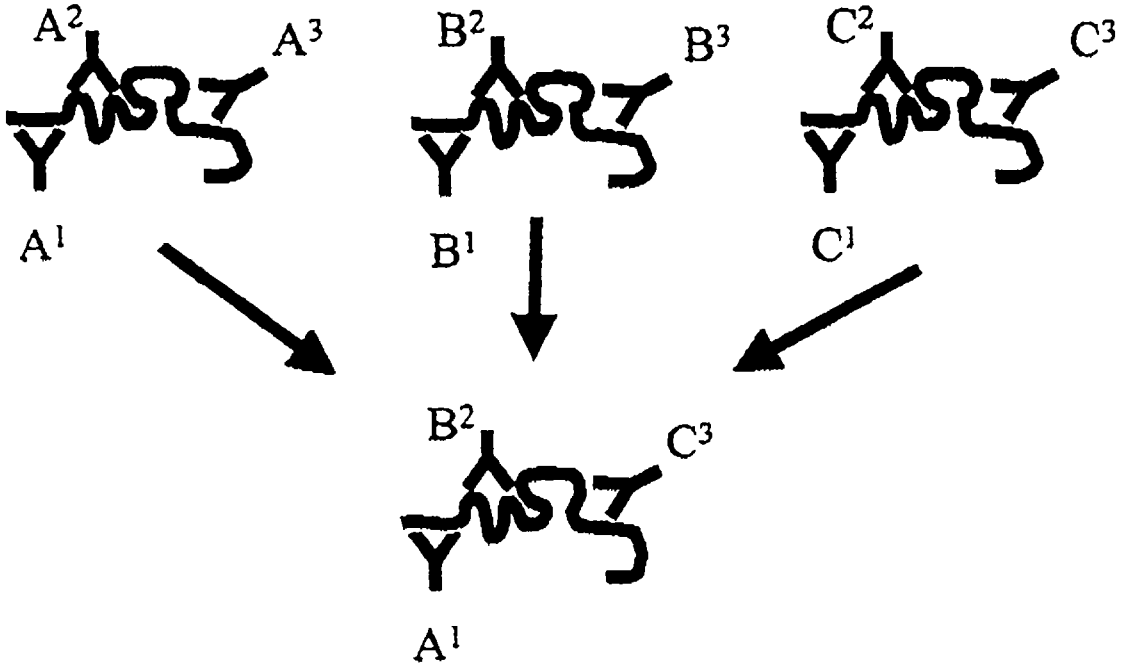
1/21
Figure 1



163163

G00002

Figure 2

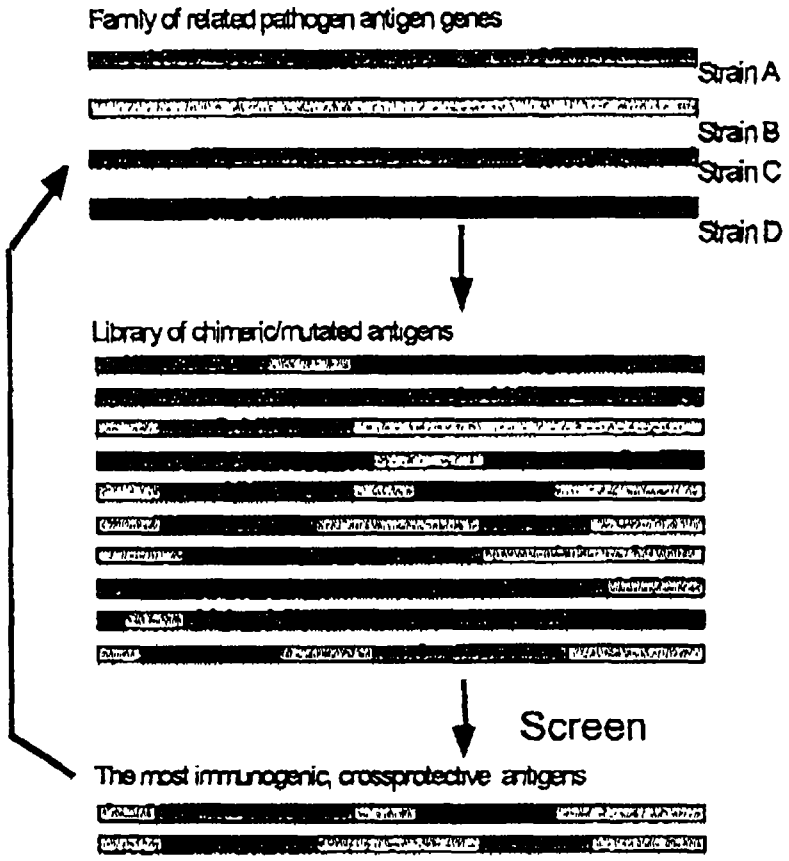


Figure 3

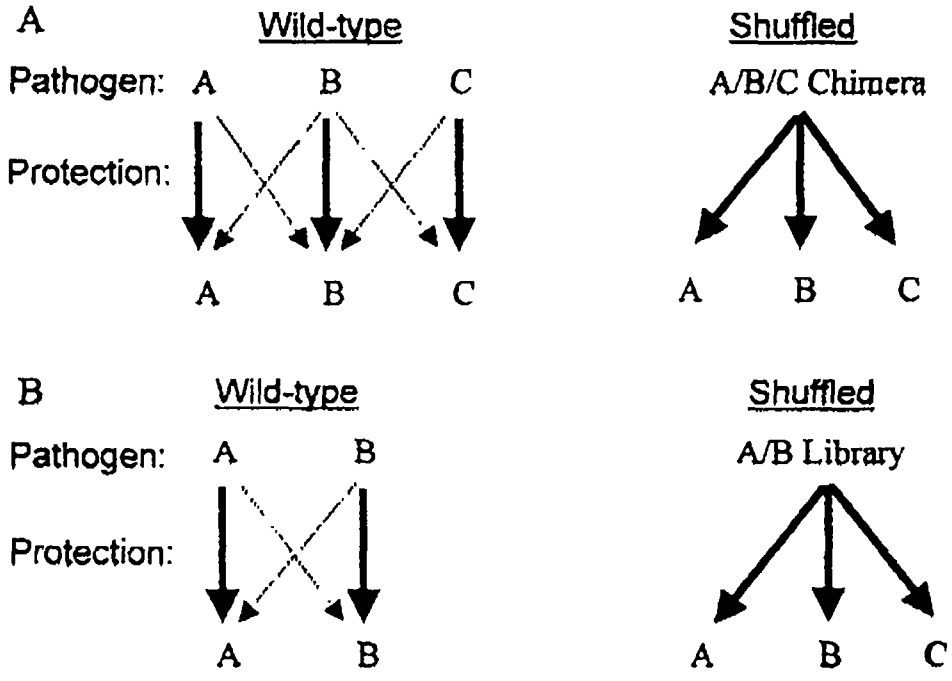


Figure 4

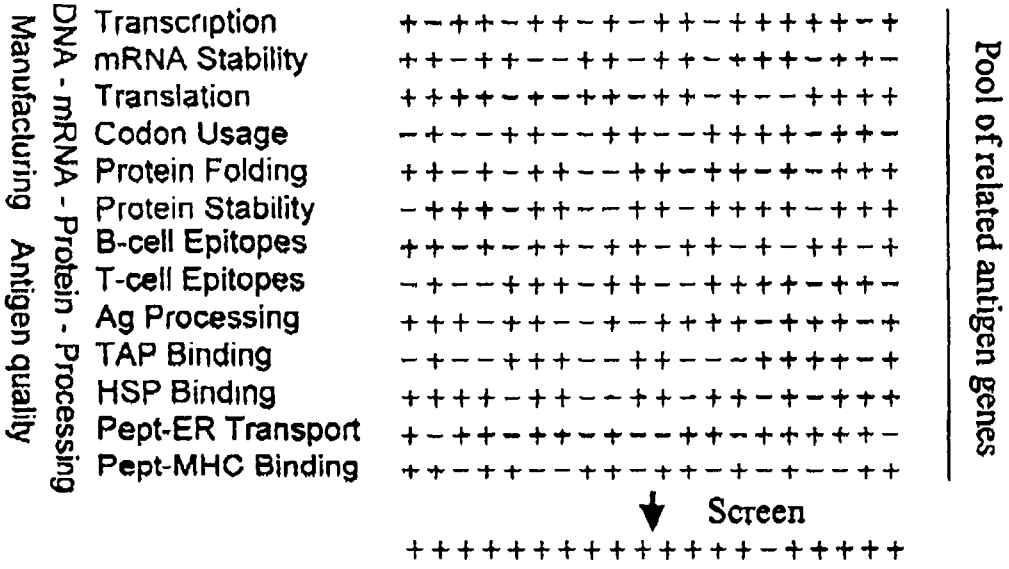

Manufacturing Antigen quality
DNA - mRNA - Protein - Processing
Transcription
mRNA Stability
Translation
Codon Usage
Protein Folding
Protein Stability
B-cell Epitopes
T-cell Epitopes
Ag Processing
TAP Binding
HSP Binding
Pept-ER Transport
Pept-MHC Binding
Screen
Pool of related antigen genes

Figure 5

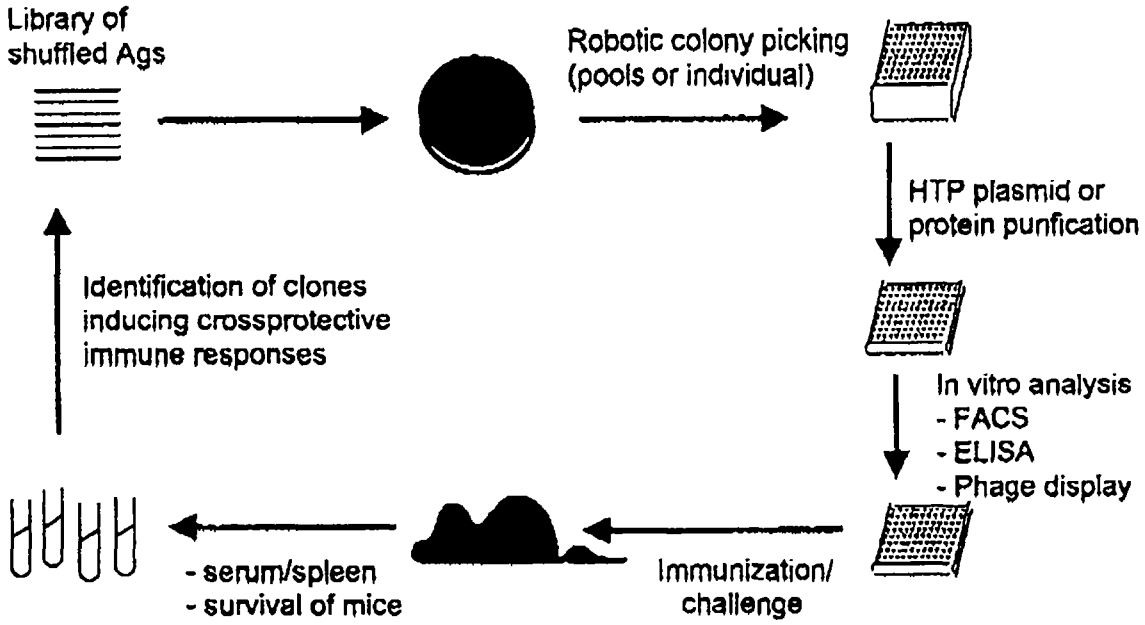


Figure 6

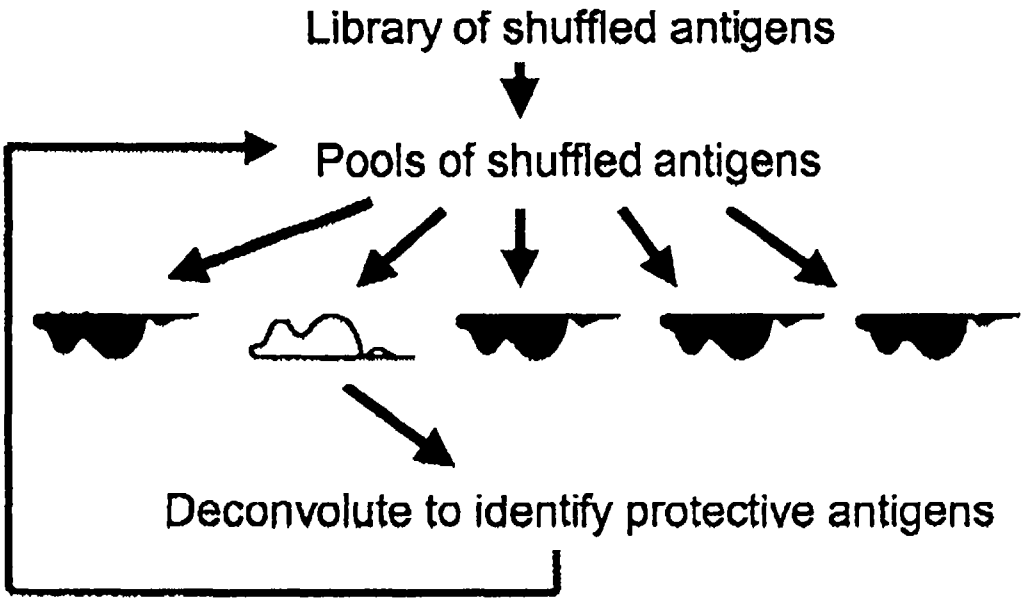


Figure 7: Alignment of glycoprotein D (gD) DNA sequences from HSV-1 and HSV-2 strains illustrating the feasibility of family shuffling when evolving gD.

```
                1                                                          60
    HSV-1_gD-1  ATGGGGGGGACTGCCGCCAGGTTGGGGGCCGTGATTTTGTTTGTCGTCATAGTGGGCCTC
  HSV-2_gD_seq  ATGGGGCGTTTGACCTCCGGCGTCGGGACGGCGGCCCTGCTAGTTGTCGCGGTGGGACTC
HSV-2_gD-2_seq  ATGGGGCGTTTGACCTCCGGCGTCGGGACGGCGGCCCTGCTAGTTGTCGCGGTGGGACTC

               61                                                         120
    HSV-1_gD-1  CATGGGGTCCGCGGCAAATATGCCTTGGCGGATGCCTCTCTCAAGATGGCCGACCCCAAT
  HSV-2_gD_seq  CGCGTCGTCTGCGCCAAATACGCCTTAGCAGACCCCTCGCTTAAGATGGCCGATCCCAAT
HSV-2_gD-2_seq  CGCGTCGTCTGCGCCAAATACGCCTTAGCAGACCCCTCGCTTAAGATGGCCGATCCCAAT

               121                                                        180
    HSV-1_gD-1  CGCTTTCGCGGCAAAGACCTTCCGGTCCTGGACCAGCTGACCGACCCTCCGGGGGGTCCGG
  HSV-2_gD_seq  CGATTTCGCGGGAAGAACCTTCCGGTTTTGGACCAGCTGACCGACCCCCCCGGGGGTGAAG
HSV-2_gD-2_seq  CGATTTCGCGGGAAGAACCTTCCGGTTTTGGACCAGCTGACCGACCCCCCCGGGGGTGAAG

               181                                                        240
    HSV-1_gD-1  CGCGTGTACCACATCCAGGCGGGCCTACCGGACCCGTTCCAGCCCCCCAGCCTCCCGATC
  HSV-2_gD_seq  CGTGTTTACCACATTCAGCCGAGCCTGGAGGACCCGTTCCAGCCCCCCAGCATCCCGATC
HSV-2_gD-2_seq  CGTGTTTACCACATTCAGCCGAGCCTGGAGGACCCGTTCCAGCCCCCCAGCATCCCGATC

               241                                                        300
    HSV-1_gD-1  ACGGTTTACTACGCCGTGTTGGAGCGCGCCTGCCGCAGCGTGCTCCTAAACGCACCGTCG
  HSV-2_gD_seq  ACTGTGTACTACGCAGTGCTGGAACGTGCCTGCCGCAGCGTGCTCCTACATGCCCCATCG
HSV-2_gD-2_seq  ACTGTGTACTACGCAGTGCTGGAACGTGCCTGCCGCAGCGTGCTCCTACATGCCCCATCG
```


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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
```


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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

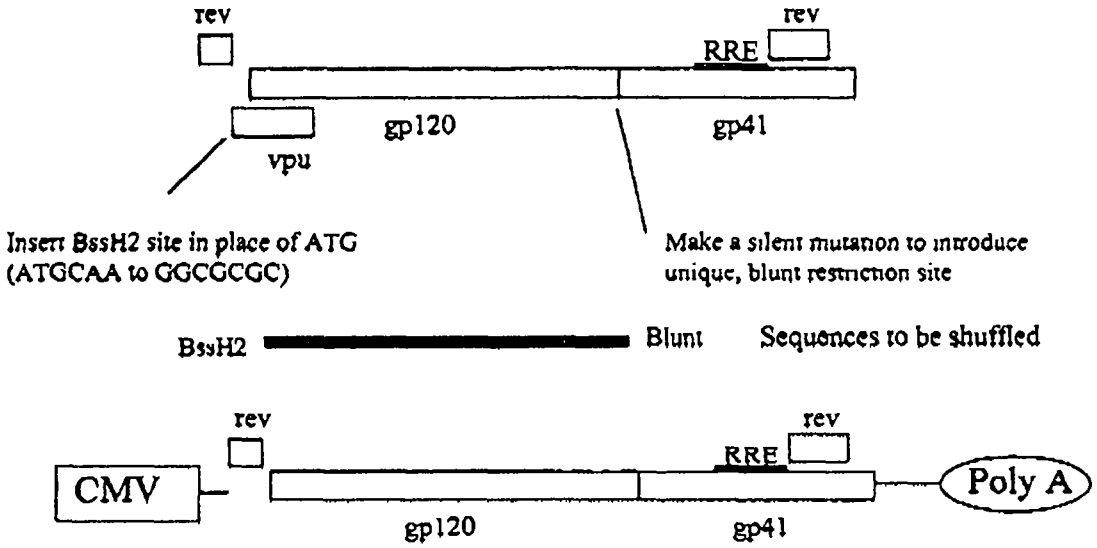


Figure 8B

PCR primers for genetic vaccine for HIV

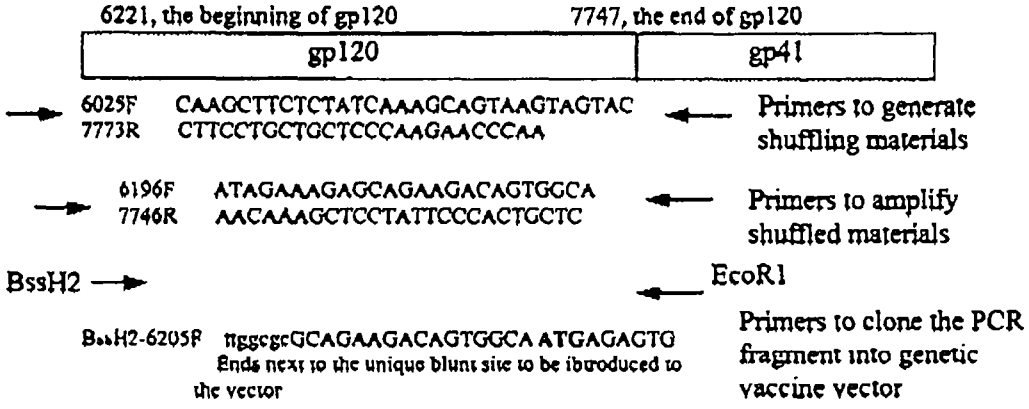


Figure 9

Domain Structure of Hepadnavirus Envelope Genes

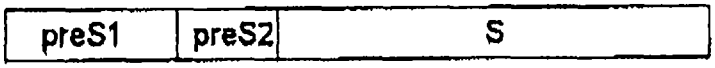


Figure 10

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

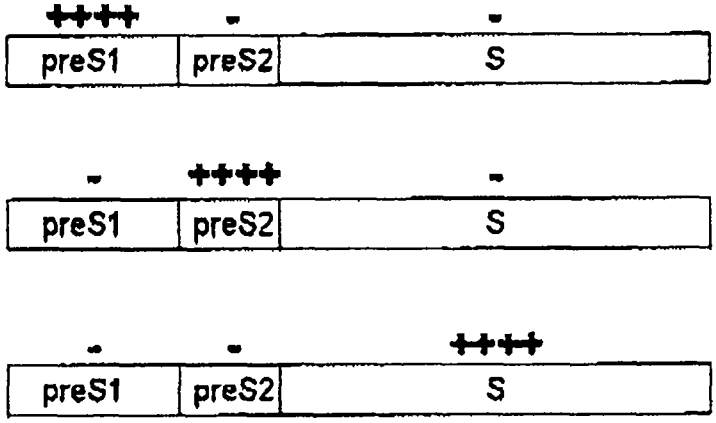


Figure 11

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

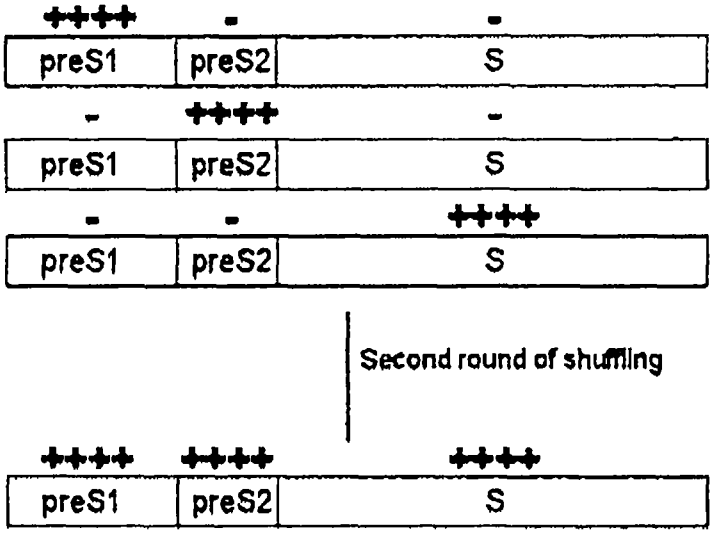


Figure 12

Transmembrane organization of the HBsAg polypeptide

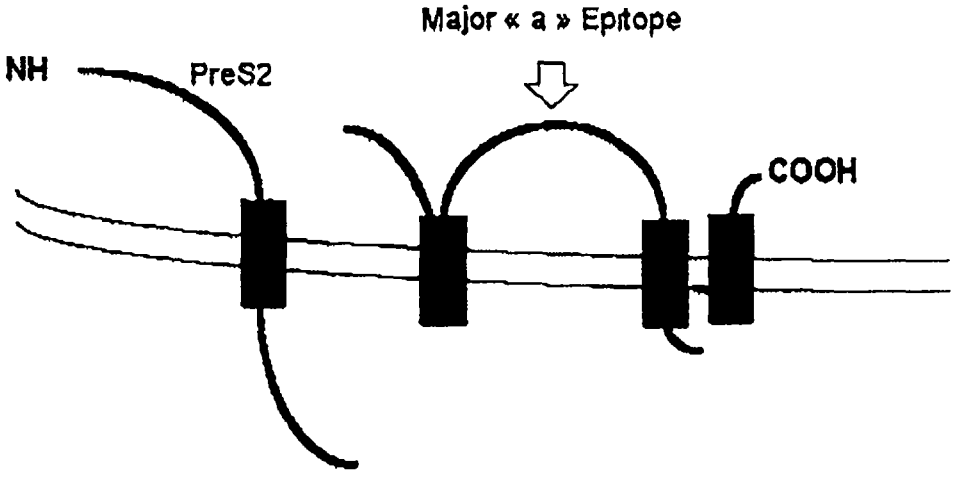
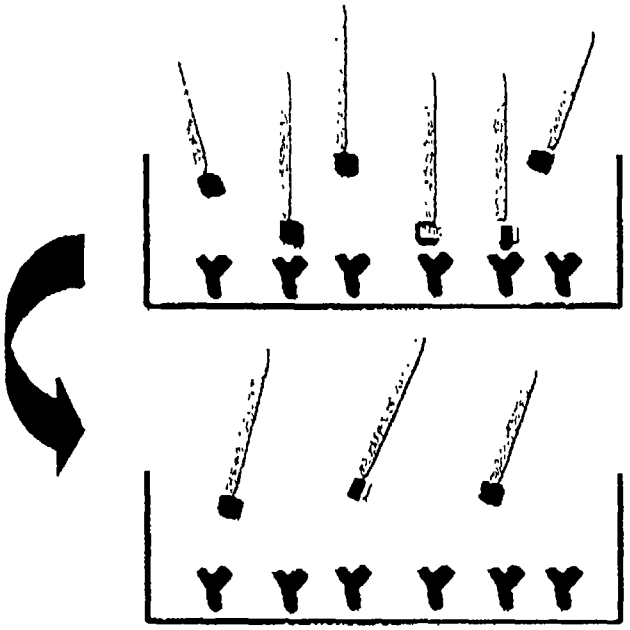


Figure 13

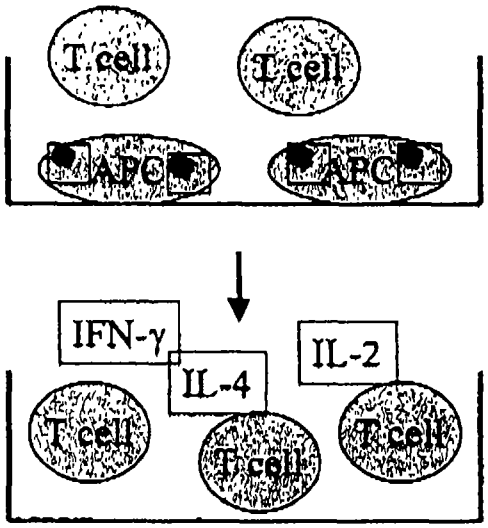
Phage Display for Allergens not Recognized by Pre-existing IgE



- 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

↓

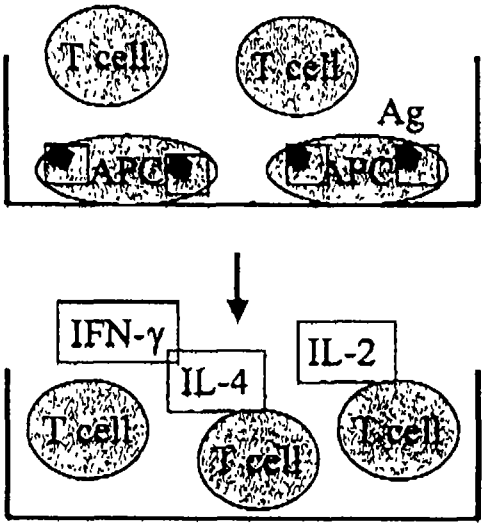
- T cell proliferation
- Cytokine synthesis (IL-2, IL-4, IFN-γ)

↓

- 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

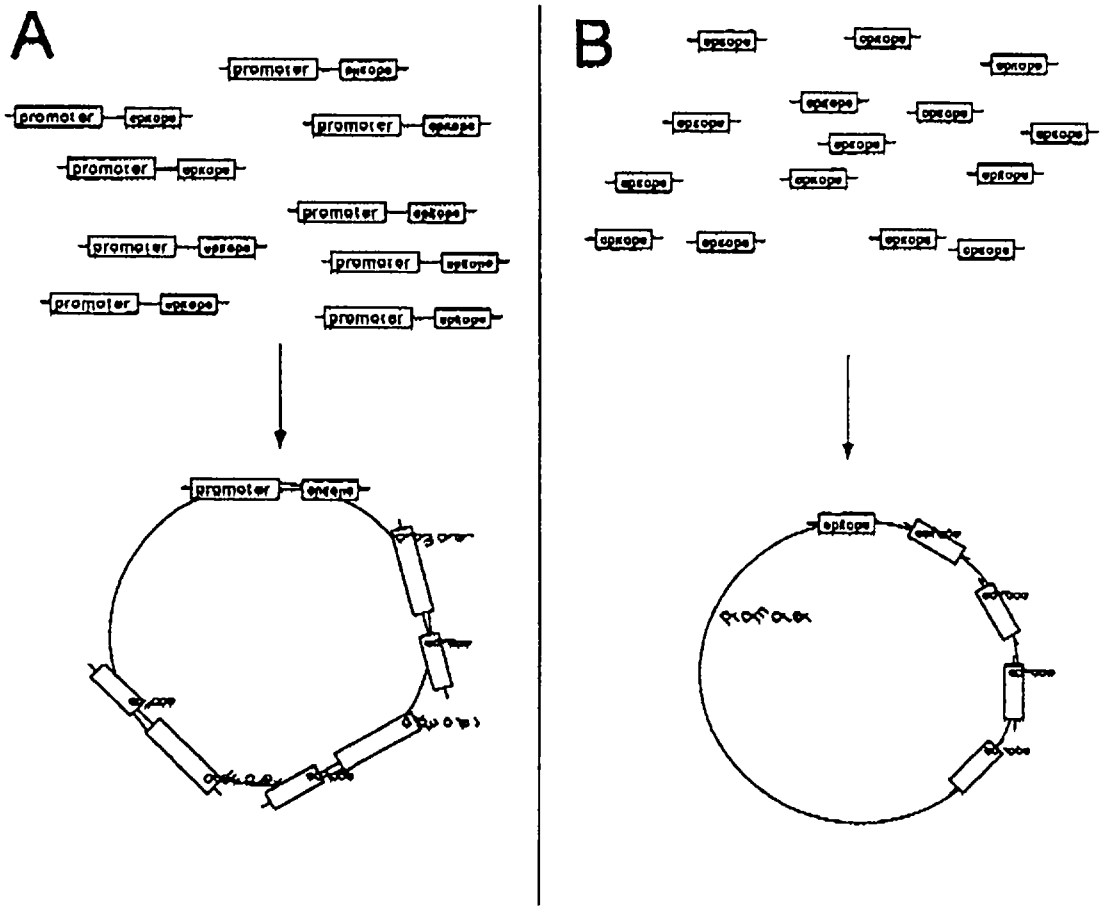


Figure 17: Sequences of PreS2-S Coding Regions of Different HBsAg (or WHsAg) Proteins

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)

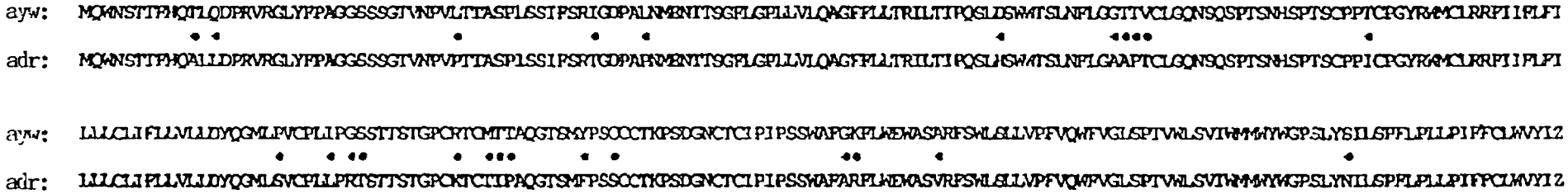
AYWSHREV (SEQ ID NO: 20)
5'-CTGCTATTGTCTTCCCAATCCTC-3' (11GC + 12AT = Tm 68)

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

