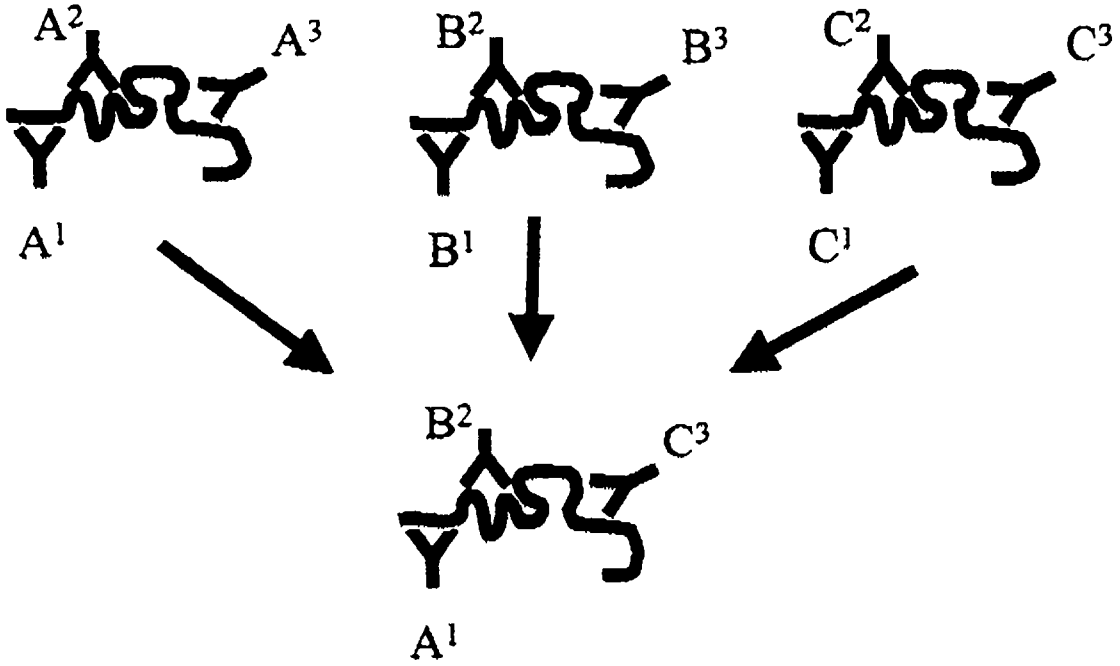
Figure 1



163163

G00002

Figure 2

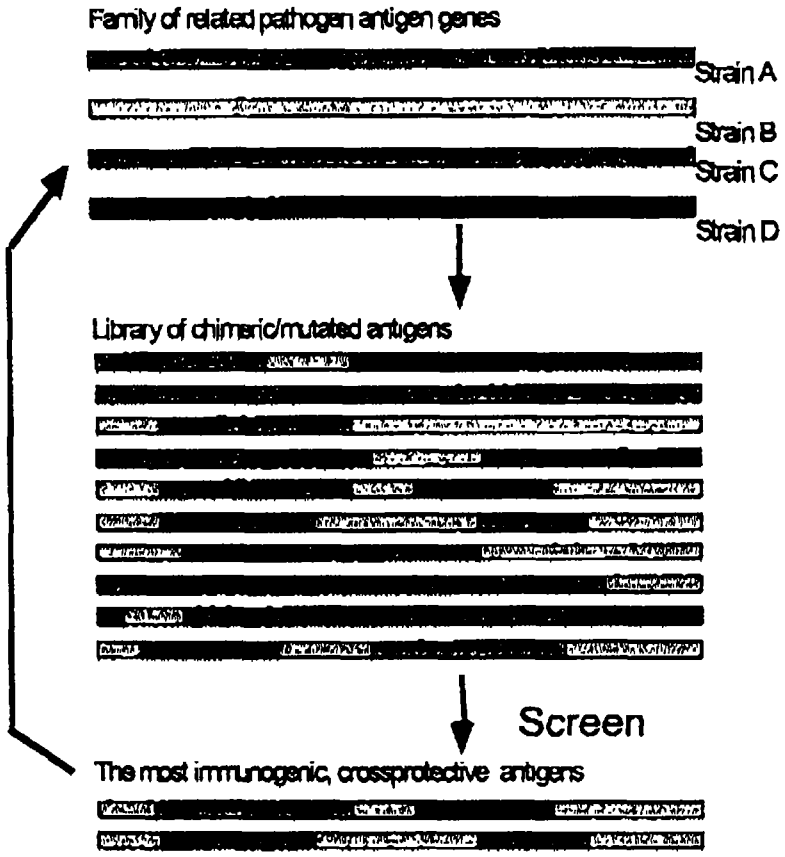


Figure 3

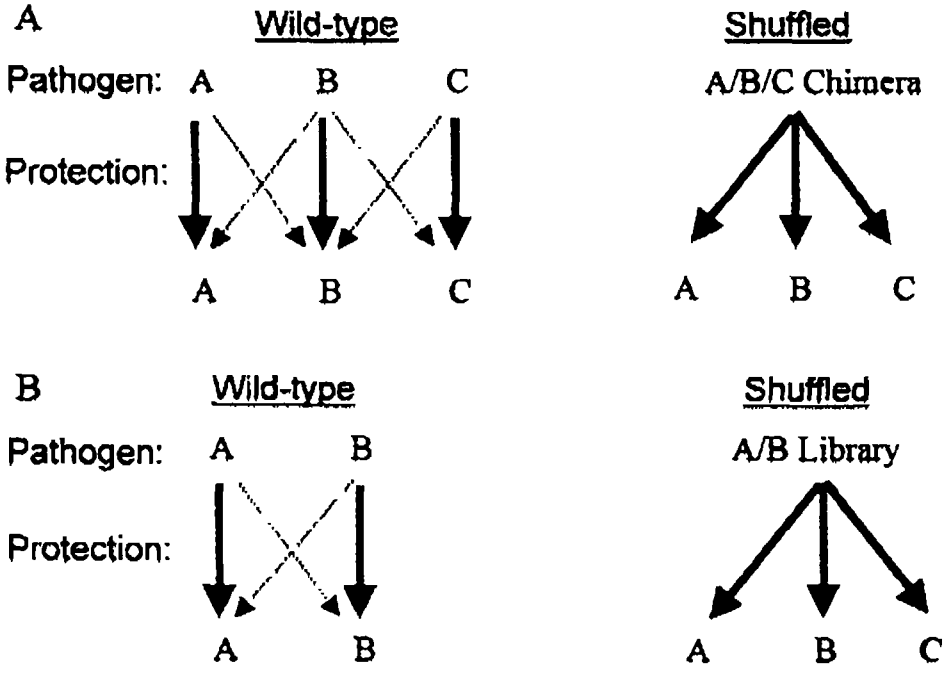


Figure 4

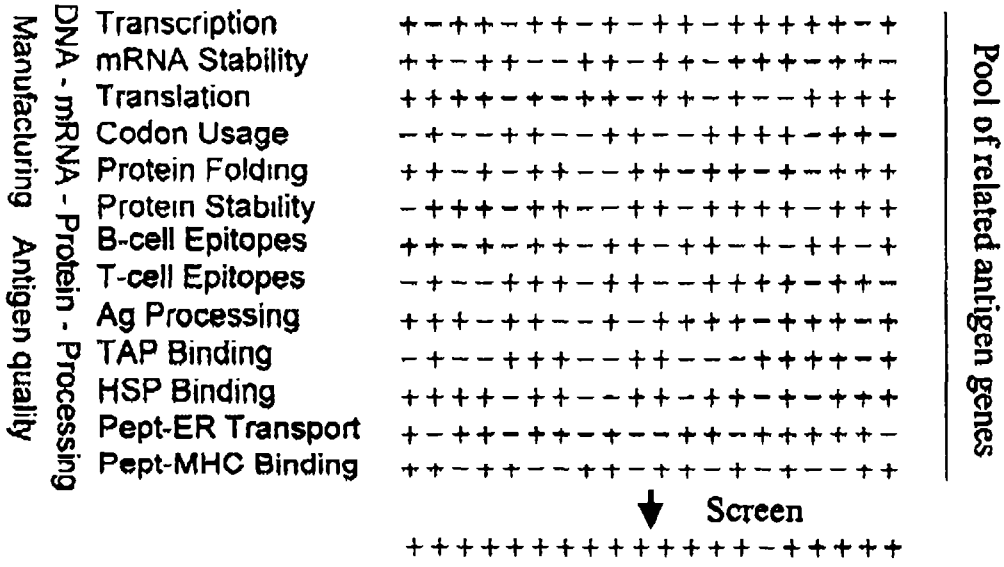

Manufacturing Antigen quality
DNA - mRNA - Protein - Processing
Transcription
mRNA Stability
Translation
Codon Usage
Protein Folding
Protein Stability
B-cell Epitopes
T-cell Epitopes
Ag Processing
TAP Binding
HSP Binding
Pept-ER Transport
Pept-MHC Binding
Screen
Pool of related antigen genes

Figure 5

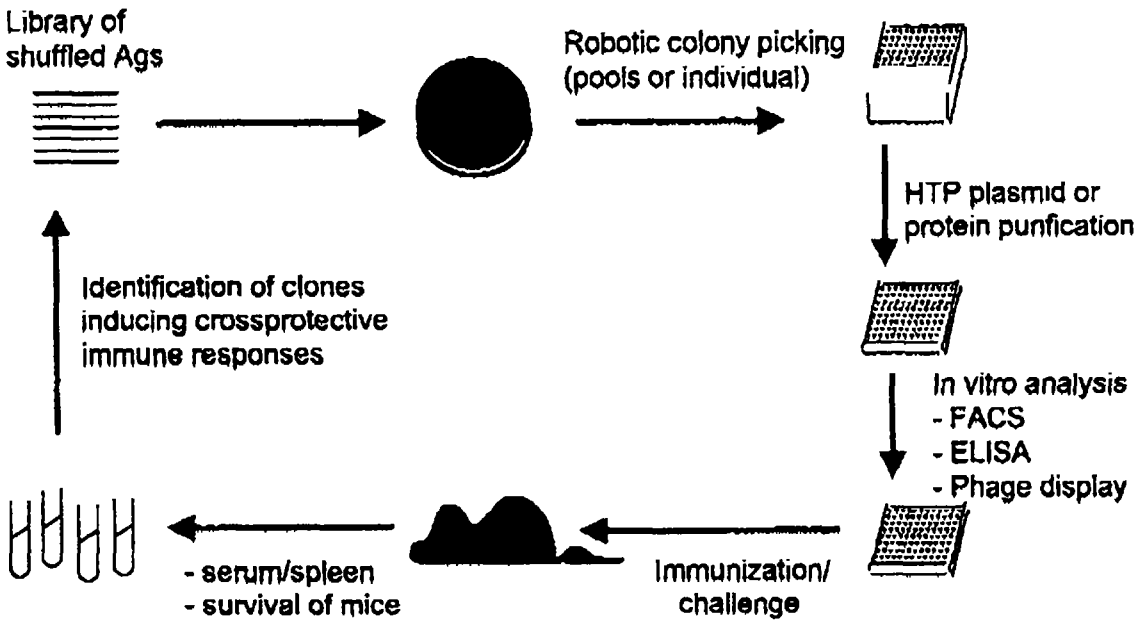


Figure 6

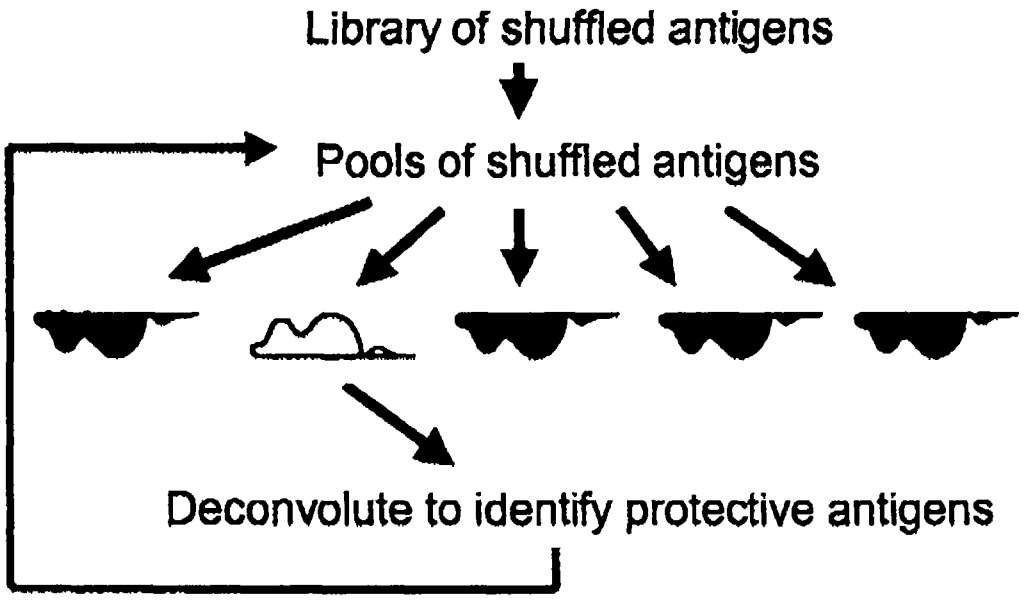


Figure 7: Alignment of glycoprotein D (gD) DNA sequences from HSV-1 and HSV-2 strains illustrating the feasibility of family shuffling when evolving gD.

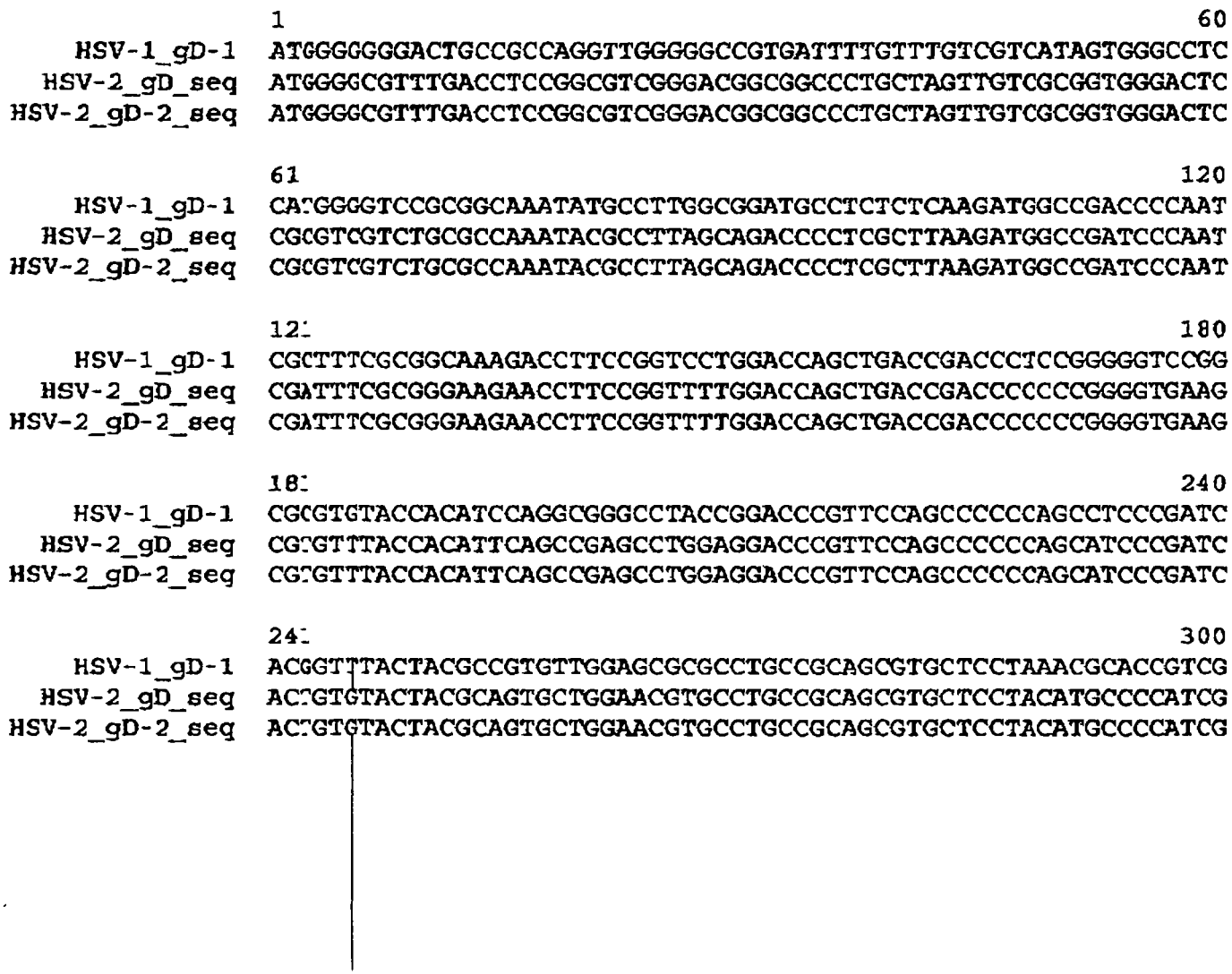


Figure 7 (continued)

```
                301                                                       360
   HSV-1_gD-1   GAGGCCCCCCAGATTGTCCGCGGGGCCTCCGAAGACGTCCGGAAACAACCCTACAACCTG
  HSV-2_gD_seq  GAGGCCCCCCAGATCGTGCGCGGGGCTTCGGACGAGGCCCGAAAGCACACGTACAACCTG
HSV-2_gD-2_seq  GAGGCCCCCCAGATCGTGCGCGGGGCTTCGGACGAGGCCCGAAAGCACACGTACAACCTG

                361                                                       420
   HSV-1_gD-1   ACCATCGCTTGGTTTCGGATGGGAGGCAACTGTGCTATCCCCATCACGGTCATGGAGTAC
  HSV-2_gD_seq  ACCATCGCCTGGTATCGCATGGGAGACAATTGCGCTATCCCCATCACGGTTATGGAATAC
HSV-2_gD-2_seq  ACCATCGCCTGGTATCGCATGGGAGACAATTGCGCTATCCCCATCACGGTTATGGAATAC

                421                                                       480
   HSV-1_gD-1   ACCGAATGCTCCTACAACAAGTCTCTGGGGGCCTGTCCCATCCGAACGCAGCCCCGCTGG
  HSV-2_gD_seq  ACCGAGTGCCCCTACAACAAGTCGTTGGGGGTCTGCCCCATCCGAACGCAGCCCCGCTGG
HSV-2_gD-2_seq  ACCGAGTGCCCCTACAACAAGTCGTTGGGGGTCTGCCCCATCCGAACGCAGCCCCGCTGG

                481                                                       540
   HSV-1_gD-1   AACTACTATGACAGCTTCAGCGCCGTCAGCGAGGATAACCTGGGGTTCCTGATGCACGCC
  HSV-2_gD_seq  AGCTACTATGACAGCTTTAGCGCCGTCAGCGAGGATAACCTGGGATTCCTGATGCACGCC
HSV-2_gD-2_seq  AGCTACTATGACAGCTTTAGCGCCGTCAGCGAGGATAACCTGGGATTCCTCATGCACGCC

                541                                                       600
   HSV-1_gD-1   CCCGCGTTTGAGACCGCCGGCACGTACCTGCGGCTCGTGAAGATAAACGACTGGACGGAG
  HSV-2_gD_seq  CCCGCCTTCGAGACCGCGGGTACGTACCTGCGGCTAGTGAAGATAAACGACTGGACGGAG
HSV-2_gD-2_seq  CCCGCCTTCGAGACCGCGGGTACGTACCTGCGGCTAGTGAAGATAAACGACTGGACGGAG

                601                                                       660
   HSV-1_gD-1   ATTACACAGTTTATCCTGGAGCACCGAGCCAAGGGCTCCTGTAAGTACGCCCTCCCGCTG
  HSV-2_gD_seq  ATCACACAATTTATCCTGGAGCACCGGGCCCGCGCCTCCTGCAAGTACGCTCTCCCCCTG
HSV-2_gD-2_seq  ATCACACAATTTATCCTGGAGCACCGGGCCCGCGCCTCCTGCAAGTACGCTCTCCCCCTG
```

Figure 7 (continued)

```
                661                                                      720
HSV-1_gD-1      CGCATCCCCCCGTCAGCCTGCCTCTCCCCCCAGGCCTACCAGCAGGGGGTGACGGTGGAC
HSV-2_gD_seq    CGCATCCCCCCGGCAGCGTGCCTCACCTCGAAGGCCTACCAACAGGGCGTGACGGTCGAC
HSV-2_gD-2_seq  CGCATCCCCCCGGCAGCGTGCCTCACCTCGAAGGCCTACCAACAGGGCGTGACGGTCGAC

                721                                                      780
HSV-1_gD-1      AGCATCGGGATGCTGCCCCGCTTCATCCCCGAGAACCAGCGCACCGTCGCCGTATACAGC
HSV-2_gD_seq    AGCATCGGGATGTTACCCCGCTTTACTCCCGAAAACCAGCGCACCGTCGCCCTATACAGC
HSV-2_gD-2_seq  AGCATCGGGATGTTACCCCGCTTTATCCCCGAAAACCAGCGCACCGTCGCCCTATACAGC

                781                                                      840
HSV-1_gD-1      TTGAAGATCGCCGGGTGGCACGGGCCCAAGGCCCCATACACGAGCACCCTGCTGCCCCCG
HSV-2_gD_seq    TTAAAAATCGCCGGGTGGCACGGCCCCAAGCCCCCGTACACCAGCACCCTGCTGCCGCCG
HSV-2_gD-2_seq  TTAAAAATCGCCGGGTGGCACGGCCCCAAGCCCCCGTACACCAGCACCCTGCTGCCGCCG

                841                                                      900
HSV-1_gD-1      GAGCTGTCCGAGACCCCCAACGCCACGCAGCCAGAACTCGCCCCGGAAGACCCCGAGGAT
HSV-2_gD_seq    GAGCTGTCCGACACCACCAACGCCACGCAACCCGAACTCGTTCCGGAAGACCCCGAGGAC
HSV-2_gD-2_seq  GAGCTGTCCGACACCACCAACGCCACGCAACCCGAACTCGTTCCGGAAGACCCCGAGGAC

                901                                                      960
HSV-1_gD-1      TCGGCCCTCTTGGAGGACCCCGTGGGGACGGTGGCGCCGCAAATCCCACCAAACTGGCAC
HSV-2_gD_seq    TCGGCCCTCTTAGAGGATCCCGCCGGGACGGTGTCTTCGCAGATCCCCCCAAACTGGCAC
HSV-2_gD-2_seq  TCGGCCCTCTTAGAGGATCCCGCCGGGACGGTGTCTTCGCAGATCCCCCCAAACTGGCAC

                961                                                     1020
HSV-1_gD-1      ATCCCGTCGATCCAGGACGCCGCGACGCCTTACCATCCCCCGGCCACCCCGAACAACATG
HSV-2_gD_seq    ATCCCGTCGATCCAGGACGTCGCGCCGC...ACCACGCCCCCGCCGCCCCAGCCAACCCG
HSV-2_gD-2_seq  ATCCCGTCGATCCAGGACGTCGCGCCGC...ACCACGCCCCCGCCGCCCCCAGCAACCCG
```

Figure 7 (continued)

```
                1021                                                1080
    HSV-1_gD-1  GGCCTGATCGCCGGCGCGGTGGGCGGCAGTCTCCTGGCAGCCCTGGTCATTTGCGGAATT
  HSV-2_gD_seq  GGCCTGATCATCGGCGCGCTGGCCGGCAGTACCCTGGCGGCGCTGGTCATCGGCGGTATT
HSV-2_gD-2_seq  GGCCTGATCATCGGCGCGCTGGCCGGCAGTACCCTGGCGGCGCTGGTCATCGGCGGTATT

                1081                                                1140
    HSV-1_gD-1  GTGTACTGGATGCACCGCCG.CACTCGGAAAGCCCCAAAGCGCATACGCCTCCCCCACAT
  HSV-2_gD_seq  GCGTTTTGGGTACGCCGCCGGCGCTCAG.TGGCCCCCAAGCGCCTACGTCTCCCCCACAT
HSV-2_gD-2_seq  GCGTTTTGGGTACGCCGCCG.CGCTCAGATGGCCCCCAAGCGCCTACGTCTCCCCCACAT

                1141                                                1200
    HSV-1_gD-1  CCGGGAAGACGACCAGCCGTCCTCGCACCAGCCCTTGTTTTACTAG.............
  HSV-2_gD_seq  CCGGGATGACGACGCGCCCCCCTCGCACCAGCCATTGTTTTACT..............
HSV-2_gD-2_seq  CCGGGATGACGACGCGCCCCCCTCGCACCAGCCATTGTTTTACTAGAGGAGTTTCCCCGT

                1201                                        1246
    HSV-1_gD-1  ..........................................
  HSV-2_gD_seq  ..........................................
HSV-2_gD-2_seq  TCCCGTGTACCTCTGGGCCCGTGTGGGAGGGTGGCCGGGGTATTTG
```


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Figure 8A

Expression of shuffled envelope gp120 protein in a genetic vaccine

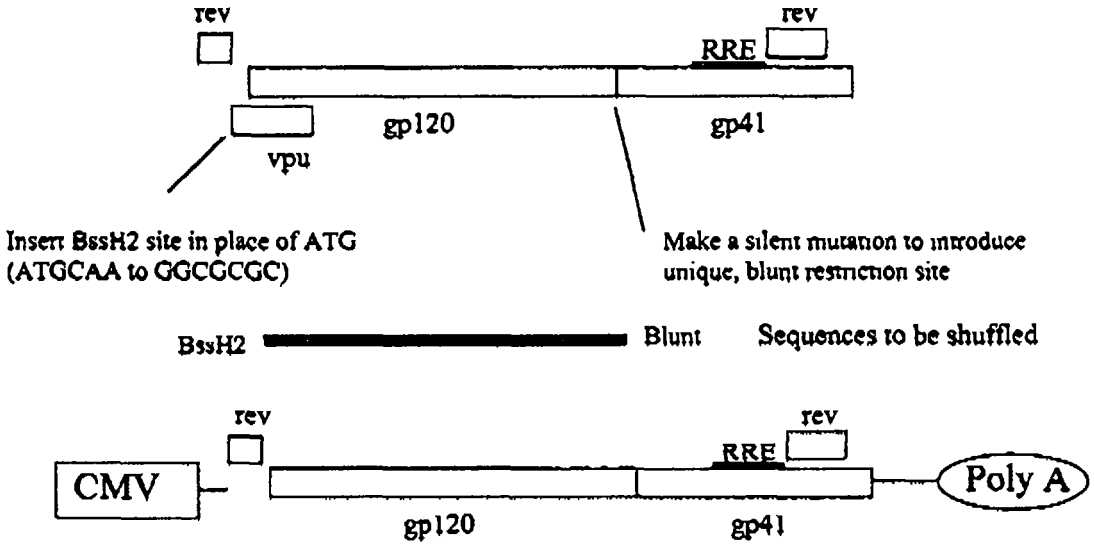


Figure 8B

PCR primers for genetic vaccine for HIV

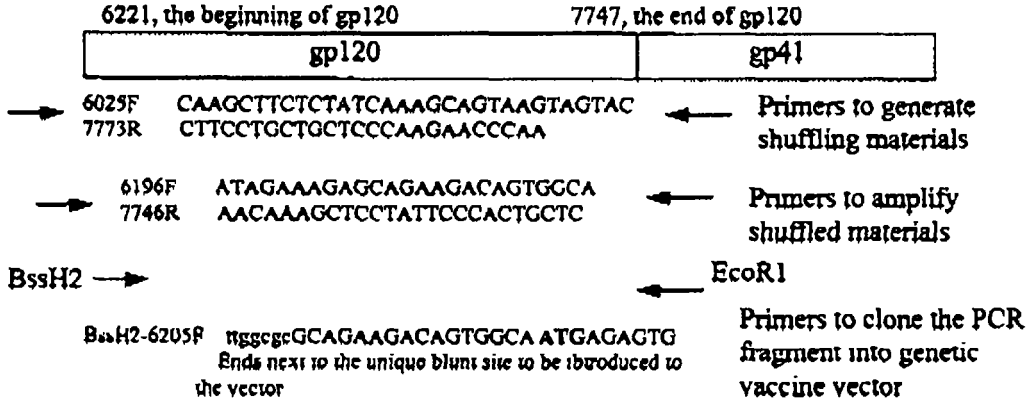


Figure 9

Domain Structure of Hepadnavirus Envelope Genes

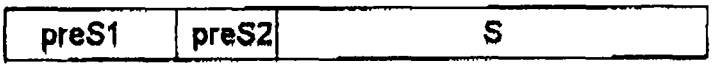


Figure 10

Isolate genes coding for proteins in which the immunogenicity of one antigenic domain is improved:

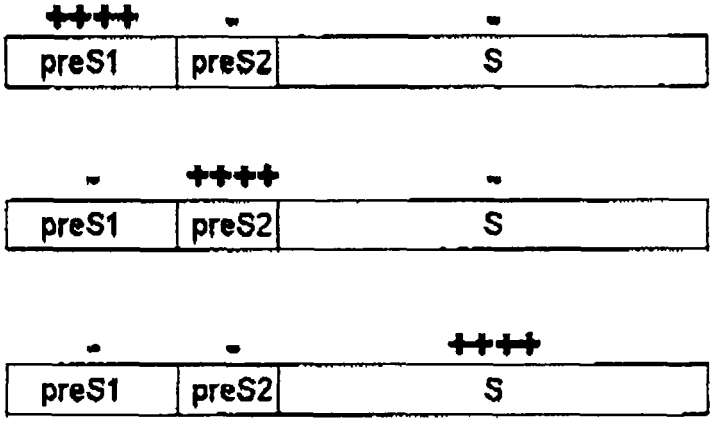


Figure 11

Shuffle genes for which the immunogenicity of one antigenic domain is improved

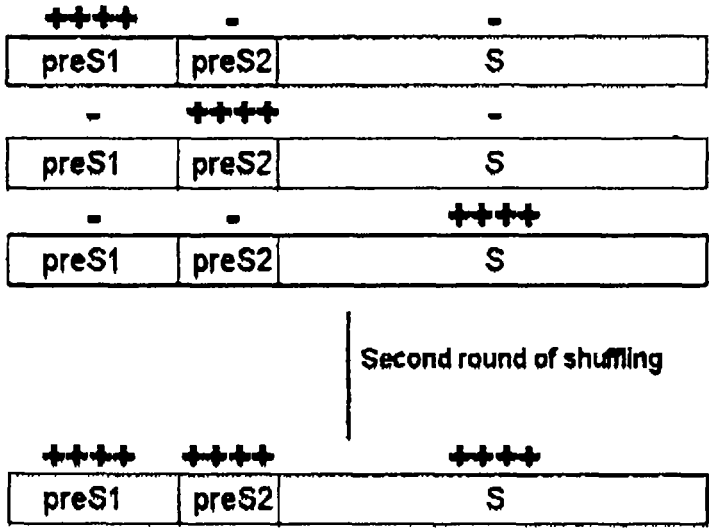


Figure 12

Transmembrane organization of the HBsAg polypeptide

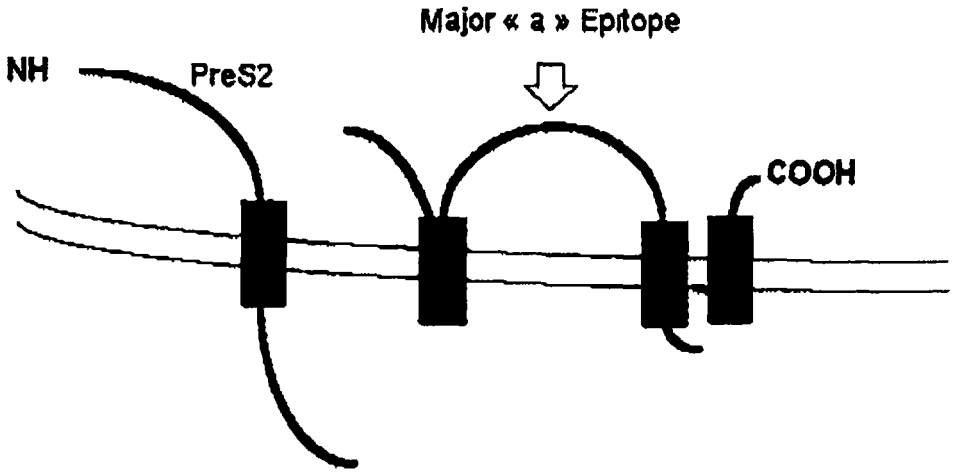
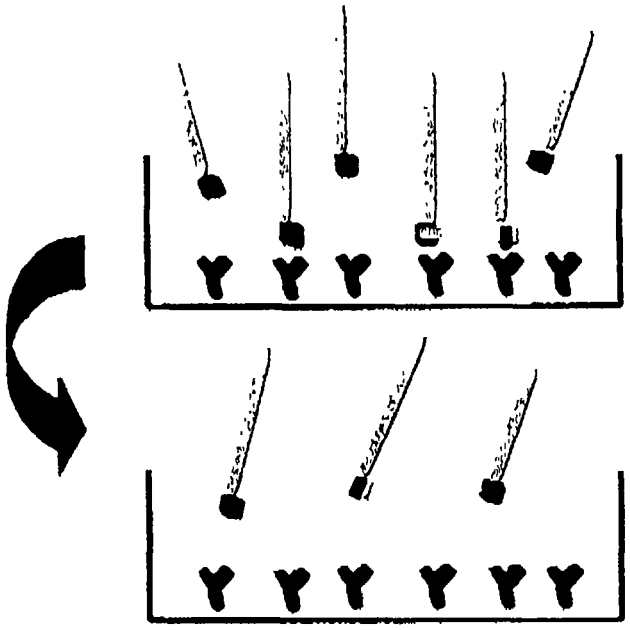


Figure 13

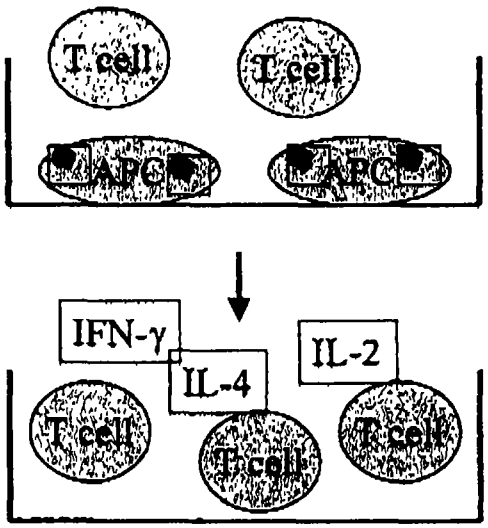
Phage Display for Allergens not Recognized by Pre-existing IgE



- Remove variants that bind to IgE derived from atopic patients
- Repeat until no binding is observed

Figure 14

Screening of Allergen Variants that Efficiently Activate Th cells from Atopics



- PBMC or T cell clones from atopic patients
- Activation with shuffled allergen variants

↓

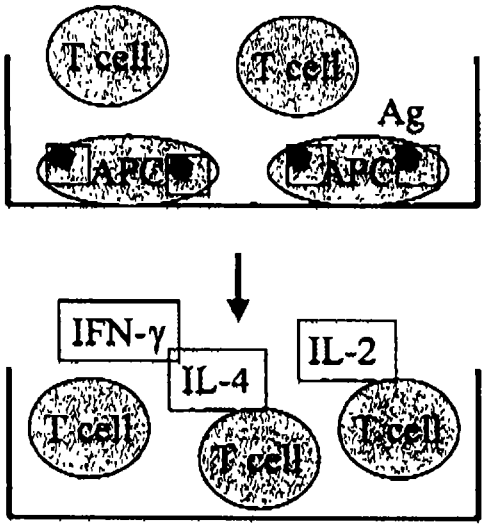
- T cell proliferation
- Cytokine synthesis (IL-2, IL-4, IFN-γ)

↓

- In vivo testing

Figure 15

Screening of Cancer Antigens that Efficiently Activate T cells from Patients



- PBMC or T cell clones from Cancer patients
- Activation with shuffled antigen variants

↓

- T cell /CTL activity
- Cytokine synthesis (IL-2, IL-4, IFN-γ)

↓

- In vivo testing

Figure 16: Generation of vectors with multiple T cell epitopes by shuffling libraries of T cell epitopes

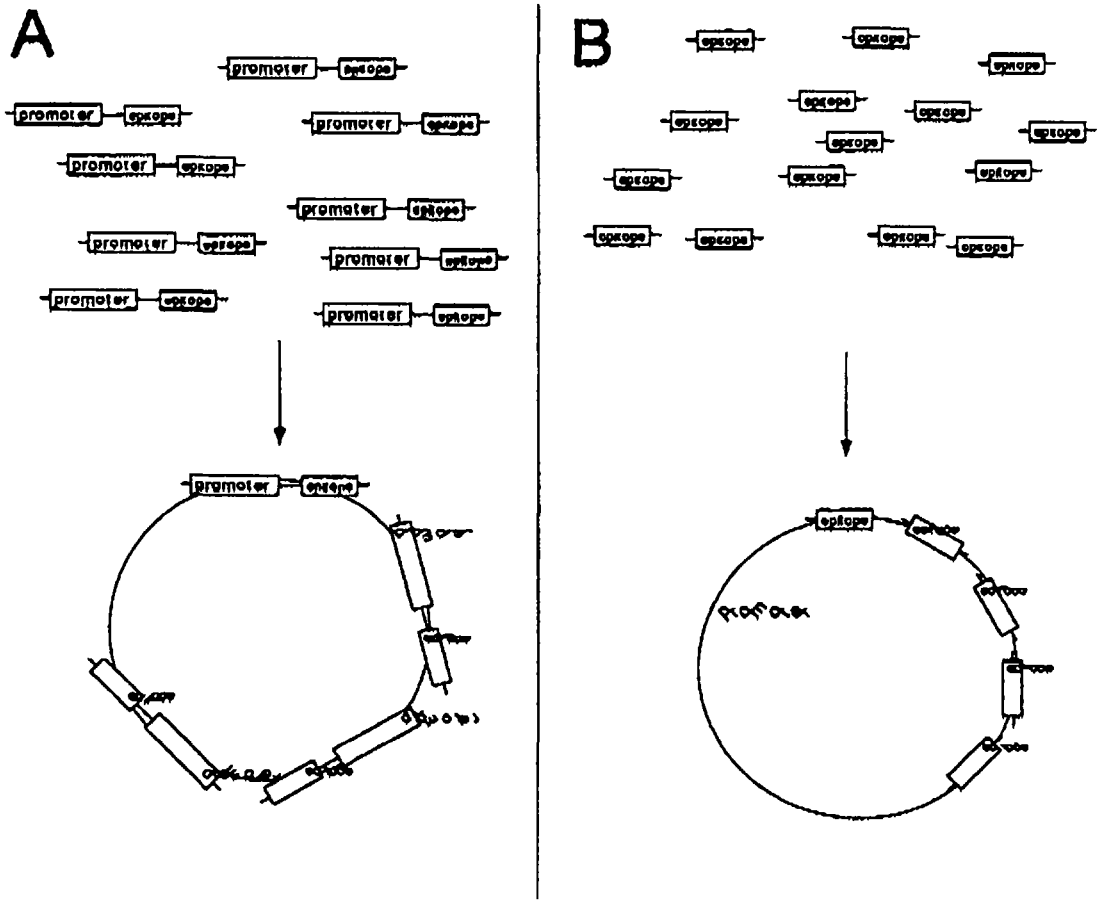


Figure 17: Sequences of PreS2-S Coding Regions of Different HBsAg (or WHsAg) Proteins

M38454, HPBADR1CG Hepatitis B virus, complete genome. Shown is HBsAg coding region (SEQ ID NO: 9); PreS2 starts at 1811 of complete genome sequence, S starts at 1976, end at 2656.

ATGCAGTGGAACTCCACAACATTCCACCAAGCTCTGCTAGACCCCAGAGTGAGGGGCCTATA
CTTTCCTGCTGGTGGCTCCAGTTCCGGAACAGTAAACCCTGTTCCGACTACTGCCTCACCCA
TATCGTCAATCTTCTCGAGGACTGGGGACCCTGCACCGAACATGGAGAACACAACATCAGGA
TTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAAT
ACCACAGAGTCTACACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGCAGCACCCACGTGTC
TTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCTTGTCCTCCAATTTGT
CCTGGTTATCGTTGGATGTGTCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATG
CCTCATCTTCTTGTTGGTTCTTCTGGACTACCAAGGTATGTTGTCTGTTTGTCCTCTACTTC
CAAGAACATCAACTACCAGCACGGGACCATGCAAGACCTGCACGATTCCTGCTCAAGGAACC
TCTATGTTTCCCTCTTCTTGCTGTACAAAACCTTCGGACGGAAACTGCACTTGTATTCCCAT
CCCATCATCTTGGGCTTTCGCAAGATTCCTATGGGAGTGGGCCTCAGTCCGTTTCTCCTGGC
TCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCA
GTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACC
TCTATTACCAATTTTCTTTTGTCTTTGGGTATACATTTGA

HBsAg Protein adr (SEQ ID NO: 10):

MQWNSTTFHQALLDPRVRGLYFPAGGSSSGTVNPVPTTASPISSIFSRTGDPAPNMENTTSG
FLGPLLVLQAGFFLLTRILTIPQSLHSWWTSLNFLGAAPTCLGQNSQSPTSNHSPTSCPPIC
PGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLSVCPLLPRTSTTSTGPCKTCTIPAQGT
SMFPSSCCTKPSDGNCTCIPIPSSWAFARFLWEWASVRFSWLSLLVPFVQWFVGLSPTVWLS
VIWMMWYWGPSLYNILSPFLPLLPIFFCLWVYIZ

Figure 17 (continued)

J02203; HPBAYW Hepatitis B Virus ayw. (SEQ ID NO: 11)

ATGCAGTGGAATTCCACAACCTTCCACCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTGTA
TTTCCCTGCTGGTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCTGACTACTGCCTCTCCCT
TATCGTCAATCTTCTCGAGGATTGGGGACCCTGCGCTGAACATGGAGAACATCACATCAGGA
TTCCTAGGACCCCTTCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAAT
ACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTC
TTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCTTGTCCTCCAACTTGT
CCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATG
CCTCATCTTCTTGTTGGTTCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTC
CAGGATCCTCAACAACCAGCACGGGACCATGCCGGACCTGCATGACTACTGCTCAAGGAACC
TCTATGTATCCCTCCTGTTGCTGTACCAAACCTTCGGACGGAAATTGCACCTGTATTCCCAT
CCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGC
TCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCA
GTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACC
GCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

Forward HBV ayw PCR Primer (with 2 natural EcoRI sites) (SEQ ID NO: 13):
5'-CCGGGAATTCCTCGACACCATGCAGTGGAATTCCACAACC-3'

Reverse HBV ayw PCR Primer (with added KpnI site) (SEQ ID NO: 14):
5'-CCGGGGTACCCAAAGACAAAAGAAAATTGGTAACAGCGG-3'

HBsAg Protein ayw (SEQ ID NO.:12):
MQWNSTTFHQTLQDPRVRGLYFPAGGSSSGTVNPVLTTASPLSSIFSRIGDPALNMENITSG
FLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSPTSNHSPTSCPPTC
PGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCMTTAQGT
SMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLS
VIWMMWYWGPSLYSILSPFLPLLPIFFCLWVYIZ

Figure 17 (continued)

J04514 OHVHEPBA Woodchuck hepatitis B virus genome (WHV8) (SEQ ID NO: 15) (PNAS 86, 1846-1849, 1989). ATCC No. 45097. EcoRI site at pos. 1 cuts the PreS1 region. HBs(S) ORF: 296-964. PreS2 starts at 116; PreS1 starts at 3039.

ATGAAAAATCAGACTTTTCATCTCCAGGGGTTCGTAGACGGATTACGAGACTTGACAACAAC
GGAACGCCAACACAATGCCTATGGAGATCCTTTTACAACACTAAGCCCTGCGGTTCCTACTG
TATCCACCATATTGTCTCCTCCCTCGACGACTGGGGACCCTGCACTGTCACCGGAGATGTCA
CCATCAAGTCTCCTAGGACTCCTCGCAGGATTACAGGTGGTGTATTTCTTGTGGACAAAAAT
CCTAACAATAGCTCAGAATCTAGATTGGTGGTGGACTTCTCTCAGTTTTCCAGGGGGCATAC
CAGAGTGCACTGGCCAAAATTCGCAGTTCCAAACTTGCAAACACTTGCCAACCTCCTGTCCA
CCAACTTGCAATGGCTTTCGTTGGATGTATCTGCGGCGTTTTATCATATACCTATTAGTCCT
GCTGCTGTGCCTCATCTTCTTGTTGGTTCTCCTGGACTGGAAAGGTTTAATACCTGTCTGTC
CTCTTCAACCCACAACAGAAACAACAGTCAATTGCAGACAATGCACAATCTCTGCACAAGAC
ATGTATACTCCTCCTTACTGTTGTTGTTTAAAACCTACGGCAGGAAATTGCACTTGTTGGCC
CATCCCTTCATCATGGGCTTTAGGAAATTACCTATGGGAGTGGGCCTTAGCCCGTTTCTCTT
GGCTCAATTTACTAGTGCCCTTGCTTCAATGGTTAGGAGGAATTTCCCTCATTGCGTGGTTT
TTGCTTATATGGATGATTTGGTTTTGGGGGCCCGCACTTCTGAGCATCTTACCGCCATTTAT
TCCCATATTTGTTCTGTTTTTCTTGATTTGGGTATACATTTGA

Forward WHV PCR Primer (with added EcoRI site) (SEQ ID NO:17):
5'-CCGGGAATTCTCATCTCCAGGGGTTCGTAGACGGATTACG-3'

Reverse WHV PCR Primer (with added KpnI site) (SEQ ID NO: 18):
5'-CCGGGGTACCCAAATCAAGAAAAACAGAACAAATATGGG-3'

WHV Surface Antigen Protein (SEQ ID NO: 16):
MKNQTFHLQGFVDGLRDLTTTERQHNAYGDPFTTLSPAVPTVSTILSPPSTTGDPALSPEMS
PSSLLGLLAGLQVVYFLWTKILTIAQNLDWWWTSLSFPGGIPECTGQNSQFQTCKHLPTSCP
PTCNGFRWMYLRRFIIYLLVLLLCLIFLLVLLDWKGLIPVCPLQPTTETTVNCRQCTISAQD
MYTPPYCCCLKPTAGNCTCWPIPSSWALGNYLWEWALARFSWLNLLVPLLQWLGGISLIAWF
LLIWMIWFWGPALLSILPPFIPIFVLFFLIWVYIZ

Figure 18

Primers for PCR amplification of the large fragments containing the S2S coding sequences (primers are about 200 bp outside the desired sequences)

AYWSHFOR (SEQ ID NO: 19)
5'-GCCGGCAGGAAGGAAATGGG-3' (13GC + 7AT = Tm 66)

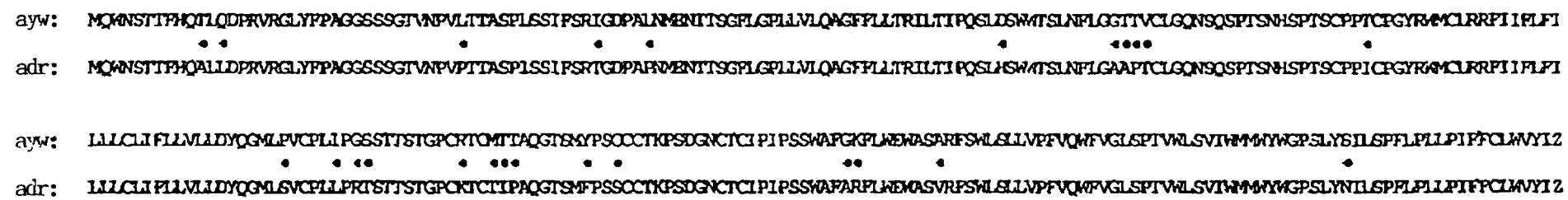
AYWSHREV (SEQ ID NO: 20)
5'-CTGCTATTGTCTTCCCAATCCTC-3' (11GC + 12AT = Tm 68)

WHVSHFOR (SEQ ID NO: 21)
5'-CGGGACATACCACGTGGTTTAG-3' (12GC + 10AT = Tm 68)

WHVSHREV (SEQ ID NO: 22)
5'-GGCATTAAAGCAGCGTATCCAC-3' (11GC + 11AT = Tm 66)

Figure 19: Alignment of amino acid sequences of different HBV subtypes.

adr (SEQ ID NO: 10) **vs. ayw** (SEQ ID NO: 12)



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Figure 20

