<400> SEQUENCE: 29

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taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct     60

ttgctagtac agtaagtgac aacagatgtt tcatcttgtt gacttccagg ttacaatagc    120

agagatattg attatcatta tgaggacttt caggattgct atttggaatc ttgacgttat    180

aataagttca atagtgagac aattatttaa gcctctaact aagaagaatt attcggagtt    240

agatgatgaa gaacctatgg agttagatta tccataaaac gaacatgaaa attattctct    300

tcctgacatt gattgtattt acatcttgcg agctatatca ctatcaggag tgtgttagag    360

gtacgactgt actactaaaa gaaccttgcc catcaggaac atacgagggc aattcaccat    420

ttcaccctct tgctgacaat aaatttgcac taacttgcac tagcacacac tttgcttttg    480

cttgtgctga cggtactcga catacctatc agctgcgtgc aagatcagtt tcaccaaaac    540

ttttcatcag acaagaggag gttcaacaag agctctactc gccacttttt ctcattgttg    600

ctgctctagt atttttaata ctttgcttca ccattaagag aaagacagaa tgaatgagct    660

cactttaatt gacttctatt tgtgcttttt agcctttctg ctattccttg ttttaataat    720

gcttattata ttttggtttt cactcgaaat ccaggatcta gaagaacctt gtaccaaagt    780

ctaaacgaac atgaaacttc tcattgtttt gacttgtatt tctctatgca gttgcatatg    840

cactgtagta cagcgctgtg catctaataa acctc atg tgc ttg aag atc ctt       893
                                       Met Cys Leu Lys Ile Leu
                                       1               5

gta agg tac aac act agg ggt aat act tat agc act gct tgg ctt tgt      941
Val Arg Tyr Asn Thr Arg Gly Asn Thr Tyr Ser Thr Ala Trp Leu Cys
            10                  15                  20

gct cta gga aag gtt tta cct ttt cat aga tgg cac act atg gtt caa      989
Ala Leu Gly Lys Val Leu Pro Phe His Arg Trp His Thr Met Val Gln
        25                  30                  35

aca tgc aca cct aat gtt act atc aac tgt caa gat cca gct ggt ggt     1037
Thr Cys Thr Pro Asn Val Thr Ile Asn Cys Gln Asp Pro Ala Gly Gly
    40                  45                  50

gcg ctt ata gct agg tgt tgg tac ctt cat gaa ggt cac caa act gct     1085
Ala Leu Ile Ala Arg Cys Trp Tyr Leu His Glu Gly His Gln Thr Ala
55                  60                  65                  70

gca ttt aga gac gta ctt gtt gtt tta aat aaa cga aca aat              1127
Ala Phe Arg Asp Val Leu Val Val Leu Asn Lys Arg Thr Asn
                75                  80

taaaatgtct gataatggac cccaatcaaa ccaacgtagt gccccccgca ttacatttgg   1187

tggacccaca gattcaactg acaataacca gaatggagga cgca                   1231
```

<210> SEQ ID NO 30
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 30

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```
Met Cys Leu Lys Ile Leu Val Arg Tyr Asn Thr Arg Gly Asn Thr Tyr
1               5                   10                  15

Ser Thr Ala Trp Leu Cys Ala Leu Gly Lys Val Leu Pro Phe His Arg
            20                  25                  30

Trp His Thr Met Val Gln Thr Cys Thr Pro Asn Val Thr Ile Asn Cys
        35                  40                  45

Gln Asp Pro Ala Gly Gly Ala Leu Ile Ala Arg Cys Trp Tyr Leu His
    50                  55                  60

Glu Gly His Gln Thr Ala Ala Phe Arg Asp Val Leu Val Val Leu Asn
65                  70                  75                  80

Lys Arg Thr Asn
```

<210> SEQ ID NO 31
<211> LENGTH: 21221
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 31

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atggagagcc ttgttcttgg tgtcaacgag aaaacacacg tccaactcag tttgcctgtc     60

cttcaggtta gagacgtgct agtgcgtggc ttcggggact ctgtggaaga ggccctatcg    120

gaggcacgtg aacacctcaa aaatggcact tgtggtctag tagagctgga aaaaggcgta    180

ctgccccagc ttgaacagcc ctatgtgttc attaaacgtt ctgatgcctt aagcaccaat    240

cacggccaca aggtcgttga gctggttgca gaaatggacg gcattcagta cggtcgtagc    300

ggtataacac tgggagtact cgtgccacat gtgggcgaaa ccccaattgc ataccgcaat    360

gttcttcttc gtaagaacgg taataaggga gccggtggtc atagctatgg catcgatcta    420

aagtcttatg acttaggtga cgagcttggc actgatccca ttgaagatta tgaacaaaac    480

tggaacacta agcatggcag tggtgcactc cgtgaactca ctcgtgagct caatggaggt    540

gcagtcactc gctatgtcga caacaatttc tgtggcccag atgggtaccc tcttgattgc    600

atcaaagatt ttctcgcacg cgcgggcaag tcaatgtgca ctctttccga acaacttgat    660

tacatcgagt cgaagagagg tgtctactgc tgccgtgacc atgagcatga aattgcctgg    720

ttcactgagc gctctgataa gagctacgag caccagacac ccttcgaaat taagagtgcc    780

aagaaatttg acactttcaa aggggaatgc ccaaagtttg tgtttcctct taactcaaaa    840

gtcaaagtca ttcaaccacg tgttgaaaag aaaaagactg agggtttcat ggggcgtata    900

cgctctgtgt accctgttgc atctccacag gagtgtaaca atatgcactt gtctaccttg    960

atgaaatgta atcattgcga tgaagtttca tggcagacgt gcgactttct gaaagccact   1020

tgtgaacatt gtggcactga aaatttagtt attgaaggac ctactacatg tgggtaccta   1080

cctactaatg ctgtagtgaa aatgccatgt cctgcctgtc aagacccaga gattggacct   1140

gagcatagtg ttgcagatta tcacaaccac tcaaacattg aaactcgact ccgcaaggga   1200

ggtaggacta gatgttttgg aggctgtgtg tttgcctatg ttggctgcta taataagcgt   1260

gcctactggg ttcctcgtgc tagtgctgat attggctcag gccatactgg cattactggt   1320

gacaatgtgg agaccttgaa tgaggatctc cttgagatac tgagtcgtga acgtgttaac   1380

attaacattg ttggcgattt tcatttgaat gaagaggttg ccatcatttt ggcatctttc   1440

tctgcttcta caagtgcctt tattgacact ataaagagtc ttgattacaa gtctttcaaa   1500

accattgttg agtcctgcgg taactataaa gttaccaagg gaaagcccgt aaaaggtgct   1560

tggaacattg gacaacagag atcagtttta acaccactgt gtggttttcc ctcacaggct   1620
```

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gctggtgtta tcagatcaat ttttgcgcgc acacttgatg cagcaaacca ctcaattcct 1680

gatttgcaaa gagcagctgt caccatactt gatggtattt ctgaacagtc attacgtctt 1740

gtcgacgcca tggtttatac ttcagacctg ctcaccaaca gtgtcattat tatggcatat 1800

gtaactggtg gtcttgtaca acagacttct cagtggttgt ctaatctttt gggcactact 1860

gttgaaaaac tcaggcctat ctttgaatgg attgaggcga aacttagtgc aggagttgaa 1920

tttctcaagg atgcttggga gattctcaaa tttctcatta caggtgtttt tgacatcgtc 1980

aagggtcaaa tacaggttgc ttcagataac atcaaggatt gtgtaaaatg cttcattgat 2040

gttgttaaca aggcactcga aatgtgcatt gatcaagtca ctatcgctgg cgcaaagttg 2100

cgatcactca acttaggtga agtcttcatc gctcaaagca agggacttta ccgtcagtgt 2160

atacgtggca aggagcagct gcaactactc atgcctctta aggcaccaaa agaagtaacc 2220

tttcttgaag gtgattcaca tgacacagta cttacctctg aggaggttgt tctcaagaac 2280

ggtgaactcg aagcactcga gacgcccgtt gatagcttca caaatggagc tatcgttggc 2340

acaccagtct gtgtaaatgg cctcatgctc ttagagatta aggacaaaga acaatactgc 2400

gcattgtctc ctggtttact ggctacaaac aatgtctttc gcttaaaagg gggtgcacca 2460

attaaaggtg taacctttgg agaagatact gtttgggaag ttcaaggtta caagaatgtg 2520

agaatcacat ttgagcttga tgaacgtgtt gacaaagtgc ttaatgaaaa gtgctctgtc 2580

tacactgttg aatccggtac cgaagttact gagtttgcat gtgttgtagc agaggctgtt 2640

gtgaagactt tacaaccagt ttctgatctc cttaccaaca tgggtattga tcttgatgag 2700

tggagtgtag ctacattcta cttatttgat gatgctggtg aagaaaactt ttcatcacgt 2760

atgtattgtt ccttttaccc tccagatgag gaagaagagg acgatgcaga gtgtgaggaa 2820

gaagaaattg atgaaacctg tgaacatgag tacggtacag aggatgatta tcaaggtctc 2880

cctctggaat ttggtgcctc agctgaaaca gttcgagttg aggaagaaga agaggaagac 2940

tggctggatg atactactga gcaatcagag attgagccag aaccagaacc tacacctgaa 3000

gaaccagtta atcagtttac tggttattta aaacttactg acaatgttgc cattaaatgt 3060

gttgacatcg ttaaggaggc acaaagtgct aatcctatgg tgattgtaaa tgctgctaac 3120

atacacctga aacatggtgg tggtgtagca ggtgcactca acaaggcaac caatggtgcc 3180

atgcaaaagg agagtgatga ttacattaag ctaaatggcc ctcttacagt aggagggtct 3240

tgtttgcttt ctggacataa tcttgctaag aagtgtctgc atgttgttgg acctaaccta 3300

aatgcaggtg aggacatcca gcttcttaag gcagcatatg aaaatttcaa ttcacaggac 3360

atcttacttg caccattgtt gtcagcaggc atatttggtg ctaaaccact tcagtcttta 3420

caagtgtgcg tgcagacggt tcgtacacag gtttatattg cagtcaatga caaagctctt 3480

tatgagcagg ttgtcatgga ttatcttgat aacctgaagc ctagagtgga agcacctaaa 3540

caagaggagc caccaaacac agaagattcc aaaactgagg agaaatctgt cgtacagaag 3600

cctgtcgatg tgaagccaaa aattaaggcc tgcattgatg aggttaccac aacactggaa 3660

gaaactaagt ttcttaccaa taagttactc ttgtttgctg atatcaatgg taagctttac 3720

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ctgtagcttc aaaaatctta ggattgccta cgcagactgt tgattcatca cagggttctg 17520

aatatgacta tgtcatattc acacaaacta ctgaaacagc acactcttgt aatgtcaacc 17580

gcttcaatgt ggctatcaca agggcaaaaa ttggcatttt gtgcataatg tctgatagag 17640

atctttatga caaactgcaa tttacaagtc tagaaatacc acgtcgcaat gtggctacat 17700

tacaagcaga aaatgtaact ggacttttta aggactgtag taagatcatt actggtcttc 17760

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ctattcgtca cgttcgtgcg tggattggct ttgatgtaga gggctgtcat gcaactagag 18000

atgctgtggg tactaaccta cctctccagc taggattttc tacaggtgtt aacttagtag 18060

ctgtaccgac tggttatgtt gacactgaaa ataacacaga attcaccaga gttaatgcaa 18120

aacctccacc aggtgaccag tttaaacatc ttataccact catgtataaa ggcttgccct 18180

ggaatgtagt gcgtattaag atagtacaaa tgctcagtga tacactgaaa ggattgtcag 18240

acagagtcgt gttcgtcctt tgggcgcatg gctttgagct tacatcaatg aagtactttg 18300

tcaagattgg acctgaaaga acgtgttgtc tgtgtgacaa acgtgcaact tgcttttcta 18360

cttcatcaga tacttatgcc tgctggaatc attctgtggg ttttgactat gtctataacc 18420

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catttatgat tgatgttcag cagtggggct ttacgggtaa ccttcagagt aaccatgacc 18480

aacattgcca ggtacatgga aatgcacatg tggctagttg tgatgctatc atgactagat 18540

gtttagcagt ccatgagtgc tttgttaagc gcgttgattg gtctgttgaa taccctatta 18600

taggagatga actgagggtt aattctgctt gcagaaaagt acaacacatg gttgtgaagt 18660

ctgcattgct tgctgataag tttccagttc ttcatgacat tggaaatcca aaggctatca 18720

agtgtgtgcc tcaggctgaa gtagaatgga agttctacga tgctcagcca tgtagtgaca 18780

aagcttacaa aatagaggaa ctcttctatt cttatgctac acatcacgat aaattcactg 18840

atggtgtttg tttgttttgg aattgtaacg ttgatcgtta cccagccaat gcaattgtgt 18900

gtaggtttga cacaagagtc ttgtcaaact tgaacttacc aggctgtgat ggtggtagtt 18960

tgtatgtgaa taagcatgca ttccacactc cagctttcga taaaagtgca tttactaatt 19020

taaagcaatt gcctttcttt tactattctg atagtccttg tgagtctcat ggcaaacaag 19080

tagtgtcgga tattgattat gttccactca aatctgctac gtgtattaca cgatgcaatt 19140

taggtggtgc tgtttgcaga caccatgcaa atgagtaccg acagtacttg gatgcatata 19200

atatgatgat ttctgctgga tttagcctat ggatttacaa acaatttgat acttataacc 19260

tgtggaatac atttaccagg ttacagagtt tagaaaatgt ggcttataat gttgttaata 19320

aaggacactt tgatggacac gccggcgaag cacctgtttc catcattaat aatgctgttt 19380

acacaaaggt agatggtatt gatgtggaga tctttgaaaa taagacaaca cttcctgtta 19440

atgttgcatt tgagctttgg gctaagcgta acattaaacc agtgccagag attaagatac 19500

tcaataattt gggtgttgat atcgctgcta atactgtaat ctgggactac aaaagagaag 19560

ccccagcaca tgtatctaca ataggtgtct gcacaatgac tgacattgcc aagaaaccta 19620

ctgagagtgc ttgttcttca cttactgtct tgtttgatgg tagagtggaa ggacaggtag 19680

acctttttag aaacgcccgt aatggtgttt taataacaga aggttcagtc aaaggtctaa 19740

caccttcaaa gggaccagca caagctagcg tcaatggagt cacattaatt ggagaatcag 19800

taaaaacaca gtttaactac tttaagaaag tagacggcat tattcaacag ttgcctgaaa 19860

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acagcacagt gaaaaattac ttcataacag atgcgcaaac aggttcatca aaatgtgtgt 20160

gttctgtgat tgatctttta cttgatgact ttgtcgagat aataaagtca caagatttgt 20220

cagtgatttc aaaagtggtc aaggttacaa ttgactatgc tgaaatttca ttcatgcttt 20280

ggtgtaagga tggacatgtt gaaaccttct acccaaaact acaagcaagt caagcgtggc 20340

aaccaggtgt tgcgatgcct aacttgtaca agatgcaaag aatgcttctt gaaaagtgtg 20400

accttcagaa ttatggtgaa aatgctgtta taccaaaagg aataatgatg aatgtcgcaa 20460

agtatactca actgtgtcaa tacttaaata cacttacttt agctgtaccc tacaacatga 20520

gagttattca ctttggtgct ggctctgata aaggagttgc accaggtaca gctgtgctca 20580

gacaatggtt gccaactggc acactacttg tcgattcaga tcttaatgac ttcgtctccg 20640

acgcagattc tactttaatt ggagactgtg caacagtaca tacggctaat aaatgggacc 20700

ttattattag cgatatgtat gaccctagga ccaaacatgt gacaaaagag aatgactcta 20760

aagaagggtt tttcacttat ctgtgtggat ttataaagca aaaactagcc ctgggtggtt 20820

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ctatagctgt aaagataaca gagcattctt ggaatgctga cctttacaag cttatgggcc 20880

atttctcatg gtggacagct tttgttacaa atgtaaatgc atcatcatcg gaagcatttt 20940

taattggggc taactatctt ggcaagccga aggaacaaat tgatggctat accatgcatg 21000

ctaactacat tttctggagg aacacaaatc ctatccagtt gtcttcctat tcactctttg 21060

acatgagcaa atttcctctt aaattaagag gaactgctgt aatgtctctt aaggagaatc 21120

aaatcaatga tatgatttat tctcttctgg aaaaaggtag gcttatcatt agagaaaaca 21180

acagagttgt ggtttcaagt gatattcttg ttaacaacta a 21221

<210> SEQ ID NO 32
<211> LENGTH: 297
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 32

atggacccca atcaaaccaa cgtagtgccc cccgcattac atttggtgga cccacagatt 60

caactgacaa taaccagaat ggaggacgca atggggcaag gccaaaacag cgccgacccc 120

aaggtttacc caataatact gcgtcttggt tcacagctct cactcagcat ggcaaggagg 180

aacttagatt ccctcgaggc cagggcgttc caatcaacac caatagtggt ccagatgacc 240

aaattggcta ctaccgaaga gctacccgac gagttcgtgg tggtgacggc aaaatga 297

<210> SEQ ID NO 33
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 33

Met Asp Pro Asn Gln Thr Asn Val Val Pro Pro Ala Leu His Leu Val
1 5 10 15

Asp Pro Gln Ile Gln Leu Thr Ile Thr Arg Met Glu Asp Ala Met Gly
20 25 30

Gln Gly Gln Asn Ser Ala Asp Pro Lys Val Tyr Pro Ile Ile Leu Arg
35 40 45

Leu Gly Ser Gln Leu Ser Leu Ser Met Ala Arg Arg Asn Leu Asp Ser
50 55 60

Leu Glu Ala Arg Ala Phe Gln Ser Thr Pro Ile Val Val Gln Met Thr
65 70 75 80

Lys Leu Ala Thr Thr Glu Glu Leu Pro Asp Glu Phe Val Val Val Thr
85 90 95

Ala Lys

<210> SEQ ID NO 34
<211> LENGTH: 213
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 34

atgctgccac cgtgctacaa cttcctcaag gaacaacatt gccaaaaggc ttctacgcag 60

agggaagcag aggcggcagt caagcctctt ctcgctcctc atcacgtagt cgcggtaatt 120

caagaaattc aactcctggc agcagtaggg gaaattctcc tgctcgaatg gctagcggag 180

gtggtgaaac tgccctcgcg ctattgctgc tag 213

<210> SEQ ID NO 35
<211> LENGTH: 70
<212> TYPE: PRT

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<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 35

Met Leu Pro Pro Cys Tyr Asn Phe Leu Lys Glu Gln His Cys Gln Lys
1 5 10 15

Ala Ser Thr Gln Arg Glu Ala Glu Ala Ala Val Lys Pro Leu Leu Ala
20 25 30

Pro His His Val Val Ala Val Ile Gln Glu Ile Gln Leu Leu Ala Ala
35 40 45

Val Gly Glu Ile Leu Leu Leu Glu Trp Leu Ala Glu Val Val Lys Leu
50 55 60

Pro Ser Arg Tyr Cys Cys
65 70

<210> SEQ ID NO 36
<211> LENGTH: 1377
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (67)..(1335)
<223> OTHER INFORMATION:

<400> SEQUENCE: 36

atgaaggtca ccaaactgct gcatttagag acgtacttgt tgttttaaat aaacgaacaa 60

attaaa atg tct gat aat gga ccc caa tca aac caa cgt agt gcc ccc 108
Met Ser Asp Asn Gly Pro Gln Ser Asn Gln Arg Ser Ala Pro
1 5 10

cgc att aca ttt ggt gga ccc aca gat tca act gac aat aac cag aat 156
Arg Ile Thr Phe Gly Gly Pro Thr Asp Ser Thr Asp Asn Asn Gln Asn
15 20 25 30

gga gga cgc aat ggg gca agg cca aaa cag cgc cga ccc caa ggt tta 204
Gly Gly Arg Asn Gly Ala Arg Pro Lys Gln Arg Arg Pro Gln Gly Leu
35 40 45

ccc aat aat act gcg tct tgg ttc aca gct ctc act cag cat ggc aag 252
Pro Asn Asn Thr Ala Ser Trp Phe Thr Ala Leu Thr Gln His Gly Lys
50 55 60

gag gaa ctt aga ttc cct cga ggc cag ggc gtt cca atc aac acc aat 300
Glu Glu Leu Arg Phe Pro Arg Gly Gln Gly Val Pro Ile Asn Thr Asn
65 70 75

agt ggt cca gat gac caa att ggc tac tac cga aga gct acc cga cga 348
Ser Gly Pro Asp Asp Gln Ile Gly Tyr Tyr Arg Arg Ala Thr Arg Arg
80 85 90

gtt cgt ggt ggt gac ggc aaa atg aaa gag ctc agc ccc aga tgg tac 396
Val Arg Gly Gly Asp Gly Lys Met Lys Glu Leu Ser Pro Arg Trp Tyr
95 100 105 110

ttc tat tac cta gga act ggc cca gaa gct tca ctt ccc tac ggc gct 444
Phe Tyr Tyr Leu Gly Thr Gly Pro Glu Ala Ser Leu Pro Tyr Gly Ala
115 120 125

aac aaa gaa ggc atc gta tgg gtt gca act gag gga gcc ttg aat aca 492
Asn Lys Glu Gly Ile Val Trp Val Ala Thr Glu Gly Ala Leu Asn Thr
130 135 140

ccc aaa gac cac att ggc acc cgc aat cct aat aac aat gct gcc acc 540
Pro Lys Asp His Ile Gly Thr Arg Asn Pro Asn Asn Asn Ala Ala Thr
145 150 155

gtg cta caa ctt cct caa gga aca aca ttg cca aaa ggc ttc tac gca 588
Val Leu Gln Leu Pro Gln Gly Thr Thr Leu Pro Lys Gly Phe Tyr Ala
160 165 170

gag gga agc aga ggc ggc agt caa gcc tct tct cgc tcc tca tca cgt 636
Glu Gly Ser Arg Gly Gly Ser Gln Ala Ser Ser Arg Ser Ser Ser Arg
175 180 185 190

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agt cgc ggt aat tca aga aat tca act cct ggc agc agt agg gga aat      684
Ser Arg Gly Asn Ser Arg Asn Ser Thr Pro Gly Ser Ser Arg Gly Asn
                195                 200                 205

tct cct gct cga atg gct agc gga ggt ggt gaa act gcc ctc gcg cta      732
Ser Pro Ala Arg Met Ala Ser Gly Gly Gly Glu Thr Ala Leu Ala Leu
            210                 215                 220

ttg ctg cta gac aga ttg aac cag ctt gag agc aaa gtt tct ggt aaa      780
Leu Leu Leu Asp Arg Leu Asn Gln Leu Glu Ser Lys Val Ser Gly Lys
        225                 230                 235

ggc caa caa caa caa ggc caa act gtc act aag aaa tct gct gct gag      828
Gly Gln Gln Gln Gln Gly Gln Thr Val Thr Lys Lys Ser Ala Ala Glu
    240                 245                 250

gca tct aaa aag cct cgc caa aaa cgt act gcc aca aaa cag tac aac      876
Ala Ser Lys Lys Pro Arg Gln Lys Arg Thr Ala Thr Lys Gln Tyr Asn
255                 260                 265                 270

gtc act caa gca ttt ggg aga cgt ggt cca gaa caa acc caa gga aat      924
Val Thr Gln Ala Phe Gly Arg Arg Gly Pro Glu Gln Thr Gln Gly Asn
                275                 280                 285

ttc ggg gac caa gac cta atc aga caa gga act gat tac aaa cat tgg      972
Phe Gly Asp Gln Asp Leu Ile Arg Gln Gly Thr Asp Tyr Lys His Trp
            290                 295                 300

ccg caa att gca caa ttt gct cca agt gcc tct gca ttc ttt gga atg     1020
Pro Gln Ile Ala Gln Phe Ala Pro Ser Ala Ser Ala Phe Phe Gly Met
        305                 310                 315

tca cgc att ggc atg gaa gtc aca cct tcg gga aca tgg ctg act tat     1068
Ser Arg Ile Gly Met Glu Val Thr Pro Ser Gly Thr Trp Leu Thr Tyr
    320                 325                 330

cat gga gcc att aaa ttg gat gac aaa gat cca caa ttc aaa gac aac     1116
His Gly Ala Ile Lys Leu Asp Asp Lys Asp Pro Gln Phe Lys Asp Asn
335                 340                 345                 350

gtc ata ctg ctg aac aag cac att gac gca tac aaa aca ttc cca cca     1164
Val Ile Leu Leu Asn Lys His Ile Asp Ala Tyr Lys Thr Phe Pro Pro
                355                 360                 365

aca gag cct aaa aag gac aaa aag aaa aag act gat gaa gct cag cct     1212
Thr Glu Pro Lys Lys Asp Lys Lys Lys Lys Thr Asp Glu Ala Gln Pro
            370                 375                 380

ttg ccg cag aga caa aag aag cag ccc act gtg act ctt ctt cct gcg     1260
Leu Pro Gln Arg Gln Lys Lys Gln Pro Thr Val Thr Leu Leu Pro Ala
        385                 390                 395

gct gac atg gat gat ttc tcc aga caa ctt caa aat tcc atg agt gga     1308
Ala Asp Met Asp Asp Phe Ser Arg Gln Leu Gln Asn Ser Met Ser Gly
    400                 405                 410

gct tct gct gat tca act cag gca taa acactcatga tgaccacaca          1355
Ala Ser Ala Asp Ser Thr Gln Ala
415                 420

aggcagatgg gctatgtaaa cg                                           1377


<210> SEQ ID NO 37
<211> LENGTH: 422
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 37

Met Ser Asp Asn Gly Pro Gln Ser Asn Gln Arg Ser Ala Pro Arg Ile
1               5                   10                  15

Thr Phe Gly Gly Pro Thr Asp Ser Thr Asp Asn Asn Gln Asn Gly Gly
            20                  25                  30

Arg Asn Gly Ala Arg Pro Lys Gln Arg Arg Pro Gln Gly Leu Pro Asn
        35                  40                  45
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Asn Thr Ala Ser Trp Phe Thr Ala Leu Thr Gln His Gly Lys Glu Glu
    50                  55                  60

Leu Arg Phe Pro Arg Gly Gln Gly Val Pro Ile Asn Thr Asn Ser Gly
65                  70                  75                  80

Pro Asp Asp Gln Ile Gly Tyr Tyr Arg Arg Ala Thr Arg Arg Val Arg
                85                  90                  95

Gly Gly Asp Gly Lys Met Lys Glu Leu Ser Pro Arg Trp Tyr Phe Tyr
            100                 105                 110

Tyr Leu Gly Thr Gly Pro Glu Ala Ser Leu Pro Tyr Gly Ala Asn Lys
        115                 120                 125

Glu Gly Ile Val Trp Val Ala Thr Glu Gly Ala Leu Asn Thr Pro Lys
    130                 135                 140

Asp His Ile Gly Thr Arg Asn Pro Asn Asn Asn Ala Ala Thr Val Leu
145                 150                 155                 160

Gln Leu Pro Gln Gly Thr Thr Leu Pro Lys Gly Phe Tyr Ala Glu Gly
                165                 170                 175

Ser Arg Gly Gly Ser Gln Ala Ser Ser Arg Ser Ser Ser Arg Ser Arg
            180                 185                 190

Gly Asn Ser Arg Asn Ser Thr Pro Gly Ser Ser Arg Gly Asn Ser Pro
        195                 200                 205

Ala Arg Met Ala Ser Gly Gly Gly Glu Thr Ala Leu Ala Leu Leu Leu
    210                 215                 220

Leu Asp Arg Leu Asn Gln Leu Glu Ser Lys Val Ser Gly Lys Gly Gln
225                 230                 235                 240

Gln Gln Gln Gly Gln Thr Val Thr Lys Lys Ser Ala Ala Glu Ala Ser
                245                 250                 255

Lys Lys Pro Arg Gln Lys Arg Thr Ala Thr Lys Gln Tyr Asn Val Thr
            260                 265                 270

Gln Ala Phe Gly Arg Arg Gly Pro Glu Gln Thr Gln Gly Asn Phe Gly
        275                 280                 285

Asp Gln Asp Leu Ile Arg Gln Gly Thr Asp Tyr Lys His Trp Pro Gln
    290                 295                 300

Ile Ala Gln Phe Ala Pro Ser Ala Ser Ala Phe Phe Gly Met Ser Arg
305                 310                 315                 320

Ile Gly Met Glu Val Thr Pro Ser Gly Thr Trp Leu Thr Tyr His Gly
                325                 330                 335

Ala Ile Lys Leu Asp Asp Lys Asp Pro Gln Phe Lys Asp Asn Val Ile
            340                 345                 350

Leu Leu Asn Lys His Ile Asp Ala Tyr Lys Thr Phe Pro Pro Thr Glu
        355                 360                 365

Pro Lys Lys Asp Lys Lys Lys Lys Thr Asp Glu Ala Gln Pro Leu Pro
    370                 375                 380

Gln Arg Gln Lys Lys Gln Pro Thr Val Thr Leu Leu Pro Ala Ala Asp
385                 390                 395                 400

Met Asp Asp Phe Ser Arg Gln Leu Gln Asn Ser Met Ser Gly Ala Ser
                405                 410                 415

Ala Asp Ser Thr Gln Ala
            420


<210> SEQ ID NO 38
<211> LENGTH: 1377
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 38
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atgaaggtca ccaaactgct gcatttagag acgtacttgt tgttttaaat aaacgaacaa 60

attaaaatgt ctgataatgg accccaatca aaccaacgta gtgccccccg cattacattt 120

ggtggaccca cagattcaac tgacaataac cagaatggag gacgcaatgg ggcaaggcca 180

aaacagcgcc gaccccaagg tttacccaat aatactgcgt cttggttcac agctctcact 240

cagcatggca aggaggaact tagattccct cgaggccagg gcgttccaat caacaccaat 300

agtggtccag atgaccaaat tggctactac cgaagagcta cccgacgagt tcgtggtggt 360

gacggcaaaa tgaaagagct cagccccaga tggtacttct attacctagg aactggccca 420

gaagcttcac ttccctacgg cgctaacaaa gaaggcatcg tatgggttgc aactgaggga 480

gccttgaata cacccaaaga ccacattggc acccgcaatc ctaataacaa tgctgccacc 540

gtgctacaac ttcctcaagg aacaacattg ccaaaaggct tctacgcaga gggaagcaga 600

ggcggcagtc aagcctcttc tcgctcctca tcacgtagtc gcggtaattc aagaaattca 660

actcctggca gcagtagggg aaattctcct gctcgaatgg ctagcggagg tggtgaaact 720

gccctcgcgc tattgctgct agacagattg aaccagcttg agagcaaagt ttctggtaaa 780

ggccaacaac aacaaggcca aactgtcact aagaaatctg ctgctgaggc atctaaaaag 840

cctcgccaaa aacgtactgc cacaaaacag tacaacgtca ctcaagcatt tgggagacgt 900

ggtccagaac aaacccaagg aaatttcggg gaccaagacc taatcagaca aggaactgat 960

tacaaacatt ggccgcaaat tgcacaattt gctccaagtg cctctgcatt ctttggaatg 1020

tcacgcattg gcatggaagt cacaccttcg ggaacatggc tgacttatca tggagccatt 1080

aaattggatg acaaagatcc acaattcaaa gacaacgtca tactgctgaa caagcacatt 1140

gacgcataca aaacattccc accaacagag cctaaaaagg acaaaaagaa aaagactgat 1200

gaagctcagc ctttgccgca gagacaaaag aagcagccca ctgtgactct tcttcctgcg 1260

gctgacatgg atgatttctc cagacaactt caaaattcca tgagtggagc ttctgctgat 1320

tcaactcagg cataaacact catgatgacc acacaaggca gatgggctat gtaaacg 1377

<210> SEQ ID NO 39
<211> LENGTH: 204
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 39

atattaggtt tttacctacc caggaaaagc caaccaacct cgatctcttg tagatctgtt 60

ctctaaacga actttaaaat ctgtgtagct gtcgctcggc tgcatgccta gtgcacctac 120

gcagtataaa caataataaa ttttactgtc gttgacaaga aacgagtaac tcgtccctct 180

tctgcagact gcttacggtt tcgt 204

<210> SEQ ID NO 40
<211> LENGTH: 809
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 40

actcaagcat ttgggagacg tggtccagaa caaacccaag gaaatttcgg ggaccaagac 60

ctaatcagac aaggaactga ttacaaacat tggccgcaaa ttgcacaatt tgctccaagt 120

gcctctgcat tctttggaat gtcacgcatt ggcatggaag tcacaccttc gggaacatgg 180

ctgacttatc atggagccat taaattggat gacaaagatc cacaattcaa agacaacgtc 240

atactgctga acaagcacat tgacgcatac aaaacattcc caccaacaga gcctaaaaag 300

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gacaaaaaga aaaagactga tgaagctcag cctttgccgc agagacaaaa gaagcagccc 360

actgtgactc ttcttcctgc ggctgacatg gatgatttct ccagacaact tcaaaattcc 420

atgagtggag cttctgctga ttcaactcag gcataaacac tcatgatgac cacacaaggc 480

agatgggcta tgtaaacgtt ttcgcaattc cgtttacgat acatagtcta ctcttgtgca 540

gaatgaattc tcgtaactaa acagcacaag taggtttagt taactttaat ctcacatagc 600

aatctttaat caatgtgtaa cattagggag gacttgaaag agccaccaca ttttcatcga 660

ggccacgcgg agtacgatcg agggtacagt gaataatgct agggagagct gcctatatgg 720

aagagcccta atgtgtaaaa ttaattttag tagtgctatc cccatgtgat tttaatagct 780

tcttaggaga atgacaaaaa aaaaaaaaa 809

<210> SEQ ID NO 41
<211> LENGTH: 448
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 41

aatgaacaca tagggctgtt caagctgggg cagtacgcct ttttccagct ctactagacc 60

acaagtgcca tttttgaggt gttcacgtgc ctccgatagg gcctcttcca cagagtcccc 120

gaagccacgc actagcacgt ctctaacctg aaggacaggc aaactgagtt ggacgtgtgt 180

tttctcgttg acaccaagaa caaggctctc catcttacct ttcggtcaca cccggacgaa 240

acctaggtat gctgatgatc gactgcaaca cggacgaaac cgtaagcagt ctgcagaaga 300

gggacgagtt actcgtttct tgtcaacgac agtaaaattt attattgttt atactgcgta 360

ggtgcactag gcatgcagcc gagcgacagc tacacagatt ttaaagttcg tttagagaac 420

agatctacaa gagatcgagg ttggttgg 448

<210> SEQ ID NO 42
<211> LENGTH: 2033
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 42

atacctaggt ttcgtccggg tgtgaccgaa aggtaagatg gagagccttg ttcttggtgt 60

caacgagaaa acacacgtcc aactcagttt gcctgtcctt caggttagag acgtgctagt 120

gcgtggcttc ggggactctg tggaagaggc cctatcggag gcacgtgaac acctcaaaaa 180

tggcacttgt ggtctagtag agctggaaaa aggcgtactg ccccagcttg aacagcccta 240

tgtgttcatt aaacgttctg atgccttaag caccaatcac ggccacaagg tcgttgagct 300

ggttgcagaa atggacggca ttcagtacgg tcgtagcggt ataacactgg gagtactcgt 360

gccacatgtg ggcgaaaccc caattgcata ccgcaatgtt cttcttcgta agaacggtaa 420

taagggagcc ggtggtcata gctatggcat cgatctaaag tcttatgact taggtgacga 480

gcttggcact gatcccattg aagattatga acaaaactgg aacactaagc atggcagtgg 540

tgcactccgt gaactcactc gtgagctcaa tggaggtgca gtcactcgct atgtcgacaa 600

caatttctgt ggcccagatg ggtaccctct tgattgcatc aaagattttc tcgcacgcgc 660

gggcaagtca atgtgcactc tttccgaaca acttgattac atcgagtcga agagaggtgt 720

ctactgctgc cgtgaccatg agcatgaaat tgcctggttc actgagcgct ctgataagag 780

ctacgagcac cagacaccct tcgaaattaa gagtgccaag aaatttgaca ctttcaaagg 840

ggaatgccca aagtttgtgt ttcctcttaa ctcaaaagtc aaagtcattc aaccacgtgt 900

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tgaaaagaaa aagactgagg gtttcatggg gcgtatacgc tctgtgtacc ctgttgcatc 960

tccacaggag tgtaacaata tgcacttgtc taccttgatg aaatgtaatc attgcgatga 1020

agtttcatgg cagacgtgcg actttctgaa agccacttgt gaacattgtg gcactgaaaa 1080

tttagttatt gaaggaccta ctacatgtgg gtacctacct actaatgctg tagtgaaaat 1140

gccatgtcct gcctgtcaag acccagagat tggacctgag catagtgttg cagattatca 1200

caaccactca aacattgaaa ctcgactccg caagggaggt aggactagat gttttggagg 1260

ctgtgtgttt gcctatgttg gctgctataa taagcgtgcc tactgggttc ctcgtgctag 1320

tgctgatatt ggctcaggcc atactggcat tactggtgac aatgtggaga ccttgaatga 1380

ggatctcctt gagatactga gtcgtgaacg tgttaacatt aacattgttg gcgattttca 1440

tttgaatgaa gaggttgcca tcattttggc atctttctct gcttctacaa gtgcctttat 1500

tgacactata aagagtcttg attacaagtc tttcaaaacc attgttgagt cctgcggtaa 1560

ctataaagtt accaagggaa agcccgtaaa aggtgcttgg aacattggac aacagagatc 1620

agttttaaca ccactgtgtg gttttccctc acaggctgct ggtgttatca gatcaatttt 1680

tgcgcgcaca cttgatgcag caaaccactc aattcctgat ttgcaaagag cagctgtcac 1740

catacttgat ggtatttctg aacagtcatt acgtcttgtc gacgccatgg tttatacttc 1800

agacctgctc accaacagtg tcattattat ggcatatgta actggtggtc ttgtacaaca 1860

gacttctcag tggttgtcta atcttttggg cactactgtt gaaaaactca ggcctatctt 1920

tgaatggatt gaggcgaaac ttagtgcagg agttgaattt ctcaaggatg cttgggagat 1980

tctcaaattt ctcattacag gtgtttttga catcgtcaag ggtcaaatac agg 2033

<210> SEQ ID NO 43
<211> LENGTH: 2018
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 43

ggattgaggc gaaacttagt gcaggagttg aatttctcaa ggatgcttgg gagattctca 60

aatttctcat tacaggtgtt tttgacatcg tcaagggtca aatacaggtt gcttcagata 120

acatcaagga ttgtgtaaaa tgcttcattg atgttgttaa caaggcactc gaaatgtgca 180

ttgatcaagt cactatcgct ggcgcaaagt tgcgatcact caacttaggt gaagtcttca 240

tcgctcaaag caagggactt taccgtcagt gtatacgtgg caaggagcag ctgcaactac 300

tcatgcctct taaggcacca aaagaagtaa cctttcttga aggtgattca catgacacag 360

tacttacctc tgaggaggtt gttctcaaga acggtgaact cgaagcactc gagacgcccg 420

ttgatagctt cacaaatgga gctatcgttg gcacaccagt ctgtgtaaat ggcctcatgc 480

tcttagagat taaggacaaa gaacaatact gcgcattgtc tcctggttta ctggctacaa 540

acaatgtctt tcgcttaaaa gggggtgcac caattaaagg tgtaaccttt ggagaagata 600

ctgtttggga agttcaaggt tacaagaatg tgagaatcac atttgagctt gatgaacgtg 660

ttgacaaagt gcttaatgaa aagtgctctg tctacactgt tgaatccggt accgaagtta 720

ctgagtttgc atgtgttgta gcagaggctg ttgtgaagac tttacaacca gtttctgatc 780

tccttaccaa catgggtatt gatcttgatg agtggagtgt agctacattc tacttatttg 840

atgatgctgg tgaagaaaac ttttcatcac gtatgtattg ttccttttac cctccagatg 900

aggaagaaga ggacgatgca gagtgtgagg aagaagaaat tgatgaaacc tgtgaacatg 960

agtacggtac agaggatgat tatcaaggtc tccctctgga atttggtgcc tcagctgaaa 1020

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cagttcgagt tgaggaagaa gaagaggaag actggctgga tgatactact gagcaatcag 1080

agattgagcc agaaccagaa cctacacctg aagaaccagt taatcagttt actggttatt 1140

taaaacttac tgacaatgtt gccattaaat gtgttgacat cgttaaggag gcacaaagtg 1200

ctaatcctat ggtgattgta aatgctgcta acatacacct gaaacatggt ggtggtgtag 1260

caggtgcact caacaaggca accaatggtg ccatgcaaaa ggagagtgat gattacatta 1320

agctaaatgg ccctcttaca gtaggagggt cttgtttgct ttctggacat aatcttgcta 1380

agaagtgtct gcatgttgtt ggacctaacc taaatgcagg tgaggacatc cagcttctta 1440

aggcagcata tgaaaatttc aattcacagg acatcttact tgcaccattg ttgtcagcag 1500

gcatatttgg tgctaaacca cttcagtctt tacaagtgtg cgtgcagacg gttcgtacac 1560

aggtttatat tgcagtcaat gacaaagctc tttatgagca ggttgtcatg gattatcttg 1620

ataacctgaa gcctagagtg gaagcaccta aacaagagga gccaccaaac acagaagatt 1680

ccaaaactga ggagaaatct gtcgtacaga agcctgtcga tgtgaagcca aaaattaagg 1740

cctgcattga tgaggttacc acaacactgg aagaaactaa gtttcttacc aataagttac 1800

tcttgtttgc tgatatcaat ggtaagcttt accatgattc tcagaacatg cttagaggtg 1860

aagatatgtc tttccttgag aaggatgcac cttacatggt aggtgatgtt atcactagtg 1920

gtgatatcac ttgtgttgta ataccctcca aaaaggctgg tggcactact gagatgctct 1980

caagagcttt gaagaaagtg ccagttgatg agtatata 2018

<210> SEQ ID NO 44
<211> LENGTH: 1442
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 44

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ttgctgatat caatggtaag ctttaccatg attctcagaa catgcttaga ggtgaagata 120

tgtctttcct tgagaaggat gcaccttaca tggtaggtga tgttatcact agtggtgata 180

tcacttgtgt tgtaataccc tccaaaaagg ctggtggcac tactgagatg ctctcaagag 240

ctttgaagaa agtgccagtt gatgagtata taaccacgta ccctggacaa ggatgtgctg 300

gttatacact tgaggaagct aagactgctc ttaagaaatg caaatctgca ttttatgtac 360

taccttcaga agcacctaat gctaaggaag agattctagg aactgtatcc tggaatttga 420

gagaaatgct tgctcatgct gaagagacaa gaaaattaat gcctatatgc atggatgtta 480

gagccataat ggcaaccatc caacgtaagt ataaaggaat taaaattcaa gagggcatcg 540

ttgactatgg tgtccgattc ttcttttata ctagtaaaga gcctgtagct tctattatta 600

cgaagctgaa ctctctaaat gagccgcttg tcacaatgcc aattggttat gtgacacatg 660

gttttaatct tgaagaggct gcgcgctgta tgcgttctct taaagctcct gccgtagtgt 720

cagtatcatc accagatgct gttactacat ataatggata cctcacttcg tcatcaaaga 780

catctgagga gcactttgta gaaacagttt ctttggctgg ctcttacaga gattggtcct 840

attcaggaca gcgtacagag ttaggtgttg aatttcttaa gcgtggtgac aaaattgtgt 900

accacactct ggagagcccc gtcgagtttc atcttgacgg tgaggttctt tcacttgaca 960

aactaaagag tctcttatcc ctgcgggagg ttaagactat aaaagtgttc acaactgtgg 1020

acaacactaa tctccacaca cagcttgtgg atatgtctat gacatatgga cagcagtttg 1080

gtccaacata cttggatggt gctgatgtta caaaaattaa acctcatgta aatcatgagg 1140

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gtaagacttt ctttgtacta cctagtgatg acacactacg tagtgaagct ttcgagtact 1200

accatactct tgatgagagt tttcttggta ggtacatgtc tgctttaaac cacacaaaga 1260

aatggaaatt tcctcaagtt ggtggtttaa cttcaattaa atgggctgat aacaattgtt 1320

atttgtctag tgttttatta gcacttcaac agcttgaagt caaattcaat gcaccagcac 1380

ttcaagaggc ttattataga gcccgtgctg gtgatgctgc taacttttgt gcactcatac 1440

tc 1442

<210> SEQ ID NO 45
<211> LENGTH: 1050
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 45

atatgtctat gacatatgga cagcagtttg gtccaacata cttggatggt gctgatgtta 60

caaaaattaa acctcatgta aatcatgagg gtaagacttt ctttgtacta cctagtgatg 120

acacactacg tagtgaagct ttcgagtact accatactct tgatgagagt tttcttggta 180

ggtacatgtc tgctttaaac cacacaaaga aatggaaatt tcctcaagtt ggtggtttaa 240

cttcaattaa atgggctgat aacaattgtt atttgtctag tgttttatta gcacttcaac 300

agcttgaagt caaattcaat gcaccagcac ttcaagaggc ttattataga gcccgtgctg 360

gtgatgctgc taacttttgt gcactcatac tcgcttacag taataaaact gttggcgagc 420

ttggtgatgt cagagaaact atgacccatc ttctacagca tgctaatttg gaatctgcaa 480

agcgagttct taatgtggtg tgtaaacatt gtggtcagaa aactactacc ttaacgggtg 540

tagaagctgt gatgtatatg ggtactctat cttatgataa tcttaagaca ggtgtttcca 600

ttccatgtgt gtgtggtcgt gatgctacac aatatctagt acaacaagag tcttcttttg 660

ttatgatgtc tgcaccacct gctgagtata aattacagca aggtacattc ttatgtgcga 720

atgagtacac tggtaactat cagtgtggtc attacactca tataactgct aaggagaccc 780

tctatcgtat tgacggagct caccttacaa agatgtcaga gtacaaagga ccagtgactg 840

atgttttcta caaggaaaca tcttacacta caaccatcaa gcctgtgtcg tataaactcg 900

atggagttac ttacacagag attgaaccaa aattggatgg gtattataaa aaggataatg 960

cttactatac agagcagcct atagaccttg taccaactca accattacca aatgcgagtt 1020

ttgataattt caaactcaca tgttctaaca 1050

<210> SEQ ID NO 46
<211> LENGTH: 1995
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 46

tttgtgcact catactcgct tacagtaata aaactgttgg cgagcttggt gatgtcagag 60

aaactatgac ccatcttcta cagcatgcta atttggaatc tgcaaagcga gttcttaatg 120

tggtgtgtaa acattgtggt cagaaaacta ctaccttaac gggtgtagaa gctgtgatgt 180

atatgggtac tctatcttat gataatctta agacaggtgt ttccattcca tgtgtgtgtg 240

gtcgtgatgc tacacaatat ctagtacaac aagagtcttc ttttgttatg atgtctgcac 300

cacctgctga gtataaatta cagcaaggta cattcttatg tgcgaatgag tacactggta 360

actatcagtg tggtcattac actcatataa ctgctaagga gaccctctat cgtattgacg 420

gagctcacct tacaaagatg tcagagtaca aaggaccagt gactgatgtt ttctacaagg 480

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aaacatctta cactacaacc atcaagcctg tgtcgtataa actcgatgga gttacttaca 540

cagagattga accaaaattg gatgggtatt ataaaaagga taatgcttac tatacagagc 600

agcctataga ccttgtacca actcaaccat taccaaatgc gagttttgat aatttcaaac 660

tcacatgttc taacacaaaa tttgctgatg atttaaatca aatgacaggc ttcacaaagc 720

cagcttcacg agagctatct gtcacattct tcccagactt gaatggcgat gtagtggcta 780

ttgactatag acactattca gcgagtttca agaaaggtgc taaattactg cataagccaa 840

ttgtttggca cattaaccag gctacaacca agacaacgtt caaaccaaac acttggtgtt 900

tacgttgtct ttggagtaca aagccagtag atacttcaaa ttcatttgaa gttctggcag 960

tagaagacac acaaggaatg gacaatcttg cttgtgaaag tcaacaaccc acctctgaag 1020

aagtagtgga aaatcctacc atacagaagg aagtcataga gtgtgacgtg aaaactaccg 1080

aagttgtagg caatgtcata cttaaaccat cagatgaagg tgttaaagta acacaagagt 1140

taggtcatga ggatcttatg gctgcttatg tggaaaacac aagcattacc attaagaaac 1200

ctaatgagct ttcactagcc ttaggtttaa aaacaattgc cactcatggt attgctgcaa 1260

ttaatagtgt tccttggagt aaaattttgg cttatgtcaa accattctta ggacaagcag 1320

caattacaac atcaaattgc gctaagagat tagcacaacg tgtgtttaac aattatatgc 1380

cttatgtgtt tacattattg ttccaattgt gtacttttac taaaagtacc aattctagaa 1440

ttagagcttc actacctaca actattgcta aaaatagtgt taagagtgtt gctaaattat 1500

gtttggatgc cggcattaat tatgtgaagt cacccaaatt ttctaaattg ttcacaatcg 1560

ctatgtggct attgttgtta agtatttgct taggttctct aatctgtgta actgctgctt 1620

ttggtgtact cttatctaat tttggtgctc cttcttattg taatggcgtt agagaattgt 1680

atcttaattc gtctaacgtt actactatgg atttctgtga aggttctttt ccttgcagca 1740

tttgtttaag tggattagac tcccttgatt cttatccagc tcttgaaacc attcaggtga 1800

cgatttcatc gtacaagcta gacttgacaa ttttaggtct ggccgctgag tgggttttgg 1860

catatatgtt gttcacaaaa ttcttttatt tattaggtct ttcagctata atgcaggtgt 1920

tctttggcta ttttgctagt catttcatca gcaattcttg gctcatgtgg tttatcatta 1980

gtattgtaca aatgg 1995

<210> SEQ ID NO 47
<211> LENGTH: 1884
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 47

aattcttggc tcatgtggtt tatcattagt attgtacaaa tggcacccgt ttctgcaatg 60

gttaggatgt acatcttctt tgcttctttc tactacatat ggaagagcta tgttcatatc 120

atggatggtt gcacctcttc gacttgcatg atgtgctata agcgcaatcg tgccacacgc 180

gttgagtgta caactattgt taatggcatg aagagatctt tctatgtcta tgcaaatgga 240

ggccgtggct tctgcaagac tcacaattgg aattgtctca attgtgacac attttgcact 300

ggtagtacat tcattagtga tgaagttgct cgtgatttgt cactccagtt taaaagacca 360

atcaacccta ctgaccagtc atcgtatatt gttgatagtg ttgctgtgaa aaatggcgcg 420

cttcacctct actttgacaa ggctggtcaa aagacctatg agagacatcc gctctcccat 480

tttgtcaatt tagacaattt gagagctaac aacactaaag gttcactgcc tattaatgtc 540

atagtttttg atggcaagtc caaatgcgac gagtctgctt ctaagtctgc ttctgtgtac 600

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tacagtcagc tgatgtgcca acctattctg ttgcttgacc aagctcttgt atcagacgtt 660

ggagatagta ctgaagtttc cgttaagatg tttgatgctt atgtcgacac cttttcagca 720

acttttagtg ttcctatgga aaaacttaag gcacttgttg ctacagctca cagcgagtta 780

gcaaagggtg tagctttaga tggtgtcctt tctacattcg tgtcagctgc ccgacaaggt 840

gttgttgata ccgatgttga cacaaaggat gttattgaat gtctcaaact ttcacatcac 900

tctgacttag aagtgacagg tgacagttgt aacaatttca tgctcaccta taataaggtt 960

gaaaacatga cgcccagaga tcttggcgca tgtattgact gtaatgcaag gcatatcaat 1020

gcccaagtag caaaaagtca caatgtttca ctcatctgga atgtaaaaga ctacatgtct 1080

ttatctgaac agctgcgtaa acaaattcgt agtgctgcca agaagaacaa catacctttt 1140

agactaactt gtgctacaac tagacaggtt gtcaatgtca taactactaa aatctcactc 1200

aagggtggta agattgttag tacttgtttt aaacttatgc ttaaggccac attattgtgc 1260

gttcttgctg cattggtttg ttatatcgtt atgccagtac atacattgtc aatccatgat 1320

ggttacacaa atgaaatcat tggttacaaa gccattcagg atggtgtcac tcgtgacatc 1380

atttctactg atgattgttt tgcaaataaa catgctggtt ttgacgcatg gtttagccag 1440

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ggttctattt cttatagtga gcttcgtcca gacactcgtt atgtgcttat ggatggttcc 1800

atcatacagt ttcctaacac ttacctggag ggttctgtta gagtagtaac aacttttgat 1860

gctgagtact gtagacatgg taca 1884

<210> SEQ ID NO 48
<211> LENGTH: 2020
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 48

cactcgttat gtgcttatgg atggttccat catacagttt cctaacactt acctggaggg 60

ttctgttaga gtagtaacaa cttttgatgc tgagtactgt agacatggta catgcgaaag 120

gtcagaagta ggtatttgcc tatctaccag tggtagatgg gttcttaata atgagcatta 180

cagagctcta tcaggagttt tctgtggtgt tgatgcgatg aatctcatag ctaacatctt 240

tactcctctt gtgcaacctg tgggtgcttt agatgtgtct gcttcagtag tggctggtgg 300

tattattgcc atattggtga cttgtgctgc ctactacttt atgaaattca gacgtgtttt 360

tggtgagtac aaccatgttg ttgctgctaa tgcacttttg tttttgatgt ctttcactat 420

actctgtctg gtaccagctt acagctttct gccgggagtc tactcagtct tttacttgta 480

cttgacattc tatttcacca atgatgtttc attcttggct caccttcaat ggtttgccat 540

gttttctcct attgtgcctt tttggataac agcaatctat gtattctgta tttctctgaa 600

gcactgccat tggttcttta acaactatct taggaaaaga gtcatgttta atggagttac 660

atttagtacc ttcgaggagg ctgctttgtg taccttttttg ctcaacaagg aaatgtacct 720

aaaattgcgt agcgagacac tgttgccact tacacagtat aacaggtatc ttgctctata 780

taacaagtac aagtatttca gtggagcctt agatactacc agctatcgtg aagcagcttg 840

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ctgccactta gcaaaggctc taaatgactt tagcaactca ggtgctgatg ttctctacca 900

accaccacag acatcaatca cttctgctgt tctgcagagt ggttttagga aaatggcatt 960

cccgtcaggc aaagttgaag ggtgcatggt acaagtaacc tgtggaacta caactcttaa 1020

tggattgtgg ttggatgaca cagtatactg tccaagacat gtcatttgca cagcagaaga 1080

catgcttaat cctaactatg aagatctgct cattcgcaaa tccaaccata gctttcttgt 1140

tcaggctggc aatgttcaac ttcgtgttat tggccattct atgcaaaatt gtctgcttag 1200

gcttaaagtt gatacttcta accctaagac acccaagtat aaatttgtcc gtatccaacc 1260

tggtcaaaca ttttcagttc tagcatgcta caatggttca ccatctggtg tttatcagtg 1320

tgccatgaga cctaatcata ccattaaagg ttctttcctt aatggatcat gtggtagtgt 1380

tggttttaac attgattatg attgcgtgtc tttctgctat atgcatcata tggagcttcc 1440

aacaggagta cacgctggta ctgacttaga aggtaaattc tatggtccat ttgttgacag 1500

acaaactgca caggctgcag gtacagacac aaccataaca ttaaatgttt tggcatggct 1560

gtatgctgct gttatcaatg gtgataggtg gtttcttaat agattcacca ctactttgaa 1620

tgactttaac cttgtggcaa tgaagtacaa ctatgaacct ttgacacaag atcatgttga 1680

catattggga cctctttctg ctcaaacagg aattgccgtc ttagatatgt gtgctgcttt 1740

gaaagagctg ctgcagaatg gtatgaatgg tcgtactatc cttggtagca ctattttaga 1800

agatgagttt acaccatttg atgttgttag acaatgctct ggtgttacct tccaaggtaa 1860

gttcaagaaa attgttaagg gcactcatca ttggatgctt ttaactttct tgacatcact 1920

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cttgccattt actcttggta ttatggcaat tgctgcatgt 2020

<210> SEQ ID NO 49
<211> LENGTH: 2040
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 49

agcatttcca gcctgaagac gtactgtagc agctaaactg cccagcacca tacctctatt 60

taggttgttt aagcctttga tgaagtacaa gtatttcact ttaggccctt ttggtgtgtc 120

tgtaacaaac ctacaaggtg gttccagttc tgtgtaaatt gtacctgtac catcactctt 180

agggaatcta gcccatttga gatcttggtg gtctgatagt aatgccagca caaacctacc 240

tcccttcgaa ttgttatagt aggcaagtgc attgtcatca gtacaagctg tttgtgtggt 300

accagccgca caggacatct gtcgtagtgc tactggactc agttcattat tctgtagttt 360

aacagctgag ttggctctta gagctgtaac aataagaggc caagccaaat ttggtgaatt 420

gtccatgtta atttcactaa gttgaacaat cttgctatcc gcatcaacaa cttgctggat 480

ttcccagagt gcagatgcat atgtaaaggt gttaccatca caagtgttct tgtaggtacc 540

ataatcaggg acaacaacca tgagtttggc tgctgtagtc aatggtatga tgttgagtgg 600

aacacaacca tcacgcgcat tgttgataat gttgttaagt gcatcattat caagcttcct 660

aagcatagtg aagagcattg tttgcatagc actagttact tttgccctct tgtcctcaga 720

tcttgcctgt ttgtacattt gggtcatagc ctgatctgcc atcttttcca acttgcgttg 780

catggcagca tcacggtcaa actcagattt agccacattc aaagatttct ttaacttttt 840

gagaacgact tcagaatcac cattagctac agcctgctca taggcctcct gggcagtggc 900

ataagcggca tatgatggta aagaactaaa ttctgaagca atagcctgaa gagtagcacg 960

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gttatcgagc atttcctcgc acaacctatt aatgtctaca gcaccctgca tggatagcaa 1020

aacagacaaa agagaaacca tcttctcgaa agcttcagtt gtgtcttttg caagaagaat 1080

atcattgtgg agttgtacac attgtgccca caatttagaa gatgactcta ctctaagttg 1140

ttgaagaacc gagagcagta ccacagatgt gcactttacg tcagacattt tagactgtac 1200

agtagcaacc ttgatacatg gtttacctcc aatacccaac aacttaatgt taagcttgaa 1260

agcatcaata ctactcttag gaggcaaaag cccctgggag ttcatatacc taaattcttg 1320

tgtagagacc aagtagtcat aaacaccaag agtaagcctg aagtaacggt tgagtaaaca 1380

gaaaaggcca aagtagcagc agcaacaata gcctaagaaa caataaacaa gcatgataca 1440

ctgtaaggtg ttgccagtaa taaataacaa tgggtaatac tcaacacaca caaacactat 1500

agctctagct aaaaacatga tagtcgtaac gacaccagaa tagttagagg ttacagaaat 1560

aactaaggcc cacatggaaa tagcttgatc taaagcatta ccatagtaga ctttgtaaac 1620

aagtgtaatg acattcatca gtgtccaaac acgtctagca gcatcatcat aaacagtgcg 1680

agctgtcatg agaataagca aaactaaagc tgaagcatac ataacacaat ccttaagcct 1740

ataaccagac aagctagtgt cagccaattc aagccatgtc atgatacgca tcacccagct 1800

agcaggcatg tagaccatat taaagtaagc aactgttgca agagaaggta acagaaacaa 1860

gcacaagaat gcgtgcttat gcttaacaag cagcatagca catgcagcaa ttgccataat 1920

accaagagta aatggcaaga aagcattctc gtaaacaaag aaaaacagtg accactgtgt 1980

actttgaaca agaatcaata gtgatgtcaa gaaagttaaa agcatccaat gatgagtgca 2040

<210> SEQ ID NO 50
<211> LENGTH: 2012
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 50

cttgtaggtt tgttacagac acaccaaaag ggcctaaagt gaaatacttg tacttcatca 60

aaggcttaaa caacctaaat agaggtatgg tgctgggcag tttagctgct acagtacgtc 120

ttcaggctgg aaatgctaca gaagtacctg ccaattcaac tgtgctttcc ttctgtgctt 180

ttgcagtaga ccctgctaaa gcatataagg attacctagc aagtggagga caaccaatca 240

ccaactgtgt gaagatgttg tgtacacaca ctggtacagg acaggcaatt actgtaacac 300

cagaagctaa catggaccaa gagtcctttg gtggtgcttc atgttgtctg tattgtagat 360

gccacattga ccatccaaat cctaaaggat tctgtgactt gaaaggtaag tacgtccaaa 420

tacctaccac ttgtgctaat gacccagtgg gttttacact tagaaacaca gtctgtaccg 480

tctgcggaat gtggaaaggt tatggctgta gttgtgacca actccgcgaa cccttgatgc 540

agtctgcgga tgcatcaacg tttttaaacg ggtttgcggt gtaagtgcag cccgtcttac 600

accgtgcggc acaggcacta gtactgatgt cgtctacagg gcttttgata tttacaacga 660

aaaagttgct ggttttgcaa agttcctaaa aactaattgc tgtcgcttcc aggagaagga 720

tgaggaaggc aatttattag actcttactt tgtagttaag aggcatacta tgtctaacta 780

ccaacatgaa gagactattt ataacttggt taaagattgt ccagcggttg ctgtccatga 840

ctttttcaag tttagagtag atggtgacat ggtaccacat atatcacgtc agcgtctaac 900

taaatacaca atggctgatt tagtctatgc tctacgtcat tttgatgagg gtaattgtga 960

tacattaaaa gaaatactcg tcacatacaa ttgctgtgat gatgattatt tcaataagaa 1020

ggattggtat gacttcgtag agaatcctga catcttacgc gtatatgcta acttaggtga 1080

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gcgtgtacgc caatcattat taaagactgt acaattctgc gatgctatgc gtgatgcagg 1140

cattgtaggc gtactgacat tagataatca ggatcttaat gggaactggt acgatttcgg 1200

tgatttcgta caagtagcac caggctgcgg agttcctatt gtggattcat attactcatt 1260

gctgatgccc atcctcactt tgactagggc attggctgct gagtcccata tggatgctga 1320

tctcgcaaaa ccacttatta agtgggattt gctgaaatat gattttacgg aagagagact 1380

ttgtctcttc gaccgttatt ttaaatattg ggaccagaca taccatccca attgtattaa 1440

ctgtttggat gataggtgta tccttcattg tgcaaacttt aatgtgttat tttctactgt 1500

gtttccacct acaagttttg gaccactagt aagaaaaata tttgtagatg gtgttccttt 1560

tgttgtttca actggatacc attttcgtga gttaggagtc gtacataatc aggatgtaaa 1620

cttacatagc tcgcgtctca gtttcaagga acttttagtg tatgctgctg atccagctat 1680

gcatgcagct tctggcaatt tattgctaga taaacgcact acatgctttt cagtagctgc 1740

actaacaaac aatgttgctt ttcaaactgt caaacccggt aattttaata aagactttta 1800

tgactttgct gtgtctaaag gtttctttaa ggaaggaagt tctgttgaac taaaacactt 1860

cttctttgct caggatggca acgctgctat cagtgattat gactattatc gttataatct 1920

gccaacaatg tgtgatatca gacaactcct attcgtagtt gaagttgttg ataaatactt 1980

tgattgttac gatggtggct gtattaatgc ca 2012

<210> SEQ ID NO 51
<211> LENGTH: 1877
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 51

gtacttcgcg tacagtggca ataccatatg acagcttaaa tgtttcctca gtggctttga 60

gcgtttctgc tgcgaaaagc ttgagtctct cagtacaagt gttggcaagt atgtaatcgc 120

cagcattagt ccaatcacat gttgctatcg cattgaagtc agtgacattg tcactgccta 180

cacatgtgtt tttgtataaa ccaaaaacct gaccattagc acataatgga aaactaatgg 240

gaggcttatg tgacttgcaa taatagctca tacctcctag atacagttgt gtcacatcag 300

tgacatcaca acctggggca ttgcaaacat agggattaac agacaacact aatttgtgtg 360

atgttgaaat gacatggtca tagcagcact tgcaacatag gaatggtctc ctaatacagg 420

caccgcaacg aagtgaagtc tgtgaattgc acaatacaca agcacctaca gcctgcaaga 480

ctgtatgtgg tgtgtacata gcctcataaa actcaggttc ccagtaccgt gaggtgttat 540

cattagttag cattacggaa tacatgtcca acatgtggcc agtaagctca tcatgtaact 600

ttctaatgta ttgtaaatac aagtgaaaga catcagcata ctcctgatta ggatgttttg 660

taagtgggta agcatcaata gccagtgaca cgaacctttc aatcataagt gtaccatctg 720

ttttgacaat atcatcgaca aaacagcctg cgcctaatat tcttgatgga tctgggtaag 780

gcaggtacac gtaatcatct ccttgtttaa ctagcattgt atgctgtgag caaaattcgt 840

gaggtccttt agtaaggtca gtctcagtcc aacattttgc ctcagacatg aacacattat 900

tttgataata aagaactgcc ttaaagttct taatgctagc tactaaacct tgagccgcat 960

agttactgtt atagcacaca acggcatcat cagaaagaat catcatggag aaatgtttac 1020

gcaggtaagc gtaaaactca tccacgaatt catgatcaac atccctattt ctatagagac 1080

actcatagag cctgtgttgt agattgcgga catacttgtc agctatctta ttaccatcag 1140

ttgaaagaag tgcatttaca ttggctgtaa cagcttgaca aatgttaaag acactattag 1200

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cataagcagt tgtagcatca ccggatgatg ttccacctgg tttaacatat agtgagccgc 1260

cacacatgac catctcactt aatacttgcg cacactcgtt agctaacctg tagaaacggt 1320

gtgataagtt acagcaagtg ttatgtttgc gagcaagaac aagagaggcc attatcctaa 1380

gcatgttagg catggctctg tcacattttg gataatccca acccataagg tgtggagttt 1440

ctacatcact gtaaacagtt tttaacatat tatgccagcc accgtaaaac ttgcttgttc 1500

caattaccac agtagctcct ctagtggcgg ctattgactt caataatttc tgatgaaact 1560

gtctatttgt catagtacta cagatagaga caccagctac ggtgcgagct ctattctttg 1620

cactaatggc atacttaaga ttcatttgag ttatagtagg gatgacatta cgcttagtat 1680

acgcgaaaag tgcatcttga tcctcataac tcattgagtc ataataaagt ctagccttac 1740

cccatttatt aaatgggaaa ccagctgatt tatccagatt gttaacgatt acttggttgg 1800

cattaataca gccaccatcg taacaatcaa agtatttatc aacaacttca actacgaata 1860

ggagttgtct gatatca 1877

<210> SEQ ID NO 52
<211> LENGTH: 2051
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 52

tcaggtccaa tcttgacaaa gtacttcatt gatgtaagct caaagccatg cgcccaaagg 60

acgaacacga ctctgtctga caatcctttc agtgtatcac tgagcatttg tactatctta 120

atacgcacta cattccaggg caagccttta tacatgagtg gtataagatg tttaaactgg 180

tcacctggtg gaggttttgc attaactctg gtgaattctg tgttattttc agtgtcaaca 240

taaccagtcg gtacagctac taagttaaca cctgtagaaa atcctagctg gagaggtagg 300

ttagtaccca cagcatctct agttgcatga cagccctcta catcaaagcc aatccacgca 360

cgaacgtgac gaatagcttc ttcgcgggtg ataaacatat tagggtaacc attgacttgg 420

taattcattt tgaaacccat catagagatg agtctacggt aggtcatgtc ctttggtatg 480

cctggtatgt caacacataa tccttcagtc ttgaacttta tatcaacgct gaggtgtgta 540

ggtgcctgtg taggatgaag accagtaatg atcttactac agtccttaaa aagtccagtt 600

acattttctg cttgtaatgt agccacattg cgacgtggta tttctagact tgtaaattgc 660

agtttgtcat aaagatctct atcagacatt atgcacaaaa tgccaatttt tgcccttgtg 720

atagccacat tgaagcggtt gacattacaa gagtgtgctg tttcagtagt ttgtgtgaat 780

atgacatagt catattcaga accctgtgat gaatcaacag tctgcgtagg caatcctaag 840

atttttgaag ctacagcgtt ctgtgaatta taaggtgaga taaaaacagc ttttctccaa 900

gcaggattgc gtgtaagaaa ttctcttaca acgcctattt gaggtctgtt gattgcagat 960

gaaacatcat gtgtaataac acctttgtag aacattttga agcattgagc tgacttatcc 1020

ttgtgtgctt ttagcttatt gtcataaact aaagcactca cagtgtcaac aatttcagca 1080

ggacaacggc gacaagttcc aaggaacatg tctggaccta ttgttttcat aagtctgcac 1140

actgaattaa aatattctgg ttctagtgtg cctttagtca gcaatgtgcg gggggctggt 1200

aattgagcag gatcgccaat atagacgtag tgttttgcac gaagtctagc attgacaaca 1260

ctcaagtcat aattagtagc catagagatt tcatcaaaga ctacaatgtc agcagttgtt 1320

tctggcaatg catttacagt gcagaaaaca tactgttcta gtgttgaatt cactttgaat 1380

ttatcaaaac actctacgcg cgcacgcgca ggtatgattc tactacattt atctatgggc 1440

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aaatatttta atgccttttc acatagggca tcaacagctg catgagagca tgccgtatac 1500

actatgcgag cagatgggta atagagagca agtccgatgg caaaatgact cttaccagta 1560

ccaggtggtc cttggagtgt agagtacttt tgcatgccga ccttttgata atttgcaaca 1620

ttgctagaaa actcatctga gatgttgagt gttgggtaca agccagtaat tctcacatag 1680

tgctcttgtg gcactagagt aggtgcacta agtggcatta cagtgtgaga tgtcaacaca 1740

aagtaatcac caacattcaa cttgtatgtc gtagtacctc tgtacacaac agcatcacca 1800

tagtcacctt tttcaaaggt gtactctcca atctgtactt tactattttt agttacacgg 1860

taaccagtaa agacatagtt tctgttcaat ggtggtctag gttttccaac ctcccatgaa 1920

agatgcaatt ctctgtcaga gagtacttcg cgtacagtgg caataccata tgacagctta 1980

aatgtttcct cagtggcttt gagcgtttct gctgcgaaaa gcttgagtct ctcagtacaa 2040

gtgttggcaa g 2051

<210> SEQ ID NO 53
<211> LENGTH: 2075
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 53

tgcttgtagt tttgggtaga aggtttcaac atgtccatcc ttacaccaaa gcatgaatga 60

aatttcagca tagtcaattg taaccttgac cacttttgaa atcactgaca aatcttgtga 120

ctttattatc tcgacaaagt catcaagtaa aagatcaatc acagaacaca cacattttga 180

tgaacctgtt tgcgcatctg ttatgaagta atttttcact gtgctgtcca tagggataaa 240

atcctctaat ttaagtggtg aatcttgtga gcgcttggct aagcctatca ttaaatgaag 300

accgccaagt tgtccatgac tgaaatctcc ataaacgatg tgttcgaagg catagccctc 360

gagcttatat cgctgtatga attcatccat agcgagctcg agaaagtcag tttccatttg 420

tgatctgggc ttaaaatcct ctaagtctct gctctgagta aagtaggttt caggcaactg 480

ttgaataatg ccgtctactt tcttaaagta gttaaactgt gtttttactg attctccaat 540

taatgtgact ccattgacgc tagcttgtgc tggtcccttt gaaggtgtta gacctttgac 600

tgaaccttct gttattaaaa caccattacg ggcgtttcta aaaaggtcta cctgtccttc 660

cactctacca tcaaacaaga cagtaagtga agaacaagca ctctcagtag gtttcttggc 720

aatgtcagtc attgtgcaga cacctattgt agatacatgt gctggggctt ctcttttgta 780

gtcccagatt acagtattag cagcgatatc aacacccaaa ttattgagta tcttaatctc 840

tggcactggt ttaatgttac gcttagccca aagctcaaat gcaacattaa caggaagtgt 900

tgtcttattt tcaaagatct ccacatcaat accatctacc tttgtgtaaa cagcattatt 960

aatgatggaa acaggtgctt cgccggcgtg tccatcaaag tgtcctttat taacaacatt 1020

ataagccaca ttttctaaac tctgtaacct ggtaaatgta ttccacaggt tataagtatc 1080

aaattgtttg taaatccata ggctaaatcc agcagaaatc atcatattat atgcatccaa 1140

gtactgtcgg tactcatttg catggtgtct gcaaacagca ccacctaaat tgcatcgtgt 1200

aatacacgta gcagatttga gtggaacata atcaatatcc gacactactt gtttgccatg 1260

agactcacaa ggactatcag aatagtaaaa gaaaggcaat tgctttaaat tagtaaatgc 1320

acttttatcg aaagctggag tgtggaatgc atgcttattc acatacaaac taccaccatc 1380

acagcctggt aagttcaagt ttgacaagac tcttgtgtca aacctacaca caattgcatt 1440

ggctgggtaa cgatcaacgt tacaattcca aaacaaacaa acaccatcag tgaatttatc 1500

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gtgatgtgta gcataagaat agaagagttc ctctattttg taagctttgt cactacatgg 1560

ctgagcatcg tagaacttcc attctacttc agcctgaggc acacacttga tagcctttgg 1620

atttccaatg tcatgaagaa ctggaaactt atcagcaagc aatgcagact tcacaaccat 1680

gtgttgtact tttctgcaag cagaattaac cctcagttca tctcctataa tagggtattc 1740

aacagaccaa tcaacgcgct taacaaagca ctcatggact gctaaacatc tagtcatgat 1800

agcatcacaa ctagccacat gtgcatttcc atgtacctgg caatgttggt catggttact 1860

ctgaaggtta cccgtaaagc cccactgctg aacatcaatc ataaatgggt tatagacata 1920

gtcaaaaccc acagaatgat tccagcaggc ataagtatct gatgaagtag aaaagcaagt 1980

tgcacgtttg tcacacagac aacacgttct ttcaggtcca atcttgacaa agtacttcat 2040

tgatgtaagc tcaaagccat gcgcccaaag gacga 2075

<210> SEQ ID NO 54
<211> LENGTH: 1891
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 54

aagattcacc acttaaatta gaggatttta tccctatgga cagcacagtg aaaaattact 60

tcataacaga tgcgcaaaca ggttcatcaa aatgtgtgtg ttctgtgatt gatcttttac 120

ttgatgactt tgtcgagata ataaagtcac aagatttgtc agtgatttca aaagtggtca 180

aggttacaat tgactatgct gaaatttcat tcatgctttg gtgtaaggat ggacatgttg 240

aaaccttcta cccaaaacta caagcaagtc aagcgtggca accaggtgtt gcgatgccta 300

acttgtacaa gatgcaaaga atgcttcttg aaaagtgtga ccttcagaat tatggtgaaa 360

atgctgttat accaaaagga ataatgatga atgtcgcaaa gtatactcaa ctgtgtcaat 420

acttaaatac acttacttta gctgtaccct acaacatgag agttattcac tttggtgctg 480

gctctgataa aggagttgca ccaggtacag ctgtgctcag acaatggttg ccaactggca 540

cactacttgt cgattcagat cttaatgact tcgtctccga cgcagattct actttaattg 600

gagactgtgc aacagtacat acggctaata aatgggacct tattattagc gatatgtatg 660

accctaggac caaacatgtg acaaaagaga atgactctaa agaagggttt ttcacttatc 720

tgtgtggatt tataaagcaa aaactagccc tgggtggttc tatagctgta aagataacag 780

agcattcttg gaatgctgac ctttacaagc ttatgggcca tttctcatgg tggacagctt 840

ttgttacaaa tgtaaatgca tcatcatcgg aagcatttt aattggggct aactatcttg 900

gcaagccgaa ggaacaaatt gatggctata ccatgcatgc taactacatt ttctggagga 960

acacaaatcc tatccagttg tcttcctatt cactctttga catgagcaaa tttcctctta 1020

aattaagagg aactgctgta atgtctctta aggagaatca aatcaatgat atgatttatt 1080

ctcttctgga aaaaggtagg cttatcatta gagaaaacaa cagagttgtg gtttcaagtg 1140

atattcttgt taacaactaa acgaacatgt ttatttttctt attatttctt actctcacta 1200

gtggtagtga ccttgaccgg tgcaccactt ttgatgatgt tcaagctcct aattacactc 1260

aacatacttc atctatgagg ggggtttact atcctgatga aatttttaga tcagacactc 1320

tttatttaac tcaggattta tttcttccat tttattctaa tgttacaggg tttcatacta 1380

ttaatcatac gtttggcaac cctgtcatac cttttaagga tggtatttat tttgctgcca 1440

cagagaaatc aaatgttgtc cgtggttggg tttttggttc taccatgaac aacaagtcac 1500

agtcggtgat tattattaac aattctacta atgttgttat acgagcatgt aactttgaat 1560

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tgtgtgacaa ccctttcttt gctgtttcta aacccatggg tacacagaca catactatga 1620

tattcgataa tgcatttaat tgcactttcg agtacatatc tgatgccttt tcgcttgatg 1680

tttcagaaaa gtcaggtaat tttaaacact tacgagagtt tgtgtttaaa aataaagatg 1740

ggtttctcta tgtttataag ggctatcaac ctatagatgt agttcgtgat ctaccttctg 1800

gttttaacac tttgaaacct atttttaagt tgcctcttgg tattaacatt acaaatttta 1860

gagccattct tacagccttt tcacctgctc a 1891

<210> SEQ ID NO 55
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: N sens primer

<400> SEQUENCE: 55

cccatatgtc tgataatgga ccccaatcaa ac 32

<210> SEQ ID NO 56
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: N antisens primer

<400> SEQUENCE: 56

cccccgggtg cctgagttga atcagcagaa gc 32

<210> SEQ ID NO 57
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sc sens primer

<400> SEQUENCE: 57

cccatatgag tgaccttgac cggtgcacca c 31

<210> SEQ ID NO 58
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SL sens primer

<400> SEQUENCE: 58

cccatatgaa accttgcacc ccacctgctc 30

<210> SEQ ID NO 59
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Sc and SL antisens primer

<400> SEQUENCE: 59

cccccgggtt taatatattg ctcatatttt ccc 33

<210> SEQ ID NO 60
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Sens set 1 primer

<400> SEQUENCE: 60

ggcatcgtat gggttg 16

<210> SEQ ID NO 61
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Antisens set 2 (28774-28759) primer

<400> SEQUENCE: 61

cagtttcacc acctcc 16

<210> SEQ ID NO 62
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Sens set 2 (28375-28390) primer

<400> SEQUENCE: 62

ggctactacc gaagag 16

<210> SEQ ID NO 63
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Antisens set 2 (28702-28687)primer

<400> SEQUENCE: 63

aattaccgcg actacg 16

<210> SEQ ID NO 64
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Probe 1/set 1 (28561-28586)

<400> SEQUENCE: 64

ggcacccgca atcctaataa caatgc 26

<210> SEQ ID NO 65
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Probe 2/set 1 (28588-28608)

<400> SEQUENCE: 65

gccaccgtgc tacaacttcc t 21

<210> SEQ ID NO 66
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Probe 1/set 2 /probe N/FL (28541-28563)

<400> SEQUENCE: 66

atacacccaa agaccacatt ggc 23

<210> SEQ ID NO 67
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Probe 2/set 2/probe SARS/N/LC705 (28565-28589)

<400> SEQUENCE: 67

cccgcaatcc taataacaat gctgc 25

<210> SEQ ID NO 68
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anchor primer 14T

<400> SEQUENCE: 68

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agatgaattc ggtacctttt ttttttttt 30

<210> SEQ ID NO 69
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: M2-14 peptide

<400> SEQUENCE: 69

Ala Asp Asn Gly Thr Ile Thr Val Glu Glu Leu Lys Gln
1 5 10

<210> SEQ ID NO 70
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: E1-12 peptide

<400> SEQUENCE: 70

Met Tyr Ser Phe Val Ser Glu Glu Thr Gly Thr Leu
1 5 10

<210> SEQ ID NO 71
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: E53-72 peptide

<400> SEQUENCE: 71

Lys Pro Thr Val Tyr Val Tyr Ser Arg Val Lys Asn Leu Asn Ser Ser
1 5 10 15

Glu Gly Val Pro Asp Leu Leu Val
20

<210> SEQ ID NO 72
<211> LENGTH: 153
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 72

gatattaggt ttttacctac ccaggaaaag ccaaccaacc tcgatctctt gtagatctgt 60

tctctaaacg aactttaaaa tctgtgtagc tgtcgctcgg ctgcatgcct agtgcaccta 120

cgcagtataa acaataataa attttactgt cgt 153

<210> SEQ ID NO 73
<211> LENGTH: 410
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 73

ttctccagac aacttcaaaa ttccatgagt ggagcttctg ctgattcaac tcaggcataa 60

acactcatga tgaccacaca aggcagatgg gctatgtaaa cgttttcgca attccgttta 120

cgatacatag tctactcttg tgcagaatga attctcgtaa ctaaacagca caagtaggtt 180

tagttaactt taatctcaca tagcaatctt taatcaatgt gtaacattag ggaggacttg 240

aaagagccac cacattttca tcgaggccac gcggagtacg atcgagggta cagtgaataa 300

tgctagggag agctgcctat atggaagagc cctaatgtgt aaaattaatt ttagtagtgc 360

tatccccatg tgattttaat agcttcttag gagaatgaca aaaaaaaaaa 410

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<210> SEQ ID NO 74
<211> LENGTH: 4382
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 74

Met Glu Ser Leu Val Leu Gly Val Asn Glu Lys Thr His Val Gln Leu
1 5 10 15

Ser Leu Pro Val Leu Gln Val Arg Asp Val Leu Val Arg Gly Phe Gly
20 25 30

Asp Ser Val Glu Glu Ala Leu Ser Glu Ala Arg Glu His Leu Lys Asn
35 40 45

Gly Thr Cys Gly Leu Val Glu Leu Glu Lys Gly Val Leu Pro Gln Leu
50 55 60

Glu Gln Pro Tyr Val Phe Ile Lys Arg Ser Asp Ala Leu Ser Thr Asn
65 70 75 80

His Gly His Lys Val Val Glu Leu Val Ala Glu Met Asp Gly Ile Gln
85 90 95

Tyr Gly Arg Ser Gly Ile Thr Leu Gly Val Leu Val Pro His Val Gly
100 105 110

Glu Thr Pro Ile Ala Tyr Arg Asn Val Leu Leu Arg Lys Asn Gly Asn
115 120 125

Lys Gly Ala Gly Gly His Ser Tyr Gly Ile Asp Leu Lys Ser Tyr Asp
130 135 140

Leu Gly Asp Glu Leu Gly Thr Asp Pro Ile Glu Asp Tyr Glu Gln Asn
145 150 155 160

Trp Asn Thr Lys His Gly Ser Gly Ala Leu Arg Glu Leu Thr Arg Glu
165 170 175

Leu Asn Gly Gly Ala Val Thr Arg Tyr Val Asp Asn Asn Phe Cys Gly
180 185 190

Pro Asp Gly Tyr Pro Leu Asp Cys Ile Lys Asp Phe Leu Ala Arg Ala
195 200 205

Gly Lys Ser Met Cys Thr Leu Ser Glu Gln Leu Asp Tyr Ile Glu Ser
210 215 220

Lys Arg Gly Val Tyr Cys Cys Arg Asp His Glu His Glu Ile Ala Trp
225 230 235 240

Phe Thr Glu Arg Ser Asp Lys Ser Tyr Glu His Gln Thr Pro Phe Glu
245 250 255

Ile Lys Ser Ala Lys Lys Phe Asp Thr Phe Lys Gly Glu Cys Pro Lys
260 265 270

Phe Val Phe Pro Leu Asn Ser Lys Val Lys Val Ile Gln Pro Arg Val
275 280 285

Glu Lys Lys Lys Thr Glu Gly Phe Met Gly Arg Ile Arg Ser Val Tyr
290 295 300

Pro Val Ala Ser Pro Gln Glu Cys Asn Asn Met His Leu Ser Thr Leu
305 310 315 320

Met Lys Cys Asn His Cys Asp Glu Val Ser Trp Gln Thr Cys Asp Phe
325 330 335

Leu Lys Ala Thr Cys Glu His Cys Gly Thr Glu Asn Leu Val Ile Glu
340 345 350

Gly Pro Thr Thr Cys Gly Tyr Leu Pro Thr Asn Ala Val Val Lys Met
355 360 365

Pro Cys Pro Ala Cys Gln Asp Pro Glu Ile Gly Pro Glu His Ser Val
370 375 380

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Ala Asp Tyr His Asn His Ser Asn Ile Glu Thr Arg Leu Arg Lys Gly
385                 390                 395                 400

Gly Arg Thr Arg Cys Phe Gly Gly Cys Val Phe Ala Tyr Val Gly Cys
                405                 410                 415

Tyr Asn Lys Arg Ala Tyr Trp Val Pro Arg Ala Ser Ala Asp Ile Gly
            420                 425                 430

Ser Gly His Thr Gly Ile Thr Gly Asp Asn Val Glu Thr Leu Asn Glu
        435                 440                 445

Asp Leu Leu Glu Ile Leu Ser Arg Glu Arg Val Asn Ile Asn Ile Val
    450                 455                 460

Gly Asp Phe His Leu Asn Glu Glu Val Ala Ile Ile Leu Ala Ser Phe
465                 470                 475                 480

Ser Ala Ser Thr Ser Ala Phe Ile Asp Thr Ile Lys Ser Leu Asp Tyr
                485                 490                 495

Lys Ser Phe Lys Thr Ile Val Glu Ser Cys Gly Asn Tyr Lys Val Thr
            500                 505                 510

Lys Gly Lys Pro Val Lys Gly Ala Trp Asn Ile Gly Gln Gln Arg Ser
        515                 520                 525

Val Leu Thr Pro Leu Cys Gly Phe Pro Ser Gln Ala Ala Gly Val Ile
    530                 535                 540

Arg Ser Ile Phe Ala Arg Thr Leu Asp Ala Ala Asn His Ser Ile Pro
545                 550                 555                 560

Asp Leu Gln Arg Ala Ala Val Thr Ile Leu Asp Gly Ile Ser Glu Gln
                565                 570                 575

Ser Leu Arg Leu Val Asp Ala Met Val Tyr Thr Ser Asp Leu Leu Thr
            580                 585                 590

Asn Ser Val Ile Ile Met Ala Tyr Val Thr Gly Gly Leu Val Gln Gln
        595                 600                 605

Thr Ser Gln Trp Leu Ser Asn Leu Leu Gly Thr Thr Val Glu Lys Leu
    610                 615                 620

Arg Pro Ile Phe Glu Trp Ile Glu Ala Lys Leu Ser Ala Gly Val Glu
625                 630                 635                 640

Phe Leu Lys Asp Ala Trp Glu Ile Leu Lys Phe Leu Ile Thr Gly Val
                645                 650                 655

Phe Asp Ile Val Lys Gly Gln Ile Gln Val Ala Ser Asp Asn Ile Lys
            660                 665                 670

Asp Cys Val Lys Cys Phe Ile Asp Val Val Asn Lys Ala Leu Glu Met
        675                 680                 685

Cys Ile Asp Gln Val Thr Ile Ala Gly Ala Lys Leu Arg Ser Leu Asn
    690                 695                 700

Leu Gly Glu Val Phe Ile Ala Gln Ser Lys Gly Leu Tyr Arg Gln Cys
705                 710                 715                 720

Ile Arg Gly Lys Glu Gln Leu Gln Leu Leu Met Pro Leu Lys Ala Pro
                725                 730                 735

Lys Glu Val Thr Phe Leu Glu Gly Asp Ser His Asp Thr Val Leu Thr
            740                 745                 750

Ser Glu Glu Val Val Leu Lys Asn Gly Glu Leu Glu Ala Leu Glu Thr
        755                 760                 765

Pro Val Asp Ser Phe Thr Asn Gly Ala Ile Val Gly Thr Pro Val Cys
    770                 775                 780

Val Asn Gly Leu Met Leu Leu Glu Ile Lys Asp Lys Glu Gln Tyr Cys
785                 790                 795                 800

Ala Leu Ser Pro Gly Leu Leu Ala Thr Asn Asn Val Phe Arg Leu Lys
                805                 810                 815
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Gly Gly Ala Pro Ile Lys Gly Val Thr Phe Gly Glu Asp Thr Val Trp
            820                 825                 830

Glu Val Gln Gly Tyr Lys Asn Val Arg Ile Thr Phe Glu Leu Asp Glu
        835                 840                 845

Arg Val Asp Lys Val Leu Asn Glu Lys Cys Ser Val Tyr Thr Val Glu
    850                 855                 860

Ser Gly Thr Glu Val Thr Glu Phe Ala Cys Val Val Ala Glu Ala Val
865                 870                 875                 880

Val Lys Thr Leu Gln Pro Val Ser Asp Leu Leu Thr Asn Met Gly Ile
                885                 890                 895

Asp Leu Asp Glu Trp Ser Val Ala Thr Phe Tyr Leu Phe Asp Asp Ala
            900                 905                 910

Gly Glu Glu Asn Phe Ser Ser Arg Met Tyr Cys Ser Phe Tyr Pro Pro
        915                 920                 925

Asp Glu Glu Glu Glu Asp Asp Ala Glu Cys Glu Glu Glu Glu Ile Asp
    930                 935                 940

Glu Thr Cys Glu His Glu Tyr Gly Thr Glu Asp Asp Tyr Gln Gly Leu
945                 950                 955                 960

Pro Leu Glu Phe Gly Ala Ser Ala Glu Thr Val Arg Val Glu Glu Glu
                965                 970                 975

Glu Glu Glu Asp Trp Leu Asp Asp Thr Thr Glu Gln Ser Glu Ile Glu
            980                 985                 990

Pro Glu Pro Glu Pro Thr Pro Glu Glu Pro Val Asn Gln Phe Thr Gly
        995                1000                1005

Tyr Leu  Lys Leu Thr Asp Asn  Val Ala Ile Lys Cys  Val Asp Ile
    1010                1015                1020

Val Lys  Glu Ala Gln Ser Ala  Asn Pro Met Val Ile  Val Asn Ala
    1025                1030                1035

Ala Asn  Ile His Leu Lys His  Gly Gly Gly Val Ala  Gly Ala Leu
    1040                1045                1050

Asn Lys  Ala Thr Asn Gly Ala  Met Gln Lys Glu Ser  Asp Asp Tyr
    1055                1060                1065

Ile Lys  Leu Asn Gly Pro Leu  Thr Val Gly Gly Ser  Cys Leu Leu
    1070                1075                1080

Ser Gly  His Asn Leu Ala Lys  Lys Cys Leu His Val  Val Gly Pro
    1085                1090                1095

Asn Leu  Asn Ala Gly Glu Asp  Ile Gln Leu Leu Lys  Ala Ala Tyr
    1100                1105                1110

Glu Asn  Phe Asn Ser Gln Asp  Ile Leu Leu Ala Pro  Leu Leu Ser
    1115                1120                1125

Ala Gly  Ile Phe Gly Ala Lys  Pro Leu Gln Ser Leu  Gln Val Cys
    1130                1135                1140

Val Gln  Thr Val Arg Thr Gln  Val Tyr Ile Ala Val  Asn Asp Lys
    1145                1150                1155

Ala Leu  Tyr Glu Gln Val Val  Met Asp Tyr Leu Asp  Asn Leu Lys
    1160                1165                1170

Pro Arg  Val Glu Ala Pro Lys  Gln Glu Glu Pro Pro  Asn Thr Glu
    1175                1180                1185

Asp Ser  Lys Thr Glu Glu Lys  Ser Val Val Gln Lys  Pro Val Asp
    1190                1195                1200

Val Lys  Pro Lys Ile Lys Ala  Cys Ile Asp Glu Val  Thr Thr Thr
    1205                1210                1215

Leu Glu  Glu Thr Lys Phe Leu  Thr Asn Lys Leu Leu  Leu Phe Ala
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    1220                1225                1230

Asp Ile  Asn Gly Lys Leu Tyr  His Asp Ser Gln Asn  Met Leu Arg
    1235                1240                1245

Gly Glu  Asp Met Ser Phe Leu  Glu Lys Asp Ala Pro  Tyr Met Val
    1250                1255                1260

Gly Asp  Val Ile Thr Ser Gly  Asp Ile Thr Cys Val  Val Ile Pro
    1265                1270                1275

Ser Lys  Lys Ala Gly Gly Thr  Thr Glu Met Leu Ser  Arg Ala Leu
    1280                1285                1290

Lys Lys  Val Pro Val Asp Glu  Tyr Ile Thr Thr Tyr  Pro Gly Gln
    1295                1300                1305

Gly Cys  Ala Gly Tyr Thr Leu  Glu Glu Ala Lys Thr  Ala Leu Lys
    1310                1315                1320

Lys Cys  Lys Ser Ala Phe Tyr  Val Leu Pro Ser Glu  Ala Pro Asn
    1325                1330                1335

Ala Lys  Glu Glu Ile Leu Gly  Thr Val Ser Trp Asn  Leu Arg Glu
    1340                1345                1350

Met Leu  Ala His Ala Glu Glu  Thr Arg Lys Leu Met  Pro Ile Cys
    1355                1360                1365

Met Asp  Val Arg Ala Ile Met  Ala Thr Ile Gln Arg  Lys Tyr Lys
    1370                1375                1380

Gly Ile  Lys Ile Gln Glu Gly  Ile Val Asp Tyr Gly  Val Arg Phe
    1385                1390                1395

Phe Phe  Tyr Thr Ser Lys Glu  Pro Val Ala Ser Ile  Ile Thr Lys
    1400                1405                1410

Leu Asn  Ser Leu Asn Glu Pro  Leu Val Thr Met Pro  Ile Gly Tyr
    1415                1420                1425

Val Thr  His Gly Phe Asn Leu  Glu Glu Ala Ala Arg  Cys Met Arg
    1430                1435                1440

Ser Leu  Lys Ala Pro Ala Val  Val Ser Val Ser Ser  Pro Asp Ala
    1445                1450                1455

Val Thr  Thr Tyr Asn Gly Tyr  Leu Thr Ser Ser Ser  Lys Thr Ser
    1460                1465                1470

Glu Glu  His Phe Val Glu Thr  Val Ser Leu Ala Gly  Ser Tyr Arg
    1475                1480                1485

Asp Trp  Ser Tyr Ser Gly Gln  Arg Thr Glu Leu Gly  Val Glu Phe
    1490                1495                1500

Leu Lys  Arg Gly Asp Lys Ile  Val Tyr His Thr Leu  Glu Ser Pro
    1505                1510                1515

Val Glu  Phe His Leu Asp Gly  Glu Val Leu Ser Leu  Asp Lys Leu
    1520                1525                1530

Lys Ser  Leu Leu Ser Leu Arg  Glu Val Lys Thr Ile  Lys Val Phe
    1535                1540                1545

Thr Thr  Val Asp Asn Thr Asn  Leu His Thr Gln Leu  Val Asp Met
    1550                1555                1560

Ser Met  Thr Tyr Gly Gln Gln  Phe Gly Pro Thr Tyr  Leu Asp Gly
    1565                1570                1575

Ala Asp  Val Thr Lys Ile Lys  Pro His Val Asn His  Glu Gly Lys
    1580                1585                1590

Thr Phe  Phe Val Leu Pro Ser  Asp Asp Thr Leu Arg  Ser Glu Ala
    1595                1600                1605

Phe Glu  Tyr Tyr His Thr Leu  Asp Glu Ser Phe Leu  Gly Arg Tyr
    1610                1615                1620
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Met Ser  Ala Leu Asn His Thr  Lys Lys Trp Lys Phe  Pro Gln Val
    1625                1630                1635

Gly Gly  Leu Thr Ser Ile Lys  Trp Ala Asp Asn Asn  Cys Tyr Leu
    1640                1645                1650

Ser Ser  Val Leu Leu Ala Leu  Gln Gln Leu Glu Val  Lys Phe Asn
    1655                1660                1665

Ala Pro  Ala Leu Gln Glu Ala  Tyr Tyr Arg Ala Arg  Ala Gly Asp
    1670                1675                1680

Ala Ala  Asn Phe Cys Ala Leu  Ile Leu Ala Tyr Ser  Asn Lys Thr
    1685                1690                1695

Val Gly  Glu Leu Gly Asp Val  Arg Glu Thr Met Thr  His Leu Leu
    1700                1705                1710

Gln His  Ala Asn Leu Glu Ser  Ala Lys Arg Val Leu  Asn Val Val
    1715                1720                1725

Cys Lys  His Cys Gly Gln Lys  Thr Thr Thr Leu Thr  Gly Val Glu
    1730                1735                1740

Ala Val  Met Tyr Met Gly Thr  Leu Ser Tyr Asp Asn  Leu Lys Thr
    1745                1750                1755

Gly Val  Ser Ile Pro Cys Val  Cys Gly Arg Asp Ala  Thr Gln Tyr
    1760                1765                1770

Leu Val  Gln Gln Glu Ser Ser  Phe Val Met Met Ser  Ala Pro Pro
    1775                1780                1785

Ala Glu  Tyr Lys Leu Gln Gln  Gly Thr Phe Leu Cys  Ala Asn Glu
    1790                1795                1800

Tyr Thr  Gly Asn Tyr Gln Cys  Gly His Tyr Thr His  Ile Thr Ala
    1805                1810                1815

Lys Glu  Thr Leu Tyr Arg Ile  Asp Gly Ala His Leu  Thr Lys Met
    1820                1825                1830

Ser Glu  Tyr Lys Gly Pro Val  Thr Asp Val Phe Tyr  Lys Glu Thr
    1835                1840                1845

Ser Tyr  Thr Thr Thr Ile Lys  Pro Val Ser Tyr Lys  Leu Asp Gly
    1850                1855                1860

Val Thr  Tyr Thr Glu Ile Glu  Pro Lys Leu Asp Gly  Tyr Tyr Lys
    1865                1870                1875

Lys Asp  Asn Ala Tyr Tyr Thr  Glu Gln Pro Ile Asp  Leu Val Pro
    1880                1885                1890

Thr Gln  Pro Leu Pro Asn Ala  Ser Phe Asp Asn Phe  Lys Leu Thr
    1895                1900                1905

Cys Ser  Asn Thr Lys Phe Ala  Asp Asp Leu Asn Gln  Met Thr Gly
    1910                1915                1920

Phe Thr  Lys Pro Ala Ser Arg  Glu Leu Ser Val Thr  Phe Phe Pro
    1925                1930                1935

Asp Leu  Asn Gly Asp Val Val  Ala Ile Asp Tyr Arg  His Tyr Ser
    1940                1945                1950

Ala Ser  Phe Lys Lys Gly Ala  Lys Leu Leu His Lys  Pro Ile Val
    1955                1960                1965

Trp His  Ile Asn Gln Ala Thr  Thr Lys Thr Thr Phe  Lys Pro Asn
    1970                1975                1980

Thr Trp  Cys Leu Arg Cys Leu  Trp Ser Thr Lys Pro  Val Asp Thr
    1985                1990                1995

Ser Asn  Ser Phe Glu Val Leu  Ala Val Glu Asp Thr  Gln Gly Met
    2000                2005                2010

Asp Asn  Leu Ala Cys Glu Ser  Gln Gln Pro Thr Ser  Glu Glu Val
    2015                2020                2025
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Val Glu  Asn Pro Thr Ile Gln  Lys Glu Val Ile Glu  Cys Asp Val
    2030                2035                2040

Lys Thr  Thr Glu Val Val Gly  Asn Val Ile Leu Lys  Pro Ser Asp
    2045                2050                2055

Glu Gly  Val Lys Val Thr Gln  Glu Leu Gly His Glu  Asp Leu Met
    2060                2065                2070

Ala Ala  Tyr Val Glu Asn Thr  Ser Ile Thr Ile Lys  Lys Pro Asn
    2075                2080                2085

Glu Leu  Ser Leu Ala Leu Gly  Leu Lys Thr Ile Ala  Thr His Gly
    2090                2095                2100

Ile Ala  Ala Ile Asn Ser Val  Pro Trp Ser Lys Ile  Leu Ala Tyr
    2105                2110                2115

Val Lys  Pro Phe Leu Gly Gln  Ala Ala Ile Thr Thr  Ser Asn Cys
    2120                2125                2130

Ala Lys  Arg Leu Ala Gln Arg  Val Phe Asn Asn Tyr  Met Pro Tyr
    2135                2140                2145

Val Phe  Thr Leu Leu Phe Gln  Leu Cys Thr Phe Thr  Lys Ser Thr
    2150                2155                2160

Asn Ser  Arg Ile Arg Ala Ser  Leu Pro Thr Thr Ile  Ala Lys Asn
    2165                2170                2175

Ser Val  Lys Ser Val Ala Lys  Leu Cys Leu Asp Ala  Gly Ile Asn
    2180                2185                2190

Tyr Val  Lys Ser Pro Lys Phe  Ser Lys Leu Phe Thr  Ile Ala Met
    2195                2200                2205

Trp Leu  Leu Leu Leu Ser Ile  Cys Leu Gly Ser Leu  Ile Cys Val
    2210                2215                2220

Thr Ala  Ala Phe Gly Val Leu  Leu Ser Asn Phe Gly  Ala Pro Ser
    2225                2230                2235

Tyr Cys  Asn Gly Val Arg Glu  Leu Tyr Leu Asn Ser  Ser Asn Val
    2240                2245                2250

Thr Thr  Met Asp Phe Cys Glu  Gly Ser Phe Pro Cys  Ser Ile Cys
    2255                2260                2265

Leu Ser  Gly Leu Asp Ser Leu  Asp Ser Tyr Pro Ala  Leu Glu Thr
    2270                2275                2280

Ile Gln  Val Thr Ile Ser Ser  Tyr Lys Leu Asp Leu  Thr Ile Leu
    2285                2290                2295

Gly Leu  Ala Ala Glu Trp Val  Leu Ala Tyr Met Leu  Phe Thr Lys
    2300                2305                2310

Phe Phe  Tyr Leu Leu Gly Leu  Ser Ala Ile Met Gln  Val Phe Phe
    2315                2320                2325

Gly Tyr  Phe Ala Ser His Phe  Ile Ser Asn Ser Trp  Leu Met Trp
    2330                2335                2340

Phe Ile  Ile Ser Ile Val Gln  Met Ala Pro Val Ser  Ala Met Val
    2345                2350                2355

Arg Met  Tyr Ile Phe Phe Ala  Ser Phe Tyr Tyr Ile  Trp Lys Ser
    2360                2365                2370

Tyr Val  His Ile Met Asp Gly  Cys Thr Ser Ser Thr  Cys Met Met
    2375                2380                2385

Cys Tyr  Lys Arg Asn Arg Ala  Thr Arg Val Glu Cys  Thr Thr Ile
    2390                2395                2400

Val Asn  Gly Met Lys Arg Ser  Phe Tyr Val Tyr Ala  Asn Gly Gly
    2405                2410                2415

Arg Gly  Phe Cys Lys Thr His  Asn Trp Asn Cys Leu  Asn Cys Asp
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    2420                2425                2430

Thr Phe  Cys Thr Gly Ser Thr  Phe Ile Ser Asp Glu  Val Ala Arg
    2435                2440                2445

Asp Leu  Ser Leu Gln Phe Lys  Arg Pro Ile Asn Pro  Thr Asp Gln
    2450                2455                2460

Ser Ser  Tyr Ile Val Asp Ser  Val Ala Val Lys Asn  Gly Ala Leu
    2465                2470                2475

His Leu  Tyr Phe Asp Lys Ala  Gly Gln Lys Thr Tyr  Glu Arg His
    2480                2485                2490

Pro Leu  Ser His Phe Val Asn  Leu Asp Asn Leu Arg  Ala Asn Asn
    2495                2500                2505

Thr Lys  Gly Ser Leu Pro Ile  Asn Val Ile Val Phe  Asp Gly Lys
    2510                2515                2520

Ser Lys  Cys Asp Glu Ser Ala  Ser Lys Ser Ala Ser  Val Tyr Tyr
    2525                2530                2535

Ser Gln  Leu Met Cys Gln Pro  Ile Leu Leu Leu Asp  Gln Ala Leu
    2540                2545                2550

Val Ser  Asp Val Gly Asp Ser  Thr Glu Val Ser Val  Lys Met Phe
    2555                2560                2565

Asp Ala  Tyr Val Asp Thr Phe  Ser Ala Thr Phe Ser  Val Pro Met
    2570                2575                2580

Glu Lys  Leu Lys Ala Leu Val  Ala Thr Ala His Ser  Glu Leu Ala
    2585                2590                2595

Lys Gly  Val Ala Leu Asp Gly  Val Leu Ser Thr Phe  Val Ser Ala
    2600                2605                2610

Ala Arg  Gln Gly Val Val Asp  Thr Asp Val Asp Thr  Lys Asp Val
    2615                2620                2625

Ile Glu  Cys Leu Lys Leu Ser  His His Ser Asp Leu  Glu Val Thr
    2630                2635                2640

Gly Asp  Ser Cys Asn Asn Phe  Met Leu Thr Tyr Asn  Lys Val Glu
    2645                2650                2655

Asn Met  Thr Pro Arg Asp Leu  Gly Ala Cys Ile Asp  Cys Asn Ala
    2660                2665                2670

Arg His  Ile Asn Ala Gln Val  Ala Lys Ser His Asn  Val Ser Leu
    2675                2680                2685

Ile Trp  Asn Val Lys Asp Tyr  Met Ser Leu Ser Glu  Gln Leu Arg
    2690                2695                2700

Lys Gln  Ile Arg Ser Ala Ala  Lys Lys Asn Asn Ile  Pro Phe Arg
    2705                2710                2715

Leu Thr  Cys Ala Thr Thr Arg  Gln Val Val Asn Val  Ile Thr Thr
    2720                2725                2730

Lys Ile  Ser Leu Lys Gly Gly  Lys Ile Val Ser Thr  Cys Phe Lys
    2735                2740                2745

Leu Met  Leu Lys Ala Thr Leu  Leu Cys Val Leu Ala  Ala Leu Val
    2750                2755                2760

Cys Tyr  Ile Val Met Pro Val  His Thr Leu Ser Ile  His Asp Gly
    2765                2770                2775

Tyr Thr  Asn Glu Ile Ile Gly  Tyr Lys Ala Ile Gln  Asp Gly Val
    2780                2785                2790

Thr Arg  Asp Ile Ile Ser Thr  Asp Asp Cys Phe Ala  Asn Lys His
    2795                2800                2805

Ala Gly  Phe Asp Ala Trp Phe  Ser Gln Arg Gly Gly  Ser Tyr Lys
    2810                2815                2820
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Asn Asp  Lys Ser Cys Pro Val  Val Ala Ala Ile Ile  Thr Arg Glu
    2825                 2830                 2835

Ile Gly  Phe Ile Val Pro Gly  Leu Pro Gly Thr Val  Leu Arg Ala
    2840                 2845                 2850

Ile Asn  Gly Asp Phe Leu His  Phe Leu Pro Arg Val  Phe Ser Ala
    2855                 2860                 2865

Val Gly  Asn Ile Cys Tyr Thr  Pro Ser Lys Leu Ile  Glu Tyr Ser
    2870                 2875                 2880

Asp Phe  Ala Thr Ser Ala Cys  Val Leu Ala Ala Glu  Cys Thr Ile
    2885                 2890                 2895

Phe Lys  Asp Ala Met Gly Lys  Pro Val Pro Tyr Cys  Tyr Asp Thr
    2900                 2905                 2910

Asn Leu  Leu Glu Gly Ser Ile  Ser Tyr Ser Glu Leu  Arg Pro Asp
    2915                 2920                 2925

Thr Arg  Tyr Val Leu Met Asp  Gly Ser Ile Ile Gln  Phe Pro Asn
    2930                 2935                 2940

Thr Tyr  Leu Glu Gly Ser Val  Arg Val Val Thr Thr  Phe Asp Ala
    2945                 2950                 2955

Glu Tyr  Cys Arg His Gly Thr  Cys Glu Arg Ser Glu  Val Gly Ile
    2960                 2965                 2970

Cys Leu  Ser Thr Ser Gly Arg  Trp Val Leu Asn Asn  Glu His Tyr
    2975                 2980                 2985

Arg Ala  Leu Ser Gly Val Phe  Cys Gly Val Asp Ala  Met Asn Leu
    2990                 2995                 3000

Ile Ala  Asn Ile Phe Thr Pro  Leu Val Gln Pro Val  Gly Ala Leu
    3005                 3010                 3015

Asp Val  Ser Ala Ser Val Val  Ala Gly Gly Ile Ile  Ala Ile Leu
    3020                 3025                 3030

Val Thr  Cys Ala Ala Tyr Tyr  Phe Met Lys Phe Arg  Arg Val Phe
    3035                 3040                 3045

Gly Glu  Tyr Asn His Val Val  Ala Ala Asn Ala Leu  Leu Phe Leu
    3050                 3055                 3060

Met Ser  Phe Thr Ile Leu Cys  Leu Val Pro Ala Tyr  Ser Phe Leu
    3065                 3070                 3075

Pro Gly  Val Tyr Ser Val Phe  Tyr Leu Tyr Leu Thr  Phe Tyr Phe
    3080                 3085                 3090

Thr Asn  Asp Val Ser Phe Leu  Ala His Leu Gln Trp  Phe Ala Met
    3095                 3100                 3105

Phe Ser  Pro Ile Val Pro Phe  Trp Ile Thr Ala Ile  Tyr Val Phe
    3110                 3115                 3120

Cys Ile  Ser Leu Lys His Cys  His Trp Phe Phe Asn  Asn Tyr Leu
    3125                 3130                 3135

Arg Lys  Arg Val Met Phe Asn  Gly Val Thr Phe Ser  Thr Phe Glu
    3140                 3145                 3150

Glu Ala  Ala Leu Cys Thr Phe  Leu Leu Asn Lys Glu  Met Tyr Leu
    3155                 3160                 3165

Lys Leu  Arg Ser Glu Thr Leu  Leu Pro Leu Thr Gln  Tyr Asn Arg
    3170                 3175                 3180

Tyr Leu  Ala Leu Tyr Asn Lys  Tyr Lys Tyr Phe Ser  Gly Ala Leu
    3185                 3190                 3195

Asp Thr  Thr Ser Tyr Arg Glu  Ala Ala Cys Cys His  Leu Ala Lys
    3200                 3205                 3210

Ala Leu  Asn Asp Phe Ser Asn  Ser Gly Ala Asp Val  Leu Tyr Gln
    3215                 3220                 3225
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Pro Pro  Gln Thr Ser Ile Thr  Ser Ala Val Leu Gln  Ser Gly Phe
    3230                3235                3240

Arg Lys  Met Ala Phe Pro Ser  Gly Lys Val Glu Gly  Cys Met Val
    3245                3250                3255

Gln Val  Thr Cys Gly Thr Thr  Thr Leu Asn Gly Leu  Trp Leu Asp
    3260                3265                3270

Asp Thr  Val Tyr Cys Pro Arg  His Val Ile Cys Thr  Ala Glu Asp
    3275                3280                3285

Met Leu  Asn Pro Asn Tyr Glu  Asp Leu Leu Ile Arg  Lys Ser Asn
    3290                3295                3300

His Ser  Phe Leu Val Gln Ala  Gly Asn Val Gln Leu  Arg Val Ile
    3305                3310                3315

Gly His  Ser Met Gln Asn Cys  Leu Leu Arg Leu Lys  Val Asp Thr
    3320                3325                3330

Ser Asn  Pro Lys Thr Pro Lys  Tyr Lys Phe Val Arg  Ile Gln Pro
    3335                3340                3345

Gly Gln  Thr Phe Ser Val Leu  Ala Cys Tyr Asn Gly  Ser Pro Ser
    3350                3355                3360

Gly Val  Tyr Gln Cys Ala Met  Arg Pro Asn His Thr  Ile Lys Gly
    3365                3370                3375

Ser Phe  Leu Asn Gly Ser Cys  Gly Ser Val Gly Phe  Asn Ile Asp
    3380                3385                3390

Tyr Asp  Cys Val Ser Phe Cys  Tyr Met His His Met  Glu Leu Pro
    3395                3400                3405

Thr Gly  Val His Ala Gly Thr  Asp Leu Glu Gly Lys  Phe Tyr Gly
    3410                3415                3420

Pro Phe  Val Asp Arg Gln Thr  Ala Gln Ala Ala Gly  Thr Asp Thr
    3425                3430                3435

Thr Ile  Thr Leu Asn Val Leu  Ala Trp Leu Tyr Ala  Ala Val Ile
    3440                3445                3450

Asn Gly  Asp Arg Trp Phe Leu  Asn Arg Phe Thr Thr  Thr Leu Asn
    3455                3460                3465

Asp Phe  Asn Leu Val Ala Met  Lys Tyr Asn Tyr Glu  Pro Leu Thr
    3470                3475                3480

Gln Asp  His Val Asp Ile Leu  Gly Pro Leu Ser Ala  Gln Thr Gly
    3485                3490                3495

Ile Ala  Val Leu Asp Met Cys  Ala Ala Leu Lys Glu  Leu Leu Gln
    3500                3505                3510

Asn Gly  Met Asn Gly Arg Thr  Ile Leu Gly Ser Thr  Ile Leu Glu
    3515                3520                3525

Asp Glu  Phe Thr Pro Phe Asp  Val Val Arg Gln Cys  Ser Gly Val
    3530                3535                3540

Thr Phe  Gln Gly Lys Phe Lys  Lys Ile Val Lys Gly  Thr His His
    3545                3550                3555

Trp Met  Leu Leu Thr Phe Leu  Thr Ser Leu Leu Ile  Leu Val Gln
    3560                3565                3570

Ser Thr  Gln Trp Ser Leu Phe  Phe Phe Val Tyr Glu  Asn Ala Phe
    3575                3580                3585

Leu Pro  Phe Thr Leu Gly Ile  Met Ala Ile Ala Ala  Cys Ala Met
    3590                3595                3600

Leu Leu  Val Lys His Lys His  Ala Phe Leu Cys Leu  Phe Leu Leu
    3605                3610                3615

Pro Ser  Leu Ala Thr Val Ala  Tyr Phe Asn Met Val  Tyr Met Pro
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    3620                3625                3630

Ala Ser  Trp Val Met Arg Ile  Met Thr Trp Leu Glu  Leu Ala Asp
    3635                3640                3645

Thr Ser  Leu Ser Gly Tyr Arg  Leu Lys Asp Cys Val  Met Tyr Ala
    3650                3655                3660

Ser Ala  Leu Val Leu Leu Ile  Leu Met Thr Ala Arg  Thr Val Tyr
    3665                3670                3675

Asp Asp  Ala Ala Arg Arg Val  Trp Thr Leu Met Asn  Val Ile Thr
    3680                3685                3690

Leu Val  Tyr Lys Val Tyr Tyr  Gly Asn Ala Leu Asp  Gln Ala Ile
    3695                3700                3705

Ser Met  Trp Ala Leu Val Ile  Ser Val Thr Ser Asn  Tyr Ser Gly
    3710                3715                3720

Val Val  Thr Thr Ile Met Phe  Leu Ala Arg Ala Ile  Val Phe Val
    3725                3730                3735

Cys Val  Glu Tyr Tyr Pro Leu  Leu Phe Ile Thr Gly  Asn Thr Leu
    3740                3745                3750

Gln Cys  Ile Met Leu Val Tyr  Cys Phe Leu Gly Tyr  Cys Cys Cys
    3755                3760                3765

Cys Tyr  Phe Gly Leu Phe Cys  Leu Leu Asn Arg Tyr  Phe Arg Leu
    3770                3775                3780

Thr Leu  Gly Val Tyr Asp Tyr  Leu Val Ser Thr Gln  Glu Phe Arg
    3785                3790                3795

Tyr Met  Asn Ser Gln Gly Leu  Leu Pro Pro Lys Ser  Ser Ile Asp
    3800                3805                3810

Ala Phe  Lys Leu Asn Ile Lys  Leu Leu Gly Ile Gly  Gly Lys Pro
    3815                3820                3825

Cys Ile  Lys Val Ala Thr Val  Gln Ser Lys Met Ser  Asp Val Lys
    3830                3835                3840

Cys Thr  Ser Val Val Leu Leu  Ser Val Leu Gln Gln  Leu Arg Val
    3845                3850                3855

Glu Ser  Ser Ser Lys Leu Trp  Ala Gln Cys Val Gln  Leu His Asn
    3860                3865                3870

Asp Ile  Leu Leu Ala Lys Asp  Thr Thr Glu Ala Phe  Glu Lys Met
    3875                3880                3885

Val Ser  Leu Leu Ser Val Leu  Leu Ser Met Gln Gly  Ala Val Asp
    3890                3895                3900

Ile Asn  Arg Leu Cys Glu Glu  Met Leu Asp Asn Arg  Ala Thr Leu
    3905                3910                3915

Gln Ala  Ile Ala Ser Glu Phe  Ser Ser Leu Pro Ser  Tyr Ala Ala
    3920                3925                3930

Tyr Ala  Thr Ala Gln Glu Ala  Tyr Glu Gln Ala Val  Ala Asn Gly
    3935                3940                3945

Asp Ser  Glu Val Val Leu Lys  Lys Leu Lys Lys Ser  Leu Asn Val
    3950                3955                3960

Ala Lys  Ser Glu Phe Asp Arg  Asp Ala Ala Met Gln  Arg Lys Leu
    3965                3970                3975

Glu Lys  Met Ala Asp Gln Ala  Met Thr Gln Met Tyr  Lys Gln Ala
    3980                3985                3990

Arg Ser  Glu Asp Lys Arg Ala  Lys Val Thr Ser Ala  Met Gln Thr
    3995                4000                4005

Met Leu  Phe Thr Met Leu Arg  Lys Leu Asp Asn Asp  Ala Leu Asn
    4010                4015                4020
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Asn Ile  Ile Asn Asn Ala Arg  Asp Gly Cys Val Pro  Leu Asn Ile
    4025                 4030                 4035

Ile Pro  Leu Thr Thr Ala Ala  Lys Leu Met Val Val  Val Pro Asp
    4040                 4045                 4050

Tyr Gly  Thr Tyr Lys Asn Thr  Cys Asp Gly Asn Thr  Phe Thr Tyr
    4055                 4060                 4065

Ala Ser  Ala Leu Trp Glu Ile  Gln Gln Val Val Asp  Ala Asp Ser
    4070                 4075                 4080

Lys Ile  Val Gln Leu Ser Glu  Ile Asn Met Asp Asn  Ser Pro Asn
    4085                 4090                 4095

Leu Ala  Trp Pro Leu Ile Val  Thr Ala Leu Arg Ala  Asn Ser Ala
    4100                 4105                 4110

Val Lys  Leu Gln Asn Asn Glu  Leu Ser Pro Val Ala  Leu Arg Gln
    4115                 4120                 4125

Met Ser  Cys Ala Ala Gly Thr  Thr Gln Thr Ala Cys  Thr Asp Asp
    4130                 4135                 4140

Asn Ala  Leu Ala Tyr Tyr Asn  Asn Ser Lys Gly Gly  Arg Phe Val
    4145                 4150                 4155

Leu Ala  Leu Leu Ser Asp His  Gln Asp Leu Lys Trp  Ala Arg Phe
    4160                 4165                 4170

Pro Lys  Ser Asp Gly Thr Gly  Thr Ile Tyr Thr Glu  Leu Glu Pro
    4175                 4180                 4185

Pro Cys  Arg Phe Val Thr Asp  Thr Pro Lys Gly Pro  Lys Val Lys
    4190                 4195                 4200

Tyr Leu  Tyr Phe Ile Lys Gly  Leu Asn Asn Leu Asn  Arg Gly Met
    4205                 4210                 4215

Val Leu  Gly Ser Leu Ala Ala  Thr Val Arg Leu Gln  Ala Gly Asn
    4220                 4225                 4230

Ala Thr  Glu Val Pro Ala Asn  Ser Thr Val Leu Ser  Phe Cys Ala
    4235                 4240                 4245

Phe Ala  Val Asp Pro Ala Lys  Ala Tyr Lys Asp Tyr  Leu Ala Ser
    4250                 4255                 4260

Gly Gly  Gln Pro Ile Thr Asn  Cys Val Lys Met Leu  Cys Thr His
    4265                 4270                 4275

Thr Gly  Thr Gly Gln Ala Ile  Thr Val Thr Pro Glu  Ala Asn Met
    4280                 4285                 4290

Asp Gln  Glu Ser Phe Gly Gly  Ala Ser Cys Cys Leu  Tyr Cys Arg
    4295                 4300                 4305

Cys His  Ile Asp His Pro Asn  Pro Lys Gly Phe Cys  Asp Leu Lys
    4310                 4315                 4320

Gly Lys  Tyr Val Gln Ile Pro  Thr Thr Cys Ala Asn  Asp Pro Val
    4325                 4330                 4335

Gly Phe  Thr Leu Arg Asn Thr  Val Cys Thr Val Cys  Gly Met Trp
    4340                 4345                 4350

Lys Gly  Tyr Gly Cys Ser Cys  Asp Gln Leu Arg Glu  Pro Leu Met
    4355                 4360                 4365

Gln Ser  Ala Asp Ala Ser Thr  Phe Leu Asn Gly Phe  Ala Val
    4370                 4375                 4380


<210> SEQ ID NO 75
<211> LENGTH: 2695
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 75
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Arg Val Cys Gly Val Ser Ala Ala Arg Leu Thr Pro Cys Gly Thr Gly
1               5                  10                  15

Thr Ser Thr Asp Val Val Tyr Arg Ala Phe Asp Ile Tyr Asn Glu Lys
            20                  25                  30

Val Ala Gly Phe Ala Lys Phe Leu Lys Thr Asn Cys Cys Arg Phe Gln
        35                  40                  45

Glu Lys Asp Glu Glu Gly Asn Leu Leu Asp Ser Tyr Phe Val Val Lys
    50                  55                  60

Arg His Thr Met Ser Asn Tyr Gln His Glu Glu Thr Ile Tyr Asn Leu
65                  70                  75                  80

Val Lys Asp Cys Pro Ala Val Ala Val His Asp Phe Phe Lys Phe Arg
                85                  90                  95

Val Asp Gly Asp Met Val Pro His Ile Ser Arg Gln Arg Leu Thr Lys
            100                 105                 110

Tyr Thr Met Ala Asp Leu Val Tyr Ala Leu Arg His Phe Asp Glu Gly
        115                 120                 125

Asn Cys Asp Thr Leu Lys Glu Ile Leu Val Thr Tyr Asn Cys Cys Asp
    130                 135                 140

Asp Asp Tyr Phe Asn Lys Lys Asp Trp Tyr Asp Phe Val Glu Asn Pro
145                 150                 155                 160

Asp Ile Leu Arg Val Tyr Ala Asn Leu Gly Glu Arg Val Arg Gln Ser
                165                 170                 175

Leu Leu Lys Thr Val Gln Phe Cys Asp Ala Met Arg Asp Ala Gly Ile
            180                 185                 190

Val Gly Val Leu Thr Leu Asp Asn Gln Asp Leu Asn Gly Asn Trp Tyr
        195                 200                 205

Asp Phe Gly Asp Phe Val Gln Val Ala Pro Gly Cys Gly Val Pro Ile
    210                 215                 220

Val Asp Ser Tyr Tyr Ser Leu Leu Met Pro Ile Leu Thr Leu Thr Arg
225                 230                 235                 240

Ala Leu Ala Ala Glu Ser His Met Asp Ala Asp Leu Ala Lys Pro Leu
                245                 250                 255

Ile Lys Trp Asp Leu Leu Lys Tyr Asp Phe Thr Glu Glu Arg Leu Cys
            260                 265                 270

Leu Phe Asp Arg Tyr Phe Lys Tyr Trp Asp Gln Thr Tyr His Pro Asn
        275                 280                 285

Cys Ile Asn Cys Leu Asp Asp Arg Cys Ile Leu His Cys Ala Asn Phe
    290                 295                 300

Asn Val Leu Phe Ser Thr Val Phe Pro Pro Thr Ser Phe Gly Pro Leu
305                 310                 315                 320

Val Arg Lys Ile Phe Val Asp Gly Val Pro Phe Val Val Ser Thr Gly
                325                 330                 335

Tyr His Phe Arg Glu Leu Gly Val Val His Asn Gln Asp Val Asn Leu
            340                 345                 350

His Ser Ser Arg Leu Ser Phe Lys Glu Leu Leu Val Tyr Ala Ala Asp
        355                 360                 365

Pro Ala Met His Ala Ala Ser Gly Asn Leu Leu Leu Asp Lys Arg Thr
    370                 375                 380

Thr Cys Phe Ser Val Ala Ala Leu Thr Asn Asn Val Ala Phe Gln Thr
385                 390                 395                 400

Val Lys Pro Gly Asn Phe Asn Lys Asp Phe Tyr Asp Phe Ala Val Ser
                405                 410                 415

Lys Gly Phe Phe Lys Glu Gly Ser Ser Val Glu Leu Lys His Phe Phe
            420                 425                 430
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Phe Ala Gln Asp Gly Asn Ala Ala Ile Ser Asp Tyr Asp Tyr Tyr Arg
        435                 440                 445

Tyr Asn Leu Pro Thr Met Cys Asp Ile Arg Gln Leu Leu Phe Val Val
    450                 455                 460

Glu Val Val Asp Lys Tyr Phe Asp Cys Tyr Asp Gly Gly Cys Ile Asn
465                 470                 475                 480

Ala Asn Gln Val Ile Val Asn Asn Leu Asp Lys Ser Ala Gly Phe Pro
                485                 490                 495

Phe Asn Lys Trp Gly Lys Ala Arg Leu Tyr Tyr Asp Ser Met Ser Tyr
            500                 505                 510

Glu Asp Gln Asp Ala Leu Phe Ala Tyr Thr Lys Arg Asn Val Ile Pro
        515                 520                 525

Thr Ile Thr Gln Met Asn Leu Lys Tyr Ala Ile Ser Ala Lys Asn Arg
    530                 535                 540

Ala Arg Thr Val Ala Gly Val Ser Ile Cys Ser Thr Met Thr Asn Arg
545                 550                 555                 560

Gln Phe His Gln Lys Leu Leu Lys Ser Ile Ala Ala Thr Arg Gly Ala
                565                 570                 575

Thr Val Val Ile Gly Thr Ser Lys Phe Tyr Gly Gly Trp His Asn Met
            580                 585                 590

Leu Lys Thr Val Tyr Ser Asp Val Glu Thr Pro His Leu Met Gly Trp
        595                 600                 605

Asp Tyr Pro Lys Cys Asp Arg Ala Met Pro Asn Met Leu Arg Ile Met
    610                 615                 620

Ala Ser Leu Val Leu Ala Arg Lys His Asn Thr Cys Cys Asn Leu Ser
625                 630                 635                 640

His Arg Phe Tyr Arg Leu Ala Asn Glu Cys Ala Gln Val Leu Ser Glu
                645                 650                 655

Met Val Met Cys Gly Gly Ser Leu Tyr Val Lys Pro Gly Gly Thr Ser
            660                 665                 670

Ser Gly Asp Ala Thr Thr Ala Tyr Ala Asn Ser Val Phe Asn Ile Cys
        675                 680                 685

Gln Ala Val Thr Ala Asn Val Asn Ala Leu Leu Ser Thr Asp Gly Asn
    690                 695                 700

Lys Ile Ala Asp Lys Tyr Val Arg Asn Leu Gln His Arg Leu Tyr Glu
705                 710                 715                 720

Cys Leu Tyr Arg Asn Arg Asp Val Asp His Glu Phe Val Asp Glu Phe
                725                 730                 735

Tyr Ala Tyr Leu Arg Lys His Phe Ser Met Met Ile Leu Ser Asp Asp
            740                 745                 750

Ala Val Val Cys Tyr Asn Ser Asn Tyr Ala Ala Gln Gly Leu Val Ala
        755                 760                 765

Ser Ile Lys Asn Phe Lys Ala Val Leu Tyr Tyr Gln Asn Asn Val Phe
    770                 775                 780

Met Ser Glu Ala Lys Cys Trp Thr Glu Thr Asp Leu Thr Lys Gly Pro
785                 790                 795                 800

His Glu Phe Cys Ser Gln His Thr Met Leu Val Lys Gln Gly Asp Asp
                805                 810                 815

Tyr Val Tyr Leu Pro Tyr Pro Asp Pro Ser Arg Ile Leu Gly Ala Gly
            820                 825                 830

Cys Phe Val Asp Asp Ile Val Lys Thr Asp Gly Thr Leu Met Ile Glu
        835                 840                 845

Arg Phe Val Ser Leu Ala Ile Asp Ala Tyr Pro Leu Thr Lys His Pro
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    850                 855                 860

Asn Gln Glu Tyr Ala Asp Val Phe His Leu Tyr Leu Gln Tyr Ile Arg
865                 870                 875                 880

Lys Leu His Asp Glu Leu Thr Gly His Met Leu Asp Met Tyr Ser Val
                885                 890                 895

Met Leu Thr Asn Asp Asn Thr Ser Arg Tyr Trp Glu Pro Glu Phe Tyr
            900                 905                 910

Glu Ala Met Tyr Thr Pro His Thr Val Leu Gln Ala Val Gly Ala Cys
        915                 920                 925

Val Leu Cys Asn Ser Gln Thr Ser Leu Arg Cys Gly Ala Cys Ile Arg
    930                 935                 940

Arg Pro Phe Leu Cys Cys Lys Cys Cys Tyr Asp His Val Ile Ser Thr
945                 950                 955                 960

Ser His Lys Leu Val Leu Ser Val Asn Pro Tyr Val Cys Asn Ala Pro
                965                 970                 975

Gly Cys Asp Val Thr Asp Val Thr Gln Leu Tyr Leu Gly Gly Met Ser
            980                 985                 990

Tyr Tyr Cys Lys Ser His Lys Pro Pro Ile Ser Phe Pro Leu Cys Ala
        995                1000                1005

Asn Gly  Gln Val Phe Gly Leu  Tyr Lys Asn Thr Cys  Val Gly Ser
    1010                1015                1020

Asp Asn  Val Thr Asp Phe Asn  Ala Ile Ala Thr Cys  Asp Trp Thr
    1025                1030                1035

Asn Ala  Gly Asp Tyr Ile Leu  Ala Asn Thr Cys Thr  Glu Arg Leu
    1040                1045                1050

Lys Leu  Phe Ala Ala Glu Thr  Leu Lys Ala Thr Glu  Glu Thr Phe
    1055                1060                1065

Lys Leu  Ser Tyr Gly Ile Ala  Thr Val Arg Glu Val  Leu Ser Asp
    1070                1075                1080

Arg Glu  Leu His Leu Ser Trp  Glu Val Gly Lys Pro  Arg Pro Pro
    1085                1090                1095

Leu Asn  Arg Asn Tyr Val Phe  Thr Gly Tyr Arg Val  Thr Lys Asn
    1100                1105                1110

Ser Lys  Val Gln Ile Gly Glu  Tyr Thr Phe Glu Lys  Gly Asp Tyr
    1115                1120                1125

Gly Asp  Ala Val Val Tyr Arg  Gly Thr Thr Thr Tyr  Lys Leu Asn
    1130                1135                1140

Val Gly  Asp Tyr Phe Val Leu  Thr Ser His Thr Val  Met Pro Leu
    1145                1150                1155

Ser Ala  Pro Thr Leu Val Pro  Gln Glu His Tyr Val  Arg Ile Thr
    1160                1165                1170

Gly Leu  Tyr Pro Thr Leu Asn  Ile Ser Asp Glu Phe  Ser Ser Asn
    1175                1180                1185

Val Ala  Asn Tyr Gln Lys Val  Gly Met Gln Lys Tyr  Ser Thr Leu
    1190                1195                1200

Gln Gly  Pro Pro Gly Thr Gly  Lys Ser His Phe Ala  Ile Gly Leu
    1205                1210                1215

Ala Leu  Tyr Tyr Pro Ser Ala  Arg Ile Val Tyr Thr  Ala Cys Ser
    1220                1225                1230

His Ala  Ala Val Asp Ala Leu  Cys Glu Lys Ala Leu  Lys Tyr Leu
    1235                1240                1245

Pro Ile  Asp Lys Cys Ser Arg  Ile Ile Pro Ala Arg  Ala Arg Val
    1250                1255                1260
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Glu Cys  Phe Asp Lys Phe Lys  Val Asn Ser Thr Leu  Glu Gln Tyr
    1265                1270                1275

Val Phe  Cys Thr Val Asn Ala  Leu Pro Glu Thr Thr  Ala Asp Ile
    1280                1285                1290

Val Val  Phe Asp Glu Ile Ser  Met Ala Thr Asn Tyr  Asp Leu Ser
    1295                1300                1305

Val Val  Asn Ala Arg Leu Arg  Ala Lys His Tyr Val  Tyr Ile Gly
    1310                1315                1320

Asp Pro  Ala Gln Leu Pro Ala  Pro Arg Thr Leu Leu  Thr Lys Gly
    1325                1330                1335

Thr Leu  Glu Pro Glu Tyr Phe  Asn Ser Val Cys Arg  Leu Met Lys
    1340                1345                1350

Thr Ile  Gly Pro Asp Met Phe  Leu Gly Thr Cys Arg  Arg Cys Pro
    1355                1360                1365

Ala Glu  Ile Val Asp Thr Val  Ser Ala Leu Val Tyr  Asp Asn Lys
    1370                1375                1380

Leu Lys  Ala His Lys Asp Lys  Ser Ala Gln Cys Phe  Lys Met Phe
    1385                1390                1395

Tyr Lys  Gly Val Ile Thr His  Asp Val Ser Ser Ala  Ile Asn Arg
    1400                1405                1410

Pro Gln  Ile Gly Val Val Arg  Glu Phe Leu Thr Arg  Asn Pro Ala
    1415                1420                1425

Trp Arg  Lys Ala Val Phe Ile  Ser Pro Tyr Asn Ser  Gln Asn Ala
    1430                1435                1440

Val Ala  Ser Lys Ile Leu Gly  Leu Pro Thr Gln Thr  Val Asp Ser
    1445                1450                1455

Ser Gln  Gly Ser Glu Tyr Asp  Tyr Val Ile Phe Thr  Gln Thr Thr
    1460                1465                1470

Glu Thr  Ala His Ser Cys Asn  Val Asn Arg Phe Asn  Val Ala Ile
    1475                1480                1485

Thr Arg  Ala Lys Ile Gly Ile  Leu Cys Ile Met Ser  Asp Arg Asp
    1490                1495                1500

Leu Tyr  Asp Lys Leu Gln Phe  Thr Ser Leu Glu Ile  Pro Arg Arg
    1505                1510                1515

Asn Val  Ala Thr Leu Gln Ala  Glu Asn Val Thr Gly  Leu Phe Lys
    1520                1525                1530

Asp Cys  Ser Lys Ile Ile Thr  Gly Leu His Pro Thr  Gln Ala Pro
    1535                1540                1545

Thr His  Leu Ser Val Asp Ile  Lys Phe Lys Thr Glu  Gly Leu Cys
    1550                1555                1560

Val Asp  Ile Pro Gly Ile Pro  Lys Asp Met Thr Tyr  Arg Arg Leu
    1565                1570                1575

Ile Ser  Met Met Gly Phe Lys  Met Asn Tyr Gln Val  Asn Gly Tyr
    1580                1585                1590

Pro Asn  Met Phe Ile Thr Arg  Glu Glu Ala Ile Arg  His Val Arg
    1595                1600                1605

Ala Trp  Ile Gly Phe Asp Val  Glu Gly Cys His Ala  Thr Arg Asp
    1610                1615                1620

Ala Val  Gly Thr Asn Leu Pro  Leu Gln Leu Gly Phe  Ser Thr Gly
    1625                1630                1635

Val Asn  Leu Val Ala Val Pro  Thr Gly Tyr Val Asp  Thr Glu Asn
    1640                1645                1650

Asn Thr  Glu Phe Thr Arg Val  Asn Ala Lys Pro Pro  Pro Gly Asp
    1655                1660                1665
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Gln Phe  Lys His Leu Ile Pro  Leu Met Tyr Lys Gly  Leu Pro Trp
    1670                1675                1680

Asn Val  Val Arg Ile Lys Ile  Val Gln Met Leu Ser  Asp Thr Leu
    1685                1690                1695

Lys Gly  Leu Ser Asp Arg Val  Val Phe Val Leu Trp  Ala His Gly
    1700                1705                1710

Phe Glu  Leu Thr Ser Met Lys  Tyr Phe Val Lys Ile  Gly Pro Glu
    1715                1720                1725

Arg Thr  Cys Cys Leu Cys Asp  Lys Arg Ala Thr Cys  Phe Ser Thr
    1730                1735                1740

Ser Ser  Asp Thr Tyr Ala Cys  Trp Asn His Ser Val  Gly Phe Asp
    1745                1750                1755

Tyr Val  Tyr Asn Pro Phe Met  Ile Asp Val Gln Gln  Trp Gly Phe
    1760                1765                1770

Thr Gly  Asn Leu Gln Ser Asn  His Asp Gln His Cys  Gln Val His
    1775                1780                1785

Gly Asn  Ala His Val Ala Ser  Cys Asp Ala Ile Met  Thr Arg Cys
    1790                1795                1800

Leu Ala  Val His Glu Cys Phe  Val Lys Arg Val Asp  Trp Ser Val
    1805                1810                1815

Glu Tyr  Pro Ile Ile Gly Asp  Glu Leu Arg Val Asn  Ser Ala Cys
    1820                1825                1830

Arg Lys  Val Gln His Met Val  Val Lys Ser Ala Leu  Leu Ala Asp
    1835                1840                1845

Lys Phe  Pro Val Leu His Asp  Ile Gly Asn Pro Lys  Ala Ile Lys
    1850                1855                1860

Cys Val  Pro Gln Ala Glu Val  Glu Trp Lys Phe Tyr  Asp Ala Gln
    1865                1870                1875

Pro Cys  Ser Asp Lys Ala Tyr  Lys Ile Glu Glu Leu  Phe Tyr Ser
    1880                1885                1890

Tyr Ala  Thr His His Asp Lys  Phe Thr Asp Gly Val  Cys Leu Phe
    1895                1900                1905

Trp Asn  Cys Asn Val Asp Arg  Tyr Pro Ala Asn Ala  Ile Val Cys
    1910                1915                1920

Arg Phe  Asp Thr Arg Val Leu  Ser Asn Leu Asn Leu  Pro Gly Cys
    1925                1930                1935

Asp Gly  Gly Ser Leu Tyr Val  Asn Lys His Ala Phe  His Thr Pro
    1940                1945                1950

Ala Phe  Asp Lys Ser Ala Phe  Thr Asn Leu Lys Gln  Leu Pro Phe
    1955                1960                1965

Phe Tyr  Tyr Ser Asp Ser Pro  Cys Glu Ser His Gly  Lys Gln Val
    1970                1975                1980

Val Ser  Asp Ile Asp Tyr Val  Pro Leu Lys Ser Ala  Thr Cys Ile
    1985                1990                1995

Thr Arg  Cys Asn Leu Gly Gly  Ala Val Cys Arg His  His Ala Asn
    2000                2005                2010

Glu Tyr  Arg Gln Tyr Leu Asp  Ala Tyr Asn Met Met  Ile Ser Ala
    2015                2020                2025

Gly Phe  Ser Leu Trp Ile Tyr  Lys Gln Phe Asp Thr  Tyr Asn Leu
    2030                2035                2040

Trp Asn  Thr Phe Thr Arg Leu  Gln Ser Leu Glu Asn  Val Ala Tyr
    2045                2050                2055

Asn Val  Val Asn Lys Gly His  Phe Asp Gly His Ala  Gly Glu Ala
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    2060                2065                2070

Pro Val  Ser Ile Ile Asn Asn  Ala Val Tyr Thr Lys  Val Asp Gly
    2075                2080                2085

Ile Asp  Val Glu Ile Phe Glu  Asn Lys Thr Thr Leu  Pro Val Asn
    2090                2095                2100

Val Ala  Phe Glu Leu Trp Ala  Lys Arg Asn Ile Lys  Pro Val Pro
    2105                2110                2115

Glu Ile  Lys Ile Leu Asn Asn  Leu Gly Val Asp Ile  Ala Ala Asn
    2120                2125                2130

Thr Val  Ile Trp Asp Tyr Lys  Arg Glu Ala Pro Ala  His Val Ser
    2135                2140                2145

Thr Ile  Gly Val Cys Thr Met  Thr Asp Ile Ala Lys  Lys Pro Thr
    2150                2155                2160

Glu Ser  Ala Cys Ser Ser Leu  Thr Val Leu Phe Asp  Gly Arg Val
    2165                2170                2175

Glu Gly  Gln Val Asp Leu Phe  Arg Asn Ala Arg Asn  Gly Val Leu
    2180                2185                2190

Ile Thr  Glu Gly Ser Val Lys  Gly Leu Thr Pro Ser  Lys Gly Pro
    2195                2200                2205

Ala Gln  Ala Ser Val Asn Gly  Val Thr Leu Ile Gly  Glu Ser Val
    2210                2215                2220

Lys Thr  Gln Phe Asn Tyr Phe  Lys Lys Val Asp Gly  Ile Ile Gln
    2225                2230                2235

Gln Leu  Pro Glu Thr Tyr Phe  Thr Gln Ser Arg Asp  Leu Glu Asp
    2240                2245                2250

Phe Lys  Pro Arg Ser Gln Met  Glu Thr Asp Phe Leu  Glu Leu Ala
    2255                2260                2265

Met Asp  Glu Phe Ile Gln Arg  Tyr Lys Leu Glu Gly  Tyr Ala Phe
    2270                2275                2280

Glu His  Ile Val Tyr Gly Asp  Phe Ser His Gly Gln  Leu Gly Gly
    2285                2290                2295

Leu His  Leu Met Ile Gly Leu  Ala Lys Arg Ser Gln  Asp Ser Pro
    2300                2305                2310

Leu Lys  Leu Glu Asp Phe Ile  Pro Met Asp Ser Thr  Val Lys Asn
    2315                2320                2325

Tyr Phe  Ile Thr Asp Ala Gln  Thr Gly Ser Ser Lys  Cys Val Cys
    2330                2335                2340

Ser Val  Ile Asp Leu Leu Leu  Asp Asp Phe Val Glu  Ile Ile Lys
    2345                2350                2355

Ser Gln  Asp Leu Ser Val Ile  Ser Lys Val Val Lys  Val Thr Ile
    2360                2365                2370

Asp Tyr  Ala Glu Ile Ser Phe  Met Leu Trp Cys Lys  Asp Gly His
    2375                2380                2385

Val Glu  Thr Phe Tyr Pro Lys  Leu Gln Ala Ser Gln  Ala Trp Gln
    2390                2395                2400

Pro Gly  Val Ala Met Pro Asn  Leu Tyr Lys Met Gln  Arg Met Leu
    2405                2410                2415

Leu Glu  Lys Cys Asp Leu Gln  Asn Tyr Gly Glu Asn  Ala Val Ile
    2420                2425                2430

Pro Lys  Gly Ile Met Met Asn  Val Ala Lys Tyr Thr  Gln Leu Cys
    2435                2440                2445

Gln Tyr  Leu Asn Thr Leu Thr  Leu Ala Val Pro Tyr  Asn Met Arg
    2450                2455                2460
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Val Ile  His Phe Gly Ala Gly  Ser Asp Lys Gly Val  Ala Pro Gly
    2465                2470                2475

Thr Ala  Val Leu Arg Gln Trp  Leu Pro Thr Gly Thr  Leu Leu Val
    2480                2485                2490

Asp Ser  Asp Leu Asn Asp Phe  Val Ser Asp Ala Asp  Ser Thr Leu
    2495                2500                2505

Ile Gly  Asp Cys Ala Thr Val  His Thr Ala Asn Lys  Trp Asp Leu
    2510                2515                2520

Ile Ile  Ser Asp Met Tyr Asp  Pro Arg Thr Lys His  Val Thr Lys
    2525                2530                2535

Glu Asn  Asp Ser Lys Glu Gly  Phe Phe Thr Tyr Leu  Cys Gly Phe
    2540                2545                2550

Ile Lys  Gln Lys Leu Ala Leu  Gly Gly Ser Ile Ala  Val Lys Ile
    2555                2560                2565

Thr Glu  His Ser Trp Asn Ala  Asp Leu Tyr Lys Leu  Met Gly His
    2570                2575                2580

Phe Ser  Trp Trp Thr Ala Phe  Val Thr Asn Val Asn  Ala Ser Ser
    2585                2590                2595

Ser Glu  Ala Phe Leu Ile Gly  Ala Asn Tyr Leu Gly  Lys Pro Lys
    2600                2605                2610

Glu Gln  Ile Asp Gly Tyr Thr  Met His Ala Asn Tyr  Ile Phe Trp
    2615                2620                2625

Arg Asn  Thr Asn Pro Ile Gln  Leu Ser Ser Tyr Ser  Leu Phe Asp
    2630                2635                2640

Met Ser  Lys Phe Pro Leu Lys  Leu Arg Gly Thr Ala  Val Met Ser
    2645                2650                2655

Leu Lys  Glu Asn Gln Ile Asn  Asp Met Ile Tyr Ser  Leu Leu Glu
    2660                2665                2670

Lys Gly  Arg Leu Ile Ile Arg  Glu Asn Asn Arg Val  Val Val Ser
    2675                2680                2685

Ser Asp  Ile Leu Val Asn Asn
    2690                2695


<210> SEQ ID NO 76
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L3/+/4932 primer

<400> SEQUENCE: 76

ccacacacag cttgtggata                                              20


<210> SEQ ID NO 77
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L4/+/6401 primer

<400> SEQUENCE: 77

ccgaagttgt aggcaatgtc                                              20


<210> SEQ ID NO 78
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L4/+/6964 primer
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<400> SEQUENCE: 78

tttggtgctc cttcttattg 20

<210> SEQ ID NO 79
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L4/-/6817 primer

<400> SEQUENCE: 79

ccggcatcca aacataattt 20

<210> SEQ ID NO 80
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/-/7633 primer

<400> SEQUENCE: 80

tggtcagtag ggttgattgg 20

<210> SEQ ID NO 81
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/-/8127 primer

<400> SEQUENCE: 81

catcctttgt gtcaacatcg 20

<210> SEQ ID NO 82
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/-/8633 primer

<400> SEQUENCE: 82

gtcacgagtg acaccatcct 20

<210> SEQ ID NO 83
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/+/7839 primer

<400> SEQUENCE: 83

atgcgacgag tctgcttcta 20

<210> SEQ ID NO 84
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/+/8785 primer

<400> SEQUENCE: 84

ttcatagtgc ctggcttacc 20

<210> SEQ ID NO 85
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/+/8255 primer

<400> SEQUENCE: 85

atcttggcgc atgtattgac 20

<210> SEQ ID NO 86
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L6/-/9422 primer

<400> SEQUENCE: 86

tgcattagca gcaacaacat 20

<210> SEQ ID NO 87
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L6/-/9966 primer

<400> SEQUENCE: 87

tctgcagaac agcagaagtg 20

<210> SEQ ID NO 88
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L6/-/10542 primer

<400> SEQUENCE: 88

cctgtgcagt ttgtctgtca 20

<210> SEQ ID NO 89
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L6/+/10677 primer

<400> SEQUENCE: 89

ccttgtggca atgaagtaca 20

<210> SEQ ID NO 90
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L6/+/10106 primer

<400> SEQUENCE: 90

atgtcatttg cacagcagaa 20

<210> SEQ ID NO 91
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L6/+/9571 primer

<400> SEQUENCE: 91

cttcaatggt ttgccatgtt 20

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<210> SEQ ID NO 92
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L7/-/11271 primer

<400> SEQUENCE: 92

tgcgagctgt catgagaata 20

<210> SEQ ID NO 93
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L7/-/11801 primer

<400> SEQUENCE: 93

aaccgagagc agtaccacag 20

<210> SEQ ID NO 94
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L7/-/12383 primer

<400> SEQUENCE: 94

tttggctgct gtagtcaatg 20

<210> SEQ ID NO 95
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L7/+/12640 primer

<400> SEQUENCE: 95

ctacgacaga tgtcctgtgc 20

<210> SEQ ID NO 96
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
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<400> SEQUENCE: 96

gagcaggctg tagctaatgg 20

<210> SEQ ID NO 97
<211> LENGTH: 20
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<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: S/L7/+/11551 primer

<400> SEQUENCE: 97

ttaggctatt gttgctgctg 20

<210> SEQ ID NO 98
<211> LENGTH: 20
<212> TYPE: DNA
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<223> OTHER INFORMATION: S/L8/-/13160 primer

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<400> SEQUENCE: 98

cagacaacat gaagcaccac 20

<210> SEQ ID NO 99
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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L8/-/13704 primer

<400> SEQUENCE: 99

cgctgacgtg atatatgtgg 20

<210> SEQ ID NO 100
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L8/-/14284 primer

<400> SEQUENCE: 100

tgcacaatga aggatacacc 20

<210> SEQ ID NO 101
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L8/+/14453 primer

<400> SEQUENCE: 101

acatagctcg cgtctcagtt 20

<210> SEQ ID NO 102
<211> LENGTH: 20
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<223> OTHER INFORMATION: S/L8/+/13968 primer

<400> SEQUENCE: 102

ggcattgtag gcgtactgac 20

<210> SEQ ID NO 103
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: S/L8/+/13401 primer

<400> SEQUENCE: 103

gtttgcggtg taagtgcag 19

<210> SEQ ID NO 104
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L9/-/15098 primer

<400> SEQUENCE: 104

tagtggcggc tattgacttc 20

<210> SEQ ID NO 105
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L9/-/15677 primer

<400> SEQUENCE: 105

ctaaaccttg agccgcatag 20

<210> SEQ ID NO 106
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: S/L9/-/16247 primer

<400> SEQUENCE: 106

catggtcata gcagcacttg 20

<210> SEQ ID NO 107
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L9/+/16323 primer

<400> SEQUENCE: 107

ccaggttgtg atgtcactga t 21

<210> SEQ ID NO 108
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L9/+/15858 primer

<400> SEQUENCE: 108

ccttacccag atccatcaag 20

<210> SEQ ID NO 109
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L9/+/15288 primer

<400> SEQUENCE: 109

cgcaaacata acacttgctg 20

<210> SEQ ID NO 110
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L10/-/16914 primer

<400> SEQUENCE: 110

agtgttgggt acaagccagt 20

<210> SEQ ID NO 111
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L10/-/17466 primer

<400> SEQUENCE: 111

gttccaagga acatgtctgg 20

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<210> SEQ ID NO 112
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
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<400> SEQUENCE: 112

aggtgcctgt gtaggatgaa 20

<210> SEQ ID NO 113
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: S/L10/+/18245 primer

<400> SEQUENCE: 113

gggctgtcat gcaactagag 20

<210> SEQ ID NO 114
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<213> ORGANISM: Artificial sequence
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<400> SEQUENCE: 114

tcttacacgc aatcctgctt 20

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: S/L10/+/17061 primer

<400> SEQUENCE: 115

tacccatctg ctcgcatagt 20

<210> SEQ ID NO 116
<211> LENGTH: 20
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<223> OTHER INFORMATION: S/L11/-/18877 primer

<400> SEQUENCE: 116

gcaagcagaa ttaaccctca 20

<210> SEQ ID NO 117
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L11/-/19396 primer

<400> SEQUENCE: 117

agcaccacct aaattgcatc 20

<210> SEQ ID NO 118
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<220> FEATURE:
<223> OTHER INFORMATION: S/L11/-/20002 primer

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<400> SEQUENCE: 118

tggtcccttt gaaggtgtta 20

<210> SEQ ID NO 119
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L11/+/20245 primer

<400> SEQUENCE: 119

tcgaacacat cgtttatgga 20

<210> SEQ ID NO 120
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L11/+/19611 primer

<400> SEQUENCE: 120

gaagcacctg tttccatcat 20

<210> SEQ ID NO 121
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L11/+/19021 primer

<400> SEQUENCE: 121

acgatgctca gccatgtagt 20

<210> SEQ ID NO 122
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L1/F3/+/800 primer

<400> SEQUENCE: 122

gaggtgcagt cactcgctat 20

<210> SEQ ID NO 123
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L1/F4/+/1391 primer

<400> SEQUENCE: 123

cagagattgg acctgagcat 20

<210> SEQ ID NO 124
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L1/F5/+/1925 primer

<400> SEQUENCE: 124

cagcaaacca ctcaattcct 20

<210> SEQ ID NO 125
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L1/R3/-/1674 primer

<400> SEQUENCE: 125

aaatgatggc aacctcttca 20

<210> SEQ ID NO 126
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L1/R4/-/1107 primer

<400> SEQUENCE: 126

cacgtggttg aatgactttg 20

<210> SEQ ID NO 127
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
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<400> SEQUENCE: 127

atttctgcaa ccagctcaac 20

<210> SEQ ID NO 128
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: SARS/L2/F3/+/2664 primer

<400> SEQUENCE: 128

cgcattgtct cctggtttac 20

<210> SEQ ID NO 129
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<213> ORGANISM: Artificial sequence
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<400> SEQUENCE: 129

gagattgagc cagaaccaga 20

<210> SEQ ID NO 130
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L2/F5/+/3746 primer

<400> SEQUENCE: 130

atgagcaggt tgtcatggat 20

<210> SEQ ID NO 131
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<220> FEATURE:
<223> OTHER INFORMATION: SARS/L2/R3/-/3579 primer

<400> SEQUENCE: 131

ctgccttaag aagctggatg 20

<210> SEQ ID NO 132
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<213> ORGANISM: Artificial sequence
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<400> SEQUENCE: 132

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<210> SEQ ID NO 133
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L2/R5/-/2529 primer

<400> SEQUENCE: 133

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<210> SEQ ID NO 134
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L3/F3/+/4708 primer

<400> SEQUENCE: 134

tctttggctg gctcttacag 20

<210> SEQ ID NO 135
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SRAS/L3/F4/+/5305 primer

<400> SEQUENCE: 135

gctggtgatg ctgctaactt 20

<210> SEQ ID NO 136
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: SARS/L3/F5/+/5822 primer

<400> SEQUENCE: 136

ccatcaagcc tgtgtcgtat 20

<210> SEQ ID NO 137
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L3/R3/-/5610 primer

<400> SEQUENCE: 137

caggtggtgc agacatcata 20

<210> SEQ ID NO 138
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L3/R4/-/4988 primer

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<400> SEQUENCE: 138

aacatcagca ccatccaagt 20

<210> SEQ ID NO 139
<211> LENGTH: 20
<212> TYPE: DNA
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<400> SEQUENCE: 139

atcggacacc atagtcaacg 20

<210> SEQ ID NO 140
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: synthetic S gene

<400> SEQUENCE: 140

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cgccccgttg acgcaaatgg gcggtaggcg tgtacggtgg gaggtctata taagcagagc 720

tcgtttagtg aaccgtcaga tcactagaag ctttattgcg gtagtttatc acagttaaat 780

tgctaacgca gtcagtgctt ctgacacaac agtctcgaac ttaagctgca gaagttggtc 840

gtgaggcact gggcaggtaa gtatcaaggt tacaagacag gtttaaggag accaatagaa 900

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cgtgaccggc ttccacacca tcaaccacac cttcggcaac cccgtgatcc ccttcaagga 1320

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caccatgaac aacaagagcc agagcgtgat catcatcaac aacagcacca acgtggtgat 1440

ccgggcctgc aacttcgagc tgtgcgacaa ccccttcttc gccgtgtcca aacccatggg 1500

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catcaacatc accaacttcc gggccatcct gaccgccttt agccctgccc aggacatctg   1800

gggcaccagc gccgccgcct acttcgtggg ctacctgaag cctaccacct tcatgctgaa   1860

gtacgacgag aacggcacca tcaccgacgc cgtggactgc agccagaacc ccctggccga   1920

gctgaagtgc agcgtgaaga gcttcgagat cgacaagggc atctaccaga ccagcaactt   1980

cagagtggtg cctagcggcg atgtggtgcg gttccccaat atcaccaacc tgtgcccctt   2040

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cgccgactac aactacaagc tgcccgacga cttcatgggc tgcgtgctgg cctggaacac   2340

ccggaacatc gacgccacaa gcaccggcaa ctacaattac aagtaccgct acctgcggca   2400

cggcaagctg cggcccttcg agcgggacat ctccaacgtg cccttcagcc ccgacggcaa   2460

gccctgcacc ccccctgccc tgaactgcta ctggcccctg aacgactacg gcttctacac   2520

caccaccggc atcggctatc agccctacag agtggtggtg ctgagcttcg agctgctgaa   2580

cgcccctgcc accgtgtgcg gccccaagct gagcaccgac ctgatcaaga accagtgcgt   2640

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caagaccagc gagatcctgg acatcagccc ctgcagcttc ggcggcgtgt ccgtgatcac   2820

ccccggcacc aacgccagca gcgaagtggc cgtgctgtac caggacgtga actgcaccga   2880

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gaacaacgtg ttccagaccc aggccggctg cctgatcggc gccgagcacg tggacaccag   3000

ctacgagtgc gacatcccca ttggcgccgg aatctgcgcc agctaccaca ccgtgagcct   3060

gctgcggagc accagccaga agtccatcgt ggcctacacc atgagcctgg gcgccgacag   3120

cagcatcgcc tacagcaaca acaccatcgc catccccacc aacttcagca tctccatcac   3180

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gaaccgggcc ctgagcggca tcgccgccga gcaggaccgg aacaccagag aagtgttcgc   3360

ccaagtgaag cagatgtata agacccccac cctgaagtac ttcgggggct tcaacttctc   3420

tcagatcctg cccgaccctc tgaagcccac caagcgctcc ttcatcgagg acctgctgtt   3480

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The invention claimed is:

1. A method for the detection of a SARS-associated coronavirus infection, from a biological sample, by indirect IgG ELISA using the SARS-associated coronavirus N protein, which comprises providing ELISA plates that have been sensitized with a solution consisting of N protein at a concentration of between 0.5 and 4 μg/ml in a 10 mM PBS buffer, pH 7.2, phenol red at 0.25 ml/l.

2. A method for the detection of a SARS-associated coronavirus infection, from a biological sample, by double epitope ELISA, comprising mixing a serum to be tested with a visualizing antigen, and contacting the resulting mixture with the antigen attached to a solid support, wherein said antigen is a SARS-associated coronavirus N protein and wherein said solid support is sensitized with a solution consisting of N protein at a concentration of between 0.5 and 4 μg/ml in a 10 mM PBS buffer, pH 7.2, phenol red at 0.25 ml/l.

3. The method as claimed in claim **2**, wherein said N protein is at a concentration of 1 μg/ml.

4. The method as claimed in claim **1**, wherein said biological sample is collected 12 days or more after said infection.

5. The method as claimed in claim **2**, wherein said biological sample is collected 12 days or more after said infection.

6. The method as claimed in claim **1**, wherein said N protein is at a concentration of 2 μg/ml.

7. The method as claimed in claim **2**, wherein said visualizing antigen consists of said SARS-associated coronavirus N protein conjugated to a visualizing molecule selected from the group consisting of a radioactive atom, a dye, a fluorescent molecule, a fluorophore, and an enzyme.

8. The method as claimed in claim **7**, wherein said enzyme is a peroxidase.

* * * * *