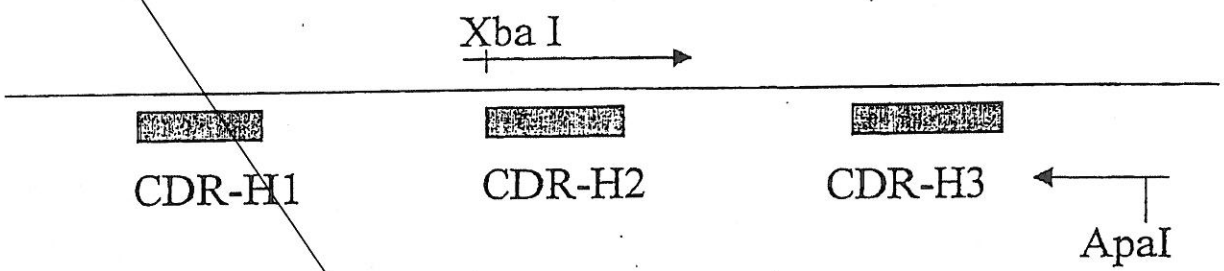
FIGURE 4: REMOVAL OF GLYCOSYLATION SITE AND REACTIVE LYSINE: PCR STRATEGY TO MUTATE CDR-H2 IN CH VECTOR



x

CDR-H2

		55 60 65
cH		GNNYTTYKRNLKG
N55Q	H2''	GNQYTTYKRNLKG
T57A	H2'	GNNYATYKRNLKG
T57V		GNNYVTYKRNLKG
K60R		GNNYTTYRRNLKG

FIGURE 5: 5/44 LIGHT CHAIN SEQUENCE GRAFT DESIGN

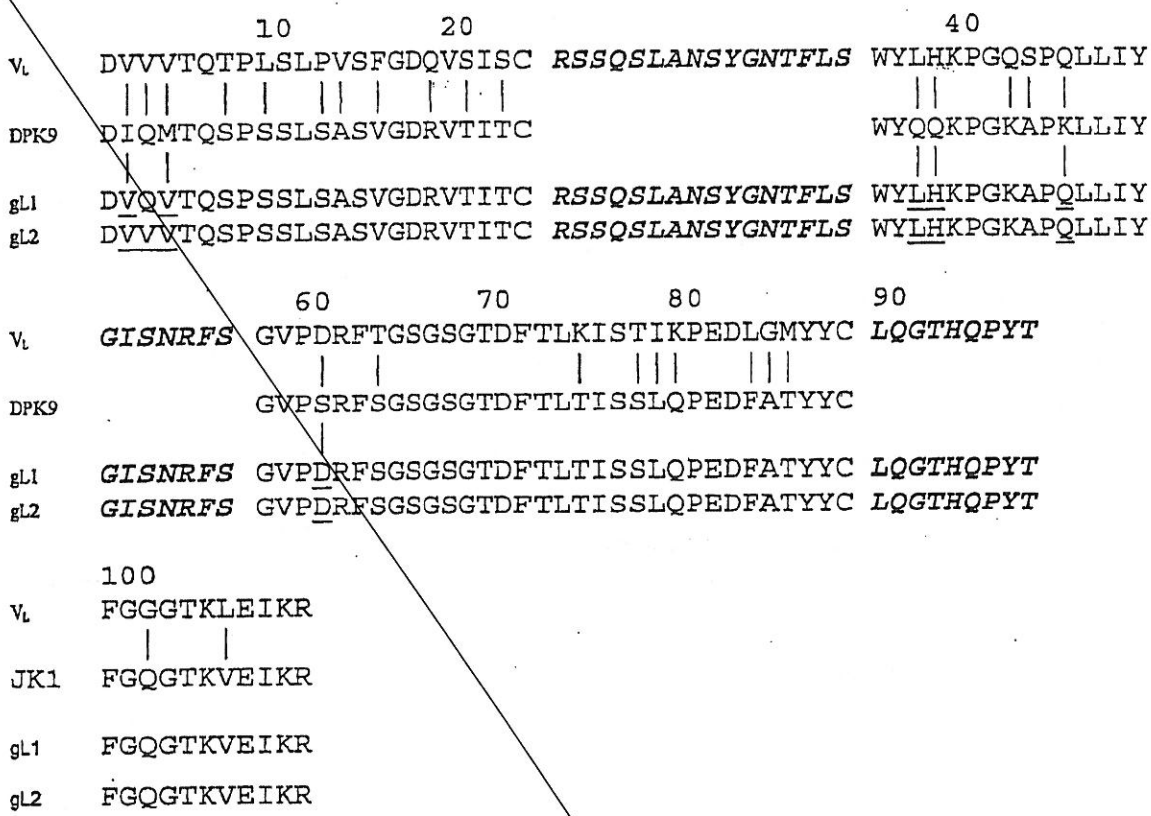


FIGURE 6: 5/44 HEAVY CHAIN SEQUENCE GRAFT DESIGN

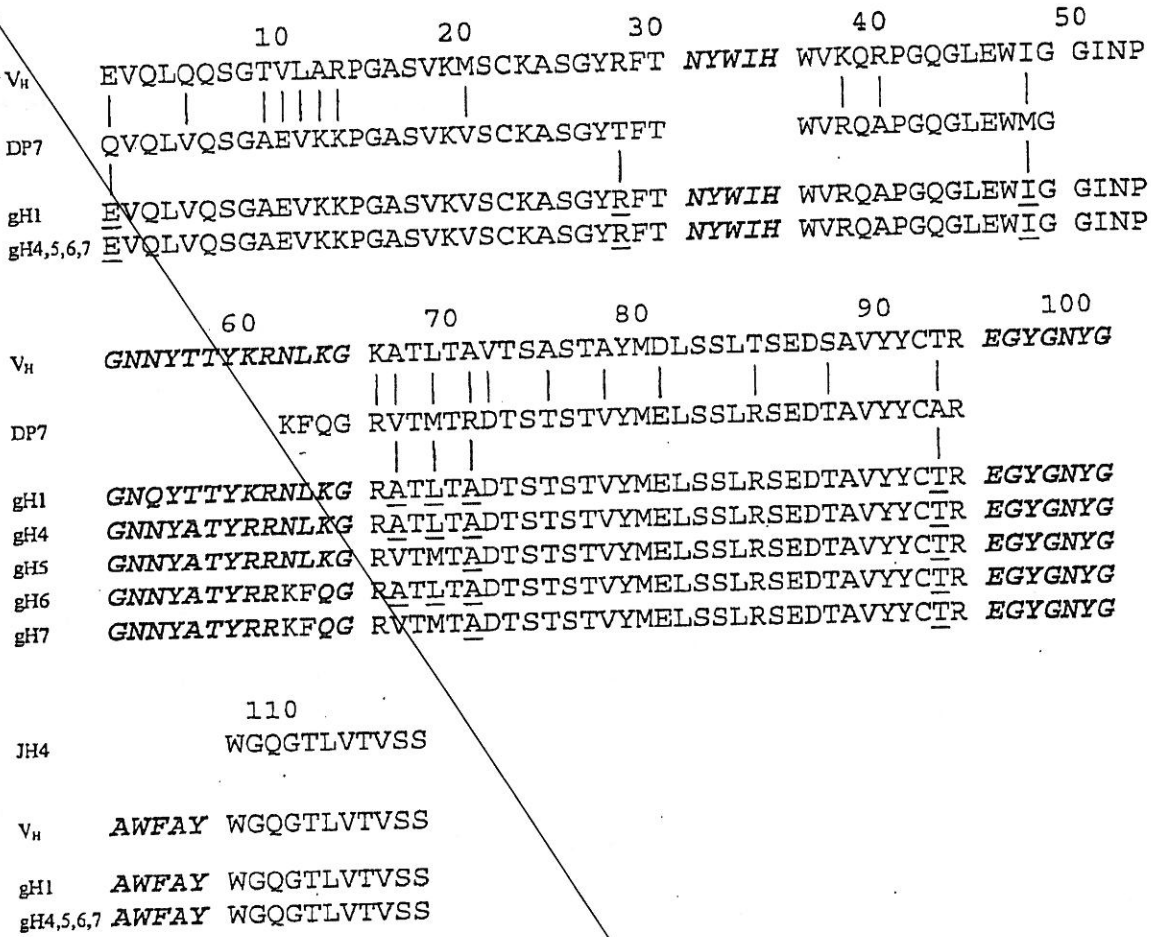


FIGURE 13: OLIGONUCLEOTIDE CASSETTES USED TO MAKE FURTHER GRAFTS

gH4

```
        XmaI       10          20          30          40          50          SacII
CC GGG AAT AAC TAC GCT ACA TAT AGG AGA AAT CTA AAG GGC AGA GCA ACG CTG ACC GC
     C TTA TTG ATG CGA TGT ATA TCC TCT TTA GAT TTC CCG TCT CGT TGC GAC TGG
P   G   N   N   Y   A   T   Y   R   R   N   L   K   G   R   A   T   L   T   A
```

gH5

```
        XmaI       10          20          30          40          50          SacII
CC GGG AAT AAC TAC GCT ACA TAT AGG AGA AAT CTA AAG GGC AGA GTT ACG ATG ACC GC
     C TTA TTG ATG CGA TGT ATA TCC TCT TTA GAT TTC CCG TCT CAA TGC TAC TGG
P   G   N   N   Y   A   T   Y   R   R   K   F   Q   G   R   V   T   M   T   A
```

gH6

```
        XmaI     10          20          30          40          50          SacII
CC GGG AAT AAC TAC GCT ACA TAT AGG AGA AAA TTC CAG GGC AGA GCA ACG CTG ACC GC
     C TTA TTG ATG CGA TGT ATA TCC TCT TTT AAG GTC CCG TCT CGT TGC GAC TGG
P   G   N   N   Y   A   T   Y   R   R   K   F   Q   G   R   A   T   L   T   A
```

gH7

```
        XmaI       10          20          30          40          50          SacII
CC GGG AAT AAC TAC GCT ACA TAT AGG AGA AAA TTC CAG GGC AGA GTT ACG ATG ACC GC
     C TTA TTG ATG CGA TGT ATA TCC TCT TTT AAG GTC CCG TCT CAA TGC TAC TGG
P   G   N   N   Y   A   T   Y   R   R   K   F   Q   G   R   V   T   M   T   A
```

gL2

```
        XmaI   10          20          30          40          50          60
BstEII
   C CGG GGT GAC GTT GTC GTG ACC CAG AGC CCA TCC AGC CTG AGC GCA TCT GTA GGA GAC CGG
       CCA CTG CAA CAG CAC TGG GTC TCG GGT AGG TCG GAC TCG CGT AGA CAT CCT CTG GCC CAG TG
 S   R   G   D   V   V   V   T   Q   S   P   S   S   L   S   A   S   V   G   D   R   V   T
```

cctgggcagg gtctagaatg gattggtggt attaatcctg gaaataatta tactacgtat 180
aagaggaact tgaagggcaa ggccacactg actgcagtca catccgccag cactgcctac 240
atggacctca gcagcctgac aagtgaggac tctgcggtct attactgtac aagagagggc 300
tatggtaact acggggcctg gtttgcttac tggggccagg ggactctggt caccgtctcc 360
tca 363

<210> 50
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> sequence within oligonucleotide primer

<400> 50
gccgccacc 9

<210> 51
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' oligonucleotide primer

<400> 51
gcgcgcaagc ttgccgccac catggacttc ggattctctc tcgtgttcct ggcactcatt 60
ctcaagggag tgcagtgtga ggtgcagctc gtcgagtctg g 101

REMOVAL OF GLYCOSYLATION SITE AND REACTIVE LYSINE: PCR STRATEGY TO MUTATE CDR-H2 IN CH VECTOR

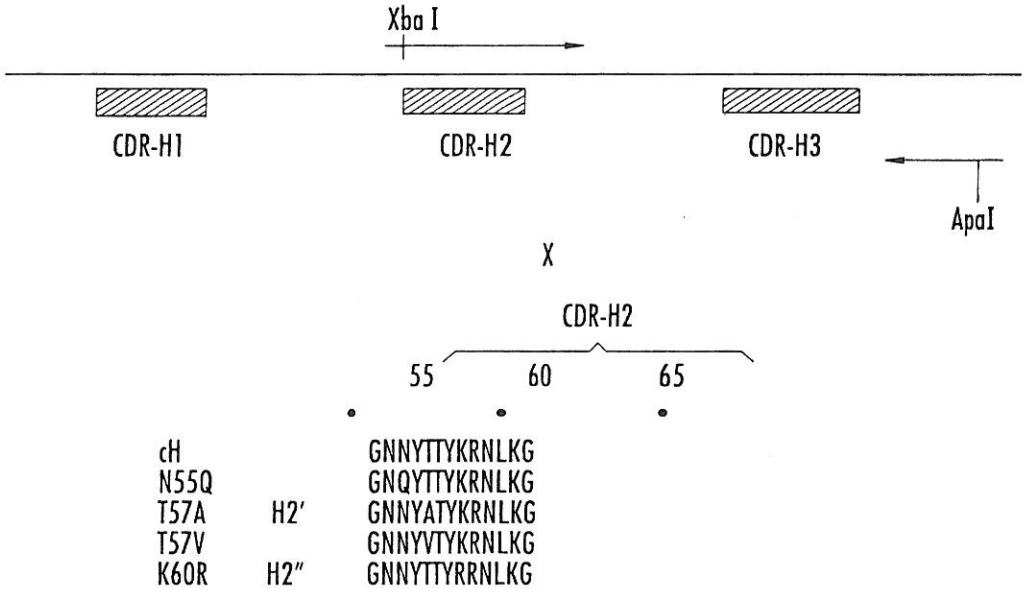


FIG. 4

5/44 LIGHT CHAIN SEQUENCE GRAFT DESIGN

```
                10        20          30          40        50
V_L   DVVVTQTPLSLPVSFGDQVSISC RSSQSLANSYGNTFLS WYLHKPGQSPQLLIY
DPK9  DIQMTQSPSSLSASVGDRVTITC                  WYQQKPGKAPKLLIY
gL1   DVQVTQSPSSLSASVGDRVTITC RSSQSLANSYGNTFLS WYLHKPGKAPQLLIY
gL2   DVVVTQSPSSLSASVGDRVTITC RSSQSLANSYGNTFLS WYLHKPGKAPQLLIY

          60        70        80        90         100
V_L   GISNRFS GVPDRFTGSGSGTDFTLKISTIKPEDLGMYYC LQGTHQPYT
DPK9          GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
gL1   GISNRFS GVPDRFSGSGSGTDFTLTISSLQPEDFATYYC LQGTHQPYT
gL2   GISNRFS GVPDRFSGSGSGTDFTLTISSLQPEDFATYYC LQGTHQPYT

           110
V_L   FGGGTKLEIKR
JK1   FGQGTKVEIKR

gL1   FGQGTKVEIKR
gL1   FGQGTKVEIKR
```

FIG.5

5/44 HEAVY CHAIN SEQUENCE GRAFT DESIGN

```
                10        20        30          40          50
VH   EVQLQQSGTVLARPGASVKMSCKASGYRFT NYWIH WVKQRPGQGLEWIG GINP
     |   |   |||||       |                  | |         |
DP7  QVQLVQSGAEVKKPGASVKVSCKASGYTFT       WVRQAPGQGLEWMG
     |                             |                    |
gH1  EVQLVQSGAEVKKPGASVKVSCKASGYRFT NYWIH WVRQAPGQGLEWIG GINP
gH4,5,6,7 EVQLVQSGAEVKKPGASVKVSCKASGYRFT NYWIH WVRQAPGQGLEWIG GINP

          60        70        80          90          100
VH   GNNYTTYKRNLKG KATLTAVTSASTAYMDLSSLTSEDSAVYYCTR EGYGNYG
                  || | || | | | |   | | |
DP7            KFQG RVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
                    | | |                       |
gH1  GNQYTTYKRNLKG RATLTADTSTSTVYMELSSLRSEDTAVYYCTR EGYGNYG
gH4  GNNYATYRRNLKG RATLTADTSTSTVYMELSSLRSEDTAVYYCTR EGYGNYG
gH5  GNNYATYRRNLKG RVTMTADTSTSTVYMELSSLRSEDTAVYYCTR EGYGNYG
gH6  GNNYATYRRKFQG RATLTADTSTSTVYMELSSLRSEDTAVYYCTR EGYGNYG
gH7  GNNYATYRRKFQG RVTMTADTSTSTVYMELSSLRSEDTAVYYCTR EGYGNYG

           110
JH4        WGQGTLVTVSS

VH   AWFAY WGQGTLVTVSS

gH1  AWFAY WGQGTLVTVSS
gH4,5,6,7 AWFAY WGQGTLVTVSS
```

FIG.6

OLIGONUCLEOTIDE CASSETTES USED TO MAKE FURTHER GRAFTS

gH4

```
XmaI        10          20          30          40          50      SacII
CC GGG AAT AAC TAC GCT ACA TAT AGG AGA AAT CTA AAG GGC AGA GCA ACG CTG ACC GC
    C TTA TTG ATG CGA TGT ATA TCC TCT TTA GAT TTC CCG TCT CGT TGC GAC TGG
P   G   N   N   Y   A   T   Y   R   R   N   L   K   G   R   A   T   L   T   A
```

gH5

```
XmaI        10          20          30          40          50      SacII
CC GGG AAT AAC TAC GCT ACA TAT AGG AGA AAT CTA AAG GGC AGA GTT ACG ATG ACC GC
    C TTA TTG ATG CGA TGT ATA TCC TCT TTA GAT TTC CCG TCT CAA TGC TAC TGG
P   G   N   N   Y   A   T   Y   R   R   N   L   K   G   R   V   T   M   T   A
```

gH6

```
XmaI        10          20          30          40          50      SacII
CC GGG AAT AAC TAC GCT ACA TAT AGG AGA AAA TTC CAG GGC AGA GCA ACG CTG ACC GC
    C TTA TTG ATG CGA TGT ATA TCC TCT TTT AAG GTC CCG TCT CGT TGC GAC TGG
P   G   N   N   Y   A   T   Y   R   R   K   F   Q   G   R   A   T   L   T   A
```

gH7

```
XmaI        10          20          30          40          50      SacII
CC GGG AAT AAC TAC GCT ACA TAT AGG AGA AAA TTC CAG GGC AGA GTT ACG ATG ACC GC
    C TTA TTG ATG CGA TGT ATA TCC TCT TTT AAG GTC CCG TCT CAA TGC TAC TGG
P   G   N   N   Y   A   T   Y   R   R   K   F   Q   G   R   V   T   M   T   A
```

gL2

```
XmaI          10          20          30          40          50          60  BstEII
  C CGG GGT GAC GTT GTC GTG ACC CAG AGC CCA TCC AGC CTG AGC GCA TCT GTA GGA GAC CGG
       CCA CTG CAA CAG CAC TGG GTC TCG GGT AGG TCG GAC TCG CGT AGA CAT CCT CTG GCC CAG TG
S   R   G   D   V   V   V   T   Q   S   P   S   S   L   S   A   S   V   G   D   R   V   T
```

FIG. 13

<213> Artificial Sequence

<220>
<223> K60R

<220>
<221>
<400> 14
Gly Asn Asn Tyr Thr Thr Tyr Arg Arg Asn Leu Lys Gly
1 5 10

<210> 15
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> CDR-H2 (K60R)~~R H'''~~ H2''

<220>
<221>

<400> 15
Gly Ile Asn Pro Gly Asn Asn Tyr Thr Thr Tyr Arg Arg Asn Leu Lys
1 5 10 15

Gly

<210> 16
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> CDR-H2 (T57A K60R) H'''

cctgggcagg gtctagaatg gattggtggt attaatcctg gaaataatta tactacgtat 180

aagaggaact tgaagggcaa ggccacactg actgcagtca catccgccag cactgcctac 240

atggacctca gcagcctgac aagtgaggac tctgcggtct attactgtac aagagagggc 300

tatggtaact acggggcctg gtttgcttac tggggccagg ggactctggt caccgtctcc 360

tca 363

<210> 50
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence within oligonucleotide primer

<400> 50
gccgccacc 9

<210> 51
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' oligonucleotide primer

<400> 51
gcgcgcaagc ttgccgccac catggacttc ggattctctc tcgtgttcct ggcactcatt 60

ctcaagggag tgcagtgtga ggtgcagctc gtcgagtctg g 101

<210> 52
<211> 339
<212> DNA
<213> Mus musculus

<220>
<223> DNA noncoding strand for mouse monoclonal 5/44 VL domain

<400> 52
ctacaacacc actgagtttg aggtgagagg gacggacagt cgaaacctct agttcaaaga 60

tagagaacgt ccagatcagt ctcagaacgt ttgtcaatac ccttgtggaa aaacagaacc 120

atggacgtgt tcggaccggt cagaggtgtc gaggagtaga taccctaaag gttgtctaaa 180

agaccccacg gtctgtccaa gtgaccgtca ccaagtccct gtctaaagtg tgagttctag 240

tcgtgttatt tcggactcct gaacccttac ataatgacga atgttccatg tgtagtcggc 300

atgtgcaagc ctccccccctg gttcgacctt tattttgca 339

<210> 53
<211> 363
<212> DNA
<213> Mus musculus

<220>
<223> DNA noncoding strand for mouse monoclonal 5/44 VH domain

<400> 53
ctccaggttg acgtcgtcag accctgacat gaccgttccg gaccccgaag gcacttctac 60

aggacgttcc gaagaccgat gtccaaatgg ttgatgacct aagtgaccca ttttgtctcc 120

ggacccgtcc cagatcttac ctaaccacca taattaggac ctttattaat atgatgcata 180

ttctccttga acttcccgtt ccggtgtgac tgacgtcagt gtaggcggtc gtgacggatg 240

tacctggagt cgtcggactg ttcactcctg agacgccaga taatgacatg ttctctcccg 300

ataccattga tgccccggac caaacgaatg accccggtcc cctgagacca gtggcagagg 360

agt 363

<210> 54
<211> 110
<212> DNA
<213> Artificial Sequence

<220>
<223> gH4 oligonucleotide

<400> 54
ccgggaataa ctacgctaca tataggagaa atctaaaggg cagagcaacg ctgaccgcct 60

tattgatgcg atgtatatcc tctttagatt tcccgtctcg ttgcgactgg 110

<210> 55
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> gH4 graft

<400> 55

Pro Gly Asn Asn Tyr Ala Thr Tyr Arg Arg Asn Leu Lys Gly Arg Ala
1 5 10 15

Thr Leu Thr Ala
20

<210> 56
<211> 109
<212> DNA
<213> Artificial Sequence

<220>
<223> gH5 oligonucleotide

<400> 56
ccgggaataa ctacgctaca tataggagaa atctaaaggg cagagttacg atgaccgcct 60

tattgatgcg atgtatatcc tctttagatt tcccgtctca atgctactgg 110

<210> 57
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> gH5 graft

<400> 57

Pro Gly Asn Asn Tyr Ala Thr Tyr Arg Arg Lys Phe Gln Gly Arg Val
1 5 10 15

Thr Met Thr Ala
20

<210> 58
<211> 110
<212> DNA
<213> Artificial Sequence

<220>
<223> gH6 oligonucleotide

<400> 58
ccgggaataa ctacgctaca tataggagaa aattccaggg cagagcaacg ctgaccgcct 60

tattgatgcg atgtatatcc tcttttaagg tcccgtctcg ttgcgactgg 110

<210> 59
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> gH6 graft

<400> 59

Pro Gly Asn Asn Tyr Ala Thr Tyr Arg Arg Lys Phe Gln Gly Arg Ala
1 5 10 15

Thr Leu Thr Ala
20

<210> 60
<211> 110
<212> DNA
<213> Artificial Sequence

<220>
<223> gH7 oligonucleotide

<400> 60
ccgggaataa ctacgctaca tataggagaa aattccaggg cagagttacg atgaccgcct 60

tattgatgcg atgtatatcc tcttttaagg tcccgtctca atgctactgg 110

<210> 61
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> gH7 graft

<400> 61

Pro Gly Asn Asn Tyr Ala Thr Tyr Arg Arg Lys Phe Gln Gly Arg Val
1 5 10 15

Thr Met Thr Ala
20

<210> 62
<211> 123
<212> DNA
<213> Artificial Sequence

<220>
<223> gL2 oligonucleotide

<400> 62
ccggggtgac gttgtcgtga cccagagccc atccagcctg agcgcatctg taggagaccg 60

gccactgcaa cagcactggg tctcgggtag gtcggactcg cgtagacatc ctctggccca 120

gtg 123

<210> 63
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> gL2 graft

<400> 63

Ser Arg Gly Asp Val Val Val Thr Gln Ser Pro Ser Ser Leu Ser Ala
1 5 10 15

Ser Val Gly Asp Arg Val Thr
20

<210> 64
<211> 781
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA noncoding strand for g5/44 light chain

<400> 64
aagcttcggc ggtggtactt caacggacaa tccgacaacc acgaagacaa caagacctaa 60

ggacgaaggg ccccactgca agttcactgg gtctcgggta ggtcggactc gcgtagacat 120

cctctggccc agtggtagtg aacatctagg tcagtctcag aacgtttgtc aataccttg 180

tggaaaaaca gaaccataga cgtgtttggt ccatttcggg gtgttaacga gtagatgcct 240

tagagattgt ctaaatcacc acatggtctg tccaagtcgc caaggccttc accatgacta 300

aagtgggagt gctagagcag agaggtcggt cttctaaagc ggtgaataat gacaaatgtt 360

ccatgtgtag tcggcatgtg taagccagtc ccatgatttc atctttagtt tgcatgccat 420

cgccggggta gacagaagta gaagggcggt agactactcg tcaactttag accttgacgg 480

agacaacaca cggacgactt attgaagata gggtctctcc ggtttcatgt caccttccac 540

ctattgcggg aggttagccc attgagggtc ctctcacagt gtctcgtcct gtcgttcctg 600

tcgtggatgt cggagtcgtc gtgggactgc gactcgtttc gtctgatgct ctttgtgttt 660

cagatgcgga cgcttcagtg ggtagtcccg gactcgagcg ggcagtgttt ctcgaagttg 720

tcccctctca caatctccct cttcacgggg gtggacgagg agtcaaggtc ggacccttaa 780

g 781

<210> 65
<211> 2160
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA noncoding strand for g5/44 heavy chain

<400> 65
ttcgaacggc ggtggtacct gaagcctaag agagagcaca aggaccgtga gtaagagttc 60

cctcacgtca cactccacgt taaccaggtc agtcctcgtc tccaattctt cggaccacga 120

aggcagtttc aaagcacatt ccgatcgccg atgtccaagt gtttaataac ctaagtaacc 180

cagtccgtcc gaggccctgt tccggacctt acctagccac cgtaattagg gcccttattg 240

atgcgatgta tatcctcttt taaggtcccg tctcaatgct actggcgcct gtggaggtgt 300

tcgtgacaga tgtacctcga cagtagagac tctaggctcc tgtggcgtca catgataaca 360

tgatctcttc cgatgccatt aatgcctcgg accaagcgga tgaccccggt cccatgggat 420

cagtgtcaga ggagtcgaag atgtttcccg ggtaggcaga aggggaccg cgggacgagg 480

tcctcgtgga ggctctcgtg tcggcgggac ccgacggacc agttcctgat gaaggggctt 540

ggccactgcc acagcacctt gagtccgcgg gactggtcgc cgcacgtgtg gaagggccga 600

caggatgtca ggagtcctga gatgagggag tcgtcgcacc actggcacgg gaggtcgtcg 660

aacccgtgct tctggatgtg gacgttgcat ctagtgttcg ggtcgttgtg gttccacctg 720

ttctctcaac cactctccgg tcgtgtccct ccctcccaca gacgaccttc ggtccgagtc 780

gggaggacgg acctgcgtgg ggccgacacg tcggggtcgg gtcccgtcgt tccgtacggg 840

gtagacagag gagtgggcct ccggagactg gtggggtgag tacgggtccc tctcccagaa 900

gacctaaaaa ggtggtccga ggcccgtcgg tgtccgacct acggggatgg ggtccgggac 960

gcgtatgtcc ccgtccacga cgcgagtctg gacggttctc ggtataggcc ctcctgggac 1020

ggggactgga ttcggttggg gtttccggtt tgagaggtga gggagtcgag tctgtggaag 1080

agaggagggt ctagactcat tgagggttag aagagagacg tctcaggttt ataccagggg 1140

gtacgggtgg tacgggtcca ttcggttggg tccggagcgg gaggtcgagt tccgccctgt 1200

ccacgggatc tcatcggacg taggtccctg tccggggtcg gcccacgact gcgtaggtgg 1260

aggtagagaa ggagtcgtgg actcaaggac ccccctggta gtcagaagga caaggggggt 1320

tttgggttcc tgtgagagta ctagagggcc tggggactcc agtgcacgca ccaccacctg 1380

cactcggtcc ttctggggct ccaggtcaag ttgaccatgc acctaccgca cctccacgta 1440

ttacggttct gtttcggcgc cctcctcgtc aagttgtcgt gcatggcaca ccagtcgcag 1500

gagtggcagg acgtggtcct gaccgacttg ccgttcctca tgttcacgtt ccagaggttg 1560

tttccggagg gcaggaggta gctcttttgg tagaggtttc ggtttccacc ctgggtgccc 1620

cacgctcccg gtgtacctgt ctccagtcga gccgggtggg agacgggacc ctcactggcg 1680

acacggttgg agacagggat gtcccgtcgg ggctctcggt gtccacatgt gggacggggg 1740

tagggtcctc ctctactggt tcttggtcca gtcggactgg acggaccagt ttccgaagat 1800

ggggtcgctg tagcggcacc tcaccctctc gttacccgtc ggcctcttgt tgatgttctg 1860

gtgcggaggg cacgacctga ggctgccgag gaagaaggag atgtcgtccg attggcacct 1920

gttctcgtcc accgtcctcc ccttacagaa gagtacgagg cactacgtac tccgagacgt 1980

gttggtgatg tgtgtcttct cggagaggga cagagaccca tttactcacg gtcccggccg 2040

ttcgggggcg aggggcccga gagccccagc gcgctcctac gaaccgtgca tggggcagat 2100

gtatgaaggg tccgtgggtc gtacctttat ttcgtgggtg gtgacgggac cgagcttaag 2160