FIGURE 1

Figure 1A

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIK
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT
EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 1B

```
gacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcacc
 D  I  Q  M  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V  T
atcacttgccgggcaagtcagagcattagcagctacttaaattggtatcagcagaaacca
 I  T  C  R  A  S  Q  S  I  S  S  Y  L  N  W  Y  Q  Q  K  P
gggaaagcccctaagctcctgatctatgctgcatccagtttgcaaagtggggtcccatca
 G  K  A  P  K  L  L  I  Y  A  A  S  S  L  Q  S  G  V  P  S
aggttcagtggcagtggatctgggacagatttcactctcaccatcagcagtctgcaacct
 R  F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  P
gaagattttgcaacttactactgtcaacagagttacagtacccctccaacgttcggccaa
 E  D  F  A  T  Y  Y  C  Q  Q  S  Y  S  T  P  P  T  F  G  Q
gggaccaaggtggagatcaaa
 G  T  K  V  E  I  K
```

Figure 1C

```
cgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatct
 R  T  V  A  A  P  S  V  F  I  F  P  P  S  D  E  Q  L  K  S
ggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacag
 G  T  A  S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q
tggaaggtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggac
 W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D
agcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaagcagactacgag
 S  K  D  S  T  Y  S  L  S  S  T  L  T  L  S  K  A  D  Y  E
aaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaag
 K  H  K  V  Y  A  C  E  V  T  H  Q  G  L  S  S  P  V  T  K
agcttcaacaggggagagtgttag
 S  F  N  R  G  E  C  -
```

Figure 1D

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK

Figure 1E

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQSYSTP

(underlining shows CDRs)

FIGURE 2

IgG heavy chains for the generation of bispecific molecules

Figure 2A

VH: dependent on the MF (target): Figure 3.

Figure 2B

CH1 Region:

```
gctagcaccaagggcccatcggtcttccccctggcaccctcctccaagagcacctctggg
 A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K  S  T  S  G
ggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcg
 G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S
tggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctacagtcctca
 W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
ggactctactccctcagcagcgtcgtgaccgtgccctccagcagcttgggcacccagacc
 G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T
tacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagagagtt
 Y  I  C  N  V  N  H  K  P  S  N  T  K  V  D  K  R  V
```

Figure 2C

Hinge Region:

```
gagcccaaatcttgtgacaaaactcacacatgcccaccgtgccca
 E  P  K  S  C  D  K  T  H  T  C  P  P  C  P
```

Figure 2D

CH2 Region:

```
gcacctgaactcctgggggaccgtcagtcttcctcttccccccaaaacccaaggacacc
 A  P  E  L  L  G  G  P  S  V  F  L  F  P  P  K  P  K  D  T
ctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagac
 L  M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H  E  D
cctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
 P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K  T  K
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcac
 P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L  H
caggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcc
 Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A
cccatcgagaaaaccatctccaaagccaaa
 P  I  E  K  T  I  S  K  A  K
```

Figure 2E

CH2 region containing L235G and G236R silencing substitutions:

```
gcacctgaactcggcaggggaccgtcagtcttcctcttccccccaaaacccaaggacacc
 A  P  E  L  G  R  G  P  S  V  F  L  F  P  P  K  P  K  D  T
ctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagac
 L  M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H  E  D
cctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
 P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K  T  K
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcac
 P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L  H
caggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcc
 Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A
cccatcgagaaaaccatctccaaagccaaa
 P  I  E  K  T  I  S  K  A  K
```

Figure 2F

CH3 domain containing substitutions L351K and T366K (KK)

```
gggcagccccgagaaccacaggtgtacaccaagcccccatcccgggaggagatgaccaag
 G  Q  P  R  E  P  Q  V  Y  T  K  P  P  S  R  E  E  M  T  K
aaccaggtcagcctgaagtgcctggtcaaaggcttctatcccagcgacatcgccgtggag
 N  Q  V  S  L  K  C  L  V  K  G  F  Y  P  S  D  I  A  V  E
tgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactcc
 W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S
gacggctccttcttcctctatagcaagctcaccgtggacaagagcaggtggcagcagggg
 D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G
aacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc
 N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S
ctctccctgtctccgggttga
 L  S  L  S  P  G  -
```

Figure 2G

CH3 domain containing substitutions L351D and L368E (DE)

```
gggcagccccgagaaccacaggtgtacaccgacccccatcccgggaggagatgaccaag
 G  Q  P  R  E  P  Q  V  Y  T  D  P  P  S  R  E  E  M  T  K
aaccaggtcagcctgacctgcgaggtcaaaggcttctatcccagcgacatcgccgtggag
 N  Q  V  S  L  T  C  E  V  K  G  F  Y  P  S  D  I  A  V  E
tgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactcc
 W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S
gacggctccttcttcctctatagcaagctcaccgtggacaagagcaggtggcagcagggg
 D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G
aacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc
 N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S
ctctccctgtctccgggttga
 L  S  L  S  P  G  -
```

FIGURE 3

Amino acid sequences of heavy chain variable regions:

Figure 3A: Heavy chain variable regions of PD-1 specific clones

MF6076

QVQLQESGPGLVKPSETLSLTCTVSTDSLGFYFWSWIRQPPGKGLEWIGYIYYSGSAN
FNPSLKSRVTMSIDTSNNQFSLKLRSVTAADTAVYFCARGGYTGYGGDWFDPWGQG
TLVTVSS

MF6226

QVQLQESGPGLVKPSETLSLTCTVSGDSIGYYFWSWIRQPPGKGLEWIGYVYYSGSNN
LNPSLKSRVTLSVDTSKNQFSLRLNSMTAADTAVYYCARGGYSGYGGDSFDLWGQGT
TVTVSS

MF6236

QVQLQESGPGLVKPSETLSLTCTVSGGSIGYYFWSWIRQPPGKGLEWIGYIYYSGSTN
FNPSLKSRVTMSVDTSKNQFSLNLRSVTTADTAVYYCARGGYTGHGGDWFDPWGQG
TLVTVSS

MF6256

EVQLVQSGAEVKKPGSSMKVSCKASGGTFSSYVISWVRQAPGQGLEWMGMIIPVFDT
SSYEKKFQGRITIIADKSTSTVYLELSSLRSEDAAVYYCARGTVEATLLFDFWGQGTLV
TVSS

MF6930

EVQLVQSGAEVKKPGSSMKVSCKASGGTFSNYVISWVRQAPGQGLEWMGMIIPVFET
ATYEKKFQGRVTIIADKSTSTVYMELSSLRSEDAAVYYCARGTVEATLLFDYWGQGTL
VTVSS

MF6932

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGMIIPFFDT
ANYAQKFQGRVTITADKSTSTASMELRSLRSEDTAVYYCARGTVSATLVFDYWGQGT
LVTVSS

MF6935

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYVISWVRQAPGQGLEWMGMIIPIFDT
ANYAQKFQGRVTITADKSTSTASMELRSLRSEDTAVYYCARGTVSGTLVFDYWGQGT
LVTVSS

MF6936

EVQLVQSGAEVKKPGSSVKVSCKASGDTFSNYVINWVRQAPGQGLEWMGMIIPVFDT
TSYERKFQGRVTITADKSTSTAYMELTSLRSEDTAVYYCARGTVGATLLFDNWGQGT
LVTVSS

MF6972

EVQLQESGPGLVKPSETLSLTCTVSGGSISTYFWSWIRQPPGKGLEWIGYIIYSGSTNY
NPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGGYSGYGGDDFDIWGQGTM
VTVSS

MF6974

EVQLVESGGDLVQPGGSLRLSCAASGFTFNSYAMSWVRQAPGKGLEWVSTISGGGA
NIYYADSVKGRFTISRDNSKSTLYLQMNSLRAEDTAVYFCASPYGSGYFDVWGQGTL
VTVSS

MF6982

EVQLVESGGDLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSTISGGGTN
IYYADSVKGRFTISRDNSKNTLFLQMNSLRAGDTAVYYCASPYGSGYLDVWGQGTLV
TVSS

Figure 3B: Heavy chain variable regions of TIM-3 specific clones

MF7676

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSKVGVGWIRQPPGKALEWLALIYWNDD
KRYSPSLKSRLTITKDTSKNQVVLTMTNMDPVDTATYYCAHRRGWGPFDYWGQGTL
VTVSS

MF7677

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALEWLAFIYSNDD
KRYSPSLKSRLTITKDTSKNQVVLTMTNMDPVDTATYYCTHRRGWGPFDYWGQGTL
VTVSS

MF7678

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALEWLVFIYSNDD
KRYSPSLKSRLTITKDTSKNQVVLTMTNMDPVDTATYYCAHRRGWGPFDYWGQGTL
VTVSS

MF7679

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALEWLALIYWNDD
KRYSPSLKSRLTITKDTSKNQVVLTMTNMDPVDTATYYCAHRRGWGPFDYWGQGTL
VTVSS

(underlining shows CDRs)

FIGURE 4

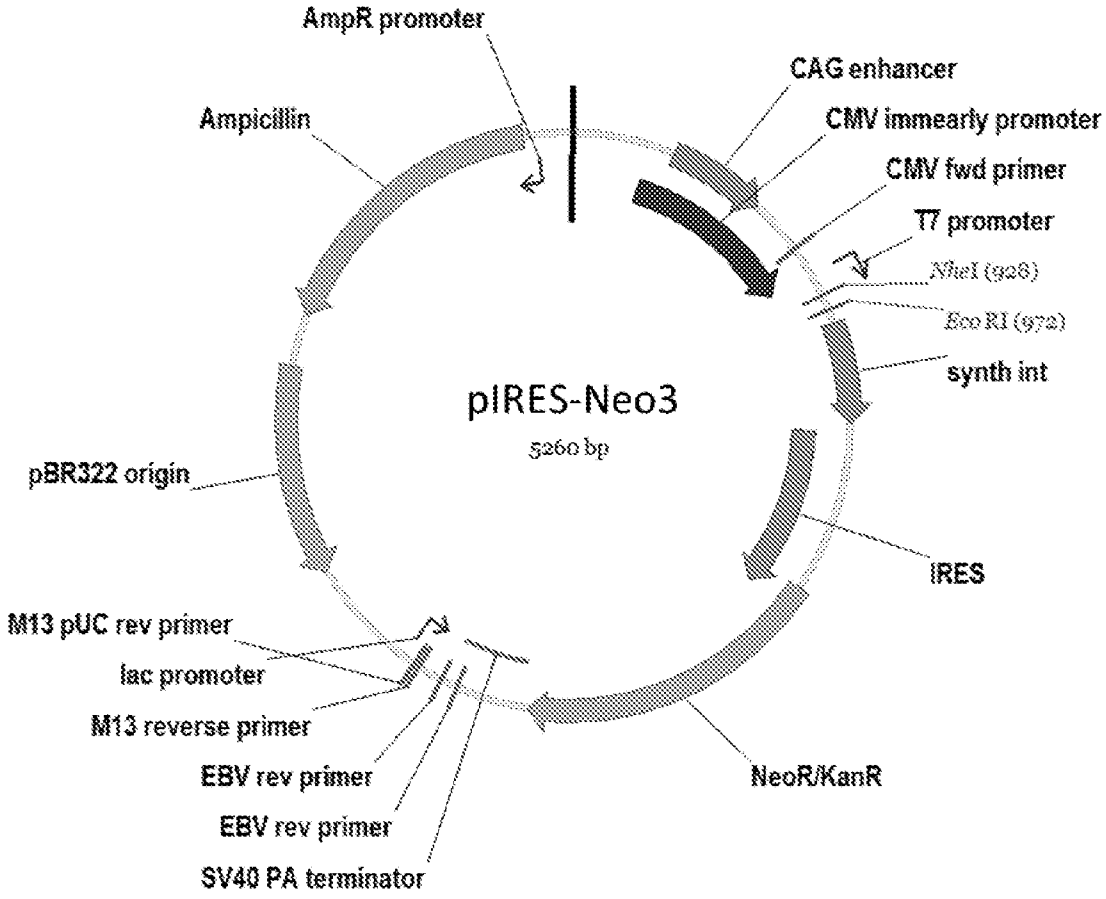

AmpR promoter
CAG enhancer
Ampicillin
CMV immearly promoter
CMV fwd primer
T7 promoter
NheI (926)
Eco RI (972)
synth int
pIRES-Neo3
5260 bp
pBR322 origin
IRES
M13 pUC rev primer
lac promoter
M13 reverse primer
EBV rev primer
EBV rev primer
SV40 PA terminator
NeoR/KanR

FIGURE 5

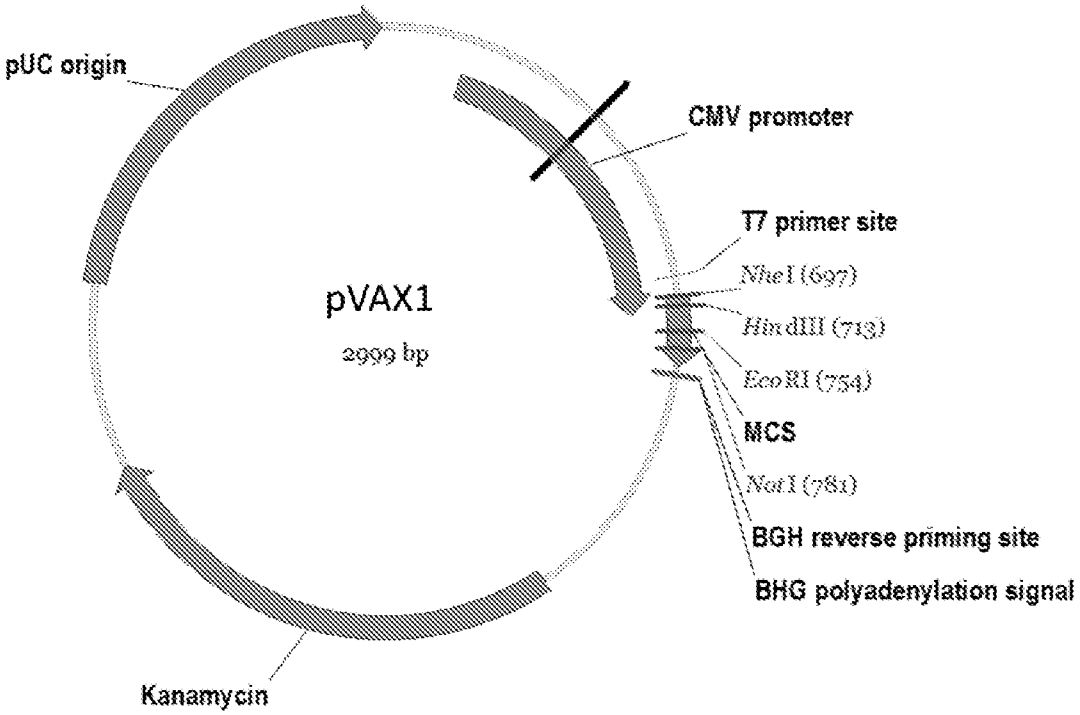

pUC origin
CMV promoter
T7 primer site
NheI (697)
HindIII (713)
EcoRI (754)
MCS
NotI (781)
BGH reverse priming site
BHG polyadenylation signal
pVAX1
2999 bp
Kanamycin

FIGURE 6

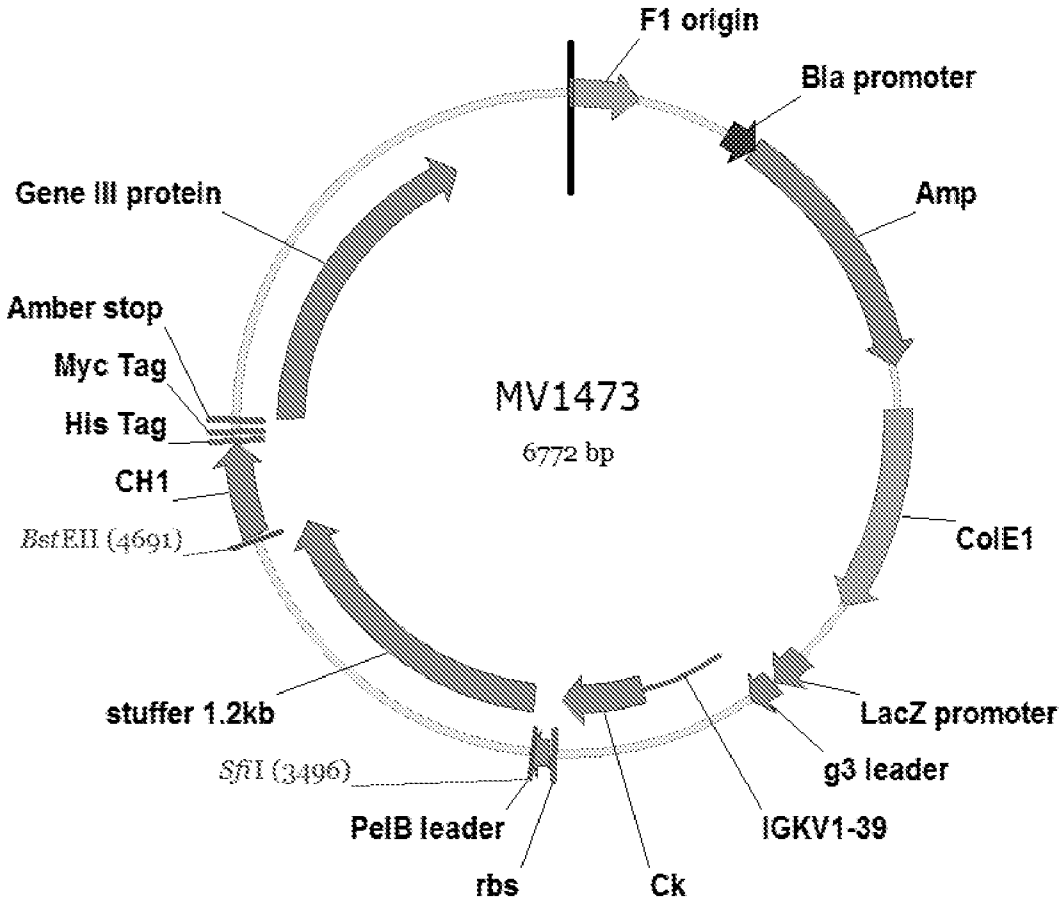

F1 origin
Bla promoter
Amp
ColE1
LacZ promoter
g3 leader
IGKV1-39
Ck
rbs
PelB leader
SfiI (3496)
stuffer 1.2kb
BstEII (4691)
CH1
His Tag
Myc Tag
Amber stop
Gene III protein
MV1473
6772 bp

FIGURE 7

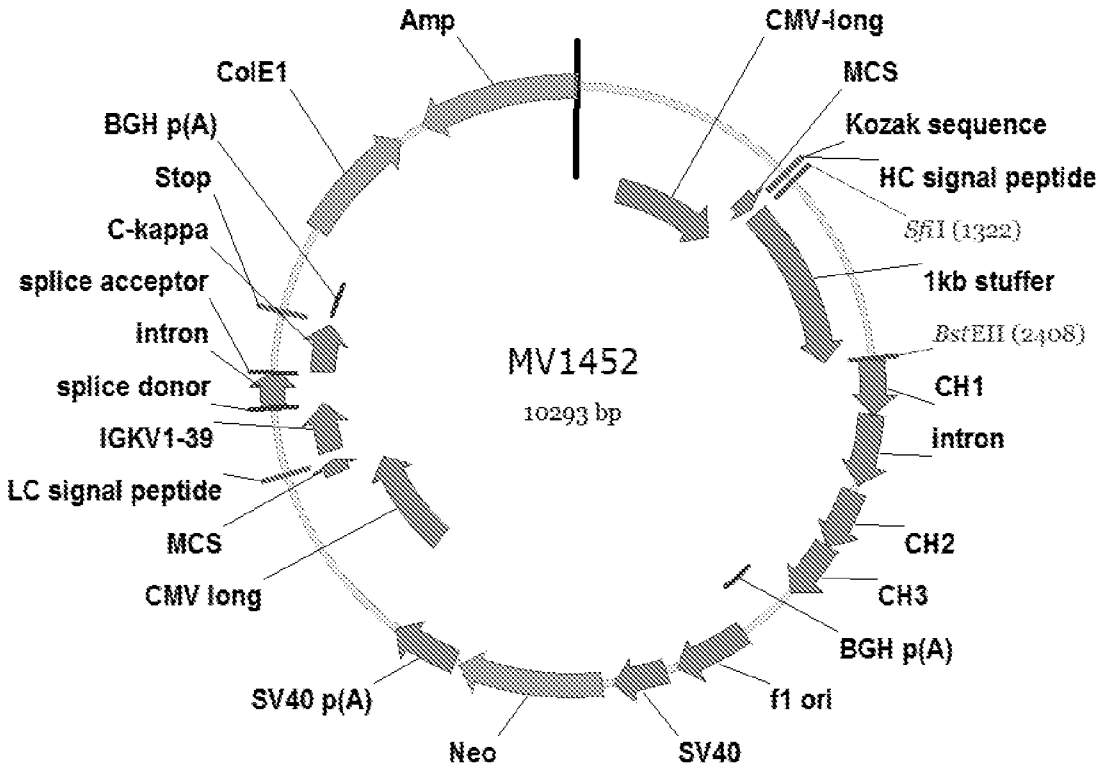

Amp
CMV-long
ColE1
MCS
BGH p(A)
Kozak sequence
Stop
HC signal peptide
C-kappa
SfiI (1322)
splice acceptor
1kb stuffer
intron
BstEII (2408)
MV1452
10293 bp
splice donor
CH1
IGKV1-39
intron
LC signal peptide
CH2
MCS
CH3
CMV long
BGH p(A)
SV40 p(A)
f1 ori
Neo
SV40

FIGURE 8

EVQLVETGAEVKKPGASVKVSCKASDYIFTKYDINWVRQAPGQGLEWMGWMSANTGNTGYAQKFQGRVTMTRDTS
INTAYMELSSLTSGDTAVYFCARSSLFKTETAPYYHFALDVWGQGTTVTVSS

FIGURE 9

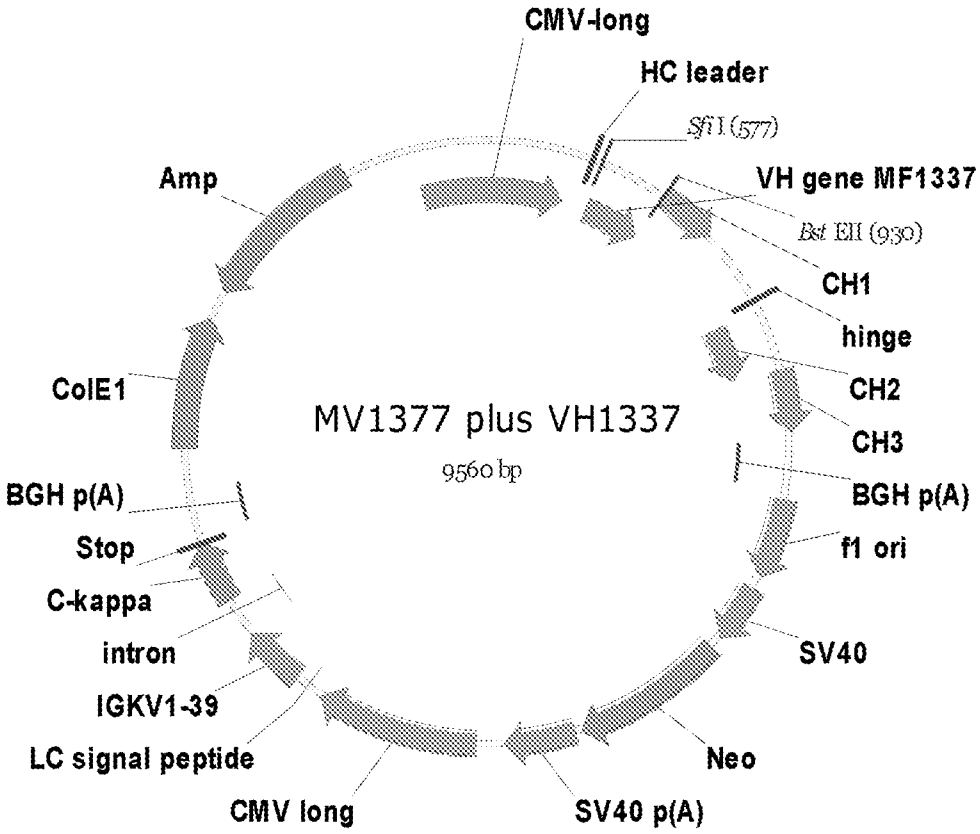

CMV-long
HC leader
Sfi I (577)
VH gene MF1337
Bst EII (930)
CH1
hinge
CH2
CH3
BGH p(A)
f1 ori
SV40
Neo
SV40 p(A)
CMV long
LC signal peptide
IGKV1-39
intron
C-kappa
Stop
BGH p(A)
ColE1
Amp
MV1377 plus VH1337
9560 bp

FIGURE 10

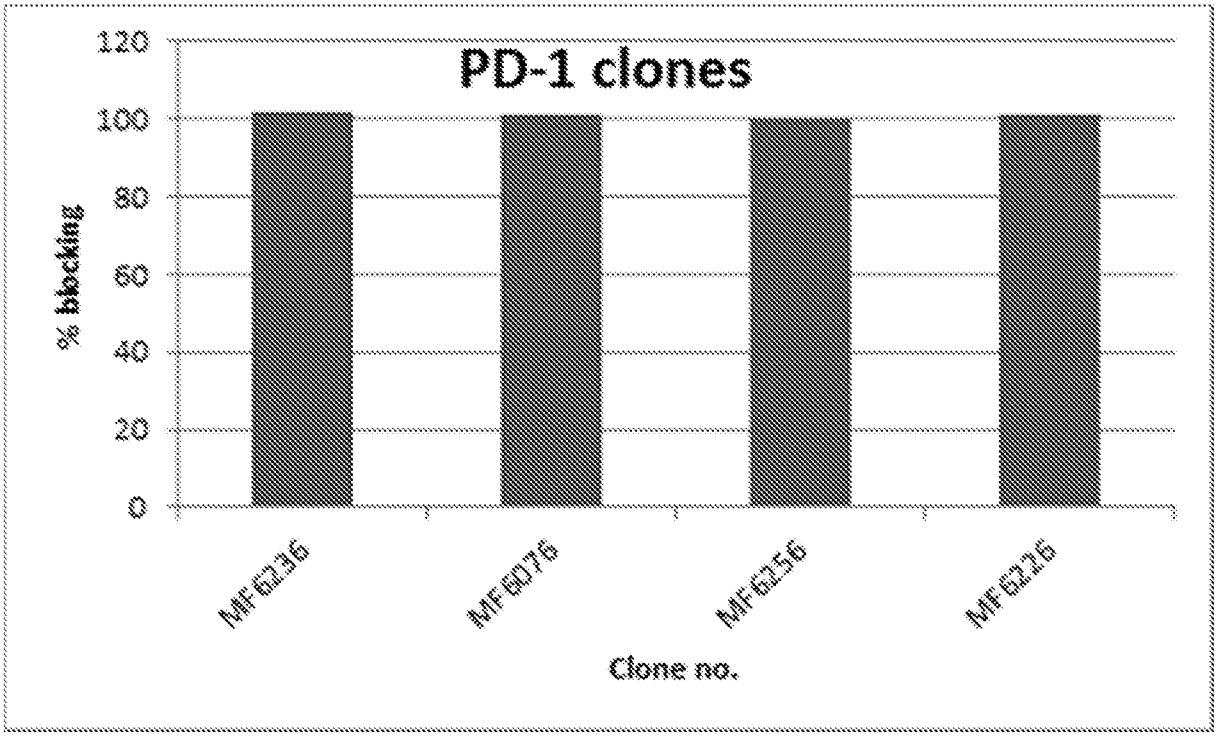
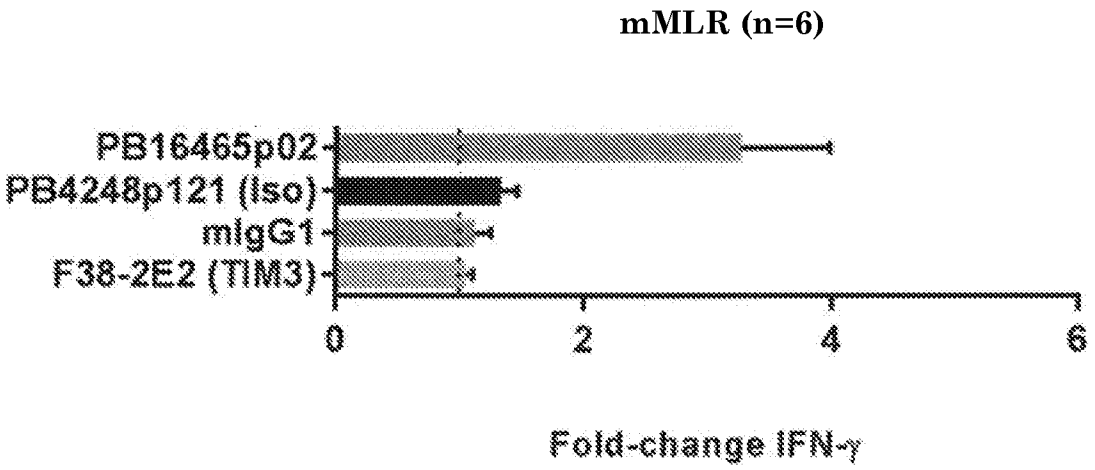

PD-1 clones
% blocking
120
100
80
60
40
20
0
MF6236
MF6076
MF6256
MF6226
Clone no.

FIGURE 11


mMLR (n=6)
PB16465p02
PB4248p121 (Iso)
mIgG1
F38-2E2 (TIM3)
0
2
4
6
Fold-change IFN-γ