

FIG. 1

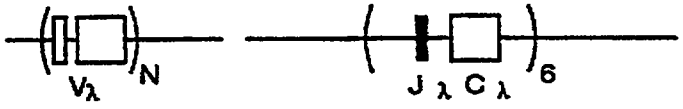


FIG. 2

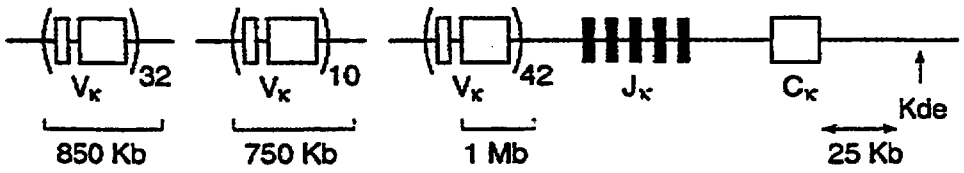


FIG. 3

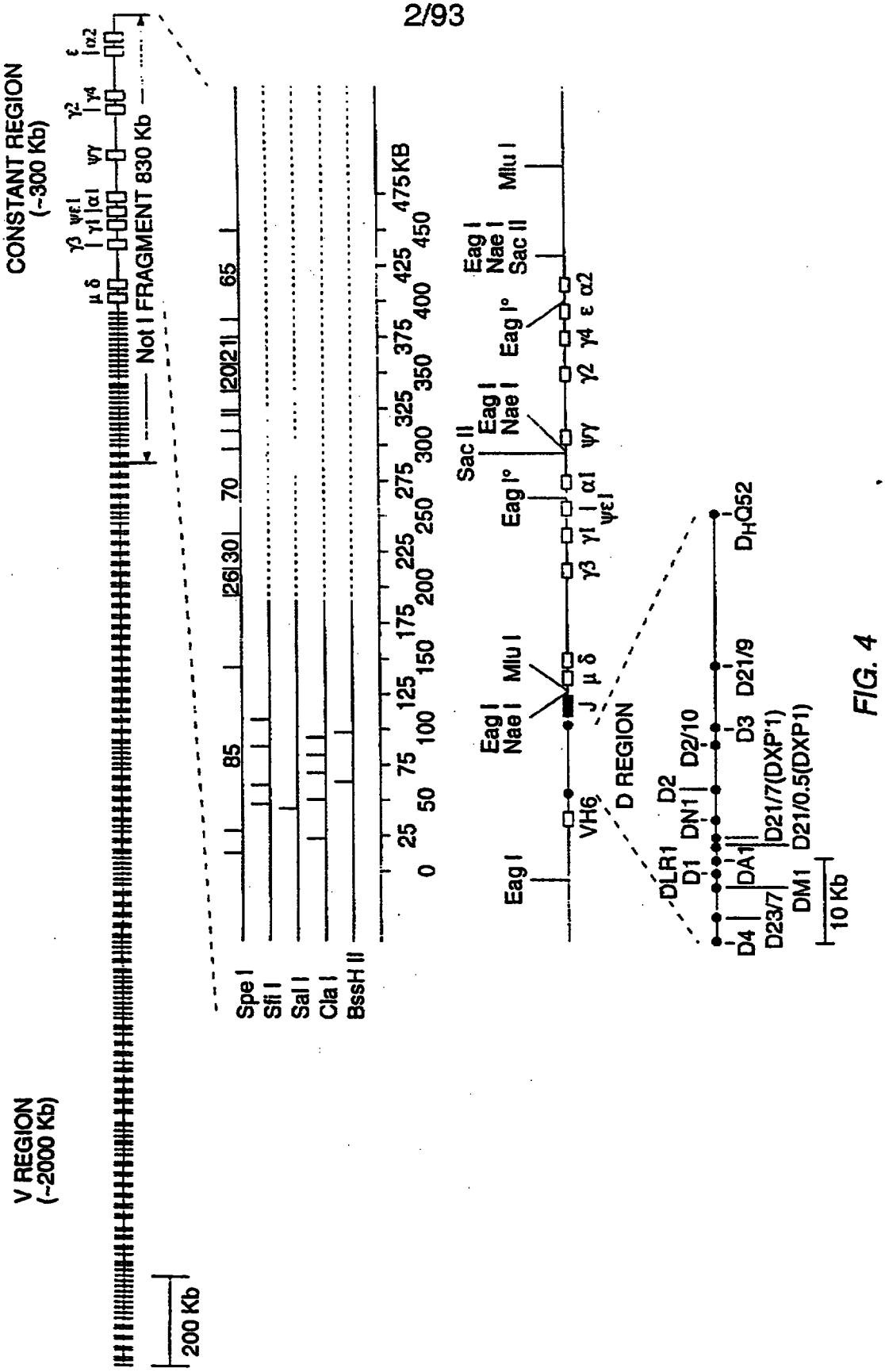

CONSTANT REGION
(~300 Kb)
V REGION
(~2000 Kb)
Not I FRAGMENT 830 Kb
200 Kb
Spe I
Sfi I
Sal I
Cla I
BssH II
475KB
Sac II
Eag I
Nae I
Mlu I
Eag I°
D REGION
DLR1
D1
DN1
D2
D2/10
D3
D21/9
D4
D23/7
DA1
D21/7(DXP'1)
DM1
D21/0.5(DXP1)
10 Kb

FIG. 4

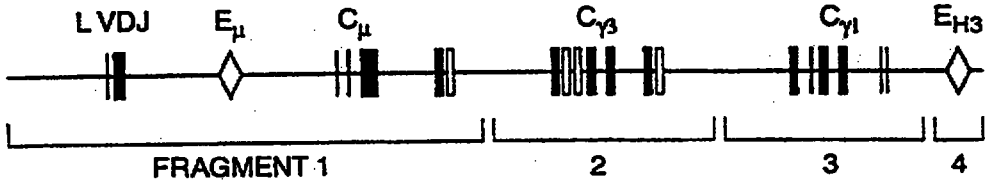

L VDJ
E_μ
C_μ
$C_{\gamma 3}$
$C_{\gamma 1}$
E_{H3}
FRAGMENT 1
2
3
4

FIG. 5

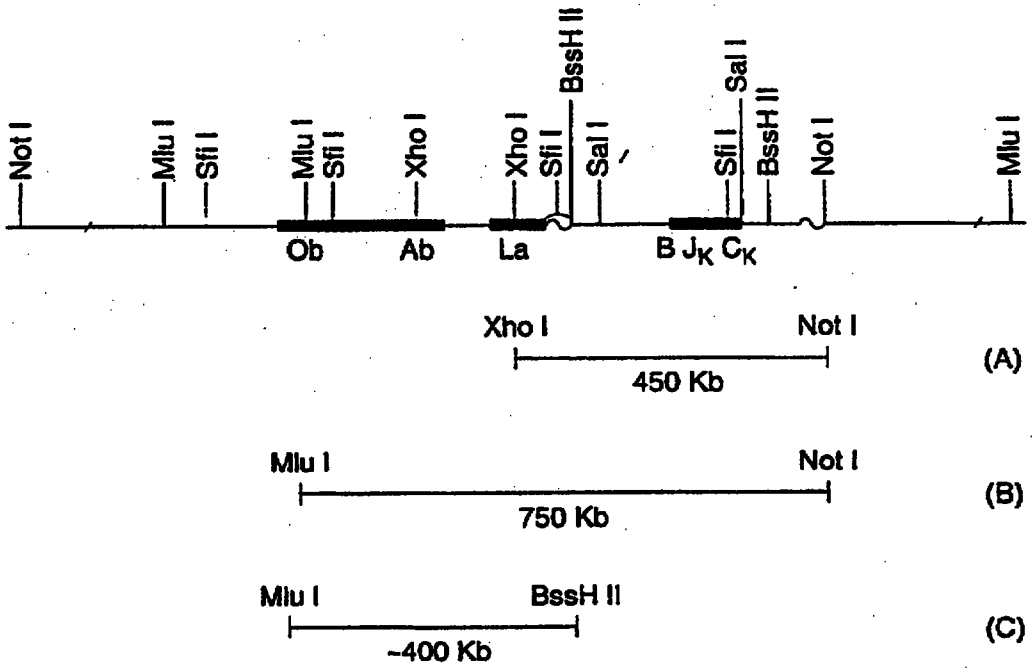

Not I
Mlu I
Sfi I
Mlu I
Sfi I
Xho I
Xho I
Sfi I
BssH II
Sal I
Sfi I
Sal I
BssH II
Not I
Mlu I
Ob
Ab
La
B J_K C_K
Xho I
Not I
450 Kb
(A)
Mlu I
Not I
750 Kb
(B)
Mlu I
BssH II
~400 Kb
(C)

FIG. 6

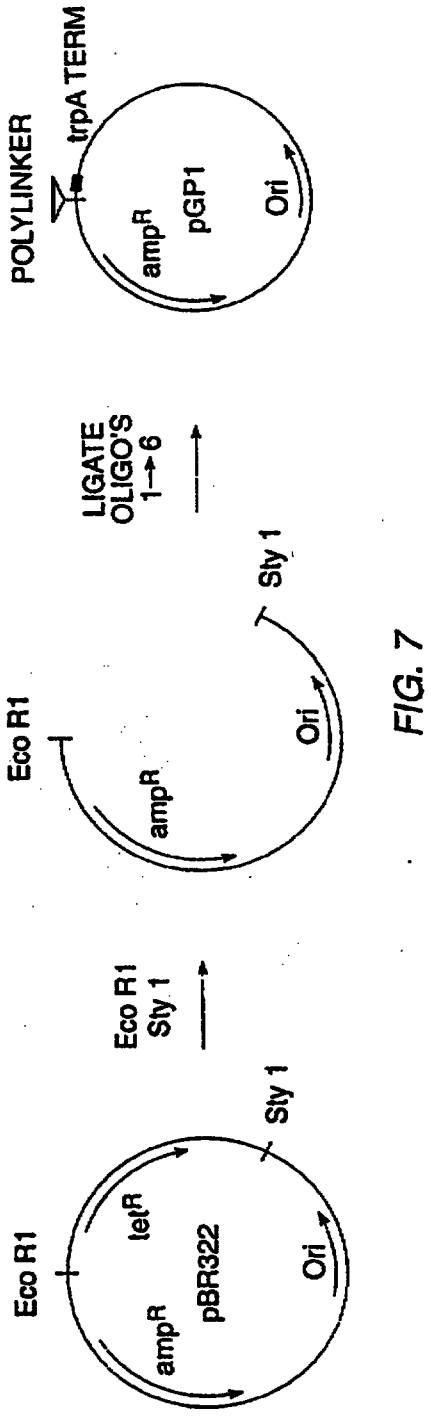
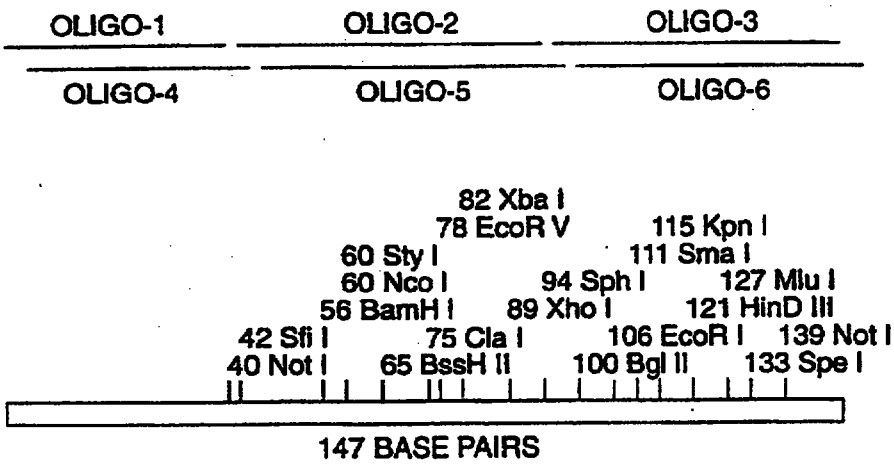
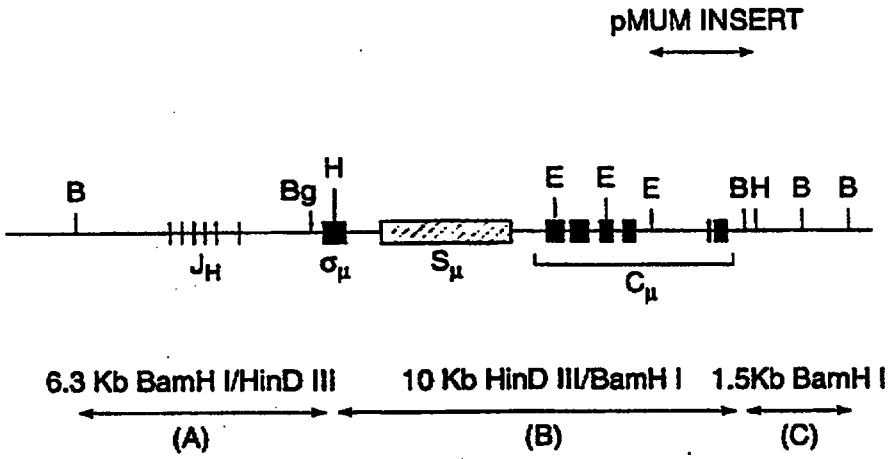

Eco R1
Sty 1
tet^R
amp^R
pBR322
Ori
Eco R1
Sty 1
LIGATE
OLIGO'S
1→6
Eco R1
Sty 1
amp^R
Ori
POLYLINKER
trpA TERM
amp^R
pGP1
Ori

FIG. 7



pGP1 POLYLINKER

FIG. 8



HUMAN μ LOCUS

FIG. 9

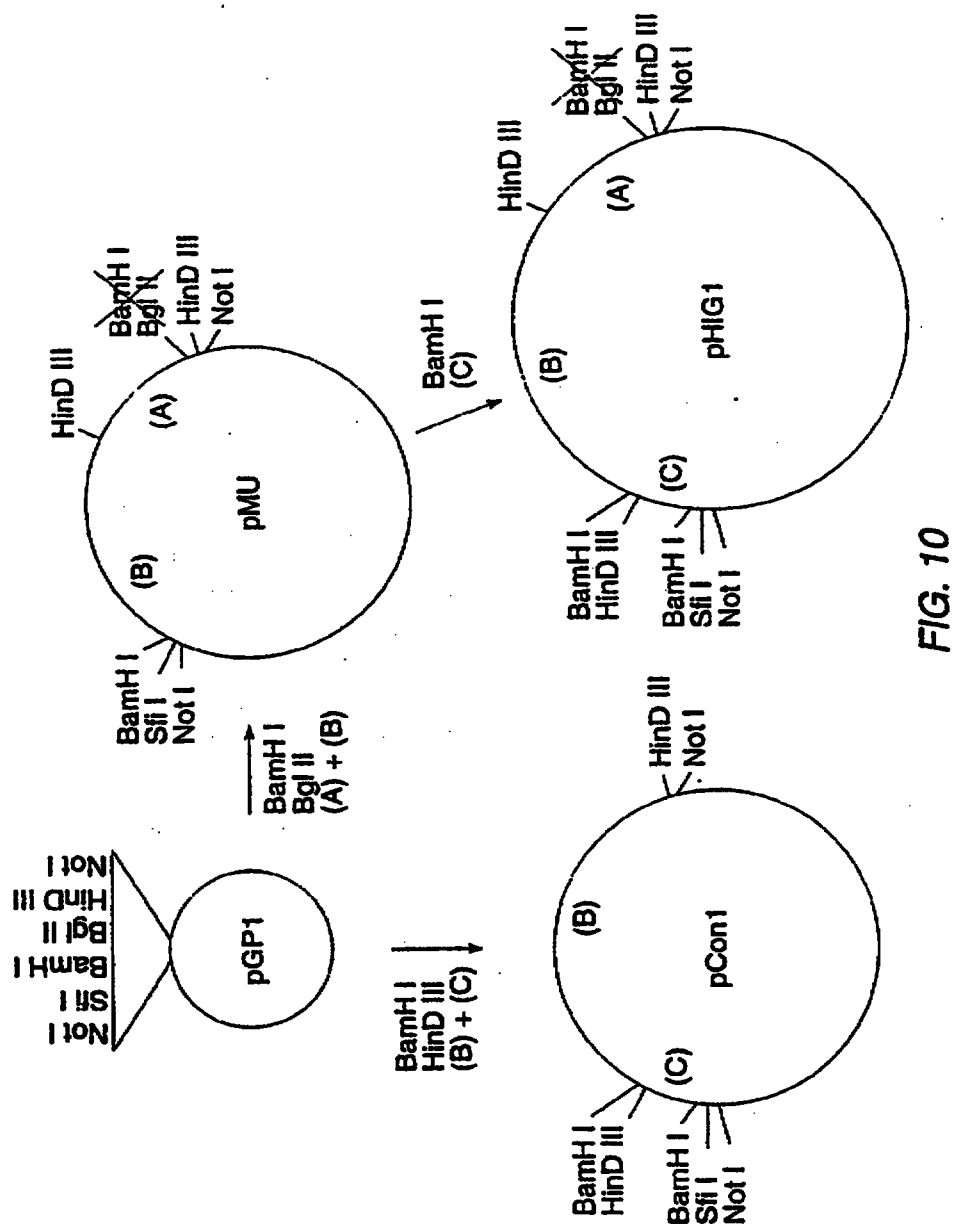

pMU
HinD III
BamH I
Bgl II
HinD III
Not I
(A)
(B)
BamH I
Sfi I
Not I
BamH I
(C)
pHIG1
BamH I
Bgl II
HinD III
Not I
(A)
(B)
(C)
BamH I
HinD III
BamH I
Sfi I
Not I
BamH I
Bgl II
(A) + (B)
pGP1
Not I
HinD III
Bgl II
BamH I
Sfi I
Not I
BamH I
HinD III
(B) + (C)
pCon1
HinD III
Not I
(B)
(C)
BamH I
HinD III
BamH I
Sfi I
Not I

FIG. 10

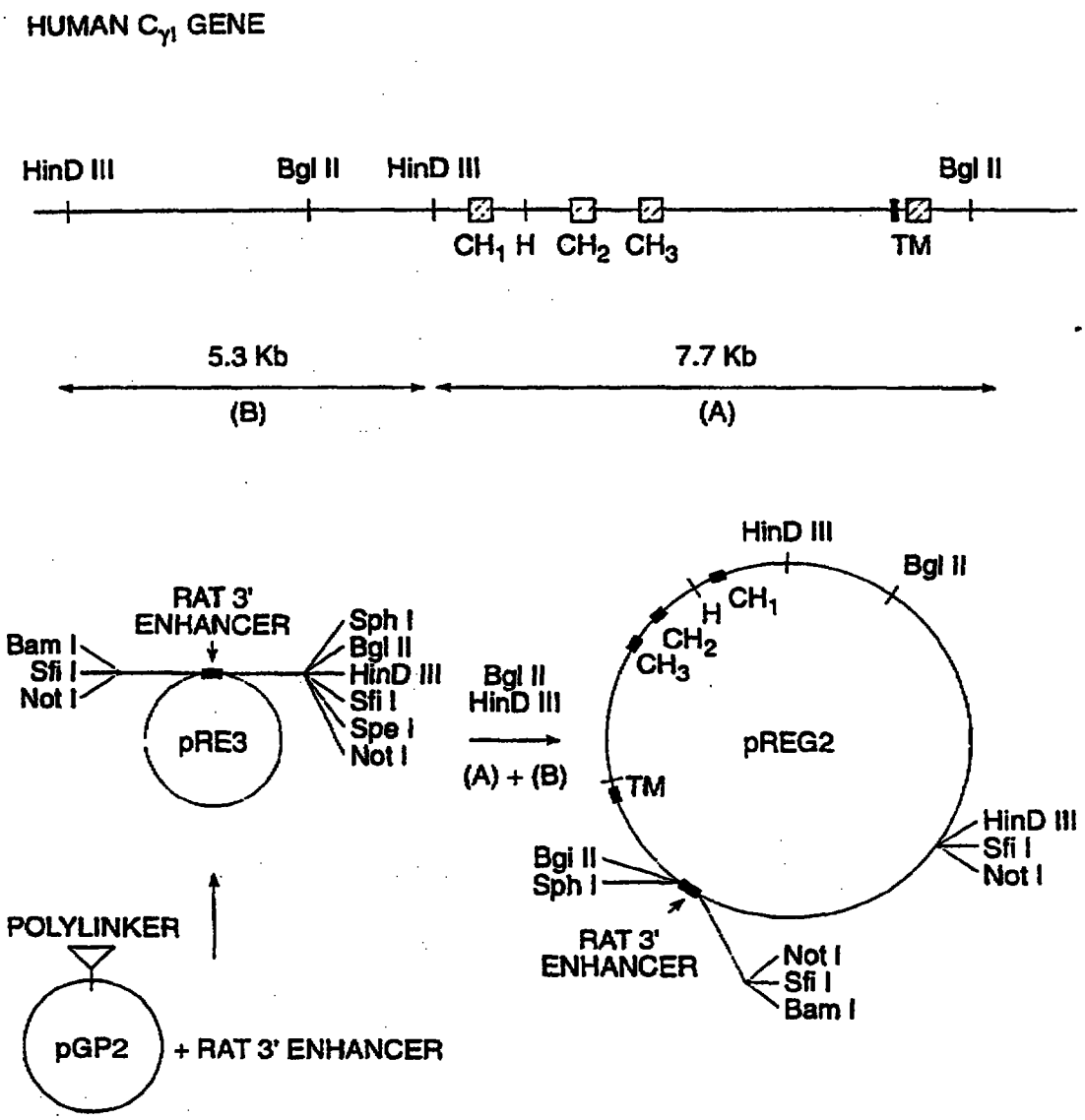

HUMAN $C_{\gamma 1}$ GENE
HinD III
Bgl II
HinD III
Bgl II
CH_1 H CH_2 CH_3
TM
5.3 Kb
7.7 Kb
(B)
(A)
RAT 3' ENHANCER
Bam I
Sfi I
Not I
Sph I
Bgl II
HinD III
Sfi I
Spe I
Not I
pRE3
Bgl II
HinD III
(A) + (B)
HinD III
Bgl II
CH_1
H
CH_2
CH_3
pREG2
TM
HinD III
Sfi I
Not I
Bgl II
Sph I
RAT 3' ENHANCER
Not I
Sfi I
Bam I
POLYLINKER
pGP2
+ RAT 3' ENHANCER

FIG. 11

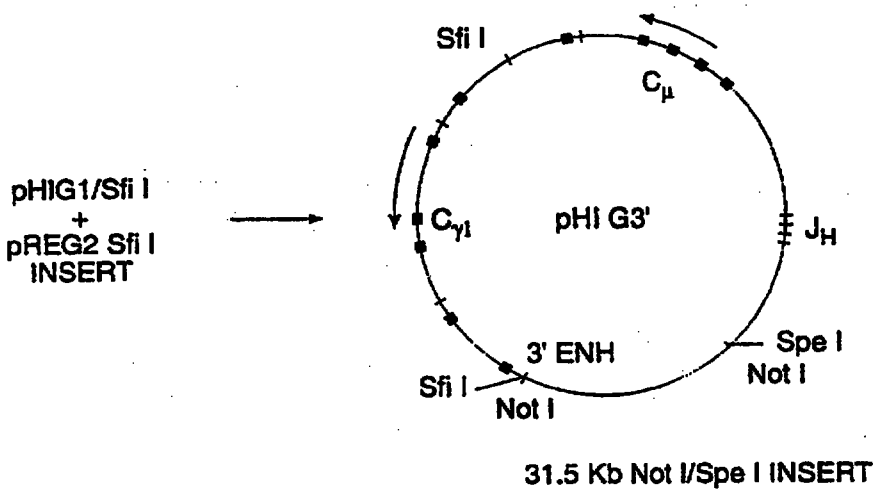

pHIG1/Sfi I
+
pREG2 Sfi I
INSERT
Sfi I
Cμ
Cγ1
pHI G3'
JH
3' ENH
Spe I
Not I
Sfi I
Not I
31.5 Kb Not I/Spe I INSERT
pCON1/Sfi I
+
pREG2 Sfi I
INSERT
Sfi I
Cμ
Cγ1
pCON
3' ENH
Spe I
Not I
Sfi I
Not I
25 Kb Not I/Spe I INSERT

FIG. 12

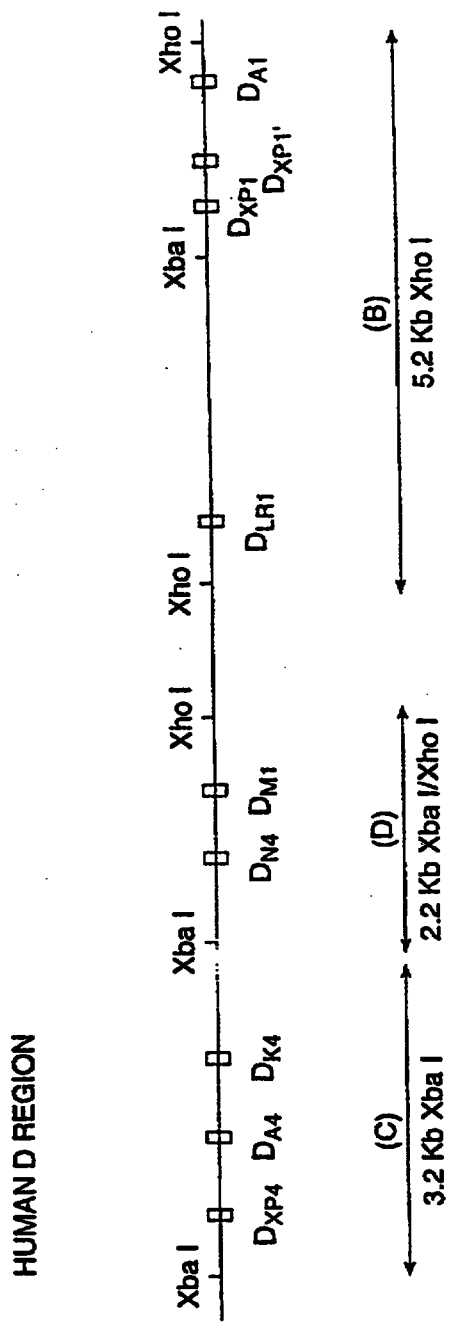

HUMAN D REGION
Xba I
Xho I
D_{XP4}
D_{A4}
D_{K4}
D_{N4}
D_{M1}
D_{LR1}
D_{XP1}
$D_{XP1'}$
D_{A1}
(C)
3.2 Kb Xba I
(D)
2.2 Kb Xba I/Xho I
(B)
5.2 Kb Xho I

FIG. 13

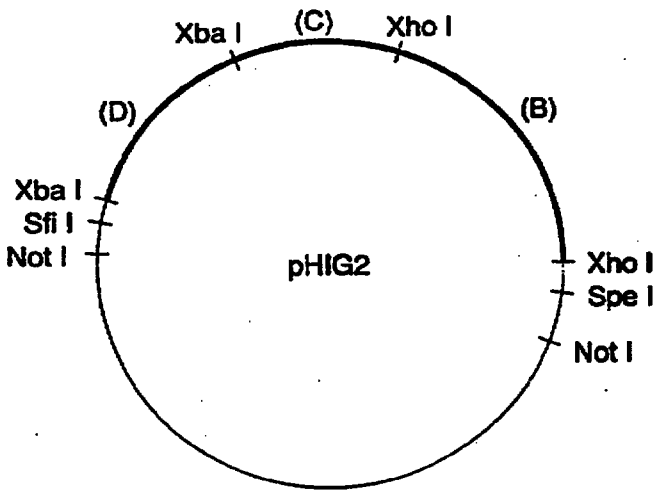


FIG. 14

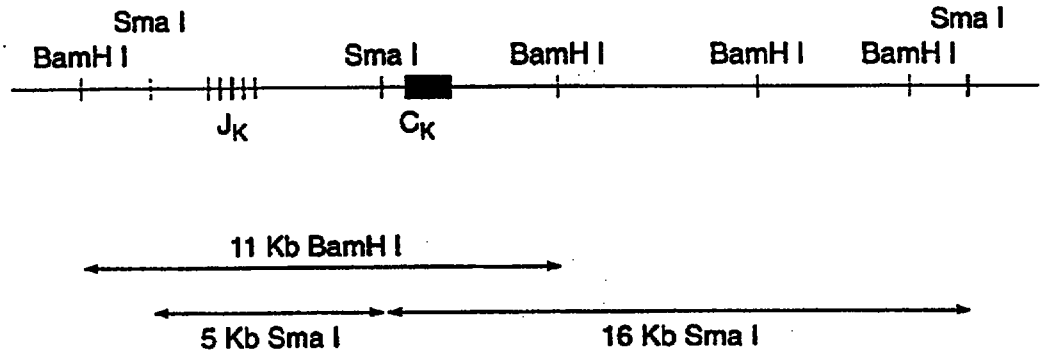

Sma I
BamH I
Sma I
BamH I
BamH I
BamH I
Sma I
J_K
C_K
11 Kb BamH I
5 Kb Sma I
16 Kb Sma I

FIG. 15

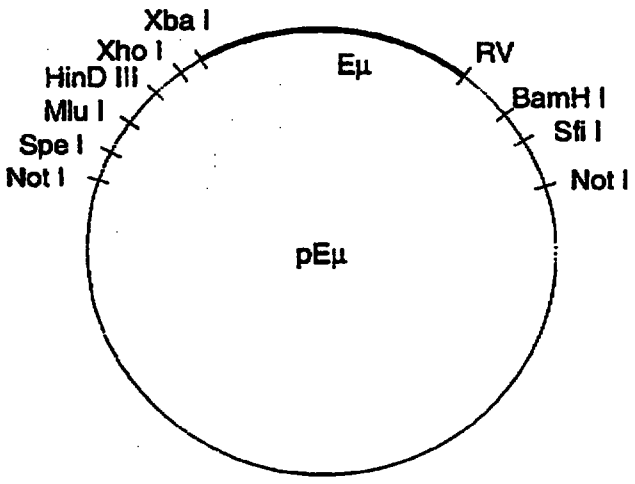

Xba I
Xho I
HinD III
Mlu I
Spe I
Not I
Eμ
RV
BamH I
Sfi I
Not I
pEμ

FIG. 16

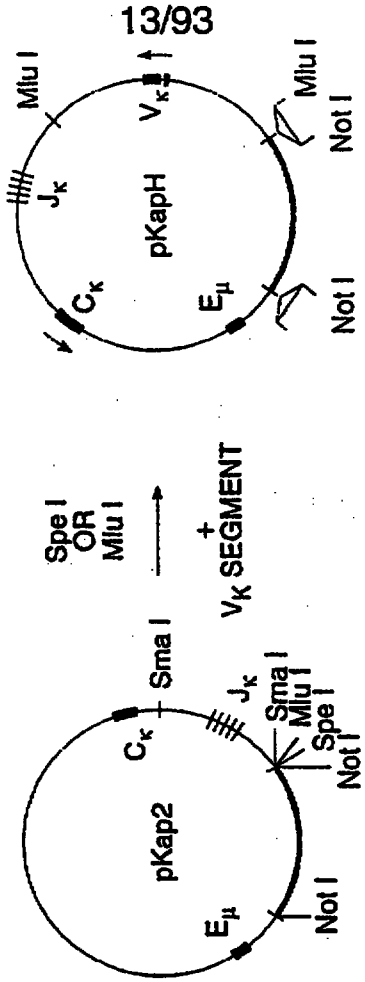

Mlu I
V_κ
Mlu I
Not I
J_κ
pKapH
C_κ
E_μ
Not I
Spe I
OR
Mlu I
+
V_K SEGMENT
Sma I
C_κ
J_κ
Sma I
Mlu I
Spe I
Not I
pKap2
E_μ
Not I
Sma I
+
5 Kb Sma I
FRAGMENT (B)
Xho I
Sma I
Mlu I
Spe I
Not I
C_κ
pKap1
E_μ
Xho I
Xba I
Eco RV
Eco RI
Sfi I
Not I

FIG. 17

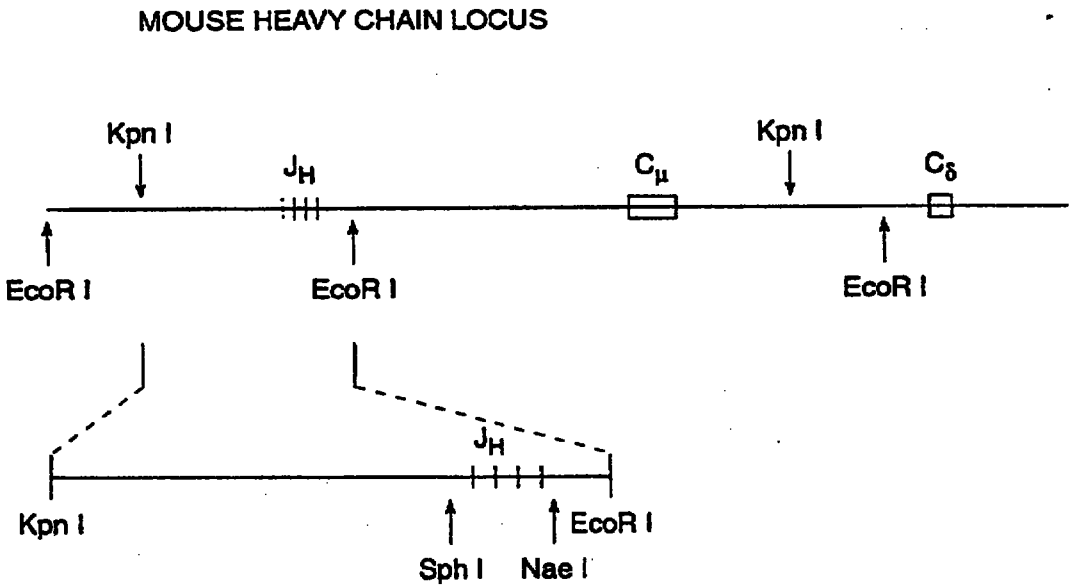

MOUSE HEAVY CHAIN LOCUS
Kpn I
J_H
C_μ
Kpn I
C_δ
EcoR I
EcoR I
EcoR I
J_H
Kpn I
EcoR I
Sph I
Nae I

FIG. 18A

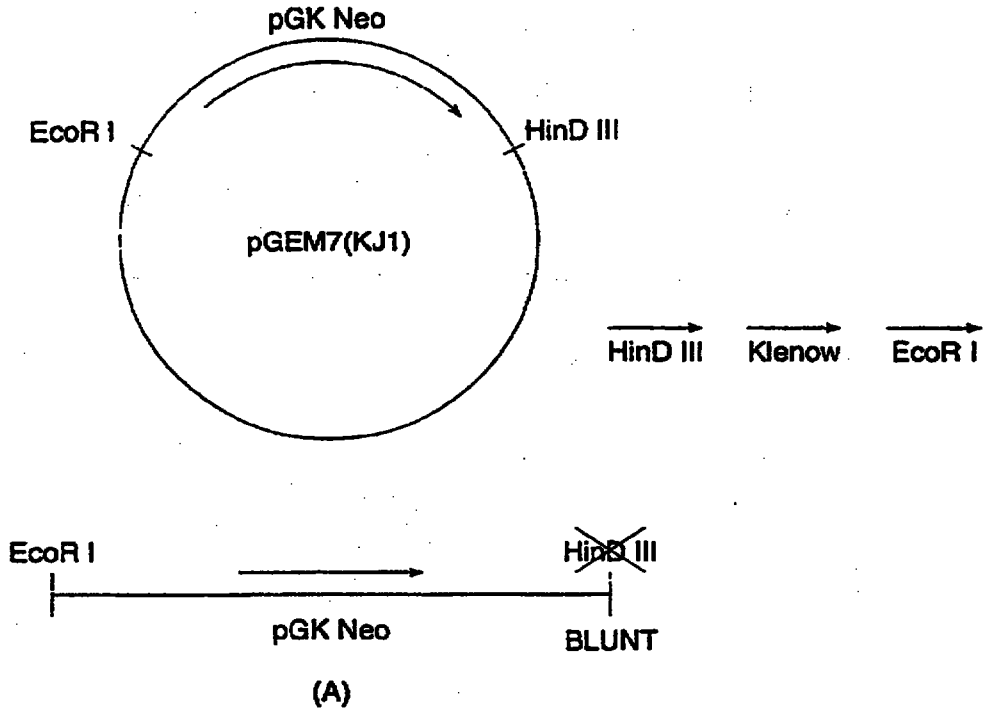

pGK Neo
EcoR I
HinD III
pGEM7(KJ1)
HinD III
Klenow
EcoR I
EcoR I
HinD III
pGK Neo
BLUNT
(A)

FIG. 18B

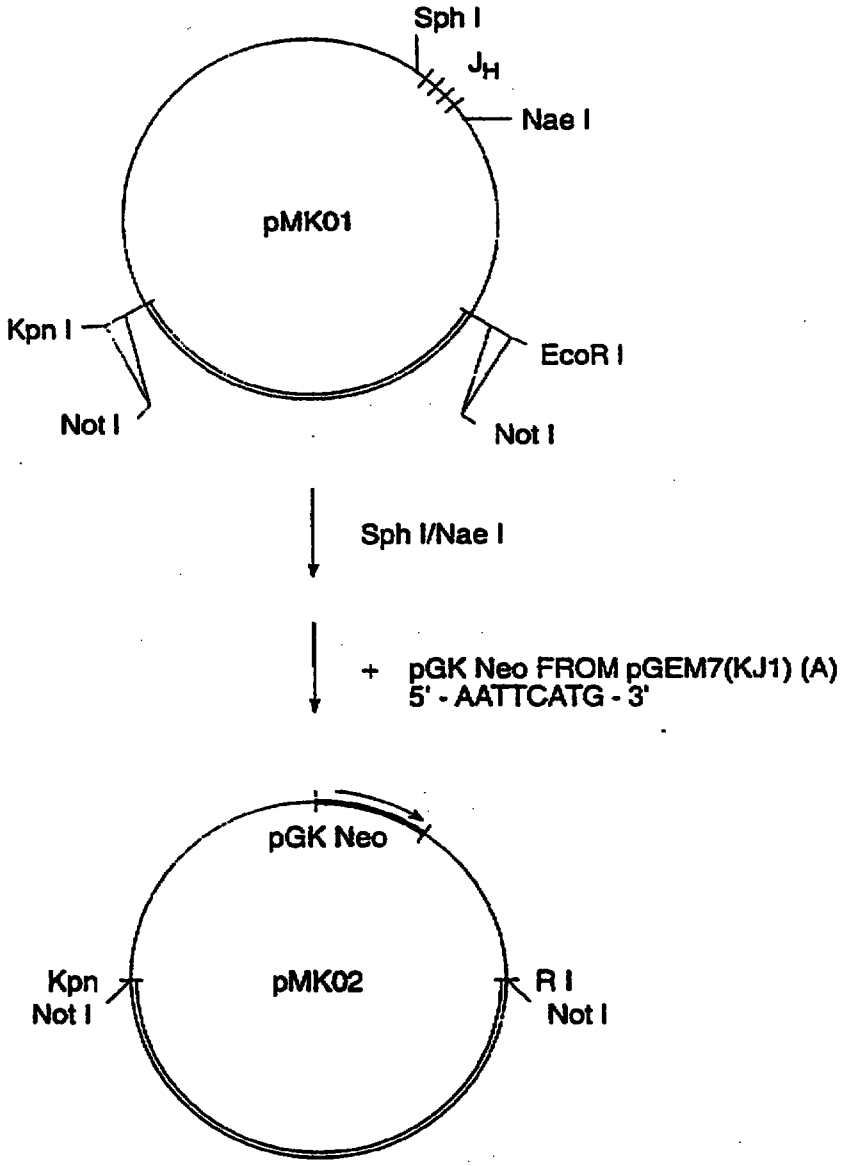

Sph I
J_H
Nae I
pMK01
Kpn I
Not I
EcoR I
Not I
Sph I/Nae I
+ pGK Neo FROM pGEM7(KJ1) (A)
5' - AATTCATG - 3'
pGK Neo
Kpn
Not I
pMK02
R I
Not I

FIG. 18C

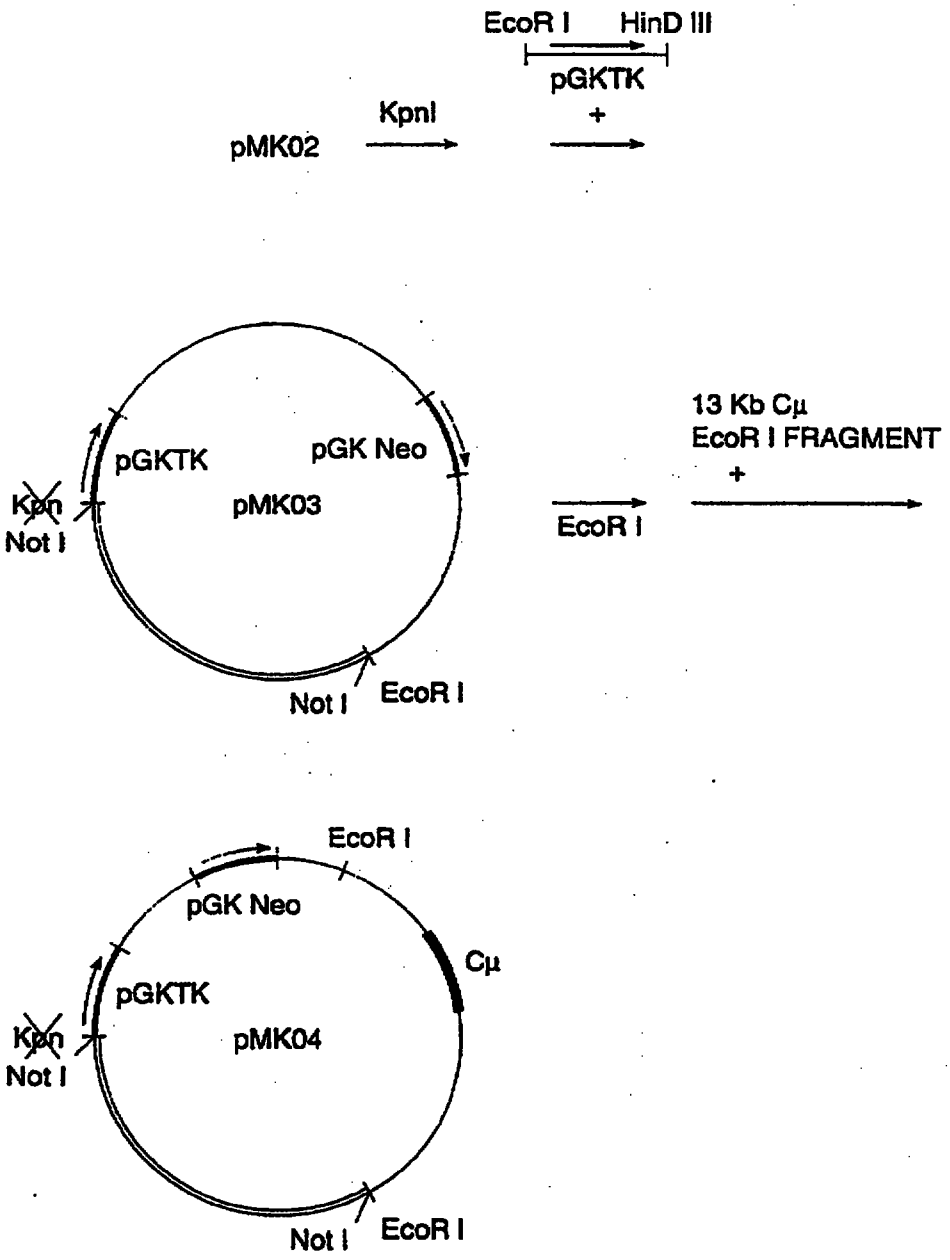

EcoR I
HinD III
pGKTK
KpnI
+
pMK02
pGKTK
pGK Neo
pMK03
Kpn
Not I
Not I
EcoR I
13 Kb Cμ
EcoR I FRAGMENT
+
EcoR I
EcoR I
pGK Neo
Cμ
pGKTK
pMK04
Kpn
Not I
Not I
EcoR I

FIG. 18D

MOUSE KAPPA GENE

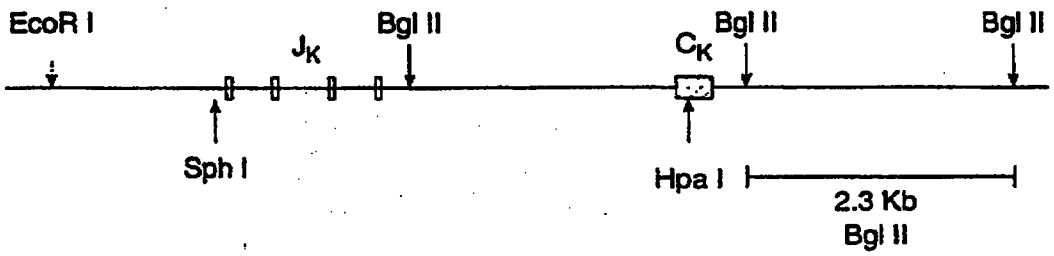


FIG. 19A

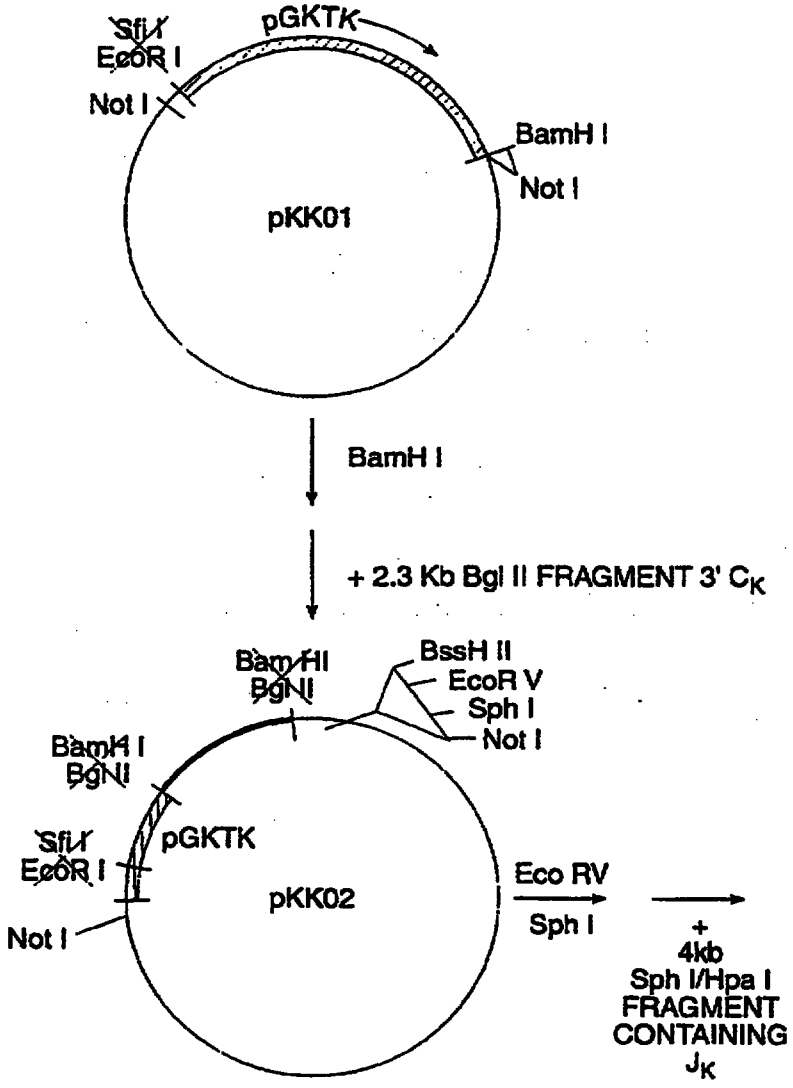

pGKTK
Sfi I
EcoR I
Not I
BamH I
Not I
pKK01
BamH I
+ 2.3 Kb Bgl II FRAGMENT 3' C_K
BamHI
BgNI
BssH II
EcoR V
Sph I
Not I
BamH I
BgNI
Sfi I
EcoR I
pGKTK
pKK02
Not I
Eco RV
Sph I
+
4kb
Sph I/Hpa I
FRAGMENT
CONTAINING
J_K

FIG. 19B

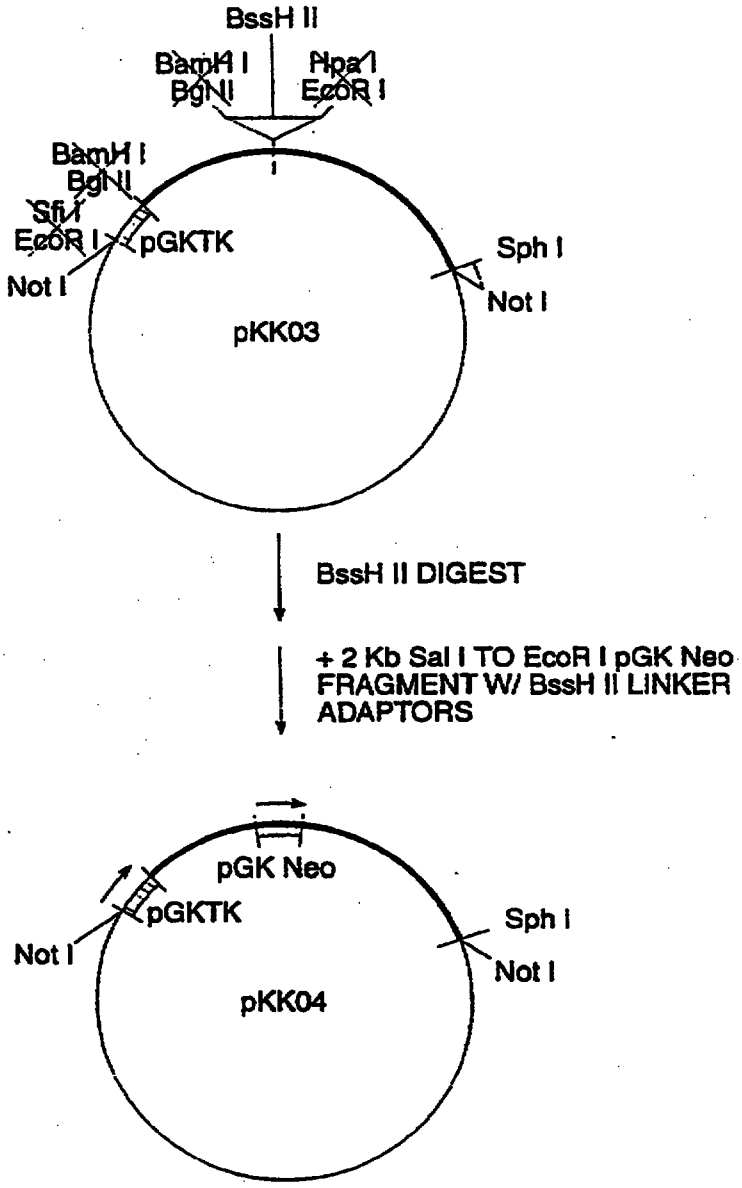

BssH II
BamH I
BglII
Hpa I
EcoR I
BamH I
BglII
Sfi I
EcoR I
pGKTK
Not I
Sph I
Not I
pKK03
BssH II DIGEST
+ 2 Kb Sal I TO EcoR I pGK Neo
FRAGMENT W/ BssH II LINKER
ADAPTORS
pGK Neo
pGKTK
Not I
Sph I
Not I
pKK04

FIG. 19C

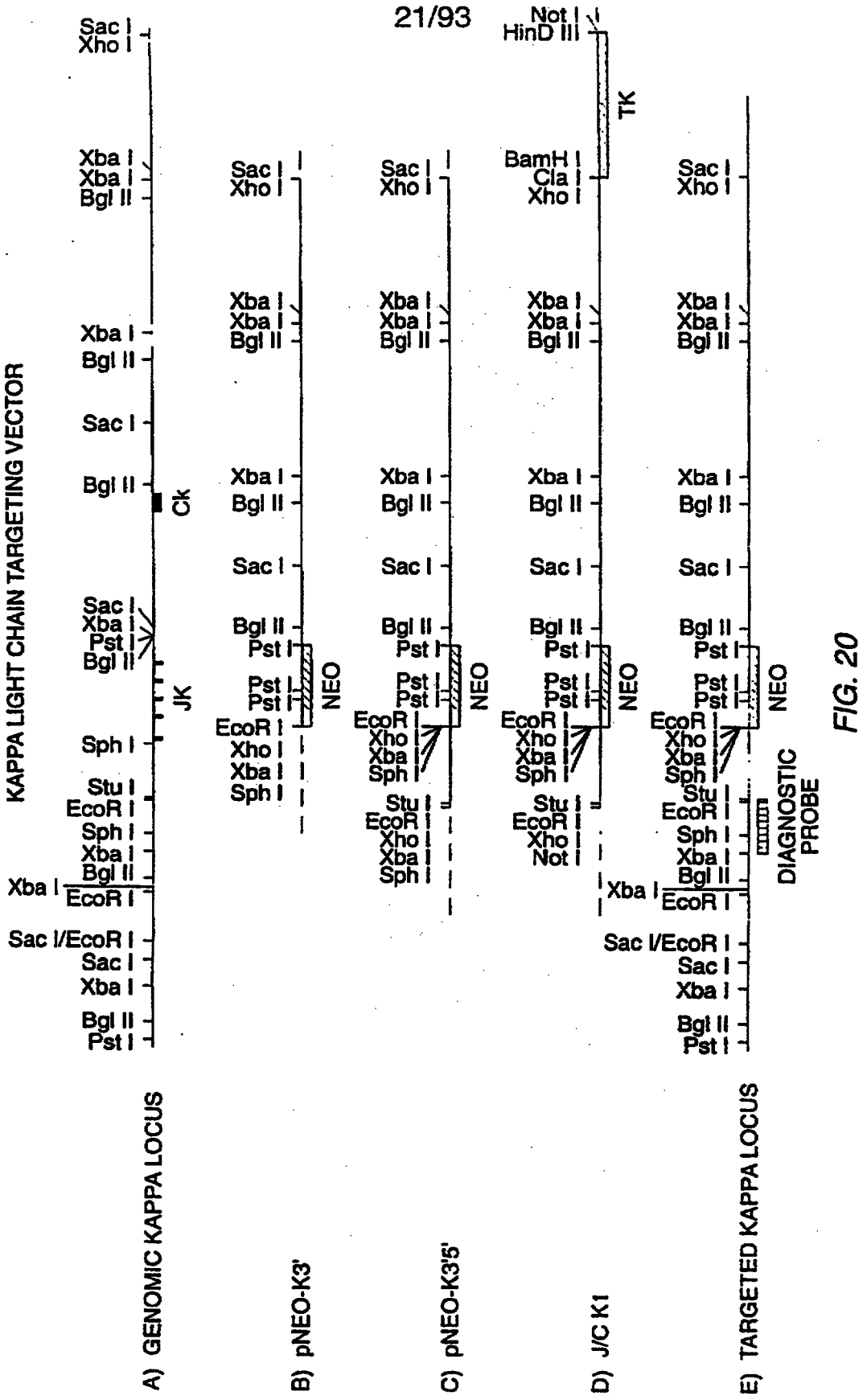

KAPPA LIGHT CHAIN TARGETING VECTOR
Sac I
Xho I
Xba I
Xba I
Bgl II
Xba I
Bgl II
Sac I
Bgl II
Ck
Sac I
Xba I
Pst I
Bgl II
JK
Sph I
Stu I
EcoR I
Sph I
Xba I
Bgl II
Xba I
EcoR I
Sac I/EcoR I
Sac I
Xba I
Bgl II
Pst I
A) GENOMIC KAPPA LOCUS
Sac I
Xho I
Xba I
Xba I
Bgl II
Xba I
Bgl II
Sac I
Bgl II
Pst I
Pst I
NEO
EcoR I
Xho I
Xba I
Sph I
B) pNEO-K3'
Sac I
Xho I
Xba I
Xba I
Bgl II
Xba I
Bgl II
Sac I
Bgl II
Pst I
Pst I
NEO
EcoR I
Xho I
Xba I
Sph I
Stu I
EcoR I
Xho I
Xba I
Sph I
C) pNEO-K3'5'
Not I
HinD III
TK
BamH I
Cla I
Xho I
Xba I
Xba I
Bgl II
Xba I
Bgl II
Sac I
Bgl II
Pst I
Pst I
NEO
EcoR I
Xho I
Xba I
Sph I
Stu I
EcoR I
Xho I
Not I
Xba I
Sac I/EcoR I
Sac I
Xba I
Bgl II
Pst I
D) J/C K1
Sac I
Xho I
Xba I
Xba I
Bgl II
Xba I
Bgl II
Sac I
Bgl II
Pst I
Pst I
NEO
EcoR I
Xho I
Xba I
Sph I
Stu I
EcoR I
Sph I
Xba I
Bgl II
Xba I
EcoR I
DIAGNOSTIC PROBE
Sac I/EcoR I
Sac I
Xba I
Bgl II
Pst I
E) TARGETED KAPPA LOCUS

FIG. 20

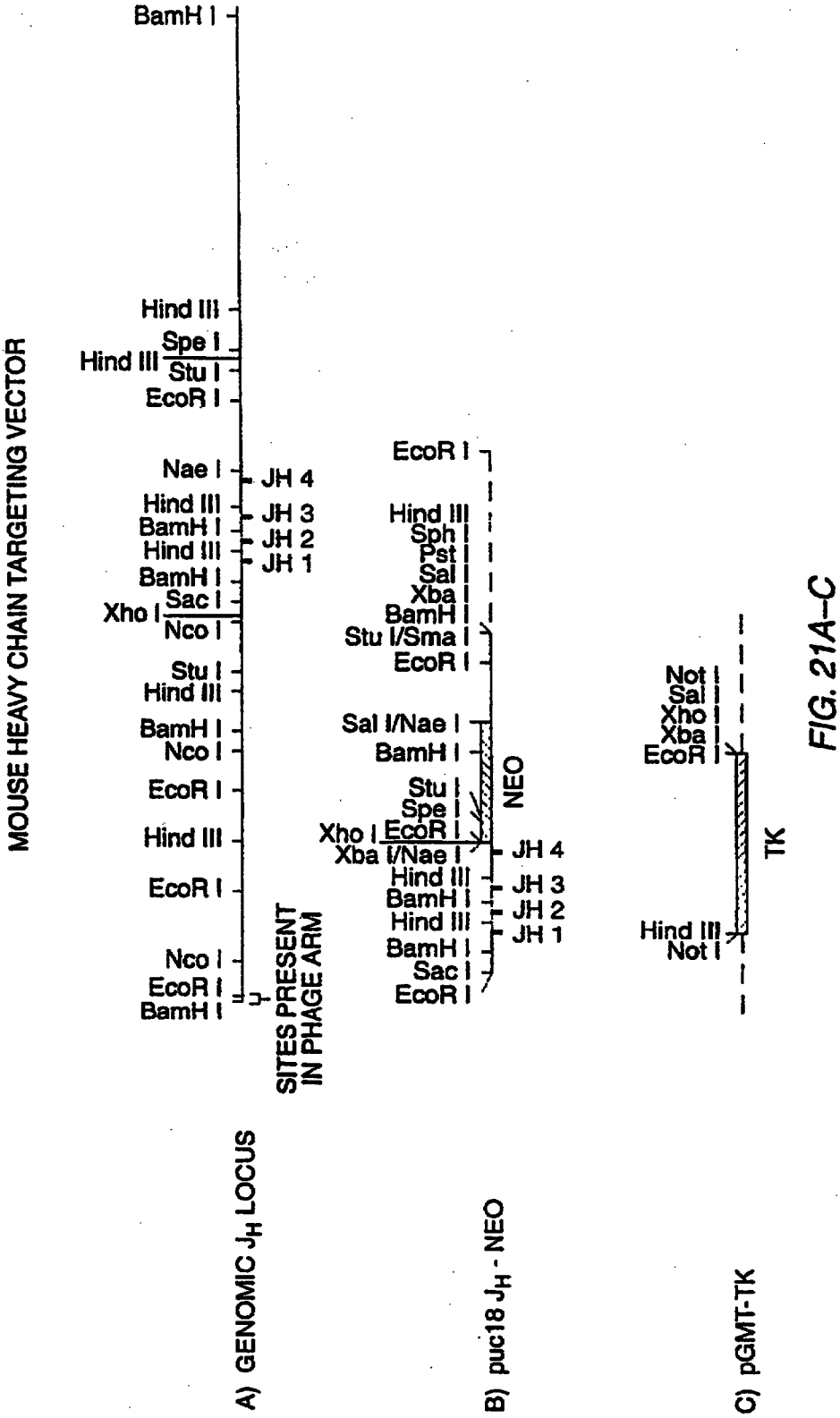

MOUSE HEAVY CHAIN TARGETING VECTOR
BamH I
Hind III
Spe I
Hind III
Stu I
EcoR I
Nae I
JH 4
Hind III
JH 3
BamH I
JH 2
Hind III
JH 1
BamH I
Sac I
Xho I
Nco I
Stu I
Hind III
BamH I
Nco I
EcoR I
Hind III
EcoR I
Nco I
EcoR I
BamH I
SITES PRESENT IN PHAGE ARM
A) GENOMIC J_H LOCUS
EcoR I
Hind III
Sph I
Pst I
Sal I
Xba I
BamH I
Stu I/Sma I
EcoR I
Sal I/Nae I
BamH I
Stu I
Spe I
Xho I
EcoR I
Xba I/Nae I
NEO
JH 4
Hind III
JH 3
BamH I
JH 2
Hind III
JH 1
BamH I
Sac I
EcoR I
B) puc18 J_H - NEO
Not I
Sal I
Xho I
Xba I
EcoR I
TK
Hind III
Not I
C) pGMT-TK

FIG. 21A–C

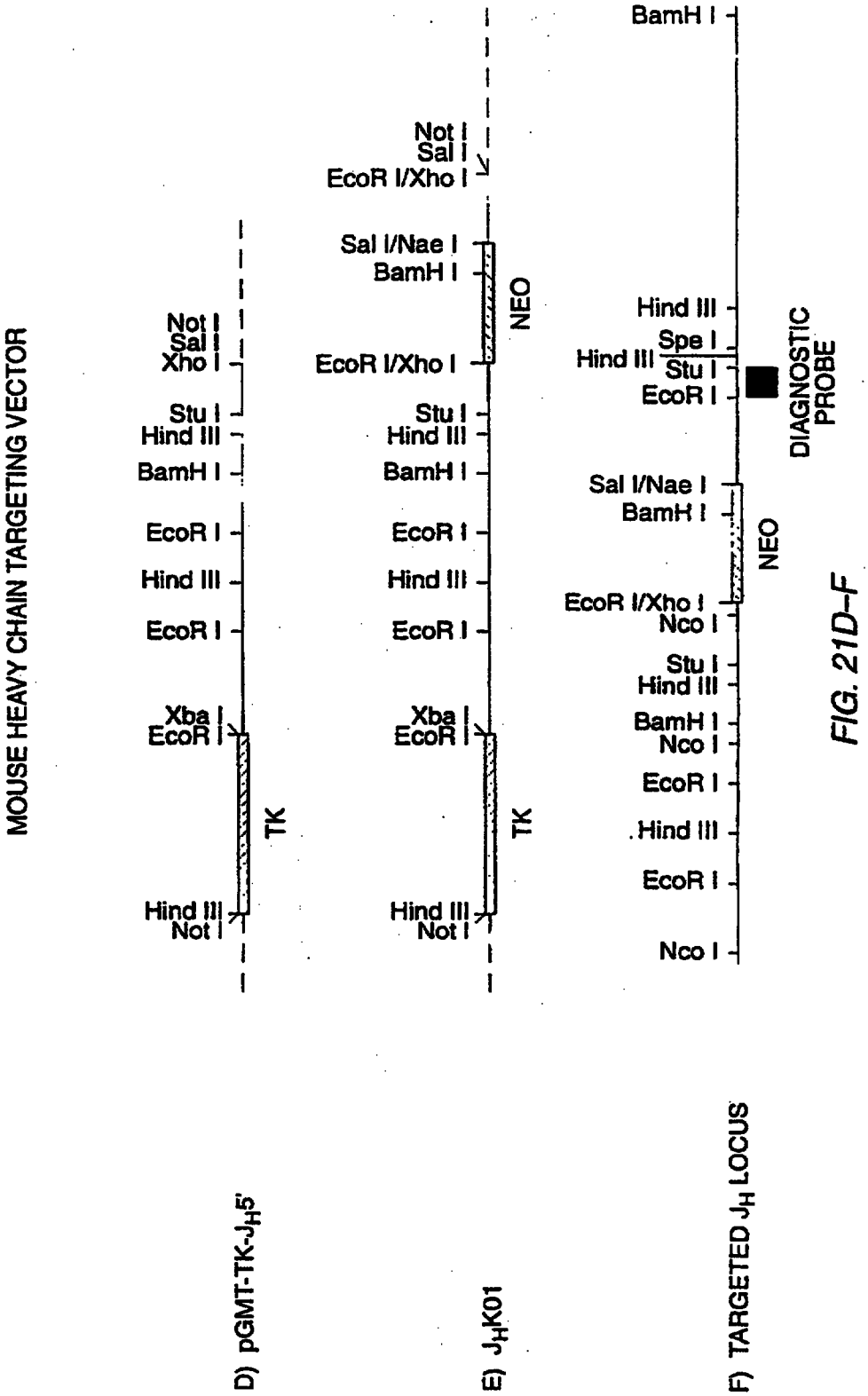

MOUSE HEAVY CHAIN TARGETING VECTOR
Not I
Sal I
Xho I
Stu I
Hind III
BamH I
EcoR I
Hind III
EcoR I
Xba I
EcoR I
TK
Hind III
Not I
D) pGMT-TK-JH5'
Not I
Sal I
EcoR I/Xho I
Sal I/Nae I
BamH I
NEO
EcoR I/Xho I
Stu I
Hind III
BamH I
EcoR I
Hind III
EcoR I
Xba I
EcoR I
TK
Hind III
Not I
E) JHKO1
BamH I
Hind III
Spe I
Hind III
Stu I
EcoR I
DIAGNOSTIC PROBE
Sal I/Nae I
BamH I
NEO
EcoR I/Xho I
Nco I
Stu I
Hind III
BamH I
Nco I
EcoR I
Hind III
EcoR I
Nco I
F) TARGETED JH LOCUS

FIG. 21D-F

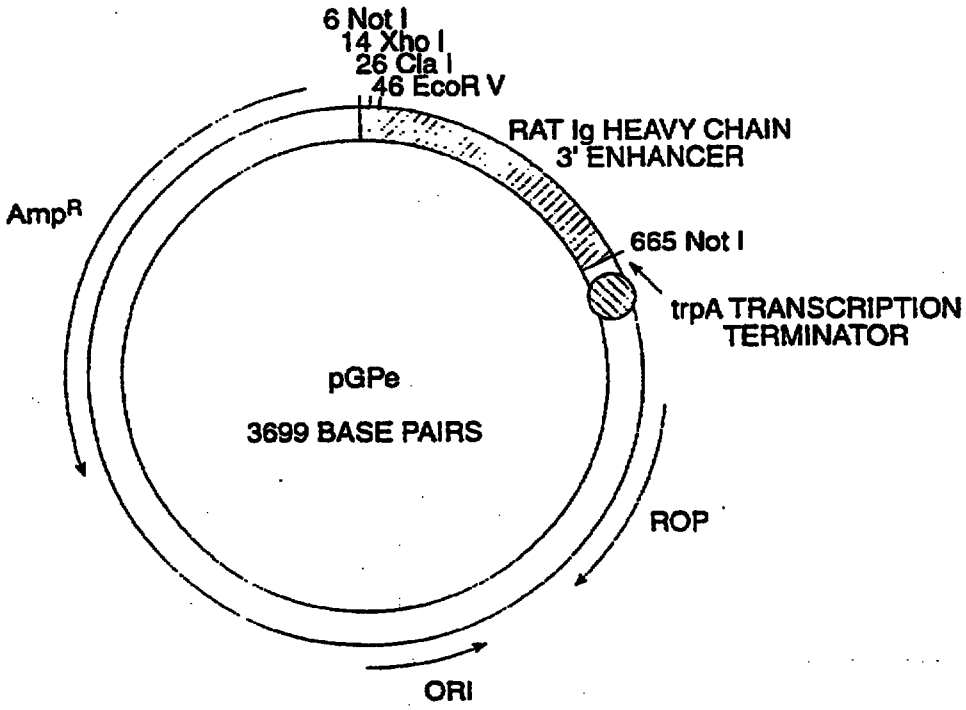

6 Not I
14 Xho I
26 Cla I
46 EcoR V
RAT Ig HEAVY CHAIN
3' ENHANCER
AmpR
665 Not I
trpA TRANSCRIPTION
TERMINATOR
pGPe
3699 BASE PAIRS
ROP
ORI

FIG. 22

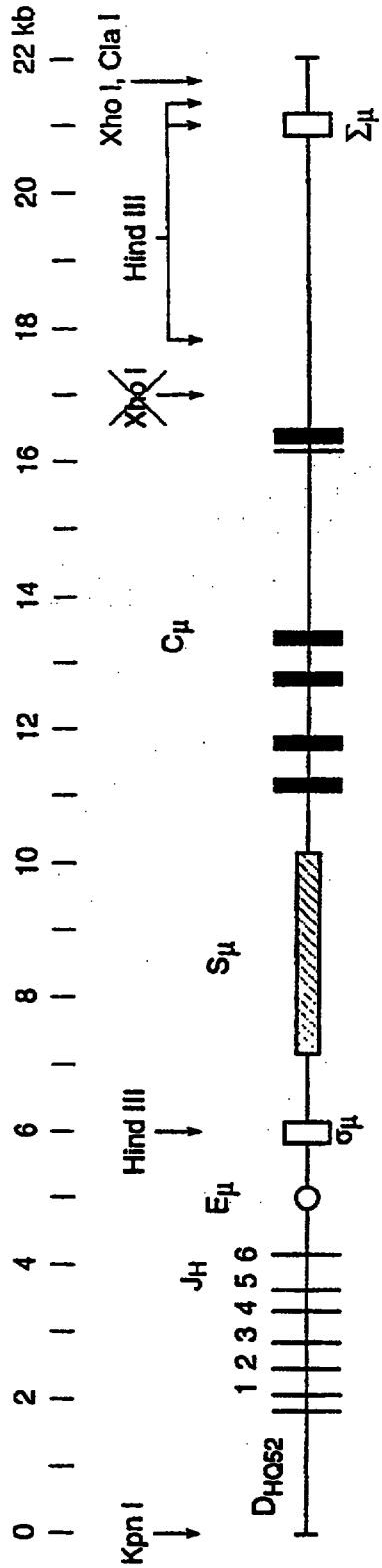

22 kb
20
18
16
14
12
10
8
6
4
2
0
Xho I, Cla I
Hind III
Xho I
Cμ
Sμ
Hind III
Eμ
JH
1 2 3 4 5 6
DHQ52
Kpn I
Σμ
σμ

FIG. 23

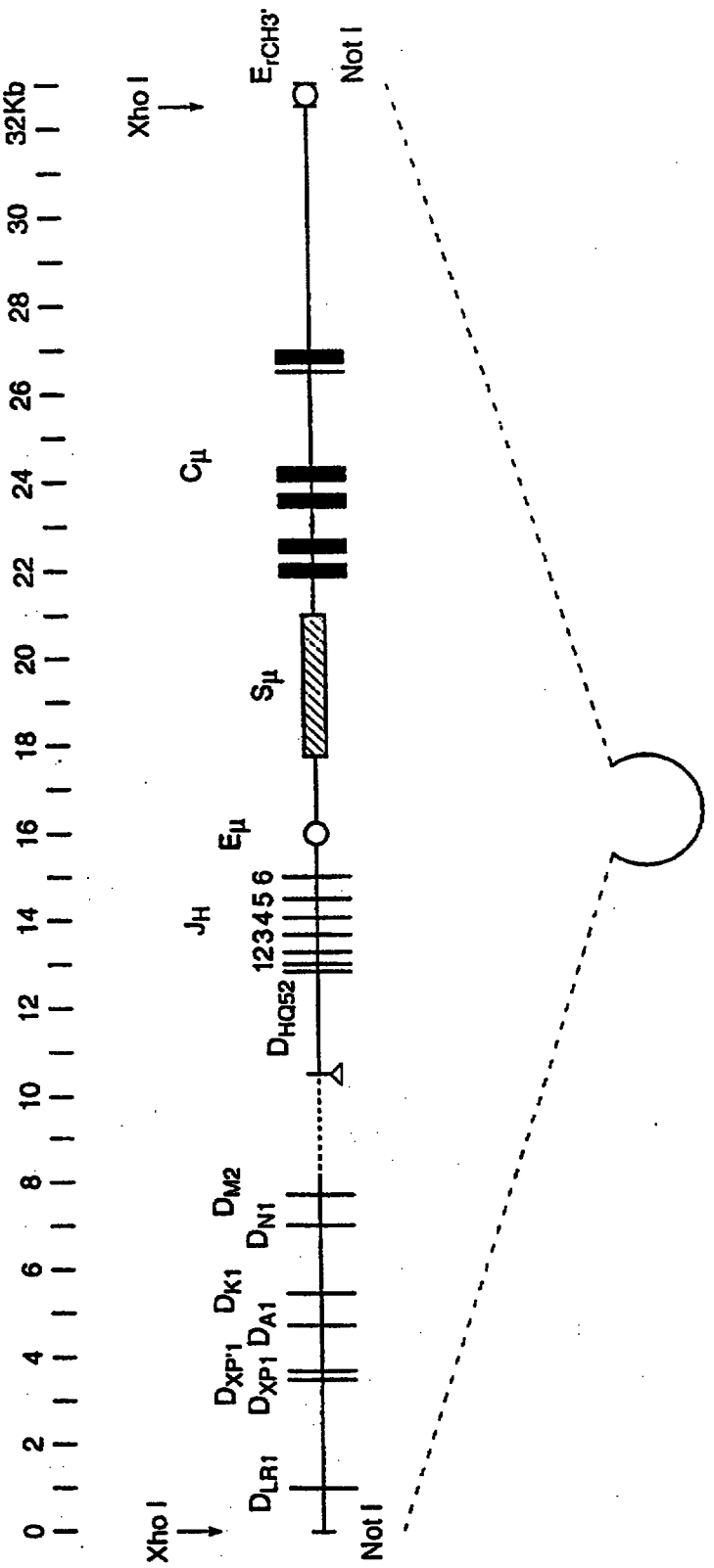

0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32Kb
Xho I
Xho I
Not I
Not I
$E_{rCH3'}$
C_μ
S_μ
E_μ
J_H
1 2 3 4 5 6
D_{HQ52}
D_{M2}
D_{N1}
D_{K1}
D_{A1}
$D_{XP'1}$
D_{XP1}
D_{LR1}

FIG. 24

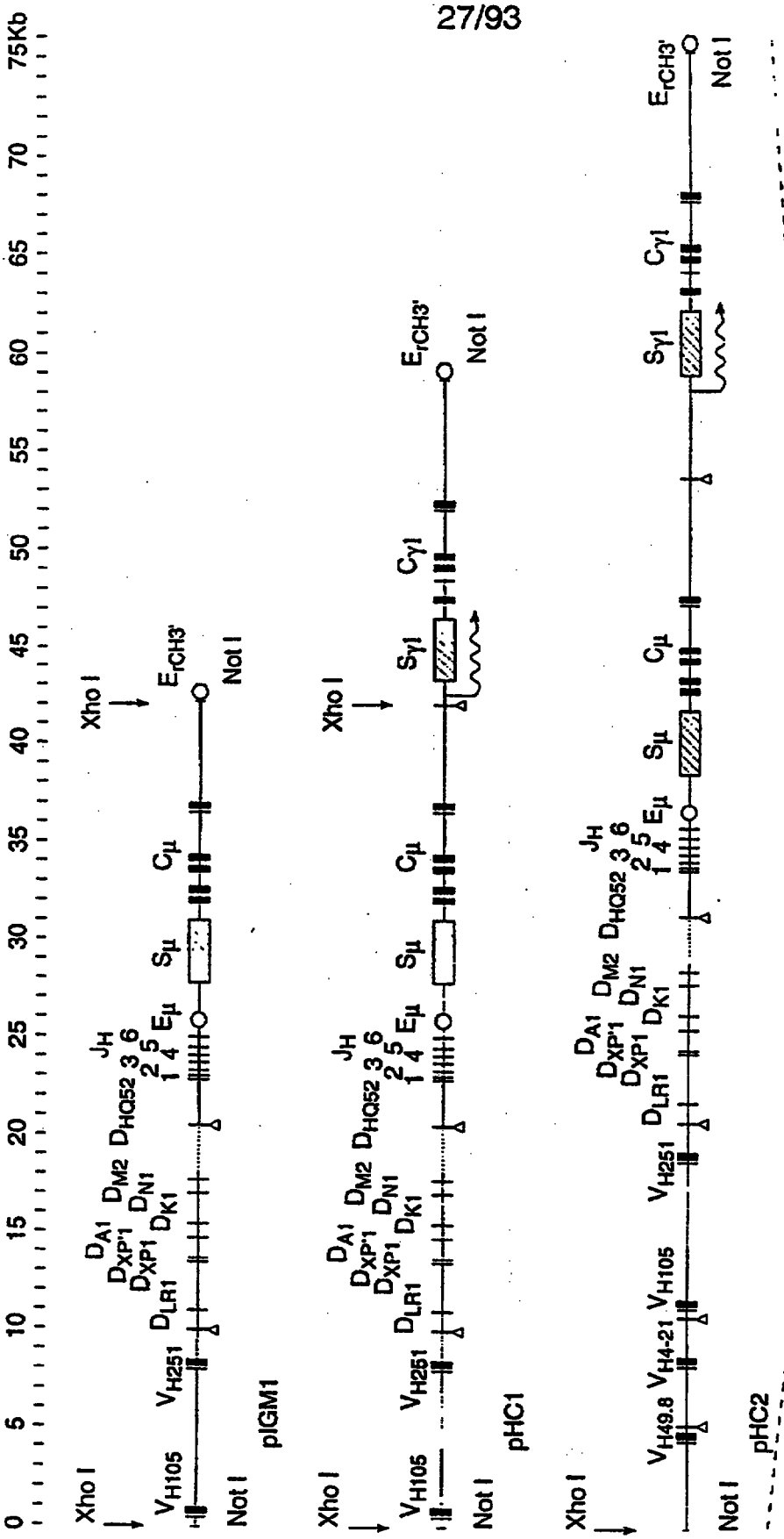

0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75Kb
Xho I
Not I
pIGM1
pHC1
pHC2
V_{H105}
V_{H251}
V_{H4-21}
$V_{H49.8}$
D_{LR1}
D_{XP1}
$D_{XP'1}$
D_{A1}
D_{K1}
D_{N1}
D_{M2}
D_{HQ52}
J_H
1 2 3 4 5 6
E_μ
S_μ
C_μ
$S_{\gamma 1}$
$C_{\gamma 1}$
$E_{rCH3'}$

FIG. 25

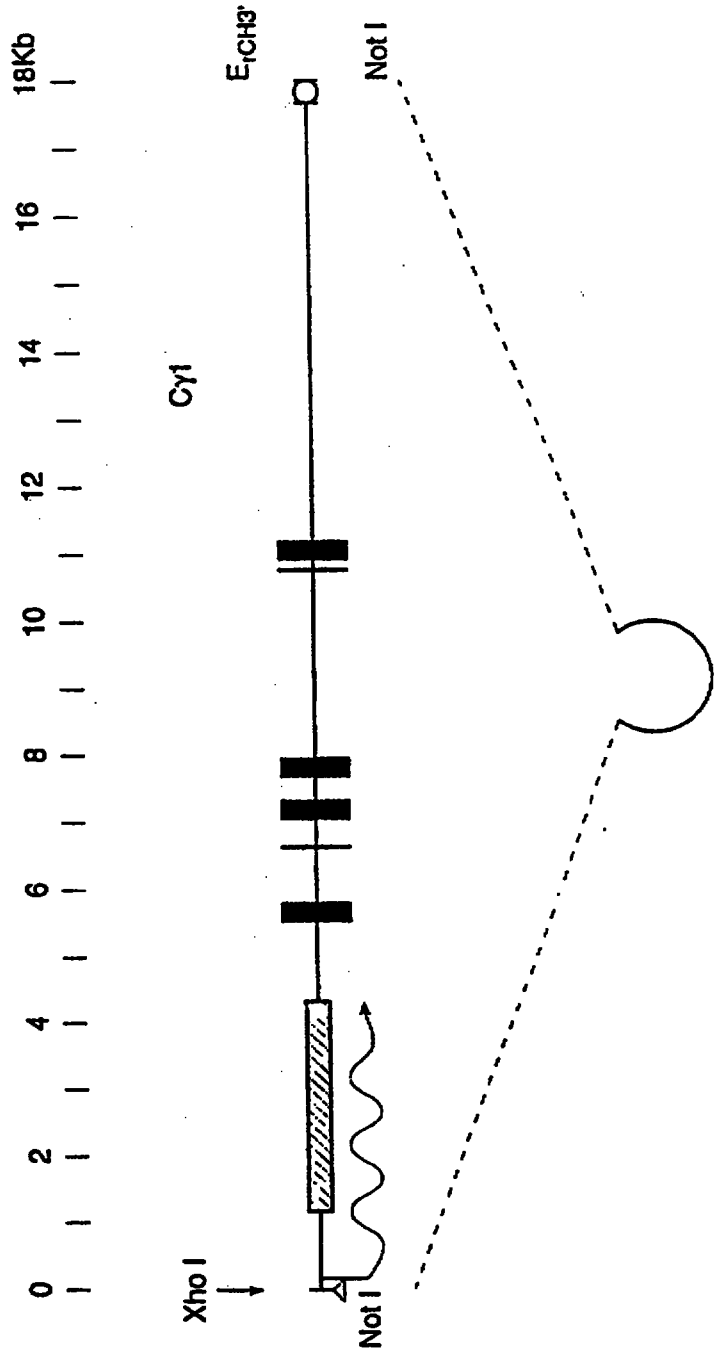


FIG. 26

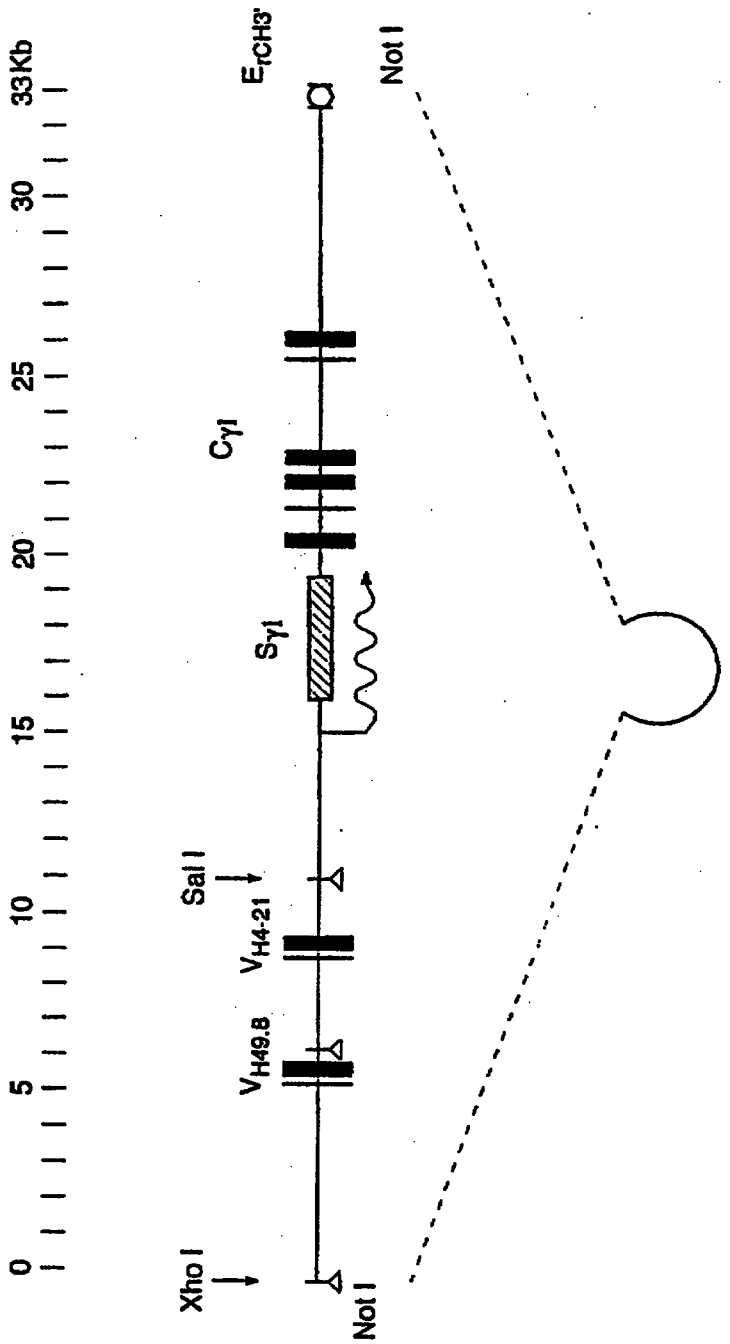

0
5
10
15
20
25
30
33Kb
Xho I
Sal I
$V_{H49.8}$
V_{H4-21}
$S_{\gamma 1}$
$C_{\gamma 1}$
$E_{rCH3'}$
Not I
Not I

FIG. 27

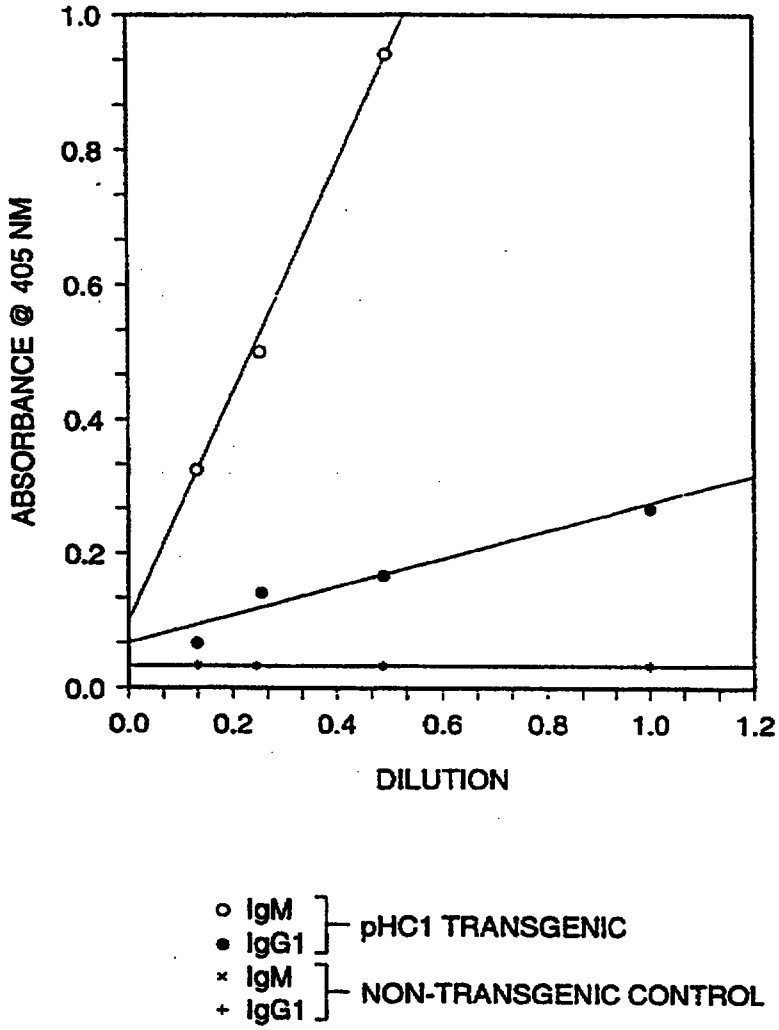

1.0
0.8
0.6
0.4
0.2
0.0
ABSORBANCE @ 405 NM
0.0
0.2
0.4
0.6
0.8
1.0
1.2
DILUTION
o IgM
• IgG1
pHC1 TRANSGENIC
× IgM
+ IgG1
NON-TRANSGENIC CONTROL

FIG. 28

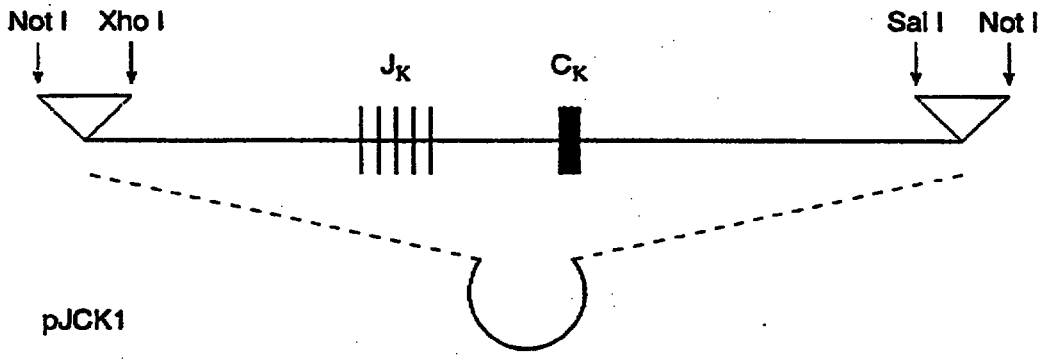


FIG. 29

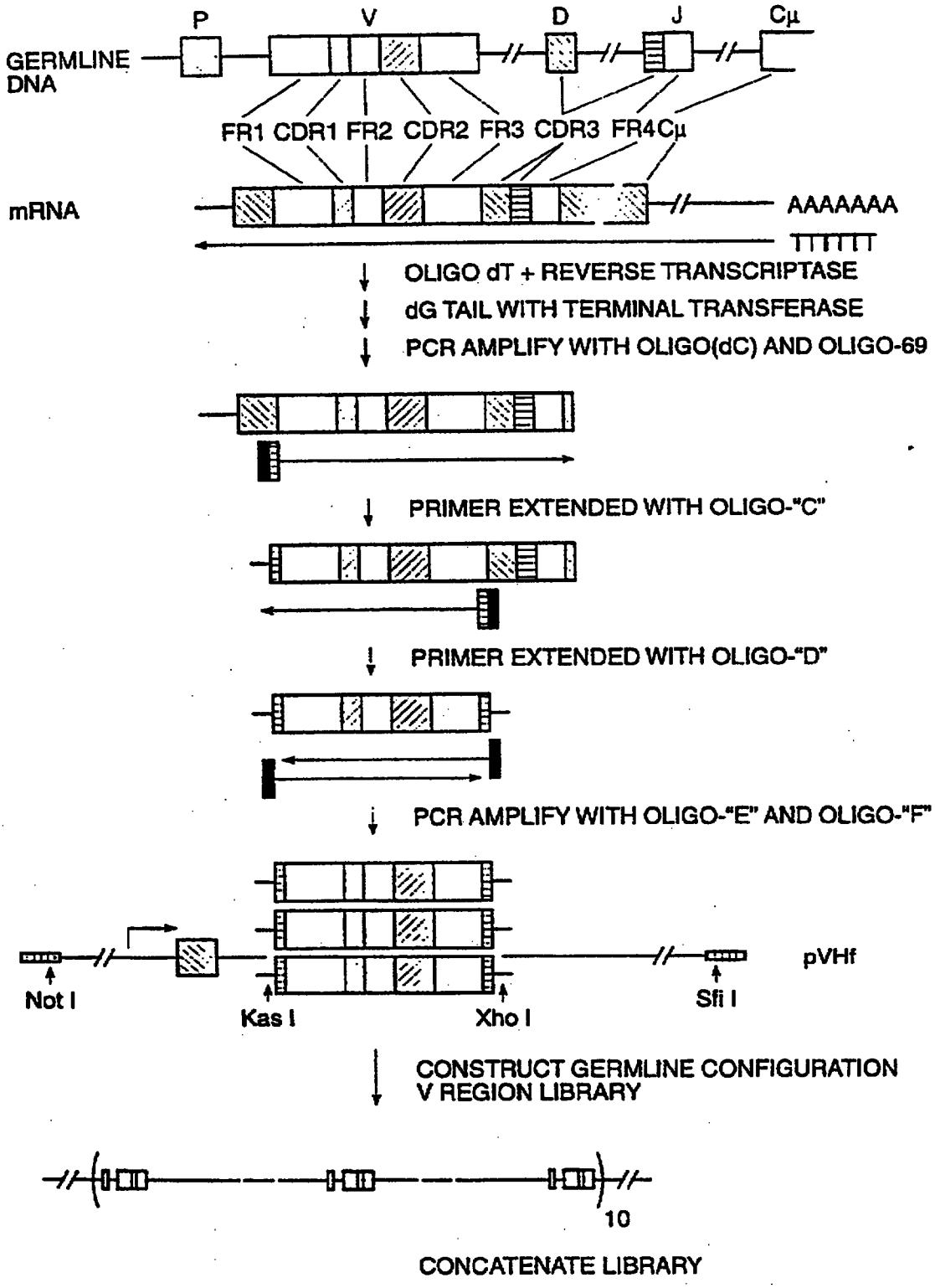

P
V
D
J
Cμ
GERMLINE DNA
FR1 CDR1 FR2 CDR2 FR3 CDR3 FR4Cμ
mRNA
AAAAAAA
TTTTTT
OLIGO dT + REVERSE TRANSCRIPTASE
dG TAIL WITH TERMINAL TRANSFERASE
PCR AMPLIFY WITH OLIGO(dC) AND OLIGO-69
PRIMER EXTENDED WITH OLIGO-"C"
PRIMER EXTENDED WITH OLIGO-"D"
PCR AMPLIFY WITH OLIGO-"E" AND OLIGO-"F"
pVHf
Not I
Kas I
Xho I
Sfi I
CONSTRUCT GERMLINE CONFIGURATION
V REGION LIBRARY
10
CONCATENATE LIBRARY
SYNTHETIC HEAVY CHAIN VARIABLE REGION

FIG. 30

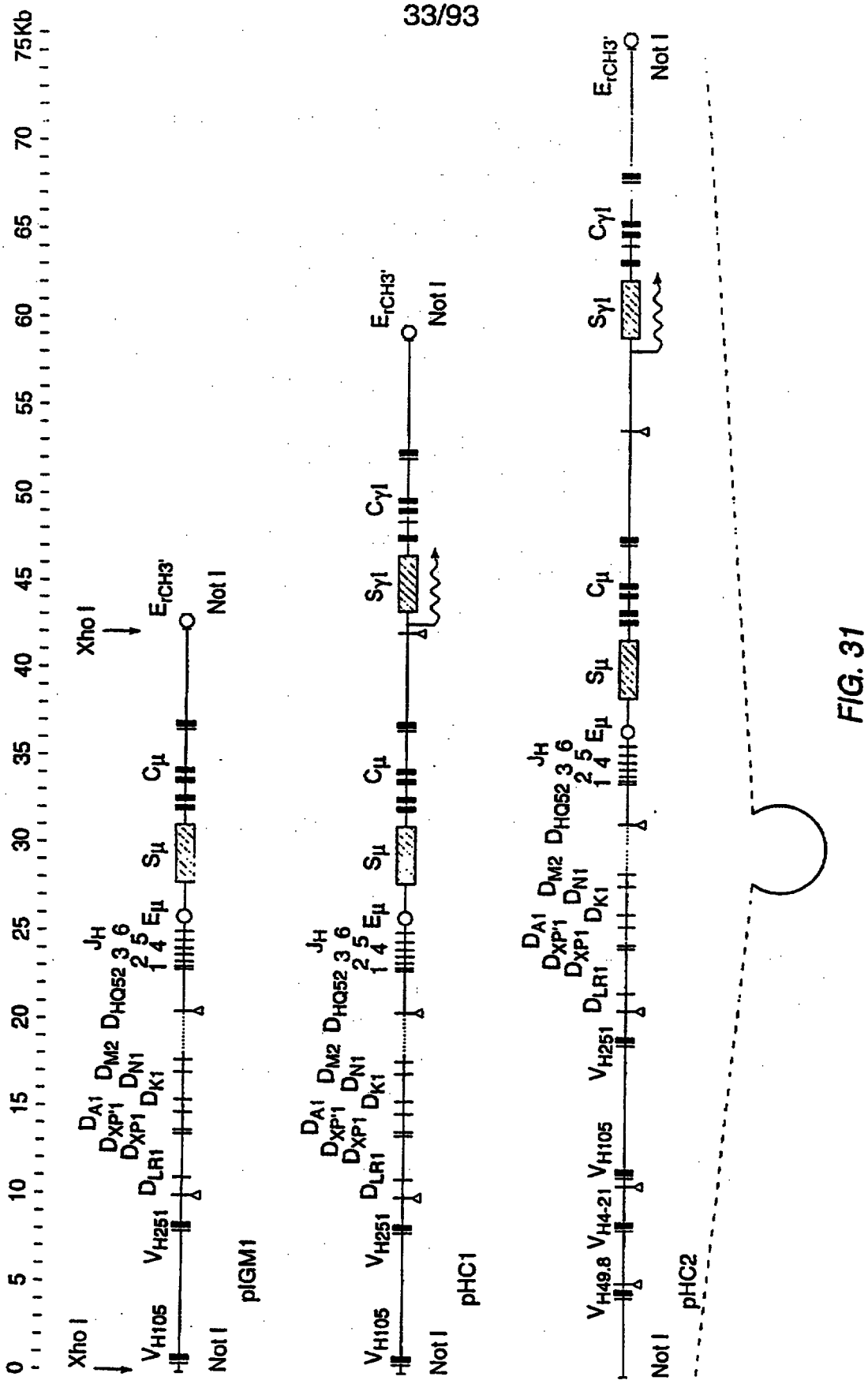

0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75Kb
Xho I
Not I
pIGM1
pHC1
pHC2
V_{H105}
V_{H251}
V_{H4-21}
$V_{H49.8}$
D_{LR1}
$D_{XP'1}$
D_{XP1}
D_{A1}
D_{K1}
D_{N1}
D_{M2}
D_{HQ52}
J_H 1 2 3 4 5 6
$E\mu$
$S\mu$
$C\mu$
$S\gamma1$
$C\gamma1$
$E_{rCH3'}$

FIG. 31

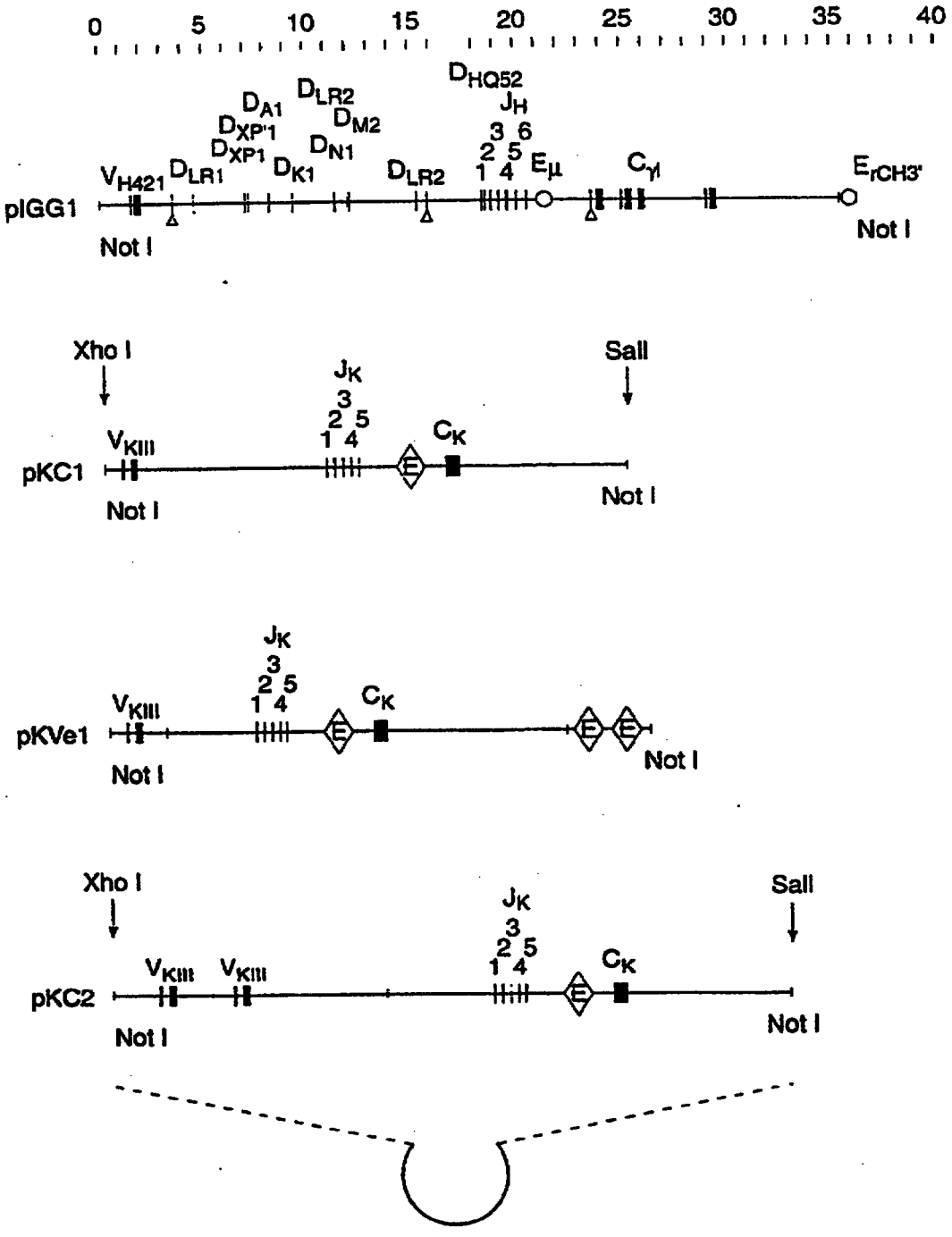

0 5 10 15 20 25 30 35 40
pIGG1
Not I
Not I
Xho I
Sall
pKC1
Not I
Not I
pKVe1
Not I
Not I
Xho I
Sall
pKC2
Not I
Not I

FIG. 32

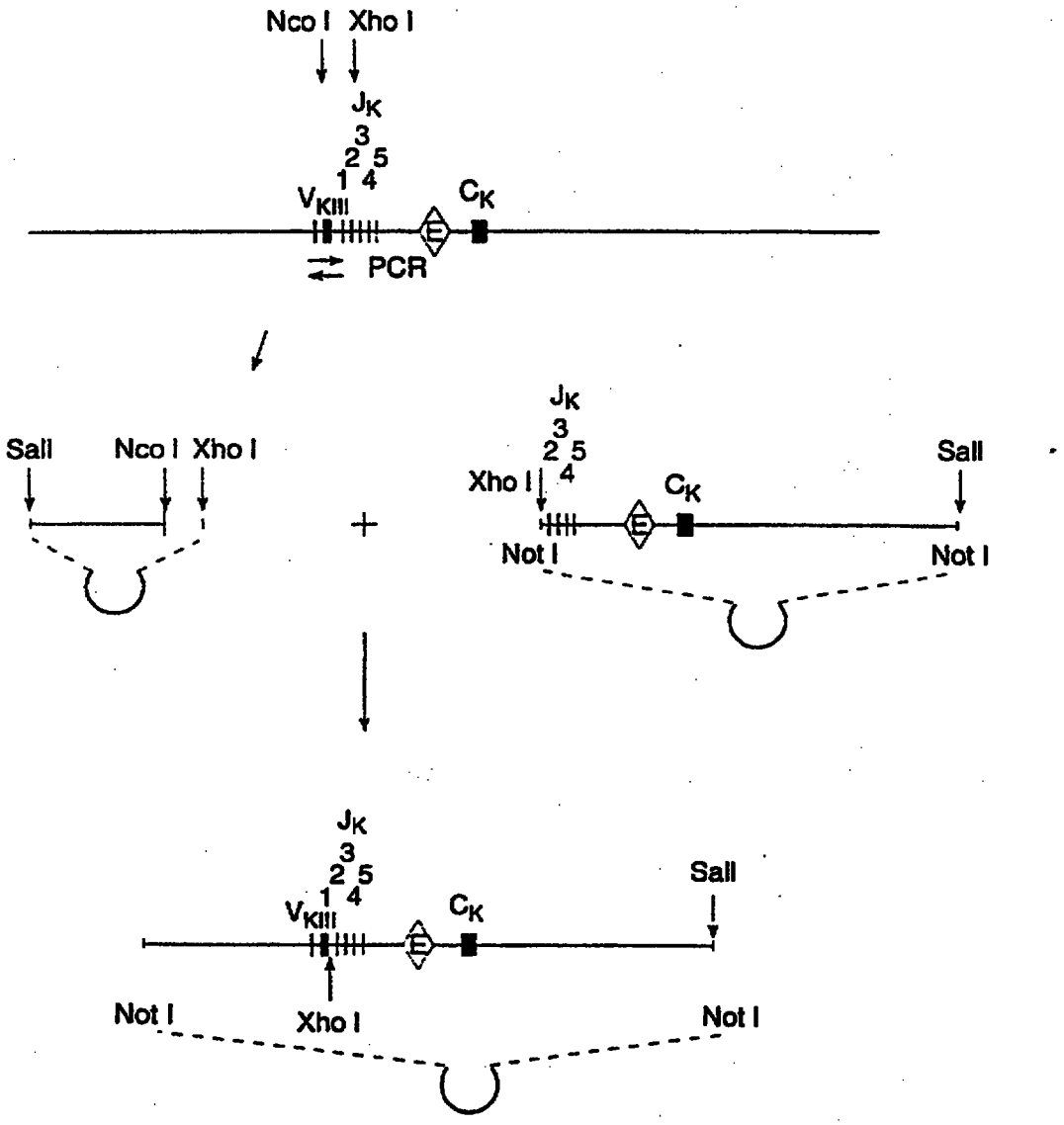

Nco I
Xho I
JK
3
2
5
1
4
VKIII
CK
PCR
SalI
Nco I
Xho I
JK
3
2
5
Xho I
4
CK
SalI
Not I
Not I
JK
3
2
5
1
4
VKIII
CK
SalI
Not I
Xho I
Not I

FIG. 33

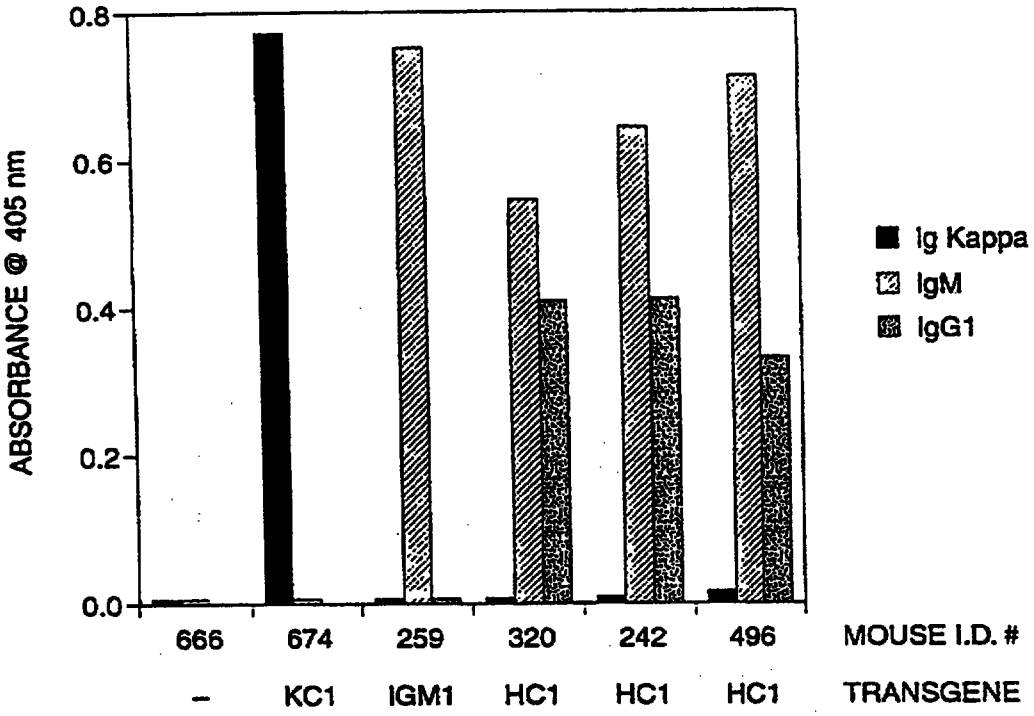

ABSORBANCE @ 405 nm
0.8
0.6
0.4
0.2
0.0
Ig Kappa
IgM
IgG1
666 674 259 320 242 496
MOUSE I.D. #
– KC1 IGM1 HC1 HC1 HC1
TRANSGENE

FIG. 34

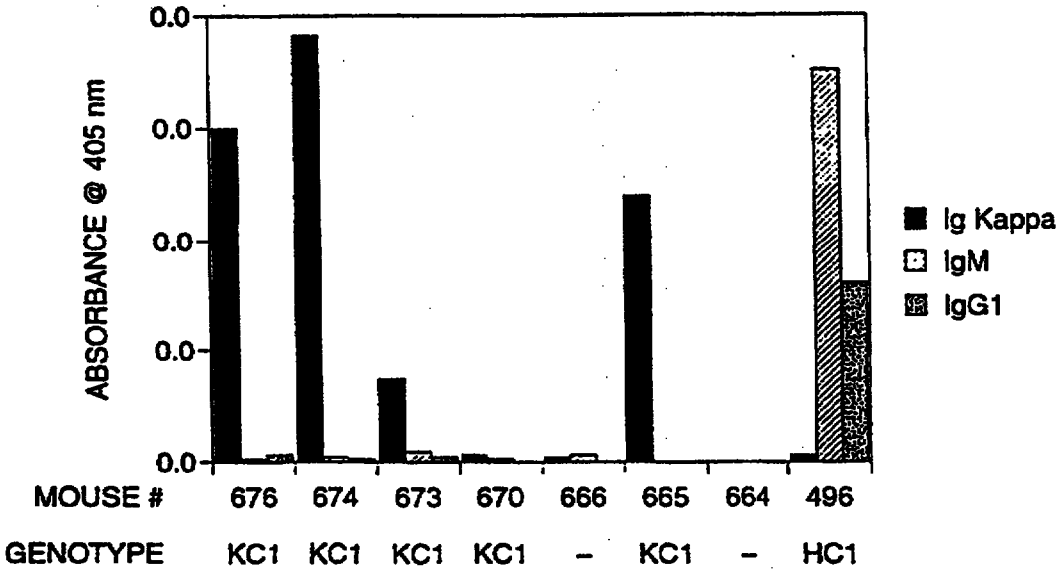

ABSORBANCE @ 405 nm
0.0
0.0
0.0
0.0
0.0
Ig Kappa
IgM
IgG1
MOUSE # 676 674 673 670 666 665 664 496
GENOTYPE KC1 KC1 KC1 KC1 – KC1 – HC1

FIG. 35

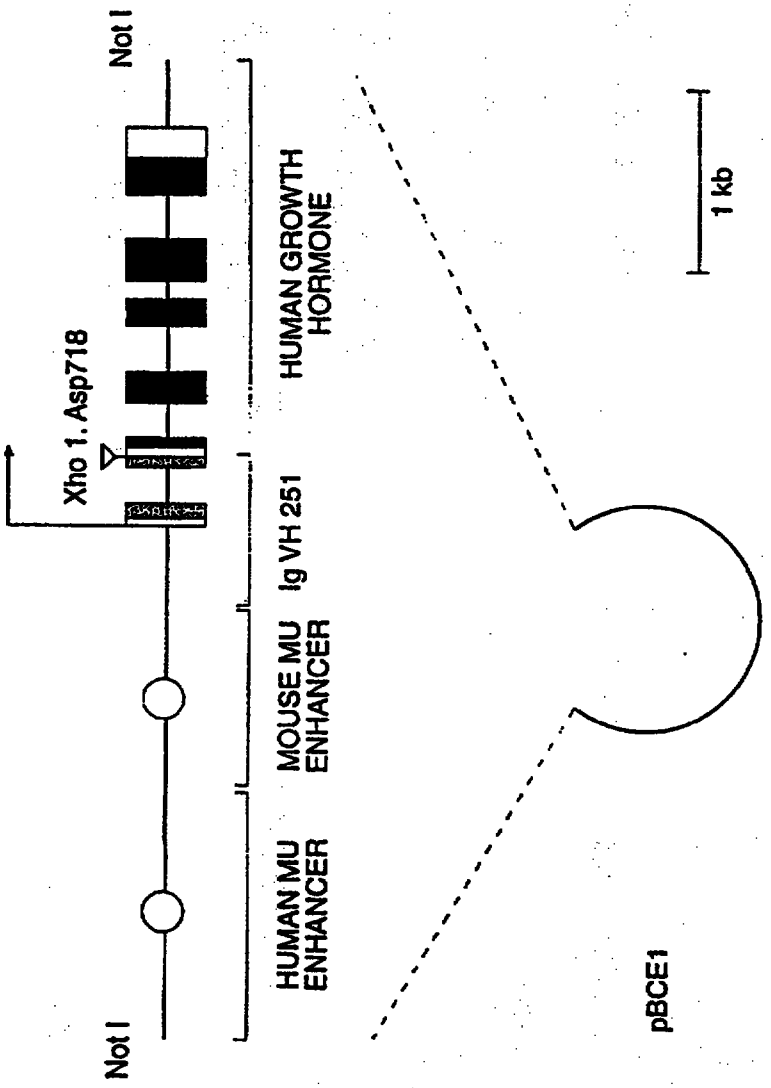

Not I
Xho 1. Asp718
HUMAN GROWTH HORMONE
Ig VH 251
MOUSE MU ENHANCER
HUMAN MU ENHANCER
Not I
1 kb
pBCE1

FIG. 36

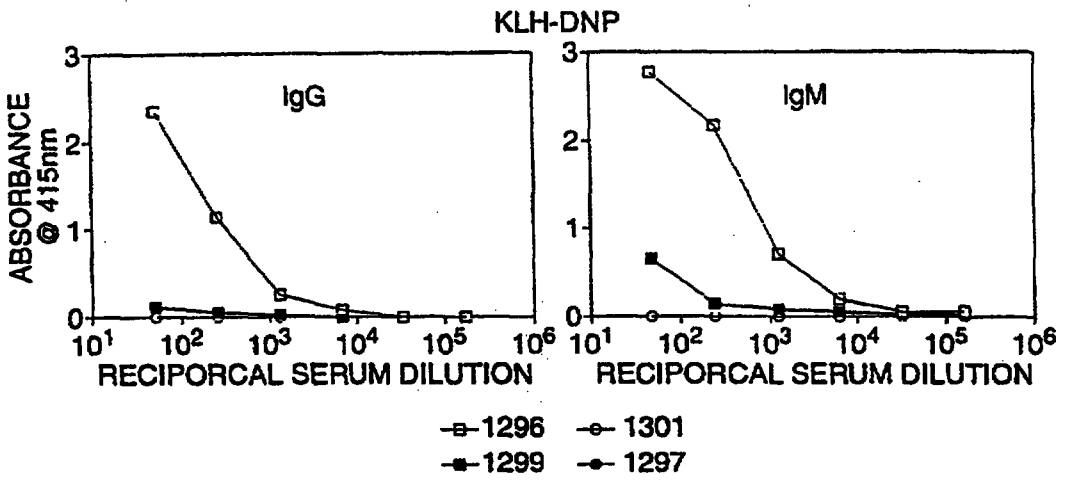


FIG. 37A

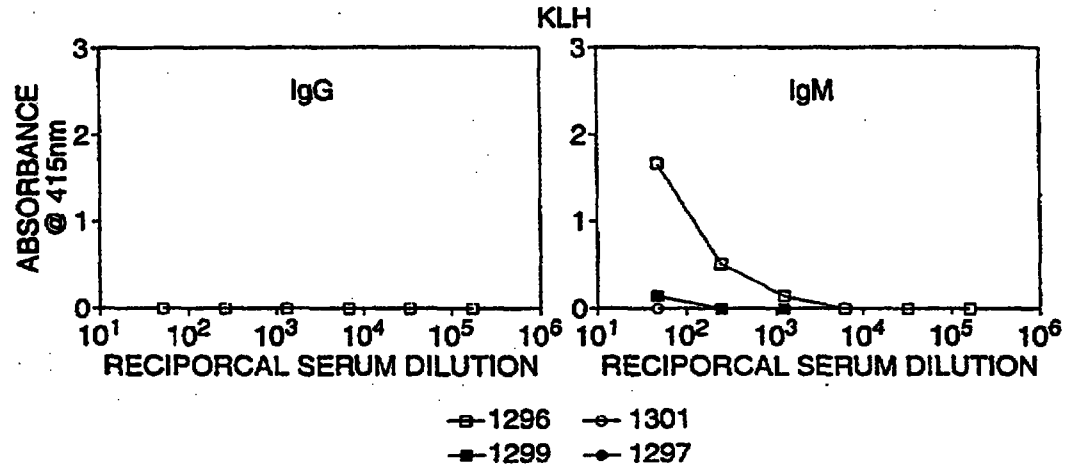


FIG. 37B

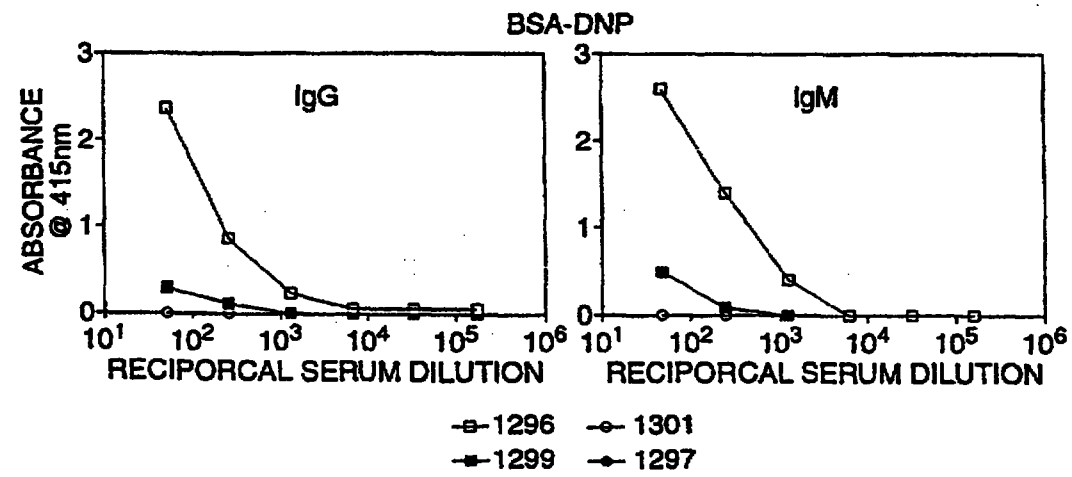


FIG. 37C

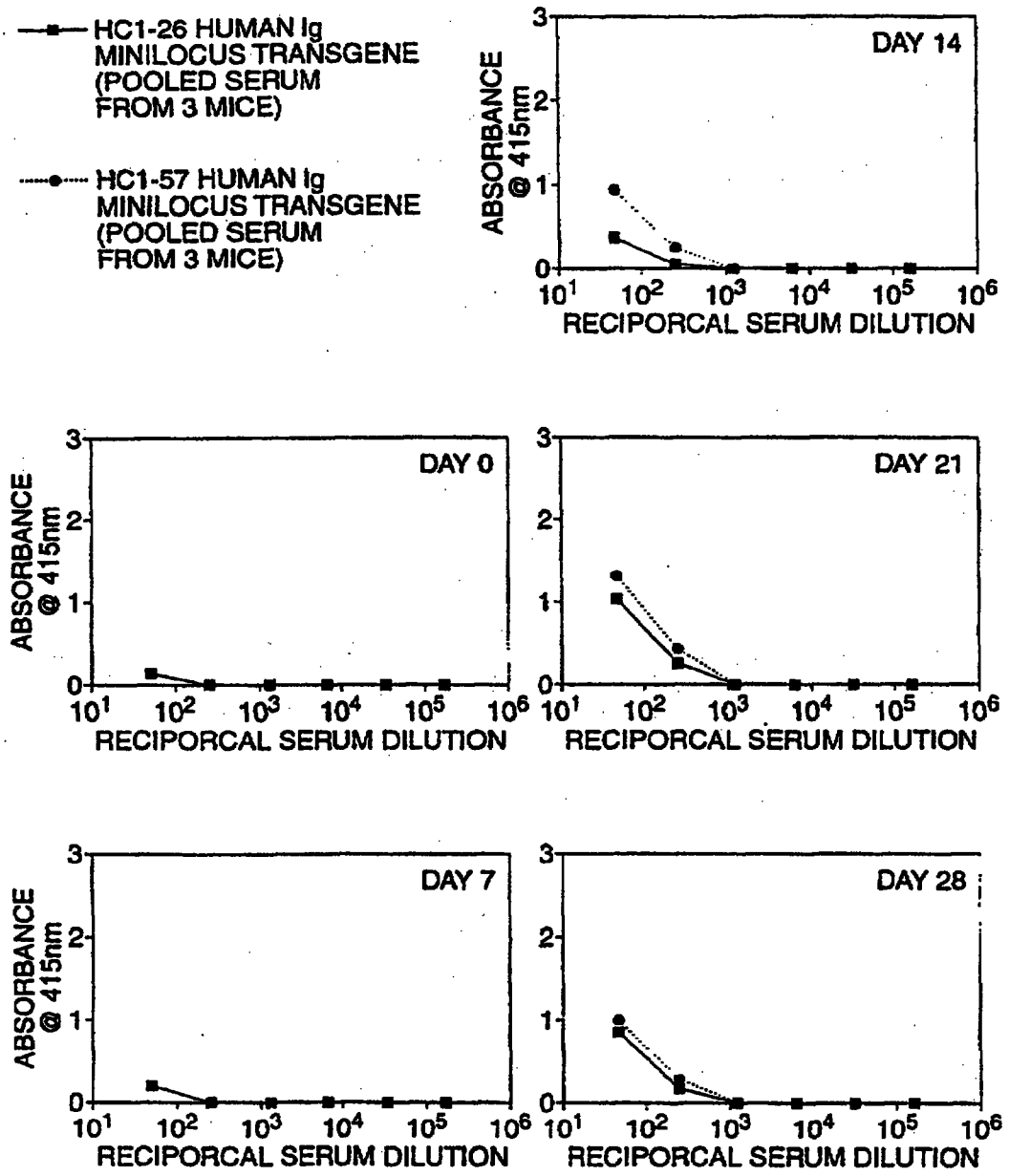

HC1-26 HUMAN Ig MINILOCUS TRANSGENE (POOLED SERUM FROM 3 MICE)
HC1-57 HUMAN Ig MINILOCUS TRANSGENE (POOLED SERUM FROM 3 MICE)
DAY 14
DAY 0
DAY 21
DAY 7
DAY 28
ABSORBANCE @ 415nm
RECIPORCAL SERUM DILUTION

FIG. 38

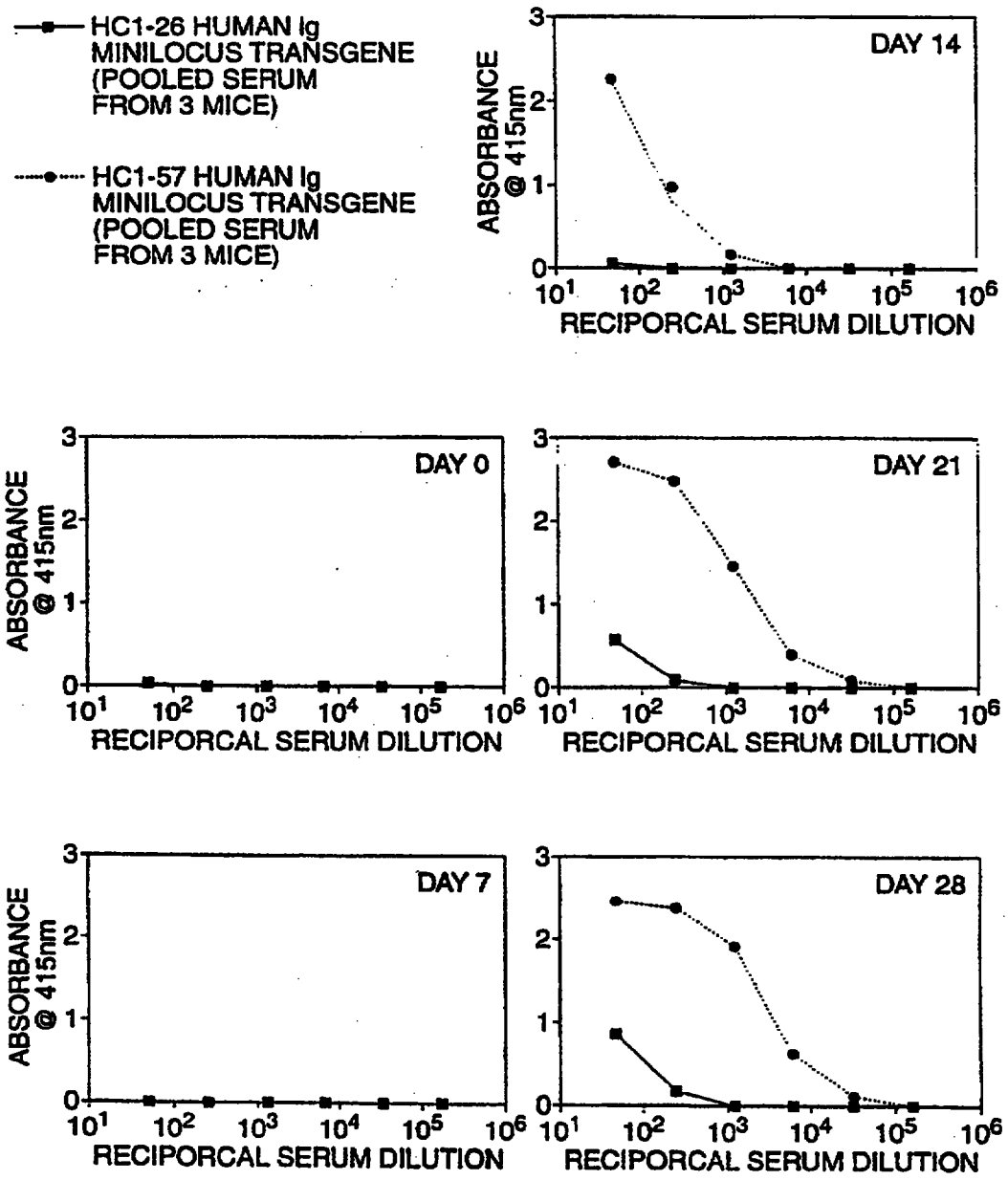

HC1-26 HUMAN Ig MINILOCUS TRANSGENE (POOLED SERUM FROM 3 MICE)
HC1-57 HUMAN Ig MINILOCUS TRANSGENE (POOLED SERUM FROM 3 MICE)
DAY 14
DAY 0
DAY 21
DAY 7
DAY 28
ABSORBANCE @ 415nm
RECIPORCAL SERUM DILUTION
0
1
2
3
10^1 10^2 10^3 10^4 10^5 10^6

FIG. 39

```
      23 24 28 29 30 31 32 35 40 52 56 60 62 64 68 71 73 75 76 79 80 8182a82b 83 84 87 88 89 91 94                      101102108
GL  AAGGGTAGCTTTACCAGCTACGGCATGTATGATAGCTCCCAAACCGCCAAGATCAGCTACCTGCAGAGCAGCAAGGCCACCGCCATGTACAGA
J2                                                                                                              TACTGGTACTTCGATCTCCTG
5.  .....C..............................G.....................CG............................ CAGGGGGGGGATA          .................
J3                                                                                                               GCTTTTGATATCATG
10. ..... ............................................................................. CATTGGctaaAtggggaT         ..............
24. ..................................................................G.....GT.T......... CGGGattacgatattttgactggttattatGCG ..............
J4                                                                                                               TACTTTGACTACCTG
1.  ........T...T..GA..............C........GT...T........A..T........A..C.G....T.............. GGgtattaTtatgAttcggggaCttattataaGTCTACCC .........T...
2.  ........................................................................................ ctaactggCCT            ....A........
3.  ........................................................................................ CATCTT                 .............A.
4.  .................C.A.......GG.......................T................................... CAAGGG                 ...........
6.  ........T...G........................................................................... CATCTT                 ............A.
11. ................A....................................................................... CAAACT                 ...........
17. ....................................................G................................... CATggtatagcagcagctggtacgtggttcGACCC ....
19. .G.........T....T....C.........A..T..................................................... CAGGGC                 ...............
23. ....................................................T.................G...........T...... CATCTT                 .............A.
27. .......................................................................................  GCCgggtataCcagcagctggtT ........T....
30. ..................................................................................CG.... CATCTT                 ............A.
32. ...........C...T........................................................................ GtggttcggggaTttattatT  .............
34. ....T.......A.....T..T.................................................................. CAAAGGGG               .............
36. .......................................................................................G GGATCGTGG              .............
J5                                                                                                               AACTGGTTCGACCCCCTG
25. ........A........C................................G..................................... CTCCCCAATGACAGT        .............
35. ........................................................................................ CGGGGGtactatggttcggggagttattat .................
J6                                                                                                               TACTACTACTACTACGGTATGGACGTCACG
18. ........A.....T..C.........A............................................................ CATGagcagctggtacAGGGT  .........................
22. ........C.........T....C................................................................ GATATGGGGGGGCCTC       .....T.....T.........
28. ...........G...A..........T....................C........................................                        C..C..G..................
33. ..........................T.......C.................A................................... CG                     .................

                CDR I        CDR II                                                          CDR III
```

FIG. 40

```
TTTTCTGGCC TGACAACCAG GGTGGCGCAG GATGCTCAGT GCAGAGAGGA   50

AGAAGCAGGT GGTCTCTGCA GCTGGAAGCT CAGCTCCCAC CCAGCTGCTT  100

TGCATGTCCC TCCCAGCTGC CCTACCTTCC AGAGCCCATA TCAATGCCTG  150

TGTCAGAGCC CTGGGGAGGA ACTGCTCAGT TAGGACCCAG AGGGAACCAT  200
                                                    Me
GGAAGCCCCA GCTCAGCTTC TCTTCCTCCT GCTACTCTGG CTCCCAGgtg  250
tGluAlaPro AlaGlnLeuL euPheLeuLe uLeuLeuTrp LeuPro      -
agggggaacc atgaggtggt tttgcacatt agtgaaaact cttgccacct  300

ctgctcagca agaaatataa ttaaaattca aagtatatca acaattttgg  350

ctctactcaa agacagttgg tttgatcttg attacatgag tgcatttctg  400

ttttatttcc aatttcagAT ACCACCGGAG AAATTGTGTT GACACAGTCT  450
                  Asp ThrThrGlyG luIleValLe uThrGlnSer
CCAGCCACCC TGTCTTTGTC TCCAGGGGAA AGAGCCACCC TCTCCTGCAG  500
ProAlaThrL euSerLeuSe rProGlyGlu ArgAlaThrL euSerCysAr
GGCCAGTCAG AGTGTTAGCA GCTACTTAGC CTGGTACCAA CAGAAACCTG  550
gAlaSerGln SerValSerS erTyrLeuAl aTrpTyrGln GlnLysProG
GCCAGGCTCC CAGGCTCCTC ATCTATGATG CATCCAACAG GGCCACTGGC  600
lyGlnAlaPr oArgLeuLeu IleTyrAspA laSerAsnAr gAlaThrGly
ATCCCAGCCA GGTTCAGTGG CAGTGGGTCT GGGACAGACT TCACTCTCAC  650
IleProAlaA rgPheSerGl ySerGlySer GlyThrAspP heThrLeuTh
CATCAGCAGC CTAGAGCCTG AAGATTTTGC AGTTTATTAC TGTCAGCAGC  700
rIleSerSer LeuGluProG luAspPheAl aValTyrTyr CysGlnGlnA
GTAGCAACTG GCCTCCCACA GTGATTCCAC ATGAAACAAA AACCCCAACA  750
rgSerAsnTr pPro
AGACCATCAG TGTTTACTAG ATTATTATAC CAGCTGCTTC CTTTACAGAC  800

AGCTAGTGGG GT                                           812
```

FIG. 41

```
AGGGCGGCGC AGATGCTCAG TGCAGAGAGA AGAAACAGGT GGTCTCTGCA    50

GCTGGAAGCT CAGCTCCCAC CCCAGCTGCT TTGCATGTCC CTCCCAGCTG   100

CCCTACCTTC CAGAGCCCAT ATCAATGCCT GGGTCAGAGC TCTGGGGAGG   150

AACTGCTCAG TTAGGACCCA GACGGAACCA TGGAAGCCCC AGCGCAGCTT   200
                                  M etGluAlaPr oAlaGlnLeu
CTCTTCCTCC TGCTACTCTG GCTCACAGgt gaggggaata tgaggtgtct   250
LeuPheLeuL euLeuLeuTr pLeuThr
ttgcacatca gtgaaaactc ctgccacctc tgctcagcaa gaaatataat   300

taaaattcaa aatagatcaa caattttggc tctactcaaa gacagtgggt   350

ttgattttga ttacatgagt gcatttctgt tttatttcca atttcagATA   400
                                                    AspT
CCACCGGAGA AATTGTGTTG ACACAGTCTC CAGCCACCCT GTCTTTGTCT   450
hrThrGlyGl uIleValLeu ThrGlnSerP roAlaThrLe uSerLeuSer
CCAGGGGAAA GAGCCACCCT CTCCTGCAGG GCCAGTCAGG GTGTTAGCAG   500
ProGlyGluA rgAlaThrLe uSerCysArg AlaSerGlnG lyValSerSe
CTACTTAGCC TGGTACCAGC AGAAACCTGG CCAGGCTCCC AGGCTCCTCA   550
rTyrLeuAla TrpTyrGlnG lnLysProGl yGlnAlaPro ArgLeuLeuI
TCTATGATGC ATCCAACAGG GCCACTGGCA TCCCAGCCAG GTTCAGTGGC   600
leTyrAspAl aSerAsnArg AlaThrGlyI lePro AlaAr gPheSerGly
AGTGGGCCTG GGACAGACTT CACTCTCACC ATCAGCAGCC TAGAGCCTGA   650
SerGlyProG lyThrAspPh eThrLeuThr IleSerSerL euGluProGl
AGATTTTGCA GTTTATTACT GTCAGCAGCG TAGCAACTGG CATCCCACAG   700
uAspPheAla ValTyrTyrC ysGlnGlnAr gSerAsnTrp His
TGATTCCACA TGAAACAAAA ACCCCAACAA GACCATCAGT GTTTACTAGA   750

TTATTATACC AGCTGCTTCC TTTACAGACA GCTAGTGGGG TGGCCACTCA   800

GTGTTAGCAT CTCAGCTCTA TTTGGCCATT TTGGAGTTCA AGTTGTCAAG   850

TCCAAAATTA CTTATGTTAG TCCATTGCAT CATACCATTT CAGTGTGGCT   900
```

FIG. 42

```
CCGCCCCAGC TGCTTTGCAT GTCCCTCCCA GCCGCCCTGC AGTCCAGAGC    50

CCATATCAAT GCCTGGGTCA GAGCTCTGGA GAAGAGCTGC TCAGTTAGGA   100

ACCCCAGAGG GAACCATGGA AACCCCAGCG CAGCTTCTCT TCCTCCTGCT   150
                 MetGl uThrProAla GlnLeuLeuP heLeuLeuLe
ACTCTGGCTC CCAGgtgagg ggaacatggg atggttttgc atgtcagtga   200
uLeuTrpLeu Pro
aaaccctctc aagtcctgtt acctggcaac tctgctcagt caatacaata   250

attaaagctc aatataaagc aataattctg gctcttctgg gaagacaatg   300

ggtttgattt agattacatg ggtgactttt ctgttttatt tccaatctca   350

gATACCACCG GAGAAATTGT GTTGACGCAG TCTCCAGGCA CCCTGTCTTT   400
AspThrThrG lyGluIleVa lLeuThrGln SerProGlyT hrLeuSerLe
GTCTCCAGGG GAAAGAGCCA CCCTCTCCTG CAGGGCCAGT CAGAGTGTTA   450
uSerProGly GluArgAlaT hrLeuSerCy sArgAlaSer GlnSerValS
GCAGCAGCTA CTTAGCCTGG TACCAGCAGA AACCTGGCCA GGCTCCCAGG   500
erSerSerTy rLeuAlaTrp TyrGlnGlnL ysProGlyGl nAlaProArg
CTCCTCATCT ATGGTGCATC CAGCAGGGCC ACTGGCATCC CAGACAGGTT   550
LeuLeuIleT yrGlyAlaSe rSerArgAla ThrGlyIleP roAspArgPh
CAGTGGCAGT GGGTCTGGGA CAGACTTCAC TCTCACCATC AGCAGACTGG   600
eSerGlySer GlySerGlyT hrAspPheTh rLeuThrIle SerArgLeuG
AGCCTGAAGA TTTTGCAGTG TATTACTGTC AGCAGTATGG TAGCTCACCT   650
luProGluAs pPheAlaVal TyrTyrCysG lnGlnTyrGl ySerSerPro
CCCACAGTGA TTCAGCTTGA AACAAAAACC TCTGCAAGAC CTTCATTGTT   700

TACTAGATTA TACCAGCTGC TTCCTTTACA GATAGCTGCT GCAATGACAA   750

CTCAATTTAG CATCTCTCTC TGCTTGGGCA TTTTGGGGAT CTTAAAAAAG   800

TAATCCCTTG ATATATTTTT GACTCTGATT CCTGCATTTT TCCTCAGACC   850

AAGATGGACA GCCAGGTTTA AGCACAGTTT CACAGTAATG GCCACTGGAT   900
```

FIG. 43

```
AAACACATTC TCTGCAGACA AATTTGAGCT ACCTTGATCT TACCTGGACA   50

GGTGGGGACA CTGAGCTGGT GCTGAGTTAC TCAGATGCGC CAGCTCTGCA  100

GCTGTGCCCA GCCTGCCCCA TCCCCTGCTC ATTTGCATGT TCCCAGAGCA  150

CAACCTCCTG CCCTGAAGCC TTATTAATAG GCTGGTCAGA CTTTGTGCAG  200

GAATCAGACC CAGTCAGGAC ACAGCATGGA CATGAGGGTC CTCGCTCAGC  250
                          MetAs pMetArgVal LeuAlaGlnL
TCCTGGGGCT CCTGCTGCTC TGTTTCCCAG gtaaggatgg agaacactag  300
euLeuGlyLe uLeuLeuLeu CysPhePro
cagtttactc agcccagggt gctcagtact gctttactat tcagggaaat  350

tctcttacaa catgattaat tgtgtggaca tttgttttta tgtttccaat  400

ctcagGTGCC AGATGTGACA TCCAGATGAC CCAGTCTCCA TCCTCACTGT  450
    GlyAla ArgCysAspI leGlnMetTh rGlnSerPro SerSerLeuS
CTGCATCTGT AGGAGACAGA GTCACCATCA CTTGTCGGGC GAGTCAGGGT  500
erAlaSerVa lGlyAspArg ValThrIleT hrCysArgAl aSerGlnGly
ATTAGCAGCT GGTTAGCCTG GTATCAGCAG AAACCAGAGA AAGCCCCTAA  550
IleSerSerT rpLeuAlaTr pTyrGlnGln LysProGluL ysAlaProLy
GTCCCTGATC TATGCTGCAT CCAGTTTGCA AAGTGGGGTC CCATCAAGGT  600
sSerLeuIle TyrAlaAlaS erSerLeuGl nSerGlyVal ProSerArgP
TCAGCGGCAG TGGATCTGGG ACAGATTTCA CTCTCACCAT CAGCAGCCTG  650
heSerGlySe rGlySerGly ThrAspPheT hrLeuThrIl eSerSerLeu
CAGCCTGAAG ATTTTGCAAC TTATTACTGC CAACAGTATA ATAGTTACCC  700
GlnProGluA spPheAlaTh rTyrTyrCys GlnGlnTyrA snSerTyrPr
ACCCACAGTG TTACACACCC AAACATAAAC CCCCAGGGAA GCAGATGTGT  750
o
GAGGCTGGGC TGCCCCAGCT GCTTCTCCTG ATGCCTCCAT CAGCTGAGAG  800

TGTTCCTCAG ATGCAGCCAC ACTCTGATGG TGTTGGTAGA TGGGGAC     847
```

FIG. 44

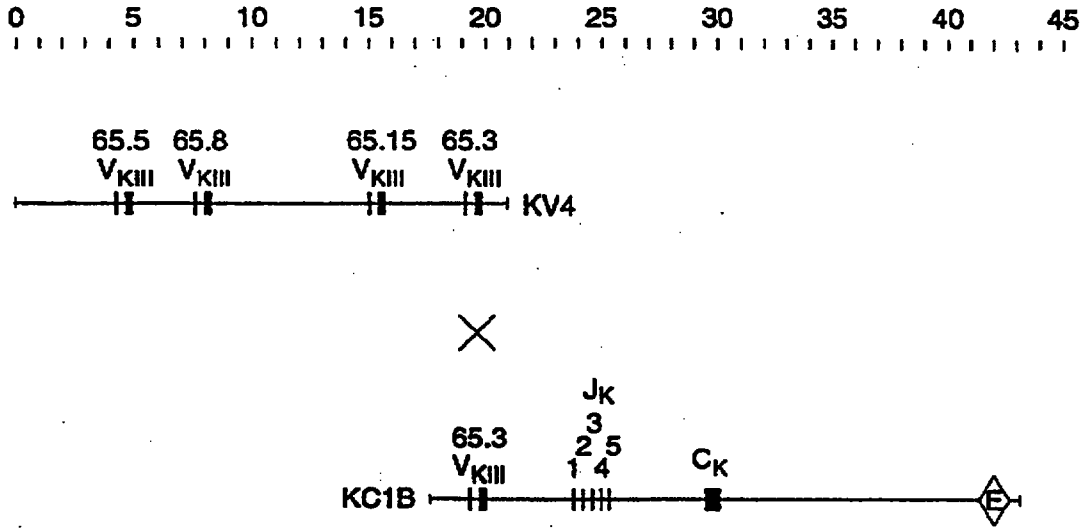

0
5
10
15
20
25
30
35
40
45
65.5 V_{KIII}
65.8 V_{KIII}
65.15 V_{KIII}
65.3 V_{KIII}
KV4
J_K
3
2
5
1
4
65.3 V_{KIII}
C_K
KC1B
E

FIG. 45

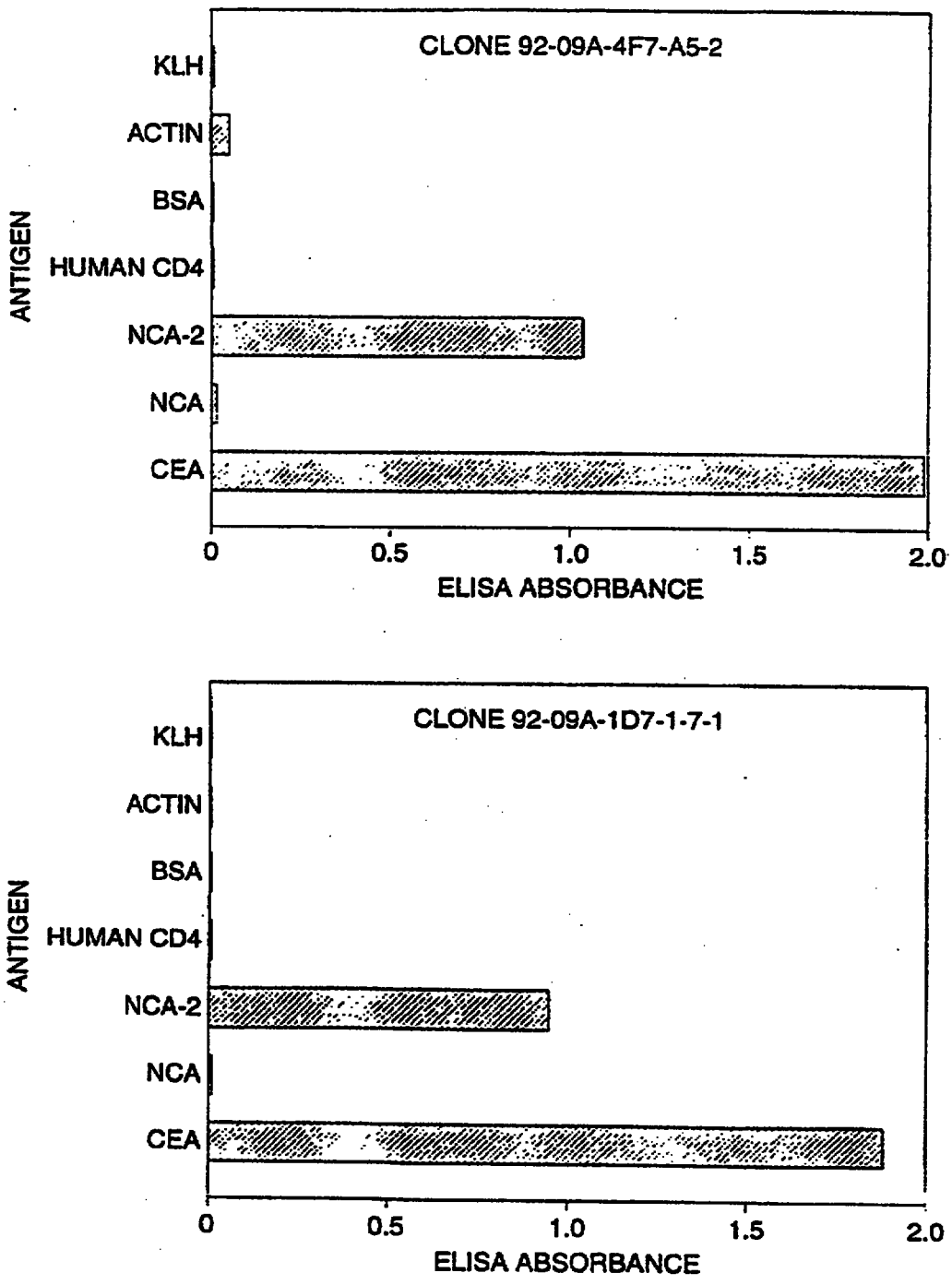

CLONE 92-09A-4F7-A5-2
KLH
ACTIN
BSA
HUMAN CD4
NCA-2
NCA
CEA
ANTIGEN
0
0.5
1.0
1.5
2.0
ELISA ABSORBANCE
CLONE 92-09A-1D7-1-7-1
KLH
ACTIN
BSA
HUMAN CD4
NCA-2
NCA
CEA
ANTIGEN
0
0.5
1.0
1.5
2.0
ELISA ABSORBANCE

FIG. 46

HUMAN
MOUSE
VH251
n D n
J
Cγ
5 DXP'1 J6 G1 GCCTCGGACACCGCCATGTATTACTGTGtGAGA cattIATGGTTCGGGGAGTTAcg CGGTgTGaACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAG CCAAAACGACACCCCATCTGTCTATCCACT
7 DHQS2 J3 G1 GCCTCGGACACCGCCATGTATTACTGTGCGAGA cACTGGGcattggat GCTcTTGATgTCTGGGGCCAAGGGACAATGcTCACCGTCTCCTTCAG CCAAACGACACCCCATCTGTCTATCCACT
2 DHQS2 J3 G2b GCCTCGGACACCGCCATGTATTACTGTGCGAGA ACTGGGGAtgat GCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAG CCAAAACAACACCCCATCAGTCTATCCACT
3 D? J3 G2b GaCTCGGACACCGCCATGTATTACTGTGCGAGA cagggagagat GCTTTAGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAG CCAAAACAACACCCCATCAGTCTATCCACT
4 DXP'1 J4 G2b GCCTCGGACACCGCCATGTATTATGTGtGAGA catgggACTATatTTCGGGGAGTTATTttcc TGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAG CCAAAACAACACCCCATCAGTCTATCCACT
10 DHQS2 J3 G2b GCCTCGGACACCGCCATGTATTACTGTGCGAGA ACTGGGGAtgat GCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAG CCAAAACAACACCCCATCAGTCTATCCACT
1 D? J3 G3 GCCTCGGACACCGCCATGTATTACTGTGCGAGA catggtctatg GATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAG CTACAACAACAGCCCCATCTGTCTATCCCTT
6 DHQS2 J4 G3 GCCTCGGACACCGCCATGTATTACTGTGCGAGA gggagcggtcACTGGGGAtcg TTTGACTATtGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAG CTACAACAACAGCCCCATCTGTCTATCCCTT
8 DIR2 J3 G3 GCCTCGGACACCGCCATGTATTACTGTGCGAGA aggtACCCCCctgat GCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAG CTACAACAACAGCCCCATCTGTCTATCCCTT
9 DIR2r J6 G3 GCCTCGGACACCGCCATGTATTACTGTGCGAGA cgGGGGCCT TACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAG CTACAACAACAGCCCCATCTGTCTATCCCTT

FIG. 47

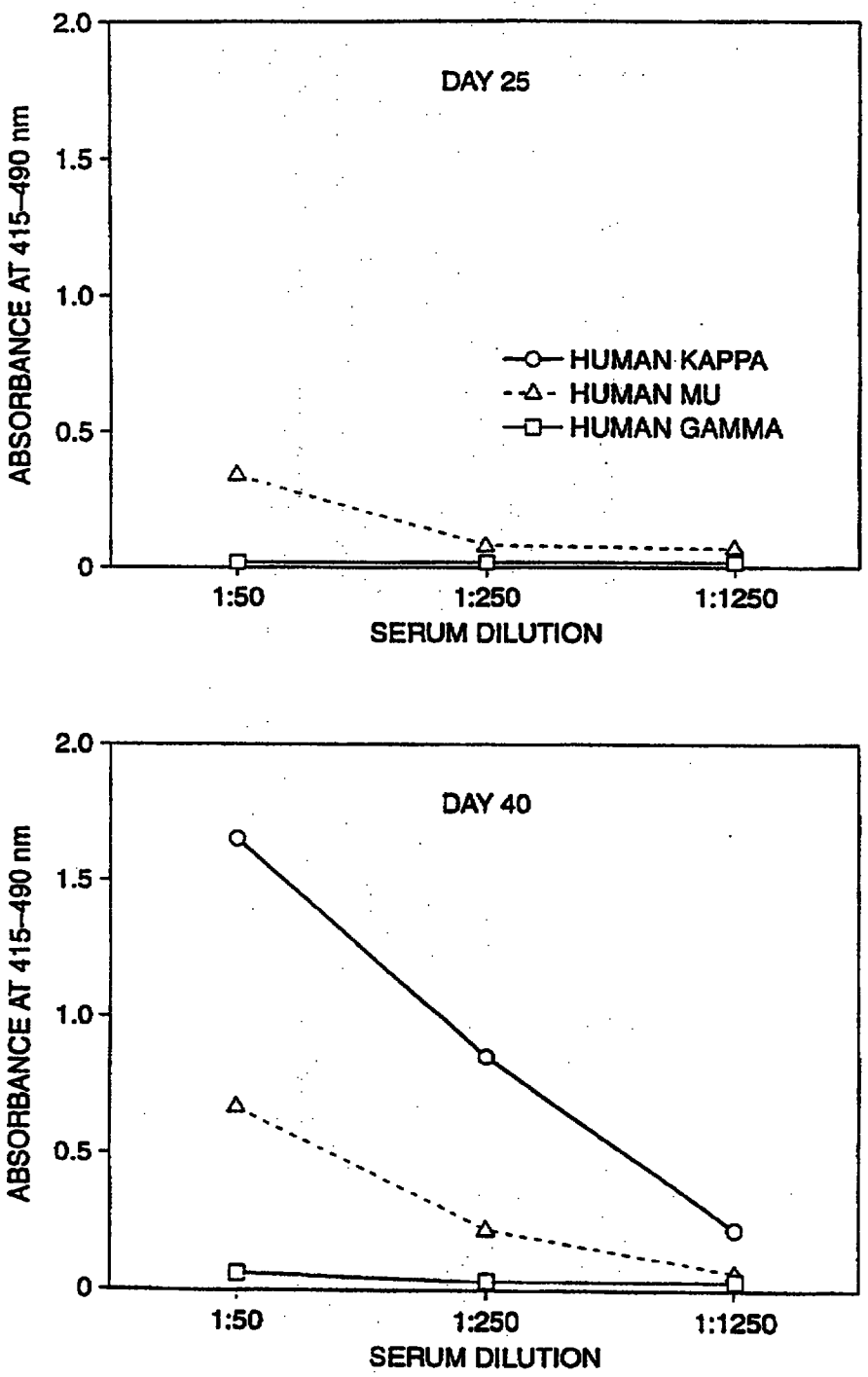

DAY 25
ABSORBANCE AT 415–490 nm
2.0
1.5
1.0
0.5
0
HUMAN KAPPA
HUMAN MU
HUMAN GAMMA
1:50
1:250
1:1250
SERUM DILUTION
DAY 40
ABSORBANCE AT 415–490 nm
2.0
1.5
1.0
0.5
0
1:50
1:250
1:1250
SERUM DILUTION

FIG. 48

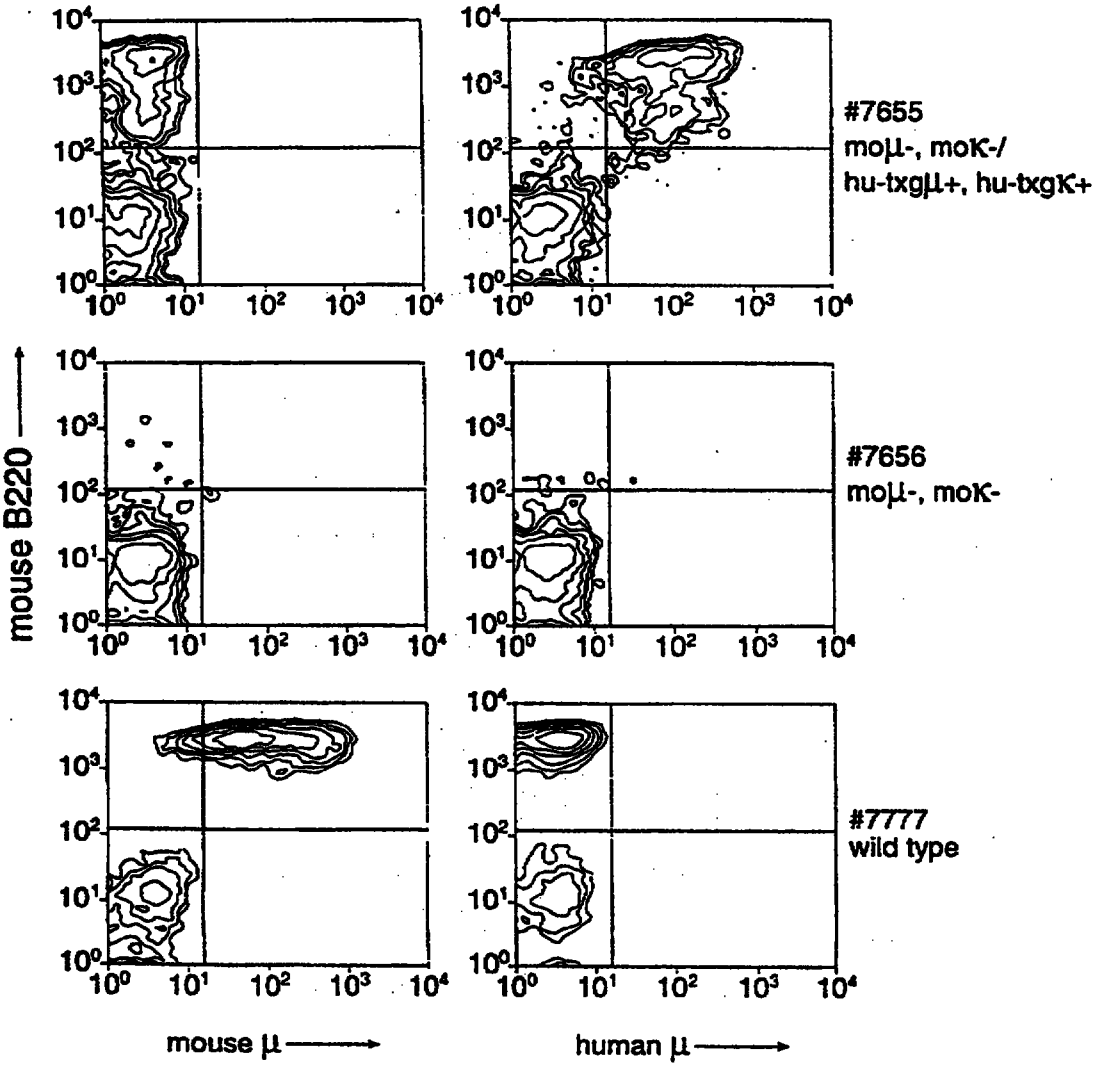

#7655
moμ-, moκ-/
hu-txgμ+, hu-txgκ+
#7656
moμ-, moκ-
#7777
wild type
mouse B220
mouse μ
human μ

FIG. 49

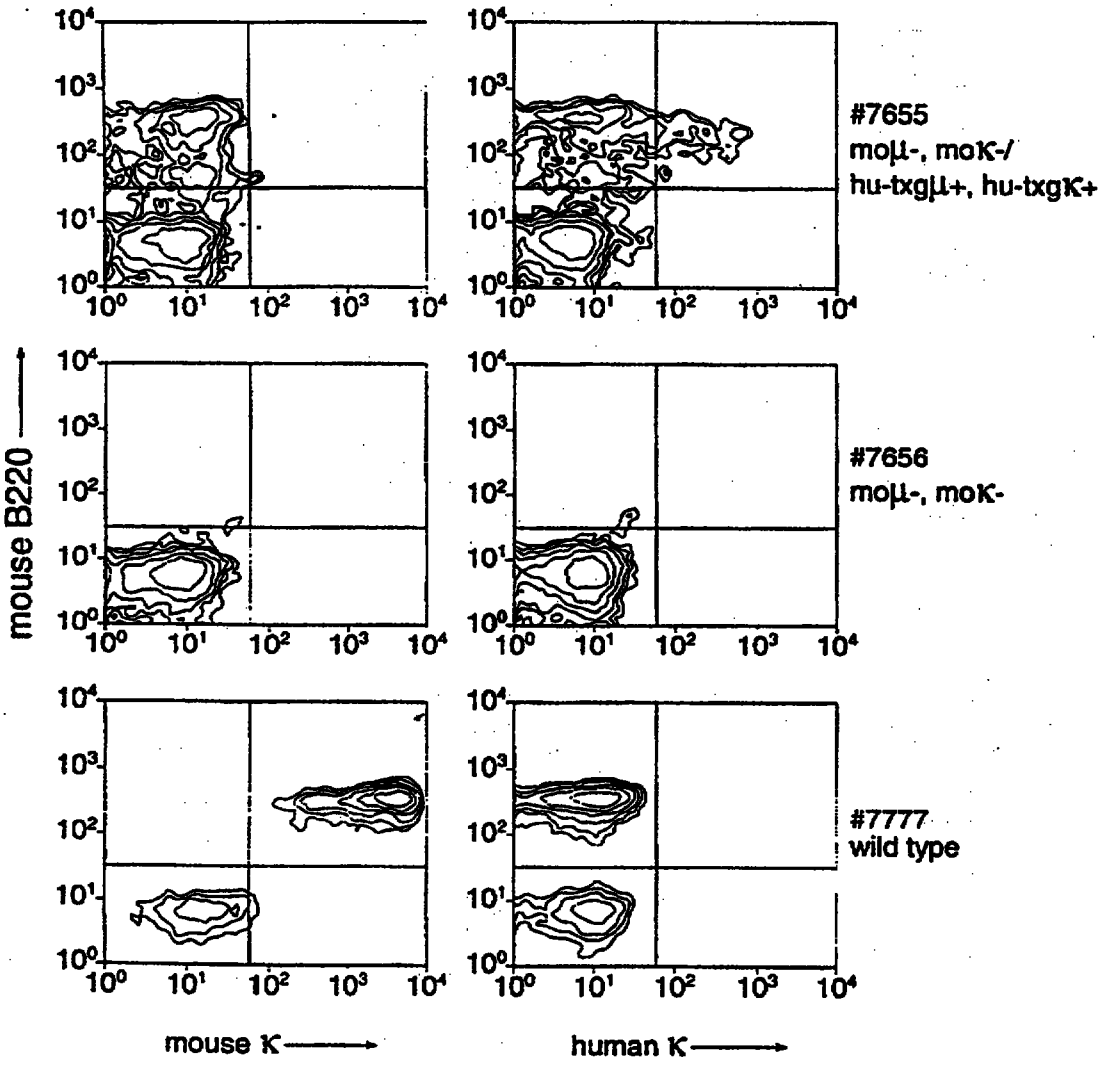

#7655
moμ-, moκ-/
hu-txgμ+, hu-txgκ+
#7656
moμ-, moκ-
#7777
wild type
mouse B220
mouse κ
human κ

FIG. 50

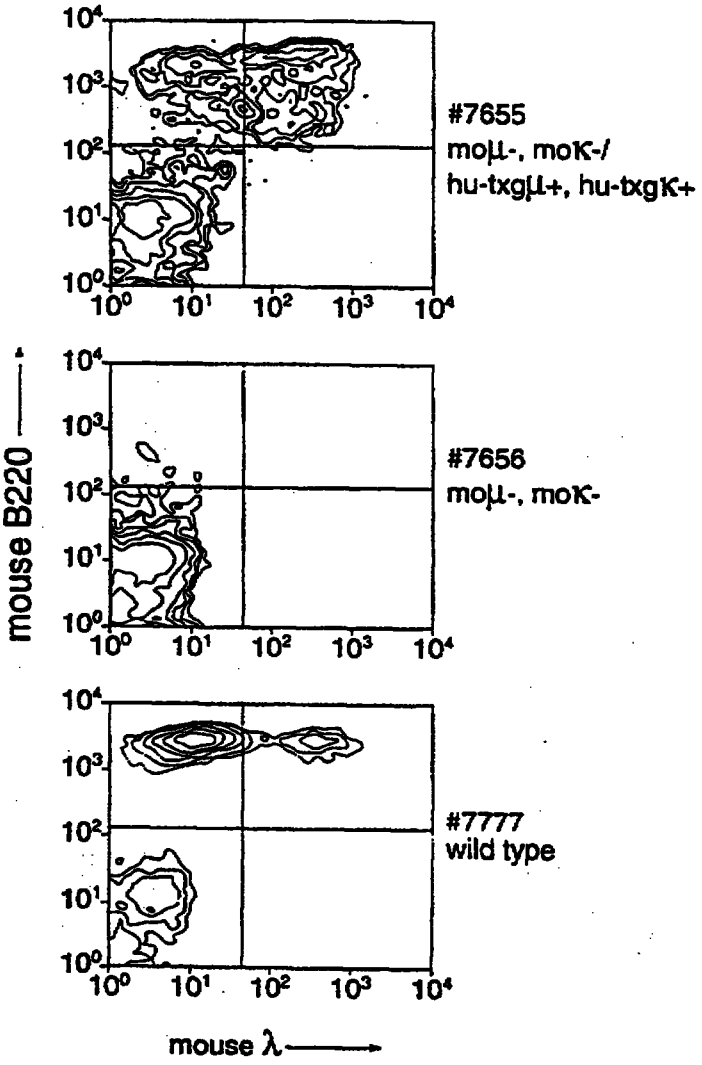

#7655
moμ-, moκ-/
hu-txgμ+, hu-txgκ+
#7656
moμ-, moκ-
#7777
wild type
mouse B220
mouse λ
10⁰
10¹
10²
10³
10⁴

FIG. 51

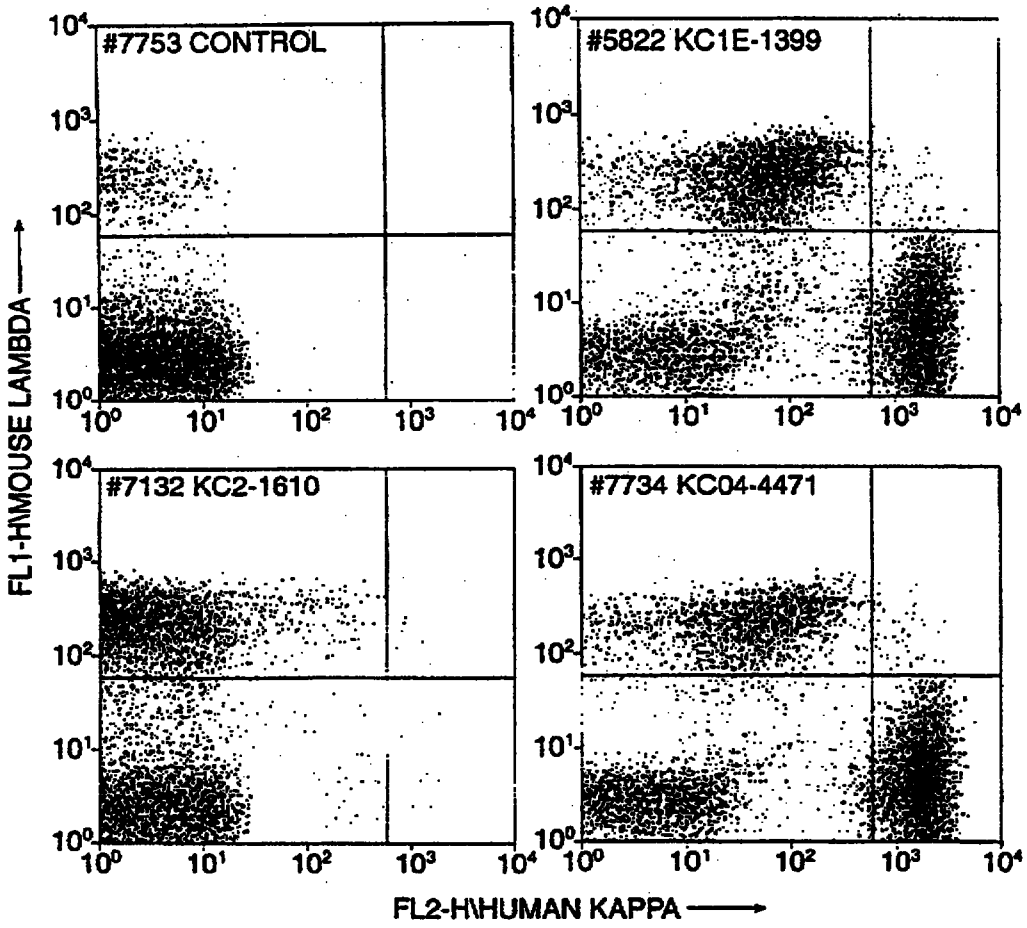

#7753 CONTROL
#5822 KC1E-1399
#7132 KC2-1610
#7734 KC04-4471
FL1-H\MOUSE LAMBDA
FL2-H\HUMAN KAPPA
10^0
10^1
10^2
10^3
10^4

FIG. 52

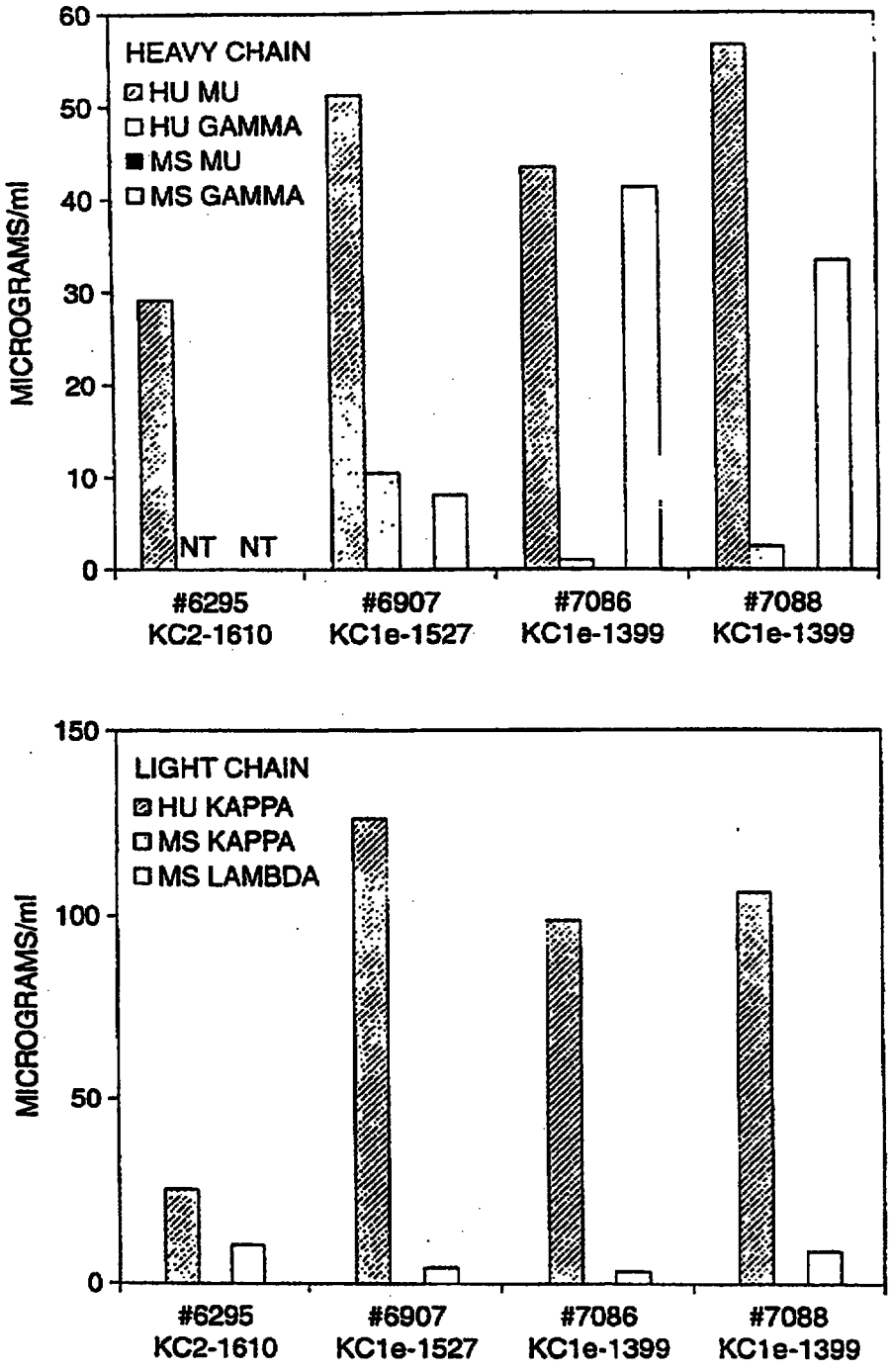

HEAVY CHAIN
HU MU
HU GAMMA
MS MU
MS GAMMA
MICROGRAMS/ml
60
50
40
30
20
10
0
NT NT
#6295 KC2-1610
#6907 KC1e-1527
#7086 KC1e-1399
#7088 KC1e-1399
LIGHT CHAIN
HU KAPPA
MS KAPPA
MS LAMBDA
MICROGRAMS/ml
150
100
50
0
#6295 KC2-1610
#6907 KC1e-1527
#7086 KC1e-1399
#7088 KC1e-1399

FIG. 53

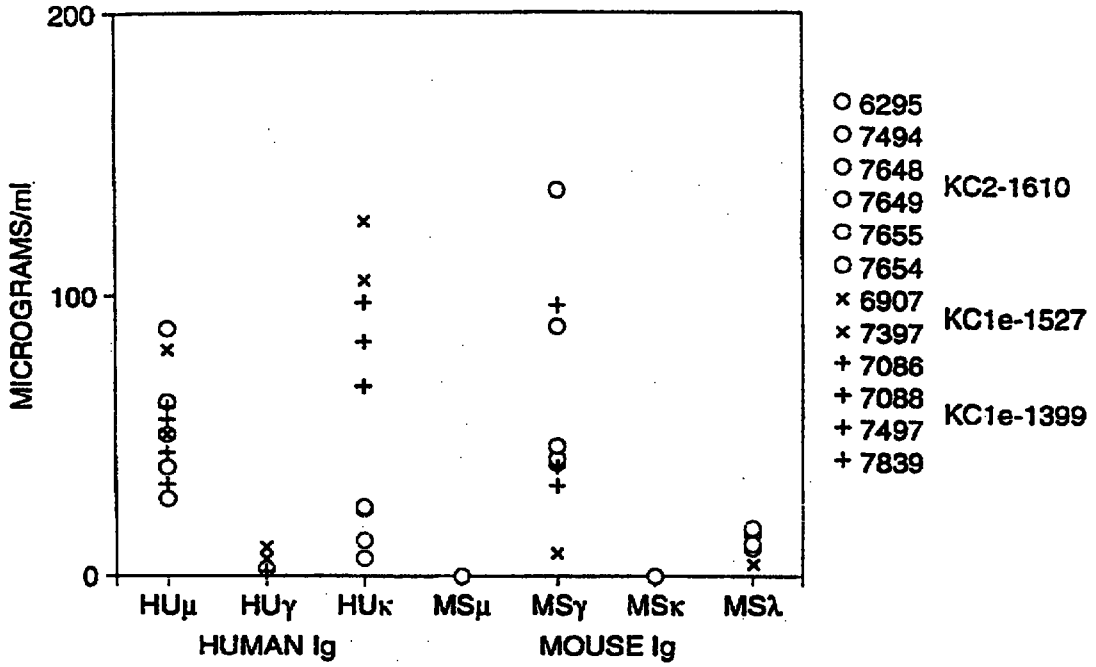

200
100
0
MICROGRAMS/ml
HUμ
HUγ
HUκ
MSμ
MSγ
MSκ
MSλ
HUMAN Ig
MOUSE Ig
6295
7494
7648
7649
7655
7654
KC2-1610
6907
7397
KC1e-1527
7086
7088
7497
7839
KC1e-1399

FIG. 54

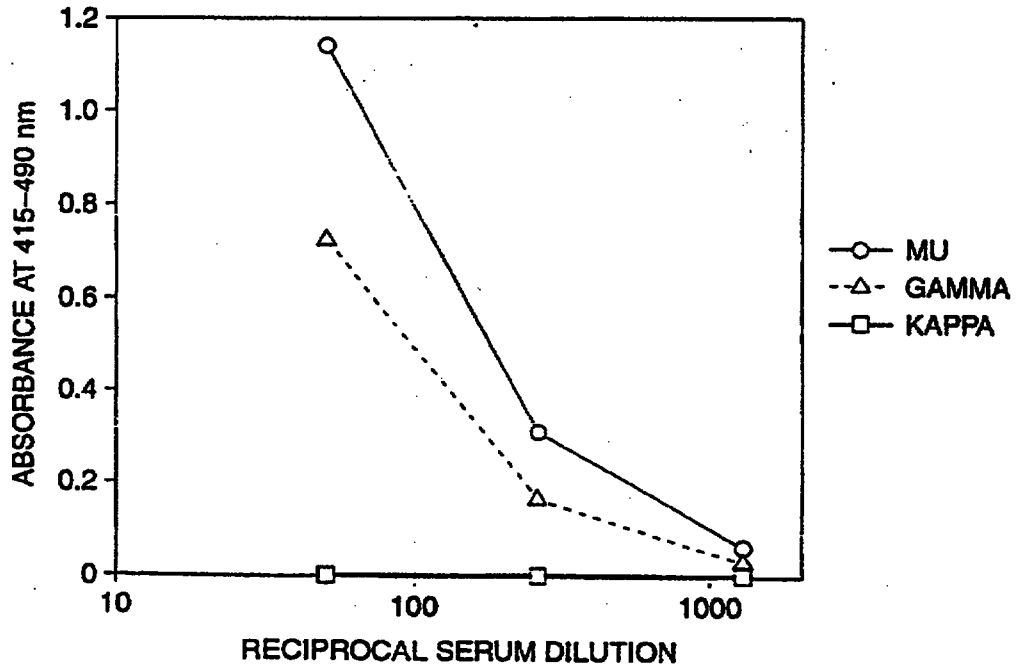
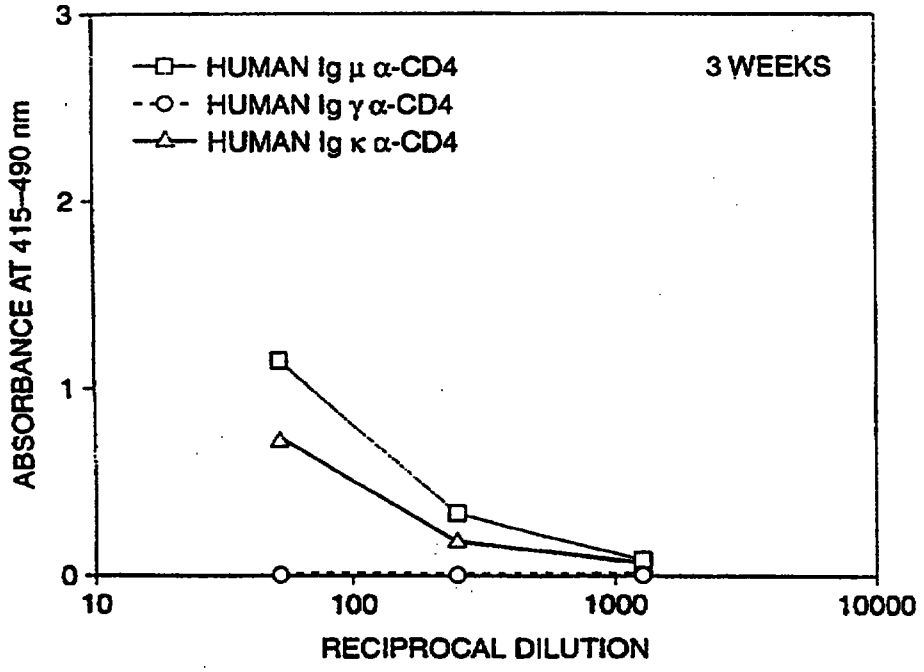

1.2
1.0
0.8
0.6
0.4
0.2
0
ABSORBANCE AT 415–490 nm
10
100
1000
RECIPROCAL SERUM DILUTION
MU
GAMMA
KAPPA

FIG. 55


HUMAN Ig μ α-CD4
HUMAN Ig γ α-CD4
HUMAN Ig κ α-CD4
3 WEEKS
ABSORBANCE AT 415–490 nm
RECIPROCAL DILUTION
0
1
2
3
10
100
1000
10000

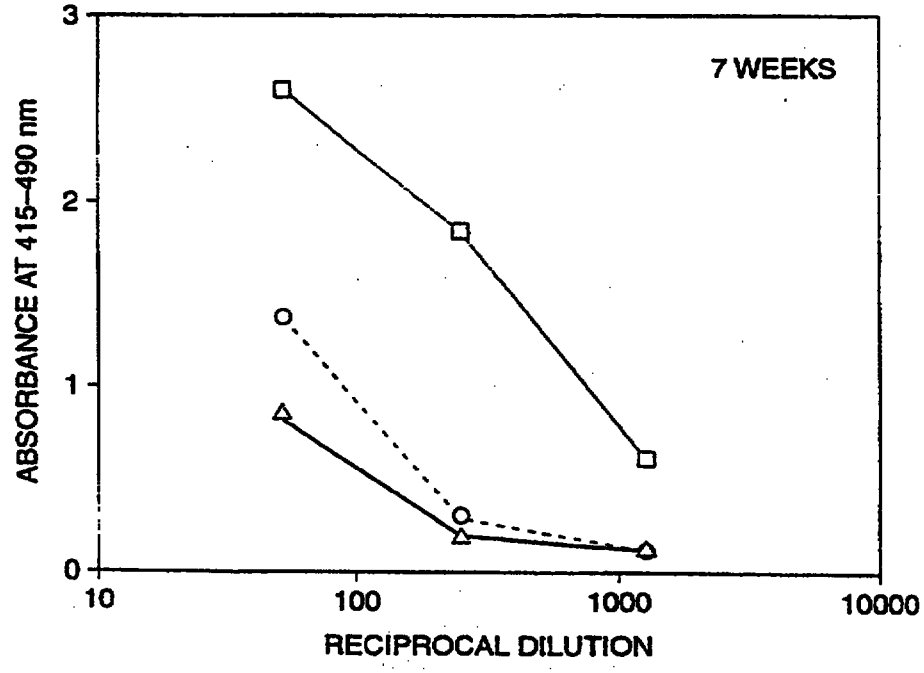

7 WEEKS
ABSORBANCE AT 415–490 nm
RECIPROCAL DILUTION
0
1
2
3
10
100
1000
10000

FIG. 56

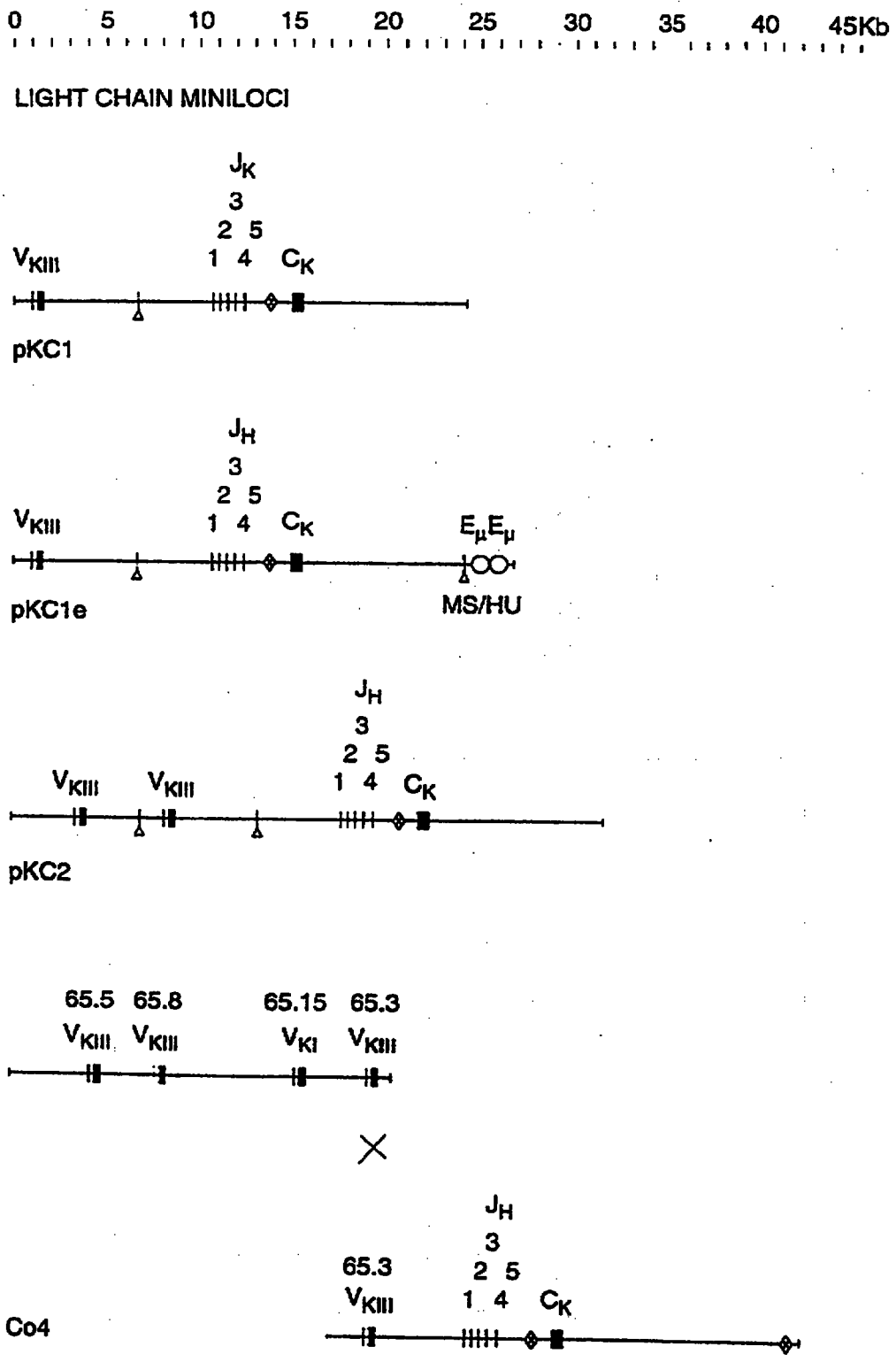

0 5 10 15 20 25 30 35 40 45Kb
LIGHT CHAIN MINILOCI
J_K
3
2 5
1 4
V_{KIII}
C_K
pKC1
J_H
3
2 5
1 4
V_{KIII}
C_K
$E_\mu E_\mu$
pKC1e
MS/HU
J_H
3
2 5
1 4
V_{KIII}
V_{KIII}
C_K
pKC2
65.5 65.8 65.15 65.3
V_{KIII} V_{KIII} V_{KI} V_{KIII}
J_H
3
2 5
1 4
65.3
V_{KIII}
C_K
Co4

FIG. 57A

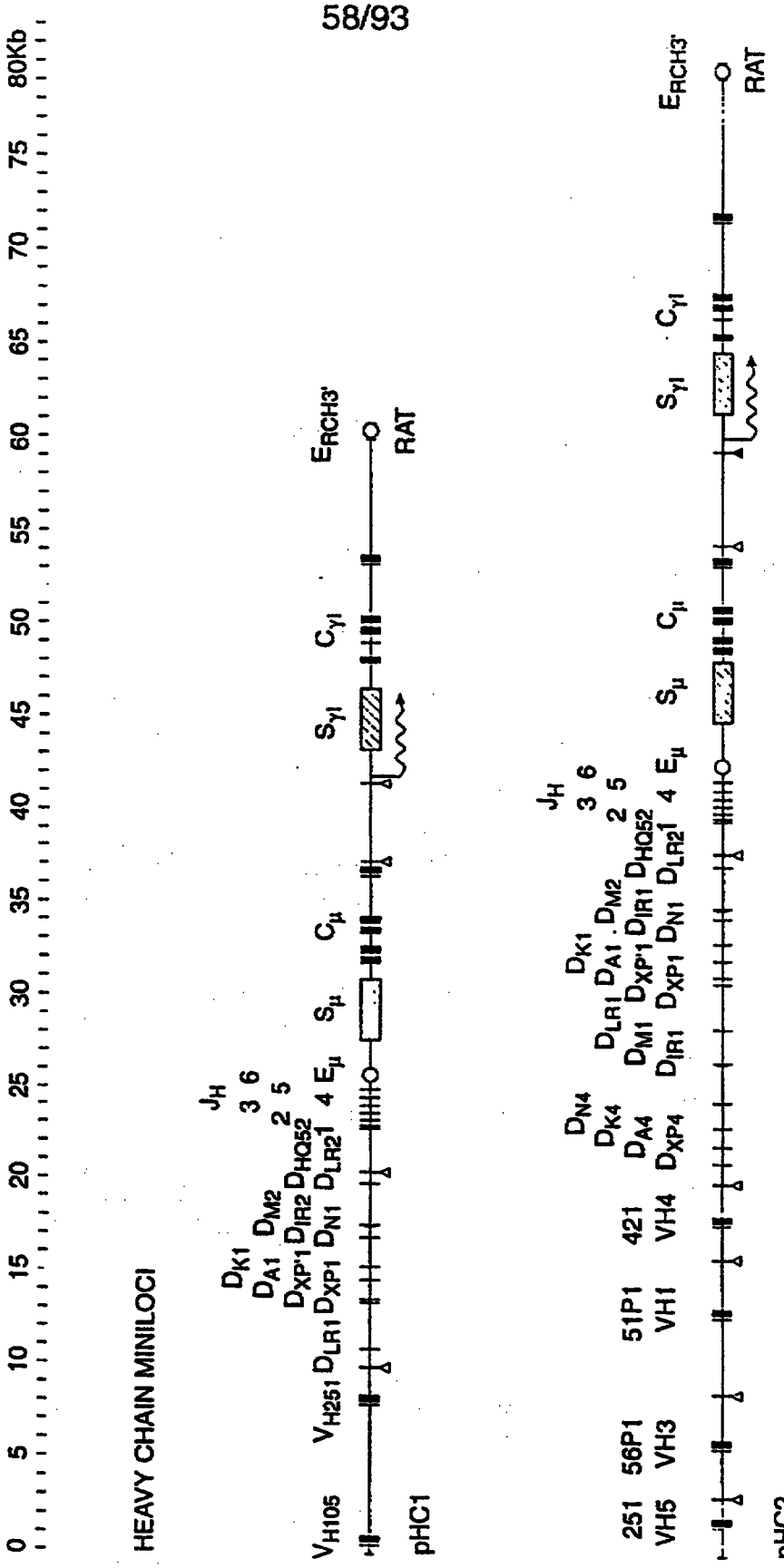

0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80Kb
HEAVY CHAIN MINILOCI
V_{H105} V_{H251} D_{LR1} D_{XP1} D_{K1} D_{A1} $D_{XP'1}$ D_{M2} D_{IR2} D_{N1} D_{HQ52} D_{LR2} J_H 3 6 2 5 4 E_μ S_μ C_μ $S_{\gamma 1}$ $C_{\gamma 1}$ $E_{RCH3'}$ RAT
pHC1
251 56P1 51P1 421 D_{N4} D_{K4} D_{A4} D_{LR1} D_{M1} D_{IR1} D_{K1} D_{A1} $D_{XP'1}$ D_{XP1} D_{M2} D_{IR1} D_{N1} D_{HQ52} D_{LR2} J_H 3 6 2 5 4 E_μ S_μ C_μ $S_{\gamma 1}$ $C_{\gamma 1}$ $E_{RCH3'}$ RAT
VH5 VH3 VH1 VH4 D_{XP4}
pHC2

FIG. 57B

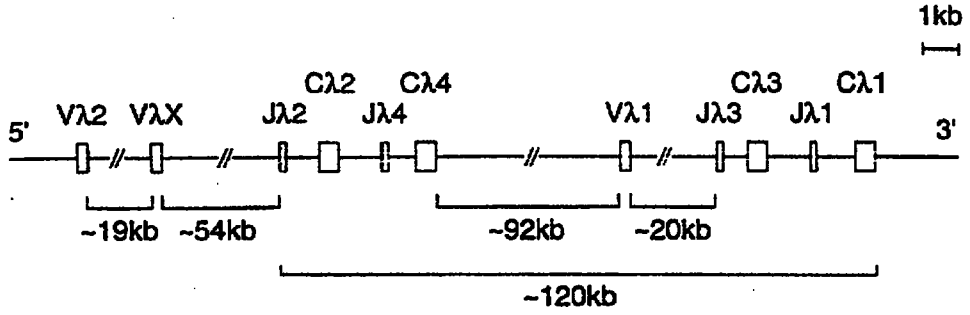

1kb
Cλ2
Cλ4
Cλ3
Cλ1
5'
Vλ2
VλX
Jλ2
Jλ4
Vλ1
Jλ3
Jλ1
3'
~19kb
~54kb
~92kb
~20kb
~120kb

FIG. 58

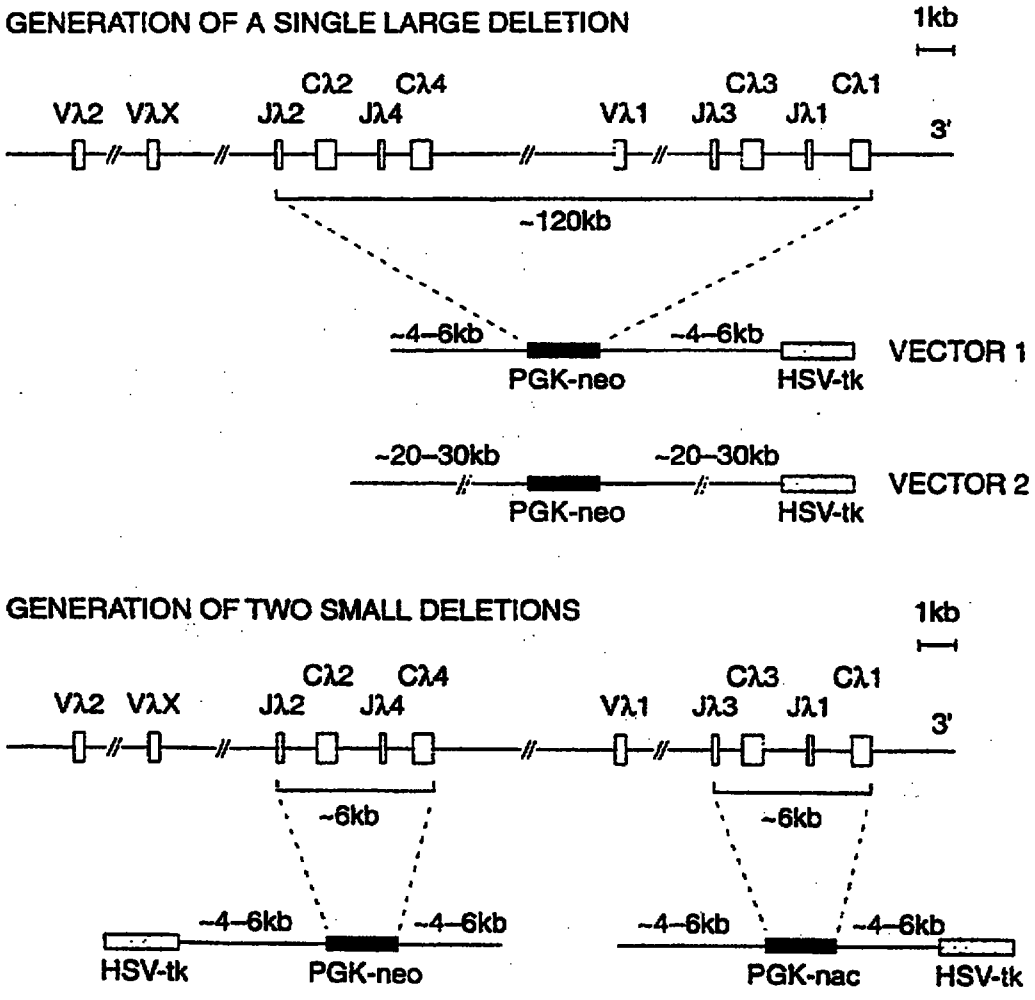

GENERATION OF A SINGLE LARGE DELETION
1kb
Cλ2
Cλ4
Cλ3
Cλ1
Vλ2
VλX
Jλ2
Jλ4
Vλ1
Jλ3
Jλ1
3'
~120kb
~4–6kb
~4–6kb
VECTOR 1
PGK-neo
HSV-tk
~20–30kb
~20–30kb
VECTOR 2
PGK-neo
HSV-tk
GENERATION OF TWO SMALL DELETIONS
1kb
Cλ2
Cλ4
Cλ3
Cλ1
Vλ2
VλX
Jλ2
Jλ4
Vλ1
Jλ3
Jλ1
3'
~6kb
~6kb
~4–6kb
~4–6kb
~4–6kb
~4–6kb
HSV-tk
PGK-neo
PGK-nac
HSV-tk

FIG. 59

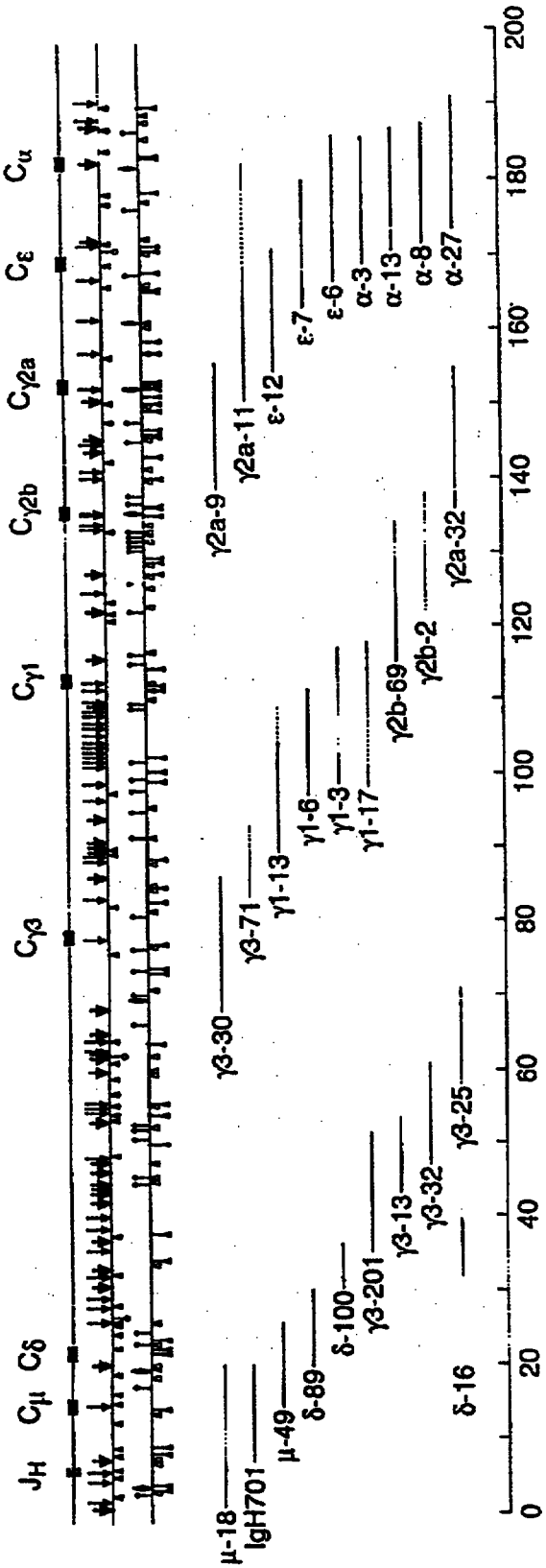

JH Cμ Cδ
Cγ3 Cγ1 Cγ2b Cγ2a Cε Cα
μ-18
IgH701
μ-49
δ-89
δ-100
γ3-201
γ3-13
γ3-32
δ-16
γ3-25
γ3-30
γ3-71
γ1-13
γ1-6
γ1-3
γ1-17
γ2b-69
γ2b-2
γ2a-32
γ2a-9
γ2a-11
ε-12
ε-7
ε-6
α-3
α-13
α-8
α-27
0 20 40 60 80 100 120 140 160 180 200
EcoR I
BamH I
Hind III
Sal I
Kpn I
Xho I
Xba I
Sac I

FIG. 60

CGAGAGGGGCGGGGGGAAGACTACTATCCCAGGCAGGTTTTAGGTTCCAGAGTCTGCGAG
AAATCCCACCATCTACCCACTGACACTCCCACCAGTCCTGTGCAGTGATCCCGTGATAAT
CGGCTGCCTGATTCACGATTACTTCCCTTTCGGCACGATGAATGTGACCTGGGGAAAGAG
TGGGAAGGATATAACCACCGTGAACTTTCCACCTGCCCTCGCCTCTGGGGGACGGTACAC
CATGAGCAGCCAGTTAACCCTGCCAGCTGTCGAGTGCCCAGAAGGAGAGTCCGTGAAATG
TTCCGTGCAACATGACTCTAACCCCGTCCAAGAATTGGATGTGAATTGCTCTGGTAAAGA
ACGTTAGGGGGTCAGCTAGGGGTGGGATAAGTCCTACCTTATCTAGATCCATATATCCCT
CTGATGCACACCCTCACAGGAATCCCTCAGAAACCTCCACTATGGGGATTGGGGGAAGGA
AGCGTAAACAGGTCTAGAAGGAGCTGGAGGCCTCAGAACATCCAGAAACGGGGACAGCAA
AGGAGACAAGGAGAATATACTGATTTGCTAGGACATCTTCTGTTACAGGTCCTACTCCTC
CTCCTCCTATTACTATTCCTTCCTGCCAGCCCAGCCTGTCACTGCAGCGGCCAGCTCTTG
AGGACCTGCTCCTGGGTTCAGATGCCAGCATCACATGTACTCTGAATGGCCTGAGAAATC
CTGAGGGAGCTGCTTTCACCTGGGAGCCCTCCACTGGGAAGGATGCAGTGCAGAAGAAAG
CTGCGCAGAATTCCTGCGGCTGCTACAGTGTGTCCAGCGTCCTGCCTGGCTGTGCTGAGC
GCTGGAACAGTGGCGCATCATTCAAGTGCACAGTTACCCATCCTGAGTCTGGCACCTTAA
CTGGCACAATTGCCAAAGTCACAGGTGAGCTCAGATGCATACCAGGACATTGTATGACGT
TCCCTGCTCACATGCCTGCTTTCTTCCTATAATACAGATGCTCAACTAACTGCTCATGTC
CTTATATCACAGAGGGAAATTGGAGCTATCTGAGGAACTGCCCAGAAGGGAAGGGCAGAG
GGGTCTTGCTCTCCTTGTCTGAGCCATAACTCTTCTTTCTACCTTCCAGTGAACACCTTC
CCACCCCAGGTCCACCTGCTACCGCCGCCGTCGGAGGAGCTGGCCCTGAATGAGCTCTTG
TCCCTGACATGCCTGGTGCGAGCTTTCAACCCTAAAGAAGTGCTGGTGCGATGGCTGCAT
GGAAATGAGGAGCTGTCCCCAGAAAGCTACCTAGTGTTTGAGCCCCTAAAGGAGCCAGGC
GAGGGAGCCACCACCTACCTGGTGACAAGCGTGTTGCGTGTATCAGCTGAAACCTGGAAA
CAGGGTGACCAGTACTCCTGCATGGTGGGCCACGAGGCCTTGCCCATGAACTTCACCCAG
AAGACCATCGACCGTCTGTCGGGTAAACCCACCAATGTCAGCGTGTCTGTGATCATGTCA
GAGGGAGATGGCATCTGCTACTGAGCCACCCTGCCTGTCCCTACTCCTAGAATAAACTCT
GTGCTCATCCAAAGTATCCCTGCACTTCCACCCAGTGCCTGTCCACCACCCTGGGGTCTA
CGAAACACAGGGAGGGGTCAGGGCCCAGGGAGGGAGAAATACCACCACCTAAGC

FIG. 61

70
80
82 a b c 83
90
ACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCCTGTGTATTACTGTGCGAGA
TACTGGTACTTCGATCTCTGGGGCCGTGGCACCCTGGTC
DXP'1
DHQS2
DXP'1
DXP1
DXP1
DK1
DK1
DXP'1
DXP'1
DA1/4
DHQS2
DHQS2
DHQS2
CDR III

FIG. 68B

```
                    20                  30                  40                  50  52 a  53                  60
VH56P1.GL  TCCCTGAGACTCTCCTGTGCAAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACTACGCAGACTCCGTGAAGGGCCGATTC
J2
5250.ln4   ....................................C.....................C.....G.....
5250.ln5   ....................................C.....................C.....G.....
5250.ln8   ...............A.A.G.A...A.....C.....................GC.....G.....G.....
5250.ln10  ...............A.A.G.A...A.....C.....................GC.....G.....G.....
5250.ln12  ....................................C.....A..G.....C.....T.G.....
5250.ln27  ...............A.A.G.A.C.A.....C.....T.....GC.....G.....
5250.ln115 ....................................C.....T.T.....C.....G.....
5250.ln118 ...............A...A.....C.....T.T.....C.....G.....
5250.ln202 ...............A.A.G.A...A.....C.....GC.....G.....
J4
5250.ln16  ....................................C.....
5250.ln19  ...............C....................C.....
5250.ln22  ....................................C.....
5250.sp26  ....................................C.....A.....T.....
5250.sp27  ...............A....................C.....
J6
5250.ln3   ....................................C.....
5250.ln24  ....................................C.....
5250.sp19  ...............A....................C.....
5250.sp22  ...............A....................G.....T.....C.A.....I.....
5250.sp28  ....................................C.....A.
VH51P1.GL  TCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGTTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTATAGCAAACTACGCACAGAAGTTCCAGGGCAGAGTC
J3
5250.sp33  ....................................C.....A.....I.....
VH4.21.GL  ACCCTGTCCCTCACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTACTGGAGCTGGATCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCAT  AGTGGAAGCACCAACTACAACCCGTCCCTCAAGAGTCGAGTC
J4
5250.ln2   ....................................
5250.sp30  ....................................
5250.sp32  ....................................
                    ____CDR I____                                                   ____CDR II____
```

FIG. 68A

70 80 82 a b c 83 90
ACCATCTCAGCCGACAAGTCCATCAGCACCGCCTACCTGCAGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGA
TACTGGTACTTCGATCTCTGGGGCCGTGGCACCCTGGTC
CAGGGGGGGGATA
GCTTTTGATATCTGGGGCCAAGGGACAATGGTC
DHQ52
CATTGGctaaAtggggaT
DXP1
CGGGattacgatatttttgactggttattatGCG
TACTTTGACTACTGGGGCCAGGGAACCCTGGTC
DXP'1
GGgtattaTtatgAttcgggggaCttattataaGTCTACCC
DHQ52
ctaactggCT
CATCTT
CAAGGG
CATCTT
CAAACT
DN1/DM2r
CATggtatagcagcagctggtacgtggttcGACCC
CAGGGC
CATCTT
DN1
GCCgggtataCcagcagctggtT
CATCTT
DXP'1
GtggttcggggaTttattatT
CAAAGGGG
GGATCGTGG
AACTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTC
DXP'1
CTtCCCAATGACAGT
CGGGGGtactatggttcgggggagttattat
TACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTC
DN1
CATGggcagctggtacAGGGT
GATATGGGGGGGCCTTC
COR III

FIG. 67B.2

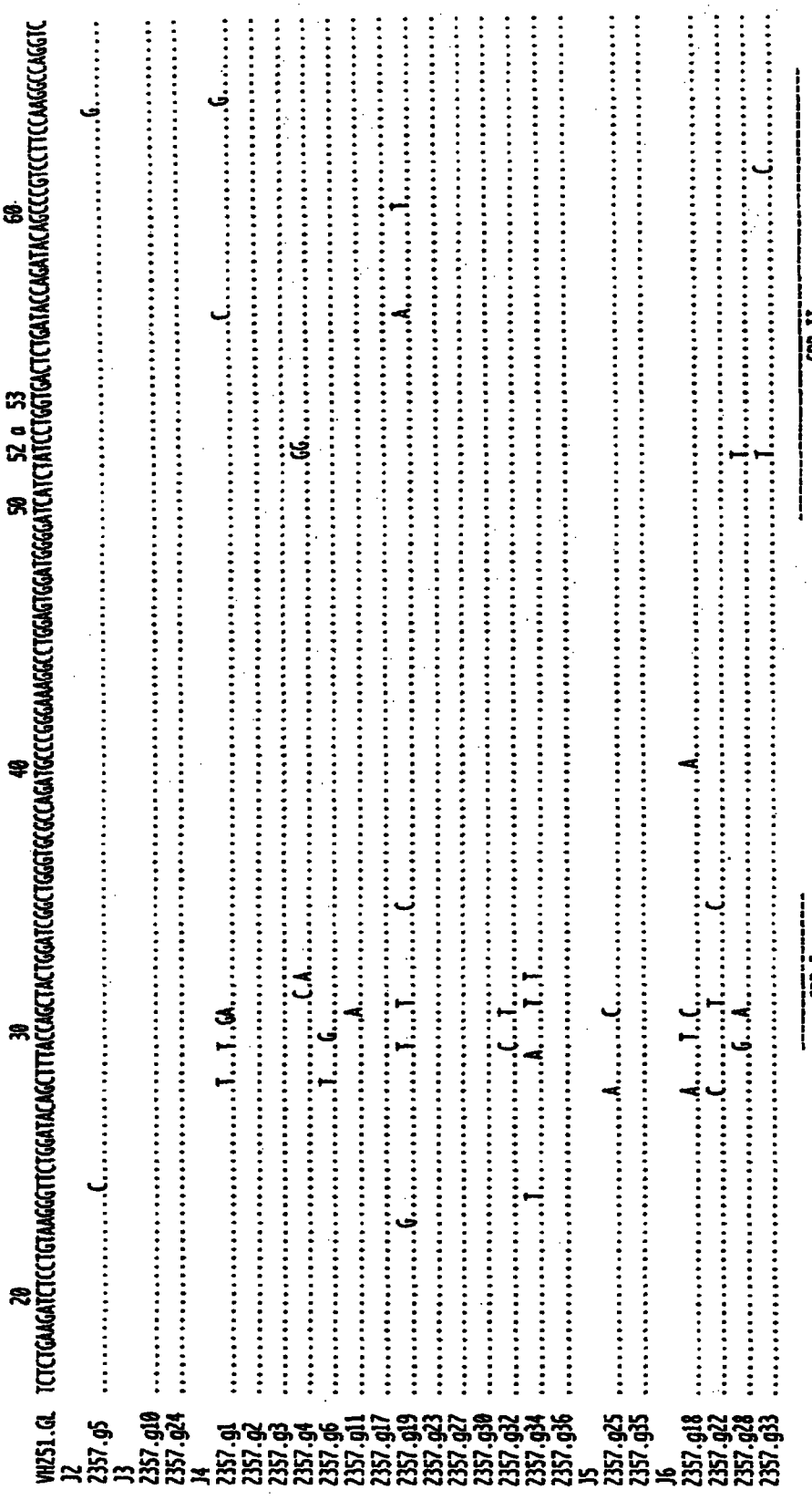

20
30
40
50
52 a 53
60
VH251.GL TCTCTGAAGATCTCCTGTAAGGGTTCTGGATACAGCTTTACCAGCTACTGGATCGGCTGGGTGCGCCAGATGCCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGATACAGCCCGTCCTTCCAAGGCCAGGTC
J2
2357.g5
J3
2357.g10
2357.g24
J4
2357.g1
2357.g2
2357.g3
2357.g4
2357.g6
2357.g11
2357.g17
2357.g19
2357.g23
2357.g27
2357.g30
2357.g32
2357.g34
2357.g36
J5
2357.g25
2357.g35
J6
2357.g18
2357.g22
2357.g28
2357.g33
CDR I
CDR II

FIG. 67B.1

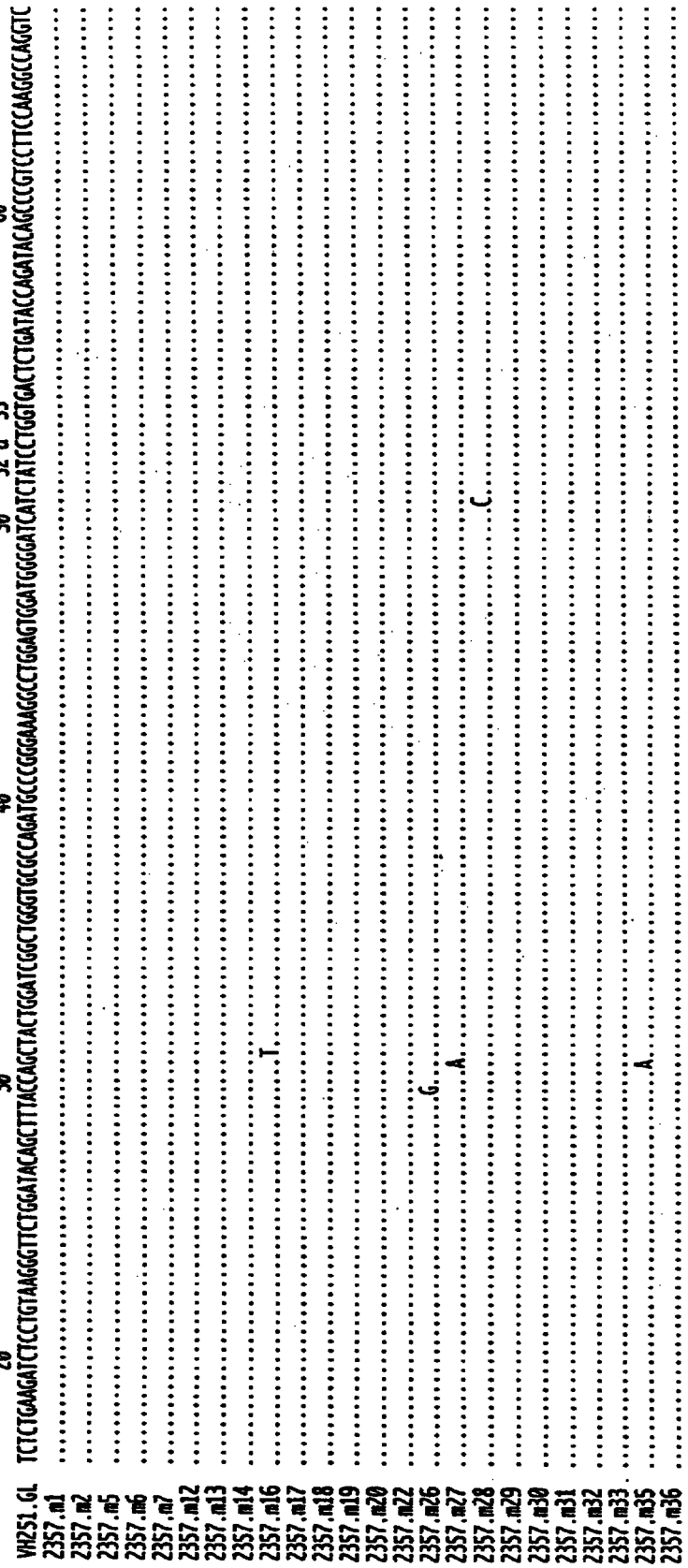

20
30
40
50
52 a 53
60
VH251.GL TCTCTGAAGATCTCCTGTAAGGGTTCTGGATACAGCTTTACCAGCTACTGGATCGGCTGGGTGCGCCAGATGCCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGATACAGCCCGTCCTTCCAAGGCCAGGTC
2357.m1
2357.m2
2357.m5
2357.m6
2357.m7
2357.m12
2357.m13
2357.m14
2357.m16
2357.m17
2357.m18
2357.m19
2357.m20
2357.m22
2357.m26
2357.m27
2357.m28
2357.m29
2357.m30
2357.m31
2357.m32
2357.m33
2357.m35
2357.m36
CDR I
CDR II

FIG. 67A

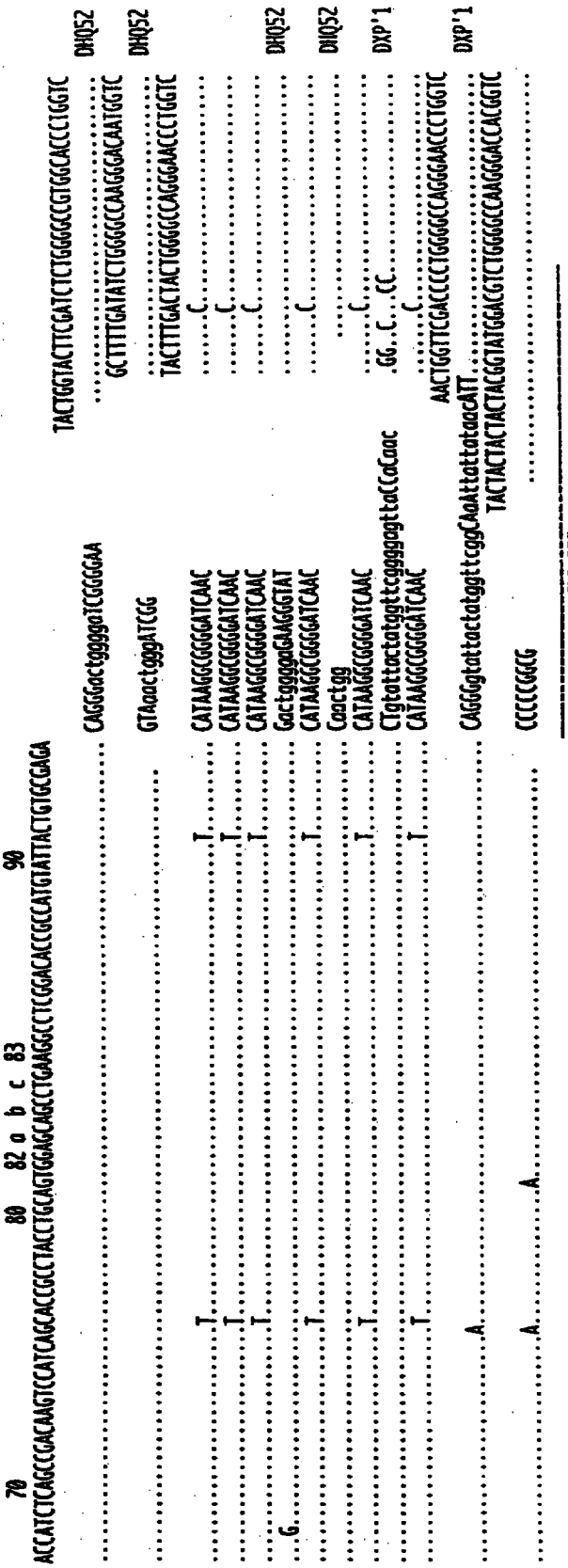

70
80 82 a b c 83 90
ACCATCTCAGCCGACAAGTCCATCAGCACCGCCTACCTGCAGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGA
CDR III
DHQ52
DXP'1

FIG. 66B.2

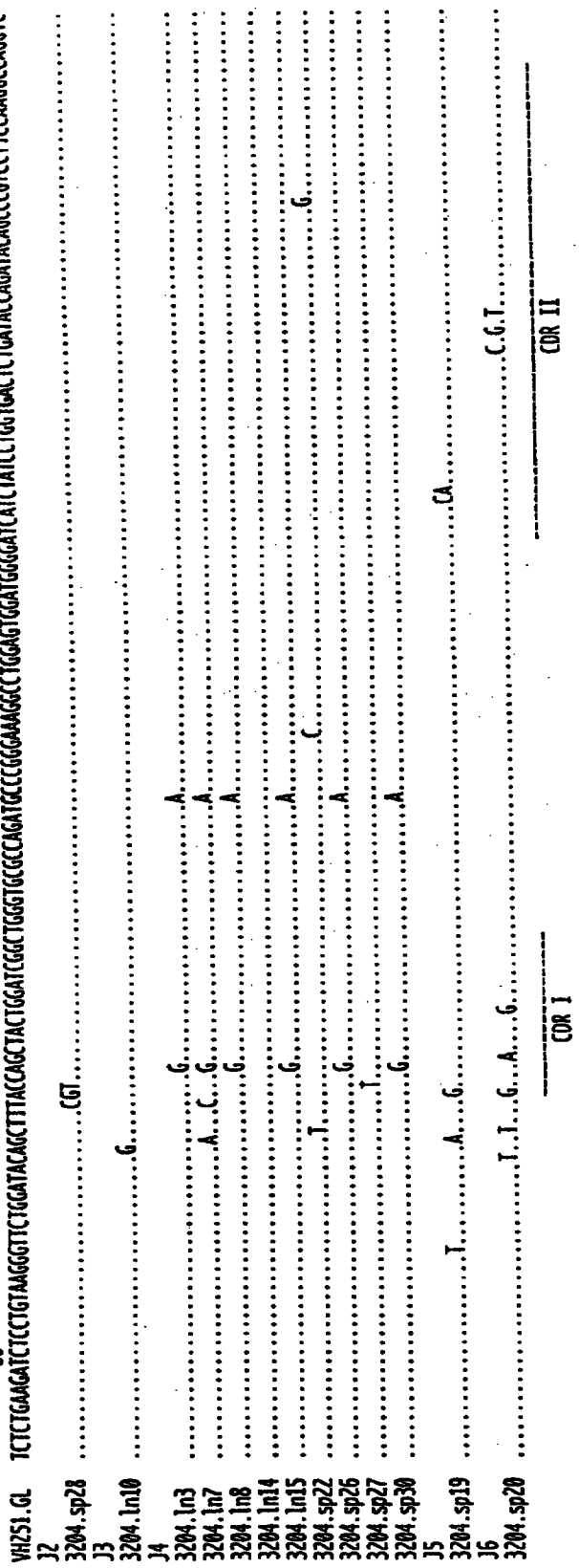

20
30
40
50
52 a 53
60
VH251.GL TCTCTGAAGATCTCCTGTAAGGGTTCTGGATACAGCTTTACCAGCTACTGGATCGGCTGGGTGCGCCAGATGCCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGATACAGCCCGTCCTTCCAAGGCCAGGTC
COR I
COR II

FIG. 66B.1

```
       70                                80  82 a b c 83                    90
ACCATCTCAGCCGACAAGTCCATCAGCACCGCCTACCTGCAGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGA
                                                                                                                      TACTGGTACTTCGATCTCTGGGGCCGTGGCAC
.........................................................................................  CAAGGGactggggaGG           ..............................  DHQ52
.........................................................................................  CAGAAGGGGGGAAGGG             ............................
.........................................................................................  CAGAAGGGGGGAAGGG             ............................
.........................................................................................  actgggTGG                ................................  DHQ52
                                                                                                                          GCTTTTGATATCTGGGGCCAAGGGAC
.........................................................................................  CATGAaactggggaTCCGGGG          ..........................  DHQ52
                                                                                                                          TACTTTGACTACTGGGGCCAGGGAAC
.........................................................................................  ACGGattacgatattttgactggttattataacCCT     ......................  DXP1
.........................................................................................  tatagcagcagcCCTT                         ......................  DN1
.........................................................................................  TCTCtactatggttcggggag                    ......................  DXP'1
.........................................................................................  CTCGattacgatattttgactggttattataacCC     .......................  DXP1
.......G.................................................................................  CTCGattacgatattttgactggttattataacCC     .......................  DXP1
.........................................................................................  CCCgtatagcagcagctggtacAGG                ......................  DN1
                                                                                                              TACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGAC
.........................................................................................  GAGGGGGGGGTTtattactatggttcggggagttatta ........................  DXP'1
.........................................................................................  GAGGGaactggggaCCAT       ....G.................................  DHQ52
                                                                                           ____________________________________________
                                                                                                           CDR III
```

FIG. 66A.2

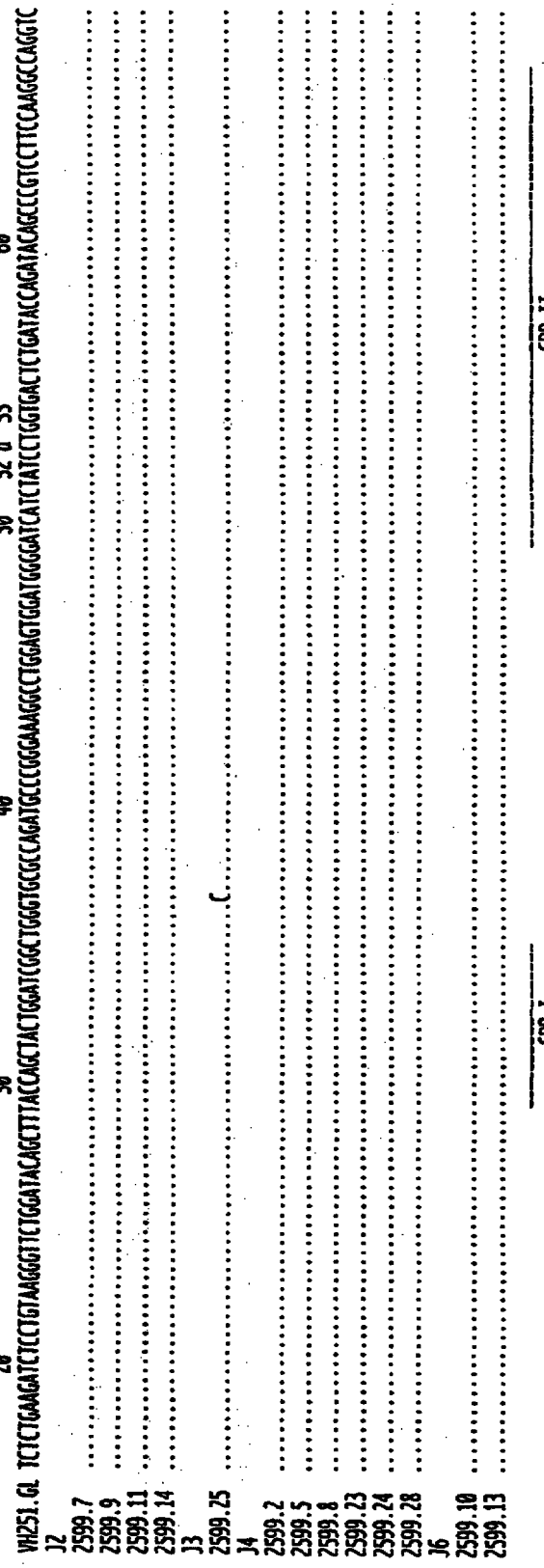

20
30
40
50
52 a 53
60
VH251.GL TCTCTGAAGATCTCCTGTAAGGGTTCTGGATACAGCTTTACCAGCTACTGGATCGGCTGGGTGCGCCAGATGCCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGATACAGCCCGTCCTTCCAAGGCCAGGTC
J2
2599.7
2599.9
2599.11
2599.14
J3
2599.25
J4
2599.2
2599.5
2599.8
2599.23
2599.24
2599.28
J6
2599.10
2599.13
COR I
COR II

FIG. 66A.1

	V_H251	N D N	J	C_γ	
2357.t5 DXP'1 J6	gcctcggacaccgccatgtattactgtgTgaga	CATTatggttcggggagttacG	cggtGtgAacgtctgggccaagggaccacggtcaccgtctcctcag	ccaaaacgacaccccatctgtctatccac	mouse γ1
2357.t7 DHQS2 J3	gcctcggacaccgccatgtattactgtgcgaga	CactggCATTGGAT	gctCttgatGtctgggccaagggacaatgCtcaccgtctcttcag	ccaaaacgacaccccatctgtctatccac	mouse γ2b
2357.t2 DHQS2 J3	gcctcggacaccgccatgtattactgtgcgaga	actggggaTGAT	gcttttgatatctgggccaagggacaatggtcaccgtctcttcag	ccaaaacaacaccccatcagtctatccac	
2357.t3 D? J3	gActcggacaccgccatgtattactgtgcgaga	CAGGGGAGAGAT	gctttagatatctgggccaagggacaatggtcaccgtctcttcag	ccaaaacaacaccccatcagtctatccac	
2357.t4 DXP'1 J4	gcctcggacaccgccatgtattaTtgtgTgaga	CATAGGGactotATttcgggggagttatttTTCC	tgactactggggccaagggacaatggtcaccgtcctcag	ccaaaacaacaccccatcagtctatccac	
2357.t10 DHQS2 J3	gcctcggacaccgccatgtattactgtgcgaga	actggggaTGAT	gcttttgatatctgggccaagggacaatggtcaccgtctcttcag	ccaaaacaacaccccatcagtctatccac	mouse γ3
2357.t1 D? J3	gcctcggacaccgccatgtattactgtgcgaga	CATGGGTCTATG	gatatctgggccaagggacaatggtcaccgtctcttcag	ctacaacaacagccccatctgtctatccct	
2357.t6 DHQS2 J4	gcctcggacaccgccatgtattactgtgcgaga	GAGAGCGGTCactgggggaTCG	tttgactaTtgggccaggggaaccctggtcaccgtctcctcag	ctacaacaacagccccatctgtctatccct	
2357.t8 DIR2 J3	gcctcggacaccgccatgtattactgtgcgaga	AGGgaccccccCTGAT	gcttttgatatctgggccaagggacaatggtcaccgtctcttcag	ctacaacaacagccccatctgtctatccct	
2357.t9 DIR2R J6	gcctcggacaccgccatgtattactgtgcgaga	CGgggggcct	tactactactacggtatggacgtctgggggccaagggaccacggtcaccgtctcctcag	ctacaacaacagccccatctgtctatccct	
			human		mouse

FIG. 65

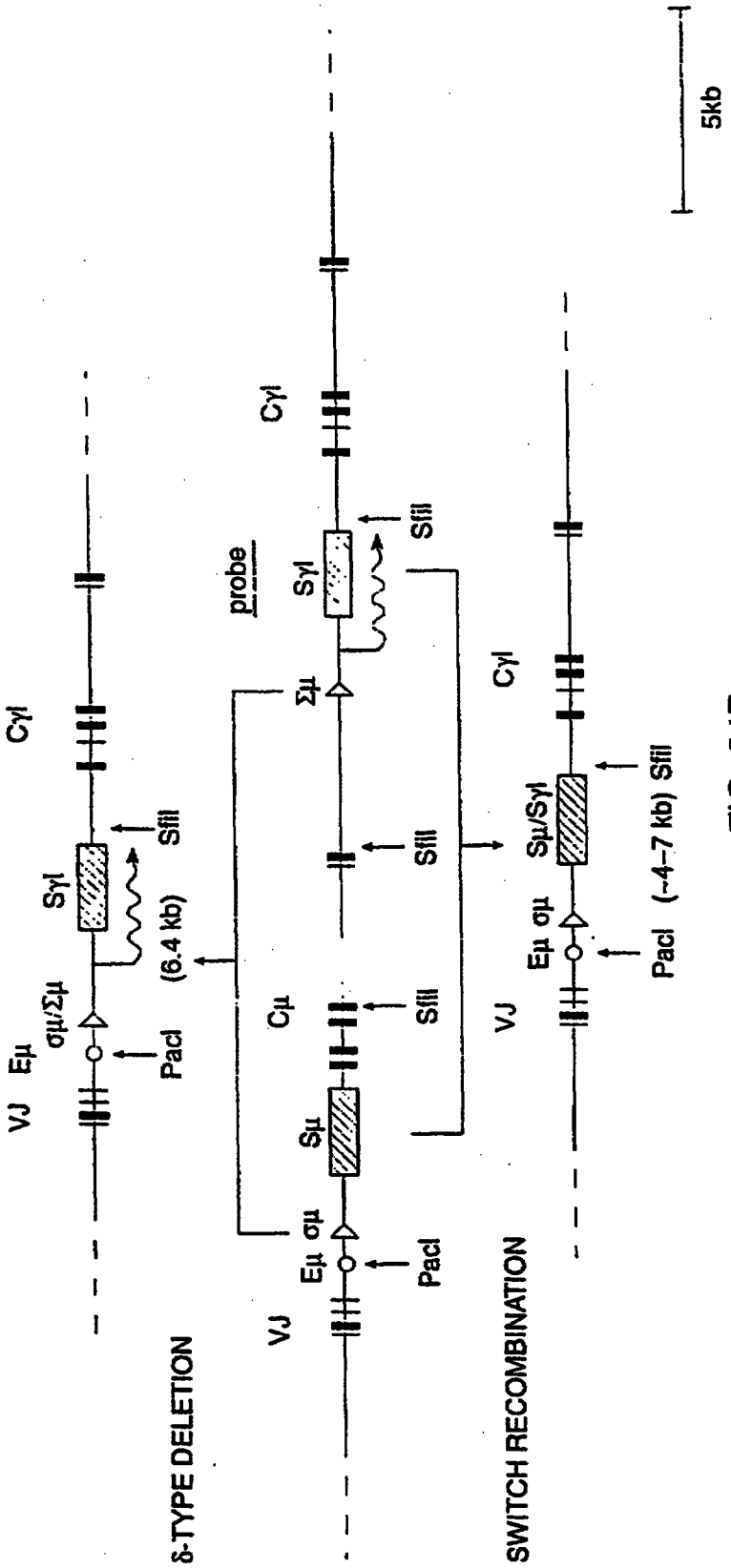

5kb
Cγl
Sγl
SfiI
probe
Σμ
Cμ
Sμ
Eμ σμ
PacI
VJ
δ-TYPE DELETION
Cγl
Sγl
SfiI
(6.4 kb)
σμ/Σμ
Eμ
PacI
VJ
Cγl
Sμ/Sγl
SfiI
Eμ σμ
PacI (~4–7 kb)
VJ
SWITCH RECOMBINATION

FIG. 64B

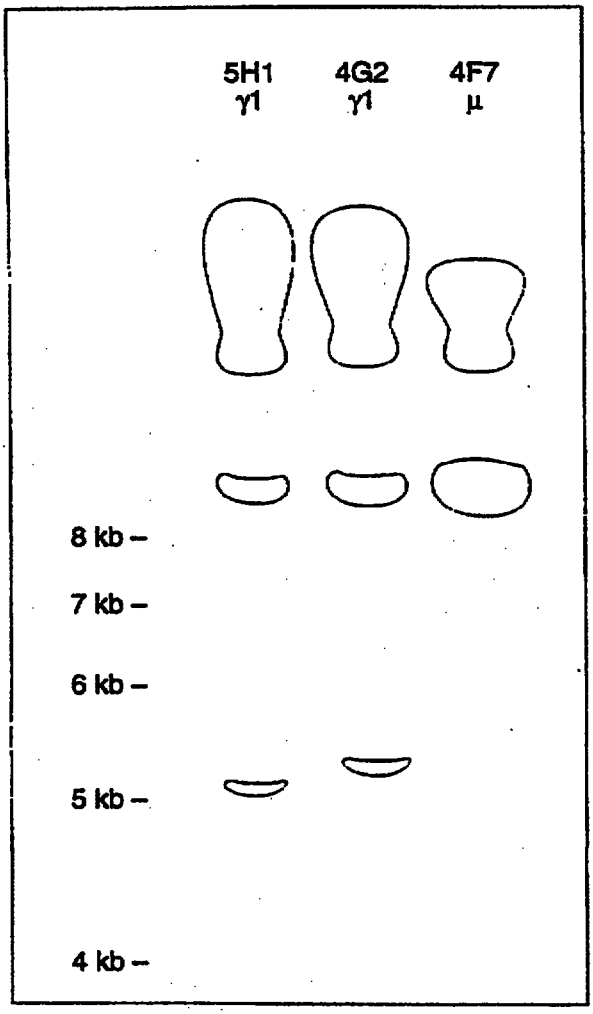


FIG. 64A

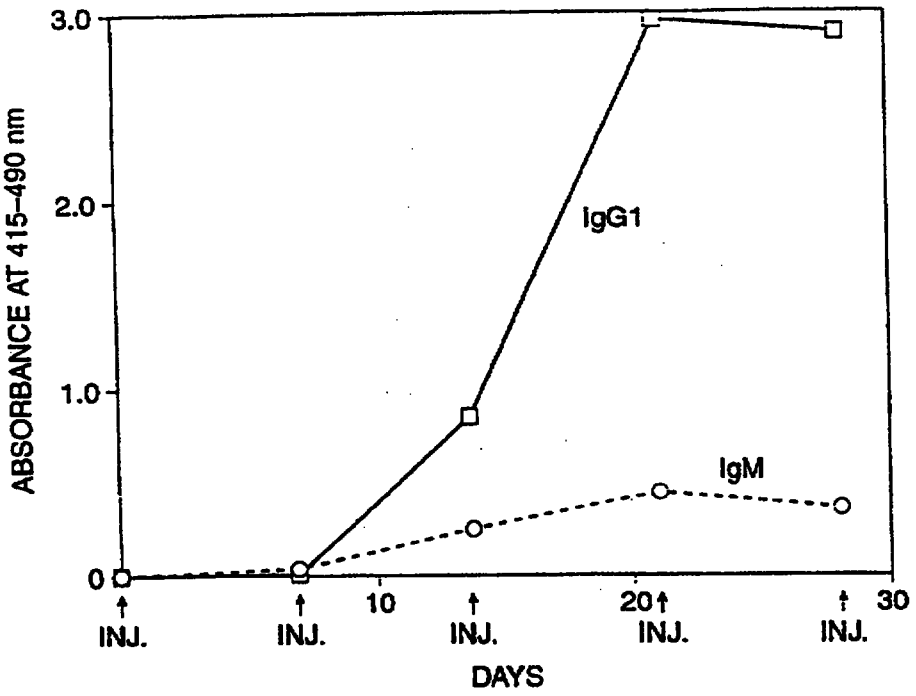

3.0
2.0
1.0
0
ABSORBANCE AT 415–490 nm
IgG1
IgM
INJ.
INJ.
10
INJ.
20
INJ.
30
INJ.
DAYS

FIG. 63

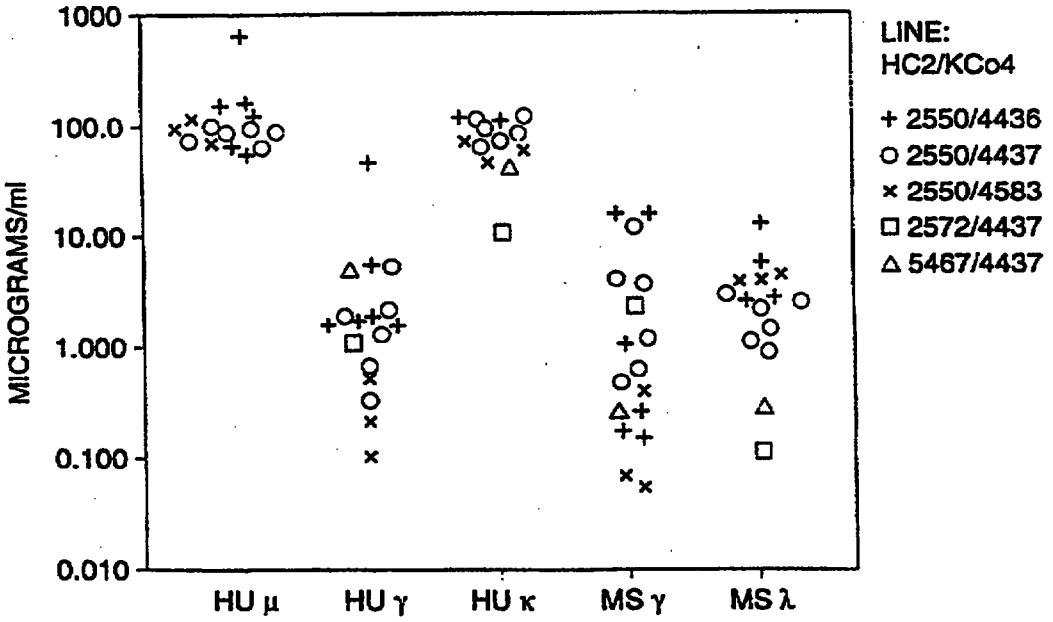

1000
100.0
10.00
1.000
0.100
0.010
MICROGRAMS/ml
HU μ
HU γ
HU κ
MS γ
MS λ
LINE:
HC2/KCo4
+ 2550/4436
○ 2550/4437
× 2550/4583
□ 2572/4437
△ 5467/4437

FIG. 70

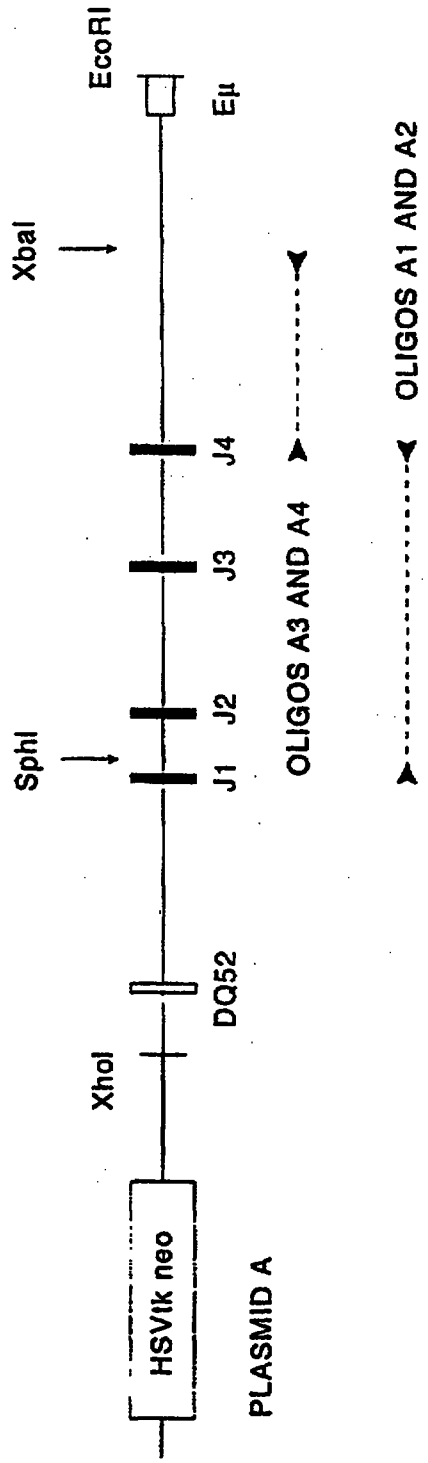

EcoRI
Eμ
XbaI
J4
J3
J2
J1
SphI
DQ52
XhoI
HSVtk neo
PLASMID A
OLIGOS A3 AND A4
OLIGOS A1 AND A2
EcoRI
Eμ
XbaI
KpnI
SphI
DQ52
XhoI
HSVtk neo
PLASMID B

FIG. 62

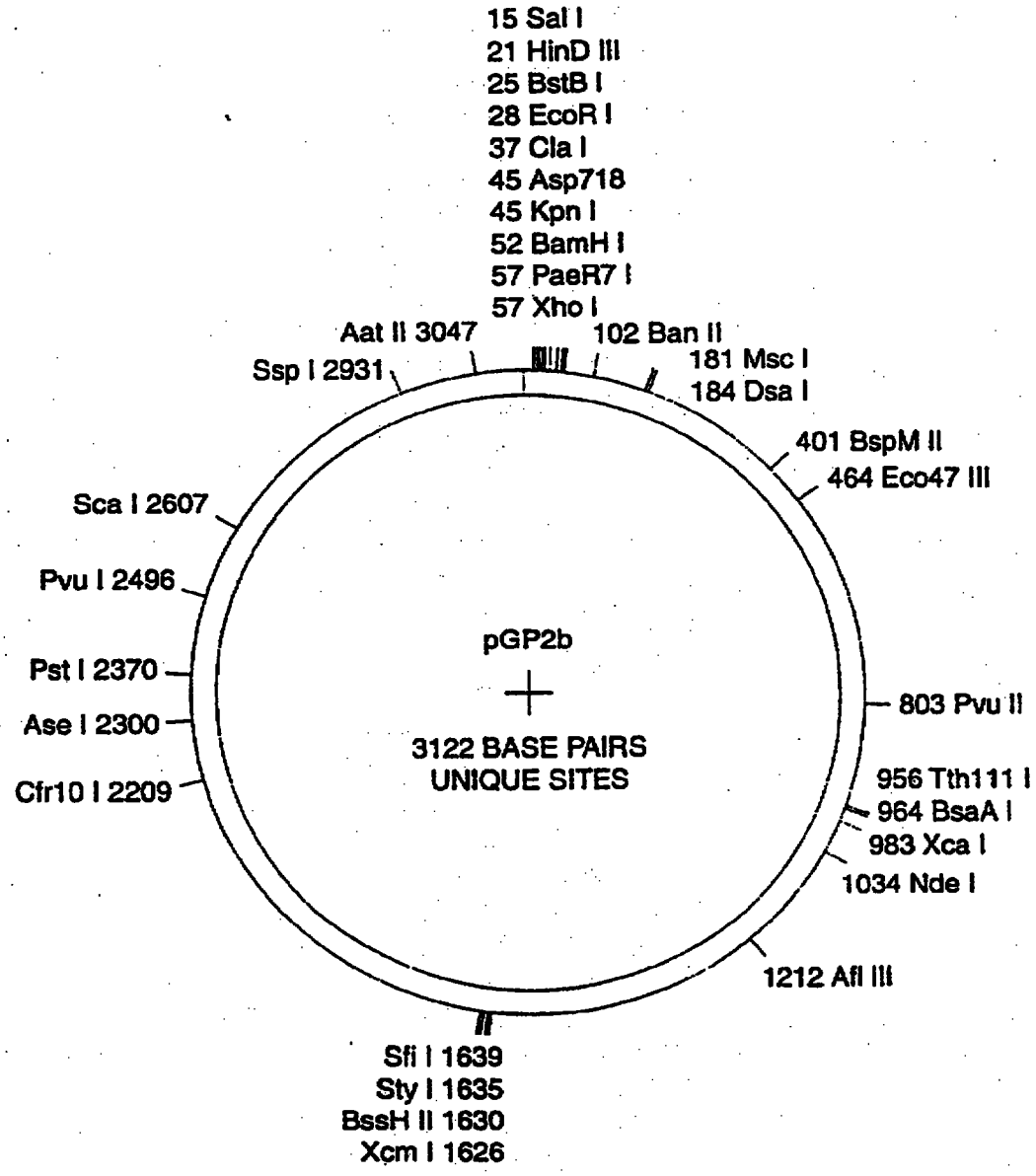

15 Sal I
21 HinD III
25 BstB I
28 EcoR I
37 Cla I
45 Asp718
45 Kpn I
52 BamH I
57 PaeR7 I
57 Xho I
Aat II 3047
102 Ban II
181 Msc I
184 Dsa I
Ssp I 2931
401 BspM II
464 Eco47 III
Sca I 2607
Pvu I 2496
pGP2b
Pst I 2370
Ase I 2300
3122 BASE PAIRS
UNIQUE SITES
803 Pvu II
Cfr10 I 2209
956 Tth111 I
964 BsaA I
983 Xca I
1034 Nde I
1212 Afl III
Sfi I 1639
Sty I 1635
BssH II 1630
Xcm I 1626

FIG. 77B

pGP2b sequence:

AATTAGCggccgctgtcgacaagcttcgaattcagtatcgatgtggtacctggatcctcgagtgcGGCCGCAGTATGCAA
AAAAAAGCCCGCTCATTAGGCGGGCTCTTGGCAGAACATATCCATCGCGTCCGCCATCTCCAGCAGCCGCACGCGGCGCA
TCTCGGGCAGCGTTGGGTCCTGGCCACGGGTGCGCATGATCGTGCTCCTGTCGTTGAGGACCCGGCTAGGCTGGCGGGGT
TGCCTTACTGGTTAGCAGAATGAATCACCGATACGCGAGCGAACGTGAAGCGACTGCTGCTGCAAAACGTCTGCGACCTG
AGCAACAACATGAATGGTCTTCGGTTTCCGTGTTTCGTAAAGTCTGGAAACGCGGAAGTCAGCGCCCTGCACCATTATGT
TCCGGATCTGCATCGCAGGATGCTGCTGGCTACCCTGTGGAACACCTACATCTGTATTAACGAAGCGCTGGCATTGACCC
TGAGTGATTTTTCTCTGGTCCCGCCGCATCCATACCGCCAGTTGTTTACCCTCACAACGTTCCAGTAACCGGGCATGTTC
ATCATCAGTAACCCGTATCGTGAGCATCCTCTCTCGTTTCATCGGTATCATTACCCCCATGAACAGAAATTCCCCCTTAC
ACGGAGGCATCAAGTGACCAAACAGGAAAAAACCGCCCTTAACATGGCCCGCTTTATCAGAAGCCAGACATTAACGCTTC
TGGAGAAACTCAACGAGCTGGACGCGGATGAACAGGCAGACATCTGTGAATCGCTTCACGACCACGCTGATGAGCTTTAC
CGCAGCTGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCGCAGCCATGACCC
AGTCACGTAGCGATAGCGGAGTGTATACTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGC
GGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTG
CGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAA
CGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATA
GGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAC
CAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCT
CCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGG
GCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGA
CACGACTTATCGCCACTGGCAGCAGCCAggcgcgccttggcctaagaggccaCTGGTAACAGGATTAGCAGAGCGAGGTA
TGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTC
TGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTT
TTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGC
TCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT
AAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCA
CCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGG
CTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGC
CAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCT
AGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTGCAGGCATCGTGGTGTCACGCTCGTCGTT
TGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTA
GCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAAT
TCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTAT
GCGGCGACCGAGTTGCTCTTGCCCGGCGTCAACACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCA
TTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCA
CCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAA
GGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATT
GTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTG
CCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCA
AG

FIG. 77A

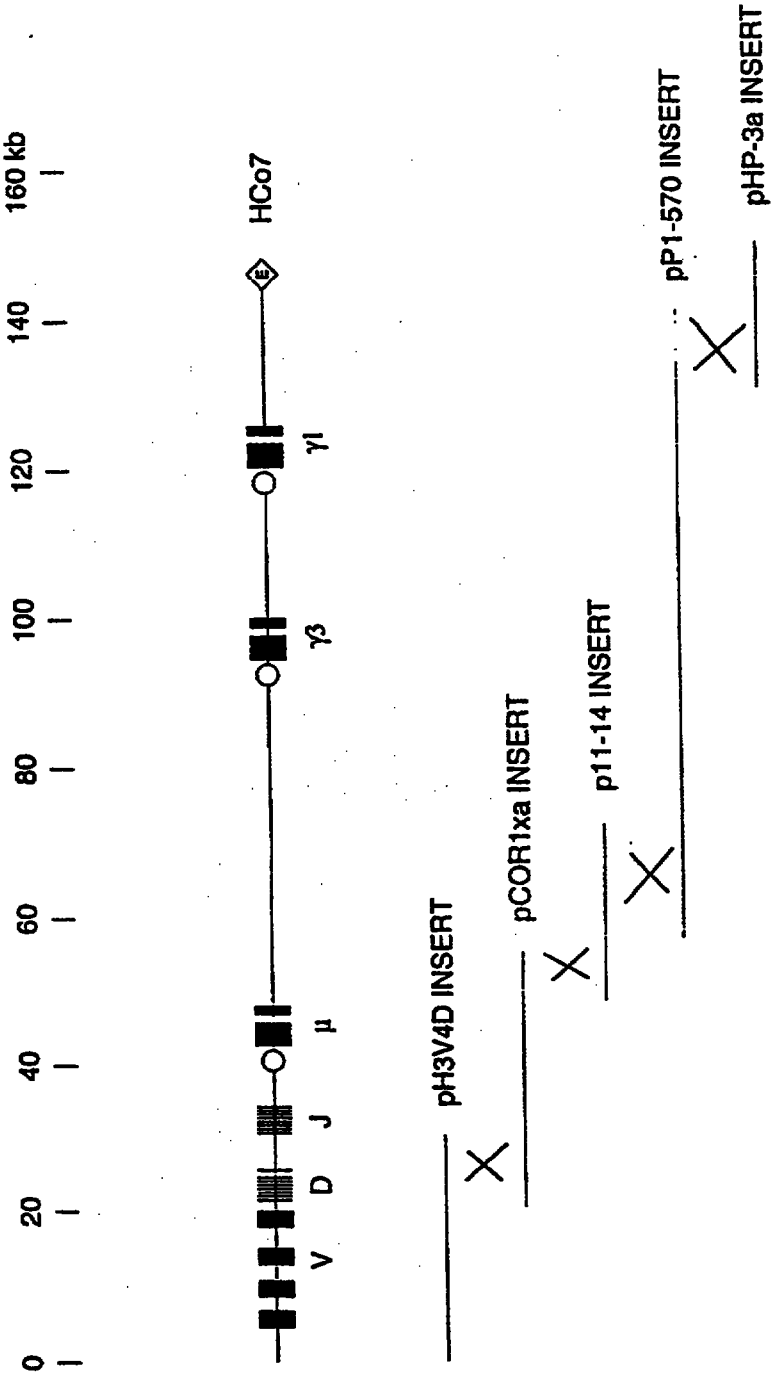
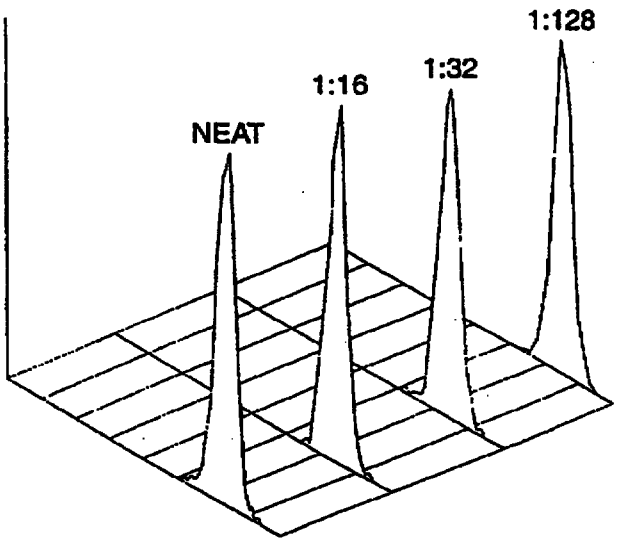

0
20
40
60
80
100
120
140
160 kb
HCo7
E
V
D
J
μ
γ3
γ1
pH3V4D INSERT
pCOR1xa INSERT
p11-14 INSERT
pP1-570 INSERT
pHP-3a INSERT

FIG. 76

RPA-T4/2C11-8
#12:BDPHARMCOMP004\FL2-H\FL2-HEIGHT



Leu-3a/2C11-8
#12:BDPHARMCOMP025\FL2-H\FL2-HEIGHT

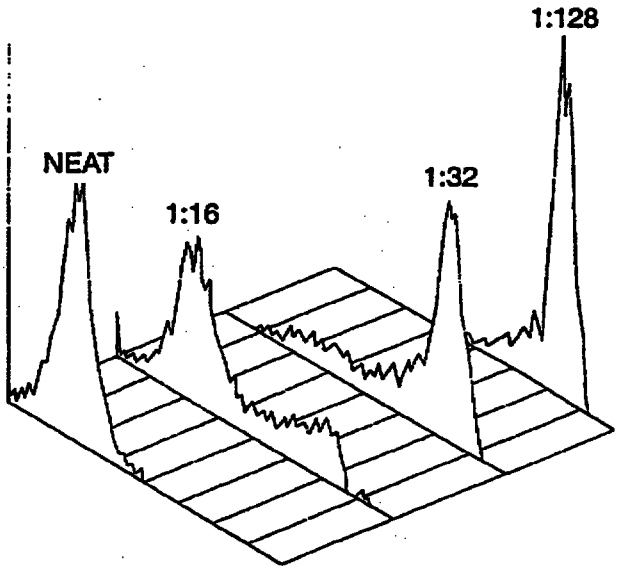
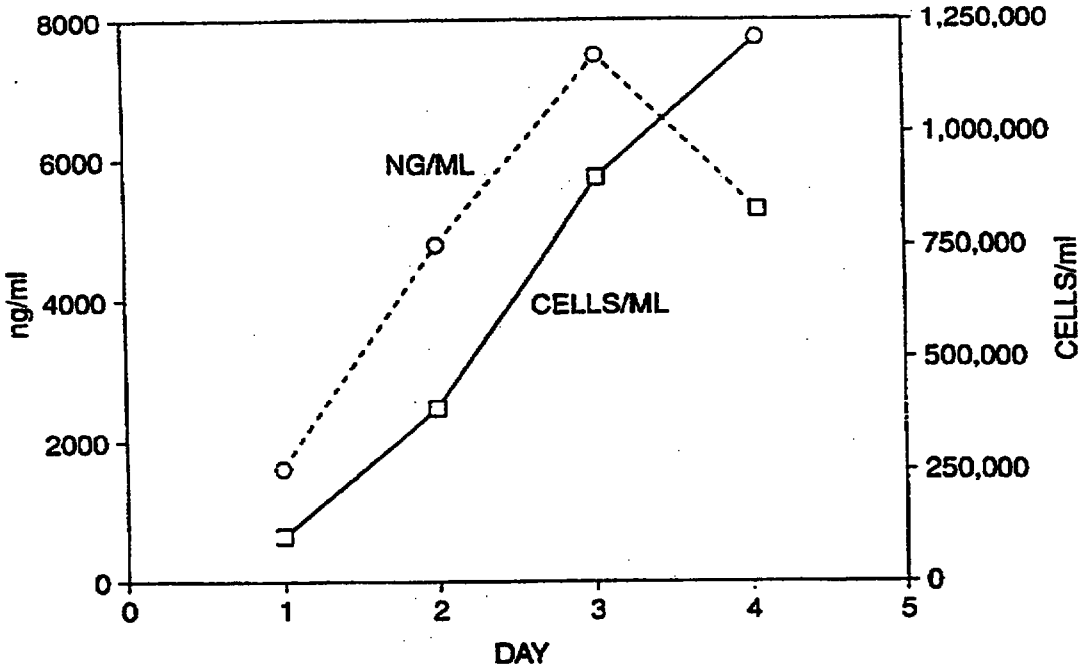


FIG. 75


8000
6000
4000
2000
0
ng/ml
NG/ML
CELLS/ML
1,250,000
1,000,000
750,000
500,000
250,000
0
CELLS/ml
0
1
2
3
4
5
DAY

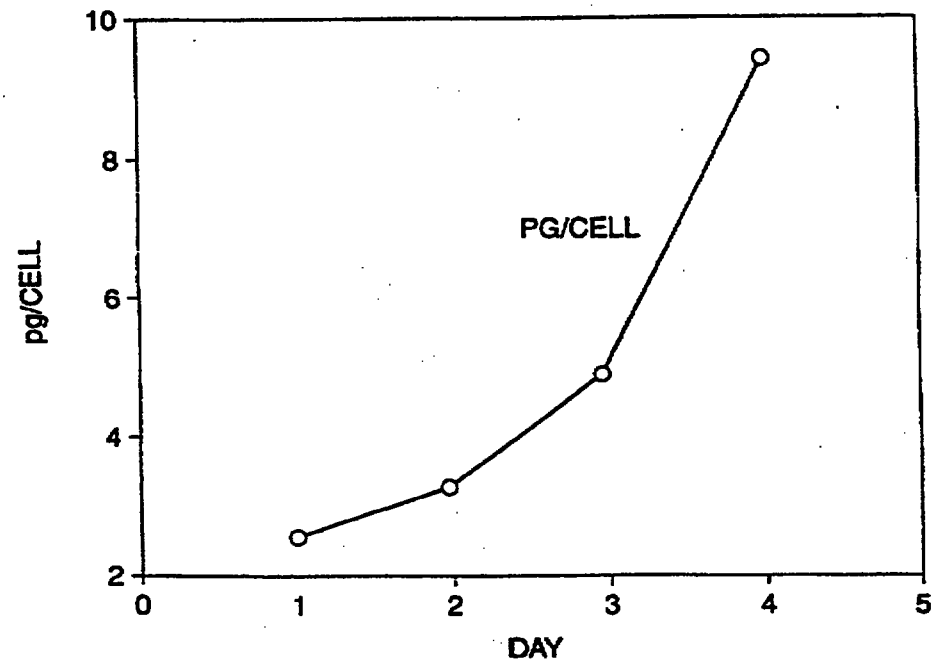

10
8
6
4
2
pg/CELL
PG/CELL
0
1
2
3
4
5
DAY

FIG. 74

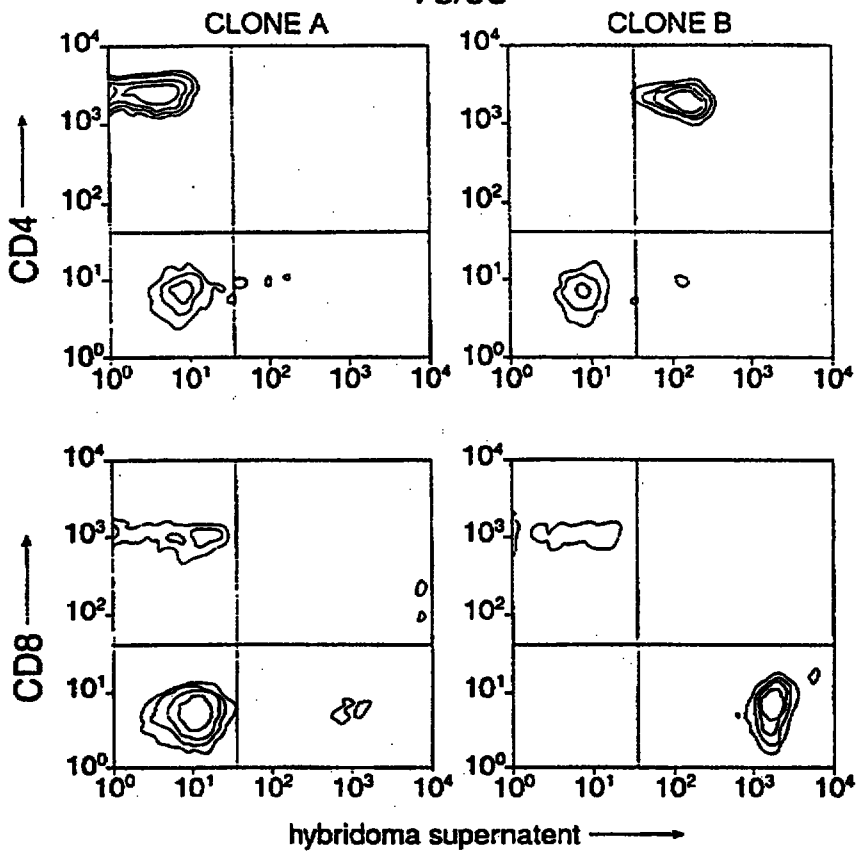

CLONE A
CLONE B
CD4
CD8
hybridoma supernatent
10^0
10^1
10^2
10^3
10^4

FIG. 72

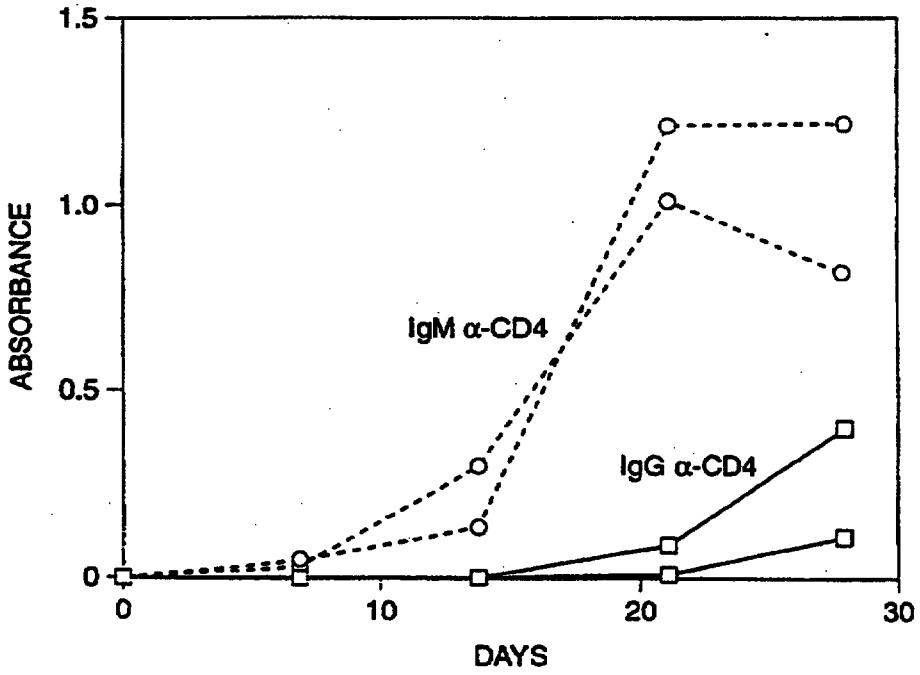

ABSORBANCE
1.5
1.0
0.5
0
IgM α-CD4
IgG α-CD4
0
10
20
30
DAYS

FIG. 73

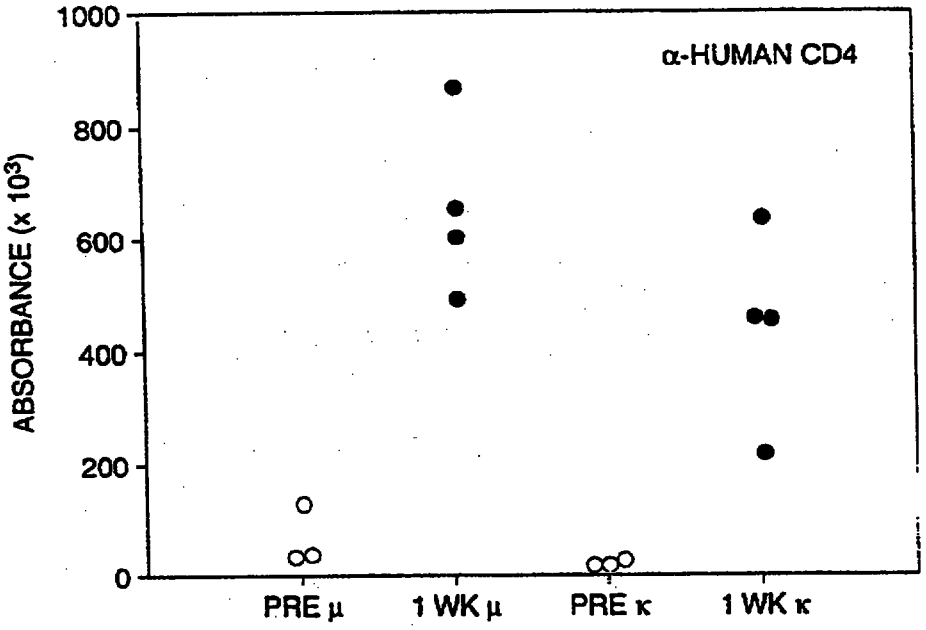


FIG. 71A

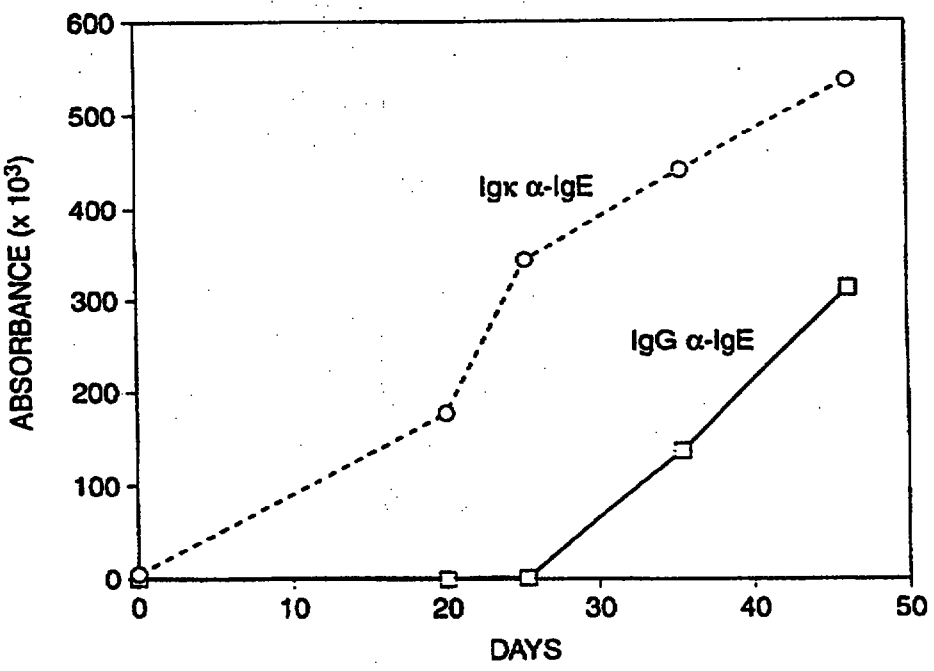


FIG. 71B

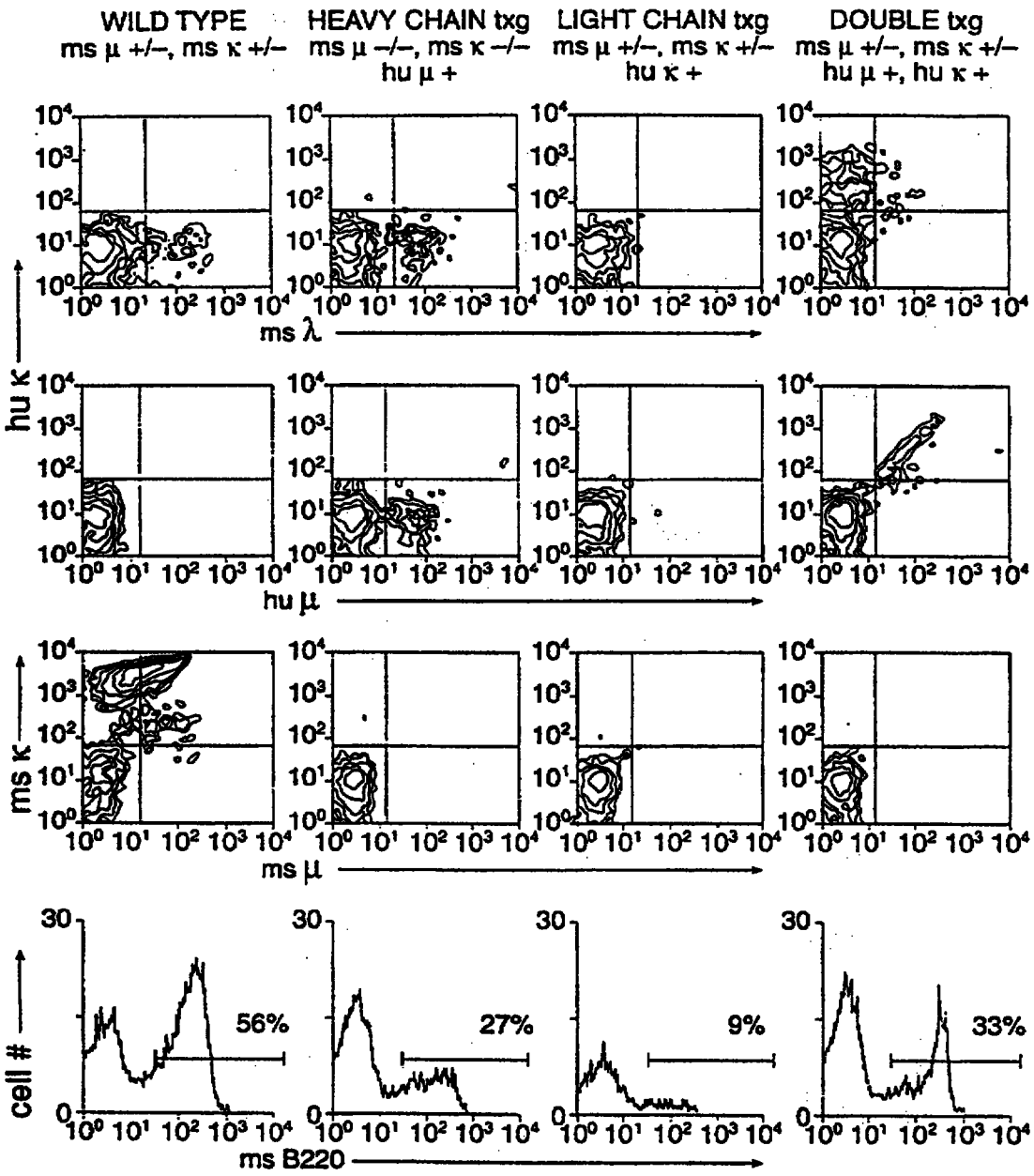

WILD TYPE
ms μ +/–, ms κ +/–
HEAVY CHAIN txg
ms μ –/–, ms κ –/–
hu μ +
LIGHT CHAIN txg
ms μ +/–, ms κ +/–
hu κ +
DOUBLE txg
ms μ +/–, ms κ +/–
hu μ +, hu κ +
hu κ
ms λ
hu μ
ms κ
ms μ
cell #
ms B220
56%
27%
9%
33%

FIG. 69

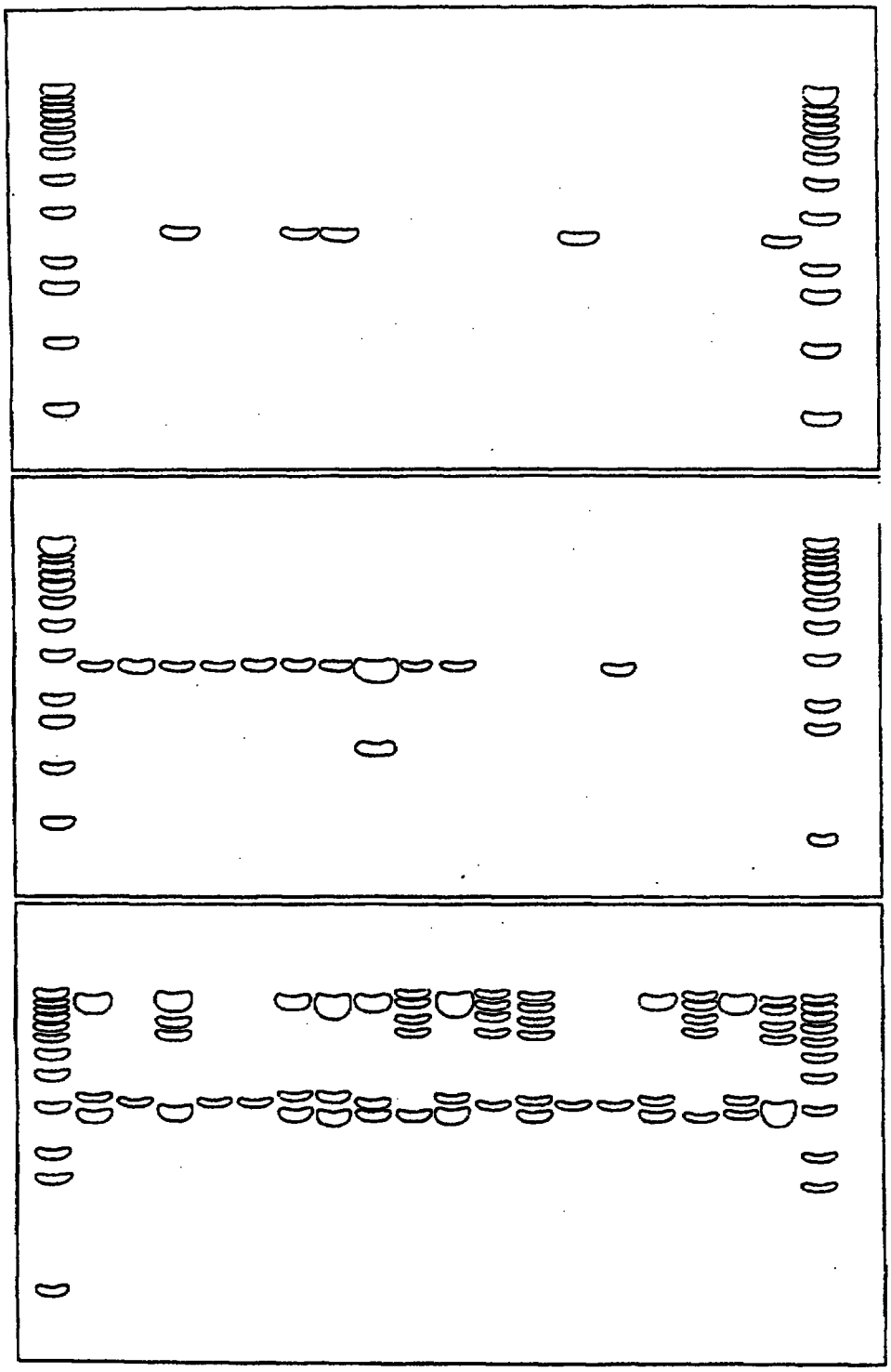


FIG. 79

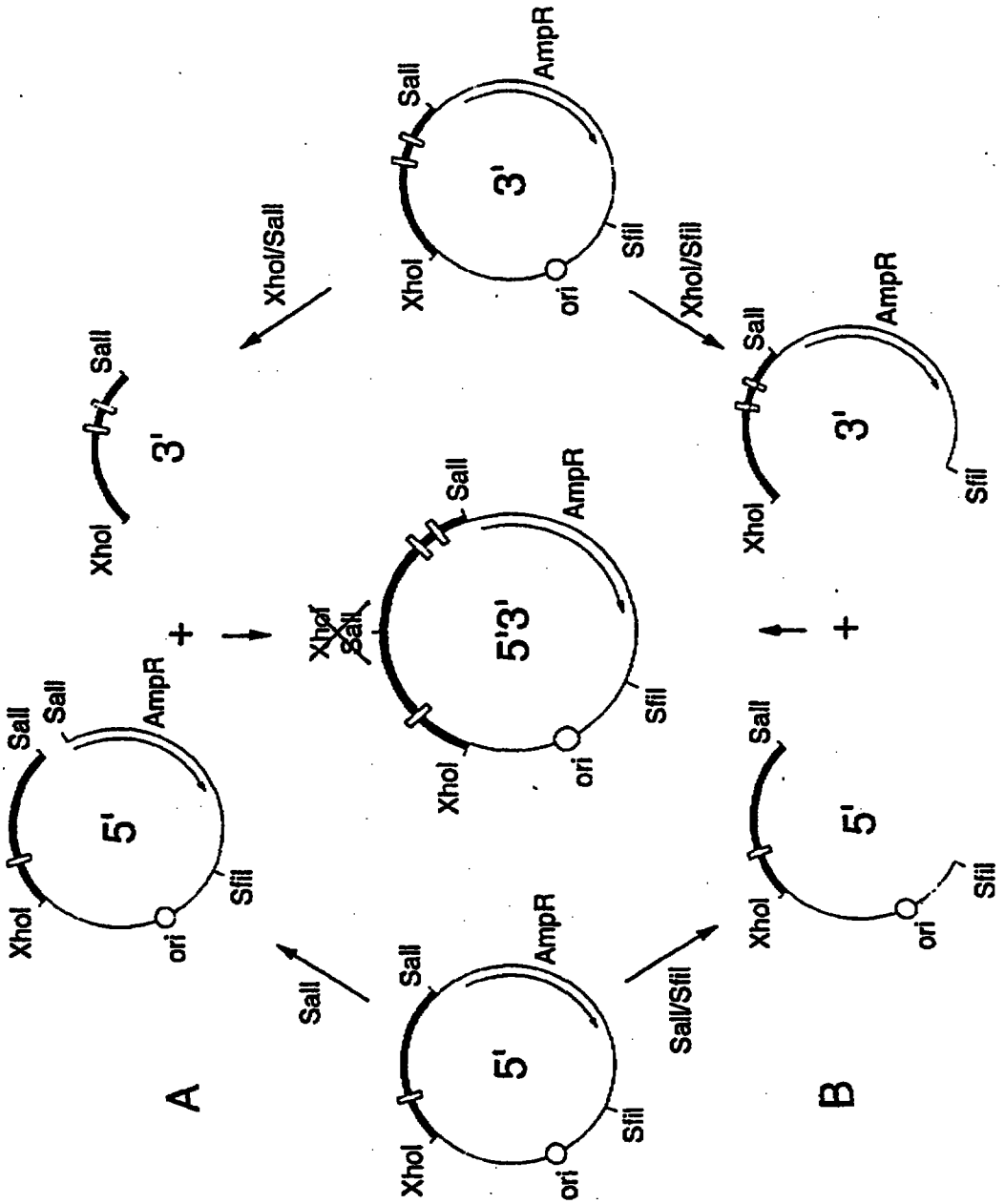

A
B
5'
3'
5'3'
XhoI
SalI
AmpR
SfiI
ori
XhoI/SalI
XhoI/SfiI
SalI/SfiI
XhoI
SalI

FIG. 78

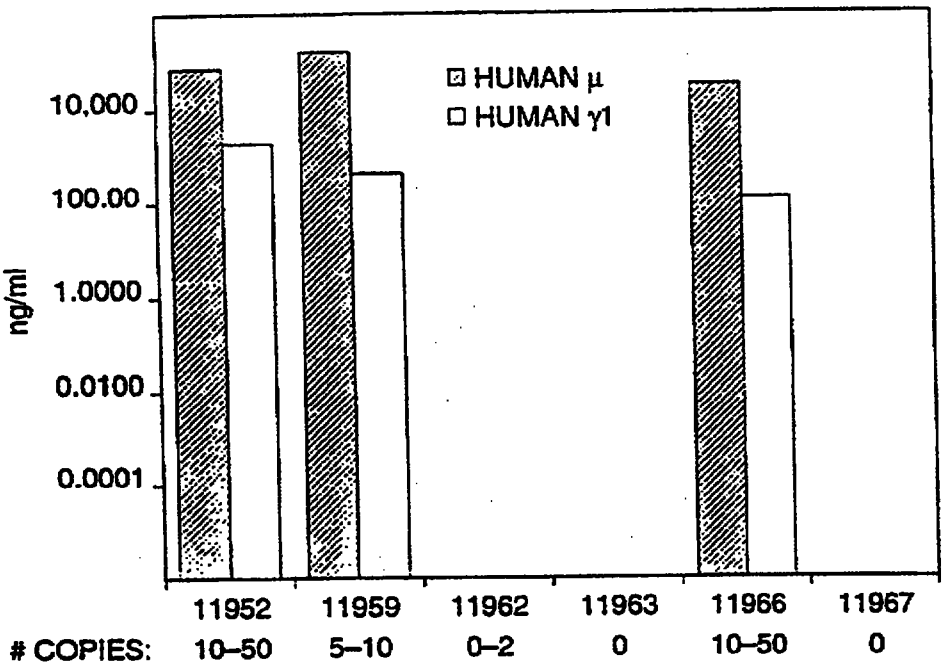

HUMAN μ
HUMAN γ1
10,000
100.00
1.0000
0.0100
0.0001
ng/ml
11952
11959
11962
11963
11966
11967
COPIES:
10–50
5–10
0–2
0
10–50
0

FIG. 81

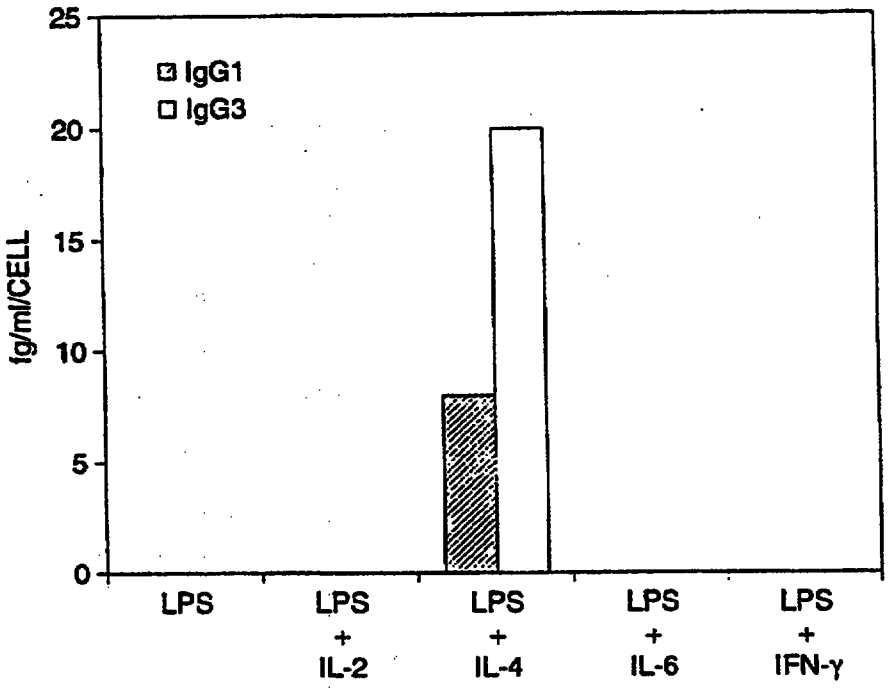

IgG1
IgG3
25
20
15
10
5
0
fg/ml/CELL
LPS
LPS + IL-2
LPS + IL-4
LPS + IL-6
LPS + IFN-γ

FIG. 84

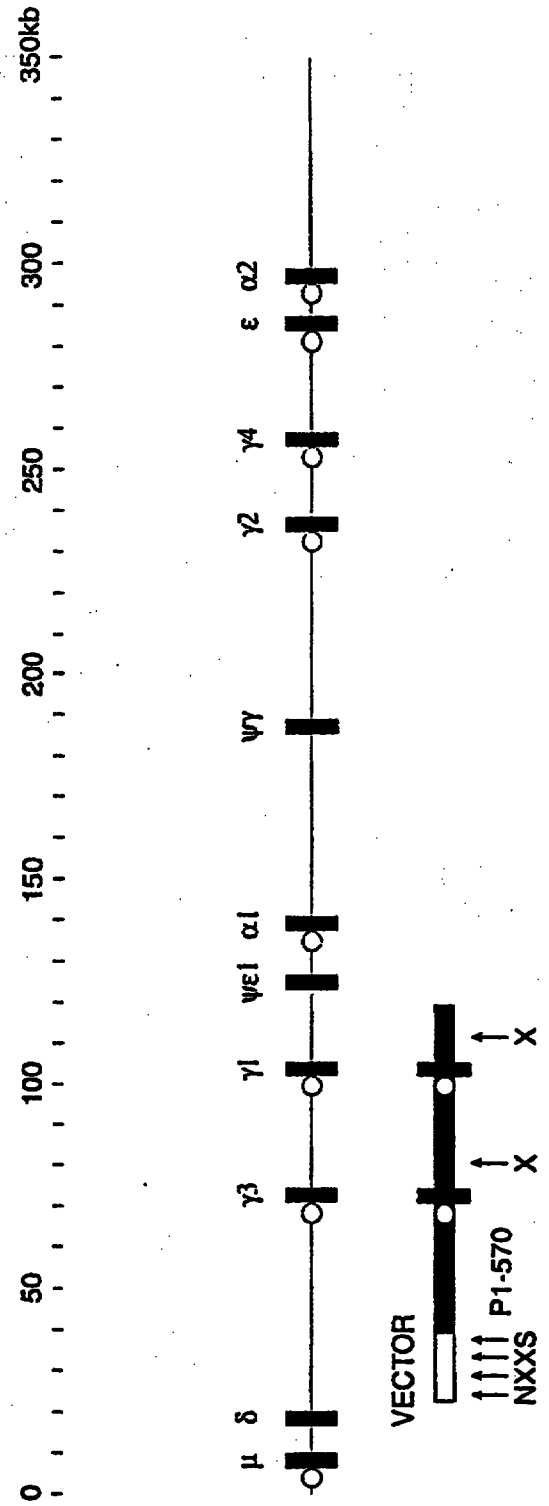

0
50
100
150
200
250
300
350kb
μ δ
γ3
γ1
ψε1 α1
ψγ
γ2
γ4
ε α2
VECTOR
NXXS
P1-570
X
X

FIG. 80

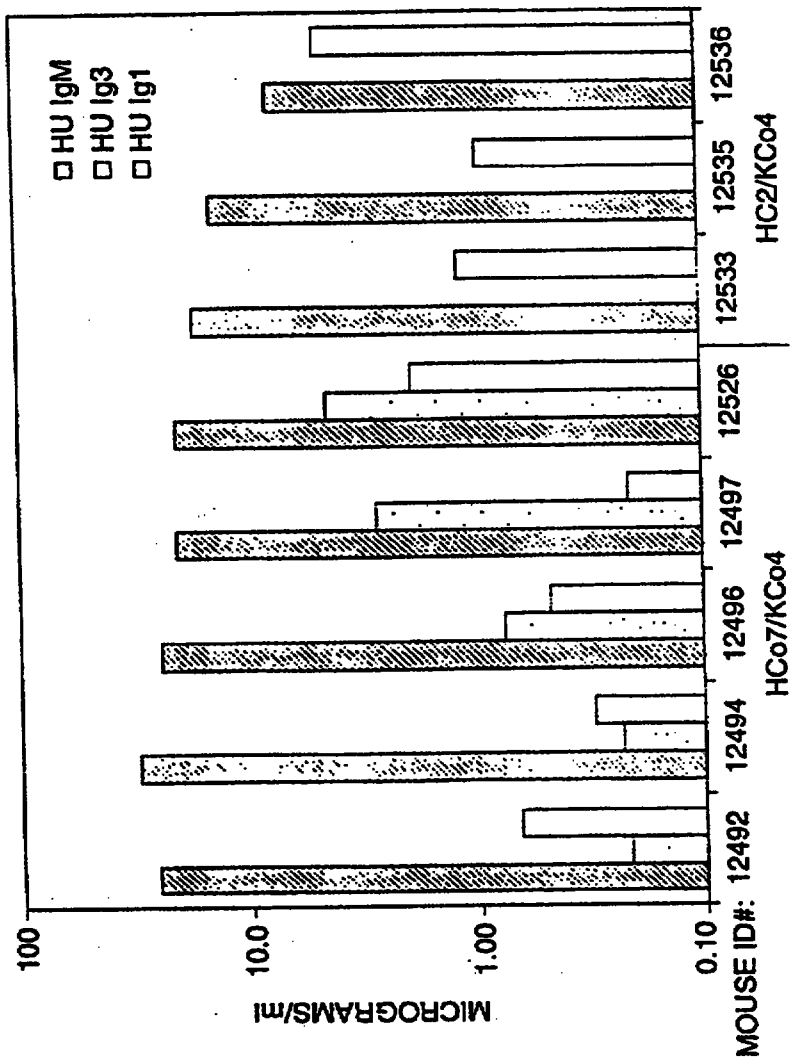

HU IgM
HU Ig3
HU Ig1
100
10.0
1.00
0.10
MICROGRAMS/ml
MOUSE ID#: 12492 12494 12496 12497 12526 12533 12535 12536
HCo7/KCo4
HC2/KCo4

FIG. 82

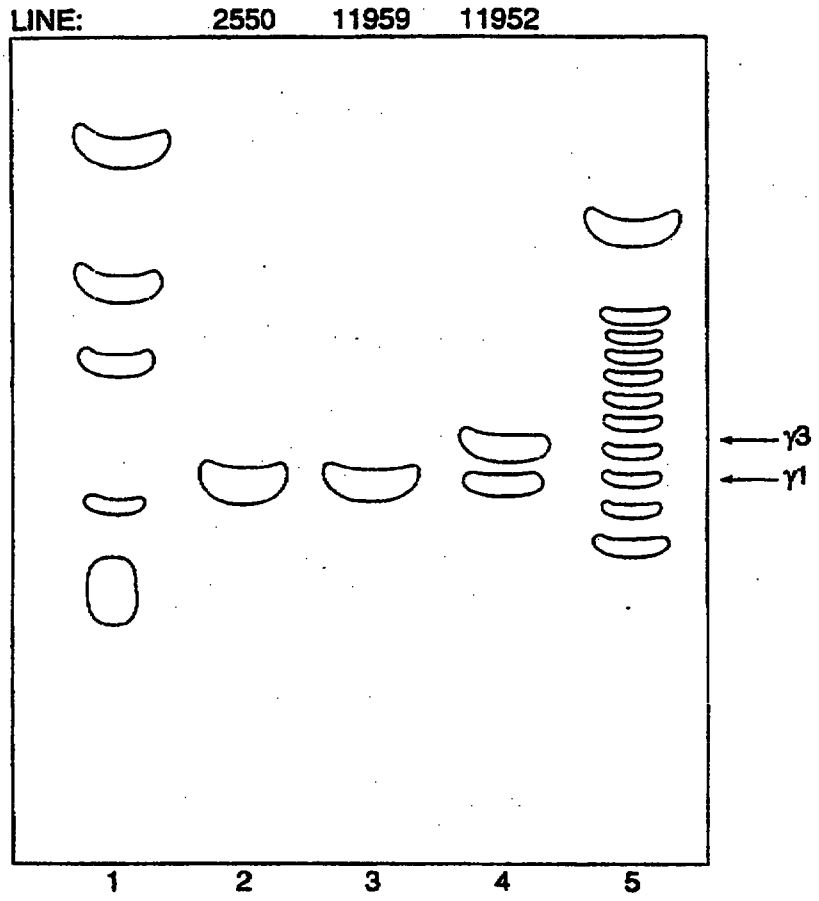


FIG. 83

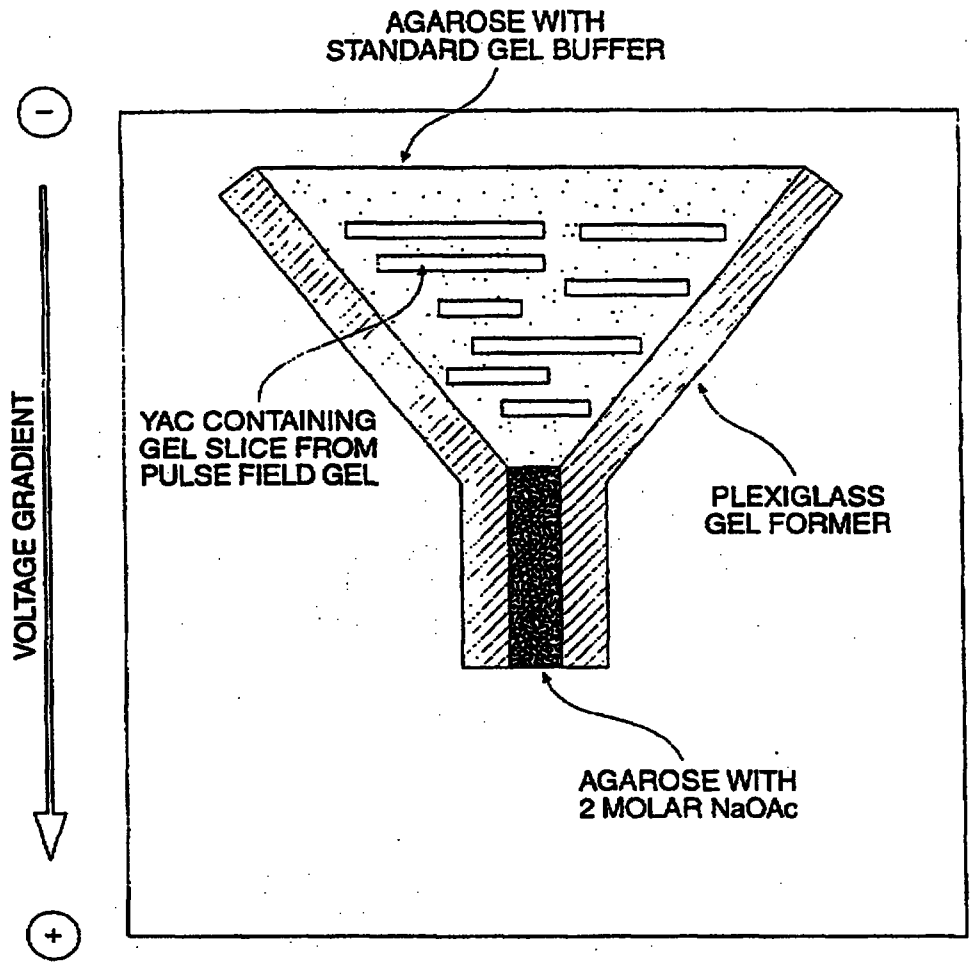

AGAROSE WITH
STANDARD GEL BUFFER
YAC CONTAINING
GEL SLICE FROM
PULSE FIELD GEL
PLEXIGLASS
GEL FORMER
AGAROSE WITH
2 MOLAR NaOAc
VOLTAGE GRADIENT
−
+

FIG. 85

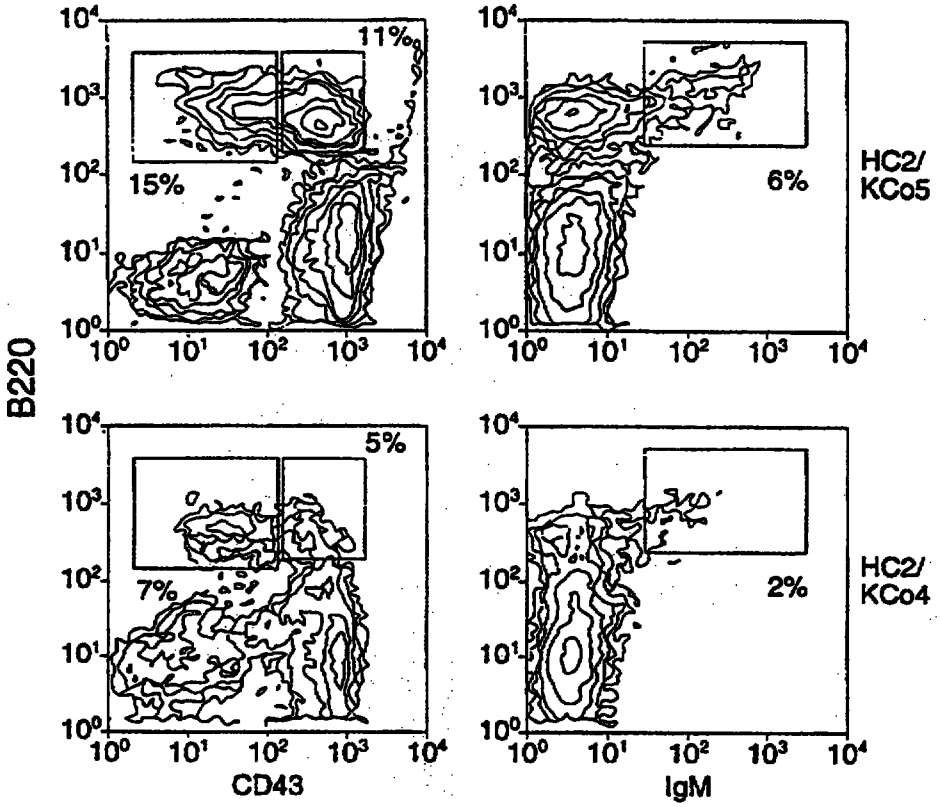

B220
CD43
IgM
11%
15%
6%
5%
7%
2%
HC2/
KCo5
HC2/
KCo4

FIG. 86

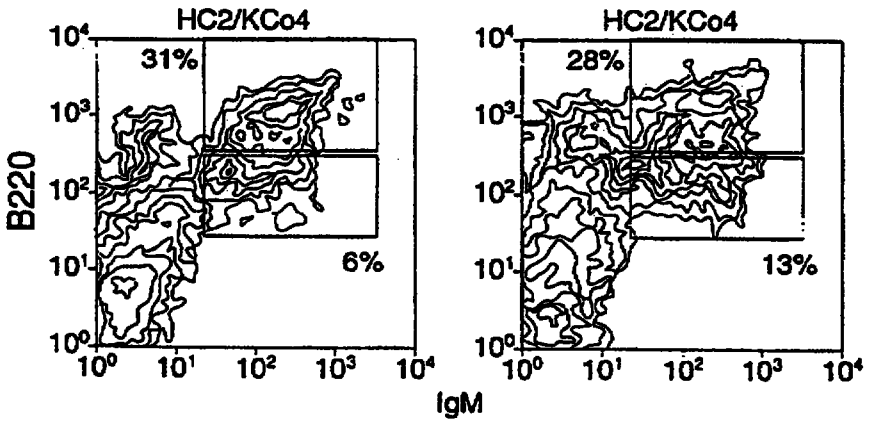

HC2/KCo4
HC2/KCo4
B220
IgM
31%
6%
28%
13%

FIG. 87

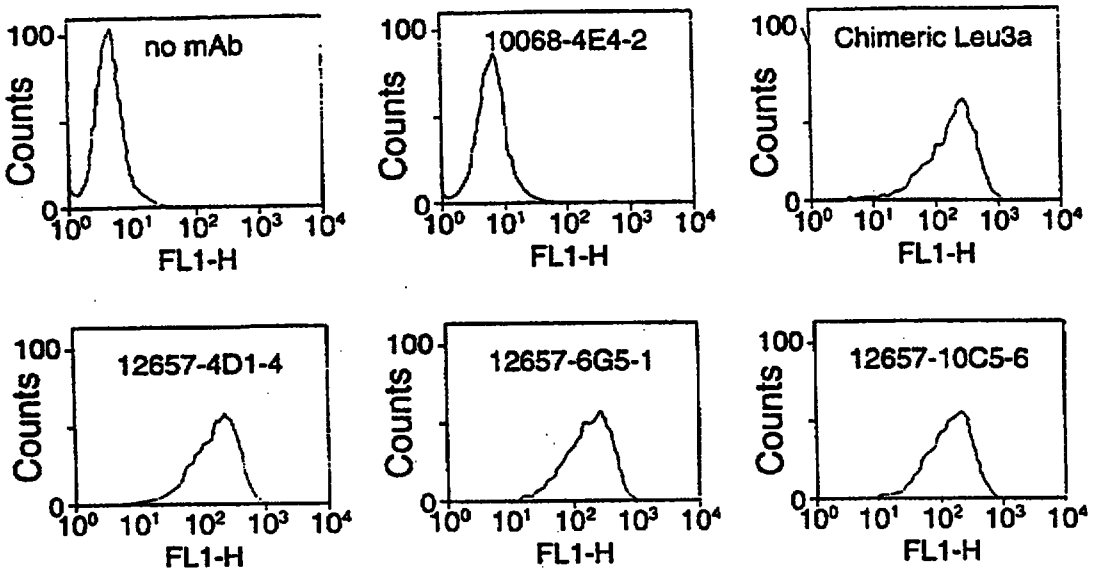

no mAb
10068-4E4-2
Chimeric Leu3a
12657-4D1-4
12657-6G5-1
12657-10C5-6
Counts
FL1-H
100
0
10^0 10^1 10^2 10^3 10^4

FIG. 88

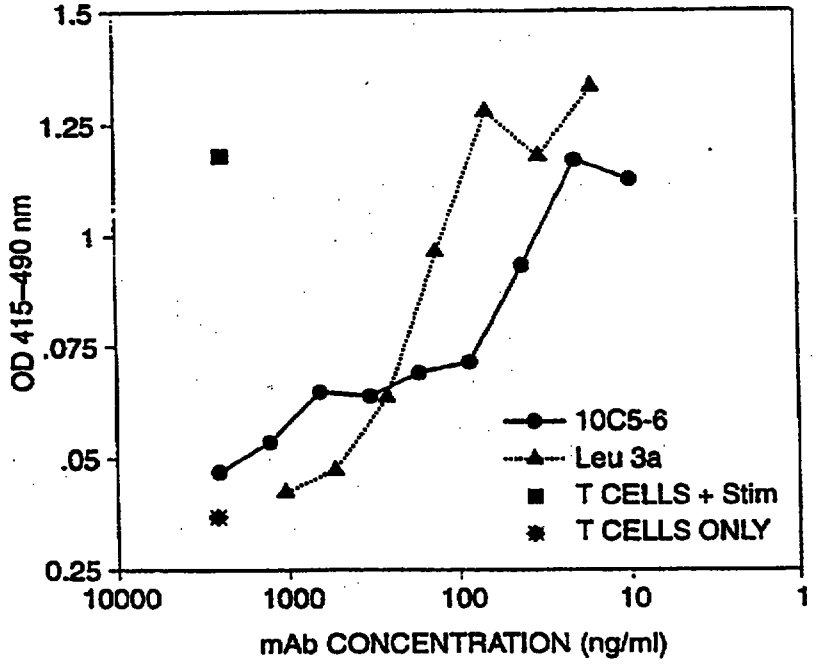

1.5
1.25
1
.075
.05
0.25
OD 415–490 nm
10000
1000
100
10
1
mAb CONCENTRATION (ng/ml)
10C5-6
Leu 3a
T CELLS + Stim
T CELLS ONLY

FIG. 90

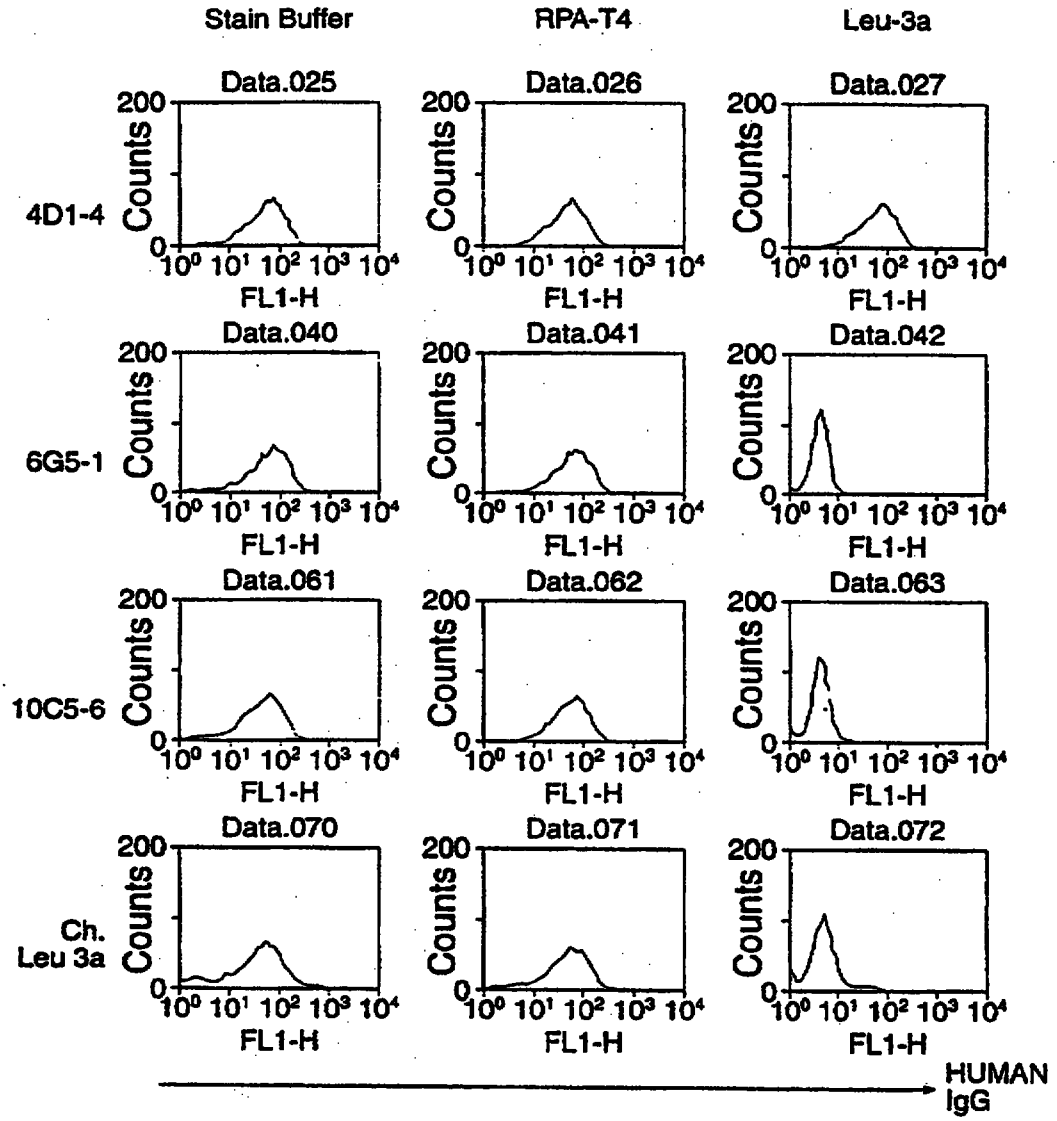

Stain Buffer
RPA-T4
Leu-3a
Data.025
Data.026
Data.027
4D1-4
Data.040
Data.041
Data.042
6G5-1
Data.061
Data.062
Data.063
10C5-6
Data.070
Data.071
Data.072
Ch. Leu 3a
Counts
200
0
10^0 10^1 10^2 10^3 10^4
FL1-H
HUMAN IgG

FIG. 89