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(54) **FAPALPHA -SPECIFIC ANTIBODY WITH IMPROVED PRODUCIBILITY**

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Related U.S. Application Data

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(30) **Foreign Application Priority Data**

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(57) **ABSTRACT**

Recombinant antibody proteins are provided that specifically bind fibroblast activation protein alpha (FAPα) and comprise framework modifications resulting in the improved producibility in host cells. The invention also relates to the use of said antibodies for diagnostic and therapeutic purposes and methods of producing said antibodies.

```
1           11          21          31          41
GACATTGTGA TGACCCAATC TCCAGACTCT TTGGCTGTGT CTCTAGGGGA
51          61          71          81          91
GAGGGCCACC ATCAACTGCA AGTCCAGTCA GAGCCTTTTA TATTCTAGAA
101         111         121         131         141
ATCAAAAGAA CTACTTGGCC TGGTATCAGC AGAAACCAGG ACAGCCACCC
151         161         171         181         191
AAACTCCTCA TCTTTTGGGC TAGCACTAGG GAATCTGGGG TACCTGATAG
201         211         221         231         241
GTTCAGTGGC AGTGGGTTTG GGACAGACTT CACCCTCACC ATTAGCAGCC
251         261         271         281         291
TGCAGGCTGA AGATGTGGCA GTTTATTACT GTCAGCAATA TTTTAGCTAT
301         311         321         331   339
CCGCTCACGT TCGGACAAGG GACCAAGGTG GAAATAAAA
```

FIG.1

```
1           11          21          31          41
DIVMTQSPDS LAVSLGERAT INCKSSQSLL YSRNQKNYLA WYQQKPGQPP
51          61          71          81          91
KLLIFWASTR ESGVPDRFSG SGFGTDFTLT ISSLQAEDVA VYYCQQYFSY
101         111
PLTFGQGTKV EIK
```

FIG.2

```
1           11          21          31          41
GACATTGTGA TGACCCAATC TCCAGACTCT TTGGCTGTGT CTCTAGGGGA
51          61          71          81          91
GAGGGCCACC ATCAACTGCA AGTCCAGTCA GAGCCTTTTA TATTCTAGAA
101         111         121         131         141
ATCAAAAGAA CTACTTGGCC TGGTTCCAGC AGAAACCAGG ACAGCCACCC
151         161         171         181         191
AAACTCCTCA TCTTTTGGGC TAGCACTAGG GAATCTGGGG TACCTGATAG
201         211         221         231         241
GTTCAGTGGC AGTGGGTTTG GGACAGACTT CACCCTCACC ATTAGCAGCC
251         261         271         281         291
TGCAGGCTGA AGATGTGGCA GTTTATGACT GTCAACAATA TTTTAGCTAT
301         311         321         331   339
CCGCTCACGT TCGGACAAGG GACCAAGGTG GAAATAAAA
```

FIG.3

```
1           11          21          31          41
DIVMTQSPDS  LAVSLGERAT  INCKSSQSLL  YSRNQKNYLA  WFQQKPGQPP
51          61          71          81          91
KLLIFWASTR  ESGVPDRFSG  SGFGTDFTLT  ISSLQAEDVA  VYDCQQYFSY
101         111
PLTFGQGTKV  EIK
```

FIG.4

```
1           11          21          31          41
GACATTGTGA  TGACCCAATC  TCCAGACTCT  TTGGCTGTGT  CTCTAGGGGA
51          61          71          81          91
GAGGGCCACC  ATCAACTGCA  AGTCCAGTCA  GAGCCTTTTA  TATTCTAGAA
101         111         121         131         141
ATCAAAAGAA  CTACTTGGCC  TGGTATCAGC  AGAAACCAGG  ACAGCCACCC
151         161         171         181         191
AAACTCCTCA  TCTATTGGGC  TAGCACTAGG  GAATCTGGGG  TACCTGATAG
201         211         221         231         241
GTTCAGTGGC  AGTGGGTTTG  GGACAGACTT  CACCCTCACC  ATTAGCAGCC
251         261         271         281         291
TGCAGGCTGA  AGATGTGGCA  GTTTATTACT  GTCAGCAATA  TTTTAGCTAT
301         311         321         331   339
CCGCTCACGT  TCGGACAAGG  GACCAAGGTG  GAAATAAAA
```

FIG.5

```
1           11          21          31          41
DIVMTQSPDS  LAVSLGERAT  INCKSSQSLL  YSRNQKNYLA  WYQQKPGQPP
51          61          71          81          91
KLLIYWASTR  ESGVPDRFSG  SGFGTDFTLT  ISSLQAEDVA  VYYCQQYFSY
101         111
PLTFGQGTKV  EIK
```

FIG.6

```
1
CAGGTGCAAC TAGTGCAGTC CGGCGCCGAA GTGAAGAAAC CCGGTGCTTC
51
CGTGAAAGTC AGCTGTAAAA CTAGTAGATA CACCTTCACT GAATACACCA
101
TACACTGGGT TAGACAGGCC CCTGGCCAAA GGCTGGAGTG GATAGGAGGT
151
ATTAATCCTA ACAATGGTAT TCCTAACTAC AACCAGAAGT TCAAGGGCCG
201
GGCCACCTTG ACCGTAGGCA AGTCTGCCAG CACCGCCTAC ATGGAACTGT
251
CCAGCCTGCG CTCCGAGGAC ACTGCAGTCT ACTACTGCGC CAGAAGAAGA
301
ATCGCCTATG GTTACGACGA GGGCCATGCT ATGGACTACT GGGGTCAAGG
351                   372
AACCCTTGTC ACCGTCTCCT CA
```

FIG.7

```
1          11         21         31         41
QVQLVQSGAE VKKPGASVKV SCKTSRYTFT EYTIHWVRQA PGQRLEWIGG
51         61         71         81         91
INPNNGIPNY NQKFKGRATL TVGKSASTAY MELSSLRSED TAVYYCARRR
101        111        121-124
IAYGYDEGHA MDYWGQGTLV TVSS
```

FIG.8

```
1
CAGGTGCAAC TAGTGCAGTC CGGCGCCGAA GTGAAGAAAC CCGGTGCTTC
51
CGTGAAAGTC AGCTGTAAAA CTAGTAGATA CACCTTCACT GAATACACCA
101
TACACTGGGT TAGACAGGCC CCTGGCCAAA GGCTGGAGTG GATAGGAGGT
151
ATTAATCCTA ACAATGGTAT TCCTAACTAC AACCAGAAGT TCAAGGGCCG
201
GGCCACCTTG ACCGTAGGCA AGTCTGCCAG CACCGCCTAC ATGGAACTGT
251
CCAGCCTGCG CTCCGAGGAC ACTGCAGTCT ACTICTGCGC CAGAAGAAGA
301
ATCGCCTATG GTTACGACGA GGGCCATGCT ATGGACTACT GGGGTCAAGG
351                   372
AACCCTTGTC ACCGTCTCCT CA
```

FIG.9

```
1           11          21          31          41
QVQLVQSGAE VKKPGASVKV SCKTSRYTFT EYTIHWVRQA PGQRLEWIGG
51          61          71          81          91
INPNNGIPNY NQKFKGRATL TVGKSASTAY MELSSLRSED TAVYFCARRR
101         111         121-124
IAYGYDEGHA MDYWGQGTLV TVSS
```

FIG.10

```
1
CAGGTGCAAC TAGTGCAGTC CGGCGCCGAA GTGAAGAAAC CCGGTGCTTC
51
CGTGAAAGTC AGCTGTAAAA CTAGTAGATA CACCTTCACT GAATACACCA
101
TACACTGGGT TAGACAGGCC CCTGGCCAAA GGCTGGAGTG GATAGGAGGT
151
ATTAATCCTA ACAATGGTAT TCCTAACTAC AACCAGAAGT TCAAGGGCCG
201
GGTCACCATC ACCGTAGACA CCTCTGCCAG CACCGCCTAC ATGGAACTGT
251
CCAGCCTGCG CTCCGAGGAC ACTGCAGTCT ACTACTGCGC CAGAAGAAGA
301
ATCGCCTATG GTTACGACGA GGGCCATGCT ATGGACTACT GGGGTCAAGG
351                     372
AACCCTTGTC ACCGTCTCCT CA
```

FIG.11

```
1           11          21          31          41
QVQLVQSGAE VKKPGASVKV SCKTSRYTFT EYTIHWVRQA PGQRLEWIGG
51          61          71          81          91
INPNNGIPNY NQKFKGRVTI TVDTSASTAY MELSSLRSED TAVYYCARRR
101         111         121-124
IAYGYDEGHA MDYWGQGTLV TVSS
```

FIG.12

```
1
CAGGTGCAAC TAGTGCAGTC CGGCGCCGAA GTGAAGAAAC CCGGTGCTTC
51
CGTGAAAGTC AGCTGTAAAA CTAGTAGATA CACCTTCACT GAATACACCA
101
TACACTGGGT TAGACAGGCC CCTGGCCAAA GGCTGGAGTG GATAGGAGGT
151
ATTAATCCTA ACAATGGTAT TCCTAACTAC AACCAGAAGT TCAAGGGCCG
201
GGTCACCATC ACCGTAGACA CCTCTGCCAG CACCGCCTAC ATGGAACTGT
251
CCAGCCTGCG CTCCGAGGAC ACTGCAGTCT ACTTCTGCGC CAGAAGAAGA
301
ATCGCCTATG GTTACGACGA GGGCCATGCT ATGGACTACT GGGGTCAAGG
351                   372
AACCCTTGTC ACCGTCTCCT CA
```

FIG.13

```
1          11         21         31         41
QVQLVQSGAE VKKPGASVKV SCKTSRYTFT EYTIHWVRQA PGQRLEWIGG
51         61         71         81         91
INPNNGIPNY NQKFKGRVTI TVDTSASTAY MELSSLRSED TAVYFCARRR
101        111        121-124
IAYGYDEGHA MDYWGQGTLV TVSS
```

FIG.14

```
1
CAGGTGCAAC TAGTGCAGTC CGGCGCCGAA GTGAAGAAAC CCGGTGCTTC
51
CGTGAAAGTC AGCTGTAAAA CTAGTGGATA CACCTTCACT GAATACACCA
101
TACACTGGGT TAGACAGGCC CCTGGCCAAA GGCTGGAGTG GATAGGAGGT
151
ATTAATCCTA ACAATGGTAT TCCTAACTAC AACCAGAAGT TCAAGGGCCG
201
GGTCACCATC ACCGTAGACA CCTCTGCCAG CACCGCCTAC ATGGAACTGT
251
CCAGCCTGCG CTCCGAGGAC ACTGCAGTCT ACTACTGCGC CAGAAGAAGA
301
ATCGCCTATG GTTACGACGA GGGCCATGCT ATGGACTACT GGGGTCAAGG
351                   372
AACCCTTGTC ACCGTCTCCT CA
```

FIG.15

```
1           11          21          31          41
QVQLVQSGAE VKKPGASVKV SCKTSGYTFT EYTIHWVRQA PGQRLEWIGG
51          61          71          81          91
INPNNGIPNY NQKFKGRVTI TVDTSASTAY MELSSLRSED TAVYYCARRR
101         111         121-124
IAYGYDEGHA MDYWGQGTLV TVSS
```

FIG.16

```
1
DIVMSQSPSS LAVSVGEKVT MSCKSSQSLL YSRNQKNYLA WFQQKPGQSP
51
KLLIFWASTR ESGVPDRFTG SGFGTDFNLT ISSVQAEDLA VYDCQQYFSY
101
PLTFGAGTKL ELKRTVAAPS VFIFPPSDEQ LKSGTASVVC LLNNFYPREA
151
KVQWKVDNAL QSGNSQESVT EQDSKDSTYS LSSTLTLSKA DYEKHKVYAC
201
EVTHQGLSSP VTKSFNRGEC
```

FIG.17

```
1
VQLQQSGPEL VKPGASVKMS CKTSRYTFTE YTIHWVRQSH GKSLEWIGGI
51
NPNNGIPNYN QKFKGRATLT VGKSSSTAYM ELRSLTSEDS AVYFCARRRI
101
AYGYDEGHAM DYWGQGTSVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC
151
LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG
201
TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP
251
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
301
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
351
EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT
401
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
451
PGK
```

FIG.18

```
340         350         360         370         380
CGTACTGTGG CTGCACCATC TGTCTTCATC TTCCCGCCAT CTGATGAGCA
390         400         410         420         430
GTTGAAATCT GGAACTGCCT CTGTTGTGTG CCTGCTGAAT AACTTCTATC
440         450         460         470         480
CCAGAGAGGC CAAAGTACAG TGGAAGGTGG ATAACGCCCT CCAATCGGGT
490         500         510         520         530
AACTCCCAGG AGAGTGTCAC AGAGCAGGAC AGCAAGGACA GCACCTACAG
540         550         560         570         580
CCTCAGCAGC ACCCTGACGC TGAGCAAAGC AGACTACGAG AAACACAAAG
590         600         610         620         630
TCTACGCCTG CGAAGTCACC CATCAGGGCC TGAGCTCGCC CGTCACAAAG
640         650         660
AGCTTCAACA GGGGAGAGTGT
```

FIG.19

```
114         124         134         144         154
RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
164         174         184         194         204
NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK
214-220
SFNRGEC
```

FIG.20

```
373
GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCT CCTCCAAGAG
423
CACCTCTGGG GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC
473
CCGAACCGGT GACGGTGTCG TGGAACTCAG GCGCCCTGAC CAGCGGCGTG
523
CACACCTTCC CGGCTGTCCT ACAGTCCTCA GGACTCTACT CCCTCAGCAG
573
CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC TACATCTGCA
623
ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC
673
AAATCTTGTG ACAAAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT
723
CCTGGGGGGA CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC
773
TCATGATCTC CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC
823
```

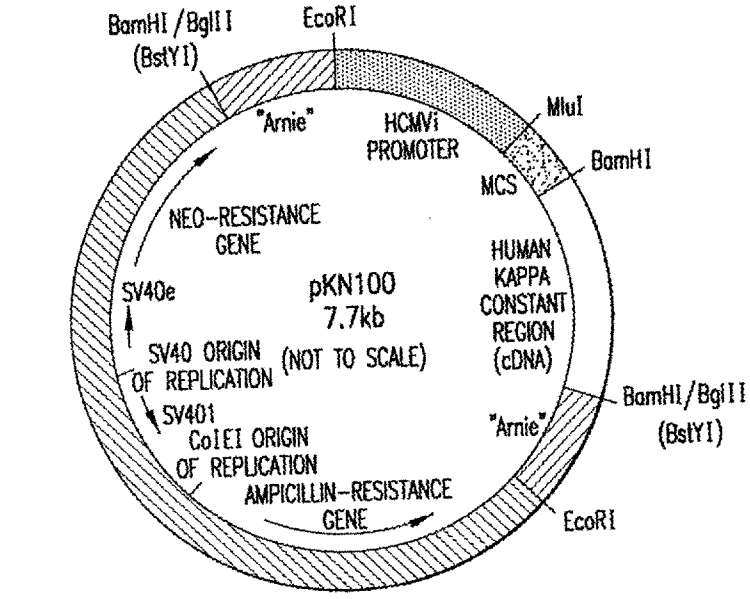
FIG.21A

```
CACGAAGACC CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT
873
GCATAATGCC AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC
923
GGGTGGTCAG CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG
973
GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA
1023
AACCATCTCC AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC
1073
TGCCCCCATC CCGGGAGGAG ATGACCAAGA ACCAGGTCAG CCTGACCTGC
1123
CTGGTCAAAG GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA
1173
TGGGCAGCCG GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG
1223
ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA CCGTGGACAA GAGCAGGTGG
1273
CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA
1323                                        1362
CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA
```

FIG.21B

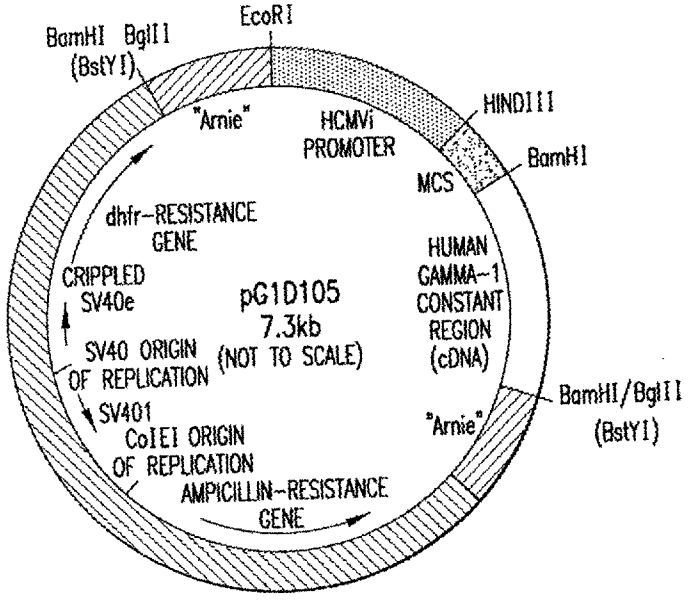
```
125
ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
175
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP
225
KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS
275
HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
325
EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC
375
LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
425                              454
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
```

FIG.22



HCMVi ENHANCER/PROMOTER/INTRON ELEMENTS
MULTIPLE CLONING SITE: 5' MluI-HindIII-SalI-XbaI-BamHI 3'
spaC2 TERMINATION SIGNAL SEQUENCE (ALSO KNOWN AS "Arnie")
pSV2neo VECTOR FRAGMENT

FIG. 23A



HCMVi ENHANCER/PROMOTER/INTRON ELEMENTS
MULTIPLE CLONING SITE: 5'MluI-HINDIII-Sse8387I-PmeI-SalI-XbaI-PacI-BamHI 3'
spaC2 TERMINATION SIGNAL SEQUENCE (ALSO KNOWN AS "Arnie")
pSV2dhfr VECTOR FRAGMENT

FIG. 23B

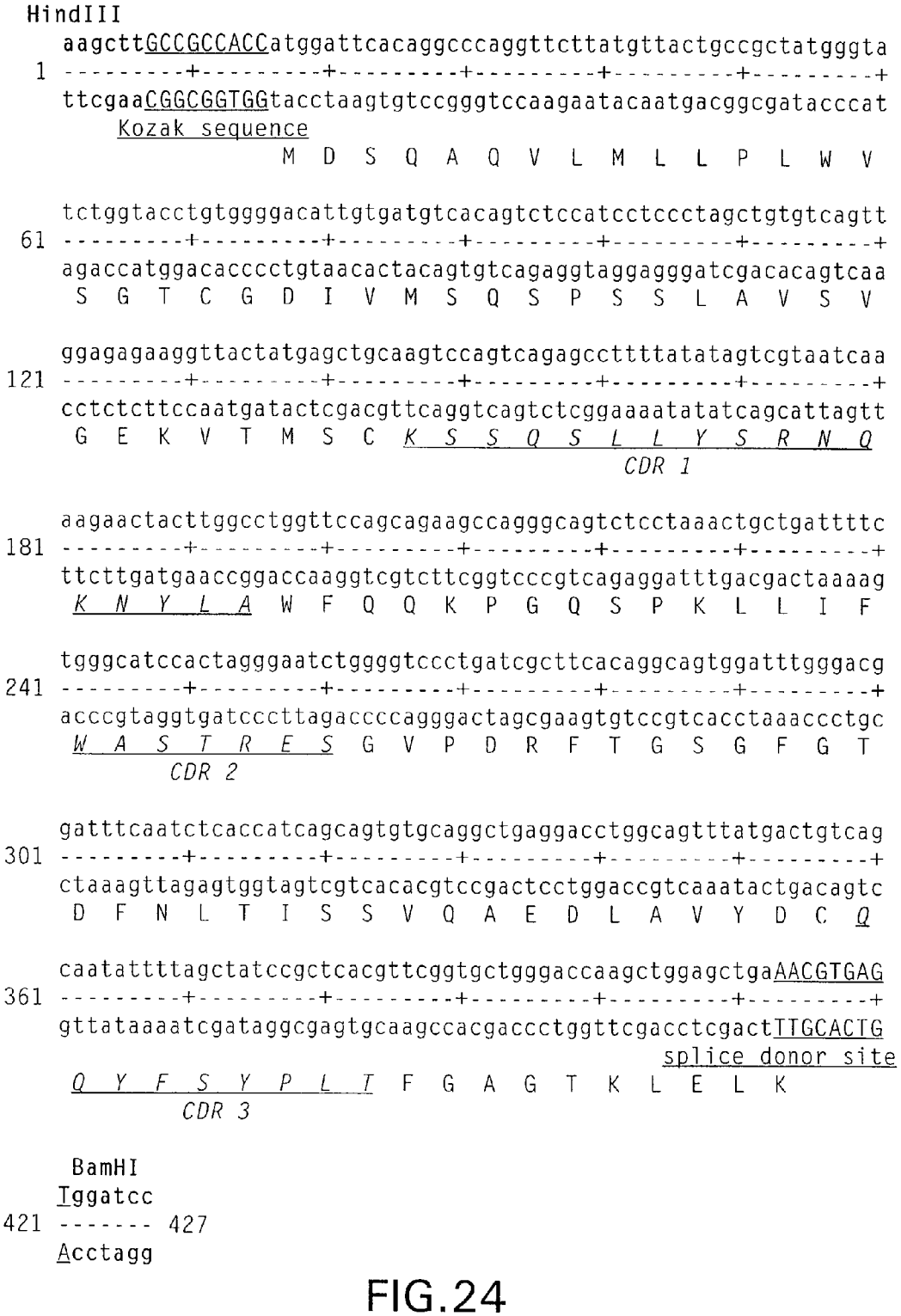

HindIII
aagcttGCCGCCACCatggattcacaggcccaggttcttatgttactgccgctatgggta
1
ttcgaaCGGCGGTGGtacctaagtgtccgggtccaagaatacaatgacggcgatacccat
Kozak sequence
M D S Q A Q V L M L L P L W V
tctggtacctgtggggacattgtgatgtcacagtctccatcctccctagctgtgtcagtt
61
agaccatggacacccctgtaacactacagtgtcagaggtaggagggatcgacacagtcaa
S G T C G D I V M S Q S P S S L A V S V
ggagagaaggttactatgagctgcaagtccagtcagagccttttatatagtcgtaatcaa
121
cctctcttccaatgatactcgacgttcaggtcagtctcggaaaatatatcagcattagtt
G E K V T M S C K S S Q S L L Y S R N Q
CDR 1
aagaactacttggcctggttccagcagaagccagggcagtctcctaaactgctgattttc
181
ttcttgatgaaccggaccaaggtcgtcttcggtcccgtcagaggatttgacgactaaaag
K N Y L A W F Q Q K P G Q S P K L L I F
tgggcatccactagggaatctggggtccctgatcgcttcacaggcagtggatttgggacg
241
acccgtaggtgatcccttagaccccagggactagcgaagtgtccgtcacctaaaccctgc
W A S T R E S G V P D R F T G S G F G T
CDR 2
gatttcaatctcaccatcagcagtgtgcaggctgaggacctggcagtttatgactgtcag
301
ctaaagttagagtggtagtcgtcacacgtccgactcctggaccgtcaaatactgacagtc
D F N L T I S S V Q A E D L A V Y D C Q
caatattttagctatccgctcacgttcggtgctgggaccaagctggagctgaAACGTGAG
361
gttataaaatcgataggcgagtgcaagccacgaccctggttcgacctcgactTTGCACTG
splice donor site
Q Y F S Y P L T F G A G T K L E L K
CDR 3
BamHI
Iggatcc
421 427
Acctagg

FIG.24

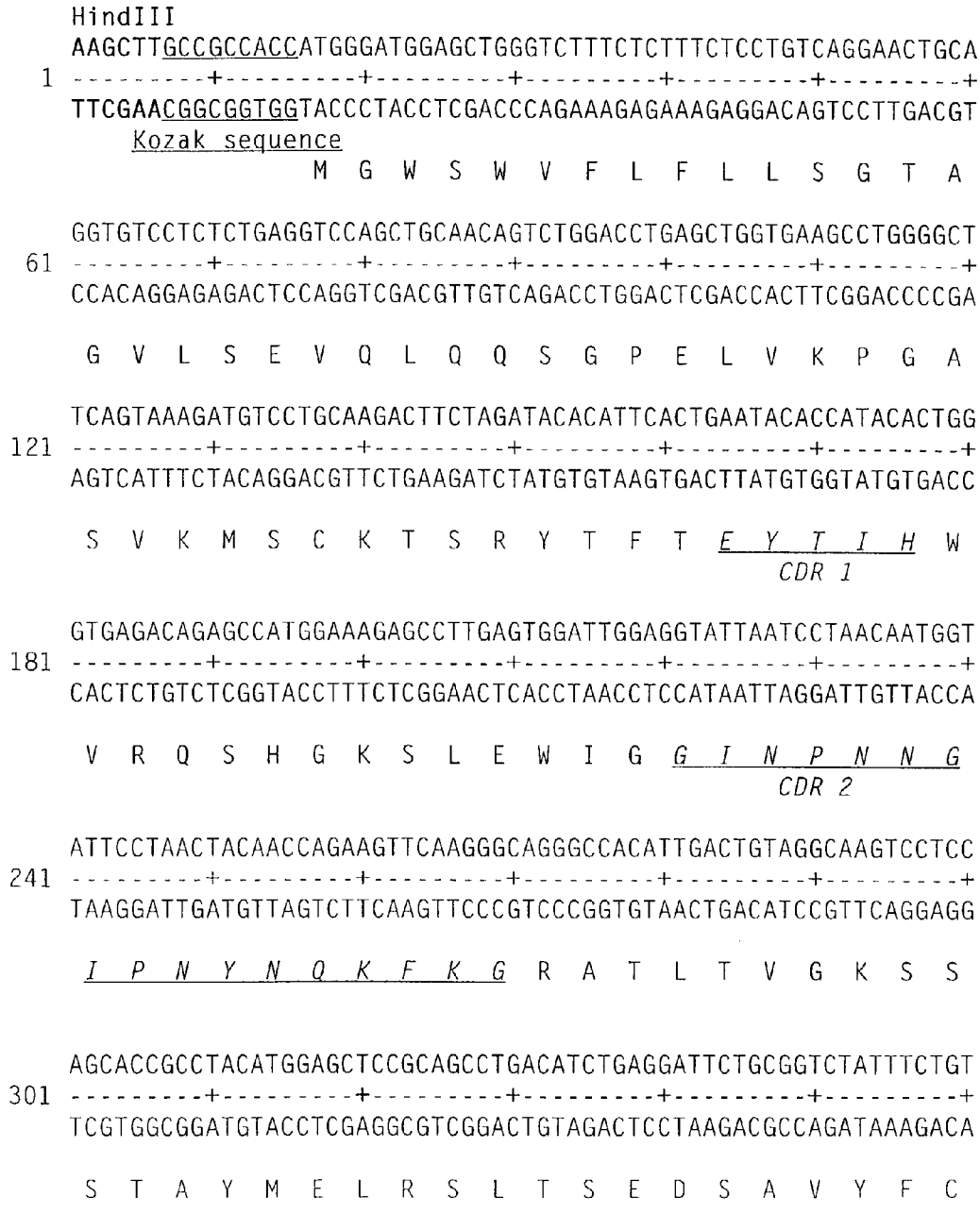

HindIII
AAGCTTGCCGCCACCATGGGATGGAGCTGGGTCTTTCTCTTTCTCCTGTCAGGAACTGCA
TTCGAACGGCGGTGGTACCCTACCTCGACCCAGAAAGAGAAAGAGGACAGTCCTTGACGT
Kozak sequence
M G W S W V F L F L L S G T A
GGTGTCCTCTCTGAGGTCCAGCTGCAACAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCT
CCACAGGAGAGACTCCAGGTCGACGTTGTCAGACCTGGACTCGACCACTTCGGACCCCGA
G V L S E V Q L Q Q S G P E L V K P G A
TCAGTAAAGATGTCCTGCAAGACTTCTAGATACACATTCACTGAATACACCATACACTGG
AGTCATTTCTACAGGACGTTCTGAAGATCTATGTGTAAGTGACTTATGTGGTATGTGACC
S V K M S C K T S R Y T F T E Y T I H W
CDR 1
GTGAGACAGAGCCATGGAAAGAGCCTTGAGTGGATTGGAGGTATTAATCCTAACAATGGT
CACTCTGTCTCGGTACCTTTCTCGGAACTCACCTAACCTCCATAATTAGGATTGTTACCA
V R Q S H G K S L E W I G G I N P N N G
CDR 2
ATTCCTAACTACAACCAGAAGTTCAAGGGCAGGGCCACATTGACTGTAGGCAAGTCCTCC
TAAGGATTGATGTTAGTCTTCAAGTTCCCGTCCCGGTGTAACTGACATCCGTTCAGGAGG
I P N Y N Q K F K G R A T L T V G K S S
AGCACCGCCTACATGGAGCTCCGCAGCCTGACATCTGAGGATTCTGCGGTCTATTTCTGT
TCGTGGCGGATGTACCTCGAGGCGTCGGACTGTAGACTCCTAAGACGCCAGATAAAGACA
S T A Y M E L R S L T S E D S A V Y F C

FIG.25A

```
    GCAAGAAGAAGAATCGCCTATGGTTACGACGAGGGCCATGCTATGGACTACTGGGGTCAA
361 ---------+---------+---------+---------+---------+---------+
    CGTTCTTCTTCTTAGCGGATACCAATGCTGCTCCCGGTACGATACCTGATGACCCCAGTT

     A  R  R  R  I  A  Y  G  Y  D  E  G  H  A  M  D  Y  W  G  Q
                                 CDR 3
                                          BamHI
    GGAACCTCAGTCACCGTCTCCTCAGGTGAGTGGATCC
421 ---------+---------+---------+-------- 468
    CCTTGGAGTCAGTGGCAGAGGAGTCCACTCACCTAGG
                       splice donor site
     G  T  S  V  T  V  S  S
```

FIG.25B

```
                                    Spe I
  1  gaattccagc acactggcgg ccgttACTAG TTATTAATAG TAATCAATTA

 51  CGGGGTCATT AGTTCATAGC CCATATATGG AGTTCCGCGT TACATAACTT

101  ACGGTAAATG GCCCGCCTGG CTGACCGCCC AACGACCCCC GCCCATTGAC

151  GTCAATAATG ACGTATGTTC CCATAGTAAC GCCAATAGGG ACTTTCCATT

201  GACGTCAATG GGTGGAGTAT TTACGGTAAA CTGCCCACTT GGCAGTACAT

251  CAAGTGTATC ATATGCCAAG TACGCCCCCT ATTGACGTCA ATGACGGTAA

301  ATGGCCCGCC TGGCATTATG CCCAGTACAT GACCTTATGG GACTTTCCTA
                     SnaB I
351  CTTGGCAGTA CATCTACGTA TTAGTCATCG CTATTACCAT GGTGATGCGG

401  TTTTGGCAGT ACATCAATGG GCGTGGATAG CGGTTTGACT CACGGGGATT

451  TCCAAGTCTC CACCCCATTG ACGTCAATGG GAGTTTGTTT TGGCACCAAA

501  ATCAACGGGA CTTTCCAAAA TGTCGTAACA ACTCCGCCCC ATTGACGCAA

551  ATGGGCGGTA GGCGTGTACG GTGGGAGGTC TATATAAGCA GAGCTCGTTT

601  AGTGAACCGT CAGATCGCCT GGAGACGCCA TCCACGCTGT TTTGACCTCC
                                    Sac II
651  ATAGAAGACA CCGGGACCGA TCCAGCCTCC GCGGCCGGGA ACGGTGCATT

701  GGAACGCGGA TTCCCCGTGC CAAGAGTGAC GTAAGTACCG CCTATAGAGT
```

FIG.26A

```
751  CTATAGGCCC ACCCCCTTGG CTTCTTATGC ATGCTATACT GTTTTTGGCT

801  TGGGGTCTAT ACACCCCCGC TTCCTCATGT TATAGGTGAT GGTATAGCTT

851  AGCCTATAGG TGTGGGTTAT TGACCATTAT TGACCACTCC CCTATTGGTG

901  ACGATACTTT CCATTACTAA TCCATAACAT GGCTCTTTGC CACAACTCTC

951  TTTATTGGCT ATATGCCAAT ACACTGTCCT TCAGAGACTG ACACGGACTC

1001 TGTATTTTTA CAGGATGGGG TCTCATTTAT TATTTACAAA TTCACATATA

1051 CAACACCACC GTCCCCAGTG CCCGCAGTTT TTATTAAACA TAACGTGGGA
                                   BspE I
1101 TCTCCACGCG AATCTCGGGT ACGTGTTCCG GACATGGGCT CTTCTCCGGT

1151 AGCGGCGGAG CTTCTACATC CGAGCCCTGC TCCCATGCCT CCAGCGACTC

1201 ATGGTCGCTC GGCAGCTCCT TGCTCCTAAC AGTGGAGGCC AGACTTAGGC

1251 ACAGCACGAT GCCCACCACC ACCAGTGTGC CGCACAAGGC CGTGGCGGTA

1301 GGGTATGTGT CTGAAAATGA GCTCggggag cgggcttgca ccgctgacgc
                 Afl II
1351 atttggaaga cttaaggcag cggcagaaga agatgcaggc agctgagttg

1401 ttgtgttctg ataagagtca gaggtaactc ccgttgcggt gctgttaacg

1451 gtggagggca gtgtagtctg agcagtactc gttgctgccg cgcgccgccac

1501 cagacataat agctgacaga ctaacagact gttcctttcc atgggtcttt
                           Mlu I        Hind III
1551 tctgcagtca ccgtccttga cacgcgtctc gggaagcttG CCGCCACCAT
                                                      M
                                                   Kpn I
1601 GGATTCACAG GCCCAGGTTC TTATGTTACT GCCGCTATGG GTATCTGGTA
       D  S  Q   A  Q  V   L  M  L  L   P  L  W   V  S  G
1651 CCTGTGGGGA CATTGTGATG TCACAGTCTC CATCCTCCCT AGCTGTGTCA
     T  C  G  D   I  V  M   S  Q  S   P  S  S  L   A  V  S
1701 GTTGGAGAGA AGGTTACTAT GAGCTGCAAG TCCAGTCAGA GCCTTTTATA
      V  G  E   K  V  T  M   S  C  K  S  S  Q  S  L  L  Y
       XbaI                                  CDR 1
1751 TTCTAGAAAT CAAAAGAACT ACTTGGCCTG GTTCCAGCAG AAGCCAGGGC
       S  R  N   Q  K  N   Y  L  A  W   F  Q  Q   K  P  G

1801 AGTCTCCTAA ACTGCTGATT TTCTGGGCAT CCACTAGGGA ATCTGGGGTC
     Q  S  P  K   L  L  I   F  W  A   S  T  R  E   S  G  V
                                  CDR 2
```

FIG.26B

```
1851  CCTGATCGCT TCACAGGCAG TGGATTTGGG ACGGATTTCA ATCTCACCAT
        P  D  R   F  T  G  S   G  F  G   T  D  F   N  L  T  I

1901  CAGCAGTGTG CAGGCTGAGG ACCTGGCAGT TTATGACTGT CAGCAATATT
        S  S  V   Q  A  E   D  L  A  V   Y  D  C   Q  Q  Y

1951  TTAGCTATCC GCTCACGTTC GGTGCTGGGA CCAAGCTGGA GCTGAAACGT
      F  S  Y  P  L  T  F   G  A  G   T  K  L  E   L  K  R
            CDR 3
         BamH I
2001  GAGTggatcc ATCTGGGATA AGCATGCTGT TTTCTGTCTG TCCCTAACAT

2051  GCCCTGTGAT TATGCGCAAA CAACACACCC AAGGGCAGAA CTTTGTTACT

2101  TAAACACCAT CCTGTTIGCT TCTTTCCTCA GGAACTGTGG CTGCACCATC
                                          T  V   A  A  P  S
2151  TGTCTTCATC TTCCCGCCAT CTGATGAGCA GTTGAAATCT GGAACTGCCT
        V  F  I   F  P  P   S  D  E  Q   L  K  S   G  T  A
2201  CTGTTGTGTG CCTGCTGAAT AACTTCTATC CCAGAGAGGC CAAAGTACAG
      S  V  V  C   L  L  N   N  F  Y   P  R  E  A   K  V  Q
2251  TGGAAGGTGG ATAACGCCCT CCAATCGGGT AACTCCCAGG AGAGTGTCAC
        W  K  V   D  N  A  L   Q  S  G   N  S  Q   E  S  V  T
2301  AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC ACCCTGACGC
        E  Q  D   S  K  D   S  T  Y  S   L  S  S   T  L  T
2351  TGAGCAAAGC AGACTACGAG AAACACAAAG TCTACGCCTG CGAAGTCACC
      L  S  K  A   D  Y  E   K  H  K   V  Y  A  C   E  V  T
2401  CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA GGGGAGAGTG
        H  Q  G   L  S  S  P   V  T  K   S  F  N   R  G  E  C
2451  TTAGAGGGAG AAGTGCCCCC ACCTGCTCCT CAGTTCCAGC CTGACCCCCT
        *
2501  CCCATCCTTT GGCCTCTGAC CCTTTTTCCA CAGGGGACCT ACCCCTATTG

2551  CGGTCCTCCA GCTCATCTTT CACCTCACCC CCCTCCTCCT CCTTGGCTTT

2601  AATTATGCTA ATGTTGGAGG AGAATGAATA AATAAAGTGA ATCTTTGCAC

2651  CTGTGGTGGA TCTAATAAAA GATATTTATT TTCATTAGAT ATGTGTGTTG

2701  GTTTTTTGTG TGCAGTGCCT CTATCTGGAG GCCAGGTAGG GCTGGCCTTG

2751  GGGGAGGGGG AGGCCAGAAT GACTCCAAGA GCTACAGGAA GGCAGGTCAG

2801  AGACCCCACT GGACAAACAG TGGCTGGACT CTGCACCATA ACACACAATC

2851  AACAGGGGAG TGAGCTGGAA ATTTGCTAGC GAATTCTTGA AGACGAAAGG

2901  GCCTCGTGAT ACGCCTATTT TTATAGGTTA ATGTCATGAT AATAATGGTT
```

FIG.26C

```
2951  TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT
3001  TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC ATGAGACAAT
3051  AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT
3101  CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT TTTGCCTTCC
3151  TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC
3201  AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA CAGCGGTAAG
3251  ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT
3301  TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGTTGAC GCCGGGCAAG
3351  AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC
3401  TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAATT
3451  ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC
                Pvu I
3501  TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT GCACAACATG
3551  GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC
3601  CATACCAAAC GACGAGCGTG ACACCACGAT GCCTGCAGCA ATGGCAACAA
3651  CGTTGCGCAA ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA
3701  CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC CACTTCTGCG
3751  CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG
3801  AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA TGGTAAGCCC
3851  TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA
3901  ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT AAGCATTGGT
3951  AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT
4001  CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT
4051  GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG
4101  TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT GCGCGTAATC
```

FIG.26D

4151 TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC

4201 GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC TTCAGCAGAG

4251 CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC

4301 TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT

4351 ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT

4401 CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG AACGGGGGGT

4451 TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA

4501 CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA GGGAGAAAGG

4551 CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG

4601 GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG TCGGGTTTCG

4651 CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA

4701 GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT CCTGGCCTTT

BspLU11I

4751 TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT

4801 GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT CGCCGCAGCC

4851 GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCTG

4901 ATGCGGTATT TTCTCCTTAC GCATCTGTGC GGTATTTCAC ACCGCATATG

Bst1107I

4951 GTGCACTCTC AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAGTATA

5001 CACTCCGCTA TCGCTACGTG ACTGGGTCAT GGCTGCGCCC CGACACCCGC

5051 CAACACCCGC TGACGCGCCC TGACGGGCTT GTCTGCTCCC GGCATCCGCT

5101 TACAGACAAG CTGTGACCGT CTCCGGGAGC TGCATGTGTC AGAGGTTTTC

5151 ACCGTCATCA CCGAAACGCG CGAGGCAGCT GTGGAATGTG TGTCAGTTAG

5201 GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG

5251 CATCTCAATT AGTCAGCAAC CAGGCTCCCC AGCAGGCAGA AGTATGCAAA

5301 GCATGCATCT CAATTAGTCA GCAACCATAG TCCCGCCCCT AACTCCGCCC

FIG.26E

```
5351  ATCCCGCCCC TAACTCCGCC CAGTTCCGCC CATTCTCCGC CCCATGGCTG
                                                 Sfi I
5401  ACTAATTTTT TTTATTTATG CAGAGGCCGA GGCCGCCTCG GCCTCTGAGC
                                           Stu I/Avr II
5451  TATTCCAGAA GTAGTGAGGA GGCTTTTTTG GAGGCCTAGG CTTTTGCAAA

5501  AAGCTAGCTT CACGCTGCCG CAAGCACTCA GGGCGCAAGG GCTGCTAAAG

5551  GAAGCGGAAC ACGTAGAAAG CCAGTCCGCA GAAACGGTGC TGACCCCGGA

5601  TGAATGTCAG CTACTGGGCT ATCTGGACAA GGGAAAACGC AAGCGCAAAG

5651  AGAAAGCAGG TAGCTTGCAG TGGGCTTACA TGGCGATAGC TAGACTGGGC

5701  GGTTTTATGG ACAGCAAGCG AACCGGAATT GCCAGCTGGG GCGCCCTCTG

5751  GTAAGGTTGG GAAGCCCTGC AAAGTAAACT GGATGGCTTT CTTGCCGCCA
                                    Bgl II/Bcl I
5801  AGGATCTGAT GGCGCAGGGG ATCAAGATCT GATCAAGAGA CAGGATGAGG

5851  ATCGTTTCGC ATGATTGAAC AAGATGGATT GCACGCAGGT TCTCCGGCCG

5901  CTTGGGTGGA GAGGCTATTC GGCTATGACT GGGCACAACA GACAATCGGC

5951  TGCTCTGATG CCGCCGTGTT CCGGCTGTCA GCGCAGGGGC GCCCGGTTCT

6001  TTTTGTCAAG ACCGACCTGT CCGGTGCCCT GAATGAACTG CAGGACGAGG
                           Msc I
6051  CAGCGCGGCT ATCGTGGCTG GCCACGACGG GCGTTCCTTG CGCAGCTGTG

6101  CTCGACGTTG TCACTGAAGC GGGAAGGGAC TGGCTGCTAT TGGGCGAAGT

6151  GCCGGGGCAG GATCTCCTGT CATCTCACCT TGCTCCTGCC GAGAAAGTAT

6201  CCATCATGGC TGATGCAATG CGGCGGCTGC ATACGCTTGA TCCGGCTACC

6251  TGCCCATTCG ACCACCAAGC GAAACATCGC ATCGAGCGAG CACGTACTCG

6301  GATGGAAGCC GGTCTTGTCG ATCAGGATGA TCTGGACGAA GAGCATCAGG

6351  GGCTCGCGCC AGCCGAACTG TTCGCCAGGC TCAAGGCGCG CATGCCCGAC

6401  GGCGAGGATC TCGTCGTGAC CCATGGCGAT GCCTGCTTGC CGAATATCAT

6451  GGTGGAAAAT GGCCGCTTTT CTGGATTCAT CGACTGTGGC CGGCTGGGTG
        Rsr II
6501  TGGCGGACCG CTATCAGGAC ATAGCGTTGG CTACCCGTGA TATTGCTGAA
```

FIG.26F

```
6551  GAGCTTGGCG GCGAATGGGC TGACCGCTTC CTCGTGCTTT ACGGTATCGC

6601  CGCTCCCGAT TCGCAGCGCA TCGCCTTCTA TCGCCTTCTT GACGAGTTCT
                              Nsp V
6651  TCTGAGCGGG ACTCTGGGGT TCGAAATGAC CGACCAAGCG ACGCCCAACC

6701  TGCCATCACG AGATTTCGAT TCCACCGCCG CCTTCTATGA AAGGTTGGGC

6751  TTCGGAATCG TTTTCCGGGA CGCCGGCTGG ATGATCCTCC AGCGCGGGGA
                                    Sma I                Nru I
6801  TCTCATGCTG GAGTTCTTCG CCCACCCCGG GCTCGATCCC CTCGCGAGTT

6851  GGTTCAGCTG CTGCCTGAGG CTGGACGACC TCGCGGAGTT CTACCGGCAG

6901  TGCAAATCCG TCGGCATCCA GGAAACCAGC AGCGGCTATC CGCGCATCCA

6951  TGCCCCCGAA CTGCAGGAGT GGGGAGGCAC GATGGCCGCT TTGGTCCCGG

7001  ATCTTTGTGA AGGAACCTTA CTTCTGTGGT GTGACATAAT TGGACAAACT

7051  ACCTACAGAG ATTTAAAGCT CTAAGGTAAA TATAAAATTT TTAAGTGTAT

7101  AATGTGTTAA ACTACTGATT CTAATTGTTT GTGTATTTTA GATTCCAACC

7151  TATGGAACTG ATGAATGGGA GCAGTGGTGG AATGCCTTTA ATGAGGAAAA

7201  CCTGTTTTGC TCAGAAGAAA TGCCATCTAG TGATGATGAG GCTACTGCTG

7251  ACTCTCAACA TTCTACTCCT CCAAAAAAGA AGAGAAAGGT AGAAGACCCC

7301  AAGGACTTTC CTTCAGAATT GCTAAGTTTT TTGAGTCATG CTGTGTTTAG

7351  TAATAGAACT CTTGCTTGCT TTGCTATTTA CACCACAAAG GAAAAAGCTG

7401  CACTGCTATA CAAGAAAATT ATGGAAAAAT ATTCTGTAAC CTTTATAAGT

7451  AGGCATAACA GTTATAATCA TAACATACTG TTTTTTCTTA CTCCACACAG

7501  GCATAGAGTG TCTGCTATTA ATAACTATGC TCAAAAATTG TGTACCTTTA

7551  GCTTTTTAAT TTGTAAAGGG GTTAATAAGG AATATTTGAT GTATAGTGCC

7601  TTGACTAGAG ATCATAATCA GCCATACCAC ATTTGTAGAG GTTTTACTTG

7651  CTTTAAAAAA CCTCCCACAC CTCCCCCTGA ACCTGAAACA TAAAATGAAT
        Mun I
7701  GCAATTGTTG TTGTTAACTT GTTTATTGCA GCTTATAATG GTTACAAATA
```

FIG.26G

```
7751  AAGCAATAGC ATCACAAATT TCACAAATAA AGCATTTTTT TCACTGCATT
7801  CTAGTTGTGG TTTGTCCAAA CTCATCAATG TATCTTATCA TGTCTGGATC
7851  TAATAAAAGA TATTTATTTT CATTAGATAT GTGTGTTGGT TTTTTGTGTG
7901  CAGTGCCTCT ATCTGGAGGC CAGGTAGGGC TGGCCTTGGG GGAGGGGGAG
7951  GCCAGAATGA CTCCAAGAGC TACAGGAAGG CAGGTCAGAG ACCCCACTGG
8001  ACAAACAGTG GCTGGACTCT GCACCATAAC ACACAATCAA CAGGGGAGTG
8051  AGCTGGAAAT TTGCTAGC
```

FIG.26H

```
   1 TTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAAT
  61 GGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTT
 121 ATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCT
 181 TCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCC
 241 CTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAA
 301 AGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGG
 361 TAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGT
 421 TCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCG
 481 CATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTAC
 541 GGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGC
                          Pvu I
 601 GGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAA
 661 CATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACC
                                                        Fsp I
 721 AAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATT
 781 AACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGA
```

FIG.27A

841 TAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAA

901 ATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAA

961 GCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAA

1021 TAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGT

1081 TTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGT

1141 GAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTG

1201 AGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGT

1261 AATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCA

1321 AGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATAC

1381 TGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTAC

1441 ATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCT

1501 TACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGG

1561 GGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACA

1621 GCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGT

1681 AAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTA

1741 TCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTC

1801 GTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGC

BspLU11I

1861 CTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAA

1921 CCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAG

1981 CGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCT

2041 GTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATA

Bst1107 I

2101 GTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACAC

2161 CCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGA

2221 CAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAA

FIG.27B

2281 CGCGCGAGGCAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCC

2341 CATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTT

Sfi I

2401 TTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGG

Stu I/Avr II

2461 AGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAGCTTACAGCTCAGGGCTGCGATT

2521 TCGCGCCAAACTTGACGGCAATCCTAGCGTGAAGGCTGGTAGGATTTTATCCCCGCTGCC

2581 ATCATGGTTCGACCATTGAACTGCATCGTCGCCGTGTCCCAAAATATGGGGATTGGCAAG

2641 AACGGAGACCTACCCTGGCCTCCGCTCAGGAACGAGTTCAAGTACTTCCAAAGAATGACC

2701 ACAACCTCTTCAGTGGAAGGTAAACAGAATCTGGTGATTATGGGTAGGAAAACCTGGTTC

2761 TCCATTCCTGAGAAGAATCGACCTTTAAAGGACAGAATTAATATAGTTCTCAGTAGAGAA

2821 CTCAAAGAACCACCACGAGGAGCTCATTTTCTTGCCAAAAGTTTGGATGATGCCTTAAGA

2881 CTTATTGAACAACCGGAATTGGCAAGTAAAGTAGACATGGTTTGGATAGTCGGAGGCAGT

2941 TCTGTTTACCAGGAAGCCATGAATCAACCAGGCCACCTCAGACTCTTTGTGACAAGGATC

3001 ATGCAGGAATTTGAAAGTGACACGTTTTTCCCAGAAATTGATTTGGGGAAATATAAACTT

3061 CTCCCAGAATACCCAGGCGTCCTCTCTGAGGTCCAGGAGGAAAAAGGCATCAAGTATAAG

3121 TTTGAAGTCTACGAGAAGAAAGACTAACAGGAAGATGCTTTCAAGTTCTCTGCTCCCCTC

Bgl II

3181 CTAAAGCTATGCATTTTTATAAGACCATGGGACTTTTGCTGGCTTTAGATCTTTGTGAAG

3241 GAACCTTACTTCTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCT

3301 AAGGTAAATATAAAATTTTTTAAGTGTATAATGTGTTAAACTACTGATTCTAATTGTTTGT

3361 GTATTTTAGATTCCAACCTATGGAACTGATGAATGGGAGCAGTGGTGGAATGCCTTTAAT

3421 GAGGAAAACCTGTTTTGCTCAGAAGAAATGCCATCTAGTGATGATGAGGCTACTGCTGAC

3481 TCTCAACATTCTACTCCTCCAAAAAAGAAGAGAAAGGTAGAAGACCCCAAGGACTTTCCT

3541 TCAGAATTGCTAAGTTTTTTGAGTCATGCTGTGTTTAGTAATAGAACTCTTGCTTGCTTT

3601 GCTATTTACACCACAAAGGAAAAAGCTGCACTGCTATACAAGAAAATTATGGAAAAATAT

3661 TCTGTAACCTTTATAAGTAGGCATAACAGTTATAATCATAACATACTGTTTTTTCTTACT

FIG.27C

3721 CCACACAGGCATAGAGTGTCTGCTATTAATAACTATGCTCAAAAATTGTGTACCTTTAGC

3781 TTTTTAATTTGTAAAGGGGTTAATAAGGAATATTTGATGTATAGTGCCTTGACTAGAGAT
BsaB I
3841 CATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCT
Mun I
3901 CCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGC

3961 TTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTC

4021 ACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCTA

4081 ATAAAAGATATTTATTTTCATTAGATATGTGTGTTGGTTTTTTGTGTGCAGTGCCTCTAT

4141 CTGGAGGCCAGGTAGGGCTGGCCTTGGGGGAGGGGGAGGCCAGAATGACTCCAAGAGCTA

4201 CAGGAAGGCAGGTCAGAGACCCCACTGGACAAACAGTGGCTGGACTCTGCACCATAACAC
EcoR I
4261 ACAATCAACAGGGGAGTGAGCTGGAAATTTGCTAGCGAATTCcagcacactggcggccgt
Spe I
4321 tACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTT

4381 CCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCC

4441 ATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACG

4501 TCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATAT

4561 GCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCA
SnaB I
4621 GTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTAT

4681 TACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACG

4741 GGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCA

4801 ACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCG

4861 TGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAG

4921 ACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGG

4981 CCGGGAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGACGTAAGTACCGCCTA

5041 TAGAGTCTATAGGCCCACCCCCTTGGCTTCTTATGCATGCTATACTGTTTTTGGCTTGGG
Bpu1102I
5101 GTCTATACACCCCCGCTTCCTCATGTTATAGGTGATGGTATAGCTTAGCCTATAGGTGTG

FIG.27D

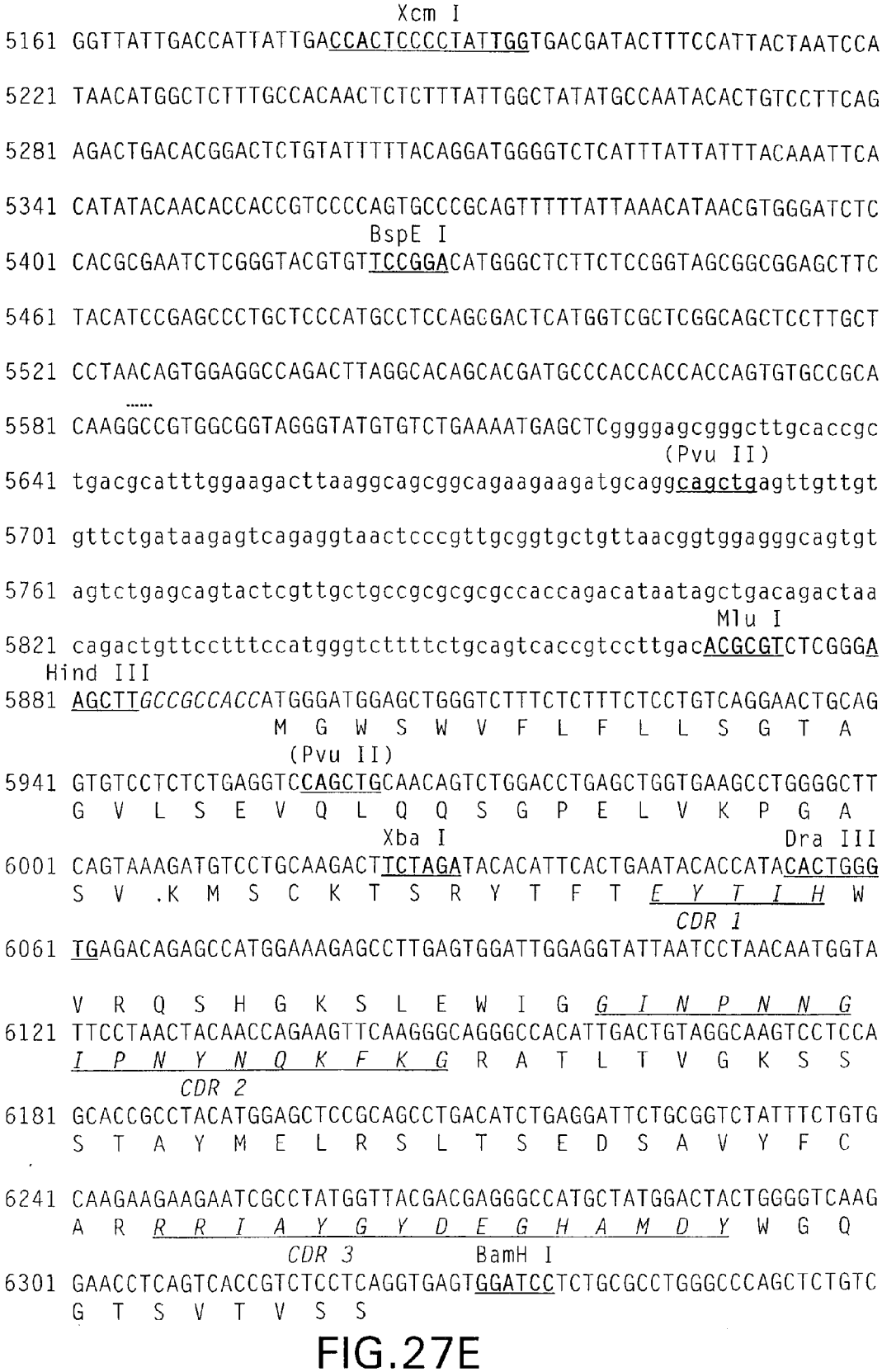

Xcm I
5161 GGTTATTGACCATTATTGACCACTCCCCTATTGGTGACGATACTTTCCATTACTAATCCA
5221 TAACATGGCTCTTTGCCACAACTCTCTTTATTGGCTATATGCCAATACACTGTCCTTCAG
5281 AGACTGACACGGACTCTGTATTTTTACAGGATGGGGTCTCATTTATTATTTACAAATTCA
5341 CATATACAACACCACCGTCCCCAGTGCCCGCAGTTTTTATTAAACATAACGTGGGATCTC
BspE I
5401 CACGCGAATCTCGGGTACGTGTTCCGGACATGGGCTCTTCTCCGGTAGCGGCGGAGCTTC
5461 TACATCCGAGCCCTGCTCCCATGCCTCCAGCGACTCATGGTCGCTCGGCAGCTCCTTGCT
5521 CCTAACAGTGGAGGCCAGACTTAGGCACAGCACGATGCCCACCACCACCAGTGTGCCGCA
5581 CAAGGCCGTGGCGGTAGGGTATGTGTCTGAAAATGAGCTCggggagcgggcttgcaccgc
(Pvu II)
5641 tgacgcatttggaagacttaaggcagcggcagaagaagatgcaggcagctgagttgttgt
5701 gttctgataagagtcagaggtaactcccgttgcggtgctgttaacggtggagggcagtgt
5761 agtctgagcagtactcgttgctgccgcgcgcgccaccagacataatagctgacagactaa
Mlu I
5821 cagactgttcctttccatgggtcttttctgcagtcaccgtccttgacACGCGTCTCGGGA
Hind III
5881 AGCTTGCCGCCACCATGGGATGGAGCTGGGTCTTTCTCTTTCTCCTGTCAGGAACTGCAG
M G W S W V F L F L L S G T A
(Pvu II)
5941 GTGTCCTCTCTGAGGTCCAGCTGCAACAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTT
G V L S E V Q L Q Q S G P E L V K P G A
Xba I
Dra III
6001 CAGTAAAGATGTCCTGCAAGACTTCTAGATACACATTCACTGAATACACCATACACTGGG
S V .K M S C K T S R Y T F T E Y T I H W
CDR 1
6061 TGAGACAGAGCCATGGAAAGAGCCTTGAGTGGATTGGAGGTATTAATCCTAACAATGGTA
V R Q S H G K S L E W I G G I N P N N G
6121 TTCCTAACTACAACCAGAAGTTCAAGGGCAGGGCCACATTGACTGTAGGCAAGTCCTCCA
I P N Y N Q K F K G R A T L T V G K S S
CDR 2
6181 GCACCGCCTACATGGAGCTCCGCAGCCTGACATCTGAGGATTCTGCGGTCTATTTCTGTG
S T A Y M E L R S L T S E D S A V Y F C
6241 CAAGAAGAAGAATCGCCTATGGTTACGACGAGGGCCATGCTATGGACTACTGGGGTCAAG
A R R R I A Y G Y D E G H A M D Y W G Q
CDR 3
BamH I
6301 GAACCTCAGTCACCGTCTCCTCAGGTGAGTGGATCCTCTGCGCCTGGGCCCAGCTCTGTC
G T S V T V S S

FIG.27E

```
6361 CCACACCGCGGTCACATGGCACCACCTCTCTTGCAGCCTCCACCAAGGGCCCATCGGTCT
                                                S  T  K  G  P  S  V

6421 TCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG
     F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L
                           Age I
6481 TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCG
     V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S

6541 GCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGG
     G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V
    BstE II
6601 TGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGC
     V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K

6661 CCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACAT
     P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D  K  T  H  T

6721 GCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAA
     C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F  P  P

6781 AACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACG
        K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D

6841 TGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATA
     V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H

6901 ATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCC
     N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V

6961 TCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACA
     L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N

7021 AAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
     K  A  L  P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E

7081 CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGA
     P  Q  V  Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V  S  L

7141 CCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC
     T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G

7201 AGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC
     Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F

7261 TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT
     L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C
```

FIG.27F

```
7321 CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
     S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P
                       NgoM I
7381 GTAAATGAGTGCGACGGCCGGCAAGCCCCGCTCCCCGGGCTCTCGCGGTCGCACGAGGAT
     G  K  *
7441 GCTTGGCACGTACCCCCTGTACATACTTCCCGGGCGCCCAGCATGGAAATAAAGCACCGG

7501 ATCTAATAAAAGATATTTATTTTCATTAGATATGTGTGTTGGTTTTTTGTGTGCAGTGCC

7561 TCTATCTGGAGGCCAGGTAGGGCTGGCCTTGGGGGAGGGGGAGGCCAGAATGACTCCAAG

7621 AGCTACAGGAAGGCAGGTCAGAGACCCCACTGGACAAACAGTGGCTGGACTCTGCACCAT

7681 AACACACAATCAACAGGGGAGTGAGCTGGaaatttgctagcgaattaattc 7731
```

FIG.27G

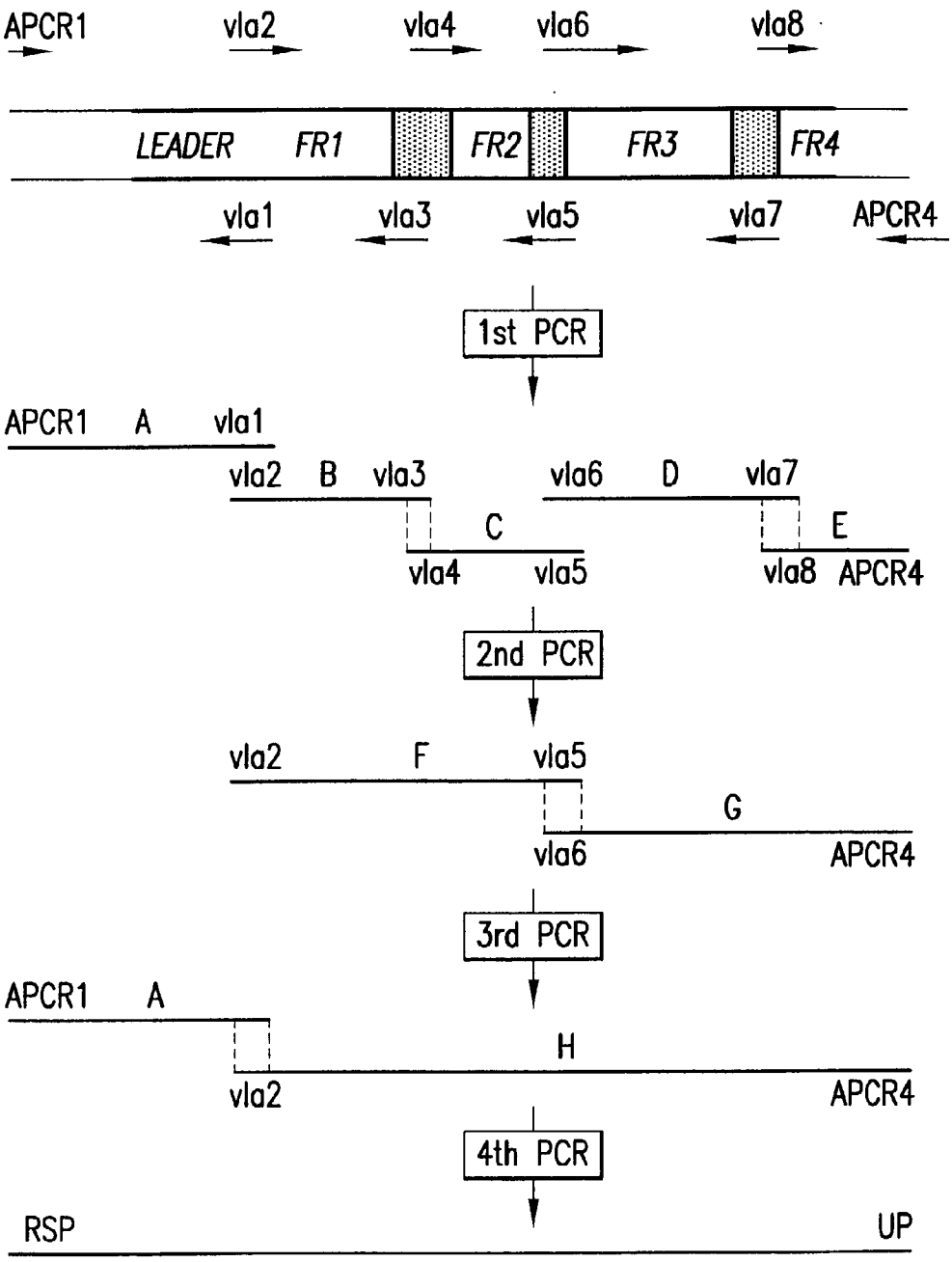

APCR1
vla2
vla4
vla6
vla8
LEADER
FR1
FR2
FR3
FR4
vla1
vla3
vla5
vla7
APCR4
1st PCR
APCR1
A
vla1
vla2
B
vla3
vla6
D
vla7
C
E
vla4
vla5
vla8
APCR4
2nd PCR
vla2
F
vla5
G
vla6
APCR4
3rd PCR
APCR1
A
H
vla2
APCR4
4th PCR
RSP
UP

FIG. 28

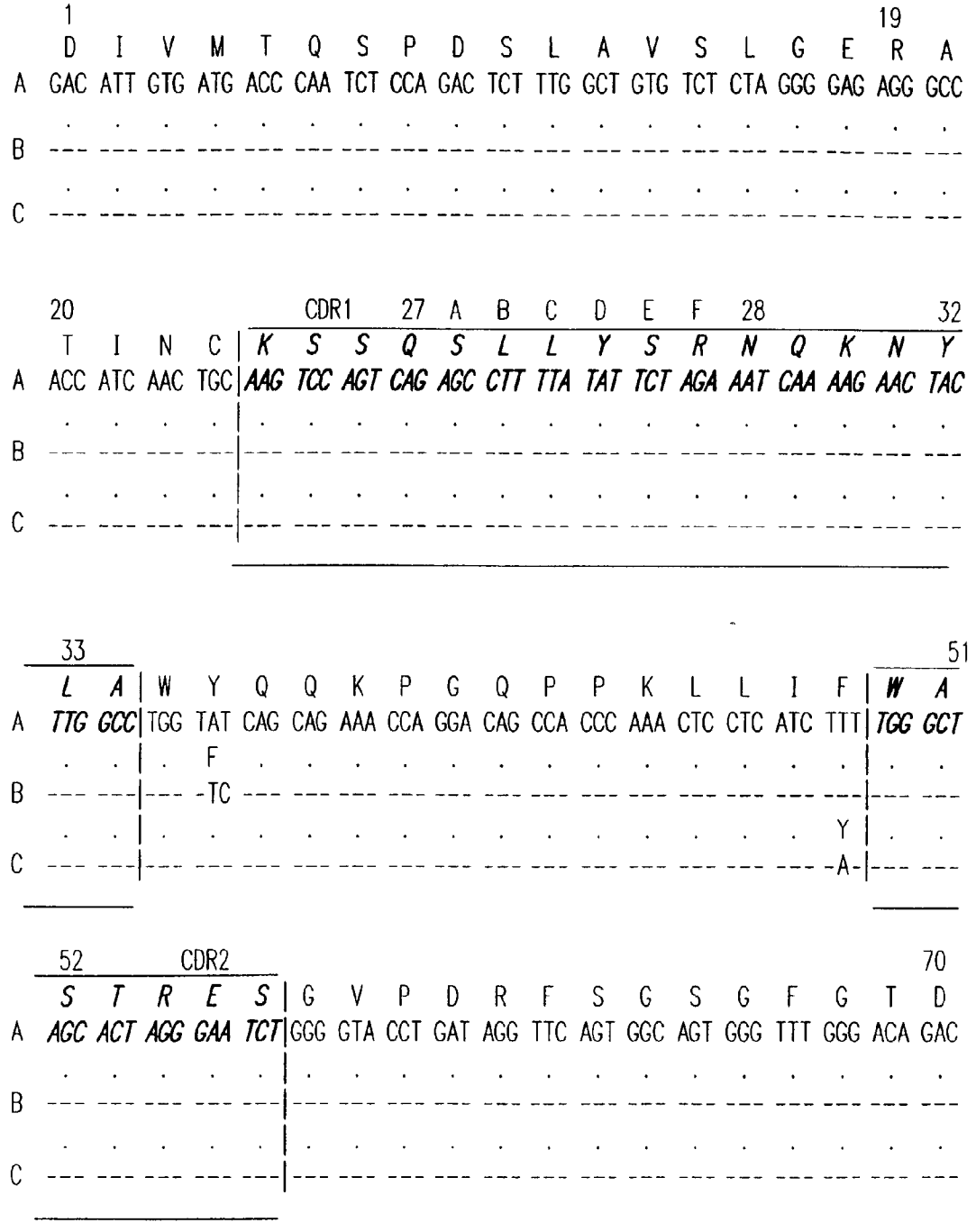

1
19
D I V M T Q S P D S L A V S L G E R A
A GAC ATT GTG ATG ACC CAA TCT CCA GAC TCT TTG GCT GTG TCT CTA GGG GAG AGG GCC
B
C
20
CDR1
27 A B C D E F 28
32
T I N C K S S Q S L L Y S R N Q K N Y
A ACC ATC AAC TGC AAG TCC AGT CAG AGC CTT TTA TAT TCT AGA AAT CAA AAG AAC TAC
B
C
33
51
L A W Y Q Q K P G Q P P K L L I F W A
A TTG GCC TGG TAT CAG CAG AAA CCA GGA CAG CCA CCC AAA CTC CTC ATC TTT TGG GCT
B -TC F
C -A- Y
52
CDR2
70
S T R E S G V P D R F S G S G F G T D
A AGC ACT AGG GAA TCT GGG GTA CCT GAT AGG TTC AGT GGC AGT GGG TTT GGG ACA GAC
B
C

FIG. 29A

	71																	88
	F	T	L	T	I	S	S	L	Q	A	E	D	V	A	V	Y	Y	C
A	TTC	ACC	CTC	ACC	ATT	AGC	AGC	CTG	CAG	GCT	GAA	GAT	GTG	GCA	GTT	TAT	TAC	TGT
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.
B	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	G--	---
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

	89					CDR3												107
	Q	*Q*	*Y*	*F*	*S*	*Y*	*P*	*L*	*T*	F	G	Q	G	T	K	V	E	I	K
A	*CAG*	*CAA*	*TAT*	*TTT*	*AGC*	*TAT*	*CCG*	*CTC*	*ACG*	TTC	GGA	CAA	GGG	ACC	AAG	GTG	GAA	ATA	AAA
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
B	--*A*	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

FIG. 29B

```
                                    Spe I
   1  gaattccagc acactggcgg ccgttACTAG TTATTAATAG TAATCAATTA

  51  CGGGGTCATT AGTTCATAGC CCATATATGG AGTTCCGCGT TACATAACTT

 101  ACGGTAAATG GCCCGCCTGG CTGACCGCCC AACGACCCCC GCCCATTGAC

 151  GTCAATAATG ACGTATGTTC CCATAGTAAC GCCAATAGGG ACTTTCCATT

 201  GACGTCAATG GGTGGAGTAT TTACGGTAAA CTGCCCACTT GGCAGTACAT

 251  CAAGTGTATC ATATGCCAAG TACGCCCCCT ATTGACGTCA ATGACGGTAA

 301  ATGGCCCGCC TGGCATTATG CCCAGTACAT GACCTTATGG GACTTTCCTA
                      SnaB I
 351  CTTGGCAGTA CATCTACGTA TTAGTCATCG CTATTACCAT GGTGATGCGG

 401  TTTTGGCAGT ACATCAATGG GCGTGGATAG CGGTTTGACT CACGGGGATT

 451  TCCAAGTCTC CACCCCATTG ACGTCAATGG GAGTTTGTTT TGGCACCAAA

 501  ATCAACGGGA CTTTCCAAAA TGTCGTAACA ACTCCGCCCC ATTGACGCAA

 551  ATGGGCGGTA GGCGTGTACG GTGGGAGGTC TATATAAGCA GAGCTCGTTT

 601  AGTGAACCGT CAGATCGCCT GGAGACGCCA TCCACGCTGT TTTGACCTCC
                                        Sac II
 651  ATAGAAGACA CCGGGACCGA TCCAGCCTCC GCGGCCGGGA ACGGTGCATT

 701  GGAACGCGGA TTCCCCGTGC CAAGAGTGAC GTAAGTACCG CCTATAGAGT

 751  CTATAGGCCC ACCCCCTTGG CTTCTTATGC ATGCTATACT GTTTTTGGCT

 801  TGGGGTCTAT ACACCCCCGC TTCCTCATGT TATAGGTGAT GGTATAGCTT

 851  AGCCTATAGG TGTGGGTTAT TGACCATTAT TGACCACTCC CCTATTGGTG

 901  ACGATACTTT CCATTACTAA TCCATAACAT GGCTCTTTGC CACAACTCTC

 951  TTTATTGGCT ATATGCCAAT ACACTGTCCT TCAGAGACTG ACACGGACTC

1001  TGTATTTTTA CAGGATGGGG TCTCATTTAT TATTTACAAA TTCACATATA

1051  CAACACCACC GTCCCCAGTG CCCGCAGTTT TTATTAAACA TAACGTGGGA
                                  (BspE I)
1101  TCTCCACGCG AATCTCGGGT ACGTGTTCCG GACATGGGCT CTTCTCCGGT

1151  AGCGGCGGAG CTTCTACATC CGAGCCCTGC TCCCATGCCT CCAGCGACTC
```

FIG.30A

```
1201 ATGGTCGCTC GGCAGCTCCT TGCTCCTAAC AGTGGAGGCC AGACTTAGGC

1251 ACAGCACGAT GCCCACCACC ACCAGTGTGC CGCACAAGGC CGTGGCGGTA

1301 GGGTATGTGT CTGAAAATGA GCTCggggag cgggcttgca ccgctgacgc
                Afl II
1351 atttggaaga cttaaggcag cggcagaaga agatgcaggc agctgagttg

1401 ttgtgttctg ataagagtca gaggtaactc ccgttgcggt gctgttaacg

1451 gtggagggca gtgtagtctg agcagtactc gttgctgccg cgcgcgccac

1501 cagacataat agctgacaga ctaacagact gttcctttcc atgggtcttt
                          Mlu I        Hind III
1551 tctgcagtca ccgtccttga cacgcgtctc gggaagcttG CCGCCACCAT
                                                    M
1601 GGAGACAGAC ACACTCCTGC TATGGGTGCT GCTGCTCTGG GTTCCAGGTT
       E  T  D   T  L  L   L  W  V  L   L  L  W   V  P  G
     (BspE I)
1651 CCTCCGGAGA CATTGTGATG ACCCAATCTC CAGACTCTTT GGCTGTGTCT
     S  S  G  D   I  V  M   T  Q  S   P  D  S  L   A  V  S

1701 CTAGGGGAGA GGGCCACCAT CAACTGCAAG TCCAGTCAGA GCCTTTTATA
      L  G  E   R  A  T  I   N  C  K  S  S  Q  S  L  L  Y
     XbaI                                          CDR 1
1751 TTCTAGAAAT CAAAAGAACT ACTTGGCCTG GTATCAGCAG AAACCAGGAC
       S  R  N   Q  K  N   Y  L  A  W   Y  Q  Q   K  P  G
                                                      KpnI
1801 AGCCACCCAA ACTCCTCATC TTTTGGGCTA GCACTAGGGA ATCTGGGGTA
     Q  P  P  K   L  L  I   F  W  A  S  T  R  E  S  G  V
                                      CDR 2
1851 CCTGATAGGT TCAGTGGCAG TGGGTTTGGG ACAGACTTCA CCCTCACCAT
      P  D  R   F  S  G  S   G  F  G   T  D  F   T  L  T  I

1901 TAGCAGCCTG CAGGCTGAAG ATGTGGCAGT TTATTACTGT CAGCAATATT
       S  S  L   Q  A  E   D  V  A  V   Y  Y  C   Q  Q  Y

1951 TTAGCTATCC GCTCACGTTC GGACAAGGGA CCAAGGTGGA AATAAAACGT
     F  S  Y  P  L  T  F   G  Q  G   T  K  V  E   I  K  R
           CDR 3
        BamH I
2001 GAGTggatcc ATCTGGGATA AGCATGCTGT TTTCTGTCTG TCCCTAACAT

2051 GCCCTGTGAT TATGCGCAAA CAACACACCC AAGGGCAGAA CTTTGTTACT

2101 TAAACACCAT CCTGTTTGCT TCTTTCCTCA GGAACTGTGG CTGCACCATC
                                    T  V   A  A  P  S
```

FIG.30B

```
2151 TGTCTTCATC TTCCCGCCAT CTGATGAGCA GTTGAAATCT GGAACTGCCT
       V  F  I   F  P  P   S  D  E  Q   L  K  S   G  T  A
2201 CTGTTGTGTG CCTGCTGAAT AACTTCTATC CCAGAGAGGC CAAAGTACAG
     S  V  V  C   L  L  N   N  F  Y   P  R  E  A   K  V  Q
2251 TGGAAGGTGG ATAACGCCCT CCAATCGGGT AACTCCCAGG AGAGTGTCAC
      W  K  V   D  N  A  L   Q  S  G   N  S  Q   E  S  V  T
2301 AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC ACCCTGACGC
       E  Q  D   S  K  D   S  T  Y  S   L  S  S   T  L  T
2351  TGAGCAAAGC AGACTACGAG AAACACAAAG TCTACGCCTG CGAAGTCACC
      L  S  K  A   D  Y  E   K  H  K   V  Y  A  C   E  V  T
2401  CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA GGGGAGAGTG
       H  Q  G   L  S  S  P   V  T  K   S  F  N   R  G  E  C
2451  TTAGAGGGAG AAGTGCCCCC ACCTGCTCCT CAGTTCCAGC CTGACCCCCT
        *                                    Psp5 II
2501  CCCATCCTTT GGCCTCTGAC CCTTTTTCCA CAGGGGACCT ACCCCTATTG

2551  CGGTCCTCCA GCTCATCTTT CACCTCACCC CCCTCCTCCT CCTTGGCTTT

2601  AATTATGCTA ATGTTGGAGG AGAATGAATA AATAAAGTGA ATCTTTGCAC

2651  CTGTGGTGGA TCTAATAAAA GATATTTATT TTCATTAGAT ATGTGTGTTG

2701  GTTTTTTGTG TGCAGTGCCT CTATCTGGAG GCCAGGTAGG GCTGGCCTTG

2751  GGGGAGGGGG AGGCCAGAAT GACTCCAAGA GCTACAGGAA GGCAGGTCAG

2801  AGACCCCACT GGACAAACAG TGGCTGGACT CTGCACCATA ACACACAATC

2851  AACAGGGGAG TGAGCTGGAA ATTTGCTAGC GAATTCTTGA AGACGAAAGG

2901  GCCTCGTGAT ACGCCTATTT TTATAGGTTA ATGTCATGAT AATAATGGTT

2951  TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT

3001  TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC ATGAGACAAT

3051  AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT

3101  CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT TTTGCCTTCC

3151  TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC

3201  AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA CAGCGGTAAG

3251  ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT

3301  TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGTTGAC GCCGGGCAAG
```

FIG.30C

```
3351  AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC

3401  TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAATT

3451  ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC
            Pvu I
3501  TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT GCACAACATG

3551  GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC

3601  CATACCAAAC GACGAGCGTG ACACCACGAT GCCTGCAGCA ATGGCAACAA

3651  CGTTGCGCAA ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA

3701  CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC CACTTCTGCG

3751  CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG

3801  AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA TGGTAAGCCC

3851  TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA

3901  ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT AAGCATTGGT

3951  AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT

4001  CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT

4051  GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG

4101  TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT GCGCGTAATC

4151  TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC

4201  GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC TTCAGCAGAG

4251  CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC

4301  TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT

4351  ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT

4401  CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG AACGGGGGGT

4451  TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA

4501  CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA GGGAGAAAGG
```

FIG.30D

```
4551  CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG

4601  GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG TCGGGTTTCG

4651  CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA

4701  GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT CCTGGCCTTT
                          BspLU11I
4751  TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT

4801  GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT CGCCGCAGCC

4851  GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCTG

4901  ATGCGGTATT TTCTCCTTAC GCATCTGTGC GGTATTTCAC ACCGCATATG
                                                      Bst1107I
4951  GTGCACTCTC AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAGTATA

5001  CACTCCGCTA TCGCTACGTG ACTGGGTCAT GGCTGCGCCC CGACACCCGC

5051  CAACACCCGC TGACGCGCCC TGACGGGCTT GTCTGCTCCC GGCATCCGCT

5101  TACAGACAAG CTGTGACCGT CTCCGGGAGC TGCATGTGTC AGAGGTTTTC

5151  ACCGTCATCA CCGAAACGCG CGAGGCAGCT GTGGAATGTG TGTCAGTTAG

5201  GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG

5251  CATCTCAATT AGTCAGCAAC CAGGCTCCCC AGCAGGCAGA AGTATGCAAA

5301  GCATGCATCT CAATTAGTCA GCAACCATAG TCCCGCCCCT AACTCCGCCC

5351  ATCCCGCCCC TAACTCCGCC CAGTTCCGCC CATTCTCCGC CCCATGGCTG
                                       Sfi I
5401  ACTAATTTTT TTTATTTATG CAGAGGCCGA GGCCGCCTCG GCCTCTGAGC
                                        Stu I/Avr II
5451  TATTCCAGAA GTAGTGAGGA GGCTTTTTTG GAGGCCTAGG CTTTTGCAAA

5501  AAGCTAGCTT CACGCTGCCG CAAGCACTCA GGGCGCAAGG GCTGCTAAAG

5551  GAAGCGGAAC ACGTAGAAAG CCAGTCCGCA GAAACGGTGC TGACCCCGGA

5601  TGAATGTCAG CTACTGGGCT ATCTGGACAA GGGAAAACGC AAGCGCAAAG

5651  AGAAAGCAGG TAGCTTGCAG TGGGCTTACA TGGCGATAGC TAGACTGGGC

5701  GGTTTTATGG ACAGCAAGCG AACCGGAATT GCCAGCTGGG GCGCCCTCTG
```

FIG.30E

```
5751  GTAAGGTTGG GAAGCCCTGC AAAGTAAACT GGATGGCTTT CTTGCCGCCA
                                  Bgl II/Bcl I
5801  AGGATCTGAT GGCGCAGGGG ATCAAGATCT GATCAAGAGA CAGGATGAGG

5851  ATCGTTTCGC ATGATTGAAC AAGATGGATT GCACGCAGGT TCTCCGGCCG

5901  CTTGGGTGGA GAGGCTATTC GGCTATGACT GGGCACAACA GACAATCGGC

5951  TGCTCTGATG CCGCCGTGTT CCGGCTGTCA GCGCAGGGGC GCCCGGTTCT

6001  TTTTGTCAAG ACCGACCTGT CCGGTGCCCT GAATGAACTG CAGGACGAGG
                        Msc I
6051  CAGCGCGGCT ATCGTGGCTG GCCACGACGG GCGTTCCTTG CGCAGCTGTG

6101  CTCGACGTTG TCACTGAAGC GGGAAGGGAC TGGCTGCTAT TGGGCGAAGT

6151  GCCGGGGCAG GATCTCCTGT CATCTCACCT TGCTCCTGCC GAGAAAGTAT

6201  CCATCATGGC TGATGCAATG CGGCGGCTGC ATACGCTTGA TCCGGCTACC

6251  TGCCCATTCG ACCACCAAGC GAAACATCGC ATCGAGCGAG CACGTACTCG

6301  GATGGAAGCC GGTCTTGTCG ATCAGGATGA TCTGGACGAA GAGCATCAGG

6351  GGCTCGCGCC AGCCGAACTG TTCGCCAGGC TCAAGGCGCG CATGCCCGAC

6401  GGCGAGGATC TCGTCGTGAC CCATGGCGAT GCCTGCTTGC CGAATATCAT

6451  GGTGGAAAAT GGCCGCTTTT CTGGATTCAT CGACTGTGGC CGGCTGGGTG
        Rsr II
6501  TGGCGGACCG CTATCAGGAC ATAGCGTTGG CTACCCGTGA TATTGCTGAA

6551  GAGCTTGGCG GCGAATGGGC TGACCGCTTC CTCGTGCTTT ACGGTATCGC

6601  CGCTCCCGAT TCGCAGCGCA TCGCCTTCTA TCGCCTTCTT GACGAGTTCT
                        Nsp V
6651  TCTGAGCGGG ACTCTGGGGT TCGAAATGAC CGACCAAGCG ACGCCCAACC

6701  TGCCATCACG AGATTTCGAT TCCACCGCCG CCTTCTATGA AAGGTTGGGC

6751  TTCGGAATCG TTTTCCGGGA CGCCGGCTGG ATGATCCTCC AGCGCGGGGA
                                  Sma I              Nru I
6801  TCTCATGCTG GAGTTCTTCG CCCACCCCGG GCTCGATCCC CTCGCGAGTT

6851  GGTTCAGCTG CTGCCTGAGG CTGGACGACC TCGCGGAGTT CTACCGGCAG

6901  TGCAAATCCG TCGGCATCCA GGAAACCAGC AGCGGCTATC CGCGCATCCA
```

FIG.30F

6951 TGCCCCCGAA CTGCAGGAGT GGGGAGGCAC GATGGCCGCT TTGGTCCCGG

7001 ATCTTTGTGA AGGAACCTTA CTTCTGTGGT GTGACATAAT TGGACAAACT

7051 ACCTACAGAG ATTTAAAGCT CTAAGGTAAA TATAAAATTT TTAAGTGTAT

7101 AATGTGTTAA ACTACTGATT CTAATTGTTT GTGTATTTTA GATTCCAACC

7151 TATGGAACTG ATGAATGGGA GCAGTGGTGG AATGCCTTTA ATGAGGAAAA

7201 CCTGTTTTGC TCAGAAGAAA TGCCATCTAG TGATGATGAG GCTACTGCTG

7251 ACTCTCAACA TTCTACTCCT CCAAAAAAGA AGAGAAAGGT AGAAGACCCC

7301 AAGGACTTTC CTTCAGAATT GCTAAGTTTT TTGAGTCATG CTGTGTTTAG

7351 TAATAGAACT CTTGCTTGCT TTGCTATTTA CACCACAAAG GAAAAAGCTG

7401 CACTGCTATA CAAGAAAATT ATGGAAAAAT ATTCTGTAAC CTTTATAAGT

7451 AGGCATAACA GTTATAATCA TAACATACTG TTTTTTCTTA CTCCACACAG

7501 GCATAGAGTG TCTGCTATTA ATAACTATGC TCAAAAATTG TGTACCTTTA

7551 GCTTTTTAAT TTGTAAAGGG GTTAATAAGG AATATTTGAT GTATAGTGCC

7601 TTGACTAGAG ATCATAATCA GCCATACCAC ATTTGTAGAG GTTTTACTTG

7651 CTTTAAAAAA CCTCCCACAC CTCCCCCTGA ACCTGAAACA TAAAATGAAT

Mun I

7701 GCAATTGTTG TTGTTAACTT GTTTATTGCA GCTTATAATG GTTACAAATA

7751 AAGCAATAGC ATCACAAATT TCACAAATAA AGCATTTTTT TCACTGCATT

7801 CTAGTTGTGG TTTGTCCAAA CTCATCAATG TATCTTATCA TGTCTGGATC

7851 TAATAAAAGA TATTTATTTT CATTAGATAT GTGTGTTGGT TTTTTGTGTG

7901 CAGTGCCTCT ATCTGGAGGC CAGGTAGGGC TGGCCTTGGG GGAGGGGGAG

7951 GCCAGAATGA CTCCAAGAGC TACAGGAAGG CAGGTCAGAG ACCCCACTGG

8001 ACAAACAGTG GCTGGACTCT GCACCATAAC ACACAATCAA CAGGGGAGTG

8051 AGCTGGAAAT TTGCTAGC

FIG.30G

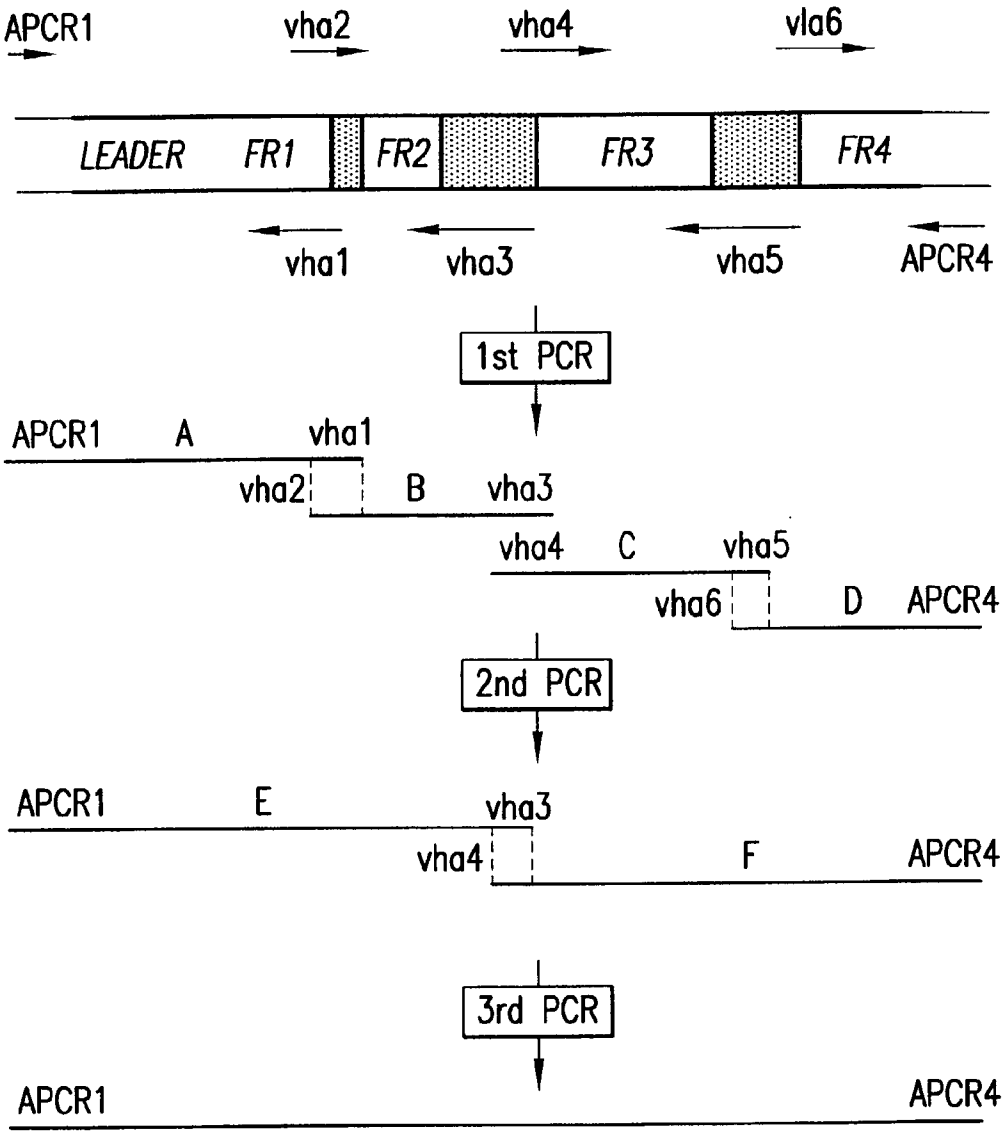

APCR1
vha2
vha4
vla6
LEADER
FR1
FR2
FR3
FR4
vha1
vha3
vha5
APCR4
1st PCR
APCR1
A
vha1
vha2
B
vha3
vha4
C
vha5
vha6
D
APCR4
2nd PCR
APCR1
E
vha3
vha4
F
APCR4
3rd PCR
APCR1
APCR4

FIG. 31

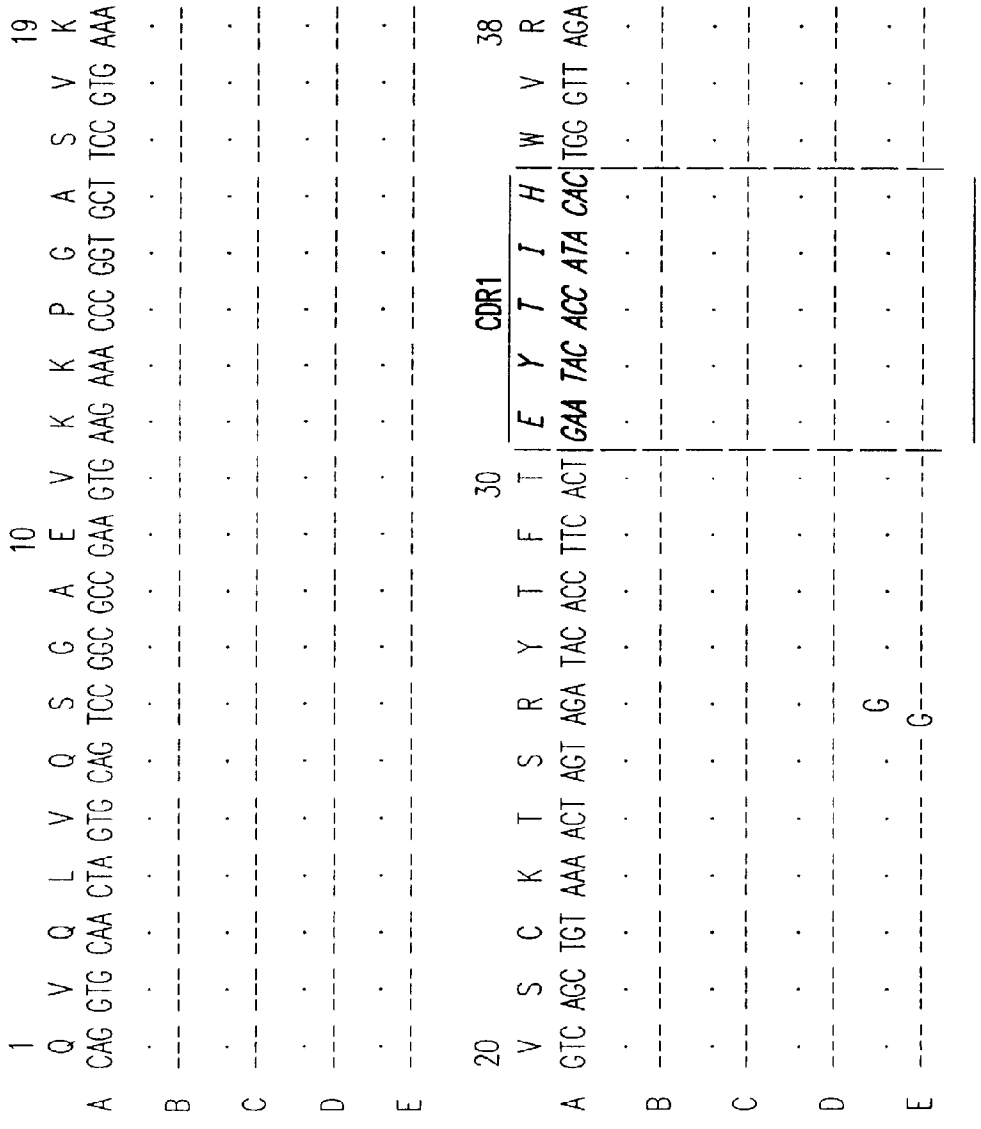

1 10 19
Q V Q L V Q S G A E V K K P G A S V K
A CAG GTG CAA CTA GTG CAG TCC GGC GCC GAA GTG AAG AAA CCC GGT GCT TCC GTG AAA
B --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
D --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
E --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
20 30 38
V S C K T S R Y T F T E Y T I H W V R
A GTC AGC TGT AAA ACT AGT AGA TAC ACC TTC ACT GAA TAC ACC ATA CAC TGG GTT AGA
B --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
D --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
E --- --- --- --- --- --- G --- --- --- --- --- --- --- --- --- --- --- ---
CDR1

FIG. 32A

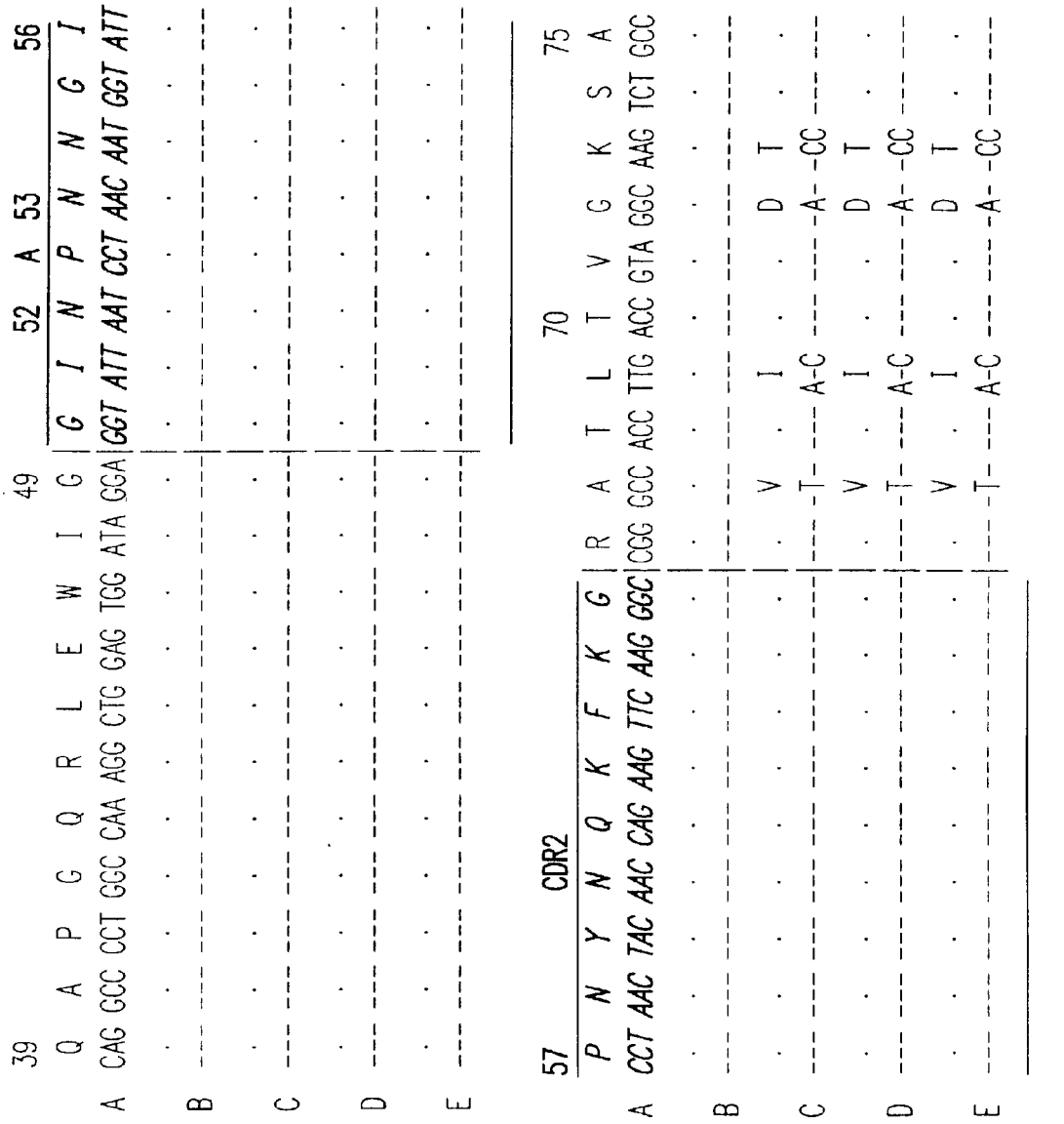

39
49
52 A 53
56
Q A P G Q R L E W I G
G I N P N N G I
CAG GCC CCT GGC CAA AGG CTG GAG TGG ATA GGA
GGT ATT AAT CCT AAC AAT GGT ATT
A
B
C
D
E
57
CDR2
70
75
P N Y N Q K F K G
R A T L T V G K S A
CCT AAC TAC AAC CAG AAG TTC AAG GGC
CGG GCC ACC TTG ACC GTA GGC AAG TCT GCC
A
B
C
D
E

FIG. 32B

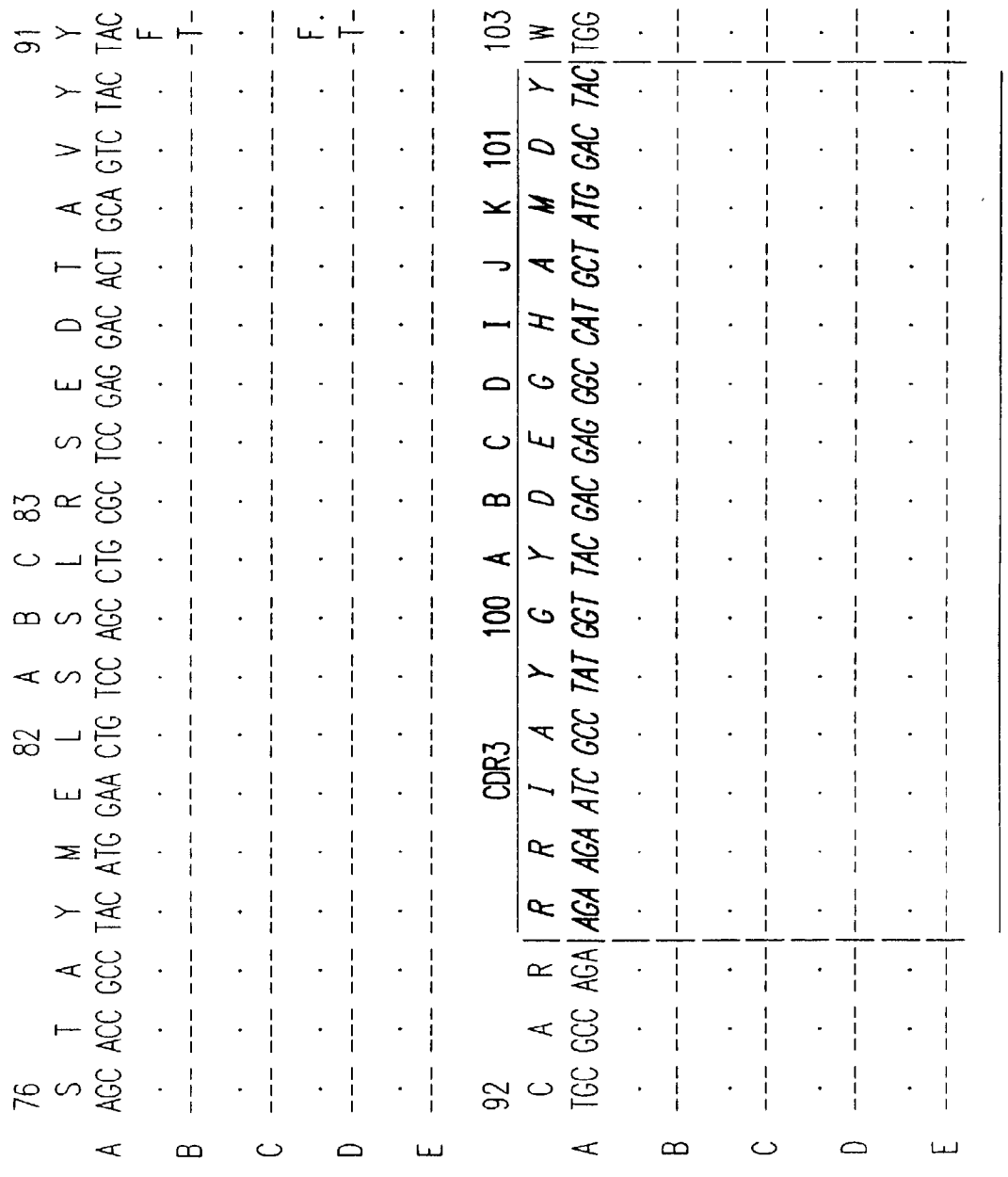

76 82 A B C 83 91
S T A Y M E L S S L R S E D T A V Y Y
A AGC ACC GCC TAC ATG GAA CTG TCC AGC CTG CGC TCC GAG GAC ACT GCA GTC TAC TAC
B ---
C ---
D ---
E ---
92 CDR3 100 A B C D I J K 101 103
C A R R R I A Y G Y D E G H A M D Y W
A TGC GCC AGA AGA AGA ATC GCC TAT GGT TAC GAC GAG GGC CAT GCT ATG GAC TAC TGG
B ---
C ---
D ---
E ---

FIG. 32C

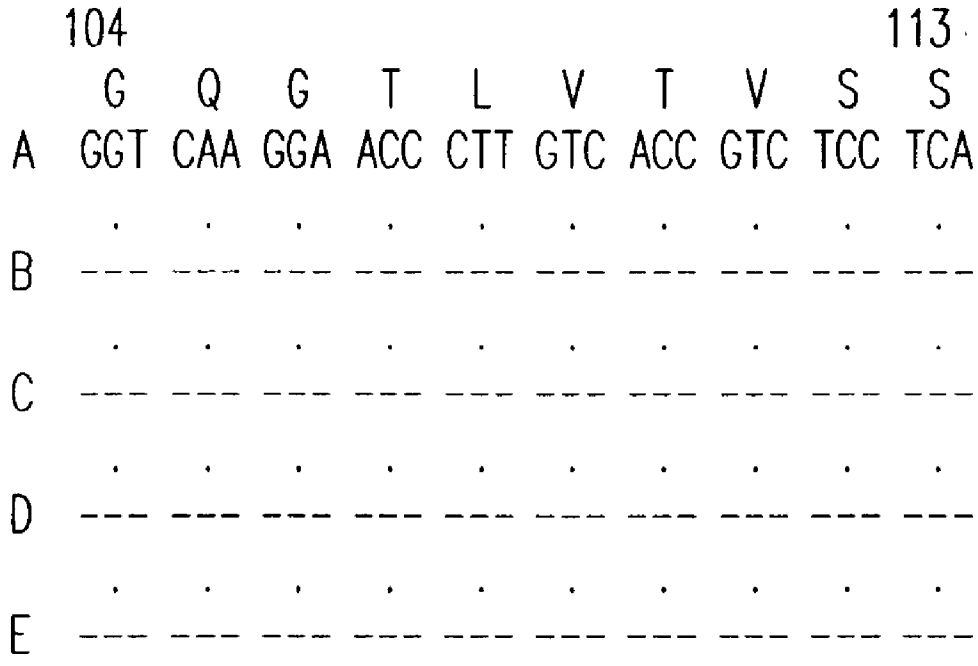

104
113
G Q G T L V T V S S
A GGT CAA GGA ACC CTT GTC ACC GTC TCC TCA
B
C
D
E

FIG. 32D

```
   1 TTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAAT
  61 GGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTT
 121 ATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCT
 181 TCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCC
 241 CTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAA
 301 AGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGG
 361 TAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGT
 421 TCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCG
 481 CATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTAC
 541 GGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGC
                          Pvu I
 601 GGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAA
 661 CATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACC
                                                    Fsp I
 721 AAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATT
 781 AACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGA
 841 TAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAA
 901 ATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAA
 961 GCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAA
1021 TAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGT
1081 TTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGT
1141 GAAGATCCTTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTG
1201 AGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGT
1261 AATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCA
1321 AGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATAC
1381 TGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTAC
```

FIG.33A

1441 ATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCT

1501 TACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGG

1561 GGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACA

1621 GCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGT

1681 AAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTA

1741 TCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTC

1801 GTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGC

BspLU11I

1861 CTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAA

1921 CCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAG

1981 CGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCT

2041 GTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATA

Bst1107 I

2101 GTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACAC

2161 CCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGA

2221 CAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAA

2281 CGCGCGAGGCAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCC

2341 CATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTT

Sfi I

2401 TTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGG

Stu I/Avr II

2461 AGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAGCTTACAGCTCAGGGCTGCGATT

2521 TCGCGCCAAACTTGACGGCAATCCTAGCGTGAAGGCTGGTAGGATTTTATCCCCGCTGCC

2581 ATCATGGTTCGACCATTGAACTGCATCGTCGCCGTGTCCCAAAATATGGGGATTGGCAAG

2641 AACGGAGACCTACCCTGGCCTCCGCTCAGGAACGAGTTCAAGTACTTCCAAAGAATGACC

2701 ACAACCTCTTCAGTGGAAGGTAAACAGAATCTGGTGATTATGGGTAGGAAAACCTGGTTC

2761 TCCATTCCTGAGAAGAATCGACCTTTAAAGGACAGAATTAATATAGTTCTCAGTAGAGAA

2821 CTCAAAGAACCACCACGAGGAGCTCATTTTCTTGCCAAAAGTTTGGATGATGCCTTAAGA

FIG.33B

2881 CTTATTGAACAACCGGAATTGGCAAGTAAAGTAGACATGGTTTGGATAGTCGGAGGCAGT

2941 TCTGTTTACCAGGAAGCCATGAATCAACCAGGCCACCTCAGACTCTTTGTGACAAGGATC

3001 ATGCAGGAATTTGAAAGTGACACGTTTTTCCCAGAAATTGATTTGGGGAAATATAAACTT

3061 CTCCCAGAATACCCAGGCGTCCTCTCTGAGGTCCAGGAGGAAAAAGGCATCAAGTATAAG

3121 TTTGAAGTCTACGAGAAGAAAGACTAACAGGAAGATGCTTTCAAGTTCTCTGCTCCCCTC

Bgl II

3181 CTAAAGCTATGCATTTTTATAAGACCATGGGACTTTTGCTGGCTTTAGATCTTTGTGAAG

3241 GAACCTTACTTCTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCT

3301 AAGGTAAATATAAAATTTTTAAGTGTATAATGTGTTAAACTACTGATTCTAATTGTTTGT

3361 GTATTTTAGATTCCAACCTATGGAACTGATGAATGGGAGCAGTGGTGGAATGCCTTTAAT

3421 GAGGAAAACCTGTTTTGCTCAGAAGAAATGCCATCTAGTGATGATGAGGCTACTGCTGAC

3481 TCTCAACATTCTACTCCTCCAAAAAAGAAGAGAAAGGTAGAAGACCCCAAGGACTTTCCT

3541 TCAGAATTGCTAAGTTTTTTGAGTCATGCTGTGTTTAGTAATAGAACTCTTGCTTGCTTT

3601 GCTATTTACACCACAAAGGAAAAAGCTGCACTGCTATACAAGAAAATTATGGAAAAATAT

3661 TCTGTAACCTTTATAAGTAGGCATAACAGTTATAATCATAACATACTGTTTTTTCTTACT

3721 CCACACAGGCATAGAGTGTCTGCTATTAATAACTATGCTCAAAAATTGTGTACCTTTAGC

3781 TTTTTAATTTGTAAAGGGGTTAATAAGGAATATTTGATGTATAGTGCCTTGACTAGAGAT

BsaB I

3841 CATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCT

Mun I

3901 CCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGC

3961 TTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTC

4021 ACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCTA

4081 ATAAAAGATATTTATTTTCATTAGATATGTGTGTTGGTTTTTTGTGTGCAGTGCCTCTAT

4141 CTGGAGGCCAGGTAGGGCTGGCCTTGGGGGAGGGGGAGGCCAGAATGACTCCAAGAGCTA

4201 CAGGAAGGCAGGTCAGAGACCCCACTGGACAAACAGTGGCTGGACTCTGCACCATAACAC

FIG.33C

EcoR I
4261 ACAATCAACAGGGGAGTGAGCTGGAAATTTGCTAGCGAATTCcagcacactggcggccgt
(Spe I)
4321 tACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTT

4381 CCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCC

4441 ATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACG

4501 TCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATAT

4561 GCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCA
SnaB I
4621 GTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTAT

4681 TACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACG

4741 GGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCA

4801 ACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCG

4861 TGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAG

4921 ACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGG

4981 CCGGGAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGACGTAAGTACCGCCTA

5041 TAGAGTCTATAGGCCCACCCCCTTGGCTTCTTATGCATGCTATACTGTTTTTGGCTTGGG
Bpu1102I
5101 GTCTATACACCCCCGCTTCCTCATGTTATAGGTGATGGTATAGCTTAGCCTATAGGTGTG
Xcm I
5161 GGTTATTGACCATTATTGACCCACTCCCCTATTGGTGACGATACTTTCCATTACTAATCCA

5221 TAACATGGCTCTTTGCCACAACTCTCTTTATTGGCTATATGCCAATACACTGTCCTTCAG

5281 AGACTGACACGGACTCTGTATTTTTACAGGATGGGGTCTCATTTATTATTTACAAATTCA

5341 CATATACAACACCACCGTCCCCAGTGCCCGCAGTTTTTATTAAACATAACGTGGGATCTC
BspE I
5401 CACGCGAATCTCGGGTACGTGTTCCGGACATGGGCTCTTCTCCGGTAGCGGCGGAGCTTC

5461 TACATCCGAGCCCTGCTCCCATGCCTCCAGCGACTCATGGTCGCTCGGCAGCTCCTTGCT

5521 CCTAACAGTGGAGGCCAGACTTAGGCACAGCACGATGCCCACCACCACCAGTGTGCCGCA

5581 CAAGGCCGTGGCGGTAGGGTATGTGTCTGAAAATGAGCTCggggagcgggcttgcaccgc
(Pvu II)
5641 tgacgcatttggaagacttaaggcagcggcagaagaagatgcaggcagctgagttgttgt

FIG.33D

```
5701 gttctgataagagtcagaggtaactcccgttgcggtgctgttaacggtggagggcagtgt

5761 agtctgagcagtactcgttgctgccgcgcgcgccaccagacataatagctgacagactaa
                                                        Mlu I
5821 cagactgttcctttccatgggtcttttctgcagtcaccgtccttgacACGCGTCTCGGGA

   Hind III
5881 AGCTTGCCGCCACCATGGACTGGACCTGGCGCGTGTTTTGCCTGCTCGCCGTGGCTCCTG
                    M  D  W  T  W  R  V  F  C  L  L  A  V  A  P

5941 GGGCCCACAGCCAGGTGCAACTGGTGCAGTCCGGCGCCGAAGTGAAGAAACCCGGTGCTT
     G  A  H  S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A
               (Pvu II)  (Spe I)
6001 CCGTGAAAGTCAGCTGTAAAACTAGTAGATACACCTTCACTGAATACACCATACACTGGG
     S  V  K  V  S  C  K  T  S  R  Y  T  F  T  E  Y  T  I  H  W
                     Msc I                              CDR 1
6061 TTAGACAGGCCCCTGGCCAAAGGCTGGAGTGGATAGGAGGTATTAATCCTAACAATGGTA
     V  R  Q  A  P  G  Q  R  L  E  W  I  G  G  I  N  P  N  N  G

6121 TTCCTAACTACAACCAGAAGTTCAAGGGCCGGGCCACCTTGACCGTAGGCAAGTCTGCCA
     I  P  N  Y  N  Q  K  F  K  G  R  A  T  L  T  V  G  K  S  A
           CDR 2
6181 GCACCGCCTACATGGAACTGTCCAGCCTGCGCTCCGAGGACACTGCAGTCTACTACTGCG
     S  T  A  Y  M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C

6241 CCAGAAGAAGAATCGCCTATGGTTACGACGAGGGCCATGCTATGGACTACTGGGGTCAAG
     A  R  R  R  I  A  Y  G  Y  D  E  G  H  A  M  D  Y  W  G  Q
                        CDR 3          BamH I
6301 GAACCCTTGTCACCGTCTCCTCAGGTGAGTGGATCCTCTGCGCCTGGGCCCAGCTCTGTC
     G  T  L  V  T  V  S  S

6361 CCACACCGCGGTCACATGGCACCACCTCTCTTGCAGCCTCCACCAAGGGCCCATCGGTCT
                                              S  T  K  G  P  S  V

6421 TCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG
     F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L
                        Age I
6481 TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCG
     V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S

6541 GCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGG
     G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V
   BstE II
6601 TGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGC
     V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K

6661 CCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACAT
     P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D  K  T  H  T
```

FIG.33E

```
6721 GCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAA
     C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F  P  P

6781 AACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACG
     K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D

6841 TGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATA
     V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H

6901 ATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCC
     N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V

6961 TCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACA
     L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N

7021 AAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
     K  A  L  P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E

7081 CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGA
     P  Q  V  Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V  S  L

7141 CCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC
     T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G

7201 AGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC
     Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F

7261 TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT
     L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C

7321 CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
     S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P
                      NgoM I
7381 GTAAATGAGTGCGACGGCCGGCAAGCCCCGCTCCCCGGGCTCTCGCGGTCGCACGAGGAT
     G  K  *
7441 GCTTGGCACGTACCCCCTGTACATACTTCCCGGGCGCCCAGCATGGAAATAAAGCACCGG

7501 ATCTAATAAAAGATATTTATTTTCATTAGATATGTGTGTTGGTTTTTTGTGTGCAGTGCC

7561 TCTATCTGGAGGCCAGGTAGGGCTGGCCTTGGGGGAGGGGGAGGCCAGAATGACTCCAAG

7621 AGCTACAGGAAGGCAGGTCAGAGACCCCACTGGACAAACAGTGGCTGGACTCTGCACCAT

7681 AACACACAATCAACAGGGGAGTGAGCTGGaaatttgctagcgaattaattc 7731
```

FIG.33F

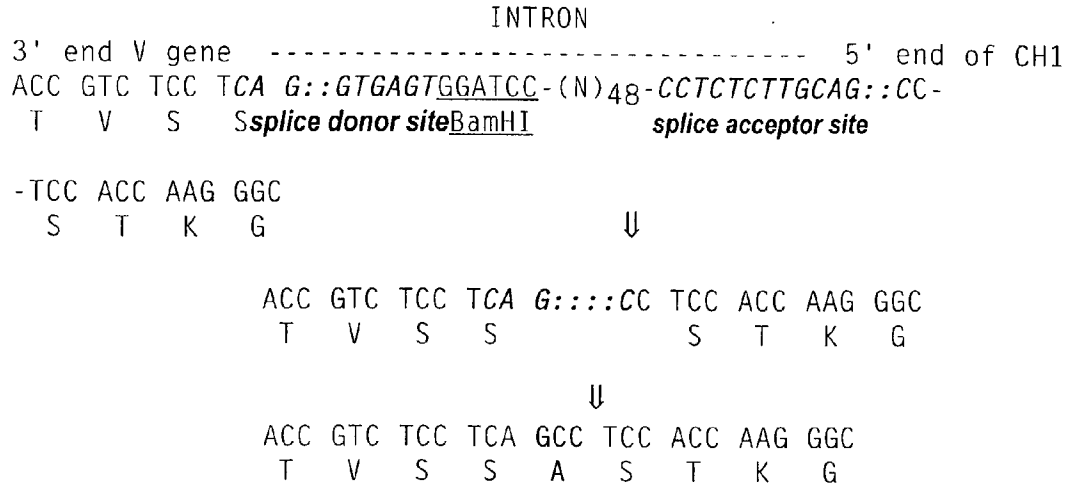


FIG.34A

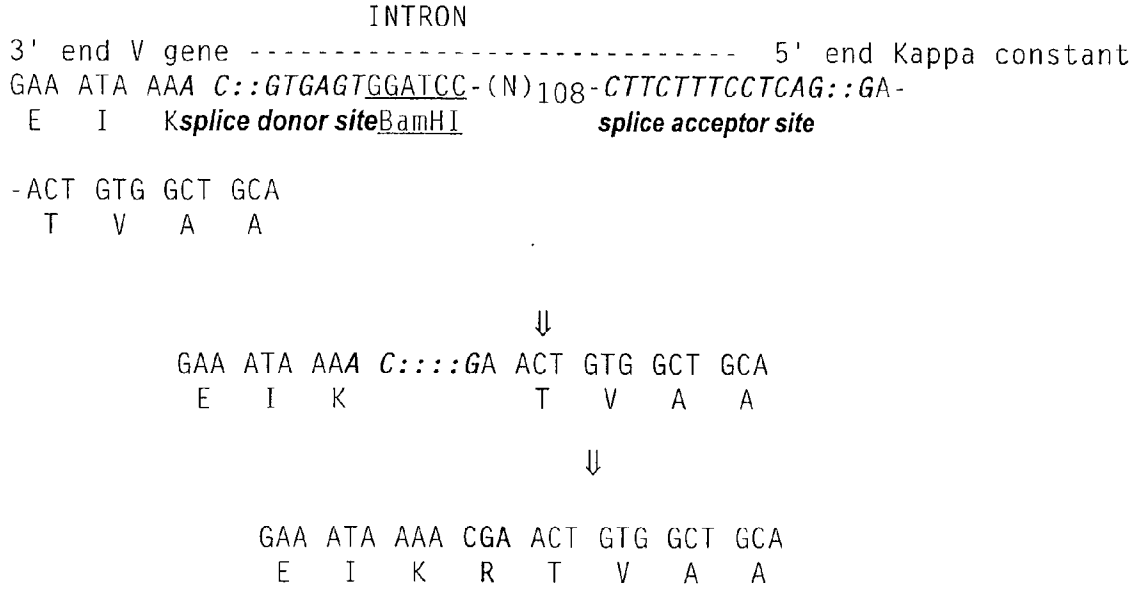


FIG.34B

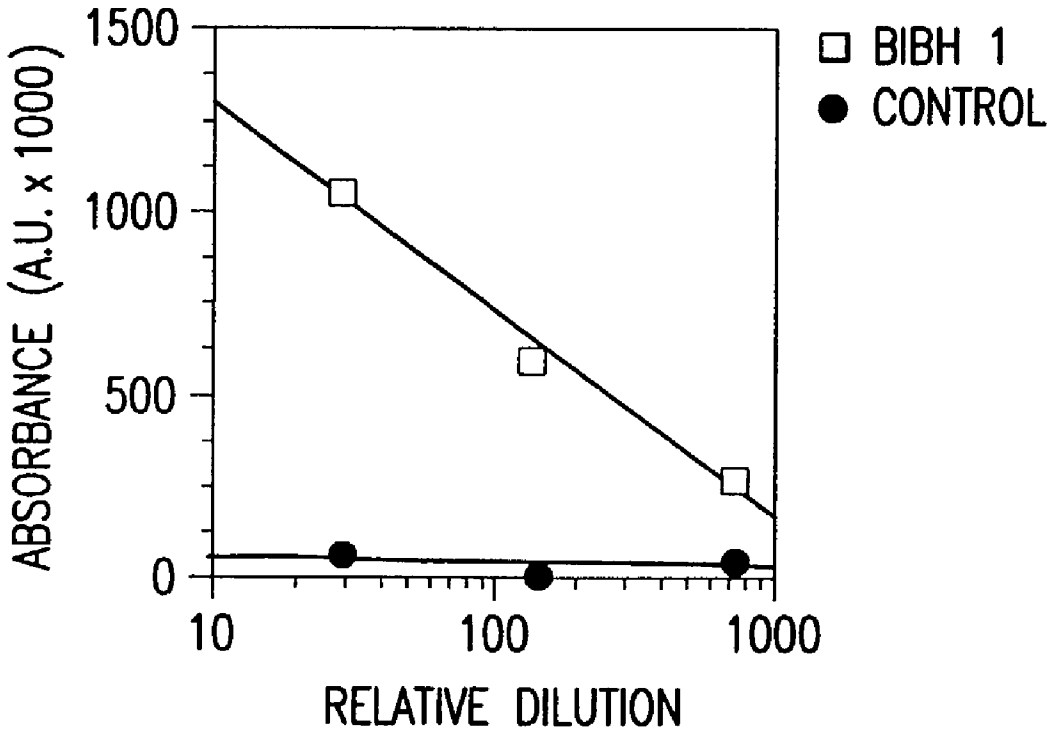

1500
1000
500
0
ABSORBANCE (A.U. x 1000)
10
100
1000
RELATIVE DILUTION
BIBH 1
CONTROL

FIG. 35

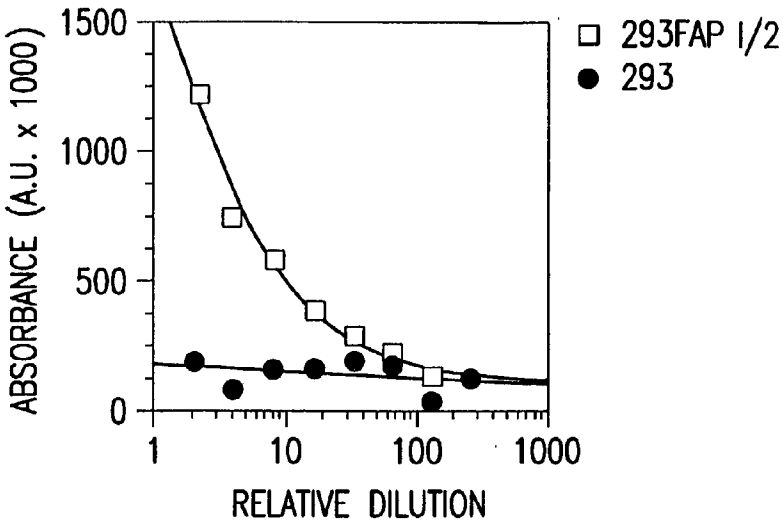

1500
1000
500
0
ABSORBANCE (A.U. x 1000)
1
10
100
1000
RELATIVE DILUTION
293FAP 1/2
293

FIG. 36

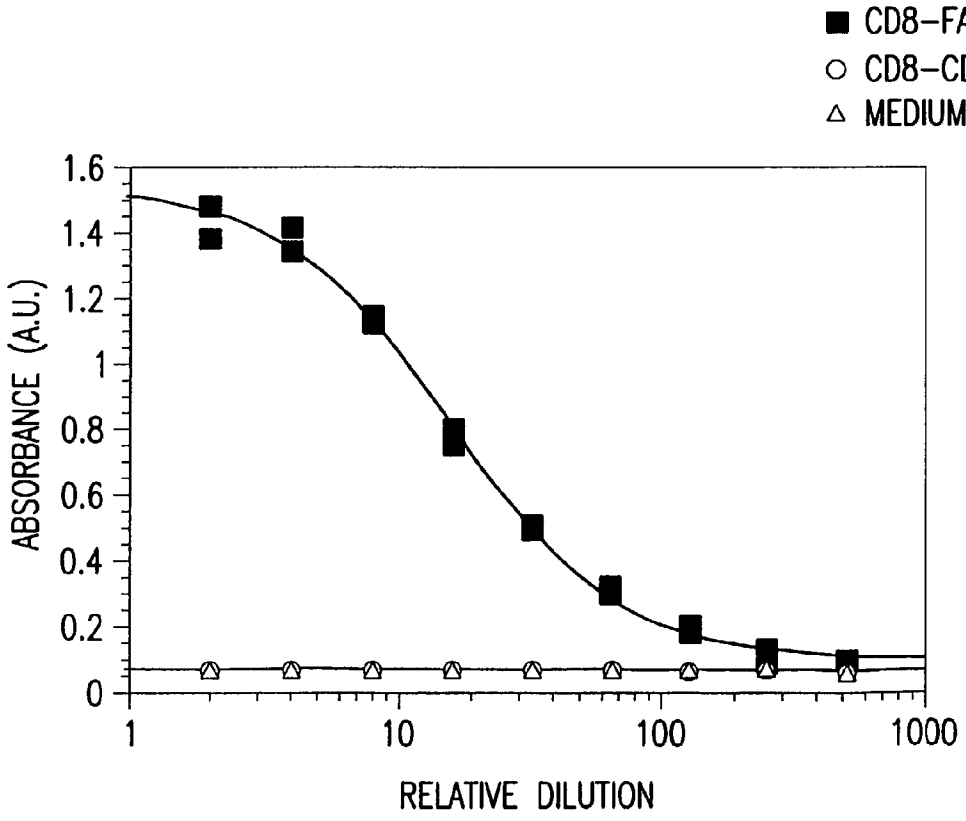

CD8-FAP
CD8-CD40L
MEDIUM
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
ABSORBANCE (A.U.)
1
10
100
1000
RELATIVE DILUTION

FIG. 37

FAPALPHA -SPECIFIC ANTIBODY WITH IMPROVED PRODUCIBILITY

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention

[0002] The present invention relates to antibody proteins that specifically bind fibroblast activation protein alpha (FAPα). The invention also relates to the use of said antibodies for diagnostic and therapeutic purposes and methods of producing said antibodies.

[0003] 2. Related Art

[0004] The invasive growth of epithelial cancers is associated with a number of characteristic cellular and molecular changes in the supporting stroma. A highly consistent molecular trait of the reactive stroma of many types of epithelial cancer is induction of the fibroblast activation protein alpha (from now on referred to as FAP), a cell surface molecule of reactive stromal fibroblasts originally identified with monoclonal antibody F19 (Garin-Chesa, P., et al., "Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers,"*Proc. Natl. Acad. Sci.* 87:7235 (1990)). Since the FAP antigen is selectively expressed in the stroma of a range of epithelial carcinomas, independent of location and histological type, a FAP-targeting concept has been developed for imaging, diagnosis and treatment of epithelial cancers and certain other conditions. For this purpose a monoclonal antibody termed F19 that specifically binds to FAP was developed and described in U.S. Pat. No. 5,059,523 and WO 93/05804, which are hereby incorporated by reference in their entirety.

[0005] One serious problem that arises when using non-human antibodies for applications in vivo in humans is that they quickly raise a human anti-non-human response that reduces the efficacy of the antibody in patients and impairs continued administration. Humanization of non-human antibodies is commonly achieved in one of two ways: (1) by constructing non-human/human chimeric antibodies, wherein the non-human variable regions are joined to human constant regions (Boulianne, G. L., et al., "Production of functional chimeric mouse/human antibody,"*Nature* 312:643 (1984)) or (2) by grafting the complementarity determining regions (CDRs) from the non-human variable regions to human variable regions and then joining these "reshaped human" variable regions to human constant regions (Riechmann L., et al., "Reshaping human antibodies for therapy,"*Nature* 332:323 (1988)). Chimeric antibodies, although significantly better than mouse antibodies, can still elicit an anti-mouse response in humans (LoBuglio, A. F., et al., "Mouse/human chimeric monoclonal antibody in man: Kinetics and immune response,"*Proc. Natl. Acad. Sci.* 86:4220 (1989)). CDR-grafted or reshaped human antibodies contain little or no protein sequences that can be identified as being derived from mouse antibodies. Although an antibody humanized by CDR-grafting may still be able to elicit some immune reactions, such as an anti-allotype or an anti-idiotypic response, as seen even with natural human antibodies, the CDR-grafted antibody will be significantly less immunogenic than a mouse antibody thus enabling a more prolonged treatment of patients.

[0006] Another serious limitation relating to the commercial use of antibodies for diagnosis, imaging and therapy is their producibility in large amounts. In many instances recombinant expression of native, chimeric and/or CDR-grafted antibodies in cell culture systems is poor. Factors contributing to poor producibility may include the choice of leader sequences and the choice of host cells for production as well as improper folding and reduced secretion. Improper folding can lead to poor assembly of heavy and light chains or a transport incompetent conformation that forbids secretion of one or both chains. It is generally accepted that the L-chain confers the ability of secretion of the assembled protein. In some instances multiple or even single substitutions can result in the increased producibility of antibodies.

[0007] Because of the clinical importance of specific immunological targeting in vitro and in vivo of specific disease-related antigens for diagnosis and therapy in humans, there is a growing need for antibodies that combine the features of antigen specificity, low immunogenicity and high producibility.

[0008] Therefore, the problem underlying the present invention was to provide antibody proteins that combine the properties of specific binding to FAP, low immunogenicity in humans, and high producibility in recombinant systems.

SUMMARY OF THE INVENTION

[0009] The technical problem is solved by the embodiments characterized in the claims.

[0010] The present invention provides new antibody proteins having the complementary determining regions of the monoclonal antibody F19 (ATCC Accession No. HB 8269), said new antibody proteins specifically binding to fibroblast activation protein (FAP), characterized in that they have framework modifications resulting in the improved producibility in host cells as compared to a chimeric antibody having the variable regions of F19 and foreign constant regions.

[0011] As used herein, an "antibody protein" is a protein with the antigen binding specificity of a monoclonal antibody.

[0012] "Complementarity determining regions of a monoclonal antibody" are understood to be those amino acid sequences involved in specific antigen binding according to Kabat (Kabat, E. A., et al., *Sequences of Proteins of Immunological Interest,* 5th Ed., NIH Publication No. 91-3242. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) in connection with Chothia and Lesk (Chothia and Lesk, *J. Mol. Biol.,* 196:901-917 (1987)).

[0013] As used herein, the term "framework modifications" refers to the exchange, deletion or addition of single or multiple amino acids in the variable regions surrounding the individual complementarity determining regions. Framework modifications may have an impact on the immunogenicity, producibility or binding specificity of an antibody protein.

[0014] "Fibroblast activation protein (FAP)", also designated fibroblast activation protein alpha (FAPα), is a membrane-bound glycoprotein belonging to the serine protease gene family (WO 97/34927). No shed or secreted form of FAP is known. FAP can be characterized by its binding to the

monoclonal antibody F19 (F19 is obtainable from the hybridoma cell line with the accession No. HB 8269 deposited at the ATCC).

[0015] The term "fibroblast activation protein specific binding" of an antibody protein is defined herein by its ability to specifically recognize and bind FAP-expressing human cells. The binding specificity of the proteins of the invention can be determined by standard methods for the evaluation of binding specificity such as described in an exemplary fashion in examples 6, 8 and 12.

[0016] The term "chimeric antibody" refers to an antibody protein having the light and heavy chain variable regions as described in **FIGS. 17 and 18** and foreign constant regions. "Foreign constant regions" as defined herein are constant regions which are different from the constant regions of F19. For comparing an antibody protein of the invention to a chimeric antibody it is to be understood that such a chimeric antibody must contain the same constant regions as said antibody protein. For the purpose of demonstration and comparison alone the human constant heavy and light chains as described in FIGS. **19** to **22** are used in an exemplary fashion.

[0017] To provide the antibody proteins of the present invention, the nucleic acid sequences of the heavy and light chain genes of the murine antibody designated F19 were determined from RNA extracted from F19 hybridoma cells (ATCC Accession No. HB 8269).

[0018] In one embodiment the present invention relates to antibody proteins having the complementary determining regions of the monoclonal antibody F19 (ATCC Accession No. HB 8269), said new antibody proteins specifically binding to fibroblast activation protein (FAP), characterized in that they have framework modifications resulting in the improved producibility in host cells as compared to a chimeric antibody having the variable regions of F19 and foreign constant regions, wherein said antibody protein is derived from the murine antibody designated F19 (ATCC Accession No. HB 8269).

[0019] To generate humanized FAP-specific antibody proteins a chimeric antibody was constructed, having variable regions of the light and heavy chains of F19 and human light and heavy constant regions, respectively. The construction and production of chimeric mouse/human antibodies is well known (Boulianne et al. (1984), referenced above) and demonstrated in an exemplary fashion in examples 1 and 2.

[0020] The variable regions of the antibody proteins of the present invention are typically linked to at least a portion of the immunoglobulin constant region (F_C), typically that of a human immunoglobulin. Human constant region DNA sequences can be isolated in accordance with well-known procedures from a variety of human cells, but preferably immortalized B cells (see Kabat et al., supra, and WO 87/02671). Hence the antibody proteins of the invention may contain all or only a portion of the constant region as long as they exhibit specific binding to the FAP antigen. The choice of the type and extent of the constant region depends on whether effector functions like complement fixation or antibody dependent cellular toxicity are desired, and on the desired pharmacological properties of the antibody protein. The antibody protein of the invention will typically be a tetramer consisting of two light chain/heavy chain pairs, but may also be dimeric, i.e., consisting of a light chain/heavy chain pair, e.g., a Fab or Fv fragment.

[0021] Therefore, in a further embodiment the invention relates to antibody proteins according to the invention, characterized in that they have a variable light chain region and a variable heavy chain region, each joined to a human constant region.

[0022] In particular, the variable region of the light chain was joined to a human kappa constant region and the variable region of the heavy chain was joined to a human gamma-1 constant region. Other human constant regions for humanizing light and heavy chains are also available to the expert.

[0023] Therefore, in one particular embodiment the antibody proteins of the invention contain a human kappa constant region.

[0024] Also, in another particular embodiment the antibody proteins of the invention contain a human gamma-1 constant region.

[0025] One particular "chimeric F19 antibody" protein (cF19) consists of the light and heavy chain variable and constant regions described in FIGS. **17** to **22**. cF19 demonstrates specific binding and high avidity to the FAP antigen. As demonstrated in example 2, the expression of cF19 in COS cells (cells derived from the kidney of an African green monkey) is poor, ranging from about 10 to 60 ng/ml, which is at least 10 fold less than most antibodies.

[0026] In an attempt to increase expression levels of cF19, the leader sequence of the F19 V_L region was changed by substitution of proline to leucine at position 9. This single change in amino acid in the leader sequence resulted in at least doubling the amount of chimeric antibody produced in COS cells. For the expression of this particular chimeric antibody in COS cells the following mutated leader sequence of the light chain: MDSQAQVMLLLLWVSGTCG, and the following leader sequence of the heavy chain: MGWSWVFLFLLSGTAGVS were used.

[0027] According to the invention the term "improved producibility" in host cells refers to the substantial improvement of expression levels and/or purified antibody yields when compared with the expression levels and/or antibody yields of a chimeric antibody without framework modifications as defined above. Two particular but not limiting examples for demonstrating improved producibility are exemplified for the COS cell expression system (in examples 2 and 5) and for the CHO cell expression system (in examples 10 and 11).

[0028] While the mutation of the leader sequence only leads to a doubling of the expression yield of the chimeric F19 antibody, a substantial improvement as defined herein refers to an improvement in expression level and/or purification yield of at least a factor of 10.

[0029] In a preferred embodiment, the invention refers to antibody proteins, characterized in that their expression levels in crude media samples as determined by ELISA and/or purified antibody yields exceed the expression levels and/or purification yields of the chimeric antibodies without framework modifications by at least a factor of 10.

[0030] In more preferred embodiment, the invention refers to antibody proteins, characterized in that their expression

levels in crude media samples as determined by ELISA and/or purified antibody yields exceed the expression levels and/or purification yields of the chimeric antibodies without framework modifications by at least a factor of 20.

[0031] In a most preferred embodiment, antibody proteins, characterized in that their expression levels in crude media samples as determined by ELISA and/or purified antibody yields exceed the expression levels and/or purification yields of the chimeric antibodies without framework modifications by at least a factor of 100.

[0032] Improved producibility of the recombinant antibody proteins of the invention can be demonstrated for eukaryotic cells in general as shown for COS and CHO (Chinese hamster ovary derived cells) eukaryotic cells (see examples 5 and 11). In a further embodiment, the present invention relates to recombinant antibody proteins characterized in that they display improved producibility in eukaryotic cells.

[0033] In a preferred embodiment the present invention relates to antibody proteins, wherein said eukaryotic cell is a Chinese hamster ovary cell (CHO cell).

[0034] It was unexpectedly found that certain framework modifications of the light chain variable regions determine the improved producibility of the antibody proteins of the invention. Three versions of reshaped light chain variable regions, designated version A, B and C, as described in FIGS. **1** to **6**, were prepared.

[0035] Light chain variable region versions A, B, and C demonstrate substantially improved producibility in CHO cells (see example 11). While light chain variable region versions A and C differ from light chain variable region version B by only two common amino acid residues they display an even further substantial improvement in producibility. There is at least another 10 fold difference in antibody secretion levels between the human reshaped F19 light chain version B and versions A or C. Reshaped human F19 light chain version A and B only differ in their amino acid sequences by two residues at positions 36 (Tyr to Phe mutation) and 87 (Tyr to Asp mutation) (nomenclature according to Kabat). This negative effect on the secretory capability of antibodies containing the light chain variable region version B could have been indirect if the Tyr to Asp and Tyr to Phe mutations, considered individually or together, merely caused improper folding of the protein. But this is unlikely to be the case since antigen binding assays show that immunoglobulins containing F19 light chain version B have similar avidities to those paired with F19 light chain version A or C, suggesting that they were not grossly misfolded.

[0036] Residue 87 in reshaped human F19 light chain version B seems particularly responsible for the reduction of secretion when compared to versions A and C.

[0037] In a preferred embodiment, the present invention relates to antibody proteins according to the invention, wherein the amino acid in Kabat position 87 of the light chain region is not asparagine.

[0038] In a more preferred embodiment, the invention relates to antibody proteins according to the invention, wherein the amino acid in Kabat position 87 of the light chain region is selected from aromatic or aliphatic amino acids.

[0039] In a most preferred embodiment, the present invention relates to antibody proteins according to the invention, wherein the aromatic amino acid in Kabat position 87 of the light chain region is a tyrosine or phenylalanine.

[0040] In a further embodiment, the present invention also pertains to antibody proteins according to the invention, wherein the amino acid in Kabat position 36 of the light chain region is selected from aromatic amino acids.

[0041] In a particular embodiment the invention relates to the specific antibody proteins that may be prepared from the individually disclosed reshaped variable regions of the light and heavy chains.

[0042] Especially light chain variable region versions A and C are particularly suitable to practice the invention because of their exceptionally high producibility, while retaining full FAP-binding specificity and achieving low immunogenicity. This holds especially true when compared to the chimeric antibody having the variable regions of F19 and the same constant regions but also when compared to light chain version B.

[0043] Therefore, in one embodiment the present invention relates to antibody proteins that contain the variable region of the light chain as set forth in SEQ ID NO:2.

[0044] In a further embodiment the invention also relates to antibody proteins, characterized in that the variable region of the light chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:1.

[0045] In one embodiment the present invention relates to antibody proteins that contain the variable region of the light chain as set forth in SEQ ID NO:6.

[0046] In a further embodiment the invention also relates to antibody proteins characterized in that the variable region of the light chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:5.

[0047] The present invention also discloses several different variable regions of the heavy chain that work particularly well with the variable regions of the light chain versions A and C in terms of improved producibility.

[0048] In one embodiment the invention relates to antibody proteins containing a variable region of the heavy chain as set forth in any one of SEQ ID NOS:8, 10, 12 and 14.

[0049] In another embodiment the invention relates to antibody proteins characterized in that the variable region of the heavy chain is encoded by a nucleotide sequence as set forth in any one of SEQ ID NOS:7, 9, 11 and 13.

[0050] In a very particular embodiment the invention relates to antibody proteins containing the variable region of the light chain as set forth in SEQ ID NO:2 and the variable region of the heavy chain as set forth in SEQ ID NO:12. Most preferably, this antibody protein additionally contains the constant region of the light chain as set forth in SEQ ID NO:20 and the constant region of the heavy chain as set forth in SEQ ID NO:22.

[0051] Thus a further aspect of the present invention is an antibody protein containing an amino acid sequence as set forth in SEQ ID NO:2. More preferably, such an antibody protein further contains an amino acid sequence as set forth

in SEQ ID NO:12. More preferably, said antibody protein further contains an amino acid sequence as set forth in SEQ ID NO:20 and an amino acid sequence as set forth in SEQ ID NO:22. A further aspect of the invention is an antibody protein as described in this paragraph which is conjugated to a radioisotope, preferably ^{131}I, ^{125}I, ^{186}Re, ^{188}Re, or ^{90}Y. An additional aspect of the present invention is a DNA molecule coding for an antibody protein as described in this paragraph. A further aspect of the invention is a host cell carrying such a DNA molecule. Accordingly, a further aspect of the invention is a method of producing an antibody protein as described in this paragraph, said method comprising the steps of cultivating such a host cell under conditions where said antibody protein is expressed by said host cell, and isolating said protein. A further aspect of the invention is a pharmaceutical composition comprising an antibody protein of the present invention and a pharmaceutically acceptable carrier.

[0052] In a further particular embodiment the invention relates to antibody proteins characterized in that the variable region of the light chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:1 and the variable region of the heavy chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:11.

[0053] In a further particular embodiment the invention relates to antibody proteins containing the variable region of the light chain as set forth in SEQ ID NO:2 and the variable region of the heavy chain as set forth in SEQ ID NO:8.

[0054] In a further particular embodiment the invention relates to antibody proteins characterized in that the variable region of the light chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:1 and the variable region of the heavy chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:7.

[0055] Humanization of the variable region of a murine antibody may be achieved employing methods known in the art. EP 0230400 discloses grafting of the CDRs of a murine variable region into the framework of a human variable region. WO 90/07861 discloses methods of reshaping a CDR-grafted variable region by introducing additional framework modifications. WO 92/11018 discloses methods of producing humanized Ig combining donor CDRs with an acceptor framework that has a high homology to the donor framework. WO 92/05274 discloses the preparation of framework mutated antibodies starting from a murine antibody. Further prior art references related to humanization of murine monoclonal antibodies are EP 0368684; EP 0438310; WO 92/07075 or WO 92/22653. Thus, the expert can produce the antibodies of the present invention starting from the publicly available murine monoclonal antibody F19 and employing techniques known in the art, e.g., from WO 92/05274; DNA molecules coding for the antibody proteins of the present invention may of course also be obtained by state-of-the-art synthetic procedures, e.g., by chemical synthesis of appropriate oligonucleotides and subsequent ligation and amplification procedures (see e.g., Frank et al., *Methods Enzymol* 154:221-249 (1987)).

[0056] In a further aspect, the present invention relates to nucleic acid molecules containing the coding information for the antibody proteins according to the invention as disclosed above. Preferably, a nucleic acid molecule according to the present invention is a nucleic acid molecule containing a nucleotide sequence selected from SEQ ID NOS:1, 3, 5, 7, 9, 11, 13 or 15.

[0057] A further aspect of the present invention is a recombinant DNA vector containing the nucleotide sequence of any one of the above-mentioned nucleic acids, especially when said nucleotide sequence is operationally linked to an expression control sequence as in expression vectors. Preferred is a recombinant DNA vector, said vector being an expression vector.

[0058] A further aspect of the present invention is a host cell carrying a vector as described, especially an expression vector. Such a host cell can be a prokaryotic or eukaryotic cell. Preferably, such a host cell is a eukaryotic cell, a yeast cell, or a mammalian cell. More preferably, said host cell is a CHO (Chinese hamster ovary) cell or a COS cell.

[0059] Accordingly, a still further aspect of the present invention is a method of producing antibody proteins according to the invention. Such a method comprises the steps of:

[0060] (a) cultivating a host cell as described above under conditions where said antibody protein is expressed by said host cell, and

[0061] (b) isolating said antibody protein.

[0062] Mammalian host cells, preferably CHO or COS cells are preferred. Host cells for producing the antibody proteins of the invention may be transfected with a single vector containing the expression units for both, the light and the heavy chain (see, e.g., WO 94/11523). In one particular embodiment the method of producing antibody proteins according to the invention pertains to host cells, wherein said host cells are cotransfected with two plasmids carrying the expression units for the light and heavy chains respectively (see, e.g., EP 0481790).

[0063] The antibody proteins of the invention provide a highly specific tool for targeting therapeutic agents to the FAP antigen. Therefore, in a further aspect, the invention relates to antibody proteins according to the invention, wherein said antibody protein is conjugated to a therapeutic agent. Of the many therapeutic agents known in the art, therapeutic agents selected from the group consisting of radioisotopes, toxins, toxoids, inflammatogenic agents, enzymes, antisense molecules, peptides, cytokines, and chemotherapeutic agents are preferred. Among the radioisotopes, gamma, beta and alpha-emitting radioisotopes may be used as a therapeutic agent. β-emitting radioisotopes are preferred as therapeutic radioisotopes. ^{186}Rhenium, ^{188}Rhenium, ^{131}Iodine and ^{90}Yttrium have been proven to be particularly useful β-emitting isotopes to achieve localized irradiation and destruction of malignant tumor cells. Therefore, radioisotopes selected from the group consisting of ^{186}Rhenium, ^{188}Rhenium, ^{131}Iodine and ^{90}Yttrium are particularly preferred as therapeutic agents conjugated to the antibody proteins of the invention. For example, for the radioiodination of an antibody of the invention, a method as disclosed in WO 93/05804, may be employed.

[0064] A further aspect of the present invention pertains to antibody proteins according to the invention, characterized in that they are labelled. Such an FAP-specific labelled antibody allows for the localization and/or detection of the FAP antigen in vitro and/or in vivo. A label is defined as a

marker that may be directly or indirectly detectable. An indirect marker is defined as a marker that cannot be detected by itself but needs a further directly detectable marker specific for the indirect marker. Preferred labels for practicing the invention are detectable markers. From the large variety of detectable markers, a detectable marker selected from the group consisting of enzymes, dyes, radioisotopes, digoxygenin, and biotin is most preferred.

[0065] A further aspect of the present invention relates to antibody proteins according to the invention, characterized in that they are conjugated to an imageable agent. A large variety of imageable agents, especially radioisotopes, are available from the state of the art. For practicing the invention gamma-emitting isotopes are more preferred. Most preferred is ^{125}Iodine.

[0066] One aspect of the present invention relates to pharmaceutical compositions containing an antibody protein according to the present invention as described above and a pharmaceutically acceptable carrier. Such pharmaceutical compositions are useful for treating tumors, wherein said tumors are associated with activated stromal fibroblasts. There are two possible effector principles for an anti-tumor stroma immunotherapy that may act synergistically: (a) an unmodified (unconjugated, "naked") antibody according to the invention may induce immune destruction or inflammatory reactions in the tumor stroma while (b) an antibody conjugated to a therapeutic agent, such as for example, a radioisotope or other toxic substance, may achieve localized irradiation and destruction of the malignant tumor cells. Accordingly, a further aspect of the present invention is the use of an antibody protein as described for the manufacture of a pharmaceutical composition, especially for the treatment of tumors.

[0067] One further embodiment are pharmaceutical compositions containing an antibody protein according to the invention conjugated to a therapeutic agent as described above and a pharmaceutically acceptable carrier useful for treating tumors, wherein said tumors are associated with activated stromal fibroblasts. Another embodiment pertains to pharmaceutical compositions containing an antibody protein according to the present invention conjugated to an imageable agent as described above and a pharmaceutically acceptable carrier useful for imaging the presence of activated stromal fibroblasts in a healing wound, inflamed skin or a tumor, in a human patient. A most preferred embodiment relates to the pharmaceutical compositions mentioned above, wherein said tumors are tumors selected from the cancer group consisting of colorectal cancers, non-small cell lung cancers, breast cancers, head and neck cancer, ovarian cancers, lung cancers, invasive bladder cancers, pancreatic cancers and cancers metastatic of the brain.

[0068] In an animal or human body, it can prove advantageous to apply the pharmaceutical compositions as described above via an intravenous or other route, e.g., systemically, locally or topically to the tissue or organ of interest, depending on the type and origin of the disease or problem treated, e.g., a tumor. For example, a systemic mode of action is desired when different organs or organ systems are in need of treatment as in e.g., systemic autoimmune diseases, or allergies, or transplantations of foreign organs or tissues, or tumors that are diffuse or difficult to localize. A local mode of action would be considered when only local manifestations of neoplastic or immunologic action are expected, such as, for example local tumors.

[0069] The antibody proteins of the present invention may be applied by different routes of application known to the expert, notably intravenous injection or direct injection into target tissues. For systemic application, the intravenous, intravascular, intramuscular, intraarterial, intraperitoneal, oral, or intrathecal routes are preferred. A more local application can be effected subcutaneously, intracutaneously, intracardially, intralobally, intramedullarly, intrapulmonarily or directly in or near the tissue to be treated (connective-, bone-, muscle-, nerve-, epithelial tissue). Depending on the desired duration and effectiveness of the treatment, pharmaceutical antibody compositions may be administered once or several times, also intermittently, for instance on a daily basis for several days, weeks or months and in different dosages.

[0070] For preparing suitable antibody preparations for the applications described above, the expert may use known injectable, physiologically acceptable sterile solutions. For preparing a ready-to-use solution for parenteral injection or infusion, aqueous isotonic solutions, such as, e.g., saline or corresponding plasma protein solutions are readily available. The pharmaceutical compositions may be present as lyophylisates or dry preparations, which can be reconstituted with a known injectable solution directly before use under sterile conditions, e.g., as a kit of parts. The final preparation of the antibody compositions of the present invention are prepared for injection, infusion or perfusion by mixing purified antibodies according to the invention with a sterile physiologically acceptable solution, that may be supplemented with known carrier substances or/and additives (e.g., serum albumin, dextrose, sodium bisulfite and EDTA).

[0071] The amount of the antibody applied depends on the nature of the disease. In cancer patients, the applied dose of a 'naked' antibody may be between 0.1 and 100 mg/m^2, preferably between 5 and 50 mg/m^2 per application. For radiolabeled antibodies, e.g., with iodine-131, the maximally tolerated dose (MTD) has to be determined which must not be exceeded in therapeutic settings. Application of radiolabeled antibody to cancer patients may then be carried out by repeated (monthly or weekly) intravenous infusion of a dose which is below the MTD (see, e.g., Welt et al., *J. Clin. Oncol.* 12:1193-1203 (1994)).

[0072] Furthermore, one aspect of the present invention relates to the use of the antibody proteins according to the invention for the treatment of cancer. In a preferred embodiment the present invention relates to the use of antibody proteins according to the invention conjugated to a therapeutic agent as described above for the treatment of cancer. In another preferred embodiment the present invention relates to the use of antibody proteins according to the invention conjugated to an imageable agent for imaging activated stromal fibroblasts. In a further preferred embodiment the present invention relates to the use of labelled antibody proteins according to the invention for detecting the presence of activated stromal fibroblasts in a sample.

[0073] One aspect of the invention relates to a method of treating tumors, wherein the tumor is associated with activated stromal fibroblasts capable of specifically forming a complex with antibody proteins according to the invention, present as naked/unmodified antibodies, modified antibody

proteins, such as, e.g., fusion proteins, or antibody proteins conjugated to a therapeutic agent, which comprises contacting the tumor with an effective amount of said antibodies. In a preferred embodiment the present invention relates to a method of treating tumors as mentioned above, wherein the tumor is a tumor having cancer cells selected from the cancer group consisting of colorectal cancers, non-small cell lung cancers, breast cancers, head and neck cancer, ovarian cancers, lung cancers, invasive bladder cancers, pancreatic cancers and metastatic cancers of the brain. The method of treating tumors as described above may be effected in vitro or in vivo.

[0074] A further aspect of the invention relates to a method of detecting the presence of activated stromal fibroblasts in wound healing, inflammation or in tumors, characterized in that

[0075] (a) a sample, possibly containing activated stromal fibroblasts, is contacted with an antibody protein according to the invention under conditions suitable for the formation of a complex between said antibody and antigen,

[0076] (b) detecting the presence of said complex, thereby detecting the presence of activated stromal fibroblasts in wound healing, inflammation or a tumor.

[0077] In a preferred embodiment, the present invention relates to a method of detecting the presence of activated stromal fibroblasts in a tumor, wherein the tumor is a tumor having cancer cells selected from the cancer group consisting of colorectal cancers, non-small cell lung cancers, breast cancers, head and neck cancer, ovarian cancers, lung cancers, bladder cancers, pancreatic cancers and metastatic cancers of the brain. Most preferred antibody proteins of the invention are those which are characterized in that they are labelled as mentioned above.

[0078] A further aspect of the invention relates to a method of imaging the presence of activated stromal fibroblasts in a healing wound, inflamed tissue (rheumatoid arthritis and cirrhosis are also positive) or a tumor, in a human patient, characterized in that

[0079] (a) an antibody protein according to the present invention conjugated to an imageable agent is administered to a human patient under conditions suitable for the formation of an antibody-antigen complex,

[0080] (b) imaging any complex formed in this manner,

[0081] (c) thereby imaging the presence of activated stromal fibroblasts in a human patient.

[0082] In a preferred embodiment the present invention relates to a method of imaging the presence of activated stromal fibroblasts as described above in tumors, wherein the tumor is a tumor having cancer cells selected from the cancer group consisting of colorectal cancers, non-small cell lung cancers, breast cancers, head and neck cancer, ovarian cancers, lung cancers, bladder cancers, pancreatic cancers and metastatic cancers of the brain.

[0083] In a further aspect the present invention relates to a method of detecting tumor-stroma, characterized in that

[0084] (a) a suitable sample is contacted with an antibody protein according to the present invention, under conditions suitable for the formation of an antibody-antigen complex,

[0085] (b) detecting the presence of any complex so formed,

[0086] (c) relating the presence of said complex to the presence of tumor-stroma.

[0087] Antibody proteins for practicing the invention are preferably labeled with a detectable marker.

[0088] In a further aspect the present invention relates to a method of imaging tumor-stroma in a human patient, which comprises

[0089] (a) administering to the patient an antibody according to the invention conjugated to an imageable agent as described above under conditions suitable for the formation of an antibody-antigen complex,

[0090] (b) imaging any complex so formed, and thereby imaging the presence of tumor-stroma in a human patient.

BRIEF DESCRIPTION OF THE FIGURES

[0091] **FIG. 1**. DNA sequence of F19 human reshaped light chain variable region version A ($hF19L_A$) SEQ ID NO:1.

[0092] **FIG. 2**. Amino acid sequence of F19 human reshaped light chain variable region version A ($hF19L_A$) SEQ ID NO:2.

[0093] **FIG. 3**. DNA sequence of F19 human reshaped light chain variable region version B ($hF19L_B$) SEQ ID NO:3. Nucleotides differing from version A are underlined and in bold type.

[0094] **FIG. 4**. Amino acid sequence of F19 human reshaped light chain variable region version B ($hF19L_B$) SEQ ID NO:4. Amino acids differing from version A are underlined and in bold type.

[0095] **FIG. 5**. DNA sequence of F19 human reshaped light chain variable region version C ($hF19L_C$) SEQ ID NO:5. Nucleotides differing from version A are underlined and in bold type.

[0096] **FIG. 6**. Amino acid sequence of F19 human reshaped light chain variable region version C ($hF19L_C$) SEQ ID NO:6. Amino acids differing from version A are underlined and in bold type.

[0097] **FIG. 7**. DNA sequence of F19 human reshaped variable region heavy chain version A ($hF19H_A$) SEQ ID NO:7.

[0098] **FIG. 8**. Amino acid sequence of F19 human reshaped heavy chain variable region version A ($hF19H_A$) SEQ ID NO:8

[0099] **FIG. 9**. DNA sequence of F19 human reshaped heavy chain variable region version B ($hF19H_B$) SEQ ID NO:9. Nucleotides differing from version A are underlined and in bold type.

[0100] **FIG. 10**. Amino acid sequence of F19 human reshaped heavy chain variable region version B ($hF19H_B$) SEQ ID NO:10. Amino acids differing from version A are underlined and in bold type.

[0101] **FIG. 11**. DNA sequence of F19 human reshaped heavy chain variable region version C ($hF19H_C$) SEQ ID NO:11. Nucleotides differing from version A are underlined and in bold type.

[0102] **FIG. 12**. Amino acid sequence of F19 human reshaped heavy chain variable region version C ($hF19H_C$) SEQ ID NO:12. Amino acids differing from version A are underlined and in bold type.

[0103] **FIG. 13**. DNA sequence of F19 human reshaped heavy chain variable region version D ($hF19H_D$) SEQ ID NO:13. Nucleotides differing from version A are underlined and in bold type.

[0104] **FIG. 14**. Amino acid sequence of F19 human reshaped heavy chain variable region version D ($hF19H_D$) SEQ ID NO:14. Amino acids differing from version A are underlined and in bold type.

[0105] **FIG. 15**. DNA sequence of F19 human reshaped heavy chain variable region version E ($hF19H_E$) SEQ ID NO:15. Nucleotides differing from version A are underlined and in bold type.

[0106] **FIG. 16**. Amino acid sequence of F19 human reshaped heavy chain variable region version E ($hF19H_E$) SEQ ID NO:16. Amino acids differing from version A are underlined and in bold type.

[0107] **FIG. 17**. Amino acid sequence of F19 chimeric light chain variable region (chF19LC) SEQ ID NO:17.

[0108] **FIG. 18**. Amino acid sequence of F19 chimeric heavy chain variable region (chF19HC) SEQ ID NO:18.

[0109] **FIG. 19**. DNA sequence of human kappa light constant chain SEQ ID NO:19.

[0110] **FIG. 20**. Amino acid sequence of human light constant chain SEQ ID NO:20.

[0111] **FIG. 21**. DNA sequence of human heavy constant chain SEQ ID NO:21.

[0112] **FIG. 22**. Amino acid sequence of human heavy constant chain SEQ ID NO:22.

[0113] **FIG. 23**. Mammalian cell expression vectors used to produce chimeric and reshaped human antibodies with human kappa light chains and human gamma-1 heavy chains.

[0114] A. Light chain expression vector: pKN100

[0115] B. Heavy chain expression vector: pG1D105

[0116] **FIG. 24**. DNA and amino acid sequences of mouse F19 light chain variable region as modified for use in the construction of chimeric F19 light chain. Restriction sites are indicated by bold letters. The Kozak sequence, CDRs 1 to 3 and the splice donor site are underlined.

[0117] **FIG. 25**. DNA and amino acid sequences of mouse F19 heavy chain variable region as modified for use in the construction of chimeric F19 heavy chain. Restriction sites are indicated by bold letters. The Kozak sequence and the splice donor site are underlined.

[0118] **FIG. 26**. DNA sequence of F19 chimeric antibody cloned into pKN100 mammalian expression vector. Restriction sites are indicated by bold letters and underlined. CDRs 1 to 3 and the splice donor site are underlined. This is the DNA sequence of the mouse F19 light chain inside the pKN100 eukaryotic expression vector. This vector has a cDNA version of the human kappa constant region gene (allotype Km(3)) terminated by a strong artificial termination sequence. In addition, the Neo selection gene is also terminated by this artificial sequence and is also in the same orientation as the kappa light chain expression cassette.

[0119] The essential components of the pKN100 eukaryotic expression vector are:

1–6	EcoRI site
7–1571	HCMVi promoter/enhancer
583–587	TATAA box
610	Start of transcription
728–736	Splice donor site
731	Beginning of intron
1557	End of intron
1544–1558	Splice acceptor site
1590–1598	Kozak sequence
1599–1658	peptide leader sequence
1659–1997	mouse F19 light chain
1996–2004	splice donor site
2011–2657	cDNA copy of human Kappa constant region (Km(3)) gene
2664–2880	Artificial spaC2 termination sequence
2887–7845	This is the pSV2neo vector DNA fragment comprising of the Amp-resistance gene (in the opposite orientation), the ColEI and SV40 origins of replication and the Neo-resistance gene (in the same orientation as the HCMVi-KCT cassette)
7852–8068	Artificial spaC2 termination signal

[0120] This sequence ends immediately upstream of the EcoRI site (position 1-6) at the beginning of the sequence. As a vector this DNA sequence would be circular.

[0121] **FIG. 27**. DNA sequence of F19 chimeric antibody cloned into pg1d105 mammalian expression vector. Restriction sites are indicated by bold letters and underlined. CDRs 1 to 3 and the splice donor site are underlined. This is the DNA sequence of the eukaryotic expression vector pG1D105 containing the mouse F19 heavy chain variable region. This vector contains a cDNA version of the human gamma-1 constant region (allotype G1m (non-a, -z, -x) also known as Gm1(17) allotype).

[0122] The essential components of the construct are:

1–2501	pBR322 based sequence including Ampicillin resistance gene and ColEI origin plus the SV40 origin and the crippled SV40 early promoter
2502–3226	dhfr gene
3233–4073	SV40 poly A sequence etc.
4074–4079	ligated BamHI and BglII site (BstYI)
4080–4302	SPA site plus C2 termination signal
4303–5867	HCMVi promoter
5879–5885	unique HindIII restriction site for cloning of immunoglobulin variable genes
5886–5894	Kozak sequence
5895–5951	signal peptide
5952–6323	mouse F19 heavy chain
6323–6330	splice donor site
6331–6336	unique BamHI restriction site for cloning of immunoglobulin variable genes
6337–7388	cDNA copy of human gamma-1 constant regions preceded by a 62 bp intron
7389–7709	Arnie termination sequence

[0123] The human gamma-1 constant region used in this construct has a G1m (non-a, -z, -x) also known as Gm1(17) allotype which is defined by a glutamic acid (E) residue at

position 356 (according to Eu numbering), and a methionine (M) residue at position 358 (according to Eu numbering) and a lysine (K) residue at position 214 (according to Eu numbering). These three residues are underlined in the sequence above.

[0124] **FIG. 28**. PCR-based method for the construction of human reshaped F19 light chain. This figure provides a schematic overview of the strategy of construction. The dotted lines indicate a complementary sequence of at least 21 bases between the primers.

[0125] **FIG. 29**. Nucleotide and deduced amino acid sequences of reshaped human F19 light chain variable regions version A, B and C. Nucleotide and deduced amino acid sequences are aligned and compared with that of version A, dashes indicate nucleotide identity, dots indicate amino acid identity with this sequence. Amino acids are numbered according to Kabat et al. (1991). The locations of CDRs are indicated in boxes.

[0126] **FIG. 30**. DNA sequence of F19 LA (human reshaped light chain version A) cloned into pKN100 mammalian expression vector. Restriction sites are indicated by bold letters and underlined. CDRs 1 to 3 and the splice donor site are underlined. This is the DNA sequence of the reshaped F19 light chain version. A cloned into pKN100 eukaryotic expression vector. This vector has a cDNA version of the human kappa constant region gene (allotype Km(3)) terminated by a strong artificial termination sequence. In addition, the Neo selection gene is also terminated by this artificial sequence and is also in the same orientation as the kappa light chain expression cassette.

[0127] The components of the vector are:

7–1571	HCMVi promoter/enhancer
583–587	TATAA box.
610	Start of transcription.
728–736	Splice donor site.
731	Beginning of intron.
1557	End of intron.
1544–1558	Splice acceptor site.
1590–1598	Kozak sequence
1599–1658	peptide leader sequence
1659–1997	reshaped F19 light chain version A
1996–2004	splice donor site
2011–2657	cDNA copy of human kappa constant region (Km(3)) gene.
2664–2880	Artificial spaC2 termination sequence.
2887–7845	This is the pSV2neo vector DNA fragment comprising of the Amp-resistance gene (in the opposite orientation), the ColEI and SV40 origins of replication and the Neo-resistance gene (in the same orientation as the HCMVi-KCT cassette).
7852–8068	Artificial spaC2 termination signal.

[0128] This sequence ends immediately upstream of the EcoRI site (position 1-6) at the beginning of the sequence below. As a vector this DNA sequence would be circular.

[0129] **FIG. 31**. PCR-based method for the construction of human reshaped F19 heavy chain. This figure provides a schematic overview of the strategy of construction. The dotted lines indicate a complementary sequence of at least 21 bases between the primers.

[0130] **FIG. 32**. Nucleotide and deduced amino acid sequences of reshaped human F19 heavy chain variable region versions a to e. Nucleotide and deduced amino acid sequences are aligned and compared with that of version A, dashes indicate nucleotide identity, dots indicate amino acid identity with this sequence. Amino acids are numbered according to Kabat et al. (1991). The location of CDRs is indicated by boxes.

[0131] **FIG. 33**. DNA sequence of F19Ha (human reshaped heavy chain version a) cloned into pg1d105 mammalian expression vector. Restriction sites are indicated by bold letters and underlined. CDRs 1 to 3 and the splice donor site are underlined. This is the DNA sequence of the eukaryotic expression vector pG1D105 containing the reshaped version A of F19 heavy chain variable region. This vector contains a cDNA version of the human gamma-1 constant region (allotype G1m (non-a,- z, -x) also known as Gm1(17) allotype).

[0132] The essential components of the construct are:

1–2501	pBR322 based sequence including Ampicillin resistance gene ColEI origin plus the SV40 origin and the crippled SV40 early promoter
2502–3226	dhfr gene
3233–4073	SV40 poly A sequence etc.
4080–4302	SPA site plus C2 termination signal
4303–5867	HCMVi promoter/enhancer
5879–5885	unique HindIII restriction site for cloning of immunoglobulin variable genes
5886–5894	Kozak sequence
5895–5951	signal peptide
5952–6323	reshaped F19 heavy chain version A
6323–6330	splice donor site
6331–6336	unique BamHI restriction site for cloning of immunoglobulin variable genes
6337–7388	cDNA copy of human gamma-1 constant regions preceded by a 62 bp intron
7389–7709	Arnie termination sequence

[0133] The human gamma-1 constant region used in this construct has a G1m (non-a, -z, -x) also known as Gm1(17) allotype which is defined by a glutamic acid (E) residue at position 356 (according to Eu numbering), a methionine (M) residue at position 358 (according to Eu numbering) and a lysine (K) residue at position 214 (according to Eu numbering). These three residues are underlined in the sequence above.

[0134] **FIG. 34**. Heavy (panel A) and light (panel B) chains RNA splicing events taking place during antibody F19 expression in mammalian cells—schematic overview.

[0135] A. Heavy chain RNA splicing

[0136] B. Kappa light chain RNA splicing

[0137] **FIG. 35**. Concentration dependence of L_AH_C supernatant binding to CD8-FAP.

[0138] **FIG. 36**. Binding of biotinylated L_AH_C to human FAP.

[0139] **FIG. 37**. CD8-FAP carries the F19 epitope as detected with cF19.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

EXAMPLES

Example 1

Construction of Mouse-human Chimeric Genes

[0140] The chimeric F19 (cF19) antibody was designed to have the mouse F19 V_L and V_H regions linked to human

kappa and gamma-1 constant regions, respectively. PCR primers were used to modify the 5'- and 3'- sequences flanking the cDNA sequences coding for the mouse F19 V_L and V_H regions (Table 1). PCR primers specific for F19 light chain V-region were designed. These adapted mouse F19 variable regions were then subcloned into mammalian cell expression vectors already containing the human kappa (pKN100 vector) or gamma-1 (pG1D105 vector) constant regions (**FIG. 23**). These vectors employ the human cytomegalovirus (HCMV) promoter/enhancer to efficiently transcribe the light and heavy chains. The vectors also contain the SV40 origin of replication to permit efficient DNA replication and subsequent protein expression in COS cells. The expression vectors were designed to have the variable regions inserted as HindIII-BamHII DNA fragments. PCR primers were designed to introduce these restrictions sites at the 5'-(HindIII) and 3'-(BamHI) ends of the cDNAs coding for the V-regions. In addition the PCR primers were designed to introduce the Kozak sequence (GCCGCCACC) at the 5'-ends of both the light and heavy chain cDNAs to allow efficient translation (Kozak, M., "At least six nucleotides preceding the AUG initiator codon enhance translation in mammalian cells,"*J. Mol. Biol.* 196:947 (1987)), and to introduce splice donor sites at the 3'-ends of both the light and heavy chain cDNAs for the variable regions to be spliced to the constant regions. The PCR primers used in the construction of the chimeric F19 light and heavy chains are shown in Table 1. The DNA and amino acid sequences of the mouse F19 V_L and V_H regions as adapted for use in the construction of chimeric F19 light and heavy chains are shown in **FIGS. 24 and 25**. The DNA sequences of mouse F19 light and heavy chains cloned into the eukaryotic expression vectors pKN100 and pG1D105, respectively, are shown in **FIGS. 26 and 27**.

TABLE 1

PCR primers for the construction of chimeric F19 antibody

A. Light chain variable region

1. Primer for the construction of the 5'-end (37 mer)
5' CAGA **AAGCTT** GCCGCCACC ATG GAT TCA CAG GCC CAG 3'
HindIII Kozak sequence M D S Q A Q

2. Primer for the construction of the 3'-end (35 mer)
5' CCGA **GGATCC** ACTCACGTTT CAG CTC CAG CTT GGT 3'
BamHI Splice donor site

B. Heavy chain variable region

1. Primer for the construction of the 5'-end (37 mer)
5' CAGA AAGCTT GCCGCCACCATG GGA TGG AGC TGG GTC 3'
HindIII Kozak sequence M G W S W V

2. Primer for the construction of the 3'-end (35 mer)
5' CCGA GGATCC ACTCACCTGA GGA GAC GGT GAC TGA 3'
BamHI Splice donor site

Example 2

Expression and Binding Activity of Chimeric F19 Antibody

[**0141**] The two plasmid DNAs coding for the chimeric F19 light and heavy chains (see example 1) were co-transfected into COS cells to look for transient expression of chimeric F19 antibody as described below. After incubation for 72 hours, the medium was collected, centrifuged to remove cellular debris, and analyzed by ELISA for the production of a human IgG1-like antibody. The COS cell supernatant containing the chimeric F19 antibody was analyzed for its ability to bind to HT 1080 cells (see example 13) expressing the FAP antigen on their surface.

Transfection of COS Cells Using Electroporation

[**0142**] The mammalian expression vectors pg1d105 and pKN100 containing the chimeric or reshaped human heavy and light chains versions, respectively, were tested in COS cells to look for transient expression of F19 antibodies. COS-7 cells were passaged routinely in DMEM (Gibco BRL cat. #41966) containing penicillin (50 IU/ml), streptomycin (50 mg/ml), L-glutamine and 10% heat-inactivated gamma globulin-free foetal calf serum (FCS, Harlan Sera-Lab cat. #D0001). The DNA was introduced into the COS cells by electroporation using the Gene Pulsar apparatus (BioRad). DNA (10 mg of each vector) was added to a 0.8 ml aliquot of 1×10^7 cells/ml in Phosphate-buffered saline (PBS, Ca^{2+} and Mg^{2+} free). A pulse was delivered at 1,900 volts, 25 mF capacitance. After a 10 minutes recovery period at ambient temperature the electroporated cells were added to 8 ml of DMEM containing 5% FCS. After incubation at 37° C. for 72 hours, the medium was collected, centrifuged to remove cellular debris, and stored under sterile conditions at 4° C. for short periods of time, or at −20° C. for longer periods.

ELISA Method for Measuring Assembled IgG1/kappa Antibody Concentrations in COS Cell Supernatants

[**0143**] Samples of antibodies produced in transfected COS cells were assayed by ELISA to determine how much chimeric or reshaped human antibody had been produced. For the detection of antibody, plates were coated with goat anti-human IgG (Fcg fragment specific) antibody (Jackson ImmunoResearch Laboratories Inc., #109-005-098). The samples from COS cells were serially diluted and added to each well. After incubation for 1 h at 37° C. and washing, horseradish peroxidase conjugated goat anti-human kappa light chain (Sigma, A-7164) was added. After incubation for

30 minutes at 37° C. and washing, K-blue substrate (a mixture of 3,3',5,5' tetramethylbenzidine and hydrogen peroxide, Bionostics Limited, #KB 175) was added for 30 minutes at room temperature. The reaction was stopped using Red Stop solution (Bionostics Limited, #RS20) and the optical density read on a microplate reader at 650 nm. Purified human IgG1/Kappa antibody (Sigma, I-3889) of known concentration was used as a standard.

[0144] The expression of chimeric F19 antibody in COS cells was poor (Table 2), between 10 and 60 ng/ml, which is at least 10 fold less than most antibodies.

[0145] In an attempt to increase expression levels of the chimeric F19 antibody, the leader sequence of F19 V_L region was changed by substitution of leucine to proline at position-9. This single change in amino acid in the leader sequence resulted in at least doubling the amount of chimeric antibody produced in COS cells.

[0146] Cell binding results show that chimeric F19 binds specifically and with the expected avidity to the FAP target.

TABLE 2

Chimeric F19 antibody concentrations in COS cell supernatants
(These are the results of three independent transfections)

Transfected Antibody components		Human γ1/K
Heavy chain	Kappa light chain	[in μg/ml]
cF19	cF19 (F19 leader sequence)	0.060
cF19	cF19 (mutated leader sequence)	0.212
cF19	cF19 (F19 leader sequence)	0.056
cF19	cF19 (mutated leader sequence)	0.108
cF19	cF19 (F19 leader sequence)	0.011
cF19	cF19 (mutated leader sequence)	0.087

Example 3

Construction of the Reshaped Human F19 Light Chain Versions A to C (L_A-L_B)

[0147] The construction of the first version of reshaped human F19 V_L region (L_A) was carried out using overlapping PCR fragments in a method similar to that described by Daugherty B. L., et al., "Polymerase chain reaction (PCR) facilitates the cloning, CDR-grafting, and rapid expression of a murine monoclonal antibody directed against the CD18 component of leukocyte integrins,"*Nucl. Acids Res.* 19:2471 (1991). Ten oligonucleotides were synthesized that consisted of five primer pairs, APCR1-vla1, vla2-vla3, vla4-vla5, vla6-vla7, and vla8-APCR4 (Table 3 and **FIG. 28**). There was an overlapping sequence of at least 21 bases between adjacent pairs (**FIG. 28**). APCR1 and APCR4 hybridized to the flanking pUC19 vector sequences. The mutagenic primers were designed such that their 5' end immediately followed the wobble position of a codon. This strategy was used to counteract the gratuitous addition of one nucleotide to the 3' end of the strand complementary to the mutagenic primer by the DNA polymerase during PCR (Sharrocks, A. D., and Shaw, P. E., "Improved primer design for PCR-based, site-directed mutagenesis,"*Nucl. Acids Res.* 20:1147 (1992)). The appropriate primer pairs (0.2 μM of each) were combined with 10 ng of version "B" of reshaped human L25VL region cDNA, and 1 unit of AmpliTaq (Perkin Elmer Cetus) DNA polymerase in 50 μl of PCR buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 μM dNTPs, and 1.5 mM $MgCl_2$. This was overlaid with mineral oil and PCR was performed for 25 cycles, each cycle consisting of a denaturation step at 94° C. for 1 minute, a primer annealing step at 55° C. for 1 minute, and an extension step at 72 ° C. for 2 minutes. This was followed by a single cycle consisting of a further elongation step at 72° C. for 10-minutes followed by cooling to 4° C. The ramp time between the primer-annealing and extension steps was 2.5 minutes. The PCR products of the five reactions (A, B, C, D and E) were then purified by gel electrophoresis followed by DNA elution using Wizard PCR preps (Promega). PCR products A, B, C, D, and E were assembled by their complementarity to one another. In the second set of PCR reactions, PCR products B and C, and D and E, (50 ng of each) were added to 50 ml PCR reactions (as described above) each containing 1 unit of AmpliTaq (Perkin Elmer Cetus) DNA polymerase. The reactions were cycled for 20 cycles as described above with the exception that the annealing temperature was raised to 60° C. In the third set of PCR reactions, PCR products F and G were PCR-amplified using 1 ml of each prior PCR reaction and the appropriate pair of PCR primers (vla2-vla5 or vla6-APCR4). The PCR reactions contained 1 unit of AmpliTaq DNA polymerase in 50 ml PCR reaction (as described above) and were amplified for 25 cycles as in the first stage. In the fourth set of PCR reactions, the PCR product H was PCR-amplified using 1 ml of each prior PCR reaction and the vla2-APCR4 pair of PCR primers. Finally, PCR products A and H were assembled by their own complementarity in a two step-PCR reaction similar to that described above using RSP and UP as the terminal primers. The fully assembled fragment representing the entire reshaped human F19 V_L region including a leader sequence was digested with HindIII and BamHI and cloned into pUC19 for sequencing. A clone having the correct DNA sequence was designated reshF19La (**FIG. 29**) and was then subcloned into the eukaryotic expression vector pKN100. The DNA sequence of reshF19La cloned into pKN100 is shown in **FIG. 30**.

[0148] The second version of reshaped human F19 V_L region (L_B) was constructed using the same scheme as that described for La but where vla4 and vla7 primers were substituted by vlb4 and vlb7 respectively (Table 3). The DNA sequence of L_B is shown in **FIG. 29**.

[0149] The third version of reshaped human F19 V_L region (L_C) was constructed using the QuikChange™ site-directed mutagenesis kit from Stratagene. The QuikChange site-directed mutagenesis method was performed according to the manufacturer's instructions, using reshF19La in pKN100 vector as double stranded DNA template. The mutagenic oligonucleotide primers F19Lc-sense and F19Lc-antisense (Table 3) for use in this protocol were designed according to the manufacturer's instructions. Briefly, both the mutagenic primers contained the desired point mutation (codon TTT at Kabat residue position 49 (Phe) changed to TAT coding for Tyr) and annealed to the same sequence on opposite strands of LA in pKN100 vector. The point mutation was verified by DNA sequencing the entire V_L region. The DNA sequence of L_C is shown in **FIG. 29**. To eliminate the possibility that random mutations occurred in the pKN100 during the PCR reaction, the V_L region was cut out of the pKN100 vector as an HindIII/BamHI fragment and re-subcloned into an unmodified pKN100 vector cut with the same two restriction enzymes beforehand.

TABLE 3

PCR primers for the construction of reshaped human F19 light chain variable regions

1. Primers for the synthesis of version "A"

F19vla1 (36 mer):
5' GTCATCACAATGTCTCCGGAGGAACCTGGAACCCAG 3'

F19vla2 (29 mer):
5' CTCCGGAGACATTGTGATGACCCAATCTC 3'

F19vla3 (45 mer):
5' GAATATAAAAGGCTCTGACTGGACTTGCAGTTGATGGTGGCCCTC 3'

F19vla4 (72 mer):
5' CAGTCAGAGCCTTTTATATTCTAGAAATCAAAAGAACTACTTGGCCTGGTATCAGCAGAAACCAGGACAGCC 3'

F19vla5 (44 mer):
5' ACCCCAGATTCCCTAGTGCTAGCCCAAAAGATGAGGAGTTTGGG 3'

F19vla6 (67 mer):
5' TAGCACTAGGGAATCTGGGGTACCTGATAGGTTCAGTGGCAGTGGGTTTGGGACAGACTTCACCCTC 3'

F19vla7 (53 mer):
5' GTCCCTTGTCCGAACGTGAGCGGATAGCTAAAATATTGCTGACAGTAATAAAC 3'

F19vla8 (33 mer):
5' GCTCACGTTCGGACAAGGGACCAAGGTGGAAAT 3'

2. Primers for the synthesis of version "B"

F19vlb4 (72 mer):
5' CAGTCAGAGCCTTTTATATTCTAGAAATCAAAAGAACTACTTGGCCTGGTTCCAGCAGAAACCAGGACAGCC 3'

F19vlb7 (57 mer):
5' TCCCTTGTCCGAACGTGAGCGGATAGCTAAAATATTGCTGACAGTCATAAACTGCC 3'

3. Primers for the synthesis of version "C"

F19Lc-sense (34 mer):
5' CCCAAACTCCTCATCTATTGGGCTAGCACTAGGG 3'

F19Lc-antisense (34 mer):
5' CCCTAGTGCTAGCCCAATAGATGAGGAGTTTGGG 3'

4. Primers hybridizing to the flanking PUC19 vector sequences

APCR1 (17 mer, sense primer): 5' TACGCAAACCGCCTCTC 3'

APCR4 (18 mer, anti-sense primer): 5' GAGTGCACCATATGCGGT 3'

RSP (-24) (16 mer, sense primer): 5' AACAGCTATGACCATG 3'

UP (-40) (17 mer, anti-sense primer): 5' GTTTTCCCAGTCACGAC 3'

Example 4

Construction of the Reshaped Human F19 Heavy Chain Versions A to E (H_A-H_E)

[0150] Version "A" of reshaped human F19 V_H regions (H_A) was constructed using the same PCR methods as described for the construction of version "A" of reshaped human F19 V_L region (L_A) (**FIG. 31**). The template DNA was version "A" of reshaped human 226 V_H (Léger, O. J. P., et al., "Humanization of a mouse antibody against human alpha-4 integrin: a potential therapeutic for the treatment of multiple sclerosis,"*Hum. Antibod.* 8:3 (1997)). Six PCR primers were designed and synthesized for the construction of version "A" of reshaped human F19 V_H region (Table 4). PCR products A, B, C, and D were obtained using APCR1-Vha1, Vha2-Vha3, Vha4-Vha5 and Vha6-APCR4 as PCR primer pairs, respectively. The PCR conditions were essentially as described for the construction of reshaped human F19 V_L region. A clone having the correct DNA sequence was designated reshF19Ha (**FIG. 32**) and was then subcloned into the eukaryotic expression vector pG1D105. The DNA sequence of reshF19Ha cloned into pG1D105 is shown in **FIG. 33**.

[0151] The third version of reshaped human F19 V_H region (H_C) was constructed using the same scheme as that described for H_A but where Vha4 primer was substituted by Vhc4 (Table 4). The DNA sequence of H_C is shown in **FIG. 32**. The second (H_B) and fourth (H_D) version of reshaped human F19 V_H region were constructed based on the PCR-

mutagenesis methods of Kamman et al. (Kamman, M., et al, "Rapid insertional mutagenesis of DNA by polymerase chain reaction (PCR),"*Nucl. Acids Res.* 17:5404 (1989)). For H_B and H_D, a mutagenic primer F19VHbd6 (Tyr-91 to Phe-91, Table 4) was used paired with APCR4 in PCR reactions with H_A and H_C as the template DNA, respectively. The PCR products VHb and VHd were restriction enzyme digested with PstI and BamHI and subcloned into reshF19Ha and reshF19Hc, respectively, previously digested with the same two restriction enzymes. The DNA sequences of H_B and H_D are shown in **FIG. 32**.

[**0152**] Version "E" of reshaped human F19 V_H region (H_E) was constructed based on the PCR-mutagenesis methods of Kamman et al. (1989) already mentioned above:

[**0153**] For reshF19He mutagenic primer F19MscIHe (Table 5) was used paired with primer F19HindIII (Table 5) in PCR reactions with H_C cloned in pg1d105 mammalian expression vector as the template DNA. The appropriate primer pairs (0.2 mM of each) were combined with 10 ng of cDNA of version "A" of reshaped human 226 VH region in 100 ml of PCR buffer containing 10 mM KCl, 10 mM $(NH_4)2SO_4$, 20 mM Tris-HCl (pH 8.8) 2 mM $MgSO_4$, 0.1% Triton X-100 and 200 mM dNTPs. Reaction mixtures were overlaid with mineral oil and kept at 94° C. for 5 minutes. Then 1 unit of Deep Vent DNA polymerase (New England Biolabs) was added ("Hot Start" PCR; Chou Q., Russell, M., et al., "Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications, "*Nucl. Acids Res.* 20:1717 (1992)) and PCR was performed for 25 cycles on a TRIO-Thermoblock Thermal Cycler (Biometra, Göttingen, Germany). Each cycle consisting of a denaturation step at 94° C. for 1 minute, a primer-annealing step at 70° C. for 1 minute, and an extension step at 72° C. for 2 minutes. This was followed by a single cycle consisting of a further elongation step at 72° C. for 10 minutes followed by cooling at 4° C. The PCR products were then extracted and purified from a TAE 1.4% standard agarose gel using a QIAquick™ gel extraction kit, following the protocol supplied by the manufacturer (QIAGEN Ltd., UK). The PCR product V_He was then restriction enzyme digested with MscI and HindIII and ligated into reshF19Hc cloned in pg1d105 previously digested with the same two restriction enzymes. The MscI restriction recognition site is unique to all the reshaped human F19 V_H region versions and is not present in the pg1d105 expression vector. The HindIII restriction recognition site is a unique site in pg1d105 for cloning of V_H immunoglobulin genes. Electroporation-competent XL-1 Blue *E. coli* cells were transformed with 1 μl of the ligated DNA and plated on agarose plates containing Ampicillin. Colonies were then screened for the presence and correct size of inserts by direct PCR on colonies (Guissow, D., and Clackson, T., "Direct clone characterization from plaques and colonies by the polymerase chain reaction,"*Nucl. Acids Res.* 17:4000 (1989)) with primers HCMi and Hucg1 hybridizing to the flanking pg1d105 vector sequences (Table 5). DNA from positive colonies was prepared using a Plasmid Midi kit, following the protocol supplied by the manufacturer (QIAGEN Ltd., UK). DNA sequencing was performed by the dideoxy chain termination method (Sanger, F., et al., "DNA sequencing with chain-terminating inhibitors,"*Proc. Natl. Acad. Sci. U.S.A.* 74:5463 (1977)) directly from circular vector DNA using conventional heat denaturation (Andersen, A., et al., "A fast and simple technique for sequencing plasmid DNA with sequenase using heat denaturation,"*Biotechniques* 13:678 (1992)) and Sequenase 2.0 (USB, Cleveland, Ohio). The DNA sequences of reshF19He is shown in **FIG. 32**.

TABLE 4

PCR primers for the construction of reshaped human F19 heavy chain variable regions versions A to D

1. Primers for the synthesis of version "A"

F19vha1 (47 mer):
5' GTGTATTCAGTGAAGGTGTATCTACTAGTTTTACAGCTGACTTTCAC 3'

F19vha2 (53 mer):
5' TAGTAGATACACCTTCACTGAATACACCATACACTGGGTTAGACAGGCCCTG 3'

F19vha3 (71 mer):
5' CCCTTGAACTTCTGGTTGTAGTTAGGAATACCATTGTTAGGATTAATACCTCCTATCCACTCCAGCCTTTG 3'

F19vha4 (71 mer):
5' TAACTACAACCAGAAGTTCAAGGGCCGGGCCACCTTGACCGTAGGCAAGTCTGCCAGCACCGCCTACATGG 3'

F19vha5 (63 mer):
5' GCATGGCCCTCGTCGTAACCATAGGCGATTCTTCTTCTGGCGCAGTAGTAGACTGCAGTGTCC 3'

F19vha6 (48 mer):
5' CTATGGTTACGACGAGGGCCATGCTATGGACTACTGGGGTCAAGGAAC 3'

2. Primers for the synthesis of version "C"

F19vhc4 (71 mer):
5' TAACTACAACCAGAAGTTCAAGGGCCGGGTCACCATCACCGTAGACACCTCTGCCAGCACCGCCTACATGG 3'

3. Primers for the synthesis of version "B" and "D"

F19vhbd6 (27 mer):
5' GGACACTGCAGTCTACTTCTGCGCCAG 3'

TABLE 4-continued

PCR primers for the construction of reshaped human F19 heavy chain variable regions versions A to D

4. Primers hybridizing to the flanking PUC19 vector sequences

APCR1 (17 mer, sense primer): 5' TACGCAAACCGCCTCTC 3'
APCR4 (18 mer, anti-sense primer): 5' GAGTGCACCATATGCGGT 3'

[0154]

TABLE 5

PCR primer for the construction of reshaped human F19 heavy chain variable regions version E

1. Primer for the synthesis of version "E"

F19MsclHe (65 mer, anti-sense):
5' CCTTTGGCCAGGGGCCTGTCTAACCCAGTGTATGGTGTATTCAGTGAAGGTGTATCCACTAGTTTCCACTAGTTT 3'
MscI

2. Primers hybridizing to the flanking pg1d105 mammalian expression vector sequences

HCMi (28 mer, sense): 5' GTCACCGTCCTTGACACGCGTCTCGGGA 3'
Hucg1 (17 mer, anti-sense): 5' TTGGAGGAGGGTGCCAG 3'

Example 5

Reshaped Human F19 Antibody Concentrations in COS Cell Supernatants

[0155] COS cells were transfected with one pair of a series of reshaped human F19 antibody constructs and the human antibody concentration was measured using the IgG1/Kappa ELISA as described in example 2.

TABLE 6

Reshaped human F19 antibody concentrations in COS cell supernatants

Transfected Antibody components		Human γ1/K
Heavy chain	Kappa light chain	Concentration [μg/ml]
H_A	L_A	2.50
H_A	L_B	0.18
H_B	L_A	1.25
H_B	L_B	0.10
H_D	L_A	1.15
H_D	L_B	0.18
H_A	L_A	1.50
H_A	L_C	1.56
H_C	L_A	1.47
H_C	L_C	1.97
cF19	L_A	1.54
cF19	L_B	0.07
cF19	L_C	2.14

[0156]

TABLE 7

Reshaped human F19 antibody concentrations in COS cell supernatants

Transfected Antibody components		Human γ1/K
Heavy chain	Kappa light chain	concentration [μg/ml]
H_A	L_A	2.00
H_A	L_C	2.50
H_C	L_A	2.90
H_C	L_C	3.00
H_E	L_A	2.80
H_E	L_C	3.50

RNA Splicing Events Required for the Expression of Immunoglobulin Genes in Mammalian Cells

[0157] Both mammalian expression vectors pKN100 and pg1d105 have an intron between the variable and the constant regions which is removed during the process of gene expression to give rise to an messenger RNA. The splicing event which consists of a DNA recombination between the heavy or light chain splice donor sites and the immunoglobulin splice acceptor site is described in **FIG. 34**.

Example 6

Flow Cytometric Analysis of the Binding of cF19 and L_AH_C to FAP-expressing Human Cells

[0158] The ability of L_AH_C to bind to both recombinant and endogenously expressed FAP on cell surface was tested.

[0159] The example was conducted to determine the binding of L_AH_C to cellular FAP. Both naturally FAP expressing

MF-SH human tumor cells (Shirasuma, K., et al., *Cancer* 55:2521-2532 (1985)) and FAP-transfected human tumor cell lines were used as cellular targets. L_AH_C was studied in cytofluorometric assays evaluating direct binding to target cells as well as by the inhibitory effect on the binding of either murine F19 or chimeric cF19 anti-FAP antibodies.

[0160] Antibodies and cell lines used were F19 (murine monoclonal anti-human FAP antibody, IgG1 subclass), mIgG (murine immunoglobulin, IgG class), cF19 (chimeric monoclonal anti-human FAP antibody, IgG1 subclass), L_AH_C (reshaped monoclonal anti-human FAP antibody, IgG1 subclass), hIgG1 (human immunoglobulin, IgG1 subclass), MF-SH (human malignant fibrous histiocytoma cell line), HT-1080 (human fibrosarcoma cell line), HT-1080FAP clone 33 (HT-1080 cell line transfected with cDNA encoding human FAP). Antibodies were biotinylated as described in examples 8 and 12.

Direct Binding of L_AH_C to FAP on the Surface of Human Tumor Cell Lines

[0161] 5×10^5 cells of the tumor cell line under investigation were incubated with the indicated concentration of test or control antibody in a total volume of 0.2 ml phosphate-buffered saline (PBS) supplemented with 1% bovine serum albumin (BSA) for 30 minutes on ice. Subsequently, cells were washed twice with 2 ml of PBS, resuspended in 0.2 ml of PBS supplemented with 1% BSA, a 1:20 dilution of mouse anti-human IgG FITC-labelled (Dianova) as secondary reagent was added and incubated for another 30 minutes on ice.

[0162] Alternatively, 5×10^5 cells of the tumor cell line under investigation were incubated with the indicated concentration of biotin-labelled cF19 in a total volume of 0.2 ml PBS supplemented with 1% BSA for 30 minutes on ice. Subsequently, cells were washed twice with 2 ml of PBS, resuspended in 0.2 ml of PBS supplemented with 1% BSA, and incubated for another 30 minutes on ice with 1:40 dilution of streptavidin-FITC (Dianova) as secondary reagent.

[0163] Cells were again washed twice with 2 ml of PBS, resuspended in a total volume of 0.5 ml of PBS supplemented with 1% paraformaldehyde (PFA) and kept on ice. Single cell fluorescence was determined cytofluorometrically by analysing the cellular green fluorescence at 488 nm in an EPICS XL (Coulter) fluorescence-activated cell analyzer.

Competition of L_AH_C for Binding of Biotinylated cF19 to Cell-surface FAP on FAP-expressing Human Cell Lines

[0164] 5×10^5 cells of the tumor cell line under investigation were incubated with the indicated amounts of unlabeled test or control antibody added together with 1 μg/ml biotin-labelled cF19 antibody. Subsequently, cells were washed twice with 2 ml of PBS, resuspended in 0.2 ml of PBS supplemented with 1% BSA, 1:40 diluted streptavidin-FITC (Dianova) as secondary reagent and incubated for another 30 minutes on ice.

[0165] Cells were then washed twice with 2 ml of PBS, resuspended in a total volume of 0.5 ml PBS supplemented with 1% PFA and kept on ice. Single cell fluorescence was determined cytofluorometrically by analysing the cellular green fluorescence at 488 nm in an EPICS XL (Coulter) fluorescence-activated cell analyzer.

[0166] Both, cF19 and L_AH_C bind in a concentration dependent manner specifically to to FAP-transfected HT-1080FAP clone33 human tumor cells (Table 8). No binding to FAP-negative HT-1080 cells was detectable (Table 9). Both cF19 and L_AH_C bound in a concentration dependent manner to human MF-SH cells endogenously expressing FAP (Table 10).

[0167] Biotinylated cF19 bound to human HT-1080FAP clone 33 (Table 11) in a concentration dependent manner. No binding was detectable to FAP-negative HT-1080 cells (Table 12).

[0168] Binding of biotinylated cF19 to HT-1080FAP clone 33 cells was inhibited by both unlabelled cF19 and unlabelled L_AH_C (Table 13).

[0169] Chimeric anti-human FAP monoclonal antibody cF19 as well as reshaped human anti-human FAP monoclonal antibody L_AH_C (example 10) were shown to bind directly to FAP expressed on human cell lines either endogenously expressing this protein or transfected with cDNA encoding for it. This binding was shown to be concentration dependent. Binding of biotinylated cF19 could be inhibited by both unlabelled cF19 and unlabelled L_AH_C.

[0170] Using cytofluorometric technology, direct binding as well as inhibition of specifically binding reagents showed specificity of chimeric cF19 and reshaped L_AH_C human monoclonal antibodies to cell surface expressed FAP.

TABLE 8

Binding of anti-FAP antibodies to HT-1080FAP clone 33 cells

Concentration of antibody	Mean fluorescence intensity		
[ng/ml]	hIgG1	cF19	L_AH_C
500	0.12	6.65	2.76
100	0.12	1.63	0.66
20	0.12	0.43	0.22
4.0	0.12	0.17	0.15
0.8	0.12	0.14	0.13

[0171]

TABLE 9

Binding of anti-FAP antibodies to non-transfected HT-1080 cells

Concentration of antibody	Mean fluorescence intensity		
[ng/ml]	hIgG1	cF19	L_AH_C
500	0.11	0.11	0.12
100.0	0.11	0.11	0.11
20.0	0.11	0.11	0.12
4.0	0.11	0.11	0.12
0.8	0.11	0.11	0.11

[0172]

TABLE 10

Binding of anti-FAP antibodies to MF-SH cells

Concentration of antibody	Mean fluorescence intensity		
[ng/ml]	hIgG1	cF19	L_AH_C
4,000	0.6	3.6	2.8
2,000	n.d.	3.3	2.5
1,000	n.d.	2.4	1.9
500	n.d.	1.8	1.3

n.d.: not done

[0173]

TABLE 11

Binding of biotinylated cF19 antibody to HT-1080FAP clone 33 cells

Concentration of antibody	Mean fluorescence intensity	
[ng/ml]	Biotinylated hIgG1	Biotinylated cF19
5,000.0	0.2	36.5
1,000.0	0.2	18.1
200.0	0.2	4.5
40.0	0.2	1.3
8.0	0.2	0.5
1.6	0.3	0.3

[0174]

TABLE 12

Binding of biotinylated cF19 antibody to non-transfected HT-1080 cells

Concentration of antibody	Mean fluorescence intensity	
[ng/ml]	Biotinylated hIgG1	Biotinylated cF19
5,000.0	0.1	0.1
1,000.0	0.1	0.1
200.0	0.1	0.1
40.0	0.1	0.1
8.0	0.1	0.1
1.6	0.1	0.1

[0175]

TABLE 13

Competition of anti-FAP antibodies with the binding of biotinylated cF19 to HT-1080FAP clone 33 cells

Competitor antibody	Concentration of competitor antibody [μg/ml]	Mean fluorescence concentration
No	0.00	11.2
hIgG1	1.00	9.0
hIgG1	3.16	11.3
hIgG1	10.00	9.8
hIgG1	31.66	10.3
cF19	1.00	7.5
cF19	3.16	4.8
cF19	10.00	1.3
cF19	31.66	1.2

TABLE 13-continued

Competition of anti-FAP antibodies with the binding of biotinylated cF19 to HT-1080FAP clone 33 cells

Competitor antibody	Concentration of competitor antibody [μg/ml]	Mean fluorescence concentration
L_AH_C	1.00	8.0
L_AH_C	3.16	5.5
L_AH_C	10.00	2.9
L_AH_C	31.66	1.7

Biotinylated cF19 was used at a concentration of 1 μg/ml in all tests shown in Table 13.

Example 7

In Vitro Immune Effector Functions of Monoclonal Antibody L_AH_C

[0176] This experiment was conducted to determine the potential of the monoclonal antibody (mAb) L_AH_C with specificity for fibroblast activation antigen (FAP) to lyse FAP-expressing targets in the presence of human complement or human mononuclear leukocytes, respectively.

[0177] In particular, the ability of L_AH_C to mediate cytotoxic effects against HT-1080FAP clone 33 cells, which expressed human FAP on the surface, was studied. Cytotoxicity was determined in vitro using the following approach: ^{51}Cr-labelled target cells were incubated in the presence of L_AH_C with human serum as source of complement or human MNC (peripheral blood mononuclear cells) as effectors. Release of ^{51}Cr was measured as measure of target-cell lysis.

[0178] Antibodies and cell lines used were L_AH_C (reshaped human anti-human FAP IgG1 antibody), hIgG1 (human IgG1 isotype control), 3S193 (murine monoclonal anti-Lewis^y IgG3 antibody), mIgG (murine IgG control), HT-1080 (human fibrosarcoma), HT-1080FAP clone 33, (HT1080 transfected with cDNA encoding human FAP), MCF-7 (human breast adenocarcinoma cell line).

Complement-mediated Lysis of Target Cells by L_AH_C

[0179] Tumor cells were radiolabelled by incubation in RPMI 1640 medium with 100 μl-Ci^{51}Cr (NEN) at 37° C. for one hour. Subsequently, cells were washed twice in ^{51}Cr-free medium and resuspended at a concentration of 2×10^5 cells per ml.

[0180] Human serum as source of complement was freshly prepared from blood of different volunteers. Blood was taken by puncturing the arm vein, remained at room temperature for one hour to allow clotting to occur, and was kept at 4° C. over night. Serum was separated by centrifugation and taken off from the sediment.

[0181] The antibody under study was diluted from the stock solution to the appropriate concentration in RPMI1640 cell culture medium.

[0182] 1×10^5 radiolabelled tumor cells of the indicated cell line were incubated for 2 h at 37° C. in an incubator (95% air and 5% CO_2) in the presence of different concen-

trations of test or control antibody and 25% (v/v) human serum as the source of human complement. Incubations were performed in U-shaped 96-well plates in a total volume of 200 μl RPMI1640 and done in triplicate. After the incubation period, plates were centrifuged, 100 μl of the supernatant was removed and radioactivity was counted in a gamma-counter. The total amount of incorporated radioactivity was determined by measuring 10^4 target cells. Spontaneous release was defined as activity released from the target cells in the absence of both antibody and complement during the described incubation period.

[0183] Specific lysis was calculated as follows:

$$\left.\begin{matrix}\text{Specific lysis}\\(\text{in \%})\end{matrix}\right\} = \frac{[\text{activity sample}] - [\text{activity spontaneous release}]}{[\text{maximum activity}] - [\text{activity spontaneous release}]} \times 100$$

Antibody-dependent Cellular Cytotoxicity (ADCC) of L_AH_C

[0184] Tumor cells were radiolabelled by incubation in RPMI1640 medium with 100 μl-$Ci^{51}Cr$ at 37° C. for one hour. Subsequently, cells were washed twice in ^{51}Cr-free medium and resuspended at a concentration of 2×10^5 cells per ml.

[0185] MNC (peripheral blood mononuclear cells) were prepared from peripheral blood taken by puncturing the arm vein of different healthy human volunteers. Clotting was prevented by the addition of 20% citrate buffer. MNC from 4 ml of this blood preparation were purified by centrifugation (30 minutes at 400 G and room temperature) on 3 ml of lymphocyte preparation medium (Boehringer Mannheim, Germany). MNC (peripheral blood mononuclear cells) were taken off from the gradient, washed three times and diluted with RPMI1640 to the appropriate concentration. Lymphocyte activated killer (LAK) cells were derived from MNC (peripheral blood mononuclear cells) by incubation for 5 days at 37° C. in an 95% air and 5% CO_2 incubator at an initial density of 1.3×10^6 cells per ml in the presence of 100 U recombinant human Interleukin-2 (IL-2). The antibody under study was diluted from the stock solution to the appropriate concentration in RPMI1640 cell culture medium.

[0186] 1×10^4 radiolabelled tumor cells of the indicated cell line were incubated for 5 h at 37° C. and 5% CO_2 in the presence of different concentrations of test or control antibody and MNC. MNC were added in amounts to reach the indicated effector:target cell ratio. Incubation was performed in U-shaped 96-well plates in a total volume of 200 μL RPMI1640 and done in duplicate.

[0187] After the incubation period, plates were centrifugated, 100 μl of the supernatant were taken off and radioactivity was determined in a gamma-counter. The total amount of incorporated radioactivity was determined by measuring 10^4 target cells. Spontaneous release was defined as activity released from the target cells in the absence of both antibody and effector cells during the described incubation period.

[0188] Specific lysis was calculated as follows:

$$\left.\begin{matrix}\text{Specific lysis}\\(\text{in \%})\end{matrix}\right\} = \frac{[\text{activity sample}] - [\text{activity spontaneous release}]}{[\text{maximum activity}] - [\text{activity spontaneous release}]} \times 100$$

Antibody-mediated Complement Lysis of Tumor Cells

[0189] No L_AH_C-specific complement-mediated lysis (above that seen with an isotype control) was observed in HT-1080FAP clone 33 cells treated with L_AH_C at concentrations up to 50 μg/ml (Table 14, Table 15a).

[0190] Lytic activity of human serum used as source of complement was shown by lysis of MCF-7 human breast carcinoma cells in the presence of 12.5 μg/ml 3S193, a murine monoclonal anti-$Lewis^y$ antibody with known complement activating ability (Table 15b).

Antibody-mediated Cellular Lysis of Tumor Cells

[0191] In the presence of L_AH_C at concentrations up to 10 μg/ml, no ADCC (antibody-dependent cellular toxicity) mediated by human MNC (Table 16) or human LAK cells (lymphokine activated killer cells, Table 17) of L_AH_C on HT-1080FAP clone 33 as measured by lysis was detectable above that seen with an isotype control at an effector:target ratio of 50:1.

[0192] In appropriate in vitro assays with either human complement or with human MNC as effector mechanisms, human anti-FAP monoclonal antibody L_AH_C revealed no detectable cytotoxic effects above isotype controls on FAP-expressing tumor cell line HT-1080FAP clone 33.

TABLE 14

Specific complement lysis (in %) of HT-1080FAP clone 33 tumor cell targets mediated by L_AH_C

Source of human serum:	HT-1080 clone 33:	
Concentration of antibody	hIgG1 isotype control	L_AH_C
A 50 μg/ml	5	4
A 10 μg/ml	5	3
B 50 μg/ml	7	5
B 10 μg/ml	6	5
0 μg/ml	0	0

Incubation: 2 hours at 37° C., 25% serum from human volunteers A or B, respectively, as source of complement.

[0193]

TABLE 15a

Specific complement lysis (in %) of HT-1080FAP clone 33 tumor cell targets mediated by human anti-FAP monoclonal antibody L_AH_C

Source of human serum:		HT-1080 clone 33:	
Concentration of antibody		hIgG1	L_AH_C
A	10.00 μg/ml	2	1
A	2.50 μg/ml	2	2

TABLE 15a-continued

Specific complement lysis (in %) of HT-1080FAP clone 33 tumor cell targets mediated by human anti-FAP monoclonal antibody L_AH_C

Source of human serum:		HT-1080 clone 33:	
Concentration of antibody		hIgG1	L_AH_C
A	0.60 μg/ml	1	1
A	0.15 μg/ml	1	2
A	0.00 μg/ml	2	2
B	10.00 μg/ml	2	2
B	2.50 μg/ml	2	2
B	0.60 μg/ml	2	2
B	0.15 μg/ml	2	2
B	0.00 μg/ml	2	2
C	10.00 μg/ml	2	2
C	2.50 μg/ml	1	1
C	0.60 μg/ml	1	1
C	0.15 μg/ml	2	1
C	0.00 μg/ml	3	3

Incubation: 2 hours at 37° C., 25% serum from human volunteers A, B or C, respectively, as source of complement.

[0194]

TABLE 15b

Specific complement lysis (in %) of MCF-7 tumor cell targets mediated by murine anti-Lewis^y monoclonal antibody 3S193

Source of human serum:		MCF-7:	
Concentration of antibody		mIgG	3S193
A	10.00 μg/ml	0	21
A	2.50 μg/ml	1	21
A	0.60 μg/ml	0	21
A	0.15 μg/ml	1	18
A	0.00 μg/ml	0	0
B	10.00 μg/ml	1	13
B	2.50 μg/ml	0	17
B	0.60 μg/ml	1	18
B	0.15 μg/ml	1	15
B	0.00 μg/ml	0	0
C	10.00 μg/ml	1	22
C	2.50 μg/ml	0	23
C	0.60 μg/ml	1	26
C	0.15 μg/ml	1	20
C	0.00 μg/ml	1	1

Incubation: 2 hours at 37° C., 25% serum from human volunteers A, B or C, as source of complement.

[0195]

TABLE 16

ADCC (antibody-dependant cellular cytotoxicity) (specific lysis in %) of HT-1080FAP clone 33 target cells by human MNC (peripheral blood mononuclear cells) mediated by L_AH_C

HT-1080FAP clone 33: Concentration of antibody:	HT-1080FAP clone 33:	
[in μg/ml]	hIgG1	L_AH_C
10	2	2
2.5	2	2
0.625	2	2

TABLE 16-continued

ADCC (antibody-dependant cellular cytotoxicity) (specific lysis in %) of HT-1080FAP clone 33 target cells by human MNC (peripheral blood mononuclear cells) mediated by L_AH_C

HT-1080FAP clone 33: Concentration of antibody:	HT-1080FAP clone 33:	
[in μg/ml]	hIgG1	L_AH_C
0.156	3	3
0	3	3

Incubation: 5 hours at 37° C., 10^4 target cells and an effector:target cell ration of 50:1.

[0196]

TABLE 17

ADCC (antibody-dependent cellular cytotoxicity, specific lysis in %) of HT-1080FAP clone 33 target cells by LAK cells (lymphokine activated killer cells) mediated by L_AH_C

Concentration of antibody:	HT-1080FAP clone 33:	
[in μg/ml]	hIgG1	L_AH_C
10	12	14
2.5	14	17
0.625	14	21
0.156	15	21
0	14	14

Incubation: 5 hours at 37° C., 10^4 target cells and an effector:target cell ration of 50:1.

Example 8

Immunohistochemical Analysis of Monoclonal Antibody L_AH_C Binding to Normal and Neoplastic Human Tissues

[0197] This experiment was performed to determine the binding characteristics of the humanized mAb L_AH_C to normal and neoplastic human tissues.

[0198] The following antibodies were used: L_AH_C, cF19, and the negative control hIgG1 were directly biotinylated according to methods of the state of the art and used at concentrations of 2.5 to 0.25 mg/ml in 2% BSA/PBS (bovine serum albumin in phosphate-buffered saline). Murine mAb F19 was used as tissue culture supernatant of the F19 hybridoma, at dilutions of 1:5 to 1:10 in 2% BSA/PBS.

[0199] The following reagents were used for immunochemical assays: Streptavidin peroxidase complex (Vector Labs., Burlingame, Calif., USA), Avidin-biotin peroxidase complex (Vector Labs.), Biotinylated horse anti-mouse (Vector Labs.), DAB (diaminobenzidine, Sigma Chemical Co., St. Louis, Mo., USA), Harris' hematoxylin.

[0200] Fresh frozen tissue samples examined included the following: Normal colon, breast, lung, stomach, pancreas, skin, larynx, urinary bladder, smooth and skeletal muscle. Among the tumors tested were carcinomas from breast, colon, lung, esophagus, uterus, ovary, pancreas, stomach, and head and neck.

[0201] An indirect immunoperoxidase method was carried out according to state of the art methods (Garin-Chesa, P., et

al., "Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers, "*Proc. Natl. Acad. Sci. USA* 87:7235-7239 (1990)) on five micrometer thickness fresh frozen sections. DAB was used as a substrate for the final reaction product. The sections were counterstained with Harris'hematoxylin and examined for antigen expression.

L_AH_C Expression in Normal Human Tissues

[**0202**] The normal tissues tested were negative for L_AH_C expression, except for the normal pancreas in which a subset of positive endocrine cells in the islets of Langerhans (A cells) were identified with L_AH_C, cF19 and F19. (Table 18). No immunoreactivity was observed with the hlgG1 (human immunoglobulin IgG1 subclass) used as a negative control.

L_AH_C Expression in Tumors

[**0203**] In the tumor samples, L_AH_C, cF19 and F19 showed an indistinguishable pattern of expression in the tumor stromal fibroblasts. A strong and homogeneous expression was found in the majority of the cases examined, especially in the cancer samples derived from breast, colon, lung, pancreas and in the squamous cell carcinomas (SQCC) of the head and neck tested (Table 18). No immunoreactivity was observed with the hlgG1 used as negative control.

[**0204**] L_AH_C, cF19 and F19 showed immunoreactivity with the tumor stromal fibroblasts in the epithelial cancer samples tested. No L_AH_C or F19 immuno-reactivity was seen with either the fibrocytes of the normal organ mesenchyme or the parenchymal cells of normal adult organs. Anti-FAP immunoreactivity was only observed in a subset of endocrine cells in the pancreatic islets, presumably glucagon-producing A cells, and in four of nine uterine samples tested, representing subsets of stromal fibroblasts in these tissues.

[**0205**] Immunohistochemical analysis of L_AH_C in normal human tissues and FAP-expressing human carcinomas showed indistinguishable patterns of binding for L_AH_C, cF19 and murine mAb F19.

TABLE 18

Immunoreactivity of mAbs L_AH_C, cF19 and F19 with normal human tumor samples

Tissue type	No.	L_AH_C	cF19	F19
Breast	4			
Epithelial cell ducts/acini		–	–	–
Myoepithelial cells		–	–	–
Connective tissue		–	–	–
Blood vessels		–	–	–
Colon	6			
Crypts of Lieberkühn		–	–	–
Connective tissue		–	–	–
Lymphoid tissue		–	–	–
Smooth muscle		–	–	–
Blood vessels		–	–	–
Myenteric plexus		–	–	–
Lung	4			
Bronchus:		–	–	–
Bronchial epithelium		–	–	–
Hyaline cartilage		–	–	–
Connective tissue		–	–	–
Mucous glands		–	–	–

TABLE 18-continued

Immunoreactivity of mAbs L_AH_C, cF19 and F19 with normal human tumor samples

Tissue type	No.	L_AH_C	cF19	F19
Alveolus:				
Pneumocytes (type I/II)		–	–	–
Alveolar phagocytes		–	–	–
Alveolar capillaries		–	–	–
Stomach	3			
Surface epithelium		–	–	–
Gastric glands		–	–	–
Chief cells		–	–	–
Parietal (oxyntic) cells		–	–	–
Mucous cells		–	–	–
Neuroendocrine cells		–	–	–
Connective tissue		–	–	–
Blood vessels		–	–	–
Smooth muscle		–	–	–
Esophagus	1			
Surface epithelium		–	–	–
Connective tissue		–	–	–
Small intestine	1			
Epithelium of villi & crypts		–	–	–
Connective tissue		–	–	–
Smooth muscle		–	–	–
Blood vessels		–	–	–
Lymphoid tissue		–	–	–
Urinary bladder	2			
Urothelium		–	–	–
Connective tissue		–	–	–
Smooth muscle		–	–	–
Blood vessels		–	–	–
Pancreas	3			
Duct epithelium		–	–	–
Acinar epithelium		–	–	–
Islets of Langerhans:		–	–	–
B-cells		–	–	–
A-cells		+*	+*	+*
D-cells		–	–	–
Connective tissue		–	–	–
Blood vessels		–	–	–
Nerves		–	–	–
Larynx	1			
Squamous epithelium		–	–	–
Mucous glands		–	–	–
Connective tissue		–	–	–
Hyaline cartilage		–	–	–
Blood vessels		–	–	–
Skeletal muscle		–	–	–
Lymph node	1			
Lymphoid cells		–	–	–
Lymph sinuses		–	–	–
Connective tissue		–	–	–
Blood vessels		–	–	–
Spleen	1			
Red & white pulp		–	–	–
Sinuses		–	–	–
Connective tissue		–	–	–
Liver	1			
Hepatocytes		–	–	–
Bile ducts		–	–	–
Portal triad		–	–	–
Thyroid gland	2			
Follicular epithelium		–	–	–
Parafollicular cells		–	–	–
Connective tissue		–	–	–
Prostate gland	1			
Glandular epithelium		–	–	–
Stroma		–	–	–
Testicle	1			
Seminiferous tubules		–	–	–
Stroma		–	–	–
Ovary	3			
Follicles		–	–	–

TABLE 18-continued

Immunoreactivity of mAbs L_AH_C, cF19 and F19 with normal human tumor samples

Tissue type	No.	L_AH_C	cF19	F19
Stroma		–	–	–
Uterine cervix	1			
Epithelium		–	–	–
Stroma		–	–	–
Uterus	9			
Endometrium:		–	–	–
glands		–	–	–
stroma		+**	+**	+**
blood vessels		–	–	–
Myometrium		–	–	–
Cerebral cortex	1			
Neurons		–	–	–
Neurological cells		–	–	–
Blood vessels		–	–	–
Cerebellum	1			
Molecular layer		–	–	–
Granular cell layer		–	–	–
Purkinje cells		–	–	–
Blood vessels		–	–	–
Skin	3			
Squamous epithelium		–	–	–
Melanocytes		–	–	–
Skin appendages		–	–	–
Connective tissue		–	–	–
Blood vessels		–	–	–

Acetone-fixed frozen sections were tested by the avidin-biotin complex immunoperoxidase procedure.
No number of tissue samples derived from different individuals tested.
*Identification of A-cells, based on morphology and location within the islets.
**Positive immunoreactivity in the stroma in 4 out of 9 samples tested. The positive samples represent early (x2) and intermediate (x2) phase proliferative endometrium.

Example 9

Species Specificity of L_AH_C Binding in Tissue Sections

[0206] This experiment was conducted to assess the reactivity of L_AH_C with tissues from mouse, rat, rabbit and cynomolgus monkeys by immunohistochemical methods.

[0207] Also used in these tests were cF19 and human IgG1 (hIgG1) as negative controls. The reagents used for immunohistochemistry were Streptavidin peroxidase complex (Vector Labs., Burlingame, Calif., USA), DAB (Sigma Chemical Co., St. Louis, Mo., USA) and Harris' hematoxylin.

[0208] The following fresh frozen tissue samples from mouse, rat, rabbit and cynomolgus were tested: Brain, liver, lung, kidney, stomach, pancreas, intestine, thymus, skin, muscle, heart, spleen, ovary, uterus and testes. As positive control, sections from normal human pancreas and a breast carcinoma sample were included in every assay.

Immunohistochemistry

[0209] An indirect immunoperoxidase method was carried out as described in the state of the art (Garin-Chesa, P., et al., "Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers, "*Proc. Natl. Acad. Sci. USA* 87:7235-7239 (1990)) on five micrometer thickness fresh frozen sections. The antibodies L_AH_C, cF19 and hIgG1 (at 1 μg/ml) were biotinylated according to the state of the art and were detected with streptavidin peroxidase complex. DAB was used as a substrate for the final reaction product. The sections were counterstained with Harris' hematoxylin and examined for antigen expression.

[0210] The normal tissues tested did not react with either L_AH_C or cF19 in the experiments (Table 1).

[0211] The normal human pancreas used as positive control showed L_AH_C and cF19 binding in a subset of endocrine cells in the islets of Langerhans as previously described for F19. In addition, binding of L_AH_C and cF19 was seen in the tumor stromal fibroblasts in the breast carcinoma sample.

[0212] Immunohistochemical analysis of normal tissues from mouse, rat, rabbit and cynomolgus failed to detect any binding of either L_AH_C or cF19, in the experiments performed.

TABLE 19

Binding of L_AH_C to tissue sections of non-human species, as determined by immunohistochemistry

Organ/Tissue type	Mouse	Rat	Rabbit	Cynomolgus
Brain				
Cerebral cortex	—	—	—	
Cerebellum	—	—	—	—
Liver				
Hepatocytes	—	—	—	—
Portal triad	—	—	—	—
Lung				
Bronchi	—	—	—	—
Alveoli	—	—	—	—
Kidney				
Glomeruli	—	—	—	—
Tubular epithelium	—	—	—	—
Stomach				
Glandular epithelium	—	—	—	—
Smooth muscle	—	—	—	—
Pancreas				
Exocrine acini	—	—	—	—
Endocrine islets	—	—	—	—
Intestine				
Glandular epithelium	—	—	—	—
Smooth muscle	—	—	—	—
Thymus				
Lymphocytes	—	—	—	—
Skin				
Keratinocytes	—	—	—	—
Sweat glands	—	—	—	—
Hair follicles	—	—	—	—
Skeletal muscle	—	—	—	—
Heart	—	—	—	—
Spleen				
Lymphocytes	—	—	—	—
Ovary				
Follicular epithelium	—	—	—	—
Stroma	—	—	—	—

TABLE 19-continued

Binding of L_AH_C to tissue sections of non-human species, as determined by immunohistochemistry

Organ/Tissue type	Mouse	Rat	Rabbit	Cynomolgus
Uterus				
Myometrium	—	—	—	—
Cervix uteri	—	—	—	—
Testis				
Tubular epithelium	nt	nt	nt	—
Connective tissue	—	—	—	—

nt = not tested

Example 10

Construction of Cell Lines Producing Chimeric and Reshaped Anti-FAP Monoclonal Antibodies

[0213] The objective of this experiment was to demonstrate stable cell lines according to the invention expressing L_AH_C, L_AH_A, L_BH_B, L_BH_D and cF19 in CHO DG44 cells. Stable cell lines transfected with humanized or chimeric F19 antibodies were produced and their identity was confirmed by PCR amplification of heavy and light variable regions using genomic DNA derived from each transfectant as template.

[0214] CHO DG44 cells maintained under serum-free conditions in SFM-II medium. Lipofectin and SFM-II serum-free medium were obtained from Gibco/BRL. Geneticin and all restriction enzymes were obtained from Boehringer Mannheim. Pfu polymerase was obtained from Stratagene.

[0215] DNA for transfections was purified from *E. coli* cells using QiaFilter Maxi Cartridges (Qiagen) as directed by the manufacturer. All DNA preparations were examined by restriction enzyme digestion. Sequences of L_AH_C variable regions in their respective vectors were confirmed using an ABI PRISM 310 Sequencer (Perkin-Elmer).

[0216] Further information regarding the vectors and DNA sequences employed is available in the prior examples.

Transfection of CHO DG44 Cells

[0217] Cells in logarithmic growth were plated into 6 well plates containing 1 ml fresh SFM-II medium. Plasmids encoding heavy and light chains of humanized or chimeric F19 versions were cotransfected into CHO DG44 cells using liposomal transfection. Liposomes were prepared using 6 μl lipofectin reagent and 0.5 μg of each vector (one for the desired heavy chain and one for the light) as described for LipofectAMINE transfections except that SFM-II medium was used to dilute all reagents. Twenty-four hours later, cells were diluted 1:10 into SFM-II medium containing 300 μg/ml Geneticin. After the initial phase of cell killing was over (10-14 days), the concentration of Geneticin was reduced to 200 mg/ml and methotrexate was added to a final concentration of 5 nM. Methotrexate concentrations were increased after 10-14 days to a final concentration of 20 nM.

PCR Amplification of Transfectant DNA

[0218] 10^7 CHO DG44 cells were centrifuged in an Eppendorf microcentrifuge briefly at full speed, washed once with PBS, and pelleted once again. Genomic DNA was prepared by ethanol precipitation after SDS lysis and Proteinase K treatment of the cell pellets.

[0219] A mixture containing one of the following primer pairs, dNTPs, buffer, and Pfu polymerase was used to amplify either the heavy or light chain variable region using genomic DNA as template. The resulting PCR products were digested with the appropriate restriction enzyme and analyzed by agarose gel electrophoresis to confirm their identity.

```
Light chain primer set:
5'-GAG ACA TTG TGA CCC AAT CTC C-3'     PKN 1690
5'-GAC AGT CAT AAA CTG CCA CAT CTT C-3' PKN.1930.R

Heavy chain primer set:
5'-TTG ACA CGC GTC TCG GGA AGC TT-3'    PG 5863
5'-GGC GCA GAG GAT CCA CTC ACC T-3'     PG 6332.R
```

[0220] The undigested heavy chain PCR product has a predicted size of 469 bp while the light chain PCR product has a predicted size of 286 bp. Verification of identity was determined by restriction enzyme digest with BstEII (heavy chain) or NlaIV (light chain).

[0221] CHO cell lines were transfected with L_AH_C, L_AH_A, L_BH_B and L_BH_D, as well as cF19. Geneticin-resistant cells were obtained and these cells were further selected for resistance to methotrexate. PCR amplification, followed by restriction enzyme cleavage of the light and heavy chain DNA produced the expected bands and confirmed the identity of L_AH_C, L_BH_B, L_AH_A and L_BH_D transfectants.

[0222] The cells described were maintained under serum-free conditions at all times and were not treated with animal-derived products such as trypsin.

[0223] Producer cell lines transfected with expressing monoclonal L_AH_C, L_AH_A, L_BH_B, L_BH_D and cF19 antibodies were produced. Their identities were confirmed using PCR amplification and restriction enzyme cleavage of the resulting PCR products of both their heavy and light chain variable regions.

Example 11

Expression of Antibody Proteins in Chinese Hamster Ovary DG 44 Cells and their Purification

[0224] The objective of this experiment was to express and purify L_AH_C, L_AH_A, L_BH_B, and L_BH_D mAbs to enable their characterization. Other goals included the establishment of a quantitative ELISA to permit measurement of antibody concentrations in both crude media samples as well as purified Ig samples and determination of relative expression levels of various humanized F19 constructs using this assay.

[0225] Serum-free CHO DG44 cells and USP-grade methotrexate were obtained from the Biotechnical Production Unit of the Dr. Karl Thomae GmbH, Biberach, Germany; both products are also commercially available. Cells were maintained under serum-free conditions at all times. SFM-II serum-free medium was obtained from Gibco/BRL. Protein A agarose was from Pierce Chemical (Indianapolis, Ind., USA). Human IgG1 standards (Cat. No. 13889), p-Nitrophenyl phosphate tablets (N 2640), bovine serum albumin

(BSA) (A 7906), and goat anti-human kappa chain specific alkaline phosphatase-conjugated antibody (A 3813) were obtained from Sigma Chemical (St. Louis, Mo., USA). Goat anti-human gamma-chain specific alkaline phosphatase-conjugated antibody was obtained from Jackson Immunoresearch Laboratories (through Stratech Scientific). Tris-buffered saline (TBS) consisted of 150 mM NaCl, 50 mM Tris, pH 7.5.

Cell Culture Conditions for Antibody Expression

[0226] Cells were cultured and maintained in T-175 flasks in SFM-II serum-free medium without agitation. The medium contained 200 μg/ml Geneticin and 20 nM methotrexate without antibiotics. Cells were passaged by dilution, were not adherent, and grew in small clusters. When the cells reached stationary phase, the medium was collected and centrifuged to remove cells and frozen at −20° C. until needed.

Purification of L_AH_C

[0227] All purification steps were carried out at 4° C. A C10/10 column (Pharmacia Fine Chemicals) was packed with Protein A agarose (3 ml bed volume). The column was washed with TBS and preeluted once with 0.1 M Na citrate, pH 3.0 to insure that no loosely bound material remained on the column. The column was then immediately reequilibrated with TBS and stored at 4° C. Spent culture supernatants were thawed and centrifuged at 10,000×g for 30 minutes prior to Protein A chromatography to remove debris and diluted with an equal volume of TBS. This material was loaded onto the Protein A column at 0.5 ml/minute using a P-1 peristaltic pump (Pharmacia) and washed with TBS until the absorbance at 280 nm was undetectable. Elution of the antibody was initiated with 0.1 M Na citrate pH 3.0 at approximately 0.2 ml/minute. The elution was monitored at 280 nm and one ml fractions of the eluted material were collected into tubes containing sufficient Tris base pH 9 to neutralize the citrate buffer. Protein-containing fractions were pooled and concentrated using an Amicon filtration apparatus with a YM-30 filter and dialyzed against PBS. The column was immediately regenerated with TBS. Protein dye-binding assays were performed with the BioRad (Hercules, Calif.) protein determination kit, according to the manufacturer's instructions, using bovine serum albumin as a standard.

Human IgG (Gamma Immunoglobulin) ELISA

[0228] ELISA plates were coated overnight with 100 μl of goat anti-human gamma-chain specific alkaline phosphatase-conjugated antibody at 0.4 mg/ml in coating buffer at 4° C. Coating antibody was removed and plates were blocked with 2% BSA in PBS for 2 hours. All subsequent steps were performed at 37° C. Blocking buffer was replaced with antibody samples or human IgG1 standard diluted in dilution buffer, serially diluted in a 200 ml volume, and incubated for one hour. Negative controls included dilution buffer and/or culture medium of nontransfected cells. Wells were washed and 100 μl of goat anti-human kappa chain specific alkaline phosphatase-conjugated antibody diluted 1:5000 was added and incubated for one hour. Wells were washed and 100 μl reaction buffer was added and incubated for 30 minutes. The reaction was stopped by addition of 1 M NaOH and absorbance read at 405 nm in an ELISA plate reader. Results were analyzed by four-parameter iterative curve fitting.

[0229] Amino acid analysis was performed according to methods available in the state of the art.

[0230] Monoclonal antibody L_AH_C was produced and purified to homogeneity using Protein A affinity chromatography. ELISA assays using human IgG1 as standard indicated L_AH_C recoveries exceeding 70%. The purity of the material was estimated to be >90% by SDS-polyacrylamide gel electrophoresis. Representative expression data and typical purification yields are shown in Table 20.

TABLE 20

Expression data and purification yields FAP antibody proteins in CHO cells

Antibody	Expression levels in crude media samples (ELISA)	Purified antibody yields	Yield improvement [purified antibody]
L_AH_C	7–10 mg/l	~5–7 mg/l	500–700
L_AH_A	5–7 mg/l	~3–4 mg/l	300–400
L_BH_B	0.5–1 mg/l	~0.2–0.5 mg/l	20–50
L_BH_D	0.8–1.5 mg/l	~0.3–0.8 mg/l	30–60
Chimeric F19	~0.02 mg/l	<0.01 mg/l	1

Representative expression data for each of the anti–FAP antibodies produced in this study are shown. Recoveries after Protein A agarose affinity chromatography were based on protein dye-binding measurements of the purified Ig using BSA as a standard.

Example 12

Binding of Monoclonal Antibody L_AH_C to Isolated Recombinant Human FAP

[0231] The objective of this study was to characterize binding of L_AH_C to isolated recombinant human FAP.

CD8-FAP ELISA

[0232] ELISA plates were coated overnight with 100 μl of mouse anti-rat antibody (Sigma Chemical R0761) at 1:2000 in coating buffer at 4° C. Coating antibody was removed and plates were blocked with 2% BSA in PBS for one hour. All subsequent steps were performed at room temperature. Blocking buffer was replaced with 100 ml of 1 μg/ml rat anti-CD8 antibody (Pharmingen 01041D) and incubated for one hour. Plates were washed and 100 μl CD8-FAP culture supernatant (see example 14) (1:2 in PBS) was added and allowed to bind for one hour. Plates were washed and antibody samples were added (two-fold serial dilutions) in a 100 μl volume and incubated for one hour. Negative controls included human IgG and/or culture medium of nontransfected cells. Wells were washed and 100 μl of horse radish peroxidase (HRP) conjugated mouse anti-human IgG1 antibody (Zymed 05-3320) diluted 1:500 in dilution buffer were added and incubated for one hour. Wells were washed and 100 μl HRP substrate, (azino-bis (3-ethylbenzthiazoline 6-sulfonic) acid, Sigma Chemical A9941), were added and incubated for 60 minutes. The reaction was stopped by addition of 1 M NaOH and absorbance read at 405/490 nm in an ELISA plate reader. Results were analyzed by four-parameter curve iterative curve fitting.

[0233] Alternatively, plates were coated directly with cF19. FAP (recombinant human FAP, see example 13) was allowed to bind to these plates as above and biotinylated

L_AH_C (~1 μg/ml) was then added. Antibody binding was detected with HRP-streptavidin conjugate as above.

Solubilization of Membrane-bound Human FAP

[0234] FAP-expressing 293FAP I/2 cells or control 293 cells were washed with PBS and lysed with 1% Triton X-114 in Tris-buffered saline. Nuclei and debris were removed by centrifugation at 10,000×g. The supernatant was phase-partitioned (Estreicher, A., et al., "Characterization of the cellular binding site for the urokinase-type plasminogen activator,"*J. Biol. Chem.* 264:1180-1189 (1989)) to enrich membrane proteins. The detergent phase was collected and diluted in buffer containing 1% Empigen BB (Calbiochem) to prevent reaggregation of the Triton X-114. This material was subjected to Concanavalin A agarose chromatography (Rettig, W. J., et al., "Regulation and heteromeric structure of the fibroblast activation protein in normal and transformed cells of mesenchymal and neuroectodermal origin, "*Cancer Res* 53:3327-3335 (1993)).

Biotinylation of L_AH_C

[0235] L_AH_C (1-2 mg) was dialyzed against 50 mM bicarbonate buffer and biotinylated with a ten-fold molar excess of sulfosuccinimidyl-6-biotinamido hexanoate (NHS-LC biotin, Pierce Chemical, Rockford, Ill., USA) for 2 hours at room temperature. Unreacted product was removed by repeated microdialysis in a microconcentrator.

Transient Transfections

[0236] COS-7 cells (American Type Tissue Culture Collection, reference number CRL 1651) were cotransfected by electroporation with the heavy and light chain vectors encoding L_AH_C.

[0237] Anti-CD8 monoclonal antibody was immobilized onto microtiter plates. CD8-FAP from medium of insect cells infected with CD8-FAP baculovirus was allowed to bind to these plates. Spent medium from COS-7 cell cultures transiently transfected with two separate vectors encoding L_AH_C was serially diluted and added to the wells containing the immobilized CD8-FAP. L_AH_C bound to isolated immobilized CD8-FAP protein (**FIG. 35**). Culture supernatants from mock-transfected COS-7 cells failed to demonstrate binding.

[0238] Recombinant membrane-bound FAP from detergent extracts of 293FAP 1/2 cells or control extracts was serially diluted and immobilized via chimeric F19 monoclonal antibody bound to microtiter plates. Biotinylated L_AH_C bound recombinant human FAP immobilized with cF19 (**FIG. 36**) in a concentration-dependent manner.

[0239] L_AH_C recognized isolated immobilized recombinant human FAP carrying the epitope for murine F19. L_AH_C bound to both CD8-FAP produced in insect cells, as well as FAP protein produced in 293FAP I/2 cells.

[0240] Culture supernatants from COS-7 cells transfected with either heavy and light chain vectors encoding L_AH_C or without DNA (Control) were collected three days posttransfection. CD8-FAP was immobilized via an anti-CD8 antibody as described in the text. Serial dilutions of the COS-7 supernatants were allowed to bind to the immobilized CD8-FAP and subsequently detected with an HRP-conjugated anti-human IgG1 antibody.

[0241] Detergent extracts of FAP-expressing 293FAP I/2 cells or control 293 cells were serially diluted and added to cF19-coated microtiter plates. Biotinylated L_AH_C was added and binding of biotinylated L_AH_C was detected with HRP-conjugated streptavidin.

Example 13

Characterization of HT-1080 Fibrosarcoma Cells and 293 Human Embryonic Kidney Cells Transfected with cDNA for Human FAP

[0242] Fibroblast activation protein (FAP) is a cell-surface, membrane-bound protein which carries the F19 epitope and is expressed on tumor stromal fibroblasts. Cell lines expressing recombinant FAP protein and matched controls lacking FAP were generated for the characterization of anti-FAP monoclonal antibodies.

[0243] Cells used were HT-1080 cells (reference number CCL 121) and 293 human embryonic kidney cells (reference number CRL 1573) were obtained from the American Type Culture Collection (Maryland, USA). Transfectam was obtained from Promega (Madison, Wis.). Geneticin and all restriction enzymes were obtained from Boehringer Mannheim. DNA for transfections was purified from *E. coli* cells using QiaFilter Maxi Cartridges (Qiagen) as directed by the manufacturer. All DNA preparations were examined by restriction enzyme digestion. Vector sequences were confirmed using an ABI PRISM 310 Sequencer.

[0244] Further information regarding the vectors and DNA sequences employed has been described in Scanlan, M. J., et al., "Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers,"*Proc. Natl. Acad. Sci. USA* 89:10832-10836 (1992). The FAP cDNA sequence has been deposited in Genbank (accession number HS09287).

Cell Culture and Immunoassays

[0245] HT-1080 cells were transfected with 1 mg DNA using Transfectam according to the manufacturer's instructions. Human embryonic kidney 293 cells were transfected by calcium phosphate transfection (Brann, M. R., et al., "Expression of cloned muscarinic receptor in A9 L cells, "*Mol. Pharmacol.* 32:450-455 (1987)) with 10 mg DNA. Twenty-four hours later, cells were diluted 1:10 into fresh medium containing 200 mg/ml Geneticin. Colonies were picked and examined by immunofluorescence for FAP expression as described in Rettig, W. J., et al., "Cell-surface glycoproteins of human sarcomas: differential expression in normal and malignant tissues and cultured cells,"*Proc. Natl. Acad. Sci. USA* 85:3110-3114 (1988).

[0246] Immunoprecipitations with cF19 were performed with metabolically labelled cells as described in Rettig, W. J., et al., "Regulation and heteromeric structure of the fibroblast activation protein in normal and transformed cells of mesenchymal and neuroectodermal origin,"*Cancer Res.* 53:3327-3335 (1993).

[0247] HT-1080 and 293 cells were tested for FAP antigen expression in immunofluorescence assays with anti-FAP antibodies and were found to be antigen-negative. Transfection of these cells with FAP.38 vector resulted in the

generation of Geneticin-resistant colonies. Isolated colonies were picked and analyzed by immunofluorescence for FAP expression. Two cell clones were identified, designated HT-1080FAP clone 33 and 293FAP I/2, which express cell surface-bound FAP protein, as recognized by cF19 antibody. Staining of nonpermeabilized HT-1080FAP clone 33 cells and 293FAP I/2 with cF19 antibody confirmed the cell surface localization of the FAP protein.

[0248] Immunoprecipitation of radiolabelled FAP protein with cF19 from extracts of 35S-methionine labelled HT-1080FAP clone 33 cells or 293FAP I/2 cells resulted in the appearance of a 93 kilodalton band after autoradiography. This band is not detectable in immunoprecipitates of parental HT-1080 or 293 cell extracts.

[0249] Two stably transfected cell lines, HT-1080FAP clone 33 and 293FAP I/2, express FAP on the cell surface as determined in immunological assays with anti-FAP mAbs. Neither parental HT-1080 cells nor parental 293 cells express detectable levels of FAP.

Example 14

Generation and Characterization of CD8-FAP Fusion Protein

[0250] A soluble form of human FAP (fibroblast activation protein) in the form of a CD8-FAP fusion protein was produced in insect cells for the characterization of L_AH_C containing the binding site for anti-FAP mAbs. Murine CD8 was chosen to permit secretion of the protein and to provide an additional epitope tag.

[0251] The cDNA encoding the extracellular domain of CD8, consisting of the first 189 amino acids of murine CD8α (Genbank M12825), was linked to that of the extracellular domain of FAP (amino acids 27 to 760), essentially as described by Lane, et al. (Lane, P., et al., "Soluble CD40 ligand can replace the normal T cell-derived CD40 ligand signal to B cells in T cell-dependent activation,"*J. Exp. Med.* 177:1209-1213 (1993)) using standard PCR protocols. The authenticity of all clones was verified by DNA sequencing. The resulting DNA was inserted into the pVL1393 vector (Invitrogen) and transfection of Sf9 cells (Invitrogen) with this vector and amplification of the resulting recombinant baculovirus were performed as described (*Baculovirus Expression Vectors. A Laboratory Manual*, O'Reilly, D. R., et al., eds., Oxford University Press:, New York (1994)). The spent medium of High Five™ cells (Invitrogen) infected with recombinant CD8-FAP baculovirus for four days was collected and cleared by ultracentrifugation.

[0252] The CD8-FAP ELISA (enzyme-linked immunosorbent assay) has been described above (example 12).

[0253] Insect cell cultures infected with CD8-FAP virus secreted a fusion protein into the medium which carries the F19 epitope and is recognized by an anti-FAP antibody (**FIG. 1**). Neither the cell culture medium alone nor medium from insect cells infected with CD8-CD40L fusion protein bound anti-FAP antibody.

[0254] Soluble CD8-FAP protein carrying the F19 epitope was secreted into the medium of infected insect cell cultures. Culture supernatant from cells infected with a control construct did not contain antigen bearing the F19 epitope.

[0255] A soluble form of FAP, CD8-FAP, was produced in insect cells and CD8-FAP was shown to carry the epitope recognized by cF19.

[0256] Supernatants from insect cells infected with recombinant baculovirus encoding either CD8-FAP or CD8-CD40L fusion protein were collected four days postinfection. Cell culture medium without cells was used as an additional control (medium). Serial dilutions of these materials were added to anti-CD8 antibody-coated microtiter plates and allowed to bind. cF19 (1 mg/ml) was subsequently added and allowed to bind. Bound cF19 was detected with horseradish peroxidase-conjugated anti-human antibody.

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Arg Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
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Pro Asp Arg Phe Ser Gly Ser Gly Phe Gly Thr Asp Phe Thr Leu Thr
 65                  70                  75                  80

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<400> SEQUENCE: 3

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gacattgtga tgacccaatc tccagactct ttggctgtgt ctctagggga gagggccacc     60

atcaactgca agtccagtca gagcctttta tattctagaa atcaaaagaa ctacttggcc    120

tggttccagc agaaaccagg acagccaccc aaactcctca tcttttgggc tagcactagg    180

gaatctgggg tacctgatag gttcagtggc agtgggtttg ggacagactt caccctcacc    240

attagcagcc tgcaggctga agatgtggca gtttatgact gtcaacaata ttttagctat    300

ccgctcacgt tcggacaagg gaccaaggtg gaaataaaa                            339
```

<210> SEQ ID NO 4
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

```
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
  1               5                  10                  15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
             20                  25                  30

Arg Asn Gln Lys Asn Tyr Leu Ala Trp Phe Gln Gln Lys Pro Gly Gln
         35                  40                  45

Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
     50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Phe Gly Thr Asp Phe Thr Leu Thr
 65                  70                  75                  80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Asp Cys Gln Gln
                 85                  90                  95

Tyr Phe Ser Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
```

-continued

100 105 110

Lys

<210> SEQ ID NO 5
<211> LENGTH: 339
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

gacattgtga tgacccaatc tccagactct ttggctgtgt ctctagggga gagggccacc 60

atcaactgca agtccagtca gagcctttta tattctagaa atcaaaagaa ctacttggcc 120

tggtatcagc agaaaccagg acagccaccc aaactcctca tctattgggc tagcactagg 180

gaatctgggg tacctgatag gttcagtggc agtgggtttg ggacagactt caccctcacc 240

attagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttttagctat 300

ccgctcacgt tcggacaagg gaccaaggtg gaaataaaa 339

<210> SEQ ID NO 6
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

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

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

Arg Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Phe Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Phe Ser Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys

<210> SEQ ID NO 7
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

caggtgcaac tagtgcagtc cggcgccgaa gtgaagaaac ccggtgcttc cgtgaaagtc 60

agctgtaaaa ctagtagata caccttcact gaatacacca tacactgggt tagacaggcc 120

cctggccaaa ggctggagtg gataggaggt attaatccta acaatggtat tcctaactac 180

aaccagaagt tcaagggccg ggccaccttg accgtaggca agtctgccag caccgcctac 240

atggaactgt ccagcctgcg ctccgaggac actgcagtct actactgcgc cagaagaaga 300

atcgcctatg gttacgacga gggccatgct atggactact ggggtcaagg aacccttgtc 360

accgtctcct ca 372

-continued

<210> SEQ ID NO 8
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Thr Ser Arg Tyr Thr Phe Thr Glu Tyr
20 25 30

Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile
35 40 45

Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Ala Thr Leu Thr Val Gly Lys Ser Ala Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His Ala Met Asp
100 105 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 9
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

caggtgcaac tagtgcagtc cggcgccgaa gtgaagaaac ccggtgcttc cgtgaaagtc 60

agctgtaaaa ctagtagata caccttcact gaatacacca tacactgggt tagacaggcc 120

cctggccaaa ggctggagtg gataggaggt attaatccta acaatggtat tcctaactac 180

aaccagaagt tcaagggccg ggccaccttg accgtaggca agtctgccag caccgcctac 240

atggaactgt ccagcctgcg ctccgaggac actgcagtct acttctgcgc cagaagaaga 300

atcgcctatg gttacgacga gggccatgct atggactact ggggtcaagg aaccttgtc 360

accgtctcct ca 372

<210> SEQ ID NO 10
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Thr Ser Arg Tyr Thr Phe Thr Glu Tyr
20 25 30

Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile
35 40 45

Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Ala Thr Leu Thr Val Gly Lys Ser Ala Ser Thr Ala Tyr

-continued

```
 65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
                 85                  90                  95

Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His Ala Met Asp
            100                 105                 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120


<210> SEQ ID NO 11
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

caggtgcaac tagtgcagtc cggcgccgaa gtgaagaaac ccggtgcttc cgtgaaagtc     60

agctgtaaaa ctagtagata caccttcact gaatacacca tacactgggt tagacaggcc    120

cctggccaaa ggctggagtg gataggaggt attaatccta acaatggtat tcctaactac    180

aaccagaagt tcaagggccg ggtcaccatc accgtagaca cctctgccag caccgcctac    240

atggaactgt ccagcctgcg ctccgaggac actgcagtct actactgcgc cagaagaaga    300

atcgcctatg gttacgacga gggccatgct atggactact ggggtcaagg aacccttgtc    360

accgtctcct ca                                                        372


<210> SEQ ID NO 12
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
  1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Thr Ser Arg Tyr Thr Phe Thr Glu Tyr
             20                  25                  30

Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile
         35                  40                  45

Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn Gln Lys Phe
     50                  55                  60

Lys Gly Arg Val Thr Ile Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr
 65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                 85                  90                  95

Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His Ala Met Asp
            100                 105                 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120


<210> SEQ ID NO 13
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

caggtgcaac tagtgcagtc cggcgccgaa gtgaagaaac ccggtgcttc cgtgaaagtc     60

agctgtaaaa ctagtagata caccttcact gaatacacca tacactgggt tagacaggcc    120
```

-continued

cctggccaaa ggctggagtg gataggaggt attaatccta acaatggtat tcctaactac 180

aaccagaagt tcaagggccg ggtcaccatc accgtagaca cctctgccag caccgcctac 240

atggaactgt ccagcctgcg ctccgaggac actgcagtct acttctgcgc cagaagaaga 300

atcgcctatg gttacgacga gggccatgct atggactact ggggtcaagg aacccttgtc 360

accgtctcct ca 372

<210> SEQ ID NO 14
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Thr Ser Arg Tyr Thr Phe Thr Glu Tyr
20 25 30

Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile
35 40 45

Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His Ala Met Asp
100 105 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 15
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

caggtgcaac tagtgcagtc cggcgccgaa gtgaagaaac ccggtgcttc cgtgaaagtc 60

agctgtaaaa ctagtggata caccttcact gaatacacca tacactgggt tagacaggcc 120

cctggccaaa ggctggagtg gataggaggt attaatccta acaatggtat tcctaactac 180

aaccagaagt tcaagggccg ggtcaccatc accgtagaca cctctgccag caccgcctac 240

atggaactgt ccagcctgcg ctccgaggac actgcagtct actactgcgc cagaagaaga 300

atcgcctatg gttacgacga gggccatgct atggactact ggggtcaagg aacccttgtc 360

accgtctcct ca 372

<210> SEQ ID NO 16
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

-continued

```
Ser Val Lys Val Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr
            20                  25                  30

Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile
         35                  40                  45

Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn Gln Lys Phe
     50                  55                  60

Lys Gly Arg Val Thr Ile Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr
 65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                 85                  90                  95

Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His Ala Met Asp
            100                 105                 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120


<210> SEQ ID NO 17
<211> LENGTH: 220
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val Gly
  1               5                  10                  15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
             20                  25                  30

Arg Asn Gln Lys Asn Tyr Leu Ala Trp Phe Gln Gln Lys Pro Gly Gln
         35                  40                  45

Ser Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
     50                  55                  60

Pro Asp Arg Phe Thr Gly Ser Gly Phe Gly Thr Asp Phe Asn Leu Thr
 65                  70                  75                  80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Asp Cys Gln Gln
                 85                  90                  95

Tyr Phe Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
            100                 105                 110

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
        115                 120                 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
    130                 135                 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
145                 150                 155                 160

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
                165                 170                 175

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
            180                 185                 190

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
        195                 200                 205

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215                 220
```

-continued

<210> SEQ ID NO 18
<211> LENGTH: 453
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

```
Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser
  1               5                  10                  15

Val Lys Met Ser Cys Lys Thr Ser Arg Tyr Thr Phe Thr Glu Tyr Thr
             20                  25                  30

Ile His Trp Val Arg Gln Ser His Gly Lys Ser Leu Glu Trp Ile Gly
         35                  40                  45

Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn Gln Lys Phe Lys
     50                  55                  60

Gly Arg Ala Thr Leu Thr Val Gly Lys Ser Ser Ser Thr Ala Tyr Met
 65                  70                  75                  80

Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala
                 85                  90                  95

Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His Ala Met Asp Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
        355                 360                 365
```

-continued

```
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445

Leu Ser Pro Gly Lys
    450
```

<210> SEQ ID NO 19
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

```
cgtactgtgg ctgcaccatc tgtcttcatc ttcccgccat ctgatgagca gttgaaatct     60

ggaactgcct ctgttgtgtg cctgctgaat aacttctatc ccagagaggc caaagtacag    120

tggaaggtgg ataacgccct ccaatcgggt aactcccagg agagtgtcac agagcaggac    180

agcaaggaca gcacctacag cctcagcagc accctgacgc tgagcaaagc agactacgag    240

aaacacaaag tctacgcctg cgaagtcacc catcagggcc tgagctcgcc cgtcacaaag    300

agcttcaaca ggggagagtg t                                              321
```

<210> SEQ ID NO 20
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

```
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
  1               5                  10                  15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
             20                  25                  30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
         35                  40                  45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
     50                  55                  60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 65                  70                  75                  80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
                 85                  90                  95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105
```

<210> SEQ ID NO 21
<211> LENGTH: 990
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

-continued

```
gcctccacca agggcccatc ggtcttcccc ctggcaccct cctccaagag cacctctggg     60

ggcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg    120

tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca    180

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc    240

tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc    300

aaatcttgtg acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga    360

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct    420

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg    480

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac    540

agcacgtacc gggtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag    600

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc    660

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgccccatc ccgggaggag    720

atgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc    780

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg    840

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg    900

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg    960

cagaagagcc tctccctgtc tccgggtaaa                                     990
```

<210> SEQ ID NO 22
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

```
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
  1               5                  10                  15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
             20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
         35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
     50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
 65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                 85                  90                  95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175
```

-continued

```
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225                 230                 235                 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330
```

<210> SEQ ID NO 23
<211> LENGTH: 427
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

```
aagcttgccg ccaccatgga ttcacaggcc caggttctta tgttactgcc gctatgggta     60

tctggtacct gtggggacat tgtgatgtca cagtctccat cctccctagc tgtgtcagtt    120

ggagagaagg ttactatgag ctgcaagtcc agtcagagcc ttttatatag tcgtaatcaa    180

aagaactact tggcctggtt ccagcagaag ccagggcagt ctcctaaact gctgattttc    240

tgggcatcca ctagggaatc tggggtccct gatcgcttca caggcagtgg atttgggacg    300

gatttcaatc tcaccatcag cagtgtgcag gctgaggacc tggcagttta tgactgtcag    360

caatatttta gctatccgct cacgttcggt gctgggacca agctggagct gaaacgtgag    420

tggatcc                                                              427
```

<210> SEQ ID NO 24
<211> LENGTH: 133
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

```
Met Asp Ser Gln Ala Gln Val Leu Met Leu Leu Pro Leu Trp Val Ser
  1               5                  10                  15

Gly Thr Cys Gly Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala
             20                  25                  30

Val Ser Val Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser
         35                  40                  45

Leu Leu Tyr Ser Arg Asn Gln Lys Asn Tyr Leu Ala Trp Phe Gln Gln
     50                  55                  60
```

-continued

```
Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg
 65                  70                  75                  80

Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Phe Gly Thr Asp
                85                  90                  95

Phe Asn Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr
            100                 105                 110

Asp Cys Gln Gln Tyr Phe Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr
        115                 120                 125

Lys Leu Glu Leu Lys
    130
```

<210> SEQ ID NO 25
<211> LENGTH: 457
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

```
aagcttgccg ccaccatggg atggagctgg gtctttctct ttctcctgtc aggaactgca     60

ggtgtcctct ctgaggtcca gctgcaacag tctggacctg agctggtgaa gcctggggct    120

tcagtaaaga tgtcctgcaa gacttctaga tacacattca ctgaatacac catacactgg    180

gtgagacaga gccatggaaa gagccttgag tggattggag gtattaatcc taacaatggt    240

attcctaact acaaccagaa gttcaagggc agggccacat tgactgtagg caagtcctcc    300

agcaccgcct acatggagct ccgcagcctg acatctgagg attctgcggt ctatttctgt    360

gcaagaagaa gaatcgccta tggttacgac gagggccatg ctatggacta ctggggtcaa    420

ggaacctcag tcaccgtctc ctcaggtgag tggatcc                             457
```

<210> SEQ ID NO 26
<211> LENGTH: 143
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

```
Met Gly Trp Ser Trp Val Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
  1               5                  10                  15

Val Leu Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
             20                  25                  30

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Thr Ser Arg Tyr Thr Phe
         35                  40                  45

Thr Glu Tyr Thr Ile His Trp Val Arg Gln Ser His Gly Lys Ser Leu
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Glu Trp Ile Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn
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Gln Lys Phe Lys Gly Arg Ala Thr Leu Thr Val Gly Lys Ser Ser Ser
                85                  90                  95

Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val
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<210> SEQ ID NO 27
<211> LENGTH: 8068
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 28

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             20                  25                  30

Val Ser Val Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser
         35                  40                  45

Leu Leu Tyr Ser Arg Asn Gln Lys Asn Tyr Leu Ala Trp Phe Gln Gln
     50                  55                  60

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg
 65                  70                  75                  80

Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Phe Gly Thr Asp
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Lys Leu Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
    130                 135                 140

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
145                 150                 155                 160

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
                165                 170                 175

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
            180                 185                 190

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
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Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
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<210> SEQ ID NO 30
<211> LENGTH: 472
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 30

Met Gly Trp Ser Trp Val Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
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Val Leu Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
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Pro Gly Ala Ser Val Lys Met Ser Cys Lys Thr Ser Arg Tyr Thr Phe
         35                  40                  45

Thr Glu Tyr Thr Ile His Trp Val Arg Gln Ser His Gly Lys Ser Leu
     50                  55                  60

Glu Trp Ile Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn
 65                  70                  75                  80

Gln Lys Phe Lys Gly Arg Ala Thr Leu Thr Val Gly Lys Ser Ser Ser
                 85                  90                  95

Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val
            100                 105                 110

Tyr Phe Cys Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His
        115                 120                 125

Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ser
    130                 135                 140

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
145                 150                 155                 160

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
                165                 170                 175

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
            180                 185                 190

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
        195                 200                 205

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
    210                 215                 220

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
225                 230                 235                 240

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
                245                 250                 255

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            260                 265                 270

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        275                 280                 285

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    290                 295                 300

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
305                 310                 315                 320

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                325                 330                 335

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            340                 345                 350

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        355                 360                 365

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
    370                 375                 380

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
385                 390                 395                 400

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                405                 410                 415
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```
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            420                 425                 430

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        435                 440                 445

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    450                 455                 460

Ser Leu Ser Leu Ser Pro Gly Lys
465                 470


<210> SEQ ID NO 31
<211> LENGTH: 339
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 31

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tggtatcagc agaaaccagg acagccaccc aaactcctca tcttttgggc tagcactagg    180

gaatctgggg tacctgatag gttcagtggc agtgggtttg ggacagactt caccctcacc    240

attagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttttagctat    300

ccgctcacgt tcggacaagg gaccaaggtg gaaataaaa                           339


<210> SEQ ID NO 32
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

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

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
             20                  25                  30

Arg Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
         35                  40                  45

Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
     50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Phe Gly Thr Asp Phe Thr Leu Thr
 65                  70                  75                  80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
                 85                  90                  95

Tyr Phe Ser Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
            100                 105                 110

Lys


<210> SEQ ID NO 33
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 33

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

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
             20                  25                  30
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```
Arg Asn Gln Lys Asn Tyr Leu Ala Trp Phe Gln Gln Lys Pro Gly Gln
         35                  40                  45

Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
     50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Phe Gly Thr Asp Phe Thr Leu Thr
 65                  70                  75                  80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Asp Cys Gln Gln
                 85                  90                  95

Tyr Phe Ser Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
            100                 105                 110

Lys
```

<210> SEQ ID NO 34
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 34

```
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
  1               5                  10                  15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
             20                  25                  30

Arg Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
         35                  40                  45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
     50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Phe Gly Thr Asp Phe Thr Leu Thr
 65                  70                  75                  80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
                 85                  90                  95

Tyr Phe Ser Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
            100                 105                 110

Lys
```

<210> SEQ ID NO 35
<211> LENGTH: 8068
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

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ctgaccgccc aacgacccccc gcccattgac gtcaataatg acgtatgttc ccatagtaac    180

gccaataggg actttccatt gacgtcaatg ggtggagtat ttacggtaaa ctgcccactt    240

ggcagtacat caagtgtatc atatgccaag tacgcccccct attgacgtca atgacggtaa    300

atggcccgcc tggcattatg cccagtacat gaccttatgg gactttccta cttggcagta    360

catctacgta ttagtcatcg ctattaccat ggtgatgcgg ttttggcagt acatcaatgg    420

gcgtggatag cggtttgact cacggggatt tccaagtctc caccccattg acgtcaatgg    480

gagtttgttt tggcaccaaa atcaacggga ctttccaaaa tgtcgtaaca actccgcccc    540

attgacgcaa atgggcggta ggcgtgtacg gtgggaggtc tatataagca gagctcgttt    600
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ccatggcgat gcctgcttgc cgaatatcat ggtggaaaat ggccgctttt ctggattcat 6480

cgactgtggc cggctgggtg tggcggaccg ctatcaggac atagcgttgg ctacccgtga 6540

tattgctgaa gagcttggcg gcgaatgggc tgaccgcttc ctcgtgcttt acggtatcgc 6600

cgctcccgat tcgcagcgca tcgccttcta tcgccttctt gacgagttct tctgagcggg 6660

actctggggt tcgaaatgac cgaccaagcg acgcccaacc tgccatcacg agatttcgat 6720

tccaccgccg ccttctatga aaggttgggc ttcggaatcg ttttccggga cgccggctgg 6780

atgatcctcc agcgcgggga tctcatgctg gagttcttcg cccaccccgg gctcgatccc 6840

ctcgcgagtt ggttcagctg ctgcctgagg ctggacgacc tcgcggagtt ctaccggcag 6900

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cttctgtggt gtgacataat tggacaaact acctacagag atttaaagct ctaaggtaaa 7080

tataaaattt ttaagtgtat aatgtgttaa actactgatt ctaattgttt gtgtatttta 7140

gattccaacc tatggaactg atgaatggga gcagtggtgg aatgccttta atgaggaaaa 7200

cctgttttgc tcagaagaaa tgccatctag tgatgatgag gctactgctg actctcaaca 7260

ttctactcct ccaaaaaaga agagaaaggt agaagacccc aaggactttc cttcagaatt 7320

gctaagtttt ttgagtcatg ctgtgtttag taatagaact cttgcttgct ttgctattta 7380

caccacaaag gaaaaagctg cactgctata caagaaaatt atggaaaaat attctgtaac 7440

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gcatagagtg tctgctatta ataactatgc tcaaaaattg tgtaccttta gctttttaat 7560

ttgtaaaggg gttaataagg aatatttgat gtatagtgcc ttgactagag atcataatca 7620

gccataccac atttgtagag gttttacttg ctttaaaaaa cctcccacac ctccccctga 7680

acctgaaaca taaaatgaat gcaattgttg ttgttaactt gtttattgca gcttataatg 7740

gttacaaata aagcaatagc atcacaaatt tcacaaataa agcatttttt tcactgcatt 7800

ctagttgtgg tttgtccaaa ctcatcaatg tatcttatca tgtctggatc taataaaaga 7860

tatttatttt cattagatat gtgtgttggt tttttgtgtg cagtgcctct atctggaggc 7920

caggtagggc tggccttggg ggaggggggag gccagaatga ctccaagagc tacaggaagg 7980

caggtcagag accccactgg acaaacagtg gctggactct gcaccataac acacaatcaa 8040

caggggagtg agctggaaat ttgctagc 8068

<210> SEQ ID NO 36
<211> LENGTH: 240
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 36

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Ser Gly Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala
20 25 30

Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser
35 40 45

Leu Leu Tyr Ser Arg Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln
50 55 60

Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg
65 70 75 80

Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Phe Gly Thr Asp
85 90 95

Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr
100 105 110

Tyr Cys Gln Gln Tyr Phe Ser Tyr Pro Leu Thr Phe Gly Gln Gly Thr
115 120 125

Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
130 135 140

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
145 150 155 160

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
165 170 175

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
180 185 190

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
195 200 205

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
210 215 220

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235 240

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<210> SEQ ID NO 37
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 37

caggtgcaac tagtgcagtc cggcgccgaa gtgaagaaac ccggtgcttc cgtgaaagtc 60

agctgtaaaa ctagtagata caccttcact gaatacacca tacactgggt tagacaggcc 120

cctggccaaa ggctggagtg gataggaggt attaatccta acaatggtat tcctaactac 180

aaccagaagt tcaagggccg ggccaccttg accgtaggca agtctgccag caccgcctac 240

atggaactgt ccagcctgcg ctccgaggac actgcagtct actactgcgc cagaagaaga 300

atcgcctatg gttacgacga gggccatgct atggactact ggggtcaagg aaccttgtc 360

accgtctcct ca 372

<210> SEQ ID NO 38
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 38

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Thr Ser Arg Tyr Thr Phe Thr Glu Tyr
20 25 30

Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile
35 40 45

Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Ala Thr Leu Thr Val Gly Lys Ser Ala Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His Ala Met Asp
100 105 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 39
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 39

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Thr Ser Arg Tyr Thr Phe Thr Glu Tyr
20 25 30

Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile
35 40 45

Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Ala Thr Leu Thr Val Gly Lys Ser Ala Ser Thr Ala Tyr
65 70 75 80

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Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
                 85                  90                  95

Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His Ala Met Asp
            100                 105                 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120


<210> SEQ ID NO 40
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 40

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
  1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Thr Ser Arg Tyr Thr Phe Thr Glu Tyr
             20                  25                  30

Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile
         35                  40                  45

Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn Gln Lys Phe
     50                  55                  60

Lys Gly Arg Val Thr Ile Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr
 65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                 85                  90                  95

Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His Ala Met Asp
            100                 105                 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120


<210> SEQ ID NO 41
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 41

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
  1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr
             20                  25                  30

Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile
         35                  40                  45

Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn Gln Lys Phe
     50                  55                  60

Lys Gly Arg Val Thr Ile Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr
 65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                 85                  90                  95

Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His Ala Met Asp
            100                 105                 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
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<210> SEQ ID NO 42
<211> LENGTH: 7731
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 42

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ggtttcttag acgtcaggtg gcacttttcg gggaaatgtg cgcggaaccc ctatttgttt 120

atttttctaa atacattcaa atatgtatcc gctcatgaga caataaccct gataaatgct 180

tcaataatat tgaaaaagga agagtatgag tattcaacat ttccgtgtcg cccttattcc 240

ctttttgcg gcattttgcc ttcctgtttt tgctcaccca gaaacgctgg tgaaagtaaa 300

agatgctgaa gatcagttgg gtgcacgagt gggttacatc gaactggatc tcaacagcgg 360

taagatcctt gagagttttc gccccgaaga acgttttcca atgatgagca cttttaaagt 420

tctgctatgt ggcgcggtat tatcccgtgt tgacgccggg caagagcaac tcggtcgccg 480

catacactat tctcagaatg acttggttga gtactcacca gtcacagaaa agcatcttac 540

ggatggcatg acagtaagag aattatgcag tgctgccata accatgagtg ataacactgc 600

ggccaactta cttctgacaa cgatcggagg accgaaggag ctaaccgctt ttttgcacaa 660

catgggggat catgtaactc gccttgatcg ttgggaaccg gagctgaatg aagccatacc 720

aaacgacgag cgtgacacca cgatgcctgc agcaatggca acaacgttgc gcaaactatt 780

aactggcgaa ctacttactc tagcttcccg gcaacaatta atagactgga tggaggcgga 840

taaagttgca ggaccacttc tgcgctcggc ccttccggct ggctggttta ttgctgataa 900

atctggagcc ggtgagcgtg ggtctcgcgg tatcattgca gcactggggc cagatggtaa 960

gccctcccgt atcgtagtta tctacacgac ggggagtcag gcaactatgg atgaacgaaa 1020

tagacagatc gctgagatag gtgcctcact gattaagcat tggtaactgt cagaccaagt 1080

ttactcatat atactttaga ttgatttaaa acttcatttt taatttaaaa ggatctaggt 1140

gaagatcctt tttgataatc tcatgaccaa aatcccttaa cgtgagtttt cgttccactg 1200

agcgtcagac cccgtagaaa agatcaaagg atcttcttga gatccttttt ttctgcgcgt 1260

aatctgctgc ttgcaaacaa aaaaaccacc gctaccagcg gtggtttgtt tgccggatca 1320

agagctacca actcttttc cgaaggtaac tggcttcagc agagcgcaga taccaaatac 1380

tgtccttcta gtgtagccgt agttaggcca ccacttcaag aactctgtag caccgcctac 1440

atacctcgct ctgctaatcc tgttaccagt ggctgctgcc agtggcgata agtcgtgtct 1500

taccgggttg gactcaagac gatagttacc ggataaggcg cagcggtcgg gctgaacggg 1560

gggttcgtgc acacagccca gcttggagcg aacgacctac accgaactga gatacctaca 1620

gcgtgagcta tgagaaagcg ccacgcttcc cgaagggaga aaggcggaca ggtatccggt 1680

aagcggcagg gtcggaacag gagagcgcac gagggagctt ccagggggaa acgcctggta 1740

tctttatagt cctgtcgggt ttcgccacct ctgacttgag cgtcgatttt tgtgatgctc 1800

gtcaggggg cggagcctat ggaaaaacgc cagcaacgcg gccttttac ggttcctggc 1860

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ccgtattacc gcctttgagt gagctgatac cgctcgccgc agccgaacga ccgagcgcag 1980

cgagtcagtg agcgaggaag cggaagagcg cctgatgcgg tattttctcc ttacgcatct 2040

gtgcggtatt tcacaccgca tatggtgcac tctcagtaca atctgctctg atgccgcata 2100

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gttaagccag tatacactcc gctatcgcta cgtgactggg tcatggctgc gccccgacac 2160

ccgccaacac ccgctgacgc gccctgacgg gcttgtctgc tcccggcatc cgcttacaga 2220

caagctgtga ccgtctccgg gagctgcatg tgtcagaggt tttcaccgtc atcaccgaaa 2280

cgcgcgaggc agcatgcatc tcaattagtc agcaaccata gtcccgcccc taactccgcc 2340

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tcgcgccaaa cttgacggca atcctagcgt gaaggctggt aggattttat ccccgctgcc 2580

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aacggagacc taccctggcc tccgctcagg aacgagttca agtacttcca aagaatgacc 2700

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tccattcctg agaagaatcg acctttaaag gacagaatta atatagttct cagtagagaa 2820

ctcaaagaac caccacgagg agctcatttt cttgccaaaa gtttggatga tgccttaaga 2880

cttattgaac aaccggaatt ggcaagtaaa gtagacatgg tttggatagt cggaggcagt 2940

tctgtttacc aggaagccat gaatcaacca ggccacctca gactctttgt gacaaggatc 3000

atgcaggaat ttgaaagtga cacgtttttc ccagaaattg atttggggaa atataaactt 3060

ctcccagaat acccaggcgt cctctctgag gtccaggagg aaaaaggcat caagtataag 3120

tttgaagtct acgagaagaa agactaacag gaagatgctt tcaagttctc tgctcccctc 3180

ctaaagctat gcatttttat aagaccatgg gacttttgct ggctttagat ctttgtgaag 3240

gaaccttact tctgtggtgt gacataattg gacaaactac ctacagagat ttaaagctct 3300

aaggtaaata taaaattttt aagtgtataa tgtgttaaac tactgattct aattgtttgt 3360

gtattttaga ttccaaccta tggaactgat gaatgggagc agtggtggaa tgcctttaat 3420

gaggaaaacc tgttttgctc agaagaaatg ccatctagtg atgatgaggc tactgctgac 3480

tctcaacatt ctactcctcc aaaaaagaag agaaaggtag aagaccccaa ggactttcct 3540

tcagaattgc taagtttttt gagtcatgct gtgtttagta atagaactct tgcttgcttt 3600

gctatttaca ccacaaagga aaaagctgca ctgctataca agaaaattat ggaaaaatat 3660

tctgtaacct ttataagtag gcataacagt tataatcata acatactgtt ttttcttact 3720

ccacacaggc atagagtgtc tgctattaat aactatgctc aaaaattgtg tacctttagc 3780

tttttaattt gtaaaggggt taataaggaa tatttgatgt atagtgcctt gactagagat 3840

cataatcagc cataccacat ttgtagaggt tttacttgct ttaaaaaacc tcccacacct 3900

ccccctgaac ctgaaacata aaatgaatgc aattgttgtt gttaacttgt ttattgcagc 3960

ttataatggt tacaaataaa gcaatagcat cacaaatttc acaaataaag catttttttc 4020

actgcattct agttgtggtt tgtccaaact catcaatgta tcttatcatg tctggatcta 4080

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acaatcaaca ggggagtgag ctggaaattt gctagcgaat tccagcacac tggcggccgt 4320

tactagttat taatagtaat caattacggg gtcattagtt catagcccat atatggagtt 4380

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gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag tcatcgctat   4680

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tgtacggtgg gaggtctata taagcagagc tcgtttagtg aaccgtcaga tcgcctggag   4920

acgccatcca cgctgttttg acctccatag aagacaccgg gaccgatcca gcctccgcgg   4980

ccgggaacgg tgcattggaa cgcggattcc ccgtgccaag agtgacgtaa gtaccgccta   5040

tagagtctat aggcccaccc ccttggcttc ttatgcatgc tatactgttt ttggcttggg   5100

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ccgtgaaagt cagctgtaaa actagtagat acaccttcac tgaatacacc atacactggg   6060

ttagacaggc ccctggccaa aggctggagt ggataggagg tattaatcct aacaatggta   6120

ttcctaacta caaccagaag ttcaagggcc gggccacctt gaccgtaggc aagtctgcca   6180

gcaccgccta catggaactg tccagcctgc gctccgagga cactgcagtc tactactgcg   6240

ccagaagaag aatcgcctat ggttacgacg agggccatgc tatggactac tggggtcaag   6300

gaacccttgt caccgtctcc tcaggtgagt ggatcctctg cgcctgggcc cagctctgtc   6360

ccacaccgcg gtcacatggc accacctctc ttgcagcctc caccaagggc ccatcggtct   6420

tccccctggc accctcctcc aagagcacct ctgggggcac agcggccctg ggctgcctgg   6480

tcaaggacta cttccccgaa ccggtgacgg tgtcgtggaa ctcaggcgcc ctgaccagcg   6540

gcgtgcacac cttcccggct gtcctacagt cctcaggact ctactccctc agcagcgtgg   6600

tgaccgtgcc ctccagcagc ttgggcaccc agacctacat ctgcaacgtg aatcacaagc   6660
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ccagcaacac caaggtggac aagaaagttg agcccaaatc ttgtgacaaa actcacacat   6720

gcccaccgtg cccagcacct gaactcctgg ggggaccgtc agtcttcctc ttcccccaa   6780

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tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgcaag gtctccaaca   7020

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tctatctgga ggccaggtag ggctggcctt gggggagggg gaggccagaa tgactccaag   7620

agctacagga aggcaggtca gagaccccac tggacaaaca gtggctggac tctgcaccat   7680

aacacacaat caacagggga gtgagctgga aatttgctag cgaattaatt c            7731
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<210> SEQ ID NO 43
<211> LENGTH: 472
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 43

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Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
  1               5                  10                  15

Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
             20                  25                  30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Thr Ser Arg Tyr Thr Phe
         35                  40                  45

Thr Glu Tyr Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu
     50                  55                  60

Glu Trp Ile Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn
 65                  70                  75                  80

Gln Lys Phe Lys Gly Arg Ala Thr Leu Thr Val Gly Lys Ser Ala Ser
                 85                  90                  95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His
        115                 120                 125

Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ser
    130                 135                 140

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
145                 150                 155                 160
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Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
                165                 170                 175

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
            180                 185                 190

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
        195                 200                 205

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
    210                 215                 220

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
225                 230                 235                 240

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
                245                 250                 255

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            260                 265                 270

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        275                 280                 285

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    290                 295                 300

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
305                 310                 315                 320

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                325                 330                 335

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            340                 345                 350

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        355                 360                 365

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
    370                 375                 380

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
385                 390                 395                 400

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                405                 410                 415

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            420                 425                 430

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        435                 440                 445

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    450                 455                 460

Ser Leu Ser Leu Ser Pro Gly Lys
465                 470


<210> SEQ ID NO 44
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 44

accgtctcct caggtgagtg gatcc                                          25


<210> SEQ ID NO 45
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
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<400> SEQUENCE: 45

cctctcttgc agcctccacc aagggc 26

<210> SEQ ID NO 46
<211> LENGTH: 14
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 46

cctctcttgc agcc 14

<210> SEQ ID NO 47
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

Thr Val Ser Ser
1

<210> SEQ ID NO 48
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 48

Ser Thr Lys Gly
1

<210> SEQ ID NO 49
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 49

accgtctcct cagcctccac caagggc 27

<210> SEQ ID NO 50
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 50

Thr Val Ser Ser Ser Thr Lys Gly
1 5

<210> SEQ ID NO 51
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 51

accgtctcct cagcctccac caagggc 27

<210> SEQ ID NO 52
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 52

Thr Val Ser Ser Ala Ser Thr Lys Gly
1 5

<210> SEQ ID NO 53
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 53

gaaataaaac gtgagtggat cc 22

<210> SEQ ID NO 54
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 54

cttctttcct caggaactgt ggctgca 27

<210> SEQ ID NO 55
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 55

Thr Val Ala Ala
1

<210> SEQ ID NO 56
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 56

gaaataaaac gaactgtggc tgca 24

<210> SEQ ID NO 57
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 57

Glu Ile Lys Thr Val Ala Ala
1 5

<210> SEQ ID NO 58
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 58

gaaataaaac gaactgtggc tgca 24

<210> SEQ ID NO 59
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 59

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Glu Ile Lys Arg Thr Val Ala Ala
1 5

<210> SEQ ID NO 60
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 60

Met Asp Ser Gln Ala Gln Val Leu Met Leu Leu Leu Leu Trp Val Ser
1 5 10 15

Gly Thr Cys Gly
20

<210> SEQ ID NO 61
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 61

Met Gly Trp Ser Trp Val Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
1 5 10 15

Val Leu Ser

<210> SEQ ID NO 62
<211> LENGTH: 9
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 62

gccgccacc 9

<210> SEQ ID NO 63
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 63

cagaaagctt gccgccacca tggattcaca ggcccag 37

<210> SEQ ID NO 64
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 64

Met Asp Ser Gln Ala Gln
1 5

<210> SEQ ID NO 65
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 65

ccgaggatcc actcacgttt cagctccagc ttggt 35

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<210> SEQ ID NO 66
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 66

cagaaagctt gccgccacca tgggatggag ctgggtc 37

<210> SEQ ID NO 67
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 67

Met Gly Trp Ser Trp Val
1 5

<210> SEQ ID NO 68
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 68

ccgaggatcc actcacctga ggagacggtg actga 35

<210> SEQ ID NO 69
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 69

gtcatcacaa tgtctccgga ggaacctgga acccag 36

<210> SEQ ID NO 70
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 70

ctccggagac attgtgatga cccaatctc 29

<210> SEQ ID NO 71
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 71

gaatataaaa ggctctgact ggacttgcag ttgatggtgg ccctc 45

<210> SEQ ID NO 72
<211> LENGTH: 72
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 72

cagtcagagc cttttatatt ctagaaatca aaagaactac ttggcctggt atcagcagaa 60

accaggacag cc 72

<210> SEQ ID NO 73
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 73

accccagatt ccctagtgct agcccaaaag atgaggagtt tggg 44

<210> SEQ ID NO 74
<211> LENGTH: 67
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 74

tagcactagg gaatctgggg tacctgatag gttcagtggc agtgggtttg ggacagactt 60

caccctc 67

<210> SEQ ID NO 75
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 75

gtcccttgtc cgaacgtgag cggatagcta aaatattgct gacagtaata aac 53

<210> SEQ ID NO 76
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 76

gctcacgttc ggacaaggga ccaaggtgga aat 33

<210> SEQ ID NO 77
<211> LENGTH: 72
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 77

cagtcagagc cttttatatt ctagaaatca aaagaactac ttggcctggt tccagcagaa 60

accaggacag cc 72

<210> SEQ ID NO 78
<211> LENGTH: 56
<212> TYPE: DNA

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<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 78

tcccttgtcc gaacgtgagc ggatagctaa aatattgctg acagtcataa actgcc 56

<210> SEQ ID NO 79
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 79

cccaaactcc tcatctattg ggctagcact aggg 34

<210> SEQ ID NO 80
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 80

ccctagtgct agcccaatag atgaggagtt tggg 34

<210> SEQ ID NO 81
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 81

tacgcaaacc gcctctc 17

<210> SEQ ID NO 82
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 82

gagtgcacca tatgcggt 18

<210> SEQ ID NO 83
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 83

aacagctatg accatg 16

<210> SEQ ID NO 84
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

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<400> SEQUENCE: 84

ccag tcacgac 17

<210> SEQ ID NO 85
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 85

tcag tgaaggtgta tctactagtt ttacagctga ctttcac 47

<210> SEQ ID NO 86
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 86

tagtagatac accttcactg aatacaccat acactgggtt agacaggccc ctg 53

<210> SEQ ID NO 87
<211> LENGTH: 71
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 87

cccttgaact tctggttgta gttaggaata ccattgttag gattaatacc tcctatccac 60

tccagccttt g 71

<210> SEQ ID NO 88
<211> LENGTH: 71
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 88

taactacaac cagaagttca agggccgggc caccttgacc gtaggcaagt ctgccagcac 60

cgcctacatg g 71

<210> SEQ ID NO 89
<211> LENGTH: 63
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 89

gcatggccct cgtcgtaacc ataggcgatt cttcttctgg cgcagtagta gactgcagtg 60

tcc 63

<210> SEQ ID NO 90
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 90

ctatggttac gacgagggcc atgctatgga ctactggggt caaggaac 48

<210> SEQ ID NO 91
<211> LENGTH: 71
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 91

taactacaac cagaagttca agggccgggt caccatcacc gtagacacct ctgccagcac 60

cgcctacatg g 71

<210> SEQ ID NO 92
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 92

ggacactgca gtctacttct gcgccag 27

<210> SEQ ID NO 93
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 93

tacgcaaacc gcctctc 17

<210> SEQ ID NO 94
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 94

gagtgcacca tatgcggt 18

<210> SEQ ID NO 95
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 95

cctttggcca ggggcctgtc taacccagtg tatggtgtat tcagtgaagg tgtatccact 60

agtttccact agttt 75

<210> SEQ ID NO 96
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 96

gtcaccgtcc ttgacacgcg tctcggga 28

<210> SEQ ID NO 97
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 97

ttggaggagg gtgccag 17

<210> SEQ ID NO 98
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 98

gagacattgt gacccaatct cc 22

<210> SEQ ID NO 99
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 99

gacagtcata aactgccaca tcttc 25

<210> SEQ ID NO 100
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 100

ttgacacgcg tctcgggaag ctt 23

<210> SEQ ID NO 101
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA Primer

<400> SEQUENCE: 101

ggcgcagagg atccactcac ct 22

<210> SEQ ID NO 102
<211> LENGTH: 339
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 102

gacattgtga tgacccaatc tccagactct ttggctgtgt ctctagggga gagggccacc 60

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atcaactgca agtccagtca gagcctttta tattctagaa atcaaaagaa ctacttggcc 120

tggttccagc agaaaccagg acagccaccc aaactcctca tcttttgggc tagcactagg 180

gaatctgggg tacctgatag gttcagtggc agtgggtttg ggacagactt caccctcacc 240

attagcagcc tgcaggctga agatgtggca gtttatgact gtcaacaata ttttagctat 300

ccgctcacgt tcggacaagg gaccaaggtg gaaataaaa 339

<210> SEQ ID NO 103
<211> LENGTH: 339
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 103

gacattgtga tgacccaatc tccagactct ttggctgtgt ctctagggga gagggccacc 60

atcaactgca agtccagtca gagcctttta tattctagaa atcaaaagaa ctacttggcc 120

tggtatcagc agaaaccagg acagccaccc aaactcctca tctattgggc tagcactagg 180

gaatctgggg tacctgatag gttcagtggc agtgggtttg ggacagactt caccctcacc 240

attagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttttagctat 300

ccgctcacgt tcggacaagg gaccaaggtg gaaataaaa 339

<210> SEQ ID NO 104
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 104

caggtgcaac tagtgcagtc cggcgccgaa gtgaagaaac ccggtgcttc cgtgaaagtc 60

agctgtaaaa ctagtagata caccttcact gaatacacca tacactgggt tagacaggcc 120

cctggccaaa ggctggagtg gataggaggt attaatccta acaatggtat tcctaactac 180

aaccagaagt tcaagggccg ggccaccttg accgtaggca agtctgccag caccgcctac 240

atggaactgt ccagcctgcg ctccgaggac actgcagtct acttctgcgc cagaagaaga 300

atcgcctatg gttacgacga gggccatgct atggactact ggggtcaagg aacccttgtc 360

accgtctcct ca 372

<210> SEQ ID NO 105
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 105

caggtgcaac tagtgcagtc cggcgccgaa gtgaagaaac ccggtgcttc cgtgaaagtc 60

agctgtaaaa ctagtagata caccttcact gaatacacca tacactgggt tagacaggcc 120

cctggccaaa ggctggagtg gataggaggt attaatccta acaatggtat tcctaactac 180

aaccagaagt tcaagggccg ggtcaccatc accgtagaca cctctgccag caccgcctac 240

atggaactgt ccagcctgcg ctccgaggac actgcagtct actactgcgc cagaagaaga 300

atcgcctatg gttacgacga gggccatgct atggactact ggggtcaagg aacccttgtc 360

accgtctcct ca 372

<210> SEQ ID NO 106

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<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 106

caggtgcaac tagtgcagtc cggcgccgaa gtgaagaaac ccggtgcttc cgtgaaagtc 60

agctgtaaaa ctagtagata caccttcact gaatacacca tacactgggt tagacaggcc 120

cctggccaaa ggctggagtg gataggaggt attaatccta acaatggtat tcctaactac 180

aaccagaagt tcaagggccg ggtcaccatc accgtagaca cctctgccag caccgcctac 240

atggaactgt ccagcctgcg ctccgaggac actgcagtct acttctgcgc cagaagaaga 300

atcgcctatg gttacgacga gggccatgct atggactact ggggtcaagg aacccttgtc 360

accgtctcct ca 372

<210> SEQ ID NO 107
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 107

caggtgcaac tagtgcagtc cggcgccgaa gtgaagaaac ccggtgcttc cgtgaaagtc 60

agctgtaaaa ctagtggata caccttcact gaatacacca tacactgggt tagacaggcc 120

cctggccaaa ggctggagtg gataggaggt attaatccta acaatggtat tcctaactac 180

aaccagaagt tcaagggccg ggtcaccatc accgtagaca cctctgccag caccgcctac 240

atggaactgt ccagcctgcg ctccgaggac actgcagtct actactgcgc cagaagaaga 300

atcgcctatg gttacgacga gggccatgct atggactact ggggtcaagg aacccttgtc 360

accgtctcct ca 372

<210> SEQ ID NO 108
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 108

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Thr Ser Arg Tyr Thr Phe Thr Glu Tyr
20 25 30

Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile
35 40 45

Gly Gly Ile Asn Pro Asn Asn Gly Ile Pro Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Arg Arg Arg Ile Ala Tyr Gly Tyr Asp Glu Gly His Ala Met Asp
100 105 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

What is claimed is:

1. An antibody protein having the complementary determining regions of the monoclonal antibody F19 (ATCC Accession No. HB 8269), said antibody protein specifically binding to fibroblast activation protein, characterised in that it has framework modifications resulting in the improved producibility in host cells as compared to a chimeric antibody having the variable regions of F19 and foreign constant regions.

2. An antibody protein characterized in that it has a variable light chain region and a variable heavy chain region according to claim 1, each joined to a human constant region.

3. The antibody protein of claim 2, wherein said human constant region of the light chain is a human kappa constant region.

4. The antibody protein of claim 2, wherein said human constant region of the heavy chain is a human gamma-1 constant region.

5. An antibody protein according to any one of claims 1 to 4, characterised in that its expression level in crude media samples as determined by ELISA and/or purified antibody yield exceeds the expression levels and/or purification yield of the chimeric antibody without framework modifications by at least a factor of 10.

6. An antibody protein according to any one of claims 1 to 4, characterised in that its expression levels in crude media samples as determined by ELISA and/or purified antibody yield exceeds the expression levels and/or purification yield of the chimeric antibody without framework modifications by at least a factor of 20.

7. An antibody protein according to any one of claims 1 to 4, characterised in that its expression level in crude media samples as determined by ELISA and/or purified antibody yield exceeds the expression levels and/or purification yield of the chimeric antibody without framework modifications by at least a factor of 100.

8. An antibody protein according to any one of claims 1 to 7, characterised in that it displays improved producibility in eukaryotic cells.

9. The antibody protein according to claim 8 wherein said eukaryotic cell is a Chinese hamster ovary cell (CHO cell).

10. An antibody protein according to any one of claims 1 to 9, wherein the amino acid in Kabat position 87 of the light chain region is not asparagine.

11. The antibody protein of claim 10, wherein the amino acid in Kabat position 87 of the light chain region is selected from aromatic or aliphatic amino acids.

12. The antibody protein of claim 11, wherein said aromatic amino acid in Kabat position 87 of the light chain region is a tyrosine or phenylalanine.

13. The antibody protein according to any one of claims 1 to 12, wherein the amino acid in Kabat position 36 of the light chain region is selected from aromatic amino acids.

14. An antibody protein according to any one of claims 1 to 13 that contains the variable region of the light chain as set forth in SEQ ID NO:2.

15. An antibody protein of claim 14 characterized in that the variable region of the light chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:1.

16. An antibody protein according to any one of claims 1 to 13 that contains the variable region of the light chain as set forth in SEQ ID NO:6.

17. An antibody protein of claim 16 characterized in that the variable region of the light chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:5.

18. An antibody protein according to any one of claims 1 to 17 containing a variable region of the heavy chain as set forth in any one of SEQ ID NOs: 8, 10, 12, 14.

19. An antibody protein according to claim 18 characterised in that the variable region of the heavy chain is encoded by a nucleotide sequence as set forth in SEQ ID NOs:7, 9, 11, 13.

20. An antibody protein according to any one of claims 1 to 14 containing the variable region of the light chain as set forth in SEQ ID NO:2 and the variable region of the heavy chain as set forth in SEQ ID NOs:12.

21. The antibody protein of claim 20 characterised in that the variable region of the light chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:1 and the variable region of the heavy chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:11.

22. An antibody protein according to claim 20 or claim 21 containing the constant region of the light chain as set forth in SEQ ID NO:20 and the constant region of the heavy chain as set forth in SEQ ID NO:22.

23. An antibody protein according to any one of claims 1 to 13, **18** or **19** containing the variable region of the light chain as set forth in SEQ ID NO:2 and the variable region of the heavy chain as set forth in SEQ ID NOs:8.

24. The antibody protein of claim 23 characterised in that the variable region of the light chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:1 and the variable region of the heavy chain is encoded by a nucleotide sequence as set forth in SEQ ID NO:7.

25. A nucleotide sequence encoding an antibody protein according to any one of claims 1 to 24.

26. A recombinant DNA vector that contains a nucleotide sequence of claim 25.

27. The recombinant DNA vector of claim 26, said vector being an expression vector.

28. A host cell carrying a vector according to claims **26** or **27**.

29. The host cell of claim 28, wherein said host cell is a eukaryotic cell.

30. The host cell of claim 29, wherein said eukaryotic host cell is a mammalian cell.

31. The host cell of claim 30, wherein said eukaryotic host cell is a CHO or a COS cell.

32. A method of producing antibody proteins according to any one of claims 1 to 24, said method comprising the steps of:

(a) cultivating a host cell according to any one of claims 28 to 31 under conditions where said antibody protein is expressed by said host cell, and

(b) isolating said antibody protein.

33. The method of claim 32, wherein said host cell is a mammalian cell, preferably a CHO or COS cell.

34. The method of claim 32 or **33**, wherein said host cell is cotransfected with two plasmids carrying the expression units for light and heavy chains respectively.

35. An antibody protein according to any one of claims 1 to 24, wherein said antibody protein is conjugated to a therapeutic agent.

36. The antibody protein of claim 35, wherein said therapeutic agent is a therapeutic agent selected from the

group consisting of radioisotopes, toxins, toxoids, inflammatory agents and chemotherapeutic agents.

37. The antibody protein of claim 36, wherein said radioisotope is a β-emitting radioisotope.

38. The antibody protein of claim 37, wherein said radioisotope is selected from the group consisting of ^{186}Rhenium, ^{188}Rhenium, ^{131}Iodine and ^{90}Yttrium.

39. An antibody protein according to any one of claims 1 to 24, characterised in that it is labelled.

40. The antibody protein of claim 39, wherein said label is a detectable marker.

41. The antibody protein of claim 40, wherein the detectable marker is a detectable marker selected from the group consisting of enzymes, dyes, radioisotopes, digoxygenin, and biotin.

42. An antibody protein according to any one of claims 1 to 24 conjugated to an imageable agent.

43. The antibody protein of claim 42, wherein the imageable agent is a radioisotope.

44. The antibody protein of claim 43, wherein said radioisotope is a γ-emitting radioisotopes.

45. The antibody protein of claim 44, wherein said radioisotope is ^{125}I.

46. A pharmaceutical composition containing an antibody protein according to any one of claims 1 to 24 and a pharmaceutically acceptable carrier.

47. A pharmaceutical composition containing an antibody protein according to any one of claims 35 to 38 and a pharmaceutically acceptable carrier.

48. A pharmaceutical composition containing an antibody protein according to any one of claims 42 to 45 and a pharmaceutically acceptable carrier.

49. The pharmaceutical composition of claims 46 to 48, for use in the treatment or imaging of tumors, wherein said tumors are associated with activated stromal fibroblasts, preferably wherein said tumors are tumors selected from the cancer group consisting of colorectal cancers, non-small cell lung cancers, breast cancers, head and neck cancer, ovarian cancers, lung cancers, bladder cancers, pancreatic cancers and metastatic cancers of the brain.

50. Use of an antibody protein according to anyone of claims 1 to 24 for the treatment of cancer.

51. Use of an antibody protein according to anyone of claims 35 to 38 for the treatment of cancer.

52. Use of an antibody protein according to anyone of claims 42 to 45 imaging activated stromal fibroblasts.

53. Use of an antibody protein according to anyone of claims 39 to 41 for detecting the presence of activated stromal fibroblasts in a sample.

54. A method of treating tumors, wherein the tumor is associated with activated stromal fibroblasts capable of specifically forming a complex with antibody proteins according to any one of claims 1 to 24 or **35** to **38**, which comprises contacting the tumor with an amount of said antibody proteins effective to treat the tumor.

55. The method of claim 54, wherein the tumor is a tumor having cancer cells selected from the cancer group consisting of colorectal cancers, non-small cell lung cancers, breast cancers, head and neck cancer, ovarian cancers, lung cancers, bladder cancers, pancreatic cancers and metastatic cancers of the brain.

56. The method of claim 54, wherein the contacting is effected in vitro.

57. The method of claim 54, wherein the contacting is effected in vivo.

58. A method of detecting the presence of activated stromal fibroblasts in wound healing, inflammation or a tumor, characterised in that

(a) a sample, possibly containing activated stromal fibroblasts, is contacted with an antibody protein according to any one of claims 1 to 24 or **39** to **41** under conditions suitable for the formation of a complex between said antibody and antigen,

(b) detecting the presence of said complex, thereby detecting the presence of activated stromal fibroblasts in wound healing, inflammation or a tumor.

59. The method of claim 58, wherein the tumor is a tumor having cancer cells selected from the cancer group consisting of colorectal cancers, non-small cell lung cancers, breast cancers, head and neck cancer, ovarian cancers, lung cancers, bladder cancers, pancreatic cancers and metastatic cancers of the brain.

60. The method of claim 58 or **59**, wherein the antibody protein is a protein according to any one of claims 39 to 41.

61. A method of imaging the presence of activated stromal fibroblasts in a healing wound, inflamed skin or a tumor, in a human patient, characterised in that

(a) an antibody protein according to any one of claims 1 to 24 conjugated to an imageable agent is administered to a human patient under conditions suitable for the formation of an antibody-antigen complex,

(b) imaging any complex formed in this manner.

62. The method of claim 61, wherein the tumor is a tumor having cancer cells selected from the cancer group consisting of colorectal cancers, non-small cell lung cancers, breast cancers, head and neck cancer, ovarian cancers, lung cancers, bladder cancers, pancreatic cancers and metastatic cancers of the brain.

63. A method of detecting tumor-stroma, characterised in that

(a) a suitable sample is contacted with an antibody protein according to any one of claims 1 to 24, under conditions suitable for the formation of an antibody-antigen complex,

(b) detecting the presence of any complex so formed,

(c) relating the presence of said complex to the presence of tumor-stroma.

64. The method of claim 62, wherein said antibody is labelled with a detectable marker.

65. A method of imaging tumor-stroma in a human patient, which comprises

(a) administering to the patient an antibody protein according to any one of claims 42 to 45, under conditions suitable for the formation of an antibody-antigen complex,

(b) imaging any complex so formed, and thereby imaging the presence of tumor-stroma in a human patient.

66. An antibody protein containing an amino acid sequence as set forth in SEQ ID NO:2.

67. An antibody protein according to claim 66 further containing an amino acid sequence as set forth in SEQ ID NO:12.

68. An antibody protein according to claim 66 or **67** further containing an amino acid sequence as set forth in SEQ ID NO:20 and an amino acid sequence as set forth in SEQ ID NO:22.

69. A DNA molecule coding for an antibody protein according to any one of claims 66 to 68.

70. A host cell carrying a DNA molecule according to claim 69.

71. A method of producing an antibody protein of any one of claims 66 to 68, said method comprising the steps of

(a) cultivating the host cell of claim 70 under conditions where said antibody protein is expressed by said host cell, and

(b) isolating said protein.

72. An antibody protein according to any one of claims 66 to 68 which is conjugated to a radioisotope, preferably ^{131}I, ^{125}I, ^{186}Re, ^{188}Re, or ^{90}Y.

73. A pharmaceutical composition comprising an antibody protein according to any one of claims 66 to 68, or **72**, and a pharmaceutically acceptable carrier.

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