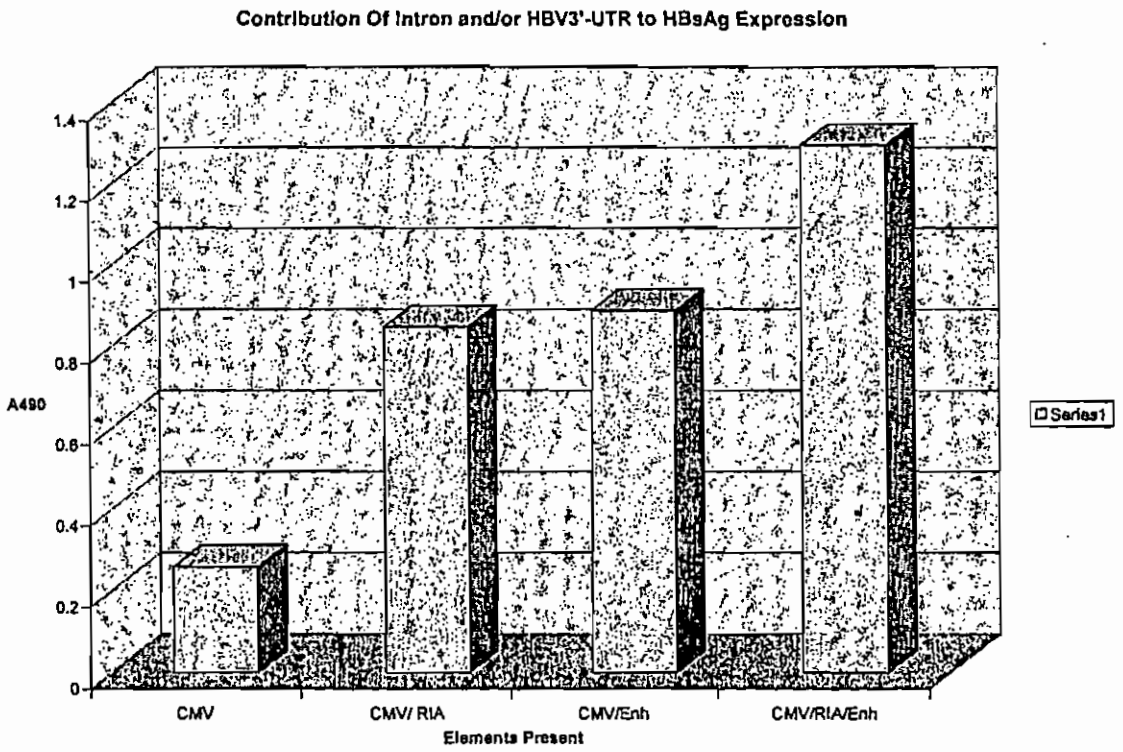
Contribution Of Intron and/or HBV3'-UTR to HBsAg Expression
A490
1.4
1.2
1
0.8
0.6
0.4
0.2
0
CMV
CMV/ RIA
CMV/Enh
CMV/RIA/Enh
Elements Present
Series1

Figure 1

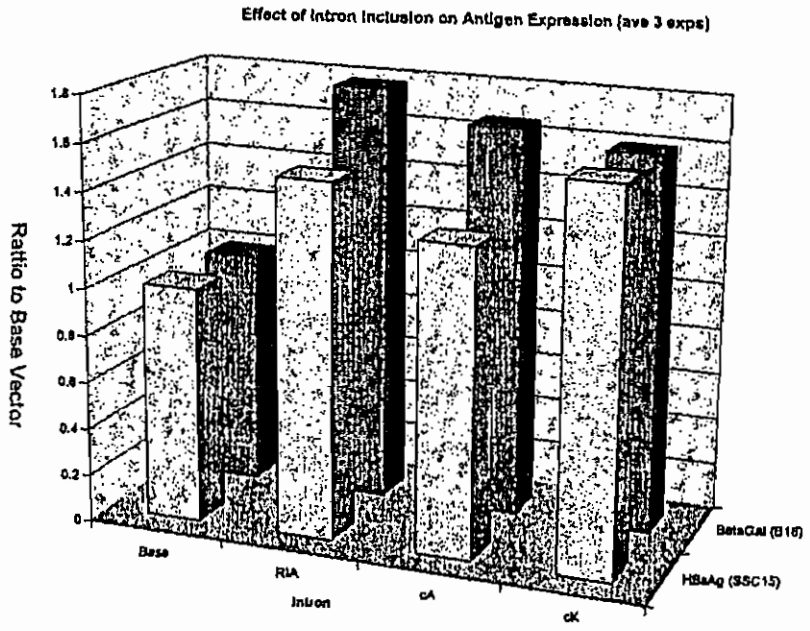
Effect of Intron Inclusion on Antigen Expression (ave 3 exps)
Ratio to Base Vector
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Base
RIA
Intron
cA
cK
BetaGal (B16)
HBsAg (S5C15)

Figure 2

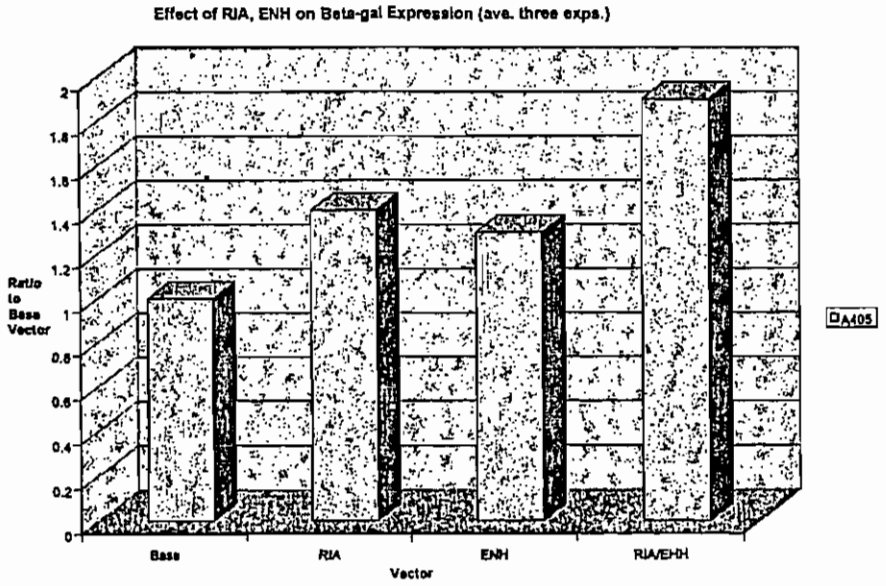
Effect of RIA, ENH on Beta-gal Expression (ave. three exps.)
Ratio to Base Vector
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Base
RIA
ENH
RIA/ENH
Vector
A405

Figure 3

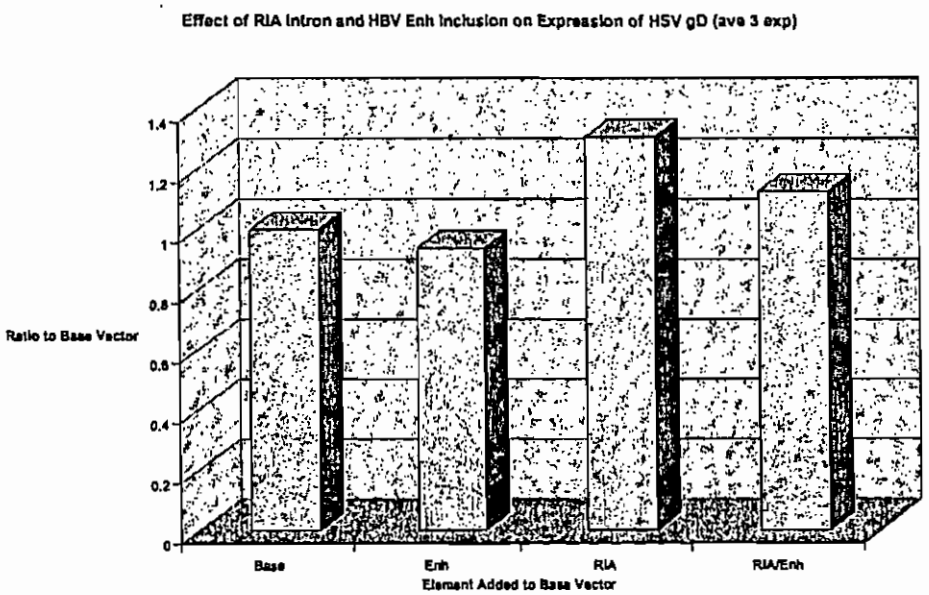
Effect of RIA Intron and HBV Enh Inclusion on Expression of HSV gD (ave 3 exp)
Ratio to Base Vector
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Base
Enh
RIA
RIA/Enh
Element Added to Base Vector

Figure 4

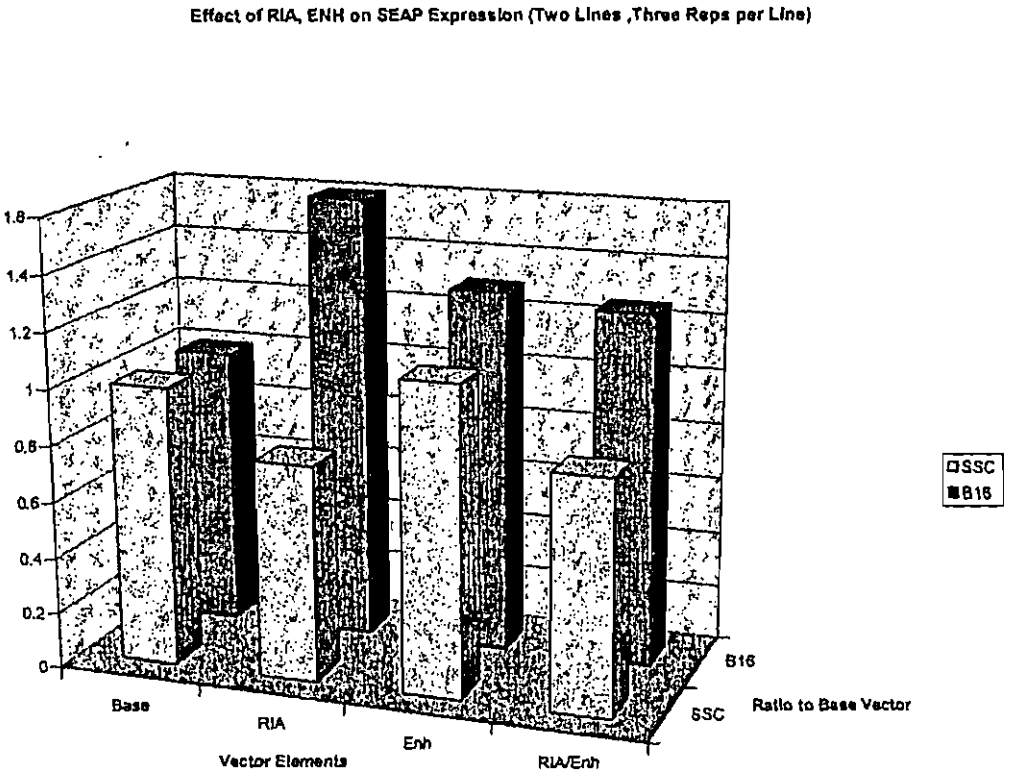
Effect of RIA, ENH on SEAP Expression (Two Lines ,Three Reps per Line)
1.8
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Base
RIA
Enh
RIA/Enh
Vector Elements
B16
SSC
Ratio to Base Vector
SSC
B16

Figure 5

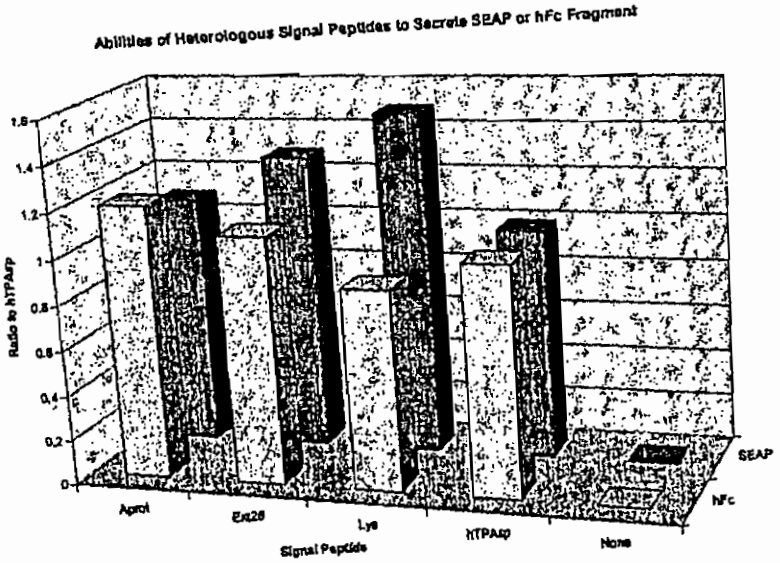
Abilities of Heterologous Signal Peptides to Secrete SEAP or hFc Fragment
Ratio to hTPAsp
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Aprot
Ext28
Lys
hTPAsp
None
Signal Peptide
SEAP
hFc

Figure 6

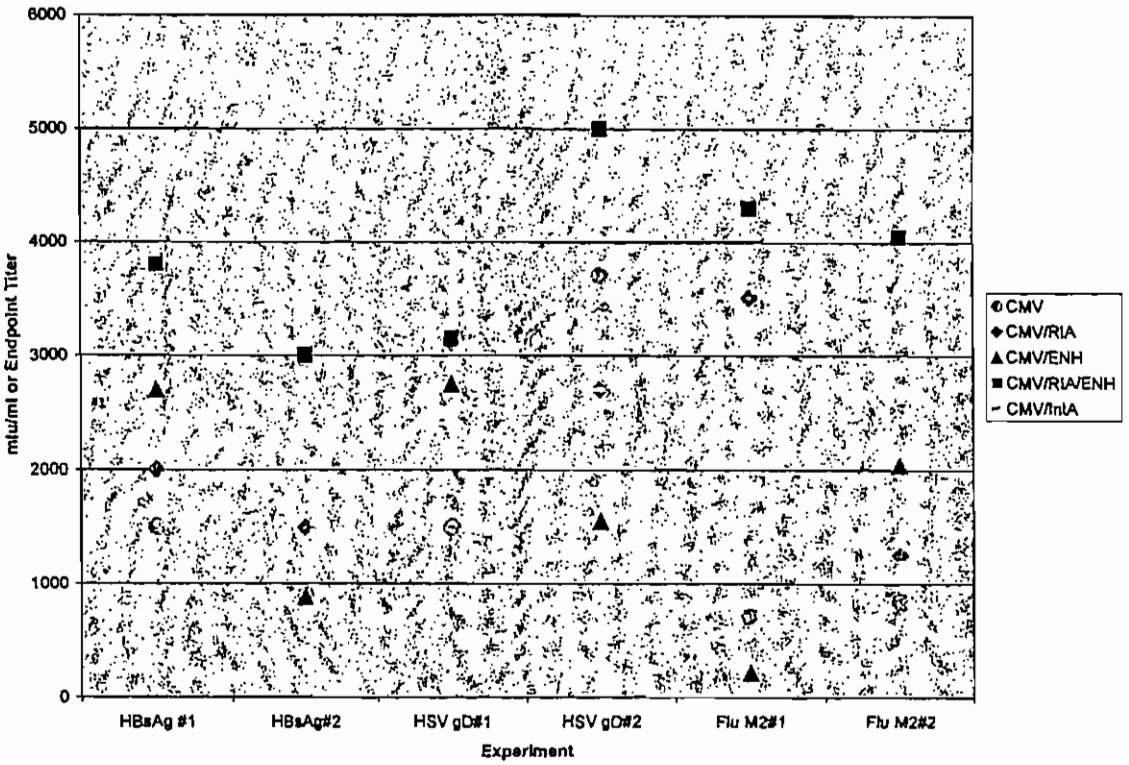
6000
5000
4000
3000
2000
1000
0
miu/ml or Endpoint Titer
HBsAg #1
HBsAg#2
HSV gD#1
HSV gD#2
Flu M2#1
Flu M2#2
Experiment
CMV
CMV/RIA
CMV/ENH
CMV/RIA/ENH
CMV/IntA

Figure 7

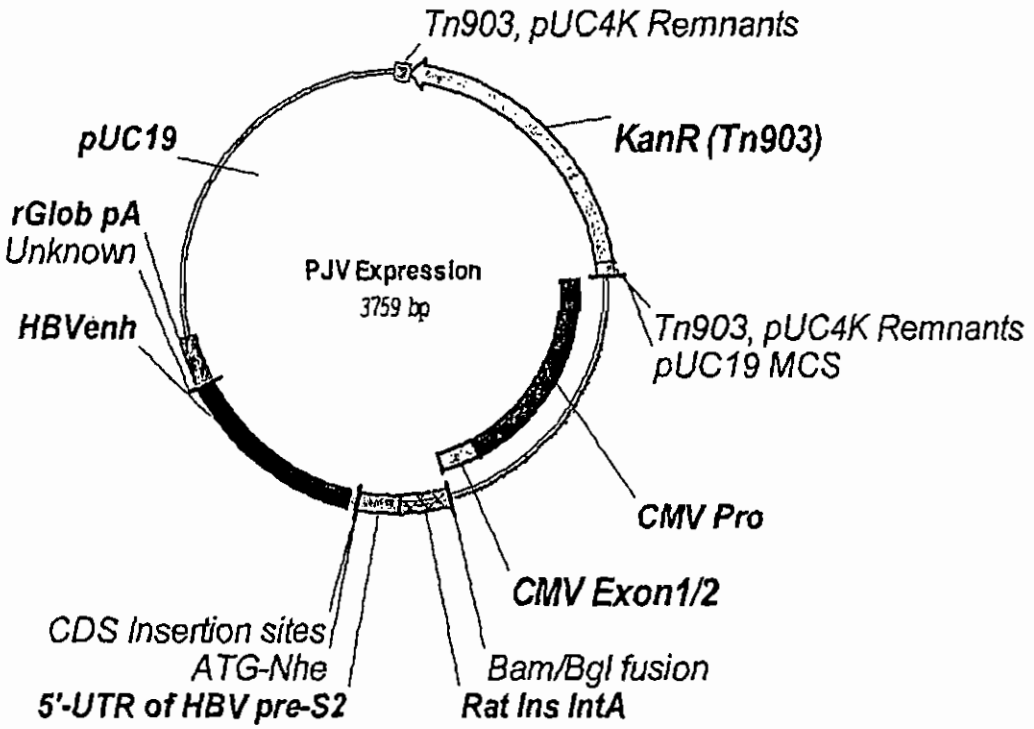
Tn903, pUC4K Remnants
KanR (Tn903)
pUC19
rGlob pA
Unknown
HBVenh
PJV Expression
3759 bp
Tn903, pUC4K Remnants
pUC19 MCS
CMV Pro
CMV Exon1/2
CDS Insertion sites
ATG-Nhe
5'-UTR of HBV pre-S2
Bam/Bgl fusion
Rat Ins IntA

Figure 8

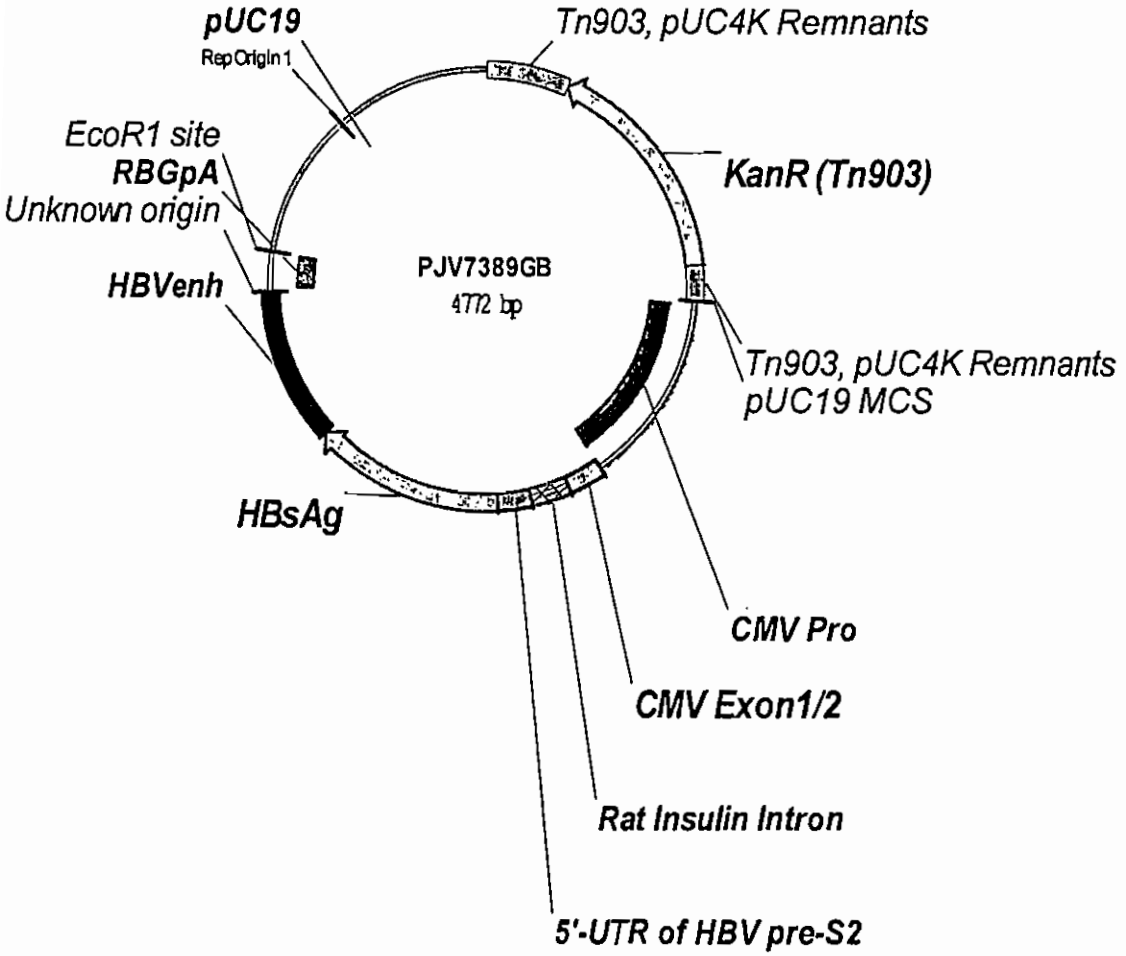
pUC19
Rep Origin 1
Tn903, pUC4K Remnants
EcoR1 site
RBGpA
Unknown origin
KanR (Tn903)
PJV7389GB
4772 bp
HBVenh
Tn903, pUC4K Remnants
pUC19 MCS
HBsAg
CMV Pro
CMV Exon1/2
Rat Insulin Intron
5'-UTR of HBV pre-S2

Figure 9

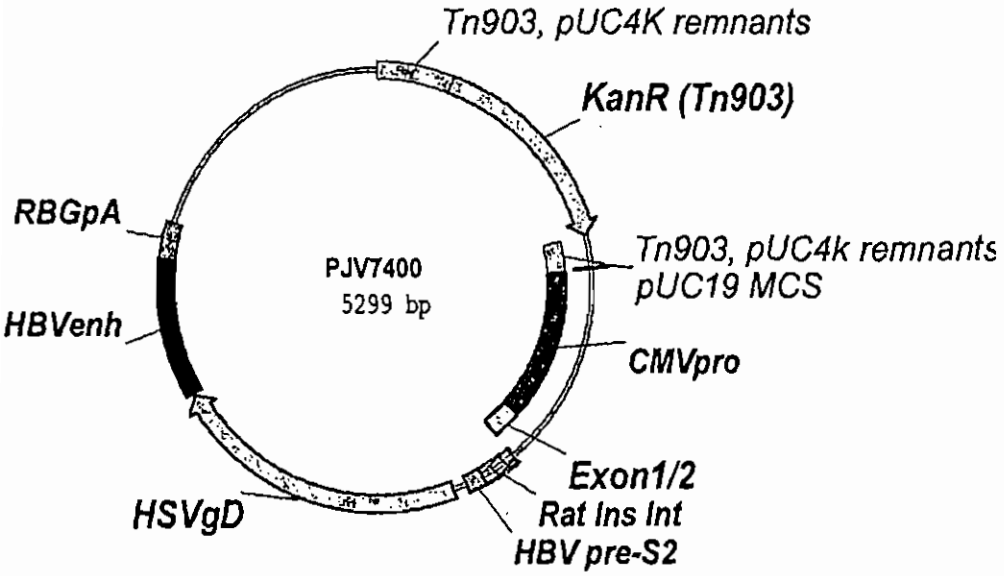
Tn903, pUC4K remnants
KanR (Tn903)
RBGpA
Tn903, pUC4k remnants
pUC19 MCS
PJV7400
5299 bp
HBVenh
CMVpro
Exon1/2
Rat Ins Int
HSVgD
HBV pre-S2

Figure 10

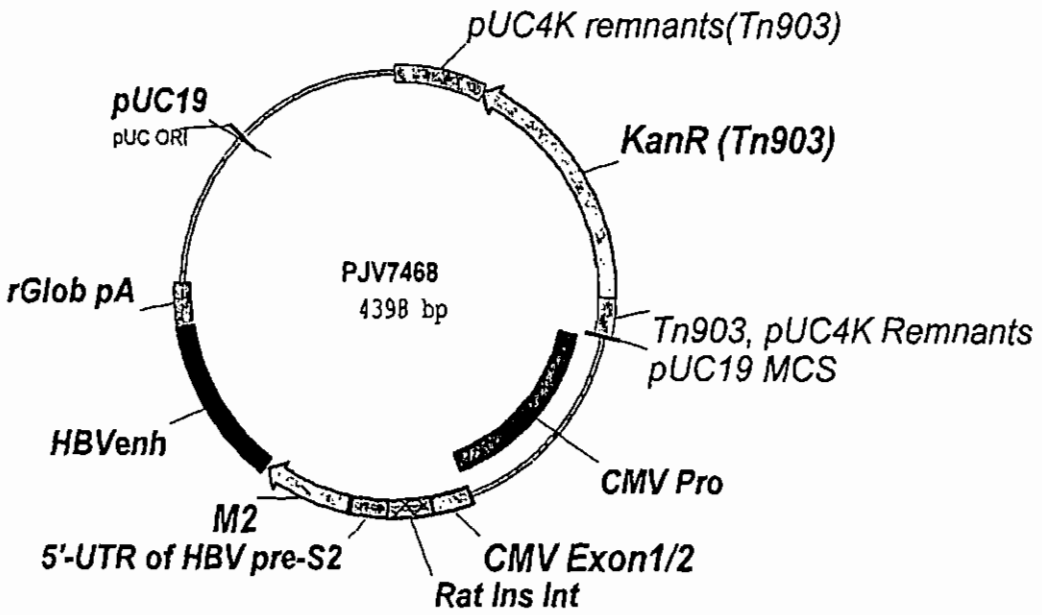
pUC4K remnants(Tn903)
pUC19
pUC ORI
KanR (Tn903)
PJV7468
4398 bp
rGlob pA
Tn903, pUC4K Remnants
pUC19 MCS
HBVenh
CMV Pro
M2
5'-UTR of HBV pre-S2
CMV Exon1/2
Rat Ins Int

Figure 11

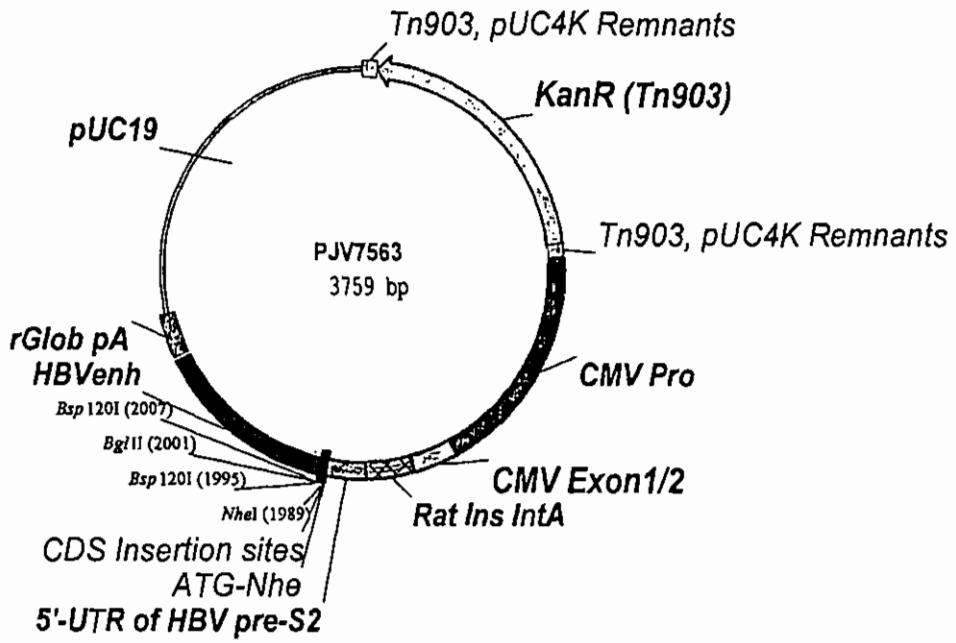
Tn903, pUC4K Remnants
KanR (Tn903)
pUC19
Tn903, pUC4K Remnants
PJV7563
3759 bp
rGlob pA
HBVenh
CMV Pro
Bsp 1201 (2007)
Bgl II (2001)
Bsp 1201 (1995)
CMV Exon1/2
Nhel (1989)
Rat Ins IntA
CDS Insertion sites
ATG-Nhe
5'-UTR of HBV pre-S2

Figure 12

Figure 13

ggcgtaatgctctgccagtgttacaaccaattaaccaattctgattagaaaaactcatcgagcatcaaatgaaactgcaatttattcatat
caggattatcaataccatatttttgaaaaagccgtttctgtaatgaaggagaaaactcaccgaggcagttccataggatggcaagatcc
tggtatcggtctgcgattccgactcgtccaacatcaatacaacctattaatttcccctcgtcaaaaataaggttatcaagtgagaaatca
ccatgagtgacgactgaatccggtgagaatggcaaaagcttatgcatttctttccagacttgttcaacaggccagccattacgctcgtc
atcaaaatcactcgcatcaaccaaaccgttattcattcgtgattgcgcctgagcgagacgaaatacgcgatcgctgttaaaaggacaa
ttacaaacaggaatcgaatgcaaccggcgcaggaacactgccagcgcatcaacaatattttcacctgaatcaggatattcttctaata
cctggaatgctgttttcccggggatcgcagtggtgagtaaccatgcatcatcaggagtacggataaaatgcttgatggtcggaagag
gcataaattccgtcagccagtttagtctgaccatctcatctgtaacatcattggcaacgctacctttgccatgtttcagaaacaactctgg
cgcatcgggcttcccatacaatcgatagattgtcgcacctgattgcccgacattatcgcgagcccatttatacccatataaatcagcat
ccatgttggaatttaatcgcggcctcgagcaagacgtttcccgttgaatatggctcataacacccttgtattactgtttatgtaagcaga
caggtcgacaatattggctattggccattgcatacgttgtatctatatcataatatgtacatttatattggctcatgtccaatatgaccgcca
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cggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaata
gggactttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtccgcc
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gacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctcgtttagtgaaccgtcagatcgcctggagacgc
catccacgctgttttgacctccatagaagacaccgggaccgatccagcctccgcggccgggaacggtgcattggaacgcggattc
cccgtgccaagagtgactcaccgtccggatctcagcaagcaggtatgtactctccagggtgggcctggcttccccagtcaagactc
cagggatttgagggacgctgtgggctcttctcttacatgtacctttgcttgcctcaaccctgactatcttccaggtcaggatcccagag
tcaggggtctgtattttcctgctggtggctccagttcaggaacagtaaaccctgctccgaatattgcctctcacatctcgtcaatctccg
cgaggactggggaccctgtgacgaacatggctagcgggcccagatctgggccctaacaaaacaaaaagatggggttattccctaa
acttcatgggttacgtaattggaagttgggggacattgccacaagatcatattgtacaaaagatcaaacactgttttagaaaacttcctg
taaacaggcctattgattggaaagtatgtcaaaggattgtgggtcttttgggctttgctgctccatttacacaatgtggatatcctgcctta
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ccccgttgctcggcaacggcctggtctgtgccaagtgtttgctgacgcaacccccactggctggggcttggccataggccatcagc
gcatgcgtggaacctttgtggctcctctgccgatccatactgcggaactcctagccgcttgttttgctcgcagccggtctggagcaaa
gctcataggaactgacaattctgtcgtcctctcgcggaaatatacatcgtttcgatctacgtatgatctttttccctctgccaaaaattatg
gggacatcatgaagccccttgagcatctgacttctggctaataaaggaaatttatttttcattgcaatagtgtgttggaatttttgtgtctct
cactcggaaggaattctgcattaatgaatcggccaacgcgcggggagaggcggtttgcgtattgggcgctcttccgcttcctcgctc
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ggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaaggccgcgttgctggcgtttttcca
taggctccgccccctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactataaagataccca
ggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcccttcgggaa
gcgtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccc
cgttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagc
cactggtaacaggattagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaa
gaacagtatttggtatctgcgctctgctgaagccagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgc
tggtagcggtggtttttttgtttgcaagcagcagattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttctacggggtct
gacgctcagtggaacgaaaactcacgttaagggattttggtcatgagattatcaaaaaggatcttcacctagatccttttaaattaaaaa
tgaagttttaaatcaatctaaagtatatatgagtaaacttggtctgacagttaccaatgcttaatcagtgaggcacctatctcagcgatct
gtctatttcgttcatccatagttgcctgactc

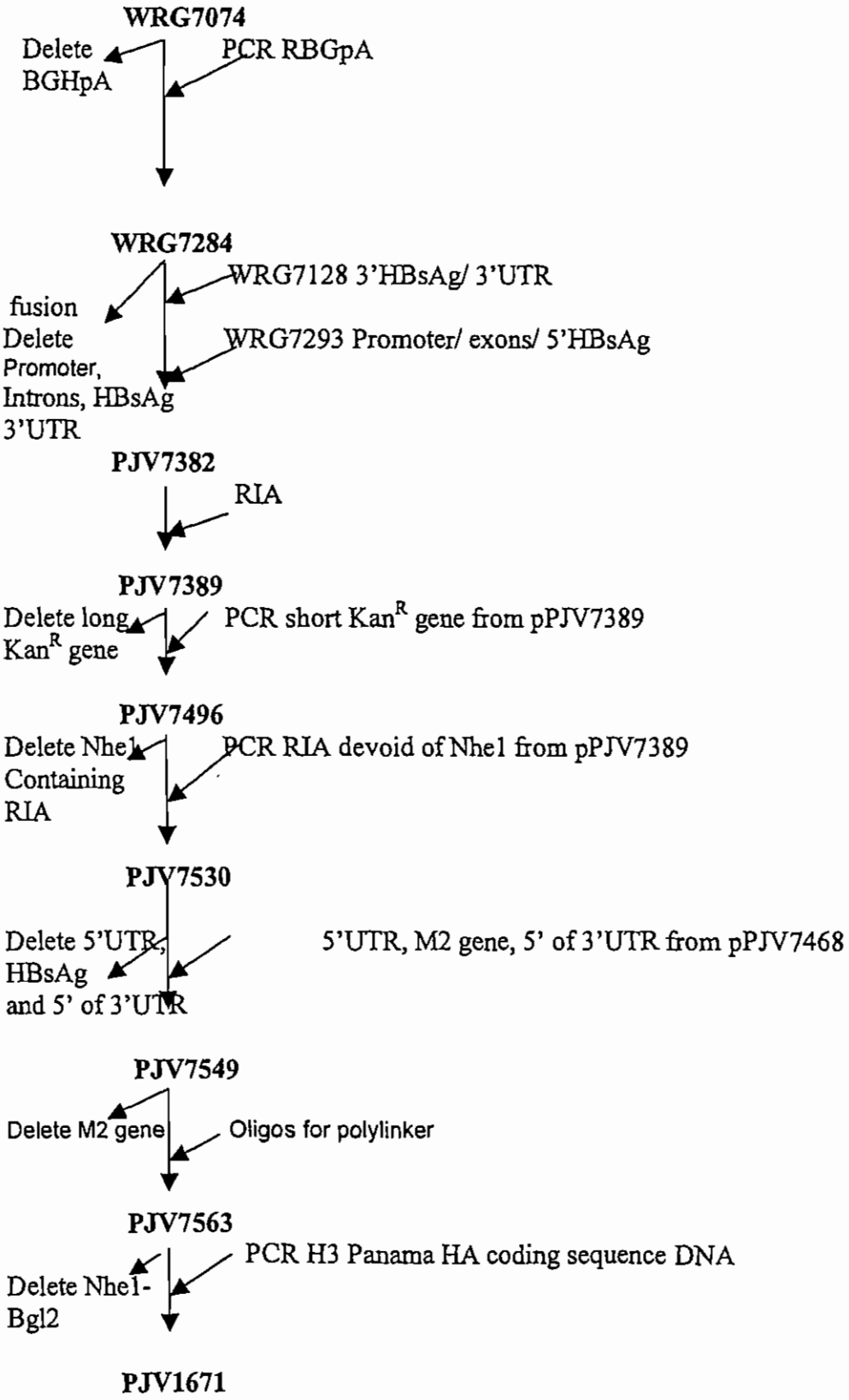

WRG7074
Delete BGHpA
PCR RBGpA
WRG7284
WRG7128 3'HBsAg/ 3'UTR
fusion
Delete Promoter, Introns, HBsAg 3'UTR
WRG7293 Promoter/ exons/ 5'HBsAg
PJV7382
RIA
PJV7389
Delete long Kan^R gene
PCR short Kan^R gene from pPJV7389
PJV7496
Delete Nhe1 Containing RIA
PCR RIA devoid of Nhe1 from pPJV7389
PJV7530
Delete 5'UTR, HBsAg and 5' of 3'UTR
5'UTR, M2 gene, 5' of 3'UTR from pPJV7468
PJV7549
Delete M2 gene
Oligos for polylinker
PJV7563
Delete Nhe1-Bgl2
PCR H3 Panama HA coding sequence DNA
PJV1671

Figure 14

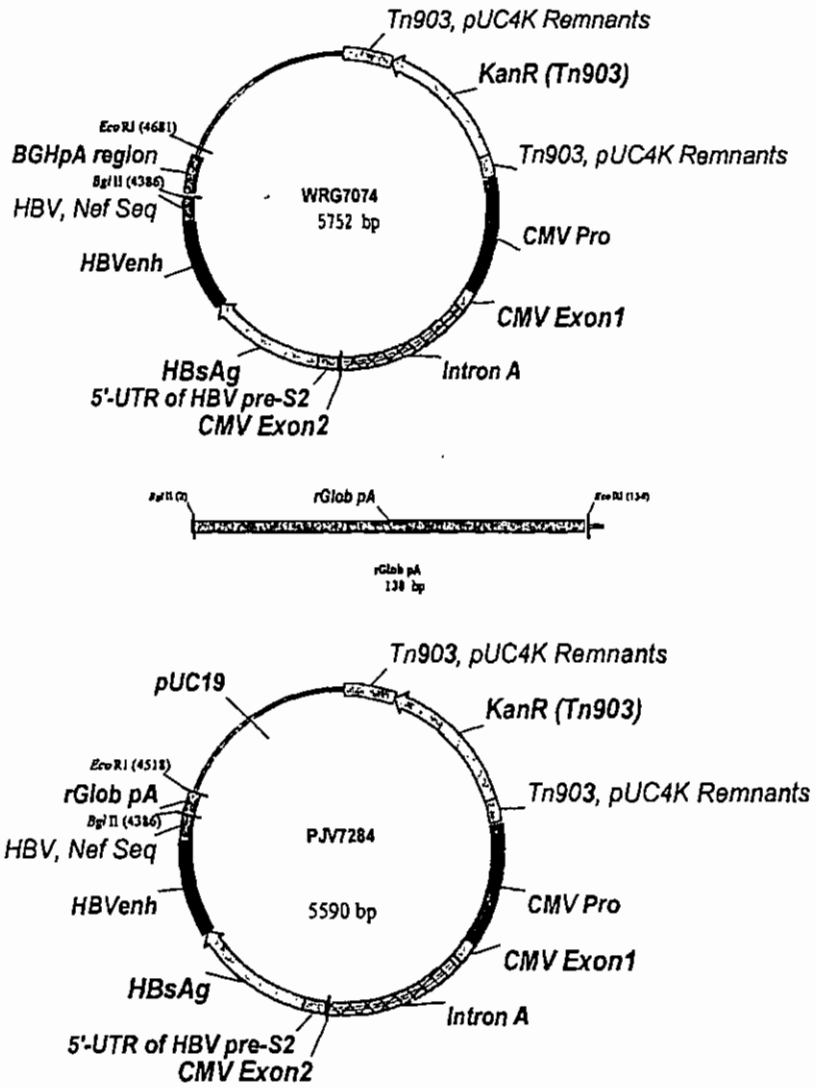
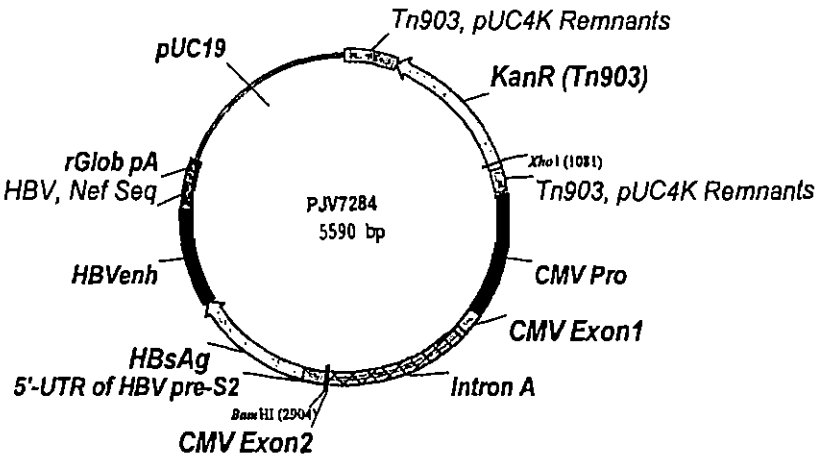
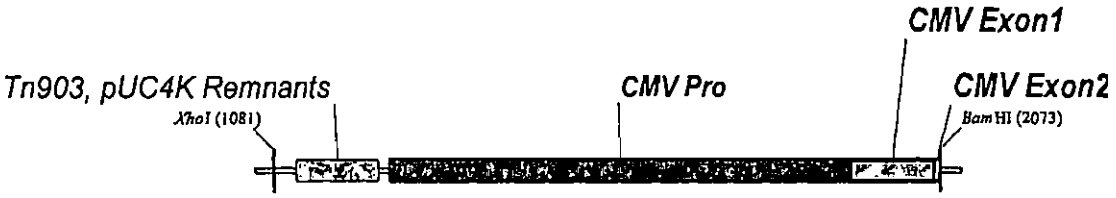

Tn903, pUC4K Remnants
KanR (Tn903)
EcoRI (4681)
BGHpA region
BglII (4386)
HBV, Nef Seq
WRG7074
5752 bp
Tn903, pUC4K Remnants
CMV Pro
HBVenh
CMV Exon1
HBsAg
Intron A
5'-UTR of HBV pre-S2
CMV Exon2
rGlob pA
rGlob pA
138 bp
Tn903, pUC4K Remnants
pUC19
KanR (Tn903)
EcoRI (4518)
rGlob pA
BglII (4386)
Tn903, pUC4K Remnants
HBV, Nef Seq
PJV7284
HBVenh
5590 bp
CMV Pro
CMV Exon1
HBsAg
Intron A
5'-UTR of HBV pre-S2
CMV Exon2

Figure 15(a)





Fragment of WRG7128
1050 bp (molecule 4759 bp)

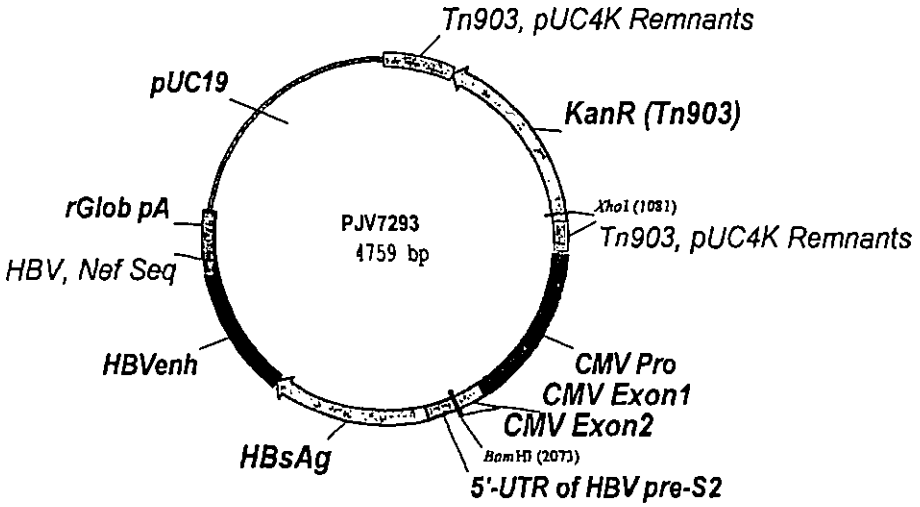


Figure 15(b)

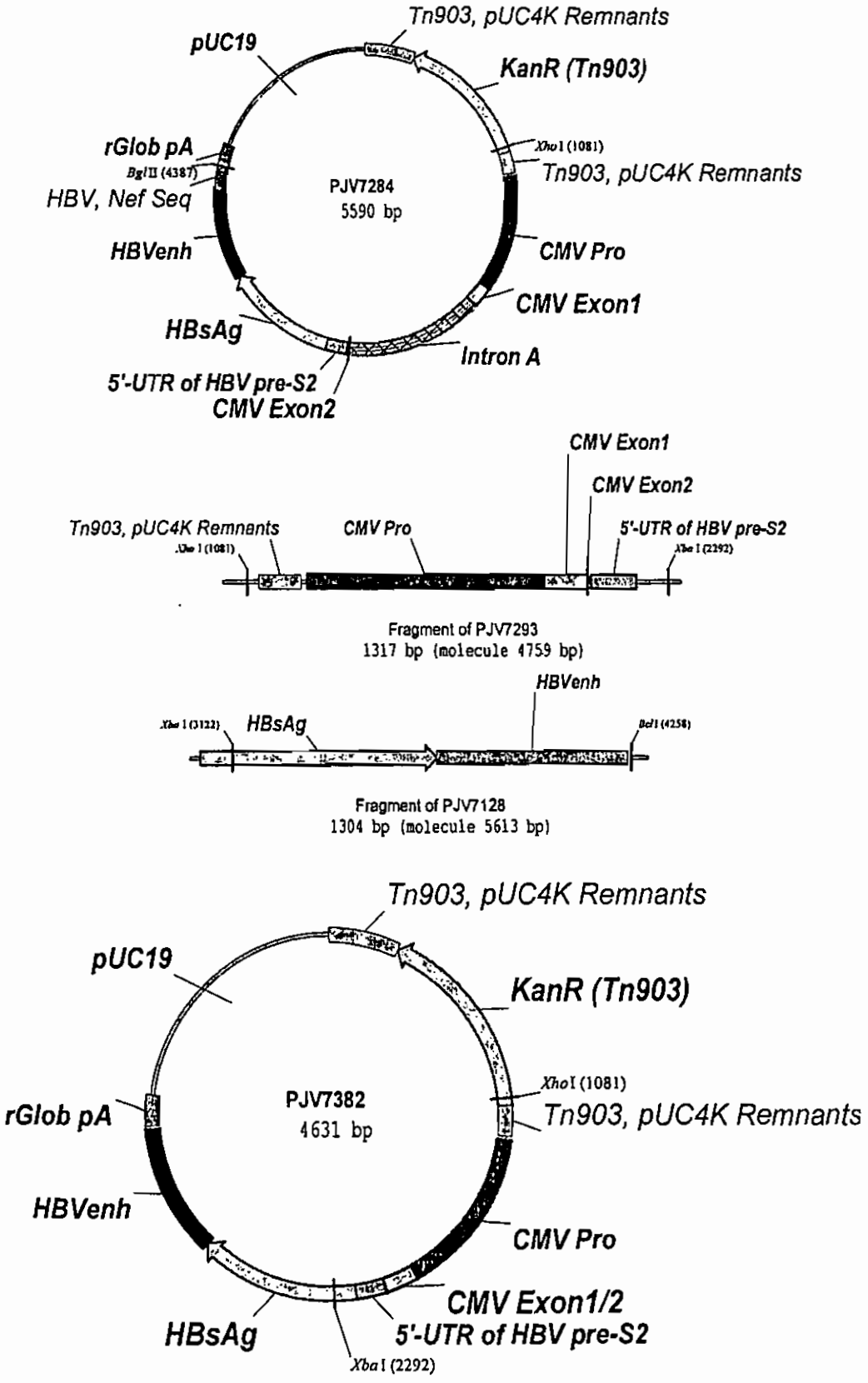

Tn903, pUC4K Remnants
pUC19
KanR (Tn903)
rGlob pA
BglII (4387)
XhoI (1081)
Tn903, pUC4K Remnants
PJV7284
5590 bp
HBV, Nef Seq
HBVenh
CMV Pro
CMV Exon1
HBsAg
Intron A
5'-UTR of HBV pre-S2
CMV Exon2
CMV Exon1
CMV Exon2
Tn903, pUC4K Remnants
CMV Pro
5'-UTR of HBV pre-S2
XhoI (1081)
XhoI (2292)
Fragment of PJV7293
1317 bp (molecule 4759 bp)
HBVenh
XhoI (3122)
HBsAg
BclI (4258)
Fragment of PJV7128
1304 bp (molecule 5613 bp)
Tn903, pUC4K Remnants
pUC19
KanR (Tn903)
XhoI (1081)
PJV7382
4631 bp
rGlob pA
Tn903, pUC4K Remnants
HBVenh
CMV Pro
CMV Exon1/2
HBsAg
5'-UTR of HBV pre-S2
XbaI (2292)

Figure 15(c)

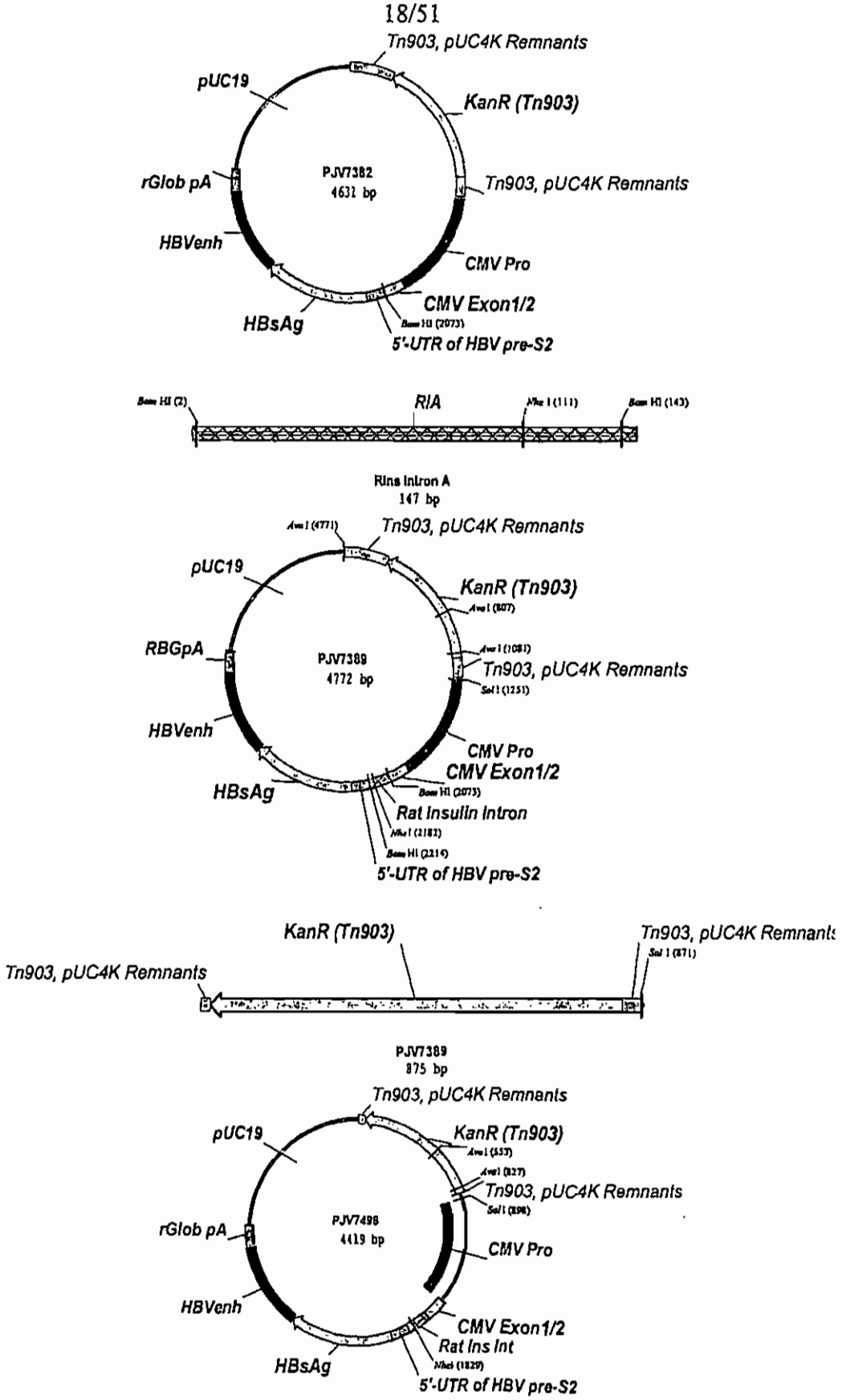

Tn903, pUC4K Remnants
pUC19
KanR (Tn903)
PJV7382
4631 bp
rGlob pA
Tn903, pUC4K Remnants
HBVenh
CMV Pro
CMV Exon1/2
HBsAg
5'-UTR of HBV pre-S2
RIA
Rins Intron A
147 bp
Tn903, pUC4K Remnants
pUC19
KanR (Tn903)
PJV7389
4772 bp
RBGpA
Tn903, pUC4K Remnants
HBVenh
CMV Pro
CMV Exon1/2
HBsAg
Rat Insulin Intron
5'-UTR of HBV pre-S2
KanR (Tn903)
Tn903, pUC4K Remnants
Tn903, pUC4K Remnants
PJV7389
875 bp
Tn903, pUC4K Remnants
pUC19
KanR (Tn903)
Tn903, pUC4K Remnants
PJV7498
4419 bp
rGlob pA
CMV Pro
HBVenh
CMV Exon1/2
Rat Ins Int
HBsAg
5'-UTR of HBV pre-S2

Figure 15(d)

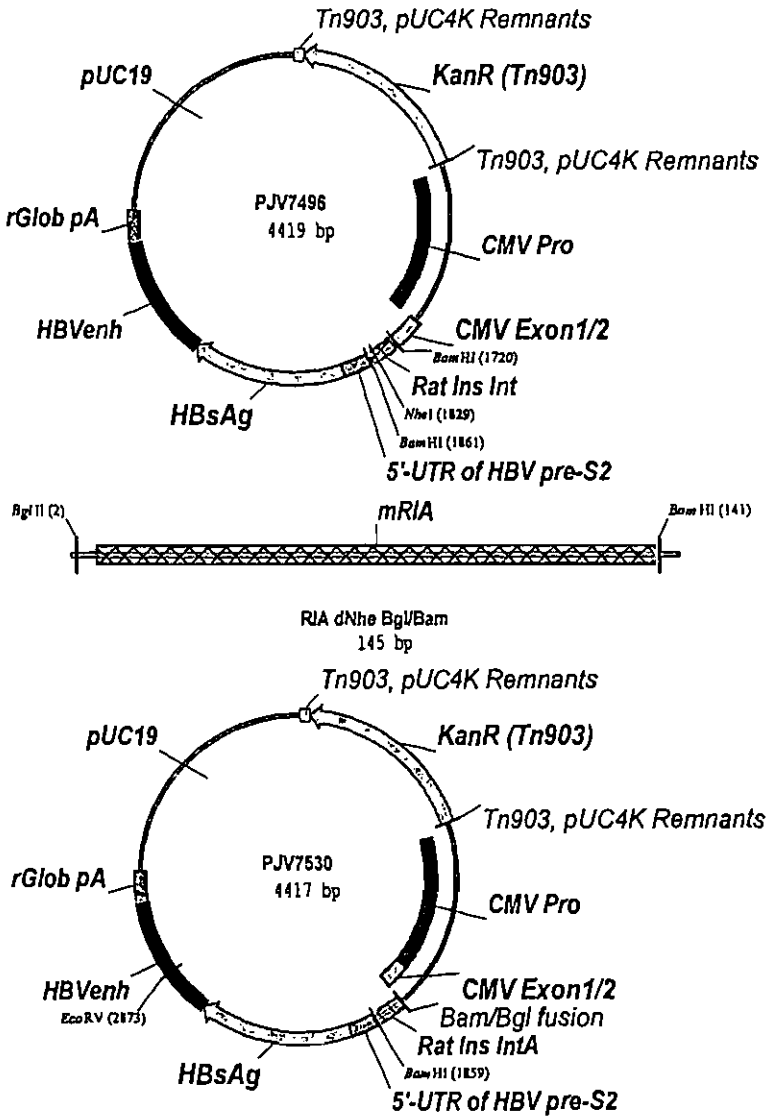

Tn903, pUC4K Remnants
pUC19
KanR (Tn903)
Tn903, pUC4K Remnants
PJV7496
4419 bp
rGlob pA
CMV Pro
HBVenh
CMV Exon1/2
BamHI (1720)
Rat Ins Int
NheI (1829)
HBsAg
BamHI (1861)
5'-UTR of HBV pre-S2
BglII (2)
mRIA
BamHI (141)
RIA dNhe Bgl/Bam
145 bp
Tn903, pUC4K Remnants
pUC19
KanR (Tn903)
Tn903, pUC4K Remnants
PJV7530
4417 bp
rGlob pA
CMV Pro
HBVenh
EcoRV (2873)
CMV Exon1/2
Bam/Bgl fusion
Rat Ins IntA
HBsAg
BamHI (1859)
5'-UTR of HBV pre-S2

Figure 15(e)

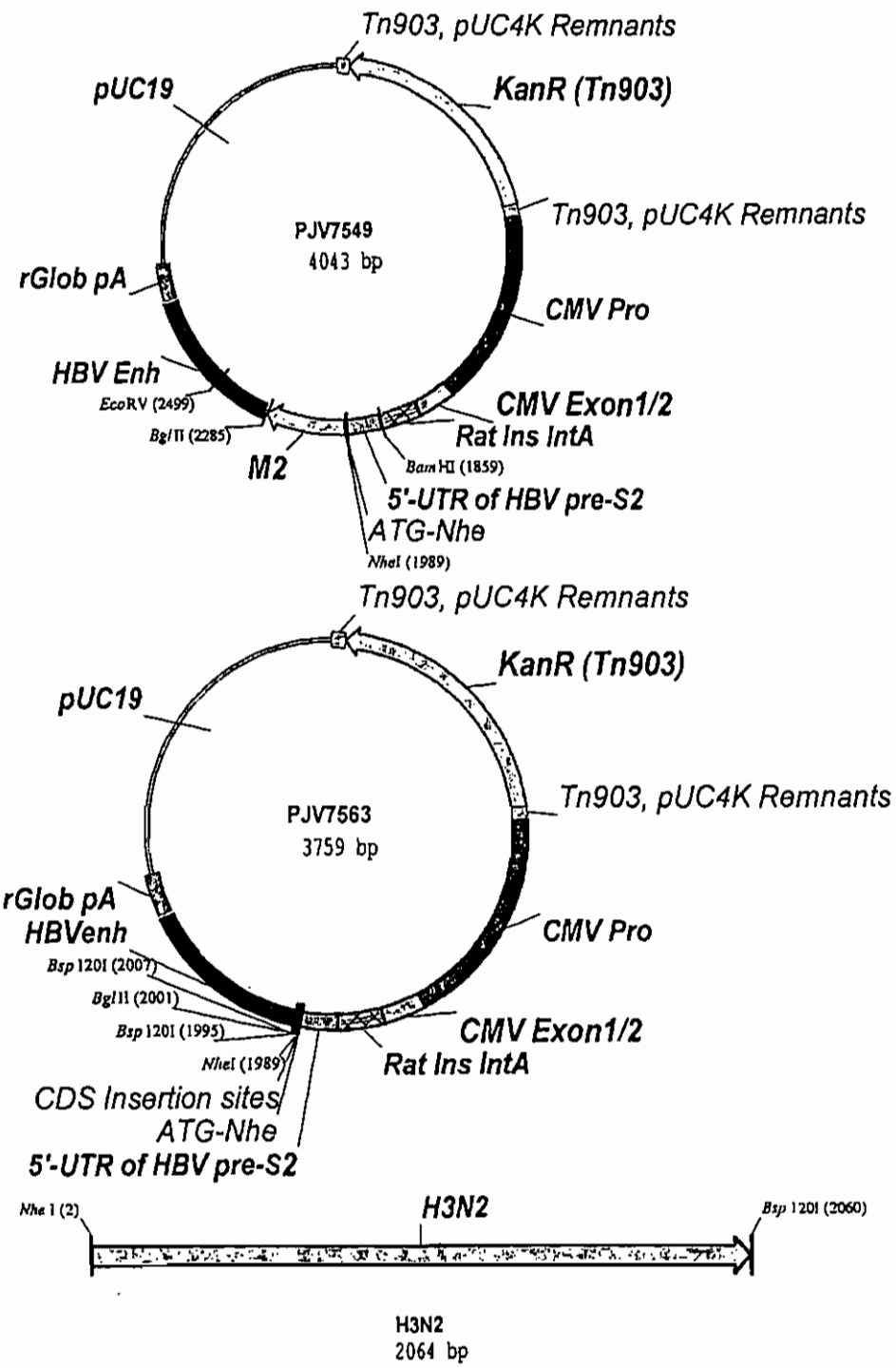
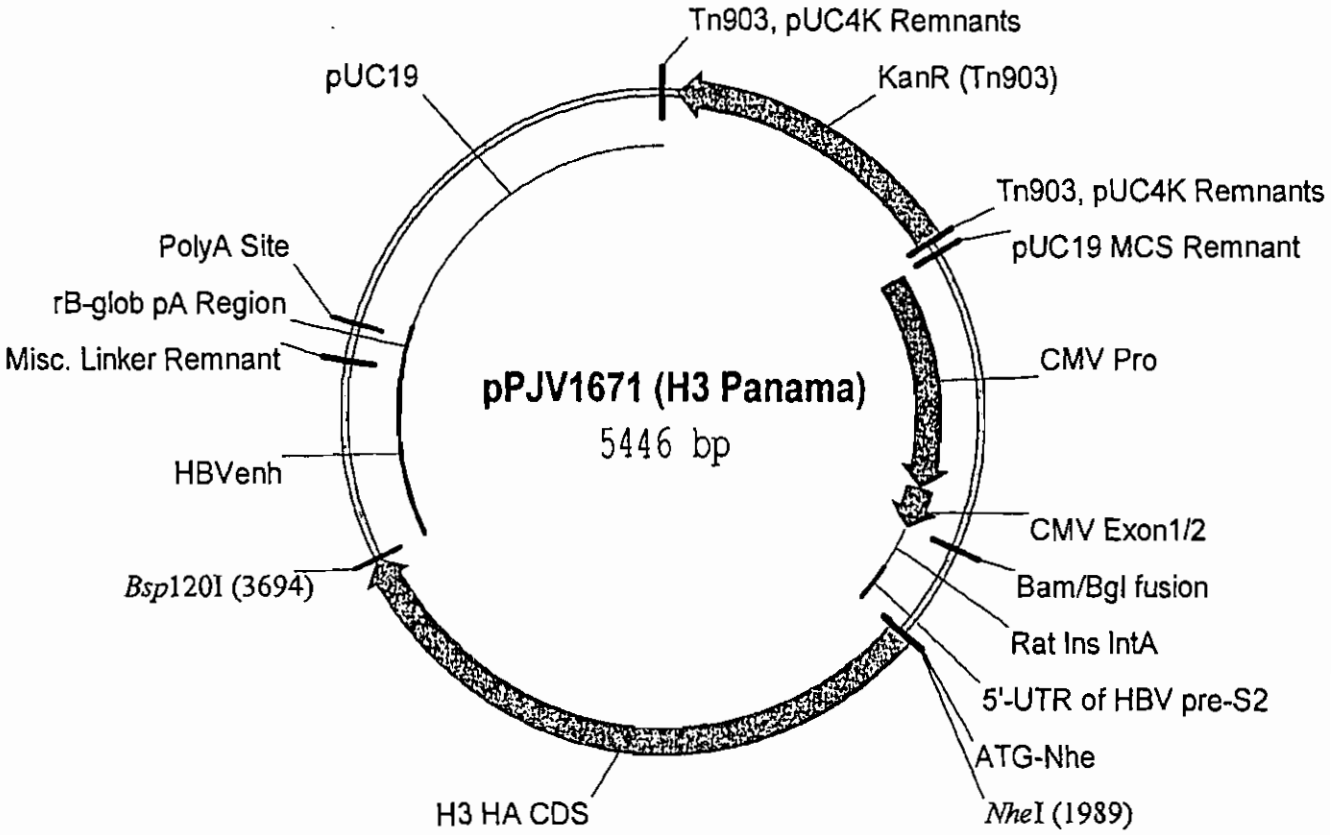

Tn903, pUC4K Remnants
pUC19
KanR (Tn903)
Tn903, pUC4K Remnants
PJV7549
4043 bp
rGlob pA
CMV Pro
HBV Enh
EcoRV (2499)
CMV Exon1/2
Bgl II (2285)
Rat Ins IntA
M2
BamHI (1859)
5'-UTR of HBV pre-S2
ATG-Nhe
NheI (1989)
Tn903, pUC4K Remnants
KanR (Tn903)
pUC19
Tn903, pUC4K Remnants
PJV7563
3759 bp
rGlob pA
HBVenh
CMV Pro
Bsp 120I (2007)
Bgl II (2001)
CMV Exon1/2
Bsp 120I (1995)
NheI (1989)
Rat Ins IntA
CDS Insertion sites
ATG-Nhe
5'-UTR of HBV pre-S2
Nhe I (2)
H3N2
Bsp 120I (2060)
H3N2
2064 bp

Figure 15(f)




```
                                  (1) 1        10        20        30        40        50        65
H3 Panama HA Natural Sequence     (1) --MKTIIALSYILCLVFAQKLPGNDNSTATLCLGHHAVSNGTLVKTITNDQIEVTNATELVQSSS
H3 Panama HA Encoded by pPJV1671  (1) MASKTIIALSYILCLVFAQKLPGNDNSTATLCLGHHAVSNGTLVKTITNDQIEVTNATELVQSSS
Consensus                         (1)    KTIIALSYILCLVFAQKLPGNDNSTATLCLGHHAVSNGTLVKTITNDQIEVTNATELVQSSS
```

Figure 16

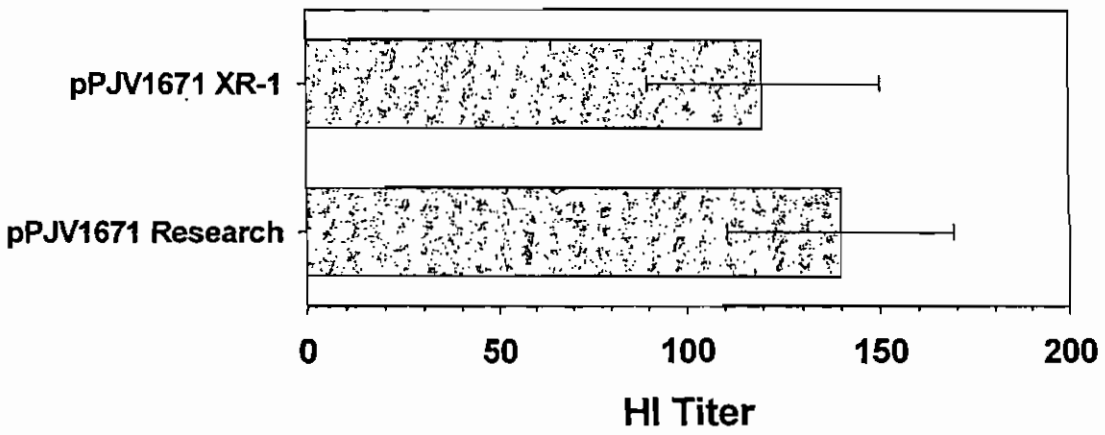


Figure 17

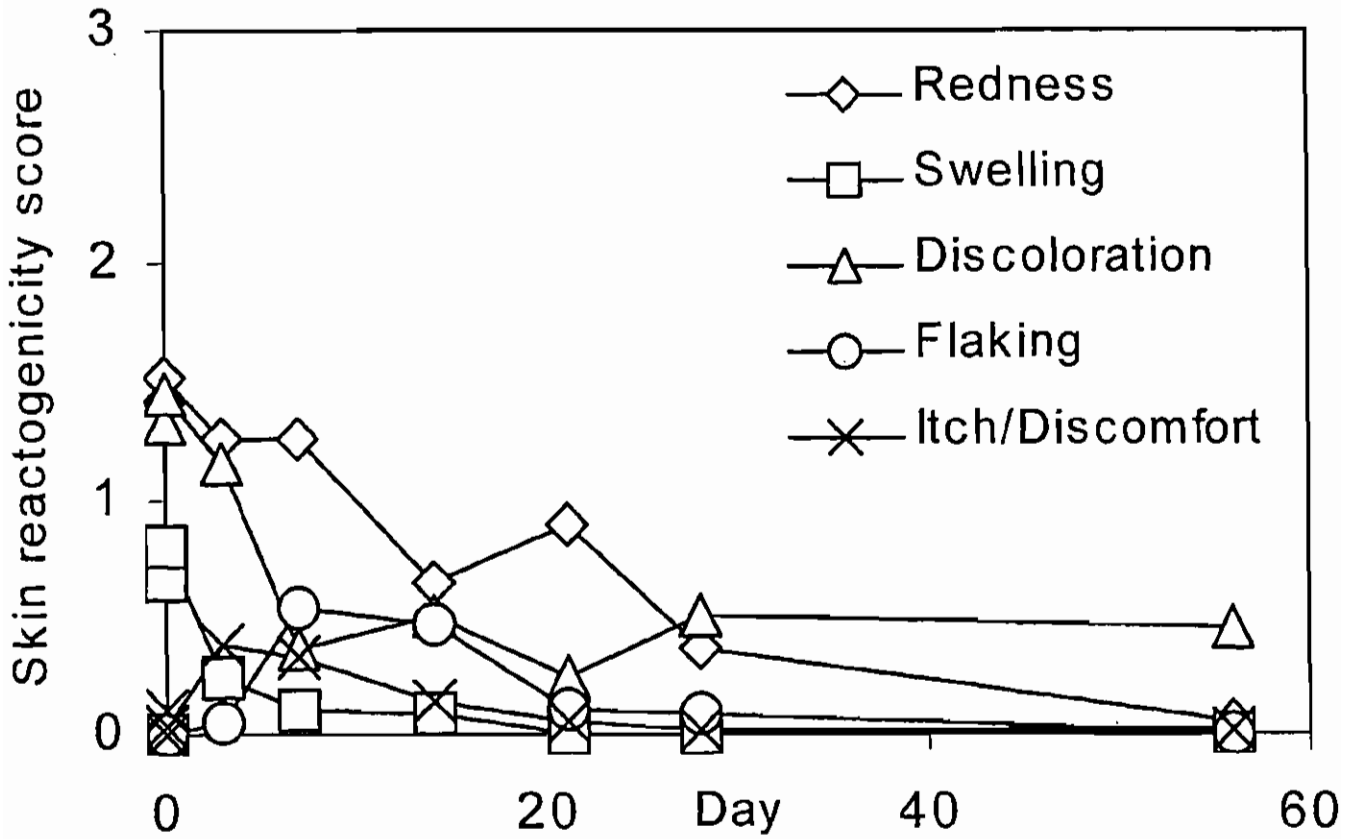

Skin reactogenicity score
3
2
1
0
0
20
Day
40
60
Redness
Swelling
Discoloration
Flaking
Itch/Discomfort

Figure 18

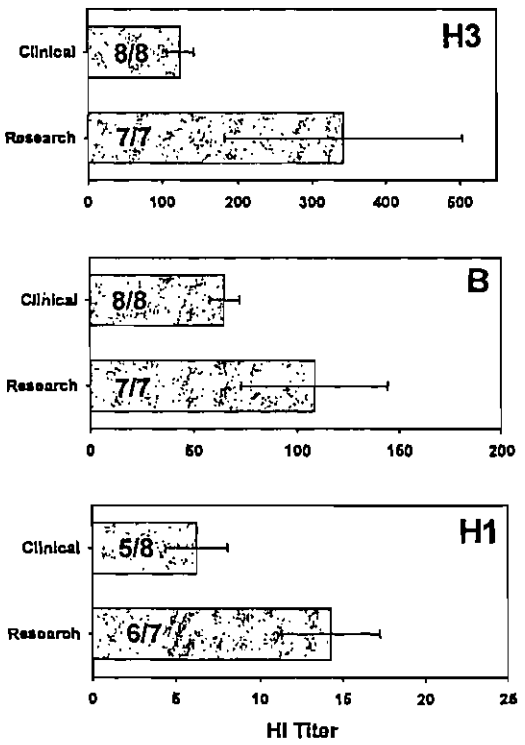

H3
Clinical
8/8
Research
7/7
0
100
200
300
400
500
B
Clinical
8/8
Research
7/7
0
50
100
150
200
H1
Clinical
5/8
Research
6/7
0
5
10
15
20
25
HI Titer

Figure 19

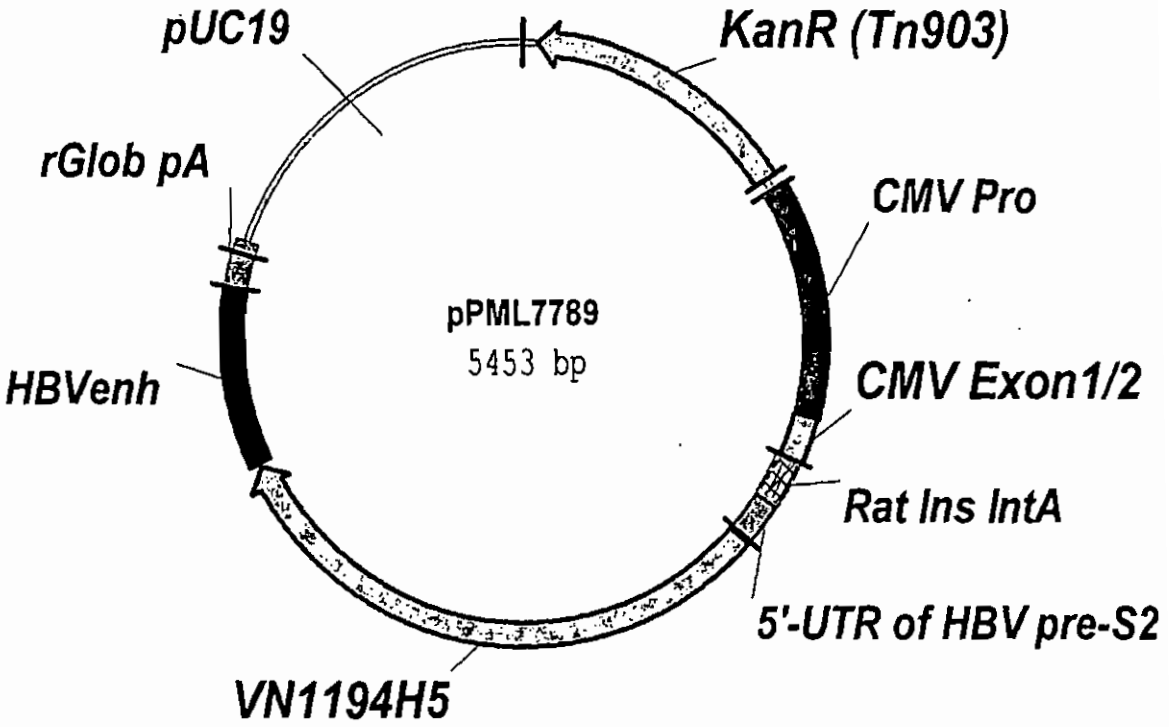

pUC19
KanR (Tn903)
rGlob pA
CMV Pro
pPML7789
5453 bp
HBVenh
CMV Exon1/2
Rat Ins IntA
5'-UTR of HBV pre-S2
VN1194H5

Figure 20

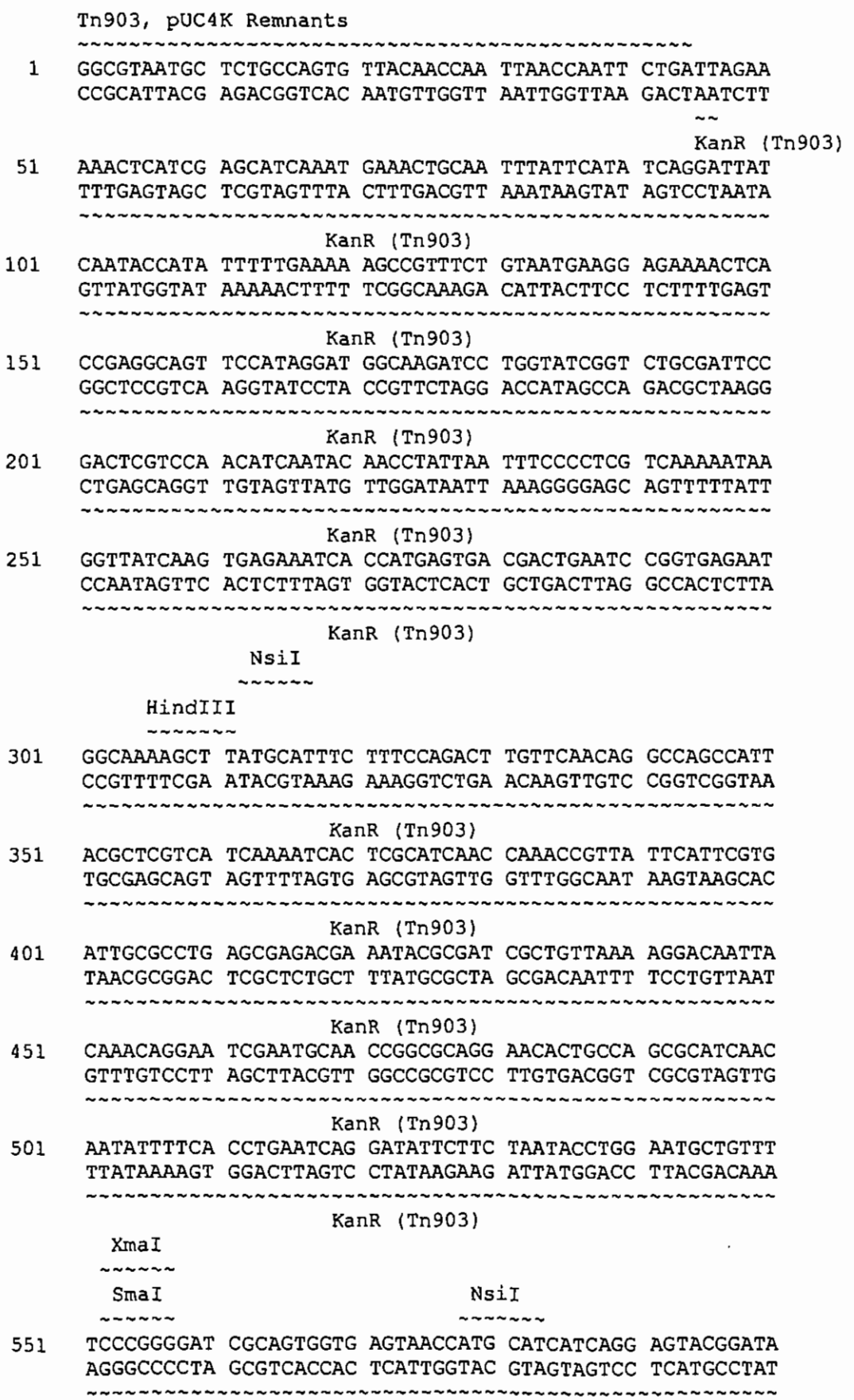

Tn903, pUC4K Remnants
1 GGCGTAATGC TCTGCCAGTG TTACAACCAA TTAACCAATT CTGATTAGAA
CCGCATTACG AGACGGTCAC AATGTTGGTT AATTGGTTAA GACTAATCTT
KanR (Tn903)
51 AAACTCATCG AGCATCAAAT GAAACTGCAA TTTATTCATA TCAGGATTAT
TTTGAGTAGC TCGTAGTTTA CTTTGACGTT AAATAAGTAT AGTCCTAATA
KanR (Tn903)
101 CAATACCATA TTTTTGAAAA AGCCGTTTCT GTAATGAAGG AGAAAACTCA
GTTATGGTAT AAAAACTTTT TCGGCAAAGA CATTACTTCC TCTTTTGAGT
KanR (Tn903)
151 CCGAGGCAGT TCCATAGGAT GGCAAGATCC TGGTATCGGT CTGCGATTCC
GGCTCCGTCA AGGTATCCTA CCGTTCTAGG ACCATAGCCA GACGCTAAGG
KanR (Tn903)
201 GACTCGTCCA ACATCAATAC AACCTATTAA TTTCCCCTCG TCAAAAATAA
CTGAGCAGGT TGTAGTTATG TTGGATAATT AAAGGGGAGC AGTTTTTATT
KanR (Tn903)
NsiI
HindIII
301 GGCAAAAGCT TATGCATTTC TTTCCAGACT TGTTCAACAG GCCAGCCATT
CCGTTTTCGA ATACGTAAAG AAAGGTCTGA ACAAGTTGTC CGGTCGGTAA
KanR (Tn903)
351 ACGCTCGTCA TCAAAATCAC TCGCATCAAC CAAACCGTTA TTCATTCGTG
TGCGAGCAGT AGTTTTAGTG AGCGTAGTTG GTTTGGCAAT AAGTAAGCAC
KanR (Tn903)
401 ATTGCGCCTG AGCGAGACGA AATACGCGAT CGCTGTTAAA AGGACAATTA
TAACGCGGAC TCGCTCTGCT TTATGCGCTA GCGACAATTT TCCTGTTAAT
KanR (Tn903)
451 CAAACAGGAA TCGAATGCAA CCGGCGCAGG AACACTGCCA GCGCATCAAC
GTTTGTCCTT AGCTTACGTT GGCCGCGTCC TTGTGACGGT CGCGTAGTTG
KanR (Tn903)
501 AATATTTTCA CCTGAATCAG GATATTCTTC TAATACCTGG AATGCTGTTT
TTATAAAAGT GGACTTAGTC CTATAAGAAG ATTATGGACC TTACGACAAA
KanR (Tn903)
XmaI
SmaI
NsiI
551 TCCCGGGGAT CGCAGTGGTG AGTAACCATG CATCATCAGG AGTACGGATA
AGGGCCCCTA GCGTCACCAC TCATTGGTAC GTAGTAGTCC TCATGCCTAT

Figure 21(a)

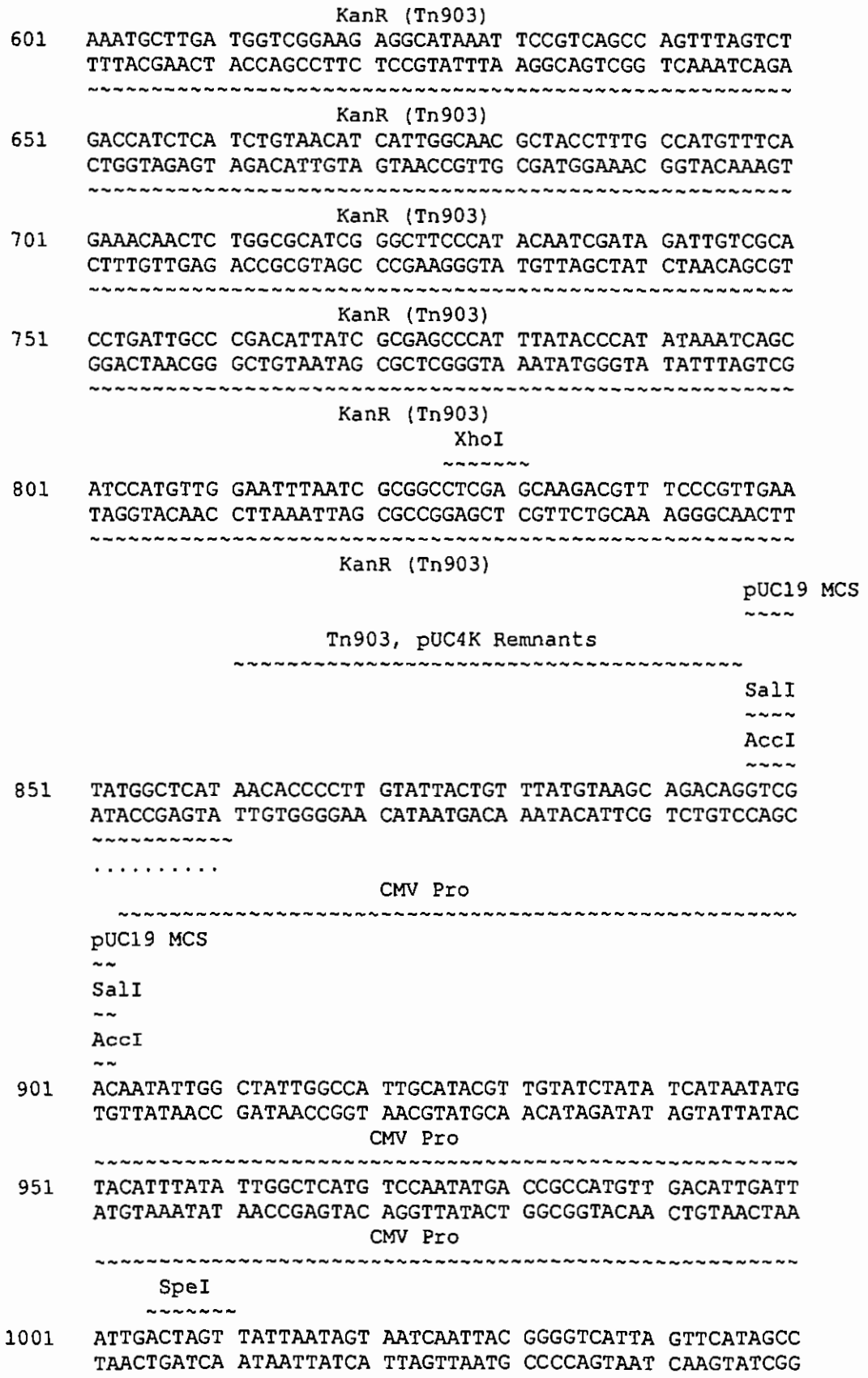

KanR (Tn903)
601 AAATGCTTGA TGGTCGGAAG AGGCATAAAT TCCGTCAGCC AGTTTAGTCT
TTTACGAACT ACCAGCCTTC TCCGTATTTA AGGCAGTCGG TCAAATCAGA
KanR (Tn903)
651 GACCATCTCA TCTGTAACAT CATTGGCAAC GCTACCTTTG CCATGTTTCA
CTGGTAGAGT AGACATTGTA GTAACCGTTG CGATGGAAAC GGTACAAAGT
KanR (Tn903)
701 GAAACAACTC TGGCGCATCG GGCTTCCCAT ACAATCGATA GATTGTCGCA
CTTTGTTGAG ACCGCGTAGC CCGAAGGGTA TGTTAGCTAT CTAACAGCGT
KanR (Tn903)
751 CCTGATTGCC CGACATTATC GCGAGCCCAT TTATACCCAT ATAAATCAGC
GGACTAACGG GCTGTAATAG CGCTCGGGTA AATATGGGTA TATTTAGTCG
KanR (Tn903)
XhoI
801 ATCCATGTTG GAATTTAATC GCGGCCTCGA GCAAGACGTT TCCCGTTGAA
TAGGTACAAC CTTAAATTAG CGCCGGAGCT CGTTCTGCAA AGGGCAACTT
KanR (Tn903)
pUC19 MCS
Tn903, pUC4K Remnants
SalI
AccI
851 TATGGCTCAT AACACCCCTT GTATTACTGT TTATGTAAGC AGACAGGTCG
ATACCGAGTA TTGTGGGGAA CATAATGACA AATACATTCG TCTGTCCAGC
CMV Pro
pUC19 MCS
SalI
AccI
901 ACAATATTGG CTATTGGCCA TTGCATACGT TGTATCTATA TCATAATATG
TGTTATAACC GATAACCGGT AACGTATGCA ACATAGATAT AGTATTATAC
CMV Pro
951 TACATTTATA TTGGCTCATG TCCAATATGA CCGCCATGTT GACATTGATT
ATGTAAATAT AACCGAGTAC AGGTTATACT GGCGGTACAA CTGTAACTAA
CMV Pro
SpeI
1001 ATTGACTAGT TATTAATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC
TAACTGATCA ATAATTATCA TTAGTTAATG CCCCAGTAAT CAAGTATCGG

Figure 21(b)

```
                          CMV Pro
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1051  CATATATGGA GTTCCGCGTT ACATAACTTA CGGTAAATGG CCCGCCTGGC
      GTATATACCT CAAGGCGCAA TGTATTGAAT GCCATTTACC GGGCGGACCG
                          CMV Pro
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1101  TGACCGCCCA ACGACCCCCG CCCATTGACG TCAATAATGA CGTATGTTCC
      ACTGGCGGGT TGCTGGGGGC GGGTAACTGC AGTTATTACT GCATACAAGG
                          CMV Pro
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1151  CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGAGTATT
      GTATCATTGC GGTTATCCCT GAAAGGTAAC TGCAGTTACC CACCTCATAA
                          CMV Pro
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                    NdeI
                                                   ~~~~~~~
1201  TACGGTAAAC TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT
      ATGCCATTTG ACGGGTGAAC CGTCATGTAG TTCACATAGT ATACGGTTCA
                          CMV Pro
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1251  CCGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC
      GGCGGGGGAT AACTGCAGTT ACTGCCATTT ACCGGGCGGA CCGTAATACG
                          CMV Pro
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1301  CCAGTACATG ACCTTACGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT
      GGTCATGTAC TGGAATGCCC TGAAAGGATG AACCGTCATG TAGATGCATA
                          CMV Pro
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                     NcoI
                    ~~~~~~~
1351  TAGTCATCGC TATTACCATG GTGATGCGGT TTTGGCAGTA CACCAATGGG
      ATCAGTAGCG ATAATGGTAC CACTACGCCA AAACCGTCAT GTGGTTACCC
                          CMV Pro
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1401  CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA
      GCACCTATCG CCAAACTGAG TGCCCCTAAA GGTTCAGAGG TGGGGTAACT
                          CMV Pro
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1451  CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT
      GCAGTTACCC TCAAACAAAA CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA
                          CMV Pro
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1501  GTCGTAATAA CCCCGCCCCG TTGACGCAAA TGGGCGGTAG GCGTGTACGG
      CAGCATTATT GGGGCGGGGC AACTGCGTTT ACCCGCCATC CGCACATGCC
                                                  CMV Exon1/2
                                                 ~~~~~~~~~~~~~
                     CMV Pro
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          SacI
                         ~~~~~~~
1551  TGGGAGGTCT ATATAAGCAG AGCTCGTTTA GTGAACCGTC AGATCGCCTG
      ACCCTCCAGA TATATTCGTC TCGAGCAAAT CACTTGGCAG TCTAGCGGAC
                       CMV Exon1/2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1601  GAGACGCCAT CCACGCTGTT TTGACCTCCA TAGAAGACAC CGGGACCGAT
      CTCTGCGGTA GGTGCGACAA AACTGGAGGT ATCTTCTGTG GCCCTGGCTA
```

Figure 21(c)

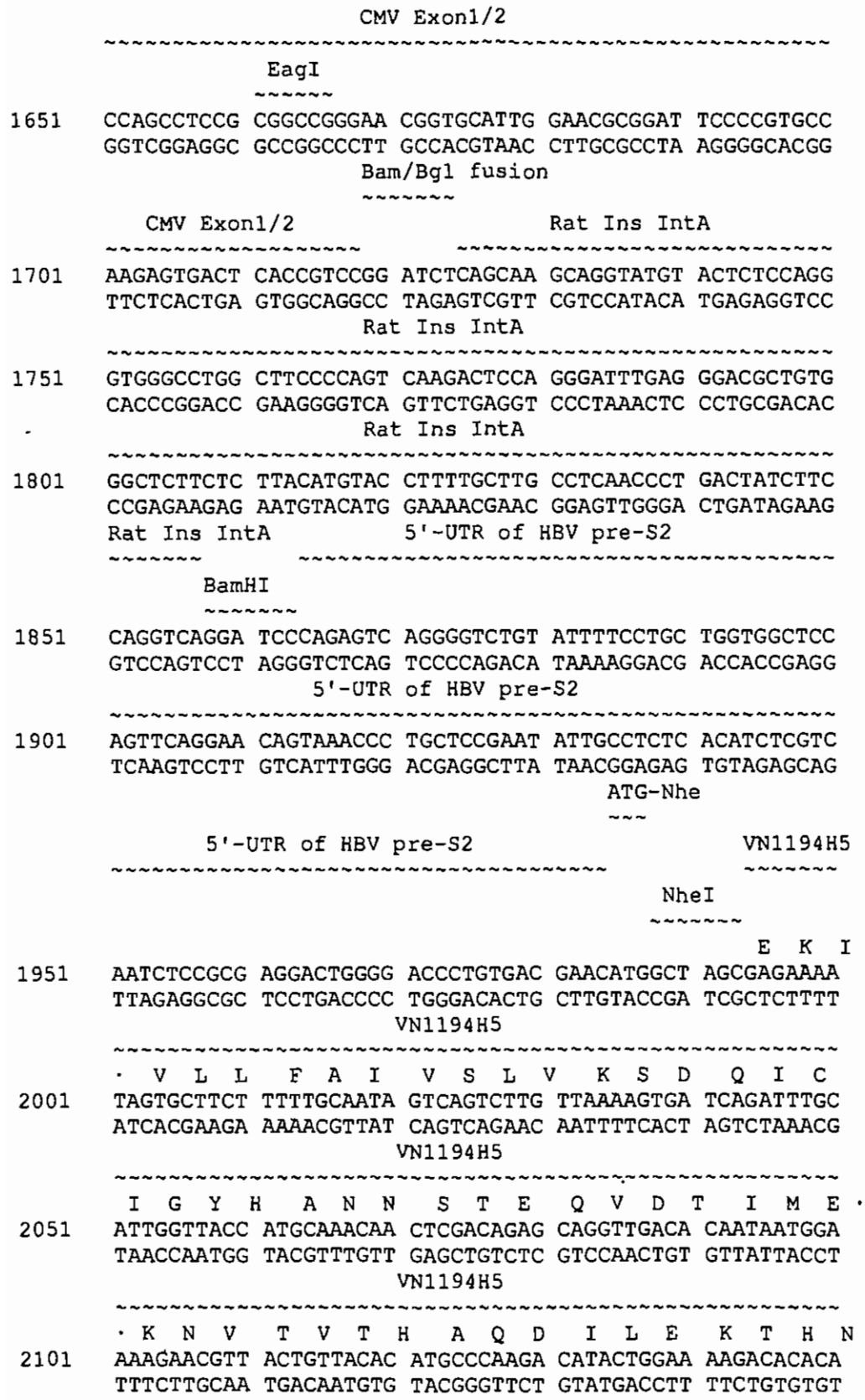

CMV Exon1/2
EagI
1651 CCAGCCTCCG CGGCCGGGAA CGGTGCATTG GAACGCGGAT TCCCCGTGCC
GGTCGGAGGC GCCGGCCCTT GCCACGTAAC CTTGCGCCTA AGGGGCACGG
Bam/Bg1 fusion
CMV Exon1/2
Rat Ins IntA
1701 AAGAGTGACT CACCGTCCGG ATCTCAGCAA GCAGGTATGT ACTCTCCAGG
TTCTCACTGA GTGGCAGGCC TAGAGTCGTT CGTCCATACA TGAGAGGTCC
Rat Ins IntA
1751 GTGGGCCTGG CTTCCCCAGT CAAGACTCCA GGGATTTGAG GGACGCTGTG
CACCCGGACC GAAGGGGTCA GTTCTGAGGT CCCTAAACTC CCTGCGACAC
Rat Ins IntA
1801 GGCTCTTCTC TTACATGTAC CTTTTGCTTG CCTCAACCCT GACTATCTTC
CCGAGAAGAG AATGTACATG GAAAACGAAC GGAGTTGGGA CTGATAGAAG
Rat Ins IntA
5'-UTR of HBV pre-S2
BamHI
1851 CAGGTCAGGA TCCCAGAGTC AGGGGTCTGT ATTTTCCTGC TGGTGGCTCC
GTCCAGTCCT AGGGTCTCAG TCCCCAGACA TAAAAGGACG ACCACCGAGG
5'-UTR of HBV pre-S2
1901 AGTTCAGGAA CAGTAAACCC TGCTCCGAAT ATTGCCTCTC ACATCTCGTC
TCAAGTCCTT GTCATTTGGG ACGAGGCTTA TAACGGAGAG TGTAGAGCAG
ATG-Nhe
5'-UTR of HBV pre-S2
VN1194H5
NheI
E K I ·
1951 AATCTCCGCG AGGACTGGGG ACCCTGTGAC GAACATGGCT AGCGAGAAAA
TTAGAGGCGC TCCTGACCCC TGGGACACTG CTTGTACCGA TCGCTCTTTT
VN1194H5
· V L L F A I V S L V K S D Q I C
2001 TAGTGCTTCT TTTTGCAATA GTCAGTCTTG TTAAAAGTGA TCAGATTTGC
ATCACGAAGA AAAACGTTAT CAGTCAGAAC AATTTTCACT AGTCTAAACG
VN1194H5
I G Y H A N N S T E Q V D T I M E ·
2051 ATTGGTTACC ATGCAAACAA CTCGACAGAG CAGGTTGACA CAATAATGGA
TAACCAATGG TACGTTTGTT GAGCTGTCTC GTCCAACTGT GTTATTACCT
VN1194H5
· K N V T V T H A Q D I L E K T H N ·
2101 AAAGAACGTT ACTGTTACAC ATGCCCAAGA CATACTGGAA AAGACACACA
TTTCTTGCAA TGACAATGTG TACGGGTTCT GTATGACCTT TTCTGTGTGT

Figure 21(d)

```
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                       XbaI
                      ~~~~~~~
      · G  K  L   C  D  L   D  G  V  K   P  L  I   L  R  D
2151  ATGGGAAGCT CTGCGATCTA GATGGAGTGA AGCCTCTAAT TTTGAGAGAT
      TACCCTTCGA GACGCTAGAT CTACCTCACT TCGGAGATTA AAACTCTCTA
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                        EcoRI
                                                       ~~~~~~
       C  S  V  A   G  W  L   L  G  N   P  M  C  D   E  F  I ·
2201  TGTAGTGTAG CTGGATGGCT CCTCGGAAAC CCAATGTGTG ACGAATTCAT
      ACATCACATC GACCTACCGA GGAGCCTTTG GGTTACACAC TGCTTAAGTA
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      ·N  V  P   E  W  S  Y   I  V  E   K  A  N   P  V  N  D ·
2251  CAATGTGCCG GAATGGTCTT ACATAGTGGA GAAGGCCAAT CCAGTCAATG
      GTTACACGGC CTTACCAGAA TGTATCACCT CTTCCGGTTA GGTCAGTTAC
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      · L  C  Y   P  G  D   F  N  D  Y   E  E  L   K  H  L
2301  ACCTCTGTTA CCCAGGGGAT TTCAATGACT ATGAAGAATT GAAACACCTA
      TGGAGACAAT GGGTCCCCTA AAGTTACTGA TACTTCTTAA CTTTGTGGAT
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       L  S  R  I   N  H  F   E  K  I   Q  I  I  P   K  S  S ·
2351  TTGAGCAGAA TAAACCATTT TGAGAAAATT CAGATCATCC CCAAAAGTTC
      AACTCGTCTT ATTTGGTAAA ACTCTTTTAA GTCTAGTAGG GGTTTTCAAG
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                      SacI
                                     ~~~~~~
      · W  S  S   H  E  A  S   L  G  V   S  S  A   C  P  Y  Q ·
2401  TTGGTCCAGT CATGAAGCCT CATTGGGGGT GAGCTCAGCA TGTCCATACC
      AACCAGGTCA GTACTTCGGA GTAACCCCCA CTCGAGTCGT ACAGGTATGG
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      · G  K  S   S  F  F   R  N  V  V   W  L  I   K  K  N
2451  AGGGAAAGTC CTCCTTTTTC AGAAATGTGG TATGGCTTAT CAAAAAGAAC
      TCCCTTTCAG GAGGAAAAAG TCTTTACACC ATACCGAATA GTTTTTCTTG
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                       BglII
                                                       ~~~
       S  T  Y  P   T  I  K   R  S  Y   N  N  T  N   Q  E  D ·
2501  AGTACATACC CAACAATAAA GAGGAGCTAC AATAATACCA ACCAAGAAGA
      TCATGTATGG GTTGTTATTT CTCCTCGATG TTATTATGGT TGGTTCTTCT
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

Figure 21(e)

```
      BglII
      ~~~
      · L  L  V   L  W  G  I   H  H  P   N  D  A   A  E  Q  T ·
2551  TCTTTTGGTA CTGTGGGGGA TTCACCATCC TAATGATGCG GCAGAGCAGA
      AGAAAACCAT GACACCCCCT AAGTGGTAGG ATTACTACGC CGTCTCGTCT
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      · K  L  Y   Q  N  P   T  T  Y  I   S  V  G   T  S  T
2601  CAAAGCTCTA TCAAAACCCA ACCACCTATA TTTCCGTTGG GACATCAACA
      GTTTCGAGAT AGTTTTGGGT TGGTGGATAT AAAGGCAACC CTGTAGTTGT
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       L  N  Q  R   L  V  P   R  I  A   T  R  S  K   V  N  G ·
2651  CTAAACCAGA GATTGGTACC AAGAATAGCT ACTAGATCCA AAGTAAACGG
      GATTTGGTCT CTAACCATGG TTCTTATCGA TGATCTAGGT TTCATTTGCC
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      · Q  S  G   R  M  E  F   F  W  T   I  L  K   P  N  D  A ·
2701  GCAAAGTGGA AGGATGGAGT TCTTCTGGAC AATTTTAAAA CCGAATGATG
      CGTTTCACCT TCCTACCTCA AGAAGACCTG TTAAAATTTT GGCTTACTAC
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                    NsiI
                                                    ~~~~~~
      · I  N  F   E  S  N   G  N  F  I   A  P  E   Y  A  Y
2751  CAATCAACTT CGAGAGTAAT GGAAATTTCA TTGCTCCAGA ATATGCATAC
      GTTAGTTGAA GCTCTCATTA CCTTTAAAGT AACGAGGTCT TATACGTATG
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       K  I  V  K   K  G  D   S  T  I   M  K  S  E   L  E  Y ·
2801  AAAATTGTCA AGAAAGGGGA CTCAACAATT ATGAAAAGTG AATTGGAATA
      TTTTAACAGT TCTTTCCCCT GAGTTGTTAA TACTTTTCAC TTAACCTTAT
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      · G  N  C   N  T  K  C   Q  T  P   M  G  A   I  N  S  S ·
2851  TGGTAACTGC AACACCAAGT GTCAAACTCC AATGGGGGCG ATAAACTCTA
      ACCATTGACG TTGTGGTTCA CAGTTTGAGG TTACCCCCGC TATTTGAGAT
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       SphI
       ~~~~~~
      · M  P  F   H  N  I   H  P  L  T   I  G  E   C  P  K
2901  GCATGCCATT CCACAATATA CACCCTCTCA CCATCGGGGA ATGCCCCAAA
      CGTACGGTAA GGTGTTATAT GTGGGAGAGT GGTAGCCCCT TACGGGGTTT
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       Y  V  K  S   N  R  L   V  L  A   T  G  L  R   N  S  P ·
2951  TATGTGAAAT CAAACAGATT AGTCCTTGCG ACTGGGCTCA GAAATAGCCC
      ATACACTTTA GTTTGTCTAA TCAGGAACGC TGACCCGAGT CTTTATCGGG
                          VN1194H5
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      · Q  R  E   R  R  R  K   K  R  G   L  F  G   A  I  A  G ·
3001  TCAAAGAGAG AGAAGAAGAA AAAAGAGAGG ATTATTTGGA GCTATAGCAG
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                          VN1194H5
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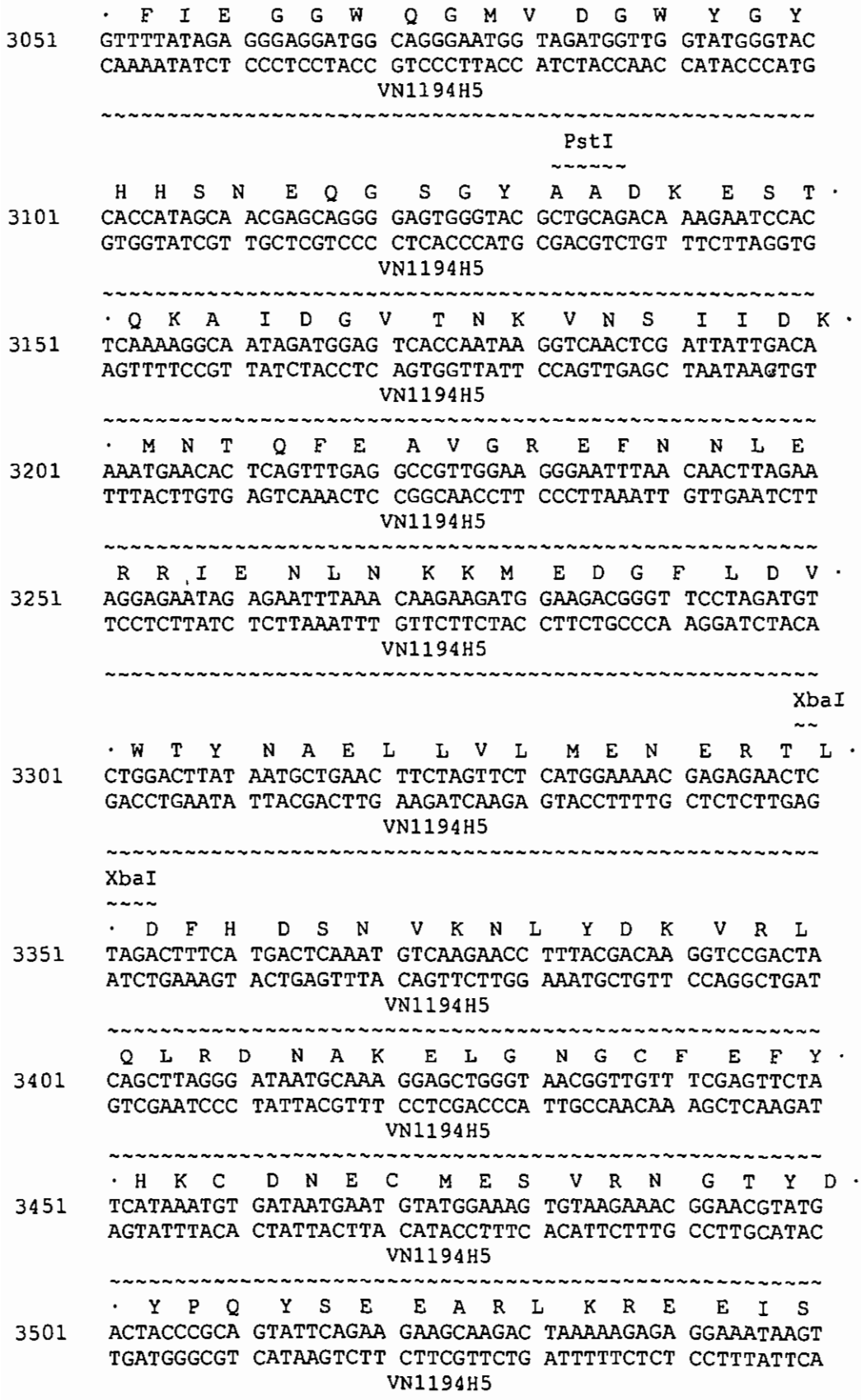
Figure 21(f)


· F I E G G W Q G M V D G W Y G Y
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CAAAATATCT CCCTCCTACC GTCCCTTACC ATCTACCAAC CATACCCATG
VN1194H5
PstI
H H S N E Q G S G Y A A D K E S T ·
3101 CACCATAGCA ACGAGCAGGG GAGTGGGTAC GCTGCAGACA AAGAATCCAC
GTGGTATCGT TGCTCGTCCC CTCACCCATG CGACGTCTGT TTCTTAGGTG
VN1194H5
· Q K A I D G V T N K V N S I I D K ·
3151 TCAAAAGGCA ATAGATGGAG TCACCAATAA GGTCAACTCG ATTATTGACA
AGTTTTCCGT TATCTACCTC AGTGGTTATT CCAGTTGAGC TAATAAGTGT
VN1194H5
· M N T Q F E A V G R E F N N L E
3201 AAATGAACAC TCAGTTTGAG GCCGTTGGAA GGGAATTTAA CAACTTAGAA
TTTACTTGTG AGTCAAACTC CGGCAACCTT CCCTTAAATT GTTGAATCTT
VN1194H5
R R I E N L N K K M E D G F L D V ·
3251 AGGAGAATAG AGAATTTAAA CAAGAAGATG GAAGACGGGT TCCTAGATGT
TCCTCTTATC TCTTAAATTT GTTCTTCTAC CTTCTGCCCA AGGATCTACA
VN1194H5
XbaI
· W T Y N A E L L V L M E N E R T L ·
3301 CTGGACTTAT AATGCTGAAC TTCTAGTTCT CATGGAAAAC GAGAGAACTC
GACCTGAATA TTACGACTTG AAGATCAAGA GTACCTTTTG CTCTCTTGAG
VN1194H5
XbaI
· D F H D S N V K N L Y D K V R L
3351 TAGACTTTCA TGACTCAAAT GTCAAGAACC TTTACGACAA GGTCCGACTA
ATCTGAAAGT ACTGAGTTTA CAGTTCTTGG AAATGCTGTT CCAGGCTGAT
VN1194H5
Q L R D N A K E L G N G C F E F Y ·
3401 CAGCTTAGGG ATAATGCAAA GGAGCTGGGT AACGGTTGTT TCGAGTTCTA
GTCGAATCCC TATTACGTTT CCTCGACCCA TTGCCAACAA AGCTCAAGAT
VN1194H5
· H K C D N E C M E S V R N G T Y D ·
3451 TCATAAATGT GATAATGAAT GTATGGAAAG TGTAAGAAAC GGAACGTATG
AGTATTTACA CTATTACTTA CATACCTTTC ACATTCTTTG CCTTGCATAC
VN1194H5
· Y P Q Y S E E A R L K R E E I S
3501 ACTACCCGCA GTATTCAGAA GAAGCAAGAC TAAAAAGAGA GGAAATAAGT
TGATGGGCGT CATAAGTCTT CTTCGTTCTG ATTTTTCTCT CCTTTATTCA
VN1194H5

Figure 21(g)

```
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
         G  V  K  L   E  S  I   G  I  Y   Q  I  L  S   I  Y  S ·
3551    GGAGTAAAAT TGGAATCAAT AGGAATTTAC CAAATATTGT CAATTTATTC
        CCTCATTTTA ACCTTAGTTA TCCTTAAATG GTTTATAACA GTTAAATAAG
                              VN1194H5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                  SacI
                  ~~~~~~~
        · T  V  A   S  S  L  A   L  A  I   M  V  A   G  L  S  L ·
3601    TACAGTGGCG AGCTCCCTAG CACTGGCAAT CATGGTAGCT GGTCTATCCT
        ATGTCACCGC TCGAGGGATC GTGACCGTTA GTACCATCGA CCAGATAGGA
                           VN1194H5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                                Bsp120I
                                                                ~
        · W  M  C   S  N  G   S  L  Q  C   R  I  C   I
3651    TATGGATGTG CTCCAATGGG TCGTTACAAT GCAGAATTTG CATTTAAATG
        ATACCTACAC GAGGTTACCC AGCAATGTTA CGTCTTAAAC GTAAATTTAC
                                 HBVenh
             ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        Bsp120I
        ~~~~~
3701    GGCCCTAACA AAACAAAAAG ATGGGGTTAT TCCCTAAACT TCATGGGTTA
        CCGGGATTGT TTTGTTTTTC TACCCCAATA AGGGATTTGA AGTACCCAAT
                              HBVenh
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3751    CGTAATTGGA AGTTGGGGGA CATTGCCACA AGATCATATT GTACAAAAGA
        GCATTAACCT TCAACCCCCT GTAACGGTGT TCTAGTATAA CATGTTTTCT
                              HBVenh
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3801    TCAAACACTG TTTTAGAAAA CTTCCTGTAA ACAGGCCTAT TGATTGGAAA
        AGTTTGTGAC AAAATCTTTT GAAGGACATT TGTCCGGATA ACTAACCTTT
                              HBVenh
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3851    GTATGTCAAA GGATTGTGGG TCTTTTGGGC TTTGCTGCTC CATTTACACA
        CATACAGTTT CCTAACACCC AGAAAACCCG AAACGACGAG GTAAATGTGT
                              HBVenh
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                        NsiI
                                        ~~~~~~~
             EcoRV                                AccI
             ~~~~~~~                              ~~~~~~~
3901    ATGTGGATAT CCTGCCTTAA TGCCTTTGTA TGCATGTATA CAAGCTAAAC
        TACACCTATA GGACGGAATT ACGGAAACAT ACGTACATAT GTTCGATTTG
                              HBVenh
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3951    AGGCTTTCAC TTTCTCGCCA ACTTACAAGG CCTTTCTAAG TAAACAGTAC
        TCCGAAAGTG AAAGAGCGGT TGAATGTTCC GGAAAGATTC ATTTGTCATG
                              HBVenh
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4001    ATGAACCTTT ACCCCGTTGC TCGGCAACGG CCTGGTCTGT GCCAAGTGTT
        TACTTGGAAA TGGGGCAACG AGCCGTTGCC GGACCAGACA CGGTTCACAA
```

Figure 21(h)

```
                          HBVenh
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                                SphI
                                                                ~~~
4051  TGCTGACGCA ACCCCCACTG GCTGGGGCTT GGCCATAGGC CATCAGCGCA
      ACGACTGCGT TGGGGGTGAC CGACCCCGAA CCGGTATCCG GTAGTCGCGT
                          HBVenh
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      SphI
      ~~~
4101  TGCGTGGAAC CTTTGTGGCT CCTCTGCCGA TCCATACTGC GGAACTCCTA
      ACGCACCTTG GAAACACCGA GGAGACGGCT AGGTATGACG CCTTGAGGAT
                          HBVenh
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4151  GCCGCTTGTT TTGCTCGCAG CCGGTCTGGA GCAAAGCTCA TAGGAACTGA
      CGGCGAACAA AACGAGCGTC GGCCAGACCT CGTTTCGAGT ATCCTTGACT
                                                 Unknown
                                                ~~~~~~~~~~~~
                    HBVenh                                    rGlob pA
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4201  CAATTCTGTC GTCCTCTCGC GGAAATATAC ATCGTTTCGA TCTACGTATG
      GTTAAGACAG CAGGAGAGCG CCTTTATATG TAGCAAAGCT AGATGCATAC
                          rGlob pA
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4251  ATCTTTTTCC CTCTGCCAAA AATTATGGGG ACATCATGAA GCCCCTTGAG
      TAGAAAAAGG GAGACGGTTT TTAATACCCC TGTAGTACTT CGGGGAACTC
                                                  PolyA_Site_1
                                                  ~
                          rGlob pA
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4301  CATCTGACTT CTGGCTAATA AAGGAAATTT ATTTTCATTG CAATAGTGTG
      GTAGACTGAA GACCGATTAT TTCCTTTAAA TAAAAGTAAC GTTATCACAC
                rGlob pA                          pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~      ~~~~~~~~~~~~~~~~
                                        EcoRI
                                        ~~~~~~
4351  TTGGAATTTT TTGTGTCTCT CACTCGGAAG GAATTCTGCA TTAATGAATC
      AACCTTAAAA AACACAGAGA GTGAGCCTTC CTTAAGACGT AATTACTTAG
                          pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4401  GGCCAACGCG CGGGGAGAGG CGGTTTGCGT ATTGGGCGCT CTTCCGCTTC
      CCGGTTGCGC GCCCCTCTCC GCCAAACGCA TAACCCGCGA GAAGGCGAAG
                          pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4451  CTCGCTCACT GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT
      GAGCGAGTGA CTGAGCGACG CGAGCCAGCA AGCCGACGCC GCTCGCCATA
                          pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4501  CAGCTCACTC AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC
      GTCGAGTGAG TTTCCGCCAT TATGCCAATA GGTGTCTTAG TCCCCTATTG
                          pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4551  GCAGGAAAGA ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA
      CGTCCTTTCT TGTACACTCG TTTTCCGGTC GTTTTCCGGT CCTTGGCATT
                          pUC19
```

Figure 21(i)

```
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4601  AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC CCTGACGAGC
      TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG GGACTGCTCG
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4651  ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC GACAGGACTA
      TAGTGTTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG CTGTCCTGAT
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4701  TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT
      ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCACG CGAGAGGACA
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4751  TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC CCTTCGGGAA
      AGGCTGGGAC GGCGAATGGC CTATGGACAG GCGGAAAGAG GGAAGCCCTT
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4801  GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG TTCGGTGTAG
      CGCACCGCGA AAGAGTATCG AGTGCGACAT CCATAGAGTC AAGCCACATC
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4851  GTCGTTCGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG TTCAGCCCGA
      CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGC AAGTCGGGCT
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4901  CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAC CCGGTAAGAC
      GGCGACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG GGCCATTCTG
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4951  ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT TAGCAGAGCG
      TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCCTA ATCGTCTCGC
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5001  AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC CTAACTACGG
      TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG GATTGATGCC
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5051  CTACACTAGA AGAACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA
      GATGTGATCT TCTTGTCATA AACCATAGAC GCGAGACGAC TTCGGTCAAT
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5101  CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA AACCACCGCT
      GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT TTGGTGGCGA
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5151  GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAAA
      CCATCGCCAC CAAAAAAACA AACGTTCGTC GTCTAATGCG CGTCTTTTTT
                              pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5201  AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT GACGCTCAGT
      TCCTAGAGTT CTTCTAGGAA ACTAGAAAAG ATGCCCCAGA CTGCGAGTCA
```

Figure 21(j)

```
                          pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5251  GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT ATCAAAAAGG
      CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA TAGTTTTTCC
                          pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5301  ATCTTCACCT AGATCCTTTT AAATTAAAAA TGAAGTTTTA AATCAATCTA
      TAGAAGTGGA TCTAGGAAAA TTTAATTTTT ACTTCAAAAT TTAGTTAGAT
                          pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5351  AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG
      TTCATATATA CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC
                          pUC19
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5401  AGGCACCTAT CTCAGCGATC TGTCTATTTC GTTCATCCAT AGTTGCCTGA
      TCCGTGGATA GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT
      pUC19
      ~~~
5451  CTC
      GAG
```

Figure 21(k)

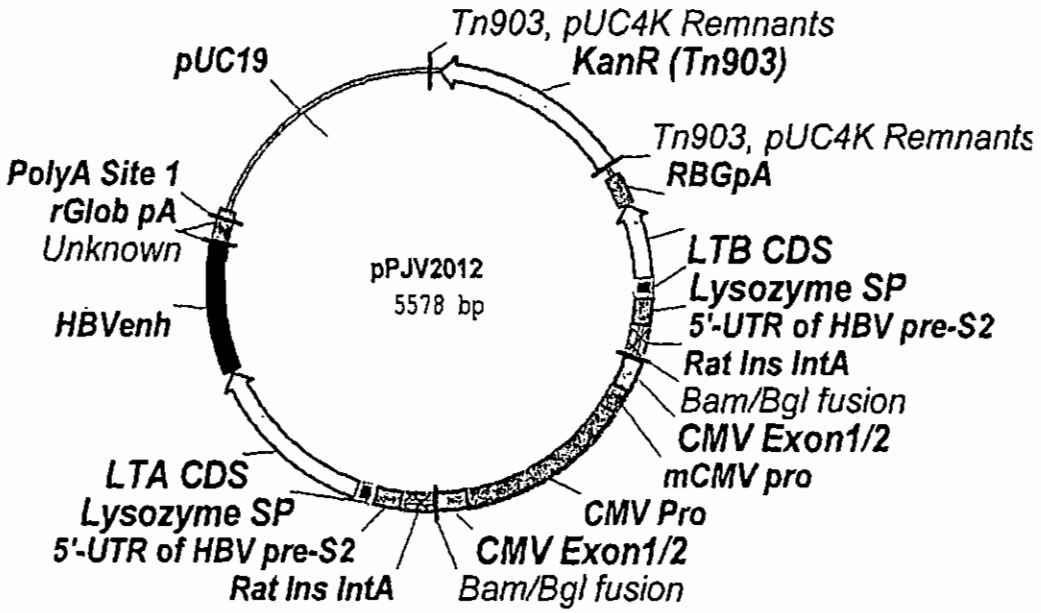
Tn903, pUC4K Remnants
KanR (Tn903)
pUC19
Tn903, pUC4K Remnants
RBGpA
PolyA Site 1
rGlob pA
Unknown
pPJV2012
5578 bp
LTB CDS
Lysozyme SP
5'-UTR of HBV pre-S2
Rat Ins IntA
Bam/Bgl fusion
HBVenh
CMV Exon1/2
mCMV pro
LTA CDS
Lysozyme SP
5'-UTR of HBV pre-S2
Rat Ins IntA
CMV Pro
CMV Exon1/2
Bam/Bgl fusion

Figure 22

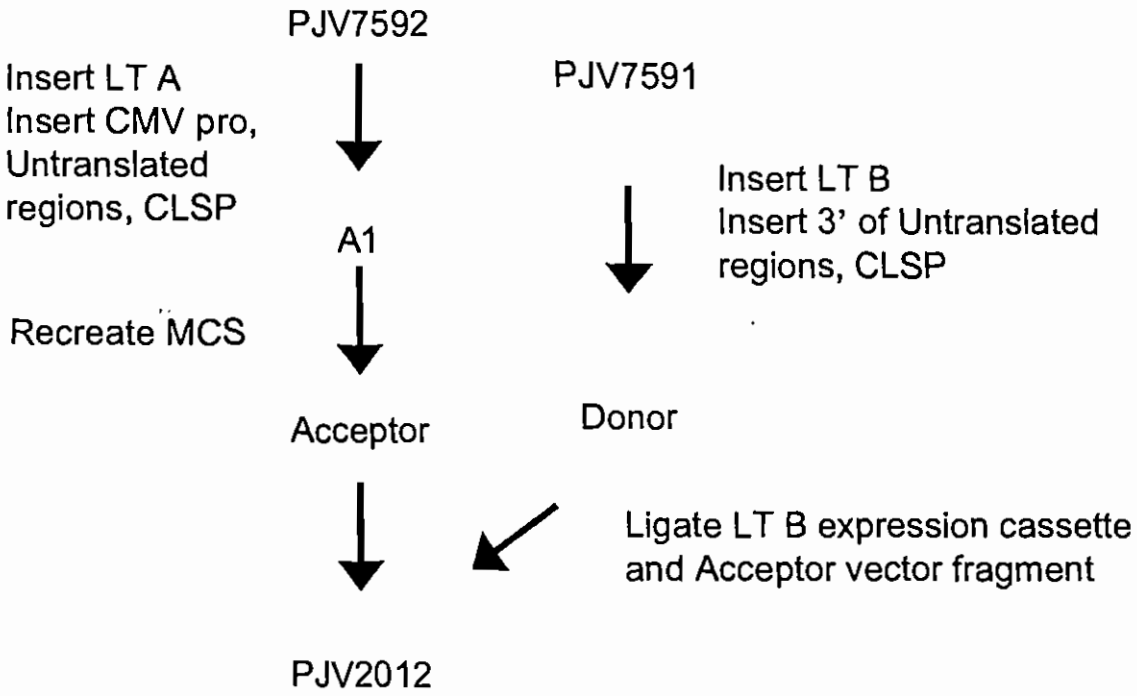
PJV7592
Insert LT A
Insert CMV pro,
Untranslated
regions, CLSP
A1
Recreate MCS
Acceptor
PJV7591
Insert LT B
Insert 3' of Untranslated
regions, CLSP
Donor
Ligate LT B expression cassette
and Acceptor vector fragment
PJV2012

Figure 23

```
  1  GGCGTAATGC TCTGCCAGTG TTACAACCAA TTAACCAATT CTGATTAGAA AAACTCATCG AGCATCAAAT GAAACTGCAA TTTATTCATA TCAGGATTAT
     CCGCATTACG AGACGGTCAC AATGTTGGTT AATTGGTTAA GACTAATCTT TTTGAGTAGC TCGTAGTTTA CTTTGACGTT AAATAAGTAT AGTCCTAATA
                                                  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                                                KanR (Tn903)
101  CAATACCATA TTTTTGAAAA AGCCGTTTCT GTAATGAAGG AGAAAACTCA CCGAGGCAGT TCCATAGGAT GGCAAGATCC TGGTATCGGT CTGCGATTCC
     GTTATGGTAT AAAAACTTTT TCGGCAAAGA CATTACTTCC TCTTTTGAGT GGCTCCGTCA AGGTATCCTA CCGTTCTAGG ACCATAGCCA GACGCTAAGG
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                  KanR (Tn903)
201  GACTCGTCCA ACATCAATAC AACCTATTAA TTTCCCCTCG TCAAAAATAA GGTTATCAAG TGAGAAATCA CCATGAGTGA CGACTGAATC CGGTGAGAAT
     CTGAGCAGGT TGTAGTTATG TTGGATAATT AAAGGGGAGC AGTTTTTATT CCAATAGTTC ACTCTTTAGT GGTACTCACT GCTGACTTAG GCCACTCTTA
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                  KanR (Tn903)
       HindIII
       ~~~~~~~
301  GGCAAAAGCT TATGCATTTC TTTCCAGACT TGTTCAACAG GCCAGCCATT ACGCTCGTCA TCAAAATCAC TCGCATCAAC CAAACCGTTA TTCATTCGTG
     CCGTTTTCGA ATACGTAAAG AAAGGTCTGA ACAAGTTGTC CGGTCGGTAA TGCGAGCAGT AGTTTTAGTG AGCGTAGTTG GTTTGGCAAT AAGTAAGCAC
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                  KanR (Tn903)
401  ATTGCGCCTG AGCGAGACGA AATACGCGAT CGCTGTTAAA AGGACAATTA CAAACAGGAA TCAAATGCAA CCGGCGCAGG AACACTGCCA GCGCATCAAC
     TAACGCGGAC TCGCTCTGCT TTATGCGCTA GCGACAATTT TCCTGTTAAT GTTTGTCCTT AGTTTACGTT GGCCGCGTCC TTGTGACGGT CGCGTAGTTG
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                  KanR (Tn903)
501  AATATTTTCA CCTGAATCAG GATATTCTTC TAATACCTGG AATGCTGTTT TCCCGGGGAT CGCAGTGGTG AGTAACCATG CATCATCAGG AGTACGGATA
     TTATAAAAGT GGACTTAGTC CTATAAGAAG ATTATGGACC TTACGACAAA AGGGCCCCTA GCGTCACCAC TCATTGGTAC GTAGTAGTCC TCATGCCTAT
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                  KanR (Tn903)
601  AAATGCTTGA TGGTCGGAAG AGGCATAAAT TCCGTCAGCC AGTTTAGTCT GACCATCTCA TCTGTAACAT CATTGGCAAC GCTACCTTTG CCATGTTTCA
     TTTACGAACT ACCAGCCTTC TCCGTATTTA AGGCAGTCGG TCAAATCAGA CTGGTAGAGT AGACATTGTA GTAACCGTTG CGATGGAAAC GGTACAAAGT
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                  KanR (Tn903)
701  GAAACAACTC TGGCGCATCG GGCTTCCCAT ACAATCGATA GATTGTCGCA CCTGATTGCC CGACATTATC GCGAGCCCAT TTATACCCAT ATAAATCAGC
     CTTTGTTGAG ACCGCGTAGC CCGAAGGGTA TGTTAGCTAT CTAACAGCGT GGACTAACGG GCTGTAATAG CGCTCGGGTA AATATGGGTA TATTTAGTCG
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                  KanR (Tn903)
801  ATCCATGTTG GAATTTAATC GCGGCCTCGA GCAAGACGTT TCCCGTTGAA TATGGCTCAT AACACCCCTT GTATTACTGT TTATGTAAGC AGACAGGTCG
     TAGGTACAAC CTTAAATTAG CGCCGGAGCT CGTTCTGCAA AGGGCAACTT ATACCGAGTA TTGTGGGGAA CATAATGACA AATACATTCG TCTGTCCAGC
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              KanR (Tn903)
901  ACAATTCCTT CCGAGTGAGA GACACAAAAA ATTCCAACAC ACTATTGCAA TGAAAATAAA TTTCCTTTAT TAGCCAGAAG TCAGATGCTC AAGGGGCTTC
     TGTTAAGGAA GGCTCACTCT CTGTGTTTTT TAAGGTTGTG TGATAACGTT ACTTTTATTT AAAGGAAATA ATCGGTCTTC AGTCTACGAG TTCCCCGAAG
```

Figure 24 (a)

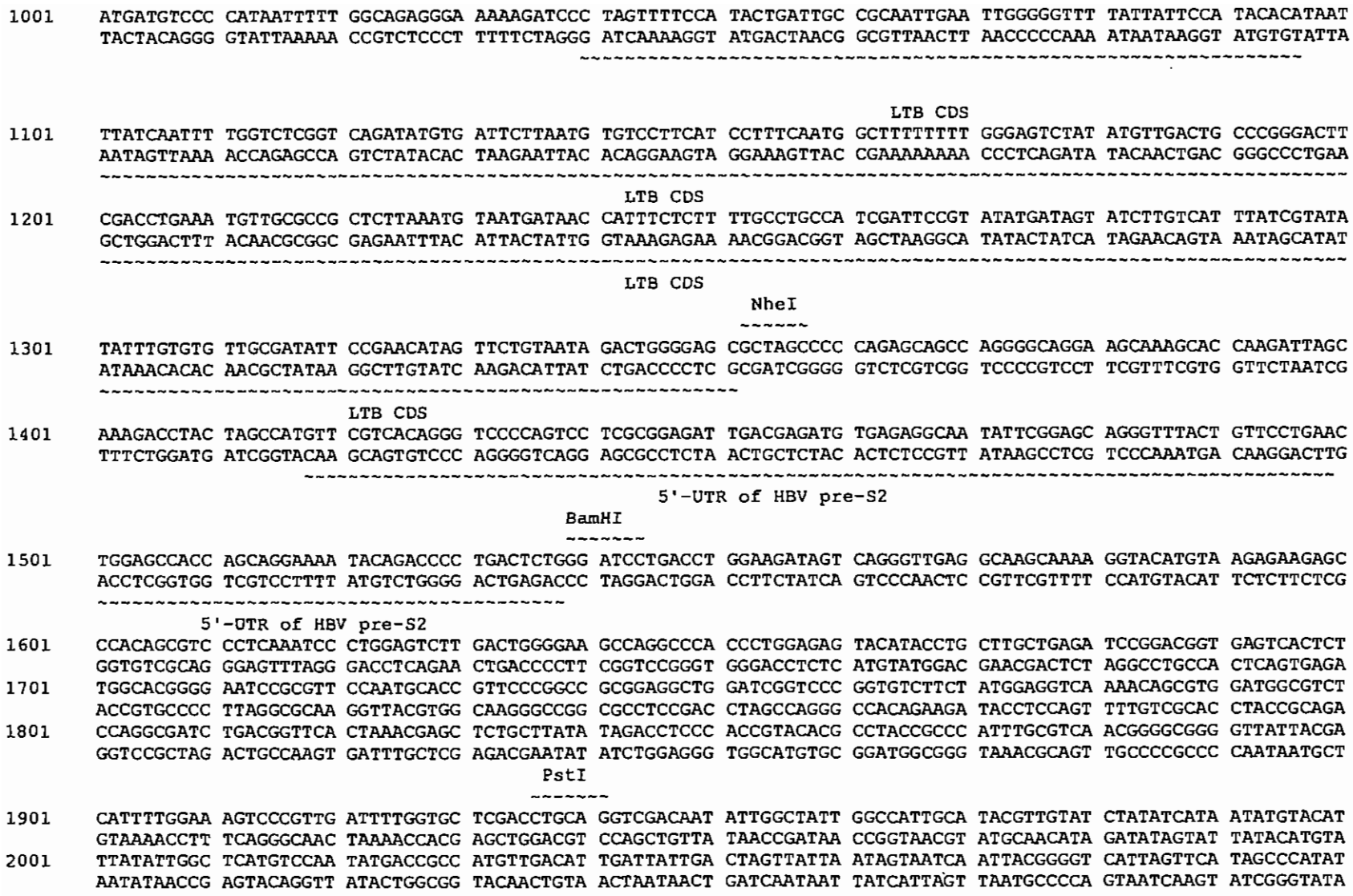
1001 ATGATGTCCC CATAATTTTT GGCAGAGGGA AAAAGATCCC TAGTTTTCCA TACTGATTGC CGCAATTGAA TTGGGGGTTT TATTATTCCA TACACATAAT
TACTACAGGG GTATTAAAAA CCGTCTCCCT TTTTCTAGGG ATCAAAAGGT ATGACTAACG GCGTTAACTT AACCCCCAAA ATAATAAGGT ATGTGTATTA
LTB CDS
1101 TTATCAATTT TGGTCTCGGT CAGATATGTG ATTCTTAATG TGTCCTTCAT CCTTTCAATG GCTTTTTTTT GGGAGTCTAT ATGTTGACTG CCCGGGACTT
AATAGTTAAA ACCAGAGCCA GTCTATACAC TAAGAATTAC ACAGGAAGTA GGAAAGTTAC CGAAAAAAAA CCCTCAGATA TACAACTGAC GGGCCCTGAA
LTB CDS
1201 CGACCTGAAA TGTTGCGCCG CTCTTAAATG TAATGATAAC CATTTCTCTT TTGCCTGCCA TCGATTCCGT ATATGATAGT ATCTTGTCAT TTATCGTATA
GCTGGACTTT ACAACGCGGC GAGAATTTAC ATTACTATTG GTAAAGAGAA AACGGACGGT AGCTAAGGCA TATACTATCA TAGAACAGTA AATAGCATAT
LTB CDS
NheI
1301 TATTTGTGTG TTGCGATATT CCGAACATAG TTCTGTAATA GACTGGGGAG CGCTAGCCCC CAGAGCAGCC AGGGGCAGGA AGCAAAGCAC CAAGATTAGC
ATAAACACAC AACGCTATAA GGCTTGTATC AAGACATTAT CTGACCCCTC GCGATCGGGG GTCTCGTCGG TCCCCGTCCT TCGTTTCGTG GTTCTAATCG
LTB CDS
1401 AAAGACCTAC TAGCCATGTT CGTCACAGGG TCCCCAGTCC TCGCGGAGAT TGACGAGATG TGAGAGGCAA TATTCGGAGC AGGGTTTACT GTTCCTGAAC
TTTCTGGATG ATCGGTACAA GCAGTGTCCC AGGGGTCAGG AGCGCCTCTA ACTGCTCTAC ACTCTCCGTT ATAAGCCTCG TCCCAAATGA CAAGGACTTG
5'-UTR of HBV pre-S2
BamHI
1501 TGGAGCCACC AGCAGGAAAA TACAGACCCC TGACTCTGGG ATCCTGACCT GGAAGATAGT CAGGGTTGAG GCAAGCAAAA GGTACATGTA AGAGAAGAGC
ACCTCGGTGG TCGTCCTTTT ATGTCTGGGG ACTGAGACCC TAGGACTGGA CCTTCTATCA GTCCCAACTC CGTTCGTTTT CCATGTACAT TCTCTTCTCG
5'-UTR of HBV pre-S2
1601 CCACAGCGTC CCTCAAATCC CTGGAGTCTT GACTGGGGAA GCCAGGCCCA CCCTGGAGAG TACATACCTG CTTGCTGAGA TCCGGACGGT GAGTCACTCT
GGTGTCGCAG GGAGTTTAGG GACCTCAGAA CTGACCCCTT CGGTCCGGGT GGGACCTCTC ATGTATGGAC GAACGACTCT AGGCCTGCCA CTCAGTGAGA
1701 TGGCACGGGG AATCCGCGTT CCAATGCACC GTTCCCGGCC GCGGAGGCTG GATCGGTCCC GGTGTCTTCT ATGGAGGTCA AAACAGCGTG GATGGCGTCT
ACCGTGCCCC TTAGGCGCAA GGTTACGTGG CAAGGGCCGG CGCCTCCGAC CTAGCCAGGG CCACAGAAGA TACCTCCAGT TTTGTCGCAC CTACCGCAGA
1801 CCAGGCGATC TGACGGTTCA CTAAACGAGC TCTGCTTATA TAGACCTCCC ACCGTACACG CCTACCGCCC ATTTGCGTCA ACGGGGCGGG GTTATTACGA
GGTCCGCTAG ACTGCCAAGT GATTTGCTCG AGACGAATAT ATCTGGAGGG TGGCATGTGC GGATGGCGGG TAAACGCAGT TGCCCCGCCC CAATAATGCT
PstI
1901 CATTTTGGAA AGTCCCGTTG ATTTTGGTGC TCGACCTGCA GGTCGACAAT ATTGGCTATT GGCCATTGCA TACGTTGTAT CTATATCATA ATATGTACAT
GTAAAACCTT TCAGGGCAAC TAAAACCACG AGCTGGACGT CCAGCTGTTA TAACCGATAA CCGGTAACGT ATGCAACATA GATATAGTAT TATACATGTA
2001 TTATATTGGC TCATGTCCAA TATGACCGCC ATGTTGACAT TGATTATTGA CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA TAGCCCATAT
AATATAACCG AGTACAGGTT ATACTGGCGG TACAACTGTA ACTAATAACT GATCAATAAT TATCATTAGT TAATGCCCCA GTAATCAAGT ATCGGGTATA

Figure 24 (b)


2101 ATGGAGTTCC GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC CCCCGCCCAT TGACGTCAAT AATGACGTAT GTTCCCATAG
TACCTCAAGG CGCAATGTAT TGAATGCCAT TTACCGGGCG GACCGACTGG CGGGTTGCTG GGGGCGGGTA ACTGCAGTTA TTACTGCATA CAAGGGTATC
2201 TAACGCCAAT AGGGACTTTC CATTGACGTC AATGGGTGGA GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC CAAGTCCGCC
ATTGCGGTTA TCCCTGAAAG GTAACTGCAG TTACCCACCT CATAAATGCC ATTTGACGGG TGAACCGTCA TGTAGTTCAC ATAGTATACG GTTCAGGCGG
2301 CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCCAGT ACATGACCTT ACGGGACTTT CCTACTTGGC AGTACATCTA CGTATTAGTC
GGGATAACTG CAGTTACTGC CATTTACCGG GCGGACCGTA ATACGGGTCA TGTACTGGAA TGCCCTGAAA GGATGAACCG TCATGTAGAT GCATAATCAG
2401 ATCGCTATTA CCATGGTGAT GCGGTTTTGG CAGTACACCA ATGGGCGTGG ATAGCGGTTT GACTCACGGG GATTTCCAAG TCTCCACCCC ATTGACGTCA
TAGCGATAAT GGTACCACTA CGCCAAAACC GTCATGTGGT TACCCGCACC TATCGCCAAA CTGAGTGCCC CTAAAGGTTC AGAGGTGGGG TAACTGCAGT
2501 ATGGGAGTTT GTTTTGGCAC CAAAATCAAC GGGACTTTCC AAAATGTCGT AATAACCCCG CCCCGTTGAC GCAAATGGGC GGTAGGCGTG TACGGTGGGA
TACCCTCAAA CAAAACCGTG GTTTTAGTTG CCCTGAAAGG TTTTACAGCA TTATTGGGGC GGGGCAACTG CGTTTACCCG CCATCCGCAC ATGCCACCCT
2601 GGTCTATATA AGCAGAGCTC GTTTAGTGAA CCGTCAGATC GCCTGGAGAC GCCATCCACG CTGTTTTGAC CTCCATAGAA GACACCGGGA CCGATCCAGC
CCAGATATAT TCGTCTCGAG CAAATCACTT GGCAGTCTAG CGGACCTCTG CGGTAGGTGC GACAAAACTG GAGGTATCTT CTGTGGCCCT GGCTAGGTCG
2701 CTCCGCGGCC GGGAACGGTG CATTGGAACG CGGATTCCCC GTGCCAAGAG TGACTCACCG TCCGGATCTC AGCAAGCAGG TATGTACTCT CCAGGGTGGG
GAGGCGCCGG CCCTTGCCAC GTAACCTTGC GCCTAAGGGG CACGGTTCTC ACTGAGTGGC AGGCCTAGAG TCGTTCGTCC ATACATGAGA GGTCCCACCC
2801 CCTGGCTTCC CCAGTCAAGA CTCCAGGGAT TTGAGGGACG CTGTGGGCTC TTCTCTTACA TGTACCTTTT GCTTGCCTCA ACCCTGACTA TCTTCCAGGT
GGACCGAAGG GGTCAGTTCT GAGGTCCCTA AACTCCCTGC GACACCCGAG AAGAGAATGT ACATGGAAAA CGAACGGAGT TGGGACTGAT AGAAGGTCCA
5'-UTR of HBV pre-S2
BamHI
2901 CAGGATCCCA GAGTCAGGGG TCTGTATTTT CCTGCTGGTG GCTCCAGTTC AGGAACAGTA AACCCTGCTC CGAATATTGC CTCTCACATC TCGTCAATCT
GTCCTAGGGT CTCAGTCCCC AGACATAAAA GGACGACCAC CGAGGTCAAG TCCTTGTCAT TTGGGACGAG GCTTATAACG GAGAGTGTAG AGCAGTTAGA
5'-UTR of HBV pre-S2
LTA CDS
NheI
3001 CCGCGAGGAC TGGGGACCCT GTGACGAACA TGGCTAGTAG GTCTTTGCTA ATCTTGGTGC TTTGCTTCCT GCCCCTGGCT GCTCTGGGGG CTAGCAATGG
GGCGCTCCTG ACCCCTGGGA CACTGCTTGT ACCGATCATC CAGAAACGAT TAGAACCACG AAACGAAGGA CGGGGACCGA CGAGACCCCC GATCGTTACC
LTA CDS
3101 CGACAAATTA TACCGTGCTG ACTCTAGACC CCCAGATGAA ATAAAACGTT CCGGAGGTCT TATGCCCAGA GGGCATAATG AGTACTTCGA TAGAGGAACT
GCTGTTTAAT ATGGCACGAC TGAGATCTGG GGGTCTACTT TATTTTGCAA GGCCTCCAGA ATACGGGTCT CCCGTATTAC TCATGAAGCT ATCTCCTTGA
LTA CDS
3201 CAAATGAATA TTAATCTTTA TGATCACGCG AGAGGAACAC AAACCGGCTT TGTCAGATAT GATGACGGAT ATGTTTCCAC TTCTCTTAGT TTGAGAAGTG
GTTTACTTAT AATTAGAAAT ACTAGTGCGC TCTCCTTGTG TTTGGCCGAA ACAGTCTATA CTACTGCCTA TACAAAGGTG AAGAGAATCA AACTCTTCAC
LTA CDS

Figure 24 (c)

```
3301    CTCACTTAGC AGGACAGTCT ATATTATCAG GATATTCCAC TTACTATATA TATGTTATAG CGACAGCACC AAATATGTTT AATGTTAATG ATGTATTAGG
        GAGTGAATCG TCCTGTCAGA TATAATAGTC CTATAAGGTG AATGATATAT ATACAATATC GCTGTCGTGG TTTATACAAA TTACAATTAC TACATAATCC
                                                  LTA CDS
        ~~~
```

```
4401  TGGGGACATC ATGAAGCCCC TTGAGCATCT GACTTCTGGC TAATAAAGGA AATTTATTTT CATTGCAATA GTGTGTTGGA ATTTTTTGTG TCTCTCACTC
      ACCCCTGTAG TACTTCGGGG AACTCGTAGA CTGAAGACCG ATTATTTCCT TTAAATAAAA GTAACGTTAT CACACAACCT TAAAAAACAC AGAGAGTGAG
            EcoRI
            ~~~~~~
4501  GGAAGGAATT CTGCATTAAT GAATCGGCCA ACGCGCGGGG AGAGGCGGTT TGCGTATTGG GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG
      CCTTCCTTAA GACGTAATTA CTTAGCCGGT TGCGCGCCCC TCTCCGCCAA ACGCATAACC CGCGAGAAGG CGAAGGAGCG AGTGACTGAG CGACGCGAGC
4601  GTCGTTCGGC TGCGGCGAGC GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG
      CAGCAAGCCG ACGCCGCTCG CCATAGTCGA GTGAGTTTCC GCCATTATGC CAATAGGTGT CTTAGTCCCC TATTGCGTCC TTTCTTGTAC ACTCGTTTTC
4701  GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA
      CGGTCGTTTT CCGGTCCTTG GCATTTTTCC GGCGCAACGA CCGCAAAAAG GTATCCGAGG CGGGGGGACT GCTCGTAGTG TTTTAGCTG CGAGTTCAGT
4801  GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC
      CTCCACCGCT TTGGGCTGTC CTGATATTTC TATGGTCCGC AAAGGGGGAC CTTCGAGGGA GCACGCGAGA GGACAAGGCT GGGACGGCGA ATGGCCTATG
4901  CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG
      GACAGGCGGA AAGAGGGAAG CCCTTCGCAC CGCGAAAGAG TATCGAGTGC GACATCCATA GAGTCAAGCC ACATCCAGCA AGCGAGGTTC GACCCGACAC
5001  TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC
      ACGTGCTTGG GGGGCCAAGTC GGGCTGGCGA CGCGGAATAG GCCATTGATA GCAGAACTCA GGTTGGGCCA TTCTGTGCTG AATAGCGGTG ACCGTCGTCG
5101  CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT
      GTGACCATTG TCCTAATCGT CTCGCTCCAT ACATCCGCCA CGATGTCTCA AGAACTTCAC CACCGGATTG ATGCCGATGT GATCTTCTTG TCATAAACCA
5201  ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA
      TAGACGCGAG ACGACTTCGG TCAATGGAAG CCTTTTTCTC AACCATCGAG AACTAGGCCG TTTGTTTGGT GGCGACCATC GCCACCAAAA AAACAAACGT
5301  AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT
      TCGTCGTCTA ATGCGCGTCT TTTTTTCCTA GAGTTCTTCT AGGAAACTAG AAAAGATGCC CCAGACTGCG AGTCACCTTG CTTTTGAGTG CAATTCCCTA
5401  TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT
      AAACCAGTAC TCTAATAGTT TTTCCTAGAA GTGGATCTAG GAAAATTTAA TTTTTACTTC AAAATTTAGT TAGATTTCAT ATATACTCAT TTGAACCAGA
5501  GACAGTTACC AATGCTTAAT CAGTGAGGCA CCTATCTCAG CGATCTGTCT ATTTCGTTCA TCCATAGTTG CCTGACTC
      CTGTCAATGG TTACGAATTA GTCACTCCGT GGATAGAGTC GCTAGACAGA TAAAGCAAGT AGGTATCAAC GGACTGAG
```

Figure 24 (e)

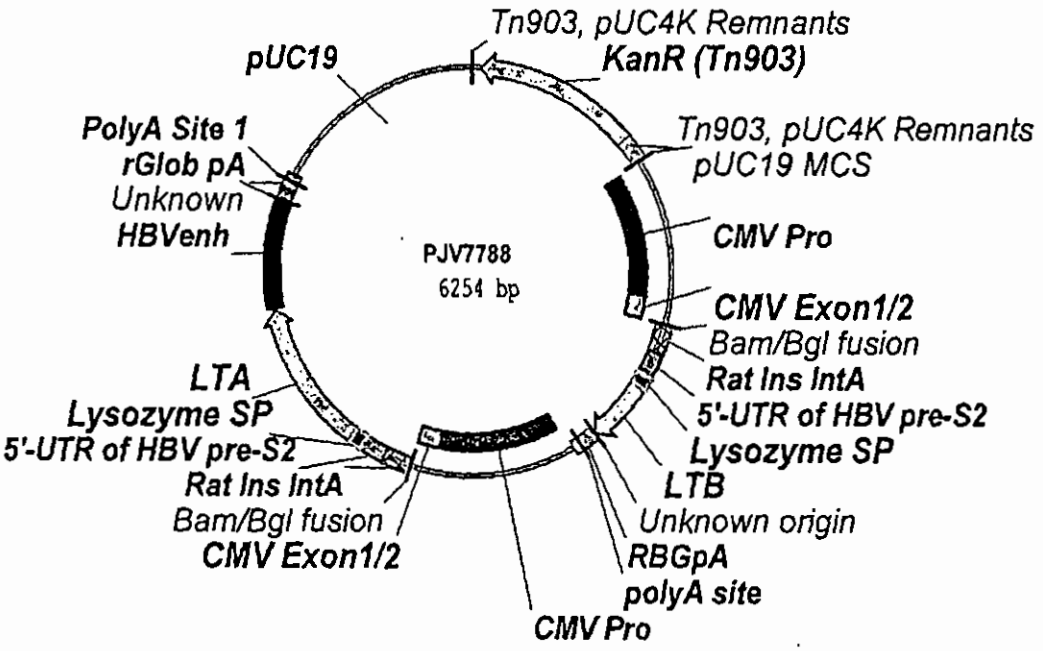
Tn903, pUC4K Remnants
KanR (Tn903)
pUC19
PolyA Site 1
rGlob pA
Unknown
HBVenh
Tn903, pUC4K Remnants
pUC19 MCS
CMV Pro
PJV7788
6254 bp
CMV Exon1/2
Bam/Bgl fusion
Rat Ins IntA
5'-UTR of HBV pre-S2
Lysozyme SP
LTB
Unknown origin
RBGpA
polyA site
CMV Pro
LTA
Lysozyme SP
5'-UTR of HBV pre-S2
Rat Ins IntA
Bam/Bgl fusion
CMV Exon1/2

Figure 25

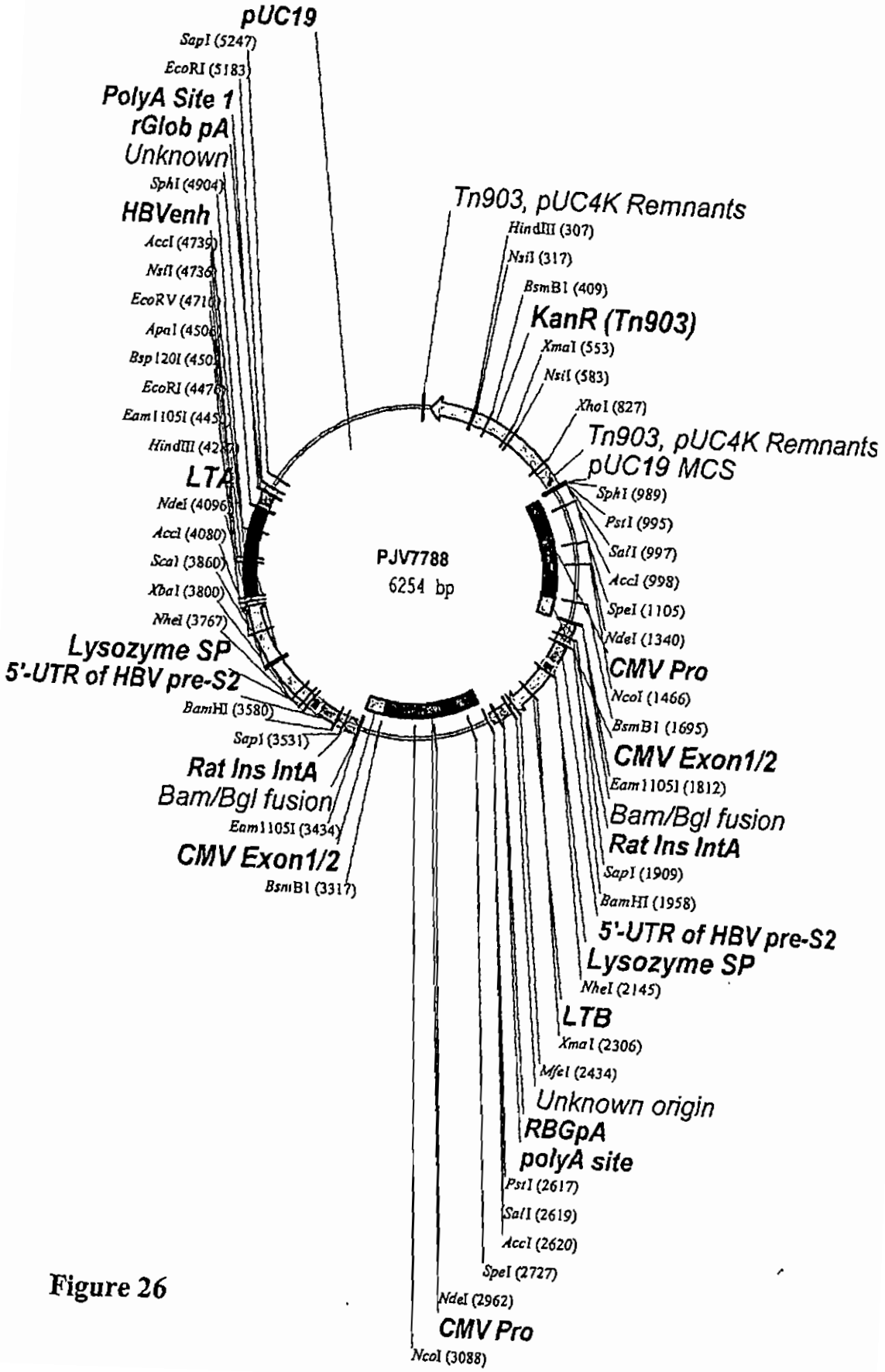
PJV7788
6254 bp
pUC19
SapI (5247)
EcoRI (5183)
PolyA Site 1
rGlob pA
Unknown
SphI (4904)
HBVenh
AccI (4739)
NsiI (4736)
EcoRV (4710)
ApaI (4506)
Bsp120I (4501)
EcoRI (4476)
Eam1105I (4454)
HindIII (4237)
LTA
NdeI (4096)
AccI (4080)
ScaI (3860)
XbaI (3800)
NheI (3767)
Lysozyme SP
5'-UTR of HBV pre-S2
BamHI (3580)
SapI (3531)
Rat Ins IntA
Bam/Bgl fusion
Eam1105I (3434)
CMV Exon1/2
BsmBI (3317)
Tn903, pUC4K Remnants
HindIII (307)
NsiI (317)
BsmBI (409)
KanR (Tn903)
XmaI (553)
NsiI (583)
XhoI (827)
Tn903, pUC4K Remnants
pUC19 MCS
SphI (989)
PstI (995)
SalI (997)
AccI (998)
SpeI (1105)
NdeI (1340)
CMV Pro
NcoI (1466)
BsmBI (1695)
CMV Exon1/2
Eam1105I (1812)
Bam/Bgl fusion
Rat Ins IntA
SapI (1909)
BamHI (1958)
5'-UTR of HBV pre-S2
Lysozyme SP
NheI (2145)
LTB
XmaI (2306)
MfeI (2434)
Unknown origin
RBGpA
polyA site
PstI (2617)
SalI (2619)
AccI (2620)
SpeI (2727)
NdeI (2962)
CMV Pro
NcoI (3088)

Figure 26

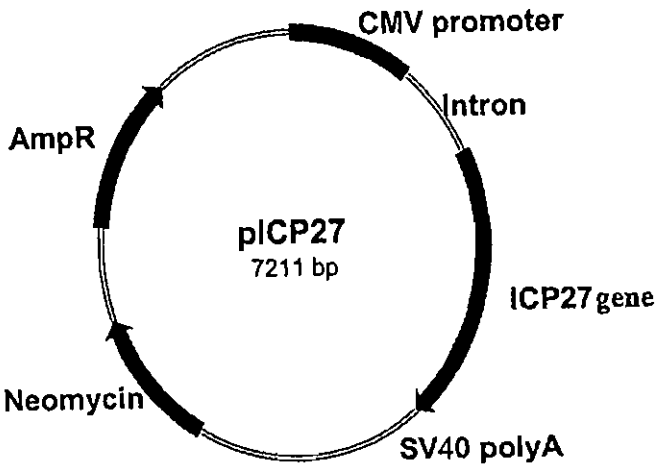
CMV promoter
Intron
AmpR
pICP27
7211 bp
ICP27 gene
Neomycin
SV40 polyA

Figure 27

MATDIDMLID LGLDLSDSEL EEDALERDEE GRRDDPESDS SGECSSSDED
MEDPCGDGGA EAIDAAIPKG PPARPEDAGT PEASTPRPAA RRGADDPPPA
TTGVWSRLGT RRSASPREPH GGKVARIQPP STKAPHPRGG RRGRRRGRGR
YGPGGADSTP NPRRRVSRNA HNQGGRHPAS ARTDGPGATH GEARRGGEQL
DVSGGPRPRG TRQAPPPLMA LSLTPPHADG RAPVPERKAP SADTIDPAVR
AVLRSISERA AVERISESFG RSALVMQDPF GGMPFPAANS PWAPVLATQA
GGFDAETRRV SWETLVAHGP SLYRTFAANP RAASTAKAMR DCVLRQENLI
EALASADETL AWCKMCIHHN LPLRPQDPII GTAAAVLENL ATRLRPFLQC
YLKARGLCGL DDLCSRRRLS DIKDIASFVL VILARLANRV ERGVSEIDYT
TVGVGAGETM HFYIPGACMA GLIEILDTHR QECSSRVCEL TASHTIAPLY
VHGKYFYCNS LF

Figure 28

A

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
BS												
BN												
CS												
CN												

	R1	R2	R3	R4	R5	R6
BS						
BN						
CS						
CN						

B

#45 RVSWETLVA**HGPSLYRTF** (SEQ ID NO:66)
#46 VA**HGPSLYRTF**AANPRAA (SEQ ID NO:67)

Figure 29

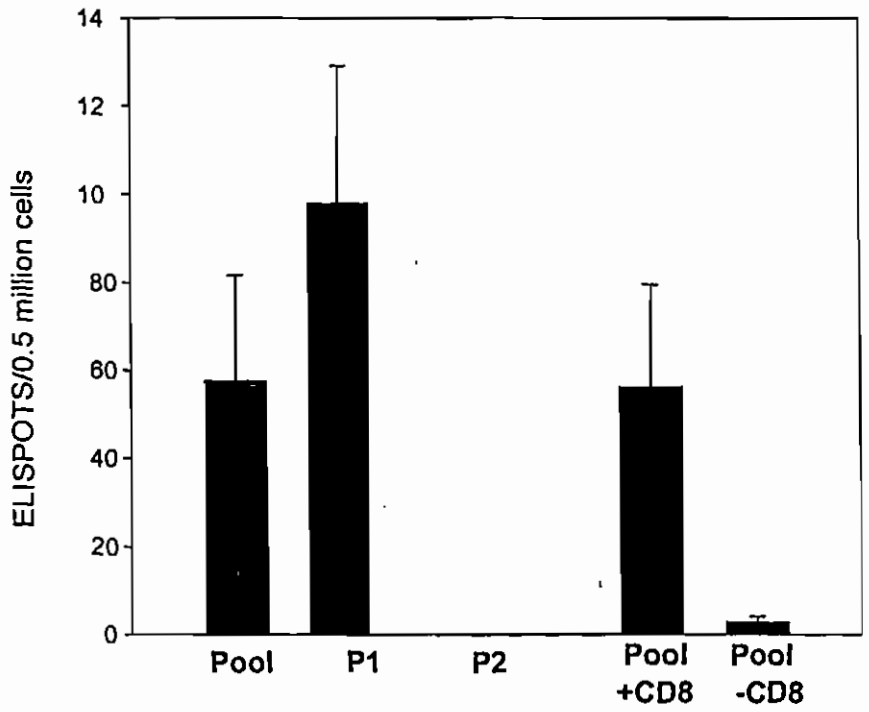
14
12
10
80
60
40
20
0
ELISPOTS/0.5 million cells
Pool
P1
P2
Pool +CD8
Pool -CD8

Figure 30

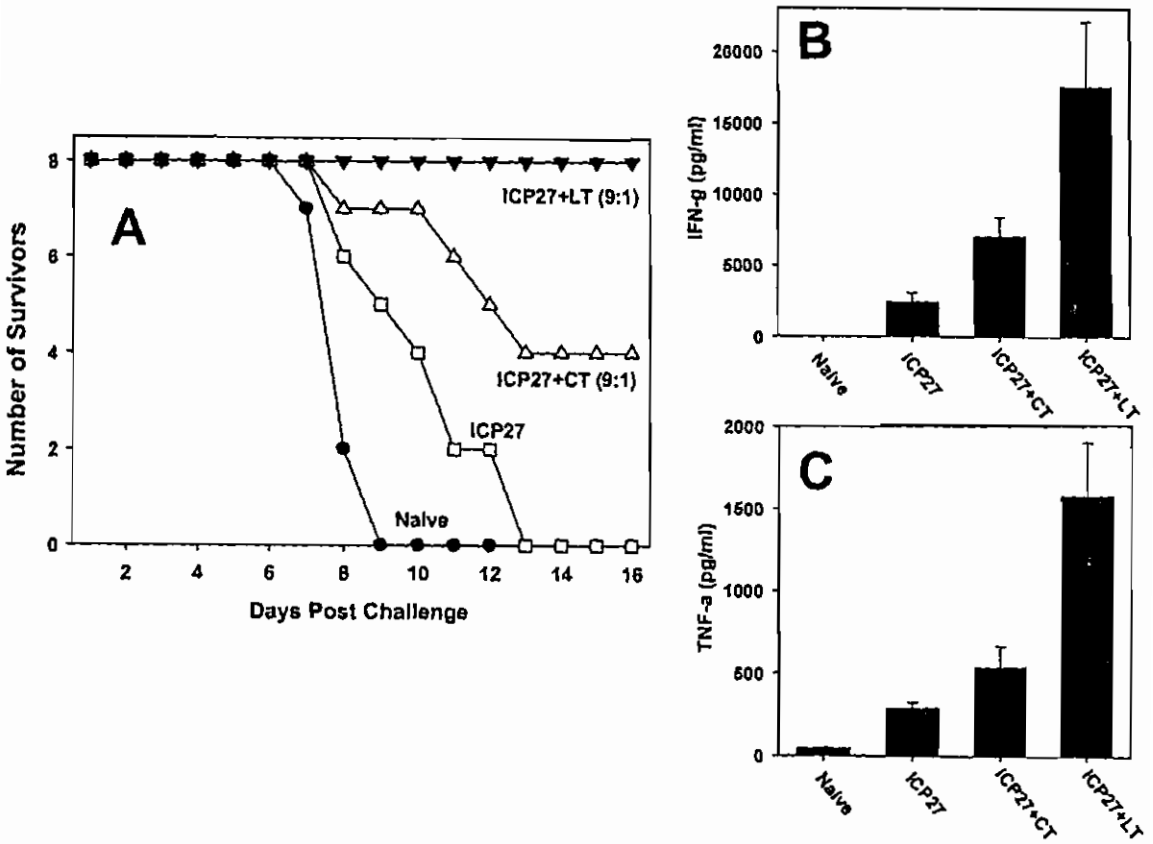
A
Number of Survivors
Days Post Challenge
ICP27+LT (9:1)
ICP27+CT (9:1)
ICP27
Naive
B
IFN-g (pg/ml)
C
TNF-a (pg/ml)
Naive
ICP27
ICP27+CT
ICP27+LT

Figure 31

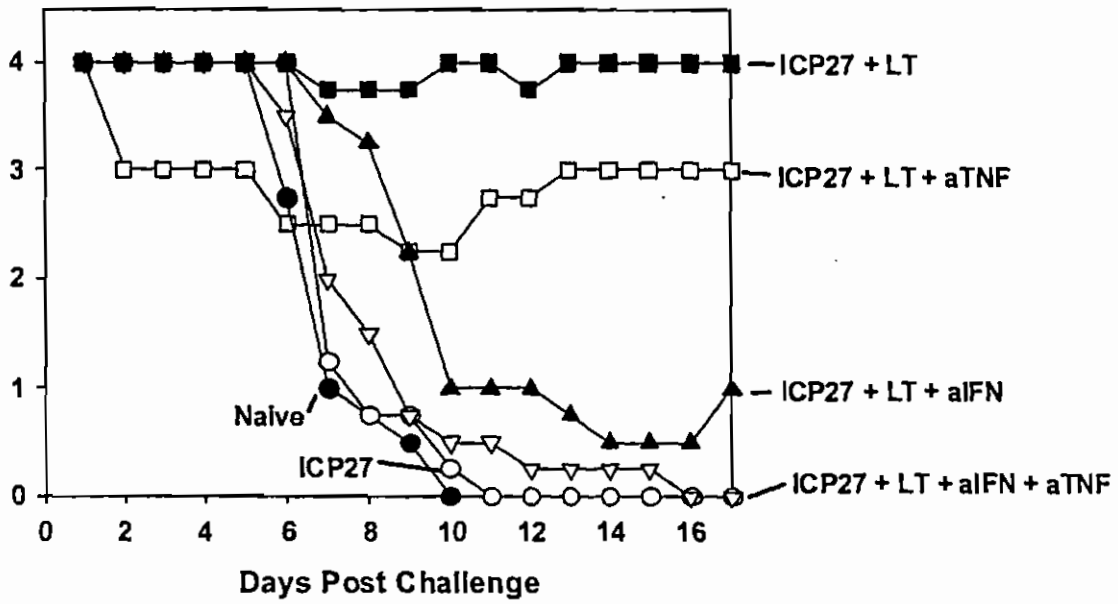
4
3
2
1
0
0
2
4
6
8
10
12
14
16
Days Post Challenge
ICP27 + LT
ICP27 + LT + aTNF
ICP27 + LT + aIFN
ICP27 + LT + aIFN + aTNF
Naive
ICP27

Figure 32

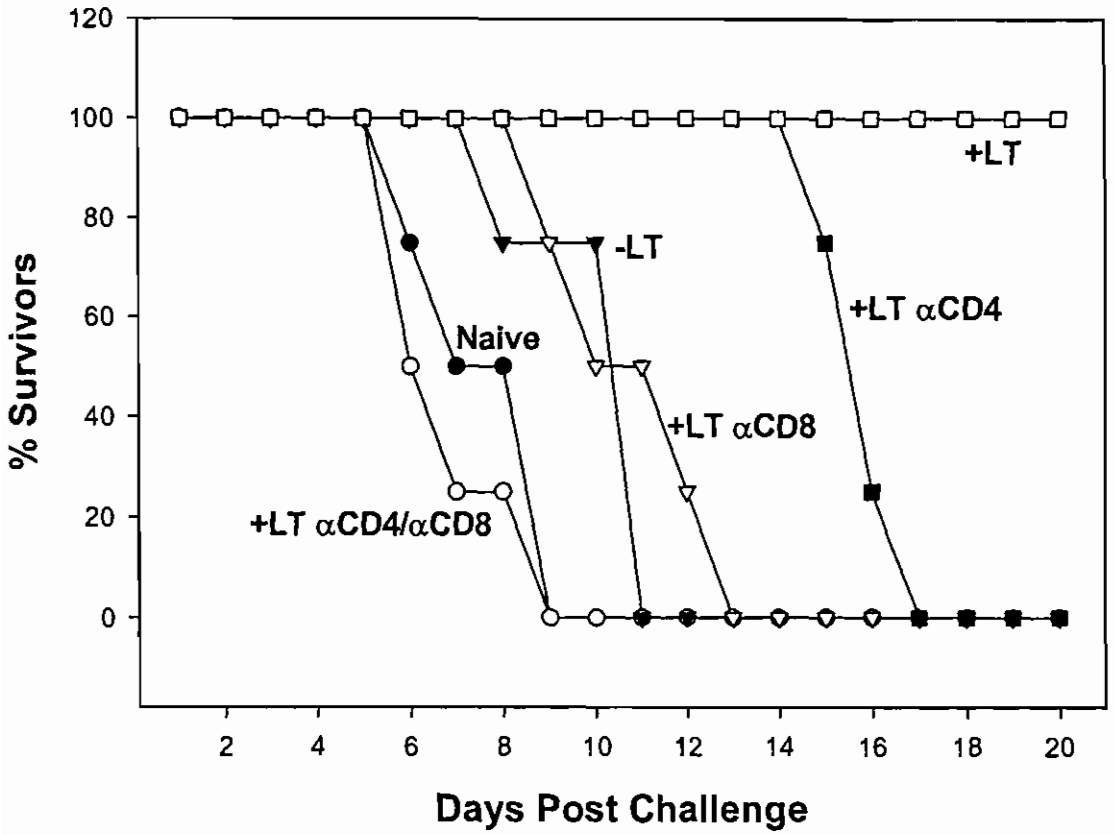
120
100
80
60
40
20
0
% Survivors
2
4
6
8
10
12
14
16
18
20
Days Post Challenge
+LT
-LT
+LT αCD4
Naive
+LT αCD8
+LT αCD4/αCD8

Figure 33