1 10 20 30 40 50 60 70 80 90 100

SfiI (70) NcoI (74)

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
AACCCCTAGCTAGGCGCGCCACCATGAAACATCTGTGGTTCTTCCTTCTCCTGGTGGCAGCGGCCCAGCCGGCCATGGCTCAGGTGCAGCTGGTGCAATCTGGAGCTG
P L A R R A T M K H L W F F L L L V A A A Q P A M A Q V Q L V Q S G A

Leader PS11 SfiI

110 120 130 140 150 160 170 180 190 200 210

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
AGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTG
E V K K P G A S V K V S C K A S G Y T F T S Y G I S W V R Q A P G Q G L

PS11

220 230 240 250 260 270 280 290 300 310 320

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
AGTGGATGGGATGGATCAGCGCTTACAATGATAACACACACTATGCACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGG
E W M G W I S A Y N D N T H Y A Q K L Q G R V T M T T D T S T S T A Y M

PS11

330 340 350 360 370 380 390 400 410 420 430

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
AGCTGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGACCCAGGCCGCTATGGACGTCTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCAGGTGGCG
E L R S L R S D D T A V Y Y C A T Q A A M D V W G Q G T L V T V S S G G

PS11

440 450 460 470 480 490 500 510 520 530 540

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
GCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCTCCTATGAGCTGACTCAGCCACCGTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCTCTTGTTCTG
G G S G G G G S G G G G S S Y E L T Q P P S A S G T P G Q R V T I S C S

PS11

550 560 570 580 590 600 610 620 630 640

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
GAAGCAGGTCCAACATCGGAGGCAATACTGTAAACTGGTACCAGCAACTCCCAGGAACGGCCCCCAAACTCCTCATCTATGCTAACGATCAGCGGCCCTCAGGGGTCC
G S R S N I G G N T V N W Y Q Q L P G T A P K L L I Y A N D Q R P S G V

PS11

650 660 670 680 690 700 710 720 730 740 750

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
CTGACCGAGTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTGAGGATGAGGCTCATTATTACTGTGCAGCATGGGATGACAGGC
P D R V S G S K S G T S A S L A I S G L Q S E D E A H Y Y C A A W D D R

PS11

760 770 780 790 800 810 820 830 840 850 860

NotI (827)

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
TGAATGGTGTGGTTTTCGGCGGAGGGACCAAACTAACCGTCCTAGGTCAGCCCAAGGCCGCCCCCTCGGCCGGCCGCAGAGCCCAAATCTTGTGACAAAACTCACACAT
L N G V V F G G G T K L T V L G Q P K A A P S A A A E P K S C D K T H T

PS11 Fc

870 880 890 900 910 920 930 940 950 960 970

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
GCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCG
C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C

Fc

980 990 1,000 1,010 1,020 1,030 1,040 1,050 1,060 1,070 1,080

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
TGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACA
V V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N

Fc

1,090 1,100 1,110 1,120 1,130 1,140 1,150 1,160 1,170 1,180

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
GCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGA
S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E
Fc

1,190 1,200 1,210 1,220 1,230 1,240 1,250 1,260 1,270 1,280 1,290

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
AAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCA
K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V
Fc

1,300 1,310 1,320 1,330 1,340 1,350 1,360 1,370 1,380 1,390 1,400

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC
K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F
Fc

1,410 1,420 1,430 1,440 1,450 1,460 1,470 1,480 1,490 1,500 1,510

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCT
L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L
Fc

1,520 1,530 1,540 1,550 1,560 1,570 1,580 1,590 1,600 1,610 1,620

XbaI (1,543)

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
CCCTGTCTCCGGGTAAATCCGGCGGCTCCTCTAGAGGAGGCACCGAGACCAGCCAGGTGGCGCCCGCCATTGAAGTTATGTATCCTCCTCCTTACCTAGACAATGAGA
S L S P G K S G G S S R G G T E T S Q V A P A I E V M Y P P P Y L D N E
Fc
C9 Tag
CD28

1,630 1,640 1,650 1,660 1,670 1,680 1,690 1,700 1,710 1,720

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
AGAGCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTGTCCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTTGGTGGAGTCC
K S N G T I I H V K G K H L C P S P L F P G P S K P F W V L V V V G G V
CD28

1,730 1,740 1,750 1,760 1,770 1,780 1,790 1,800 1,810 1,820 1,830

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
TGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAATAGAGTTAGGCAGGGAT
L A C Y S L L V T V A F I I F W V R S K R S R L L H S D Y M N R V R Q G
gp41(713-720)
CD28

1,840 1,850 1,861

PacI (1,850)

pcDNA-CMV-PS11FcCD28gp41(70...
Frame 3
ATTCATAGTTAATTAATCGCGAAAG
Y S * L I N R E
PacI
gp41(713-720)