## Figure 1: Sequence of CDRs of VR165

**Heavy Chain**

| | |
|---|---|
| **H1** SYGMS | (SEQ. ID. NO. 1) |
| **H2** TITSGGSYTYYPDTVKG | (SEQ. ID. NO. 2) |
| **H3** IGEDALDY | (SEQ. ID. NO. 3) |

**Light Chain**

| | |
|---|---|
| **L1** RASQDIAGSLN | (SEQ. ID. NO. 4) |
| **L2** ATSSLDS | (SEQ. ID. NO. 5) |
| **L3** LQYGSFPPT | (SEQ. ID. NO. 6) |

**Figure 2: Protein sequence of mouse monoclonal antibody VR165 variable domains**

**Heavy Chain** (SEQ. ID. NO. 7)

VR165 EVQLVESGGDLVKPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLQWVATITSGGSYTYYPDTVKG

VR165 RFTISRDNAENTLYLQMSSLKSEDTAMYYCVRIGEDALDYWGQGTSVTVSS

**Light Chain** (SEQ. ID. NO. 8)

VR165 DIQMTQSPSSLSASLGERVSLTCRASQDIAGSLNWLRQEPDGTIKRLIYATSSLDS

VR165 GVPKRFSGSRSGSDYSLTISSLESEDFVDYYCLQYGSFPPTFGGGSKLEIKR

**Figure 3: Comparison of VR165 V-region to Chosen framework V-regions**

Seq. Id. No. 9: VH3-7 GL V-region

```
VH3-7 GL   EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYYVDSVKG
                    |  |     |                    | | | |
VR165      EVQLVESGGDLVKPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLQWVATITSGGSYTYYPDTVKG


VH3-7 GL   RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR        WGQGTLVTVSS
                  | |     |  ||    |   |            |
VR165      RFTISRDNAENTLYLQMSSLKSEDTAMYYCVRIGEDALDYWGQGTSVTVSS
```

Seq. Id. No.10: A30 GL V-region

```
A30 GL     DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQS
                       | |  ||              || | ||||
VR165      DIQMTQSPSSLSASLGERVSLTCRASQDIAGSLNWLRQEPDGTIKRLIYATSSLDS


A30 GL    GVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYP  FGQGTKVEIKR
            |     |  ||||      ||   ||              | | |
VR165     GVPKRFSGSRSGSDYSLTISSLESEDFVDYYCLQYGSFPPTFGGGSKLEIKR
```

## Figure 4: Light Chain, including signal sequences

```
(Seq. Id. No. 13)  ATG AAA AAG ACA GCT ATC GCA ATT GCA
                   TAC TTT TTC TGT CGA TAG CGT TAA CGT

(Seq. Id. No. 11)   M   K   K   T   A   I   A   I   A>


(Seq. Id. No. 13)  GTG GCC TTG GCT GGT TTC GCT ACC GTA GCG CAA GCT GAT ATC CAG ATG ACC CAG AGT
                   CAC CGG AAC CGA CCA AAG CGA TGG CAT CGC GTT CGA CTA TAG GTC TAC TGG GTC TCA

(Seq. Id. No. 11)   V   A   L   A   G   F   A   T   V   A   Q   A   D   I   Q   M   T   Q   S>


(Seq. Id. No. 13)  CCA AGC AGT CTC TCC GCC AGC GTA GGC GAT CGT GTG ACT ATT ACC TGT CGT GCC AGT
                   GGT TCG TCA GAG AGG CGG TCG CAT CCG CTA GCA CAC TGA TAA TGG ACA GCA CGG TCA

(Seq. Id. No. 11)   P   S   S   L   S   A   S   V   G   D   R   V   T   I   T   C   R   A   S>


(Seq. Id. No. 13)  CAG GAC ATC GCG GGT AGC CTG AAC TGG TTG CAG CAA AAA CCG GGC AAA GCC ATC AAG
                   GTC CTG TAG CGC CCA TCG GAC TTG ACC AAC GTC GTT TTT GGC CCG TTT CGG TAG TTC

(Seq. Id. No. 11)   Q   D   I   A   G   S   L   N   W   L   Q   Q   K   P   G   K   A   I   K>


(Seq. Id. No. 13)  CGC CTC ATC TAT GCG ACG TCC AGC CTG GAT AGC GGT GTG CCA AAA CGT TTC AGT GGC
                   GCG GAG TAG ATA CGC TGC AGG TCG GAC CTA TCG CCA CAC GGT TTT GCA AAG TCA CCG
(Seq. Id. No. 11)   R   L   I   Y   A   T   S   S   L   D   S   G   V   P   K   R   F   S   G>


(Seq. Id. No. 13)  AGT CGC AGC GGT TCT GAC TAT ACC CTC ACA ATT TCG TCT CTC CAG CCG GAA GAT TTC
                   TCA GCG TCG CCA AGA CTG ATA TGG GAG TGT TAA AGC AGA GAG GTC GGC CTT CTA AAG

(Seq. Id. No. 11)   S   R   S   G   S   D   Y   T   L   T   I   S   S   L   Q   P   E   D   F>


(Seq. Id. No. 13)  GCC ACT TAC TAT TGT CTG CAA TAT GGC AGC TTC CCT CCG ACC TTC GGT CAG GGC ACT
                   CGG TGA ATG ATA ACA GAC GTT ATA CCG TCG AAG GGA GGC TGG AAG CCA GTC CCG TGA

(Seq. Id. No. 11)   A   T   Y   Y   C   L   Q   Y   G   S   F   P   P   T   F   G   Q   G   T>


(Seq. Id. No. 13)  AAA GTA GAA ATC AAA CGT ACG GTA GCG GCC CCA TCT GTC TTC ATC TTC CCG CCA TCT
                   TTT CAT CTT TAG TTT GCA TGC CAT CGC CGG GGT AGA CAG AAG TAG AAG GGC GGT AGA

(Seq. Id. No. 11)   K   V   E   I   K   R   T   V   A   A   P   S   V   F   I   F   P   P   S>


(Seq. Id. No. 13)  GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT
                   CTA CTC GTC AAC TTT AGA CCT TGA CGG AGA CAA CAC ACG GAC GAC TTA TTG AAG ATA

(Seq. Id. No. 11)   D   E   Q   L   K   S   G   T   A   S   V   V   C   L   L   N   N   F   Y>


(Seq. Id. No. 13)  CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC
                   GGG TCT CTC CGG TTT CAT GTC ACC TTC CAC CTA TTG CGG GAG GTT AGC CCA TTG AGG

(Seq. Id. No. 11)   P   R   E   A   K   V   Q   W   K   V   D   N   A   L   Q   S   G   N   S>


(Seq. Id. No. 13)  CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC
                   GTC CTC TCA CAG TGT CTC GTC CTG TCG TTC CTG TCG TGG ATG TCG GAG TCG TCG TGG

(Seq. Id. No. 11)   Q   E   S   V   T   E   Q   D   S   K   D   S   T   Y   S   L   S   S   T>


(Seq. Id. No. 13)  CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC
                   GAC TGC GAC TCG TTT CGT CTG ATG CTC TTT GTG TTT CAG ATG CGG ACG CTT CAG TGG

(Seq. Id. No. 11)   L   T   L   S   K   A   D   Y   E   K   H   K   V   Y   A   C   E   V   T>


(Seq. Id. No. 13)  CAT CAG GGC CTG AGC TCA CCA GTA ACA AAA AGT TTT AAT AGA GGG GAG TGT TAA
                   GTA GTC CCG GAC TCG AGT GGT CAT TGT TTT TCA AAA TTA TCT CCC CTC ACA ATT

(Seq. Id. No. 11)   H   Q   G   L   S   S   P   V   T   K   S   F   N   R   G   E   C   *>
```

## Figure 5: Heavy Chain, including signal sequences

```
(Seq. Id. No. 14)  ATG AAG AAG ACT GCT ATA GCA ATT GCA GTG GCG CTA GCT GGT TTC GCC ACC GTG GCG CAA
                   TAC TTC TTC TGA CGA TAT CGT TAA CGT CAC CGC GAT CGA CCA AAG CGG TGG CAC CGC GTT
(Seq. Id. No. 12)   M   K   K   T   A   I   A   I   A   V   A   L   A   G   F   A   T   V   A   Q>

(Seq. Id. No. 14)  GCT GAG GTT CAG CTG GTC GAG TCT GGA GGC GGG CTT GTC CAG CCT GGA GGG AGC CTG
                   CGA CTC CAA GTC GAC CAG CTC AGA CCT CCG CCC GAA CAG GTC GGA CCT CCC TCG GAC
(Seq. Id. No. 12)   A   E   V   Q   L   V   E   S   G   G   G   L   V   Q   P   G   G   S   L>

(Seq. Id. No. 14)  CGT CTC TCT TGT GCA GCA AGC GGC TTC ACC TTT TCC TCT TAC GGT ATG TCC TGG GTG
                   GCA GAG AGA ACA CGT CGT TCG CCG AAG TGG AAA AGG AGA ATG CCA TAC AGG ACC CAC
(Seq. Id. No. 12)   R   L   S   C   A   A   S   G   F   T   F   S   S   Y   G   M   S   W   V>

(Seq. Id. No. 14)  CGG CAG GCA CCT GGG AAG GGC CTG GAG TGG GTG GCA ACC ATT ACG TCC GGA GGC AGC
                   GCC GTC CGT GGA CCC TTC CCG GAC CTC ACC CAC CGT TGG TAA TGC AGG CCT CCG TCG
(Seq. Id. No. 12)   R   Q   A   P   G   K   G   L   E   W   V   A   T   I   T   S   G   G   S>

(Seq. Id. No. 14)  TAT ACA TAC TAC GTG GAC AGC GTC AAG GGC CGT TTC ACC ATT TCC CGG GAC AAT GCA
                   ATA TGT ATG ATG CAC CTG TCG CAG TTC CCG GCA AAG TGG TAA AGG GCC CTG TTA CGT
(Seq. Id. No. 12)   Y   T   Y   Y   V   D   S   V   K   G   R   F   T   I   S   R   D   N   A>

(Seq. Id. No. 14)  AAG AAT ACC CTT TAC CTC CAG ATG AAC TCT CTC CGC GCA GAG GAC ACA GCA GTC TAT
                   TTC TTA TGG GAA ATG GAG GTC TAC TTG AGA GAG GCG CGT CTC CTG TGT CGT CAG ATA
(Seq. Id. No. 12)   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D   T   A   V   Y>

(Seq. Id. No. 14)  TAC TGT GTA CGG ATC GGC GAA GAC GCG TTG GAC TAC TGG GGA CAG GGG ACC CTT GTG
                   ATG ACA CAT GCC TAG CCG CTT CTG CGC AAC CTG ATG ACC CCT GTC CCC TGG GAA CAC
(Seq. Id. No. 12)   Y   C   V   R   I   G   E   D   A   L   D   Y   W   G   Q   G   T   L   V>

(Seq. Id. No. 14)  ACA GTC TCC TCT GCT TCT ACA AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC
                   TGT CAG AGG AGA CGA AGA TGT TTC CCG GGT AGC CAG AAG GGG GAC CGT GGG AGG AGG
(Seq. Id. No. 12)   T   V   S   S   A   S   T   K   G   P   S   V   F   P   L   A   P   S   S>

(Seq. Id. No. 14)  AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC
                   TTC TCG TGG AGA CCC CCG TGT CGC CGG GAC CCG ACG GAC CAG TTC CTG ATG AAG GGG
(Seq. Id. No. 12)   K   S   T   S   G   G   T   A   A   L   G   C   L   V   K   D   Y   F   P>

(Seq. Id. No. 14)  GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC
                   CTT GGC CAC TGC CAC AGC ACC TTG AGT CCG CGG GAC TGG TCG CCG CAC GTG TGG AAG
(Seq. Id. No. 12)   E   P   V   T   V   S   W   N   S   G   A   L   T   S   G   V   H   T   F>

(Seq. Id. No. 14)  CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC
                   GGC CGA CAG GAT GTC AGG AGT CCT GAG ATG AGG GAG TCG TCG CAC CAC TGG CAC GGG
(Seq. Id. No. 12)   P   A   V   L   Q   S   S   G   L   Y   S   L   S   S   V   V   T   V   P>

(Seq. Id. No. 14)  TCC AGC AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC
                   AGG TCG TCG AAC CCG TGG GTC TGG ATG TAG ACG TTG CAC TTA GTG TTC GGG TCG TTG
(Seq. Id. No. 12)   S   S   S   L   G   T   Q   T   Y   I   C   N   V   N   H   K   P   S   N>

(Seq. Id. No. 14)  ACC AAG GTC GAC AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC GCC
                   TGG TTC CAG CTG TTC TTT CAA CTC GGG TTT AGA ACA CTG TTT TGA GTG TGT ACG CGG
(Seq. Id. No. 12)   T   K   V   D   K   K   V   E   P   K   S   C   D   K   T   H   T   C   A>

(Seq. Id. No. 14)  GCG TGA
                   CGC ACT
(Seq. Id. No. 12)   A   *>
```

FIGURE 6

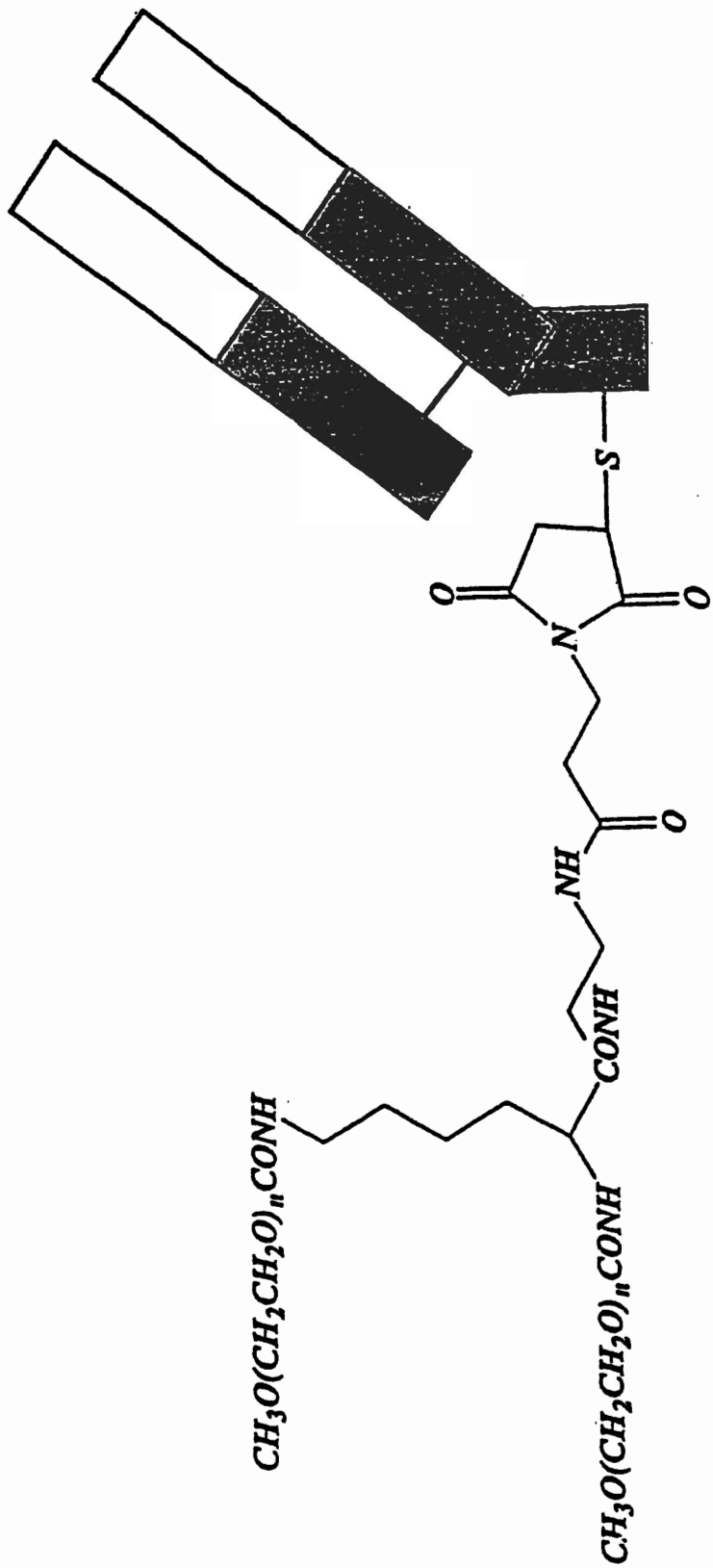


**Figure 7:**

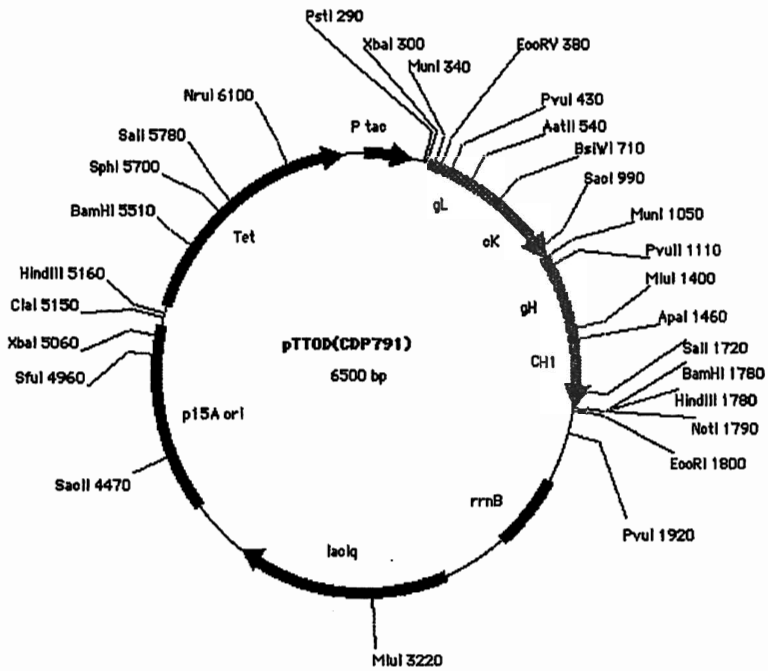
**gH3 (SEQ ID NO: 15)**

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATIT
SGGSYTYYVDSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCVRIGEDAL
DYWGQGTLVTVSS

**gL3 (SEQ ID NO: 16)**

DIQMTQSPSSLSASVGDRVTITCRASQDIAGSLNWLQQKPGKAIKRLIYATSSL
DSGVPKRFSGSRSGSDYTLTISSLQPEDFATYYCLQYGSFPPTFGQGTKVEIKR

**Figure 8: pTTOD(CDP791)**



## Figure 9: Protein sequence of the designed VH and VL grafts

### Heavy Chain

(Seq.Id.No. 17)

gH1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATITSGGSYTYYPDTVKG
gH1 RFTISRDNAKNSLYLQMNSLRAEDTAVYYCARIGEDALDYWGQGTLVTVSS

(Seq.Id.No. 18)

gH2 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATITSGGSYTYYPDTVKG
gH2 RFTISRDNAKNTLYLQMNSLRAEDTAVYYCVRIGEDALDYWGQGTLVTVSS

(Seq.Id.No. 19)

gH3 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATITSGGSYTYYVDSVKG
gH3 RFTISRDNAKNTLYLQMNSLRAEDTAVYYCVRIGEDALDYWGQGTLVTVSS

### Light Chain

(Seq.Id.No. 20)

gL1 DIQMTQSPSSLSASVGDRVTITCRASQDIAGSLNWYQQKPGKAPKRLIYATSSLDS
gL1 GVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQYGSFPPTFGQGTKVEIKR

(Seq.Id.No. 21)

gL2 DIQMTQSPSSLSASVGDRVTITCRASQDIAGSLNWYQQKPGKAPKRLIYATSSLDS
gL2 GVPKRFSGSRSGSDYTLTISSLQPEDFATYYCLQYGSFPPTFGQGTKVEIKR

(Seq.Id.No. 22)

gL3 DIQMTQSPSSLSASVGDRVTITCRASQDIAGSLNWLQQKPGKAIKRLIYATSSLDS
gL3 GVPKRFSGSRSGSDYTLTISSLQPEDFATYYCLQYGSFPPTFGQGTKVEIKR

**Figure 10: Gene design**

gH1 sequence **(SEQ ID NO:23)**

```
        HindIII     20            30            40            50            60
GAATAAAAGC TTGCCGCCAC C ATG AAG ATG TGG TTA AAC TGG GTT TTC CTT GCC CTC ATT
CTTATTTTCG AACGGCGGTG G TAC TTC TAC ACC AAT TTG ACC CAA AAG GAA CGG GAG TAA
                        M   K   M   W   L   N   W   V   F   L   A   L   I>

            70          80        PvuII           100           110
TTA AAA GGT GTC CAG TGT GAG GTG CAG CTG GTC GAG TCT GGA GGC GGG CTT GTC CAG
AAT TTT CCA CAG GTC ACA CTC CAC GTC GAC CAG CTC AGA CCT CCG CCC GAA CAG GTC
 L   K   G   V   Q   C   E   V   Q   L   V   E   S   G   G   G   L   V   Q>

120           130           140           150           160           170
CCT GGA GGG AGC CTG CGT CTC TCT TGT GCA GCA AGC GGC TTC ACC TTT TCC TCT TAC
GGA CCT CCC TCG GAC GCA GAG AGA ACA CGT CGT TCG CCG AAG TGG AAA AGG AGA ATG
 P   G   G   S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>

    180           190           200           210           220           230
GGT ATG TCC TGG GTG CGG CAG GCA CCT GGG AAG GGC CTG GAG TGG GTG GCA ACC ATT
CCA TAC AGG ACC CAC GCC GTC CGT GGA CCC TTC CCG GAC CTC ACC CAC CGT TGG TAA
 G   M   S   W   V   R   Q   A   P   G   K   G   L   E   W   V   A   T   I>

      BspEI           250           260           270           280
ACG TCC GGA GGC AGC TAT ACA TAC TAC CCG GAC ACC GTC AAG GGC CGT TTC ACC ATT
TGC AGG CCT CCG TCG ATA TGT ATG ATG GGC CTG TGG CAG TTC CCG GCA AAG TGG TAA
 T   S   G   G   S   Y   T   Y   Y   P   D   T   V   K   G   R   F   T   I>

290  XmaI    300           310           320           330           340
TCC CGG GAC AAT GCA AAG AAT AGC CTT TAC CTC CAG ATG AAC TCT CTC CGC GCA GAG
AGG GCC CTG TTA CGT TTC TTA TCG GAA ATG GAG GTC TAC TTG AGA GAG GCG CGT CTC
 S   R   D   N   A   K   N   S   L   Y   L   Q   M   N   S   L   R   A   E>

   350           360           370           380     MluI 390           400
GAC ACA GCA GTC TAT TAC TGT GCA CGG ATC GGC GAA GAC GCG TTG GAC TAC TGG GGA
CTG TGT CGT CAG ATA ATG ACA CGT GCC TAG CCG CTT CTG CGC AAC CTG ATG ACC CCT
 D   T   A   V   Y   Y   C   A   R   I   G   E   D   A   L   D   Y   W   G>

       410           420           430           440  ApaI     450
CAG GGG ACC CTT GTG ACA GTC TCC TCT GCT TCT ACA AAG GGC CCA AGA AA
GTC CCC TGG GAA CAC TGT CAG AGG AGA CGA AGA TGT TTC CCG GGT TCT TT
 Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P>
```

gL1 sequence **(SEQ ID NO:24)**

```
          SfuI      20            30            40            50            60
GGATGATTCG AAGCCGCCAC C ATG AGG ACC CCT GCT CAG ATT CTT GGC TTC TTG TTG CTC
CCTACTAAGC TTCGGCGGTG G TAC TCC TGG GGA CGA GTC TAA GAA CCG AAG AAC AAC GAG
                        M   R   T   P   A   Q   I   L   G   F   L   L   L>

            70          80   EcoRV   90           100           110
TTG TTT CCA GGT ACC AGA TGT GAT ATC CAG ATG ACC CAG AGT CCA AGC AGT CTC TCC
AAC AAA GGT CCA TGG TCT ACA CTA TAG GTC TAC TGG GTC TCA GGT TCG TCA GAG AGG
 L   F   P   G   T   R   C   D   I   Q   M   T   Q   S   P   S   S   L   S>

120           130           140           150           160           170
GCC AGC GTA GGC GAT CGT GTG ACT ATT ACC TGT CGT GCC AGT CAG GAC ATC GCG GGT
CGG TCG CAT CCG CTA GCA CAC TGA TAA TGG ACA GCA CGG TCA GTC CTG TAG CGC CCA
 A   S   V   G   D   R   V   T   I   T   C   R   A   S   Q   D   I   A   G>

    180           190           200           210           220           230
AGC CTG AAC TGG TAT CAG CAA AAA CCG GGC AAA GCC CCC AAG CGC CTC ATC TAT GCG
TCG GAC TTG ACC ATA GTC GTT TTT GGC CCG TTT CGG GGG TTC GCG GAG TAG ATA CGC
 S   L   N   W   Y   Q   Q   K   P   G   K   A   P   K   R   L   I   Y   A>

AatII   240           250           260           270           280
ACG TCC AGC CTG GAT AGC GGT GTG CCA TCT CGT TTC AGT GGC AGT GGC AGC GGT ACT
TGC AGG TCG GAC CTA TCG CCA CAC GGT AGA GCA AAG TCA CCG TCA CCG TCG CCA TGA
 T   S   S   L   D   S   G   V   P   S   R   F   S   G   S   G   S   G   T>

290           300           BsmBI         320           330           340
GAA TTT ACC CTC ACA ATT TCG TCT CTC CAG CCG GAA GAT TTC GCC ACT TAC TAT TGT
CTT AAA TGG GAG TGT TAA AGC AGA GAG GTC GGC CTT CTA AAG CGG TGA ATG ATA ACA
 E   F   T   L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C>
```

```
   350          360          370          380          390          400
CTG CAA TAT GGC AGC TTC CCT CCG ACC TTC GGT CAG GGC ACT AAA GTA GAA ATC AAA
GAC GTT ATA CCG TCG AAG GGA GGC TGG AAG CCA GTC CCG TGA TTT CAT CTT TAG TTT
 L   Q   Y   G   S   F   P   P   T   F   G   Q   G   T   K   V   E   I   K>

 BsiWI 410
CGT ACG GC GTGC
GCA TGC CG CACG
 R   T>
```

**Figure 11: Oligonucleotides for gene assembly**

**gL1 F1 (SEQ ID NO:25)**
GGATGATTCGAAGCCGCCAC

**gL1 F2 (SEQ ID NO:26)**
TCCAGGTACCAGATGTGATATCCAGATGACCCAGAGTCCAAGCAGTCTCTCCGCCAGCGTAGGCGATCGTG
TGACTATTACCTGTC

**gL1 F3 (SEQ ID NO:27)**
CAAAAACCGGGCAAAGCCCCCAAGCGCCTCATCTATGCGACGTCCAGCCTGGATAGCGGTGTGCCATCTCG
TTTCAGTGGCAGTGGC

**gL1 F4 (SEQ ID NO:28)**
AGATTTCGCCACTTACTATTGTCTGCAATATGGCAGCTTCCCTCCGACCTTCGGTCAGGGCACTAAAGTAGA
AATCAAACGTACGGCGTGC

**gL1 R1 (SEQ ID NO:29)**
GCACGCCGTACGTTTGATTTC

**gL1 R2 (SEQ ID NO:30)**
GACAATAGTAAGTGGCGAAATCTTCCGGCTGGAGAGACGAAATTGTGAGGGTAAATTCAGTACCGCTGCCA
CTGCCACTGAAACGAG

**gL1 R3 (SEQ ID NO:31)**
GGGGCTTTGCCCGGTTTTTGCTGATACCAGTTCAGGCTACCCGCGATGTCCTGACTGGCACGACAGGTAAT
AGTCACACGATC

**gL1 R4 (SEQ ID NO:32)**
GATATCACATCTGGTACCTGGAAACAAGAGCAACAAGAAGCCAAGAATCTGAGCAGGGGTCCTCATGGTGG
CGGCTTCGAATCATCC

**gH1 F1 (SEQ ID NO:33)**
GAATAAAAGCTTGCCGCCACC

**gH1 F2 (SEQ ID NO:34)**
TCCAGTGTGAGGTGCAGCTGGTCGAGTCTGGAGGCGGGCTTGTCCAGCCTGGAGGGAGCCTGCGTCTCTC
TTGTGCAGCAAGCGGCTTCAC

**gH1 F3 (SEQ ID NO:35)**
AGTGGGTGGCAACCATTACGTCCGGAGGCAGCTATACATACTACCCGGACACCGTCAAGGGCCGTTTCACC
ATTTCCCGGGACAATGCAA

**gH1 F4 (SEQ ID NO:36)**
CTATTACTGTGCACGGATCGGCGAAGACGCGTTGGACTACTGGGGACAGGGGACCCTTGTGACAGTCTCCT
CTGCTTCTACAAAGGGCCCAAGAAA

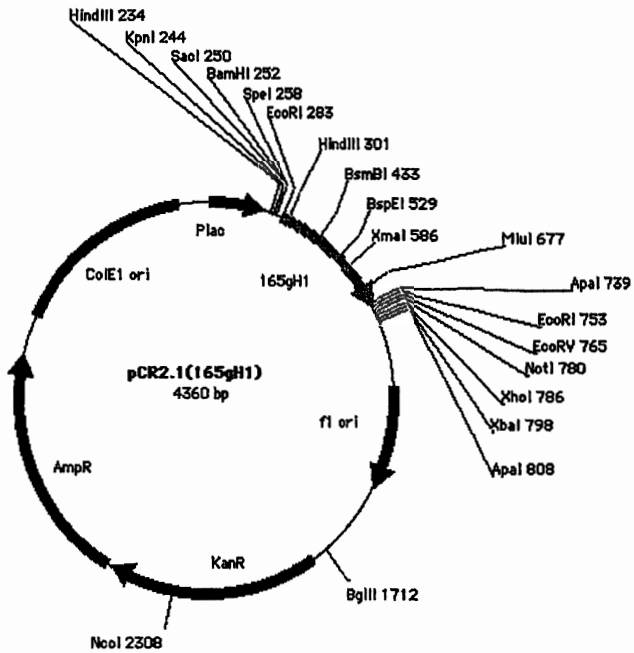
**gH1 R1 (SEQ ID NO:37)**
TTTCTTGGGCCCTTTGTAGAAG

**gH1 R2 (SEQ ID NO:38)**
CCGATCCGTGCACAGTAATAGACTGCTGTGTCCTCTGCGCGGAGAGAGTTCATCTGGAGGTAAAGGCTATT
CTTTGCATTGTCCCGGGAAATGG

**gH1 R3 (SEQ ID NO:39)**
ACGTAATGGTTGCCACCCACTCCAGGCCCTTCCCAGGTGCCTGCCGCACCCAGGACATACCGTAAGAGGAA
AAGGTGAAGCCGCTTGCTGCACA

**gH1 R4 (SEQ ID NO:40)**
CAGCTGCACCTCACACTGGACACCTTTTAAAATGAGGGCAAGGAAAACCCAGTTTAACCACATCTTCATGGT
GGCGGCAAGCTTTTATTC

**Figure 12: Plasmid pCR2.1(gH1) and pCR2.1(gL1)**



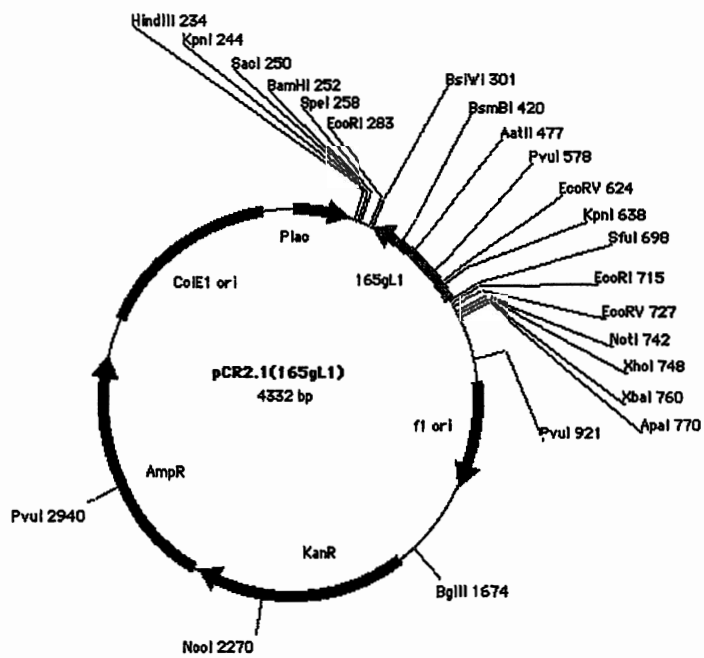



**Figure 13: Oligonucleotide cassettes for construction of gH2, gH3, gL2, gH3**

**gH2 cassette** **(SEQ ID NO: 41)**

```
 XmaI      10          20          30          40          50
TCC CGG GAC AAT GCA AAG AAT ACC CTT TAC CTC CAG ATG AAC TCT CTC CGC GCA GAG
AGG GCC CTG TTA CGT TTC TTA TGG GAA ATG GAG GTC TAC TTG AGA GAG GCG CGT CTC
 S   R   D   N   A   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E>

 60          70          80          90       MluI
GAC ACA GCA GTC TAT TAC TGT GTA CGG ATC GGC GAA GAC GCG TTG
CTG TGT CGT CAG ATA ATG ACA CAT GCC TAG CCG CTT CTG CGC AAC
 D   T   A   V   Y   Y   C   V   R   I   G   E   D   A   L>
```

**gH3 cassette** **(SEQ ID NO:42)**

```
 BspEI     10          20          30          40          50
TCC GGA GGC AGC TAT ACA TAC TAC GTG GAC AGC GTC AAG GGC CGT TTC ACC ATT TCC
AGG CCT CCG TCG ATA TGT ATG ATG CAC CTG TCG CAG TTC CCG GCA AAG TGG TAA AGG
 S   G   G   S   Y   T   Y   Y   V   D   S   V   K   G   R   F   T   I   S>

XmaI
CGG GAC
GCC CTG
 R   D>
```

**gL2 cassette** **(SEQ ID NO:43)**

```
   AatII   10          20          30          40          50
GCG ACG TCC AGC CTG GAT AGC GGT GTG CCA AAA CGT TTC AGT GGC AGT CGC AGC GGT
CGC TGC AGG TCG GAC CTA TCG CCA CAC GGT TTT GCA AAG TCA CCG TCA GCG TCG CCA
 A   T   S   S   L   D   S   G   V   P   K   R   F   S   G   S   R   S   G>

 60          70          80 BsmBI    90
TCT GAC TAT ACC CTC ACA ATT TCG TCT CTC CAG
AGA CTG ATA TGG GAG TGT TAA AGC AGA GAG GTC
 S   D   Y   T   L   T   I   S   S   L   Q>
```

**gL3 cassette** **(SEQ ID NO:44)**

```
   PvuI    10          20          30          40          50
GGC GAT CGT GTG ACT ATT ACC TGT CGT GCC AGT CAG GAC ATC GCG GGT AGC CTG AAC
CCG CTA GCA CAC TGA TAA TGG ACA GCA CGG TCA GTC CTG TAG CGC CCA TCG GAC TTG
 G   D   R   V   T   I   T   C   R   A   S   Q   D   I   A   G   S   L   N>

 60          70          80          90          100      AatII
TGG TTG CAG CAA AAA CCG GGC AAA GCC ATC AAG CGC CTC ATC TAT GCG ACG TCC
ACC AAC GTC GTT TTT GGC CCG TTT CGG TAG TTC GCG GAG TAG ATA CGC TGC AGG
 W   L   Q   Q   K   P   G   K   A   I   K   R   L   I   Y   A   T   S>
```

Underlined residues represent changed amino acids compared to the parent sequence.

## Figure 14: Oligonucleotides pairs for gH2, gH3, gL2, gL3 construction

**gH2T (SEQ ID NO:45)**
CCGGGACAATGCAAAGAATACCCTTTACCTCCAGATGAACTCTCTCCGCGCAGAGGACACAGCAGT
CTATTACTGTGTACGGATCGGCGAAGA

**gH2B (SEQ ID NO:46)**
CGCGTCTTCGCCGATCCGTACACAGTAATAGACTGCTGTGTCCTCTGCGCGGAGAGAGTTCATCTG
GAGGTAAAGGGTATTCTTTGCATTGTC

**gH3T (SEQ ID NO:47)**
CCGGAGGCAGCTATACATACTACGTGGACAGCGTCAAGGGCCGTTTCACCATTTC

**gH3B (SEQ ID NO:48)**
CCGGGAAATGGTGAAACGGCCCTTGACGCTGTCCACGTAGTATGTATAGCTGCCT

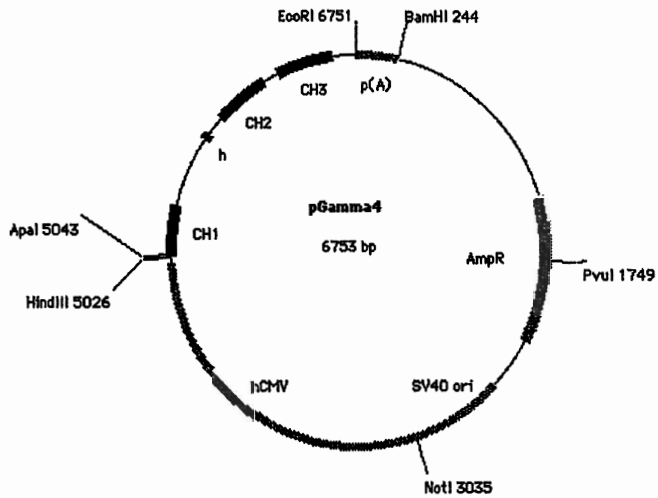
**gL2T (SEQ ID NO:49)**
CCAGCCTGGATAGCGGTGTGCCAAAACGTTTCAGTGGCAGTCGCAGCGGTTCTGACTATACCCTC
ACAATTTCGTCTCT

**gL2B (SEQ ID NO:50)**
CTGGAGAGACGAAATTGTGAGGGTATAGTCAGAACCGCTGCGACTGCCACTGAAACGTTTTGGCA
CACCGCTATCCAGGCTGGACGT

**gL3T (SEQ ID NO:51)**
CGTGTGACTATTACCTGTCGTGCCAGTCAGGACATCGCGGGTAGCCTGAACTGGTTGCAGCAAAA
ACCGGGCAAAGCCATCAAGCGCCTCATCTATGCGACGT

**gH3B (SEQ ID NO:52)**
CGCATAGATGAGGCGCTTGATGGCTTTGCCCGGTTTTTGCTGCAACCAGTTCAGGCTACCCGCGAT
GTCCTGACTGGCACGACAGGTAATAGTCACACGAT

**Figure 15: CHO expression plasmids**



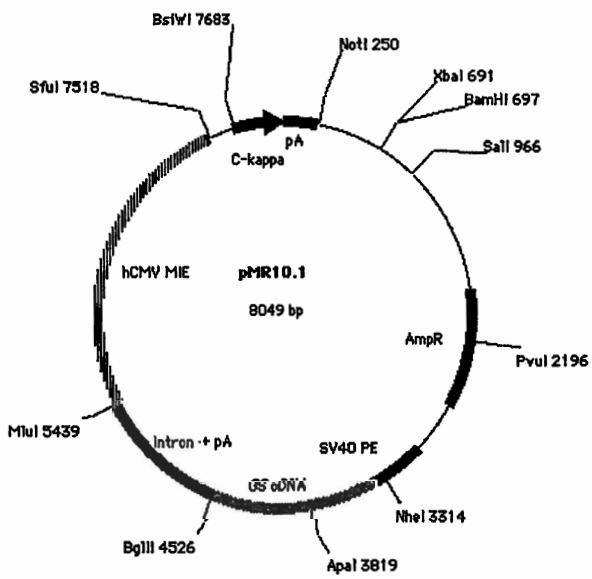
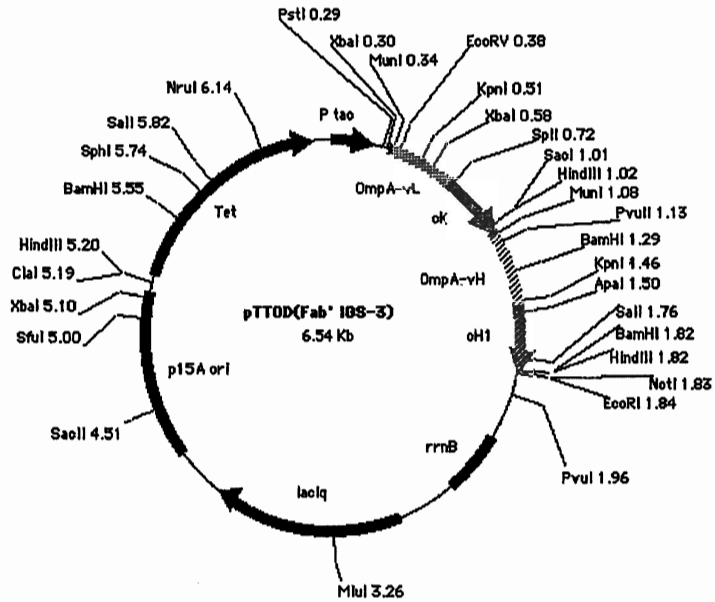



**Figure 16: pTTOD(Fab' IGS-3) map**



## Figure 17: IGS Sequences

**(SEQ ID NO:53)**

**IGS-1;** Intergenic space = -1

G,AGC,TCA,CCA,GTA,ACA,AAA,AGT,TTT,AAT,AGA,GGA,GAG,TGT,TAATG,AAG,AAG,ACT,GCT,ATA,GCA,ATT,G

S S P V T K S F N R G E C * M K K T A I A I

*End of c-Kappa sequence ->* *Start of OmpA sequence ->*

**(SEQ ID NO:54)**

**IGS-2;** Intergenic space = +1

G,AGC,TCA,CCA,GTA,ACA,AAA,AGT,TTT,AAT,AGA,GGG,GAG,TGT,TAA AATG,AAG,AAG,ACT,GCT,ATA,GCA,ATT,G

S S P V T K S F N R G E C * M K K T A I A I

**(SEQ ID NO:55)**

**IGS-3;** Intergenic space = +13

G,AGC,TCA,CCA,GTA,ACA,AAA,AGC,TTT,AAT,AGA,GGA,GAG,TGT,TGA GGAGGAAAAAAAAATG,AAG,AAA,ACT,GCT,ATA,GCA,ATT,G

S S P V T K S F N R G E C * M K K T A I A I

**Figure 18: Fermentation of Fab'**

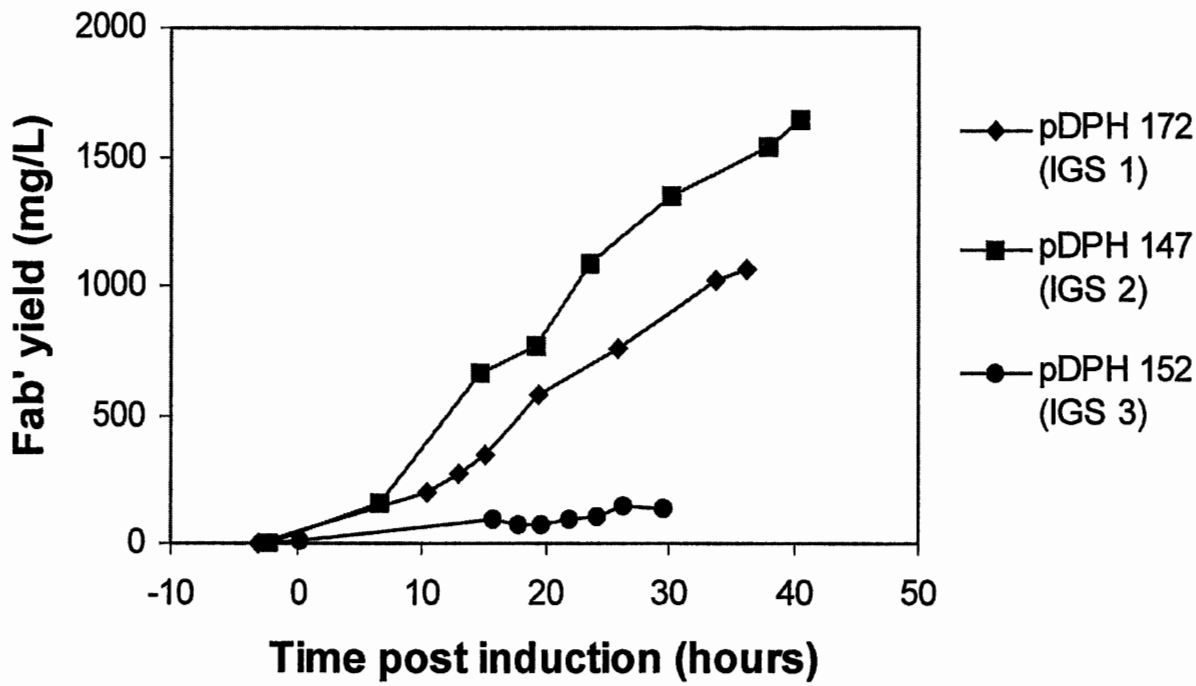


**Figure 19: Coding and flanking sequence of CDP791 Fab' (SEQ ID NO:56)**

```
        10         20         30         40         50         60         70
AATTCTCATG TTTGACAGCT TATCATCGAC TGCACGGTGC ACCAATGCTT CTGGCGTCAG GCAGCCATCG
TTAAGAGTAC AAACTGTCGA ATAGTAGCTG ACGTGCCACG TGGTTACGAA GACCGCAGTC CGTCGGTAGC

        80         90        100        110        120        130        140
GAAGCTGTGG TATGGCTGTG CAGGTCGTAA ATCACTGCAT AATTCGTGTC GCTCAAGGCG CACTCCCGTT
CTTCGACACC ATACCGACAC GTCCAGCATT TAGTGACGTA TTAAGCACAG CGAGTTCCGC GTGAGGGCAA

       150        160        170        180        190        200        210
CTGGATAATG TTTTTTGCGC CGACATCATA ACGGTTCTGG CAAATATTCT GAAATGAGCT GTTGACAATT
GACCTATTAC AAAAAACGCG GCTGTAGTAT TGCCAAGACC GTTTATAAGA CTTTACTCGA CAACTGTTAA

       220        230        240        250        260        270        280
AATCATCGGC TCGTATAATG TGTGGAATTG TGAGCGGATA ACAATTTCAC ACAGGAAACA GCGATGAGCT
TTAGTAGCCG AGCATATTAC ACACCTTAAC ACTCGCCTAT TGTTAAAGTG TGTCCTTTGT CGCTACTCGA

       290        300        310        320        330        340
TGGCTGCAGG TCGAGTTCTA GATAACGAGG CGTAAAAA ATG AAA AAG ACA GCT ATC GCA ATT GCA
ACCGACGTCC AGCTCAAGAT CTATTGCTCC GCATTTTT TAC TTT TTC TGT CGA TAG CGT TAA CGT
                                      M   K   K   T   A   I   A   I   A>

   350        360        370        380        390        400
GTG GCC TTG GCT GGT TTC GCT ACC GTA GCG CAA GCT GAT ATC CAG ATG ACC CAG AGT
CAC CGG AAC CGA CCA AAG CGA TGG CAT CGC GTT CGA CTA TAG GTC TAC TGG GTC TCA
 V   A   L   A   G   F   A   T   V   A   Q   A   D   I   Q   M   T   Q   S>

       410        420        430        440        450
CCA AGC AGT CTC TCC GCC AGC GTA GGC GAT CGT GTG ACT ATT ACC TGT CGT GCC AGT
GGT TCG TCA GAG AGG CGG TCG CAT CCG CTA GCA CAC TGA TAA TGG ACA GCA CGG TCA
 P   S   S   L   S   A   S   V   G   D   R   V   T   I   T   C   R   A   S>

460        470        480        490        500        510
CAG GAC ATC GCG GGT AGC CTG AAC TGG TTG CAG CAA AAA CCG GGC AAA GCC ATC AAG
GTC CTG TAG CGC CCA TCG GAC TTG ACC AAC GTC GTT TTT GGC CCG TTT CGG TAG TTC
 Q   D   I   A   G   S   L   N   W   L   Q   Q   K   P   G   K   A   I   K>

   520        530        540        550        560        570
CGC CTC ATC TAT GCG ACG TCC AGC CTG GAT AGC GGT GTG CCA AAA CGT TTC AGT GGC
GCG GAG TAG ATA CGC TGC AGG TCG GAC CTA TCG CCA CAC GGT TTT GCA AAG TCA CCG
 R   L   I   Y   A   T   S   S   L   D   S   G   V   P   K   R   F   S   G>

       580        590        600        610        620        630
AGT CGC AGC GGT TCT GAC TAT ACC CTC ACA ATT TCG TCT CTC CAG CCG GAA GAT TTC
TCA GCG TCG CCA AGA CTG ATA TGG GAG TGT TAA AGC AGA GAG GTC GGC CTT CTA AAG
 S   R   S   G   S   D   Y   T   L   T   I   S   S   L   Q   P   E   D   F>

           640        650        660        670        680
GCC ACT TAC TAT TGT CTG CAA TAT GGC AGC TTC CCT CCG ACC TTC GGT CAG GGC ACT
CGG TGA ATG ATA ACA GAC GTT ATA CCG TCG AAG GGA GGC TGG AAG CCA GTC CCG TGA
 A   T   Y   Y   C   L   Q   Y   G   S   F   P   P   T   F   G   Q   G   T>

690        700        710        720        730        740
AAA GTA GAA ATC AAA CGT ACG GTA GCG GCC CCA TCT GTC TTC ATC TTC CCG CCA TCT
TTT CAT CTT TAG TTT GCA TGC CAT CGC CGG GGT AGA CAG AAG TAG AAG GGC GGT AGA
 K   V   E   I   K   R   T   V   A   A   P   S   V   F   I   F   P   P   S>

    750        760        770        780        790        800
GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT
CTA CTC GTC AAC TTT AGA CCT TGA CGG AGA CAA CAC ACG GAC GAC TTA TTG AAG ATA
 D   E   Q   L   K   S   G   T   A   S   V   V   C   L   L   N   N   F   Y>

       810        820        830        840        850
CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC
GGG TCT CTC CGG TTT CAT GTC ACC TTC CAC CTA TTG CGG GAG GTT AGC CCA TTG AGG
 P   R   E   A   K   V   Q   W   K   V   D   N   A   L   Q   S   G   N   S>

860        870        880        890        900        910
CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC
GTC CTC TCA CAG TGT CTC GTC CTG TCG TTC CTG TCG TGG ATG TCG GAG TCG TCG TGG
 Q   E   S   V   T   E   Q   D   S   K   D   S   T   Y   S   L   S   S   T>
```

```
   920          930          940          950          960          970
CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC
GAC TGC GAC TCG TTT CGT CTG ATG CTC TTT GTG TTT CAG ATG CGG ACG CTT CAG TGG
 L   T   L   S   K   A   D   Y   E   K   H   K   V   Y   A   C   E   V   T>

       980          990         1000         1010         1020         1030
CAT CAG GGC CTG AGC TCA CCA GTA ACA AAA AGT TTT AAT AGA GGG GAG TGT TAA A ATG
GTA GTC CCG GAC TCG AGT GGT CAT TGT TTT TCA AAA TTA TCT CCC CTC ACA ATT T TAC
 H   Q   G   L   S   S   P   V   T   K   S   F   N   R   G   E   C   *>
                                                                       M>

         1040         1050         1060         1070         1080
AAG AAG ACT GCT ATA GCA ATT GCA GTG GCG CTA GCT GGT TTC GCC ACC GTG GCG CAA
TTC TTC TGA CGA TAT CGT TAA CGT CAC CGC GAT CGA CCA AAG CGG TGG CAC CGC GTT
 K   K   T   A   I   A   I   A   V   A   L   A   G   F   A   T   V   A   Q>

1090         1100         1110         1120         1130         1140
GCT GAG GTT CAG CTG GTC GAG TCT GGA GGC GGG CTT GTC CAG CCT GGA GGG AGC CTG
CGA CTC CAA GTC GAC CAG CTC AGA CCT CCG CCC GAA CAG GTC GGA CCT CCC TCG GAC
 A   E   V   Q   L   V   E   S   G   G   G   L   V   Q   P   G   G   S   L>

   1150         1160         1170         1180         1190         1200
CGT CTC TCT TGT GCA GCA AGC GGC TTC ACC TTT TCC TCT TAC GGT ATG TCC TGG GTG
GCA GAG AGA ACA CGT CGT TCG CCG AAG TGG AAA AGG AGA ATG CCA TAC AGG ACC CAC
 R   L   S   C   A   A   S   G   F   T   F   S   S   Y   G   M   S   W   V>

       1210         1220         1230         1240         1250
CGG CAG GCA CCT GGG AAG GGC CTG GAG TGG GTG GCA ACC ATT ACG TCC GGA GGC AGC
GCC GTC CGT GGA CCC TTC CCG GAC CTC ACC CAC CGT TGG TAA TGC AGG CCT CCG TCG
 R   Q   A   P   G   K   G   L   E   W   V   A   T   I   T   S   G   G   S>

1260         1270         1280         1290         1300         1310
TAT ACA TAC TAC GTG GAC AGC GTC AAG GGC CGT TTC ACC ATT TCC CGG GAC AAT GCA
ATA TGT ATG ATG CAC CTG TCG CAG TTC CCG GCA AAG TGG TAA AGG GCC CTG TTA CGT
 Y   T   Y   Y   V   D   S   V   K   G   R   F   T   I   S   R   D   N   A>

  1320         1330         1340         1350         1360         1370
AAG AAT ACC CTT TAC CTC CAG ATG AAC TCT CTC CGC GCA GAG GAC ACA GCA GTC TAT
TTC TTA TGG GAA ATG GAG GTC TAC TTG AGA GAG GCG CGT CTC CTG TGT CGT CAG ATA
 K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D   T   A   V   Y>

       1380         1390         1400         1410         1420
TAC TGT GTA CGG ATC GGC GAA GAC GCG TTG GAC TAC TGG GGA CAG GGG ACC CTT GTG
ATG ACA CAT GCC TAG CCG CTT CTG CGC AAC CTG ATG ACC CCT GTC CCC TGG GAA CAC
 Y   C   V   R   I   G   E   D   A   L   D   Y   W   G   Q   G   T   L   V>

1430         1440         1450         1460         1470         1480
ACA GTC TCC TCT GCT TCT ACA AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC
TGT CAG AGG AGA CGA AGA TGT TTC CCG GGT AGC CAG AAG GGG GAC CGT GGG AGG AGG
 T   V   S   S   A   S   T   K   G   P   S   V   F   P   L   A   P   S   S>

  1490         1500         1510         1520         1530         1540
AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC
TTC TCG TGG AGA CCC CCG TGT CGC CGG GAC CCG ACG GAC CAG TTC CTG ATG AAG GGG
 K   S   T   S   G   G   T   A   A   L   G   C   L   V   K   D   Y   F   P>

       1550         1560         1570         1580         1590         1600
GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC
CTT GGC CAC TGC CAC AGC ACC TTG AGT CCG CGG GAC TGG TCG CCG CAC GTG TGG AAG
 E   P   V   T   V   S   W   N   S   G   A   L   T   S   G   V   H   T   F>

         1610         1620         1630         1640         1650
CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC
GGC CGA CAG GAT GTC AGG AGT CCT GAG ATG AGG GAG TCG TCG CAC CAC TGG CAC GGG
 P   A   V   L   Q   S   S   G   L   Y   S   L   S   S   V   V   T   V   P>

1660         1670         1680         1690         1700         1710
TCC AGC AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC
AGG TCG TCG AAC CCG TGG GTC TGG ATG TAG ACG TTG CAC TTA GTG TTC GGG TCG TTG
 S   S   S   L   G   T   Q   T   Y   I   C   N   V   N   H   K   P   S   N>

   1720         1730         1740         1750         1760         1770
ACC AAG GTC GAC AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC GCC
TGG TTC CAG CTG TTC TTT CAA CTC GGG TTT AGA ACA CTG TTT TGA GTG TGT ACG CGG
 T   K   V   D   K   K   V   E   P   K   S   C   D   K   T   H   T   C   A>
```

```
      1780       1790       1800       1810       1820       1830       1840
GCG TGA TGA GGATCCAAGC TTGCGGCCGC GAATTCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA
CGC ACT ACT CCTAGGTTCG AACGCCGGCG CTTAAGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT
 A   *>

      1850       1860       1870       1880       1890       1900       1910
AACCCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTCGC CAGCTCGCGT AATAGCGAAG
TTGGGACCGC AATGGGTTGA ATTAGCGGAA CGTCGTGTAG GGGGAAAGCG GTCGAGCGCA TTATCGCTTC

      1920       1930       1940       1950       1960       1970       1980
AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGCAGCCT GAATGGCGAA TGGCGCCTGA TGCGGTATTT
TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCGTCGGA CTTACCGCTT ACCGCGGACT ACGCCATAAA

      1990       2000
TCTCCTTACG CATCTGTGCG
AGAGGAATGC GTAGACACGC
```

**Figure 20: Radioimmunoassay Results**

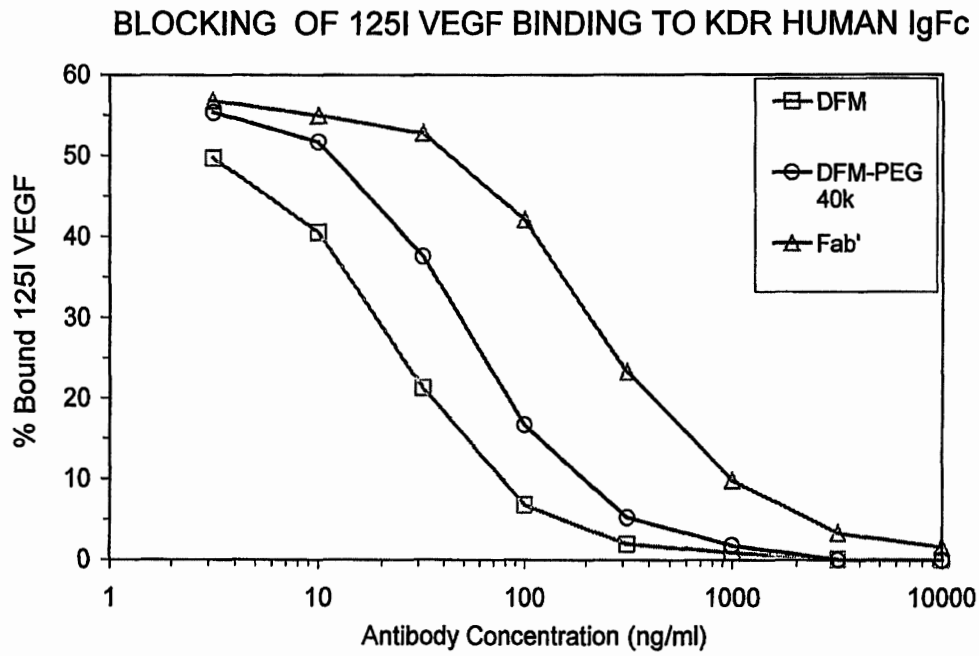


Figure 21: Protein sequence of gH3-grafted heavy chain

**Heavy Chain antibody sequence (SEQ ID NO:57)**

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATITSGGSY
TYYVDSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCVRIGEDALDYWGQGTLVT
VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPA
PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE
PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK*