```
1   2   3   4   5   6   7   8   9   10  11  12  13  14
Pro-Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-

15  16  17  18  19  20
Pro-Gly-Ser-Thr-Ala-Pro
```

FIG. 1

```
1   2   3   4   5   6   7   8   9   10  11  12  13  14
Pro-Ala-His-Gly-Val-Val-Ala-Ala-Pro-Asp-Ile-Arg-Pro-Ala-

15  16  17  18  19  20
Pro-Gly-Ala-Val-Ala-Pro
```

5′ CCG GCG CAT GGT GTC GTA GCT GCC CCA GAC ATC CGG CCC GCA CCG GGT GCG GTT GCC CCT 3′

FIG. 2

```
1   2   3   4   5   6   7   8   9   10  11  12  13  14
Pro-Ala-His-Gly-Val-Val-Ala-Ala-Pro-Asp-Val-Arg-Pro-Ala-

15  16  17  18  19  20
Pro-Gly-Ala-Val-Ala-Pro
```

5′CCG GCG CAT GGT GTC GTA GCT GCC CCA GAC GTG CGG CCC GCA CCG GGT GCG GTT GCC CCT 3′

FIG. 3

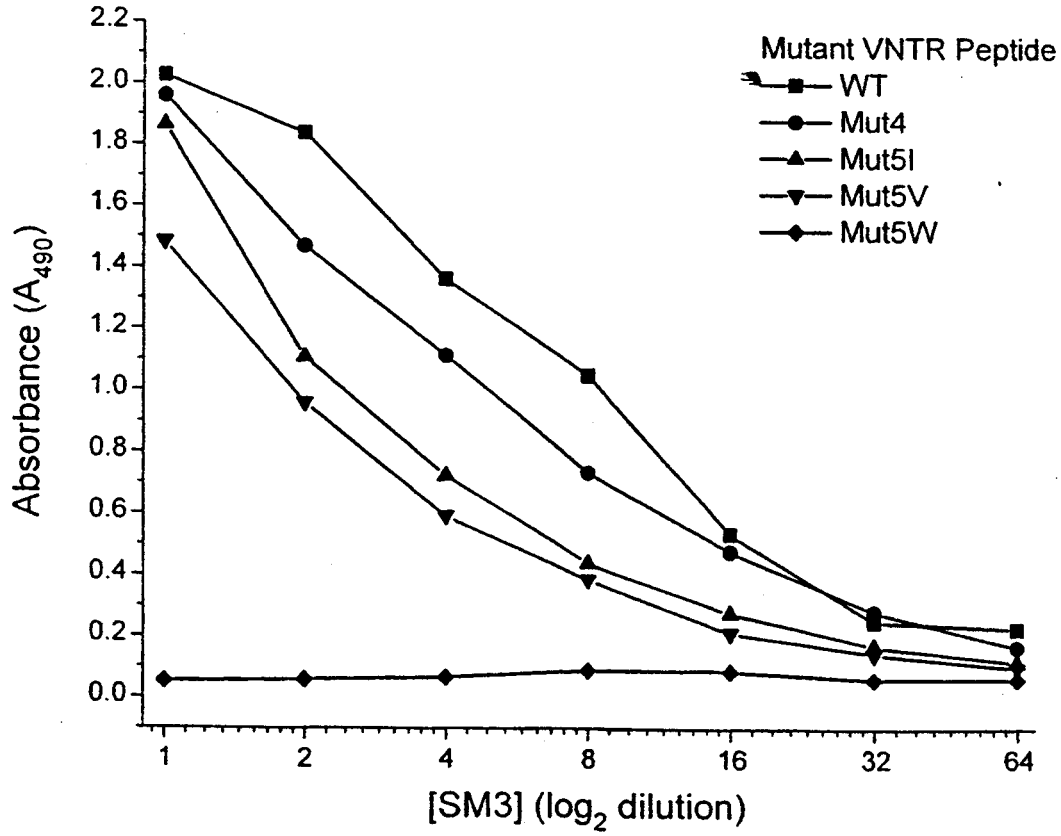
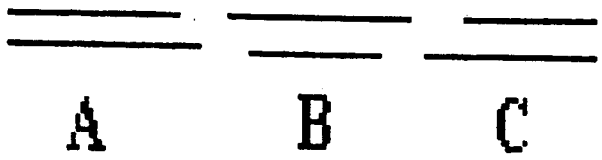

Mutant VNTR Peptide
WT
Mut4
Mut5I
Mut5V
Mut5W
Absorbance (A_{490})
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1
2
4
8
16
32
64
[SM3] (log_2 dilution)

FIG. 4

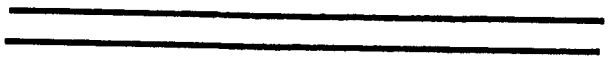
FIG. 5

CLONING STRATEGY FOR 5 REPEAT MUC1 CONSTRUCTS



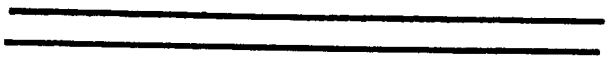
Anneal

(A) 86 bp Sgr A I site + 1.5 repeats
(B) 144 bp 2 repeats
(C) 145 bp 1.5 repeats + Hinge + N-terminus FrC + Sal I site



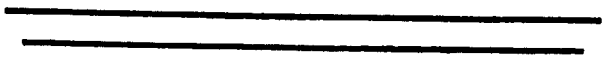
Ligate

A + B + C



Gel Purify

375 bp A + B + C



Digest

SgrA I / Sal I
Gel purify

Clone
pVAC-1stc SgrA I / Sal I
'in frame' FrC fusion

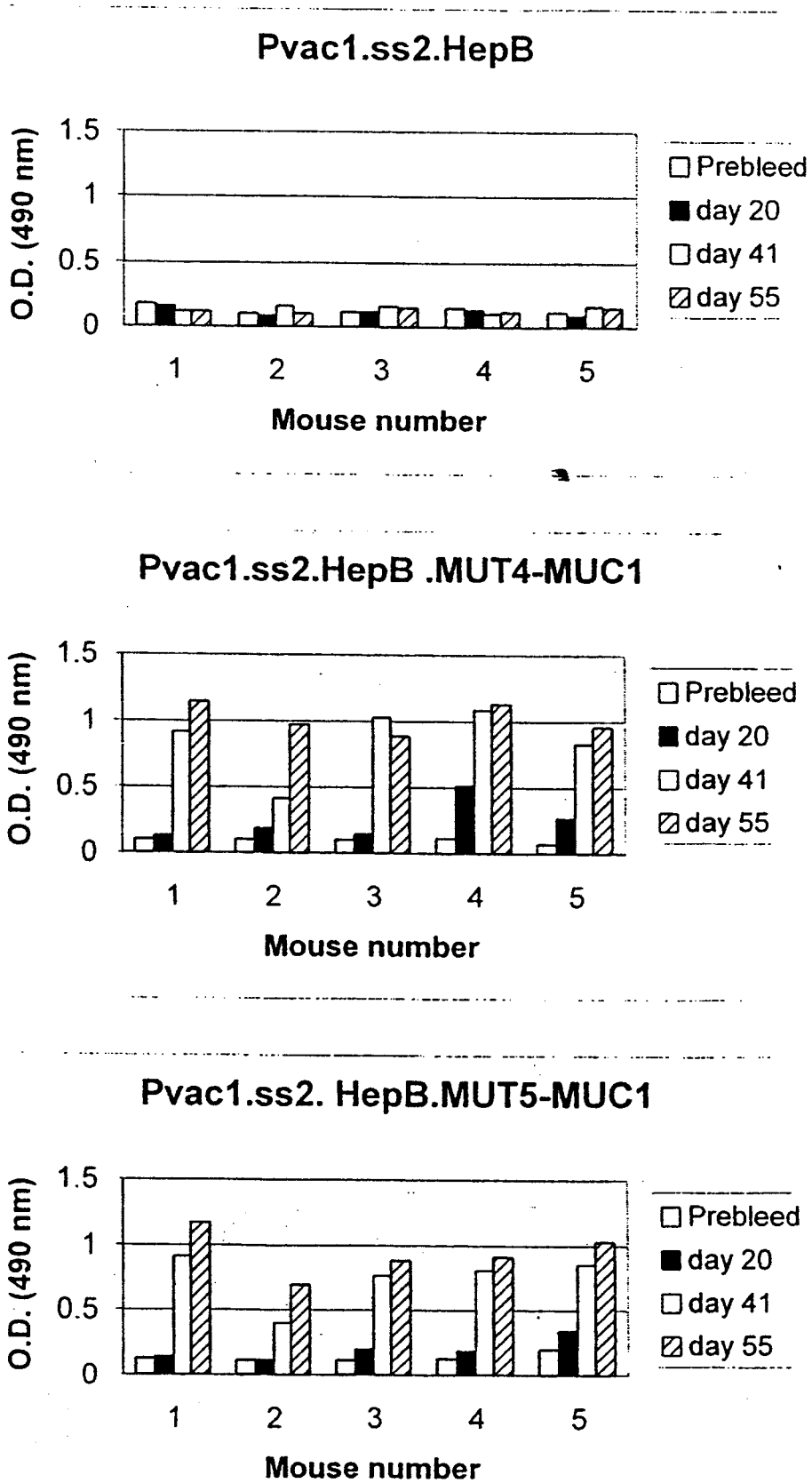
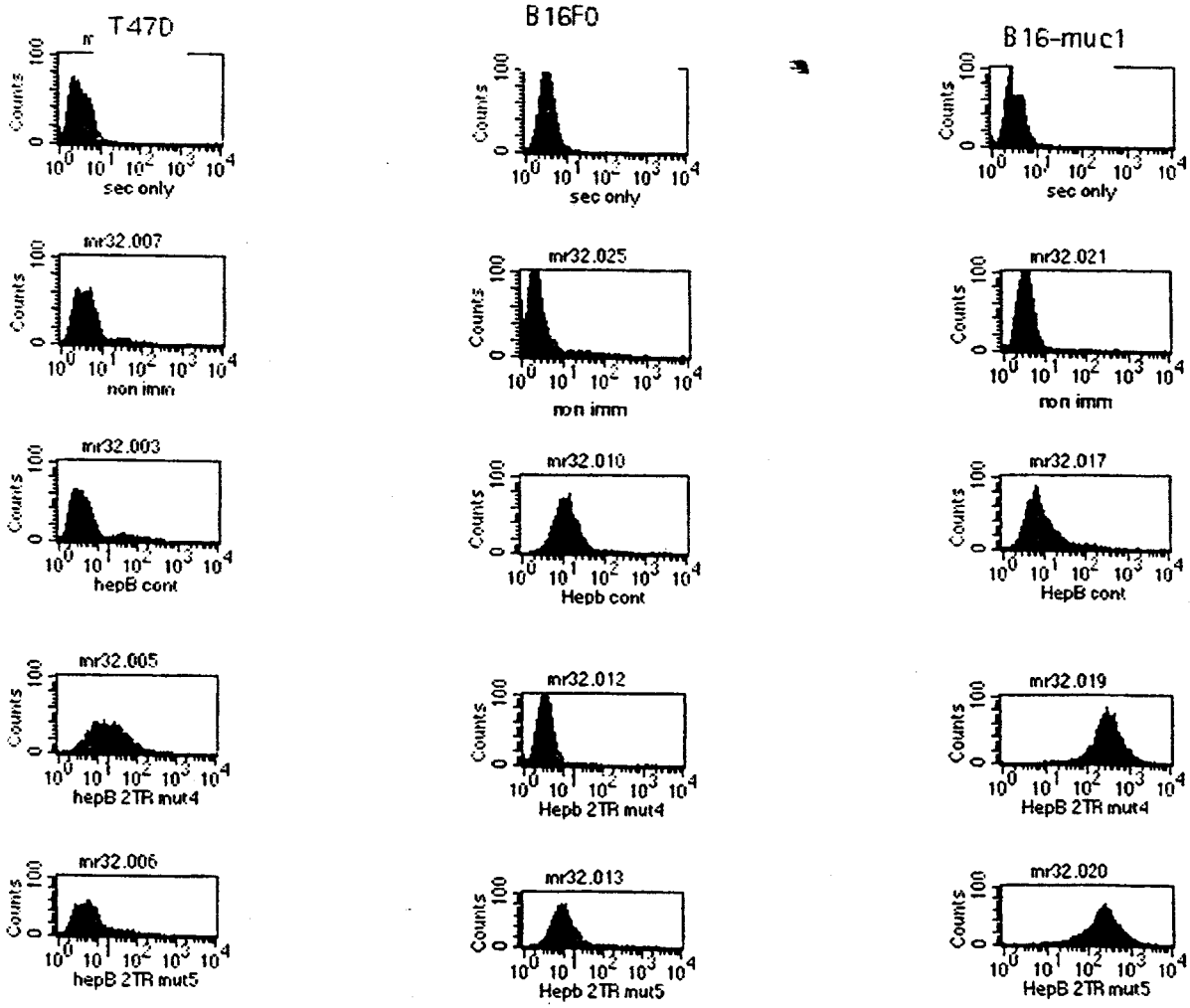

Pvac1.ss2.HepB
O.D. (490 nm)
1.5
1
0.5
0
1
2
3
4
5
Mouse number
Prebleed
day 20
day 41
day 55
Pvac1.ss2.HepB .MUT4-MUC1
O.D. (490 nm)
1.5
1
0.5
0
1
2
3
4
5
Mouse number
Prebleed
day 20
day 41
day 55
Pvac1.ss2. HepB.MUT5-MUC1
O.D. (490 nm)
1.5
1
0.5
0
1
2
3
4
5
Mouse number
Prebleed
day 20
day 41
day 55

FIG. 6

FIG. 7

FACS ANALYSIS OF SERA FROM ANIMALS IMMUNISED WITH PLASMIDS CONTAINING MUTANT MUC1



Rsr II
1 CTAGCCGGAC CGTACAGTTA CTCAGCACAC AGCACCTCAC CATGGGATGG
M G W

51 AGCTGTATCA TCCTCTTCTT GGTAGCAACA GCTACAGGTG TCCACTCCCA
S C I I L F L V A T A T G V H S
Secretory signal

SgrA I
101 GGTCCAAAGA TCGCCGGCGG GACCCGGACC TATGAAAAAC TTAGACTGTT
Q V Q R S P A G P G P M K N L D C
Hinge Fr C

Sal I
151 GGGTCGACAA CGAAGAAGAC
W V D

FIG. 8

A Top
*ATCCTACGCCGGGCGCATGGTGTC*gtcgccGCCCCGGACACCAGGCCGGCCCCGGGCgctgttGCCC
CCCCCGCTCACGGAGTTgtg

A Bottom
AGCggccacAACTCCGTGAGCGGGGGGGGCaacagcGCCCGGGGCCGGCCTGGTGTCCGGGGCgg
cgacGACACCATGCGCCGGCGTAGGAT

B Top
gccGCTCCCGATACTCGACCTGCACCCGGAgctgtcGCTCCACCTGCACACGGCGTGgttgcaGCAC
CTGACACACGTCCGGCGCCAGGCgcggtgGCGCCCCCAGCCCACGGGGTAgtagccGCGCCGGACACGCGC
CCCGCC

B Bottom
GCGCGTGTCCGGCGCggctacTACCCCATGGGCTGGGGGCGCcaccgcGCCTGGCGCCGGACGTG
TGTCAGGTGCtgcaacCACGCCGTGTGCAGGTGGAGCgacagcTCCGGGTGCAGGTCGAGTATCGGG

C Top
CCGGGAgccgtcGCCCCCCCAGCCCACGGAGTTgttgctGCCCCCGACACCCGGCCCGCACCGGGAg
cagtgGCCCCTCCTGCAGGACCCGGACCTATGAAAAACTTAGACTGTTGGGTCGACGTCGGCCATAGCGGC
CGCGGAA

C Bottom
TTCCGCGGCCGCTATGGCCGACGTCGACCCAACAGTCTAAGTTTTTCATAGGTCCGGGTCCT
GCAGGAGGGGCcactgcTCCCGGTGCGGGCCGGGTGTCGGGGGCagcaacAACTCCGTGGGCTGGGGGGGC
gacggcTCCCGGGGCGGG

FIG. 9

A Top
*ATCCTA*CGCCGGCG*CATGGTGTC*gtcgccGCCCCGGACaatAGGCCGGCCCCGGGCgctgttGCCCC
CCCCGCTCACGGAGTTgtg

A Bottom
AGCggccacAACTCCGTGAGCGGGGGGGGCaacagcGCCCGGGGCCGGCCTattGTCCGGGGCggcga
cGACACCATGCGCCGGCGTAGGAT

B Top
GccGCTCCCGATaatCGACCTGCACCCGGAgctgtcGCTCCACCTGCACACGGCGTGgttgcaGCACC
TGACaacCGTCCGGCGCCAGGCgcggtgGCGCCCCCAGCCCACGGGGTAgtagccGCGCCGGACaatCGCC
CCGCC

B Bottom
GCGattGTCCGGCGCggctacTACCCCATGGGCTGGGGGCGCcaccgcGCCTGGCGCCGGACGgttGT
CAGGTGCtgcaacCACGCCGTGTGCAGGTGGAGCgacagcTCCGGGTGCAGGTCGattATCGGG

C Top
CCGGGAgccgtcGCCCCCCCAGCCCACGGAGTTgttgctGCCCCCGACaatCGGCCCGCACCGGGAgc
agtgGCCCCTCCTGCAGGACCCGGACCTATGAAAAACTTAGACTGTTGGGTCGACGTCGGCCATAGCGGCC
GCGGAA

C Bottom
*TTCCGCGGCCGCTATGGCCGACGT*CGACCCAACAGTCTAAGTTTTTCATAGGTCCGGGTCCT
GCAGGAGGGGCcactgcTCCCGGTGCGGGCCGattGTCGGGGGCagcaacAACTCCGTGGGCTGGGGGGGC
gacggcTCCCGGGGCGGG

FIG. 10