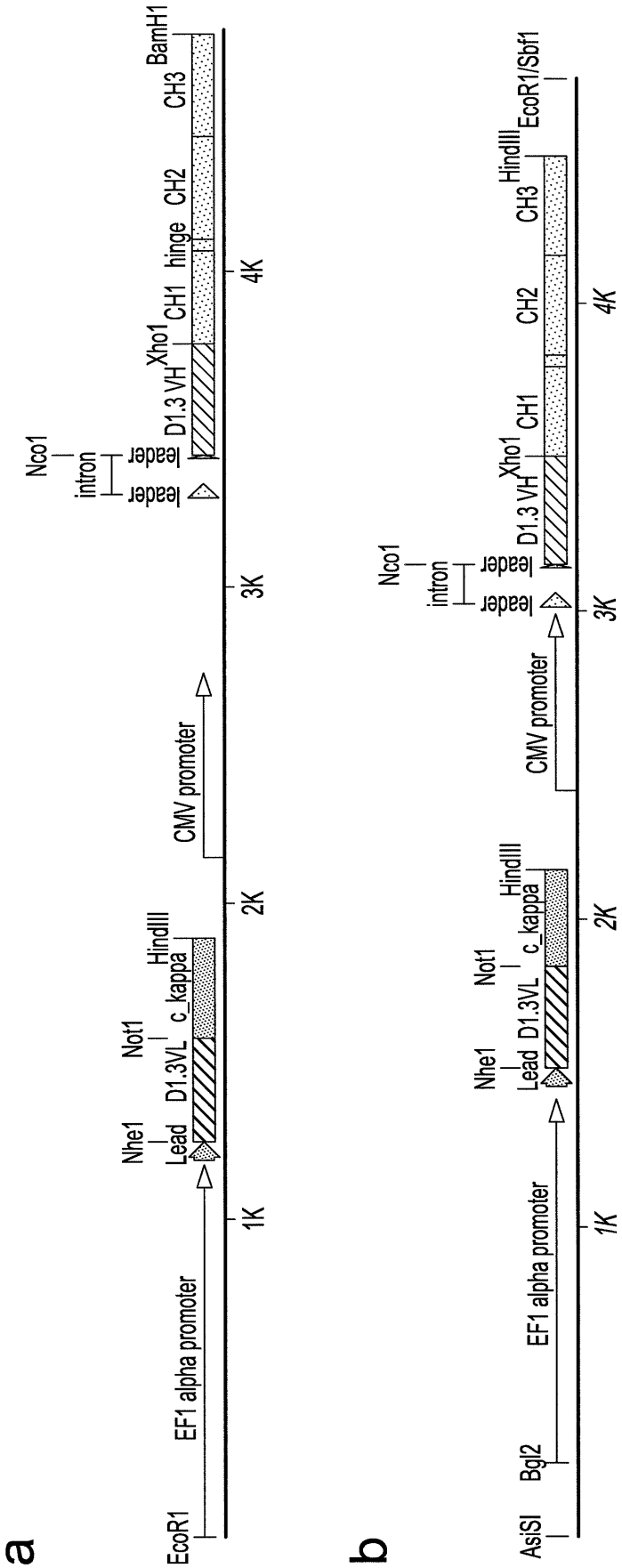

a
EcoR1
EF1 alpha promoter
Nhe1
Lead
D1.3VL
Not1
c_kappa
HindIII
1K
2K
CMV promoter
3K
Nco1
intron
leader
leader
D1.3 VH
Xho1
CH1
hinge
CH2
CH3
BamH1
4K
b
AsiSI
Bgl2
EF1 alpha promoter
Nhe1
Lead
D1.3VL
Not1
c_kappa
HindIII
1K
2K
CMV promoter
3K
Nco1
intron
leader
leader
D1.3 VH
Xho1
CH1
CH2
CH3
HindIII
EcoR1/Sbf1
4K

Fig. 1

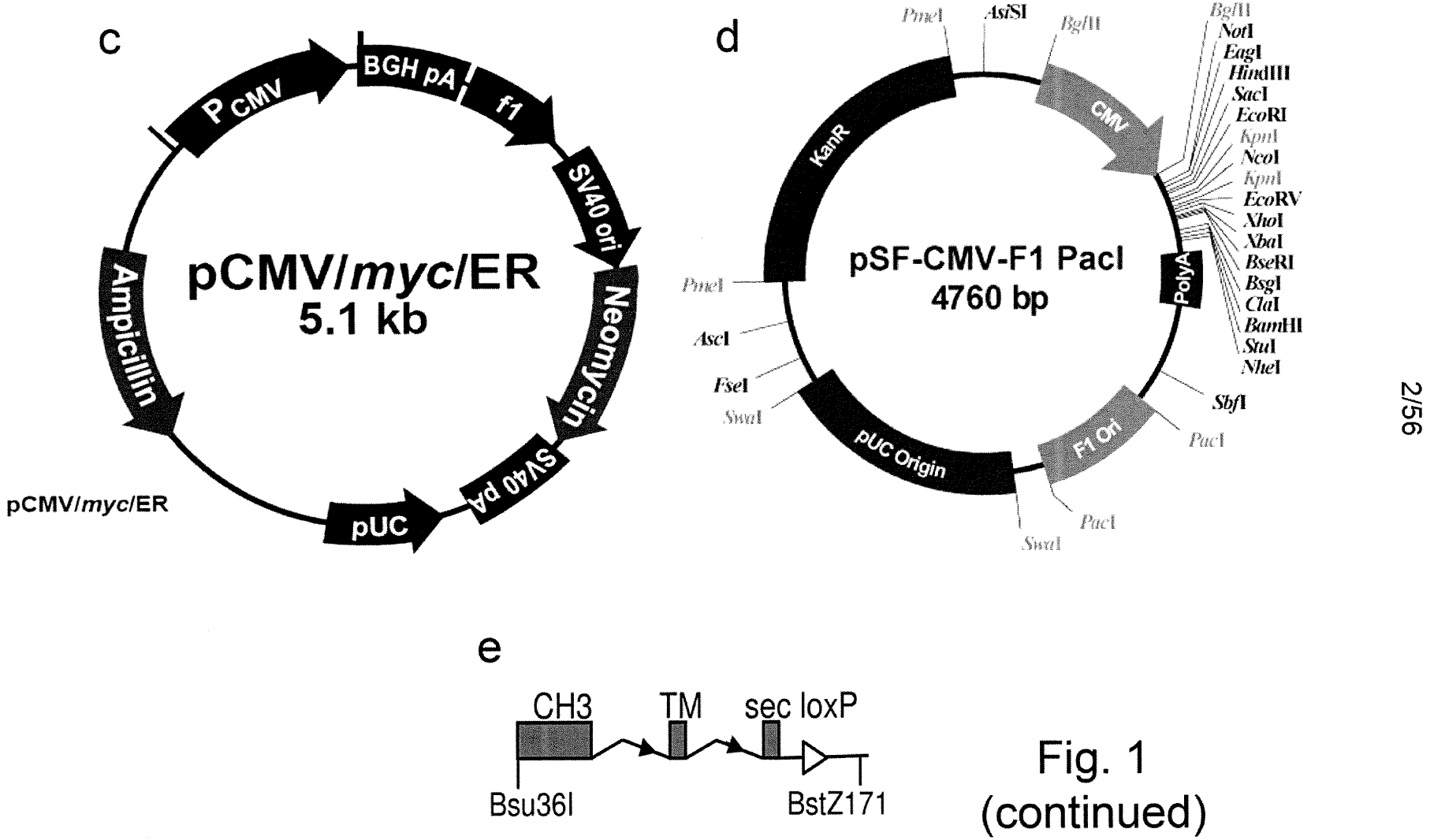



Fig. 1
(continued)

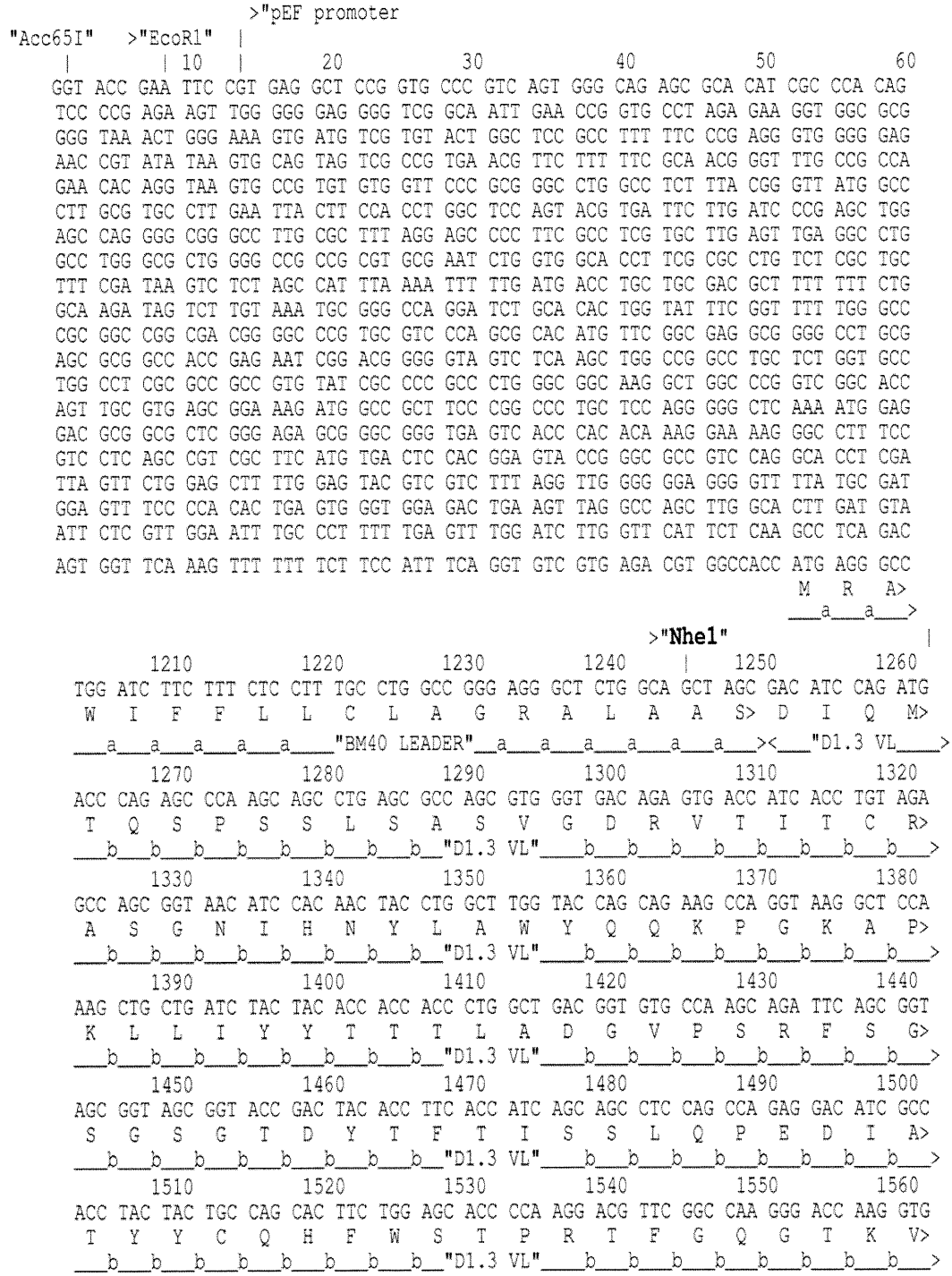

>"pEF promoter
"Acc65I" >"EcoR1"
GGT ACC GAA TTC CGT GAG GCT CCG GTG CCC GTC AGT GGG CAG AGC GCA CAT CGC CCA CAG
TCC CCG AGA AGT TGG GGG GAG GGG TCG GCA ATT GAA CCG GTG CCT AGA GAA GGT GGC GCG
GGG TAA ACT GGG AAA GTG ATG TCG TGT ACT GGC TCC GCC TTT TTC CCG AGG GTG GGG GAG
AAC CGT ATA TAA GTG CAG TAG TCG CCG TGA ACG TTC TTT TTC GCA ACG GGT TTG CCG CCA
GAA CAC AGG TAA GTG CCG TGT GTG GTT CCC GCG GGC CTG GCC TCT TTA CGG GTT ATG GCC
CTT GCG TGC CTT GAA TTA CTT CCA CCT GGC TCC AGT ACG TGA TTC TTG ATC CCG AGC TGG
AGC CAG GGG CGG GCC TTG CGC TTT AGG AGC CCC TTC GCC TCG TGC TTG AGT TGA GGC CTG
GCC TGG GCG CTG GGG CCG CCG CGT GCG AAT CTG GTG GCA CCT TCG CGC CTG TCT CGC TGC
TTT CGA TAA GTC TCT AGC CAT TTA AAA TTT TTG ATG ACC TGC TGC GAC GCT TTT TTT CTG
GCA AGA TAG TCT TGT AAA TGC GGG CCA GGA TCT GCA CAC TGG TAT TTC GGT TTT TGG GCC
CGC GGC CGG CGA CGG GGC CCG TGC GTC CCA GCG CAC ATG TTC GGC GAG GCG GGG CCT GCG
AGC GCG GCC ACC GAG AAT CGG ACG GGG GTA GTC TCA AGC TGG CCG GCC TGC TCT GGT GCC
TGG CCT CGC GCC GCC GTG TAT CGC CCC GCC CTG GGC GGC AAG GCT GGC CCG GTC GGC ACC
AGT TGC GTG AGC GGA AAG ATG GCC GCT TCC CGG CCC TGC TCC AGG GGG CTC AAA ATG GAG
GAC GCG GCG CTC GGG AGA GCG GGC GGG TGA GTC ACC CAC ACA AAG GAA AAG GGC CTT TCC
GTC CTC AGC CGT CGC TTC ATG TGA CTC CAC GGA GTA CCG GGC GCC GTC CAG GCA CCT CGA
TTA GTT CTG GAG CTT TTG GAG TAC GTC GTC TTT AGG TTG GGG GGA GGG GTT TTA TGC GAT
GGA GTT TCC CCA CAC TGA GTG GGT GGA GAC TGA AGT TAG GCC AGC TTG GCA CTT GAT GTA
ATT CTC GTT GGA ATT TGC CCT TTT TGA GTT TGG ATC TTG GTT CAT TCT CAA GCC TCA GAC
AGT GGT TCA AGG TTT TTT TCT TCC ATT TCA GGT GTC GTG AGA CGT GGCCACC ATG AGG GCC
M R A>
___a___a___>
>"Nhe1"
1210 1220 1230 1240 1250 1260
TGG ATC TTC TTT CTC CTT TGC CTG GCC GGG AGG GCT CTG GCA GCT AGC GAC ATC CAG ATG
W I F F L L C L A G R A L A A S> D I Q M>
___a___a___a___a___a____"BM40 LEADER"__a___a___a___a___a___a___><___"D1.3 VL____>
1270 1280 1290 1300 1310 1320
ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT GAC AGA GTG ACC ATC ACC TGT AGA
T Q S P S S L S A S V G D R V T I T C R>
___b___b___b___b___b___b___b___b__"D1.3 VL"____b___b___b___b___b___b___b___b___>
1330 1340 1350 1360 1370 1380
GCC AGC GGT AAC ATC CAC AAC TAC CTG GCT TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA
A S G N I H N Y L A W Y Q Q K P G K A P>
___b___b___b___b___b___b___b___b__"D1.3 VL"____b___b___b___b___b___b___b___b___>
1390 1400 1410 1420 1430 1440
AAG CTG CTG ATC TAC TAC ACC ACC ACC CTG GCT GAC GGT GTG CCA AGC AGA TTC AGC GGT
K L L I Y Y T T T L A D G V P S R F S G>
___b___b___b___b___b___b___b___b__"D1.3 VL"____b___b___b___b___b___b___b___b___>
1450 1460 1470 1480 1490 1500
AGC GGT AGC GGT ACC GAC TAC ACC TTC ACC ATC AGC AGC CTC CAG CCA GAG GAC ATC GCC
S G S G T D Y T F T I S S L Q P E D I A>
___b___b___b___b___b___b___b___b__"D1.3 VL"____b___b___b___b___b___b___b___b___>
1510 1520 1530 1540 1550 1560
ACC TAC TAC TGC CAG CAC TTC TGG AGC ACC CCA AGG ACG TTC GGC CAA GGG ACC AAG GTG
T Y Y C Q H F W S T P R T F G Q G T K V>
___b___b___b___b___b___b___b___b__"D1.3 VL"____b___b___b___b___b___b___b___b___>

Fig. 2

```
                    >"Not1"                                                   |
        1570        | 1580        1590        1600        1610        1620
GAA ATC AAA CGT ACC GCG  GCC GCC CCT TCC GTG TTC ATC TTC CCT CCC TCC GAC GAG CAG
 E   I   K   R   T> A    A   A   P   S   V   F   I   F   P   P   S   D   E   Q>
___b__"D1.3 VL"b__>c___c___"CODON OPTIMISED HUMAN_C_KAPPA"_c___c___c___c___c
        1630        1640        1650        1660        1670        1680
CTG AAG TCC GGC ACC GCC TCT GTG GTG TGC CTG CTG AAC AAC TTC TAC CCT CGG GAG GCC
 L   K   S   G   T   A   S   V   V   C   L   L   N   N   F   Y   P   R   E   A>
___c___c___c___c___c___"CODON OPTIMISED HUMAN_C_KAPPA"_c___c___c___c___c___c___>
        1690        1700        1710        1720        1730        1740
AAG GTG CAG TGG AAG GTG GAC AAC GCC CTG CAG TCC GGC AAC TCC CAG GAA TCC GTC ACC
 K   V   Q   W   K   V   D   N   A   L   Q   S   G   N   S   Q   E   S   V   T>
___c___c___c___c___c___"CODON OPTIMISED HUMAN_C_KAPPA"_c___c___c___c___c___c___>
        1750        1760        1770        1780        1790        1800
GAG CAG GAC TCC AAG GAC TCT ACC TAC TCC CTG TCC TCC ACC CTG ACC CTG TCC AAG GCC
 E   Q   D   S   K   D   S   T   Y   S   L   S   S   T   L   T   L   S   K   A>
___c___c___c___c___c___"CODON OPTIMISED HUMAN_C_KAPPA"_c___c___c___c___c___c___>
        1810        1820        1830        1840        1850        1860
GAC TAC GAG AAG CAC AAG CTG TAC GCC TGC GAA GTG ACC CAC CAG GGC CTG TCC TCT CCC
 D   Y   E   K   H   K   L   Y   A   C   E   V   T   H   Q   G   L   S   S   P>
___c___c___c___c___c___"CODON OPTIMISED HUMAN_C_KAPPA"_c___c___c___c___c___c___>
                                          >"HindIII"      >"BclI"   >"BGH_pA→
        1870        1880        1890        1900        1910        | 1920
GTG ACC AAG TCC TTC AAC CGG GGC GAG TGC TA ATA AAA GCT TAC GAC GTG ATC AGC CTC
 V   T   K   S   F   N   R   G   E   C>
___"CODON OPTIMISED HUMAN_C_KAPPA"_c___>
        1930        1940        1950        1960        1970        1980
GAC TGT GCC TTC TAG TTG CCA GCC ATC TGT TGT TTG CCC CTC CCC CGT GCC TTC CTT GAC
        1990        2000        2010        2020        2030        2040
CCT GGA AGG TGC CAC TCC CAC TGT CCT TTC CTA ATA AAA TGA GGA AAT TGC ATC GCA TTG
        2050        2060        2070        2080        2090        2100
TCT GAG TAG GTG TCA TTC TAT TCT GGG GGG TGG GGT GGG GCA GGA CAG CAA GGG GGA GGA
                                                  >"SpeI CMV_promoter"→
        2110        2120        2130        2140    |   | 2150        2160
TTG GGA AGA CAA TAG CAG GCA TGC TGG GGA ACA TTG ATT ATT GAC TAG TTA TTA ATA GTA
ATC AAT TAC GGG GTC ATT AGT TCA TAG CCC ATA TAT GGA GTT CCG CGT TAC ATA ACT TAC
GGT AAA TGG CCC GCC TGG CTG ACC GCC CAA CGA CCC CCG CCC ATT GAC GTC AAT AAT GAC
GTA TGT TCC CAT AGT AAC GCC AAT AGG GAC TTT CCA TTG ACG TCA ATG GGT GGA GTA TTT
ACG GTA AAC TGC CCA CTT GGC AGT ACA TCA AGT GTA TCA TAT GCC AAG TCC GCC CCC TAT
TGA CGT CAA TGA CGG TAA ATG GCC CGC CTG GCA TTA TGC CCA GTA CAT GAC CTT ACG GGA
                                                  >"ex-Nco_site"
        2470        2480        2490        2500    |   2510        2520
CTT TCC TAC TTG GCA GTA CAT CTA CGT ATT AGT CAT CGC TAT TAC CAT AGT GAT GCG GTT
TTG GCA GTA CAC CAA TGG GCG TGG ATA GCG GTT TGA CTC ACG GGG ATT TCC AAG TCT CCA
CCC CAT TGA CGT CAA TGG GAG TTT GTT TTG GCA CCA AAA TCA ACG GGA CTT TCC AAA ATG
TCG TAA TAA CCC CGC CCC GTT GAC GCA AAT GGG CGG TAG GCG TGT ACG GTG GGA GGT CTA
                          ← end CMV promoter-II-Tri-partite leader→
        2650        2660        2670        2680        2690        2700
TAT AAG CAG AGC TCG TTT AGT GAA CCG TCA GATC CT CAC TCT CTT CCG CAT CGC TGT CTG
CGA GGG CCA GCT GTT GGG CTC GCG GTT GAG GAC AAA CTC TTC GCG GTC TTT CCA GTA CTC
TTG GAT CGG AAA CCC GTC GGC CTC CGA ACG GTA CTC CGC CAC CGA GGG ACC TGA GCG AGT
                          >"ex-XhoI_site"
        2890        2900        | 2910        2920        2930        2940
CCG CAT CGA CCG GAT CGG AAA ACC TCT CGT GAA AGG CGT CTA ACC AGT CAC AGT CGC AAG
GTA GGC TGA GCA CCG TGG CGG GCG GCA GCG GGT GGC GGT CGG GGT TGT TTC TGG CGG AGG
TGC TGC TGA TGA TGT AAT TAA AGT AGG CGG TCT TGA GAC GGC GGA TGG TCG AGG TGA GGT
GTG GCA GGC TTG AGA TCC AGC TGT TGG GGT GAG TAC TCC CTC TCA AAA GCG GGC ATT ACT
TCT GCG CTA AGA TTG TCA GTT TCC AAA AAC GAG GAG GAT TTG ATA TTC ACC TGG CCC GAT
CTG GCC ATA CAC TTG AGT GAC AAT GAC ATC CAC TTT GCC TTT CTC TCC ACA GGT GTC CAC
```

Fig. 2 (continued)

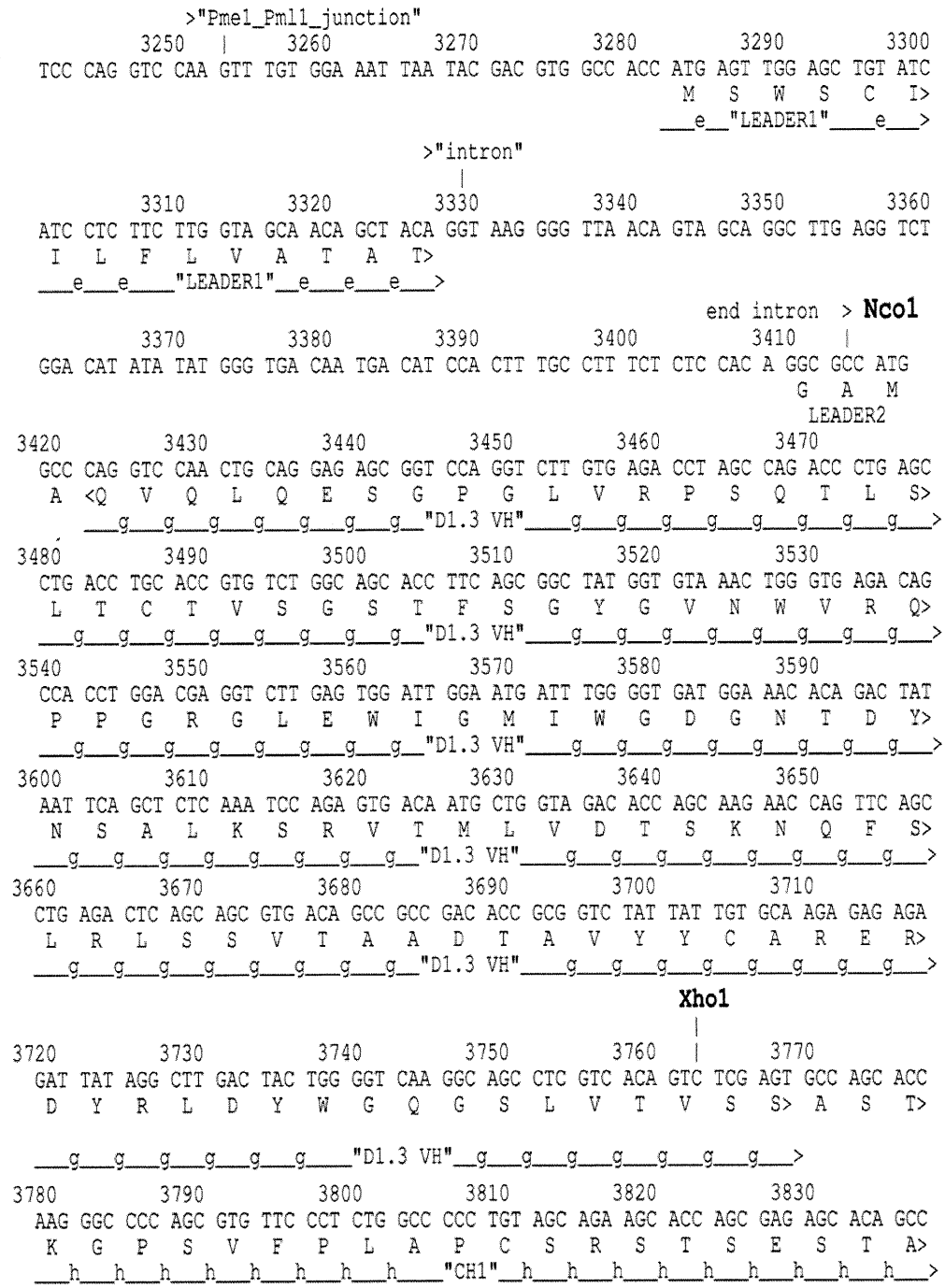



Fig. 2 (continued)

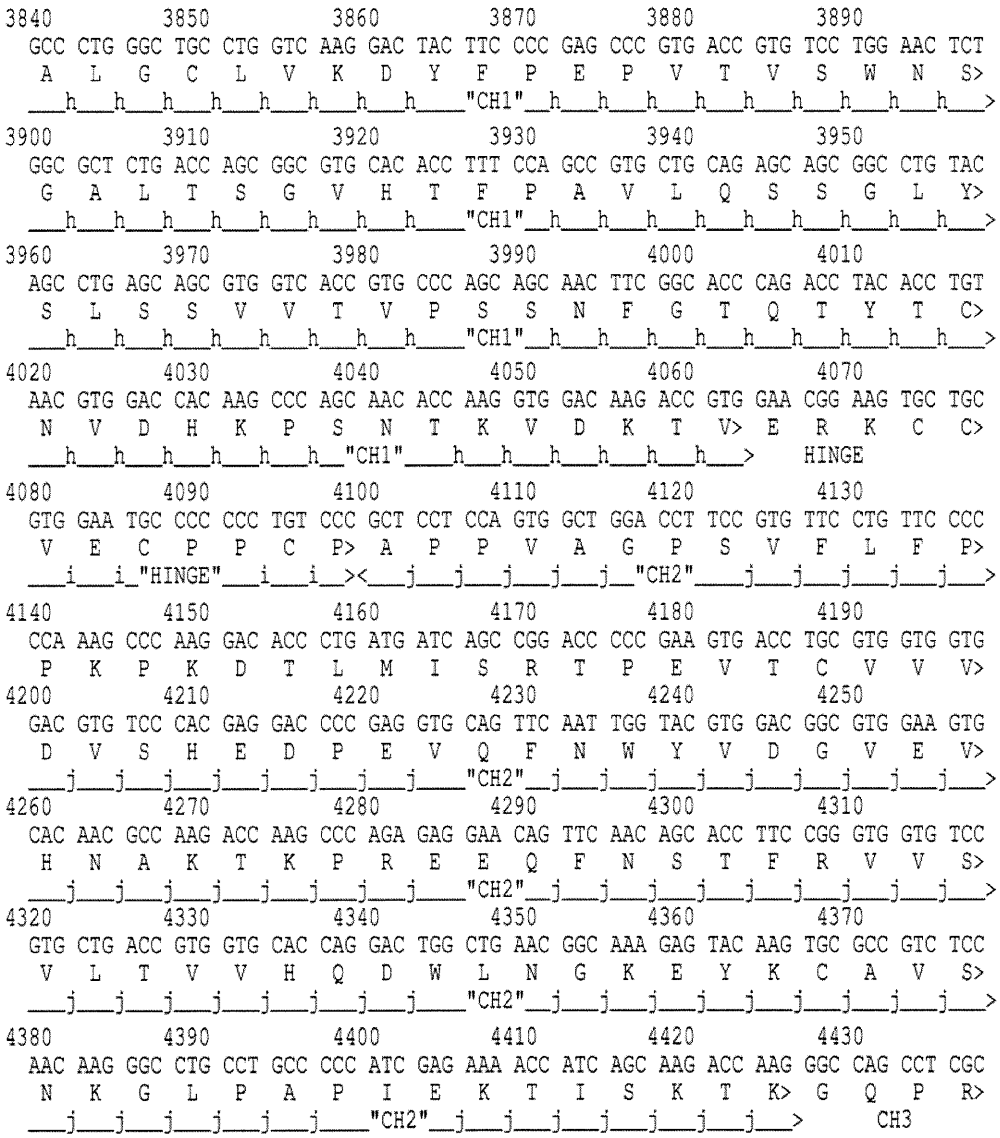



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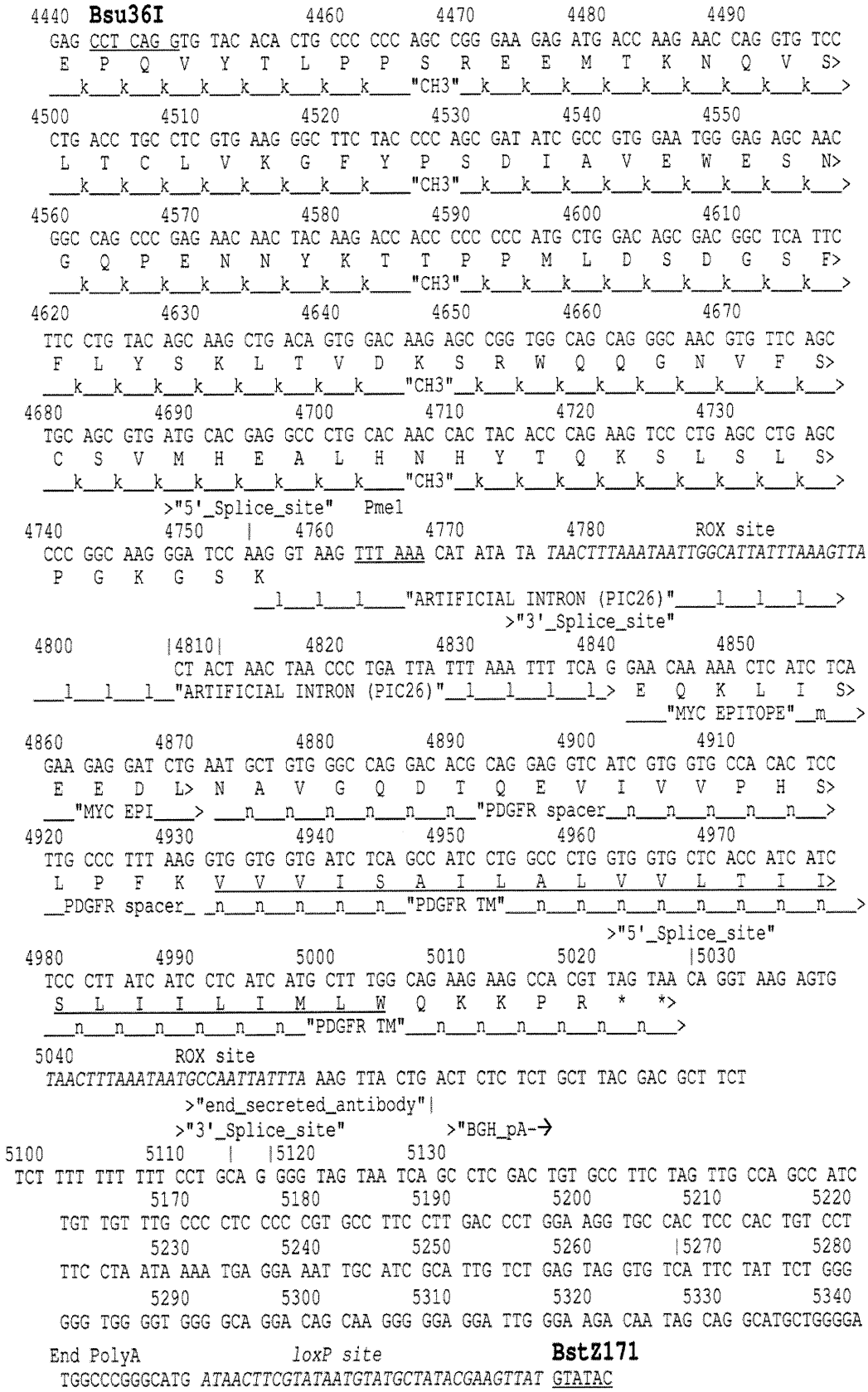



Fig. 2 (continued)

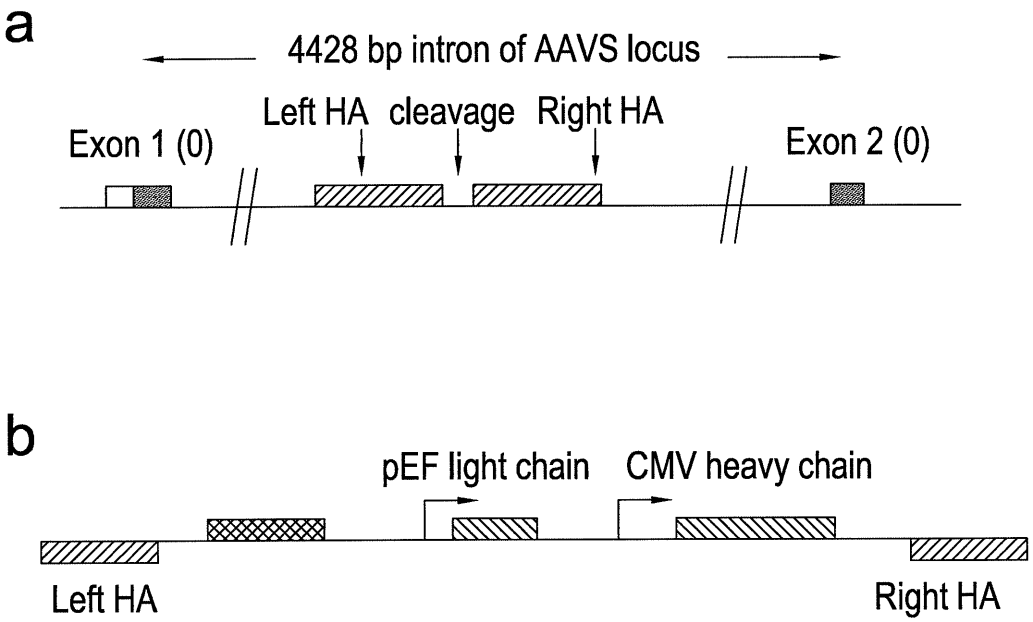

a
4428 bp intron of AAVS locus
Left HA
cleavage
Right HA
Exon 1 (0)
Exon 2 (0)
b
pEF light chain
CMV heavy chain
Left HA
Right HA

Fig. 3

C

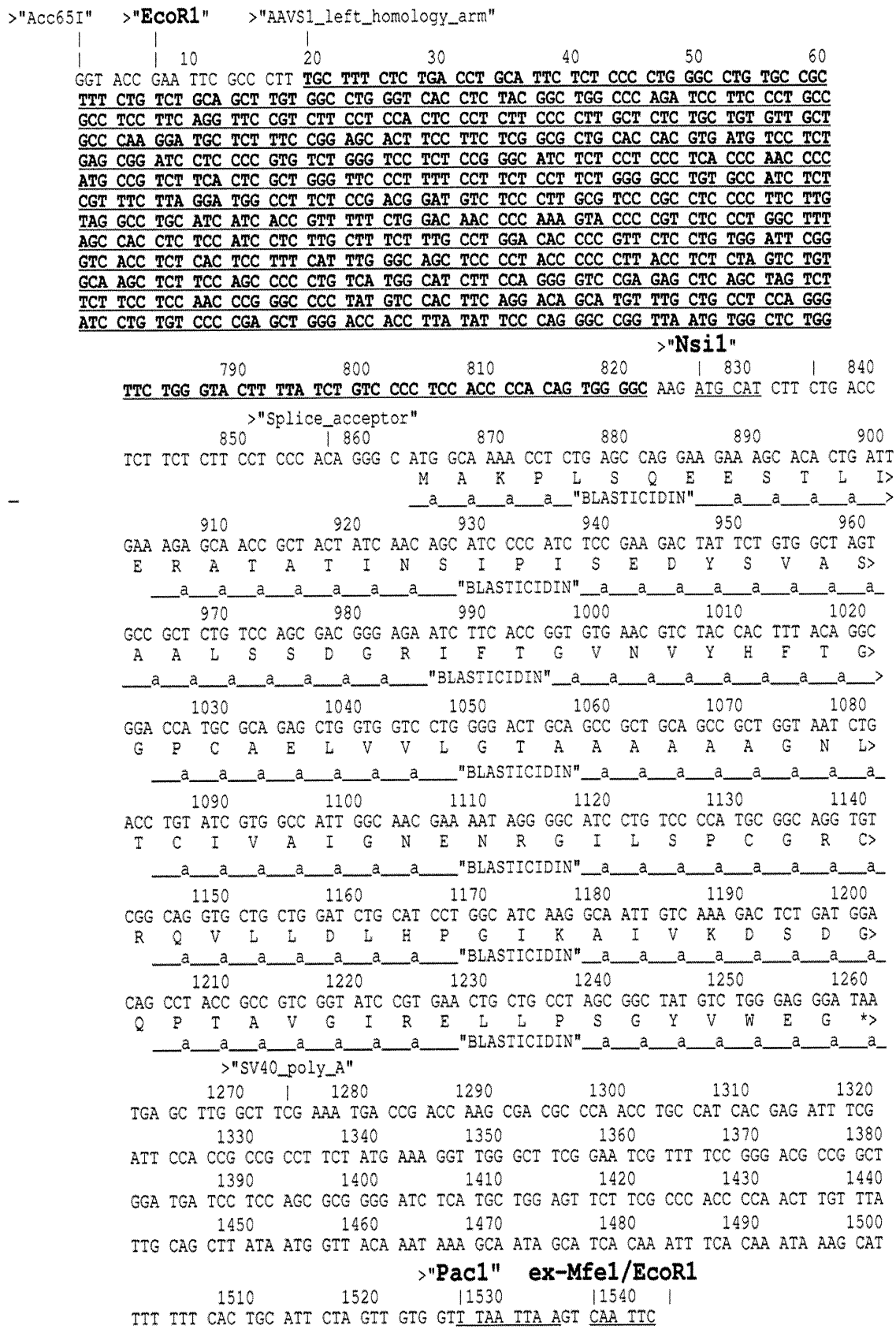



Fig. 3 (continued)

←D1.3 ANTIBODY EXPRESSION CASSETTE→

6887 loxP site **BstZ171** **AscI** AAVS1_right_HA

TGGCCCGGGCATG *ATAACTTCGTATAATGTATGCTATACGAAGTTATG* TATAC GGCGCGCCC **ACTAGGGACAGGATTGGTGACA**

6960 6970 6980 6990 7000 7010

GAA AAG CCC CAT CCT TAG GCC TCC TCC TTC CTA GTC TCC TGA TAT TGG GTC TAA CCC CCA
CCT CCT GTT AGG CAG ATT CCT TAT CTG GTG ACA CAC CCC CAT TTC CTG GAG CCA TCT CTC
TCC TTG CCA GAA CCT CTA AGG TTT GCT TAC GAT GGA GCC AGA GAG GAT CCT GGG AGG GAG
AGC TTG GCA GGG GGT GGG AGG GAA GGG GGG GAT GCG TGA CCT GCC CGG TTC TCA GTG GCC
ACC CTG CGC TAC CCT CTC CCA GAA CCT GAG CTG CTC TGA CGC GGC TGT CTG GTG CGT TTC
ACT GAT CCT GGT GCT GCA GCT TCC TTA CAC TTC CCA AGA GGA GAA GCA GTT TGG AAA AAC
AAA ATC AGA ATA AGT TGG TCC TGA GTT CTA ACT TTG GCT CTT CAC CTT TCT AGT CCC CAA
TTT ATA TTG TTC CTC CGT GCG TCA GTT TTA CCT GTG AGA TAA GGC CAG TAG CCA GCC CCG
TCC TGG CAG GGC TGT GGT GAG GAG GGG GGT GTC CGT GTG GAA AAC TCC CTT TGT GAG AAT
GGT GCG TCC TAG GTG TTC ACC AGG TCG TGG CCG CCT CTA CTC CCT TTC TCT TTC TCC ATC
CTT CTT TCC TTA AAG AGT CCC CAG TGC TAT CTG GGA CAT ATT CCT CCG CCC AGA GCA GGG
TCC CGC TTC CCT AAG GCC CTG CTC TGG GCT TCT GGG TTT GAG TCC TTG GCA AGC CCA GGA
GAG GCG CTC AGG CTT CCC TGT CCC CCT TCC TCG TCC ACC ATC TCA TGC CCC TGG CTC TCC

7740 7750 End AAVS1 right Homol Arm **MluI** **BstZ1** 7790

TGC CCC TTC CCT ACA GGG GTT CCT GGC TCT GCT CTG ACG CGT GTATAC TCG ATC TTT CCG

Fig. 3 (continued)

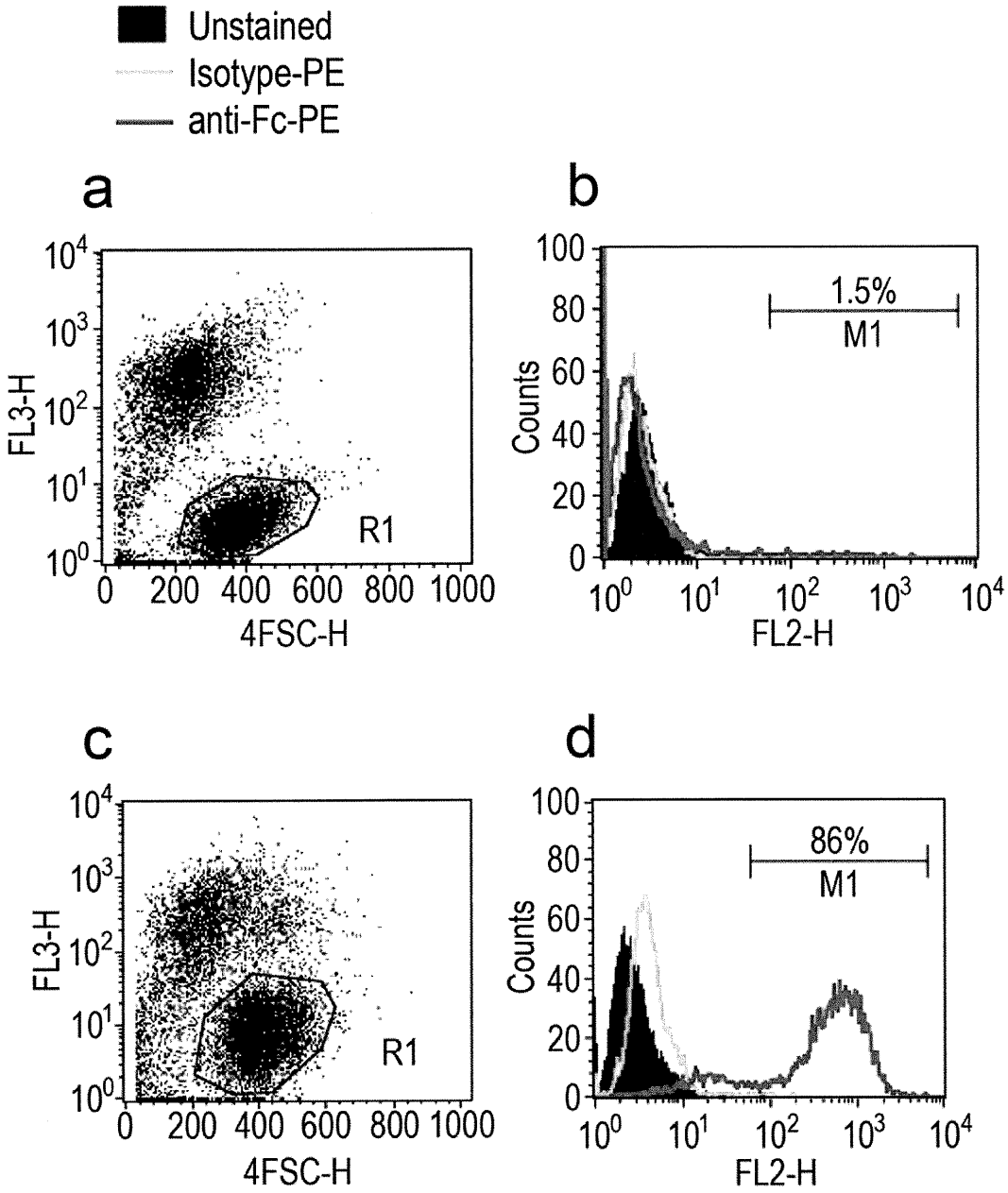

Unstained
Isotype-PE
anti-Fc-PE
a
b
c
d
FL3-H
4FSC-H
R1
Counts
FL2-H
1.5%
M1
86%
M1

Fig. 4

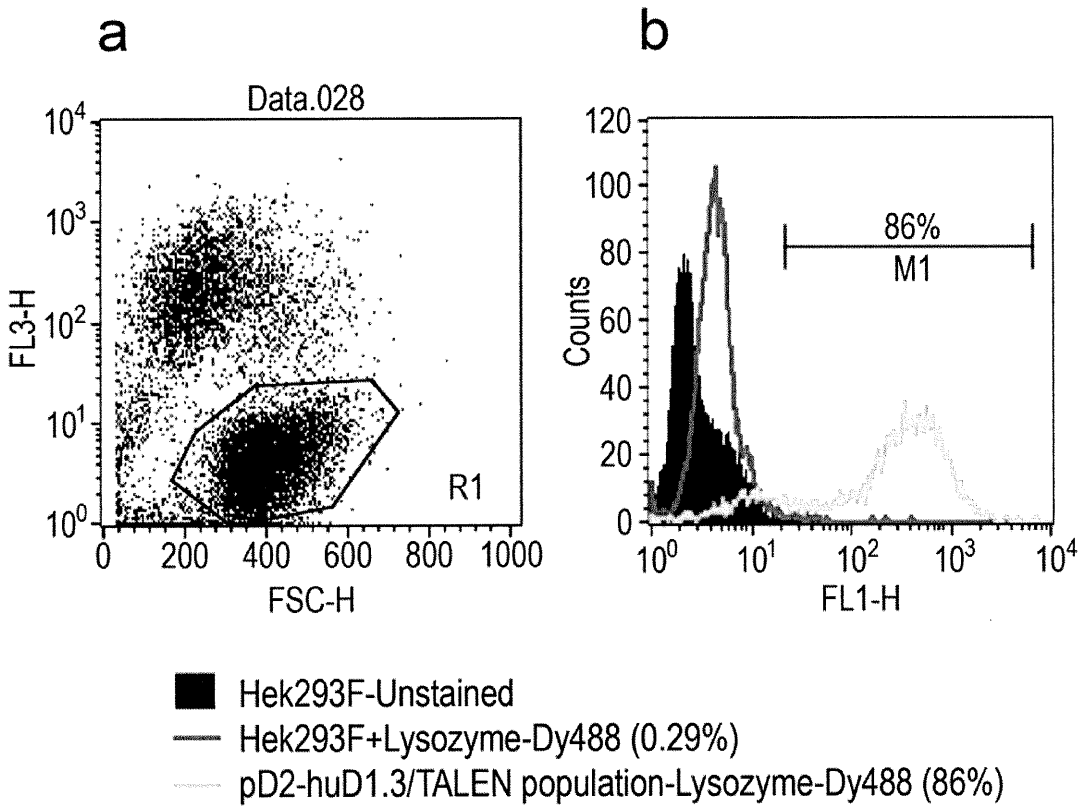

a
b
Data.028
FL3-H
FSC-H
R1
Counts
FL1-H
86%
M1
Hek293F-Unstained
Hek293F+Lysozyme-Dy488 (0.29%)
pD2-huD1.3/TALEN population-Lysozyme-Dy488 (86%)

Fig. 5

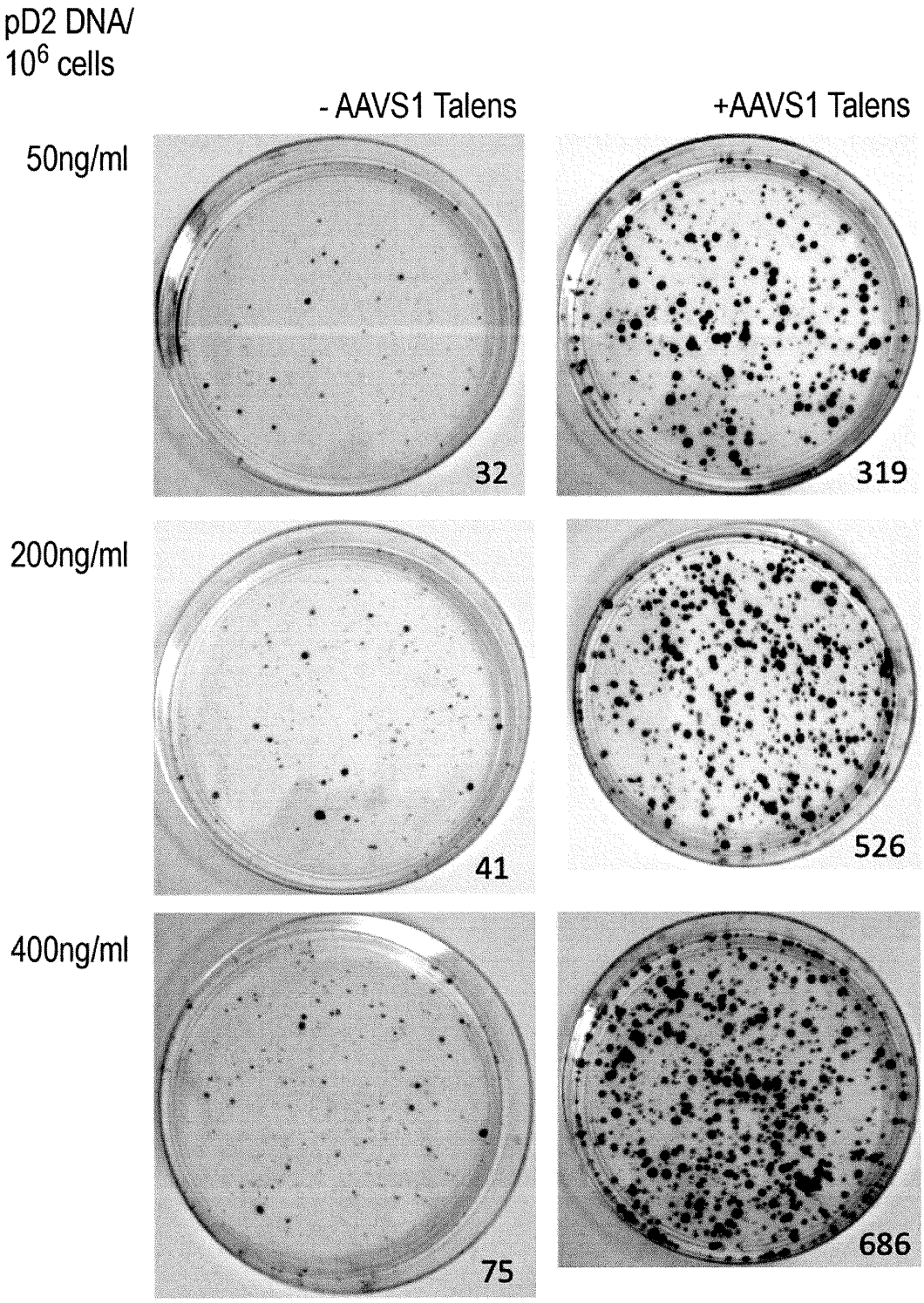
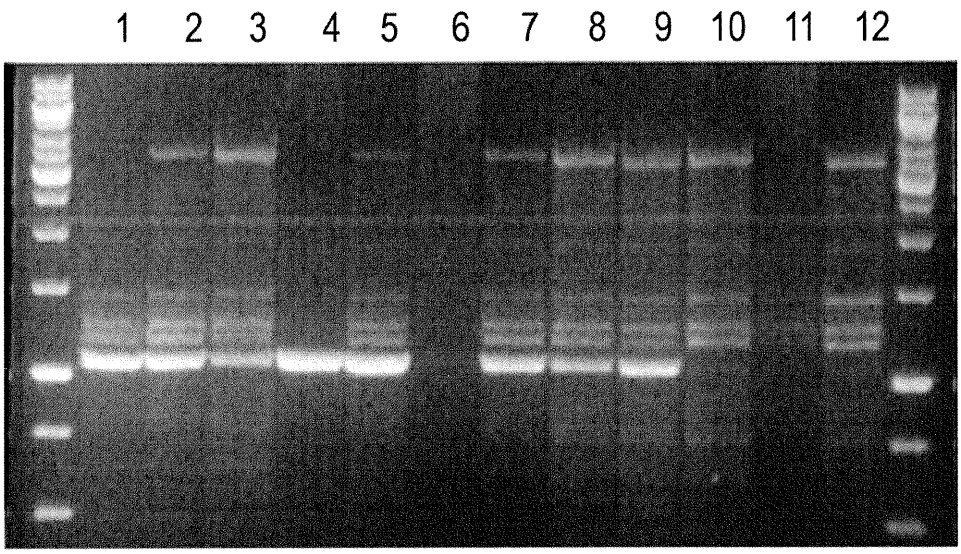

pD2 DNA/
10^6 cells
- AAVS1 Talens
+AAVS1 Talens
50ng/ml
32
319
200ng/ml
41
526
400ng/ml
75
686

Fig. 6

a




b

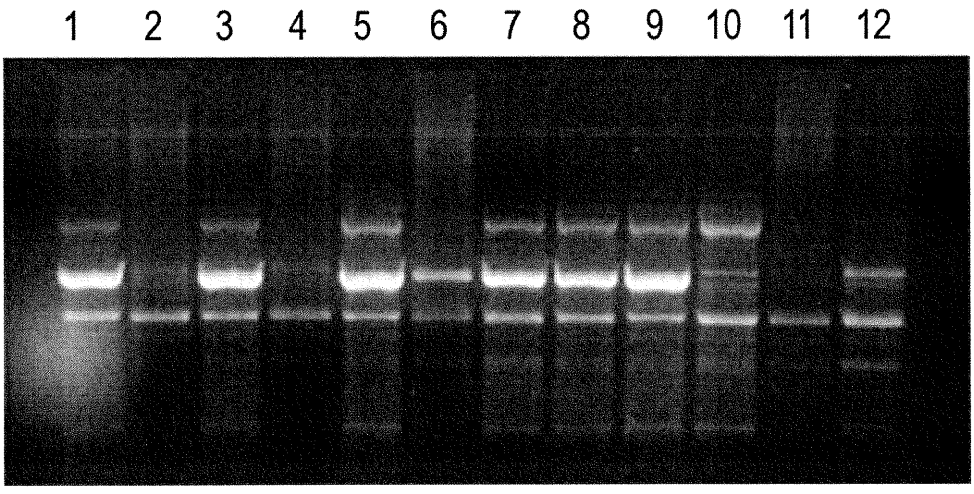
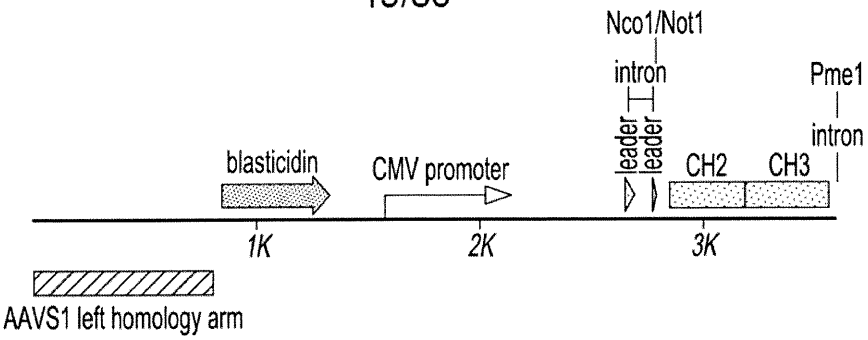



Fig. 7

a




b

```
>"Nco1"          >"Not1"
    |                |
    |    10          20          30          40          50          60
GGC GCC ATG GCC CAG GTC GCG GCC GCC GTG GAA TGC CCC CCC TGT CCC GCT CCT CCA GTG
 G   A   M   A>                                                  A   P   P   V>
___"LEADER"____>                                                ____"CH2"__c___>
          70          80          90          100         110         120
GCT GGA CCT TCC GTG TTC CTG TTC CCC CCA AAG CCC AAG GAC ACC CTG ATG ATC AGC CGG
 A   G   P   S   V   F   L   F   P   P   K   P   K   D   T   L   M   I   S   R>
___c___c___c___c___c___c___c___c____"CH2"__c___c___c___c___c___c___c___c___c___>
          130         140         150         160         170         180
ACC CCC GAA GTG ACC TGC GTG GTG GTG GAC GTG TCC CAC GAG GAC CCC GAG GTG CAG TTC
 T   P   E   V   T   C   V   V   V   D   V   S   H   E   D   P   E   V   Q   F>
___c___c___c___c___c___c___c___c____"CH2"__c___c___c___c___c___c___c___c___c___>
          190         200         210         220         230         240
AAT TGG TAC GTG GAC GGC GTG GAA GTG CAC AAC GCC AAG ACC AAG CCC AGA GAG GAA CAG
 N   W   Y   V   D   G   V   E   V   H   N   A   K   T   K   P   R   E   E   Q>
___c___c___c___c___c___c___c___c____"CH2"__c___c___c___c___c___c___c___c___c___>
                2624 primer
          250     |   260         270         280         290         300
TTC AAC AGC ACC TTC CGG GTG GTG TCC GTG CTG ACC GTG GTG CAC CAG GAC TGG CTG AAC
 F   N   S   T   F   R   V   V   S   V   L   T   V   V   H   Q   D   W   L   N>
___c___c___c___c___c___c___c___c____"CH2"__c___c___c___c___c___c___c___c___c___>
          310         320         330         340         350         360
GGC AAA GAG TAC AAG TGC GCC GTC TCC AAC AAG GGC CTG CCT GCC CCC ATC GAG AAA ACC
 G   K   E   Y   K   C   A   V   S   N   K   G   L   P   A   P   I   E   K   T>
___c___c___c___c___c___c___c___c____"CH2"__c___c___c___c___c___c___c___c___c___>
          370         380         390         400         410         420
ATC AGC AAG ACC AAG GGC CAG CCT CGC GAG CCT CAG GTG TAC ACA CTG CCC CCC AGC CGG
 I   S   K   T   K>< G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R>
___c__"CH2"____c___>__d___d___d___d____"CH3"__d___d___d___d___d___d___d___d___
          430         440         450         460         470         480
GAA GAG ATG ACC AAG AAC CAG GTG TCC CTG ACC TGC CTC GTG AAG GGC TTC TAC CCC AGC
 E   E   M   T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S>
___d___d___d___d___d___d___d___d____"CH3"__d___d___d___d___d___d___d___d___d___>
          490         500         510         520         530         540
GAT ATC GCC GTG GAA TGG GAG AGC AAC GGC CAG CCC GAG AAC AAC TAC AAG ACC ACC CCC
 D   I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T   P>
___d___d___d___d___d___d___d___d____"CH3"__d___d___d___d___d___d___d___d___d___>
          550         560         570         580         590         600
CCC ATG CTG GAC AGC GAC GGC TCA TTC TTC CTG TAC AGC AAG CTG ACA GTG GAC AAG AGC
 P   M   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V   D   K   S>
___d___d___d___d___d___d___d___d____"CH3"__d___d___d___d___d___d___d___d___d___>
          610         620         630         640         650         660
CGG TGG CAG CAG GGC AAC GTG TTC AGC TGC AGC GTG ATG CAC GAG GCC CTG CAC AAC CAC
 R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E   A   L   H   N   H>
___d___d___d___d___d___d___d___d____"CH3"__d___d___d___d___d___d___d___d___d___>
          670         680         690         700         710         720
TAC ACC CAG AAG TCC CTG AGC CTG AGC CCC GGC AAG GGA TCC AAG GTAAGTTTAAACATA
 Y   T   Q   K   S   L   S   L   S   P   G   K   G   S   K>    INTRON
___d___d___d___d____"CH3"__d___d___d___d___d___>
          Pme1|
          730
TATATAACTTTAAA
  INTRON
```

Fig. 8

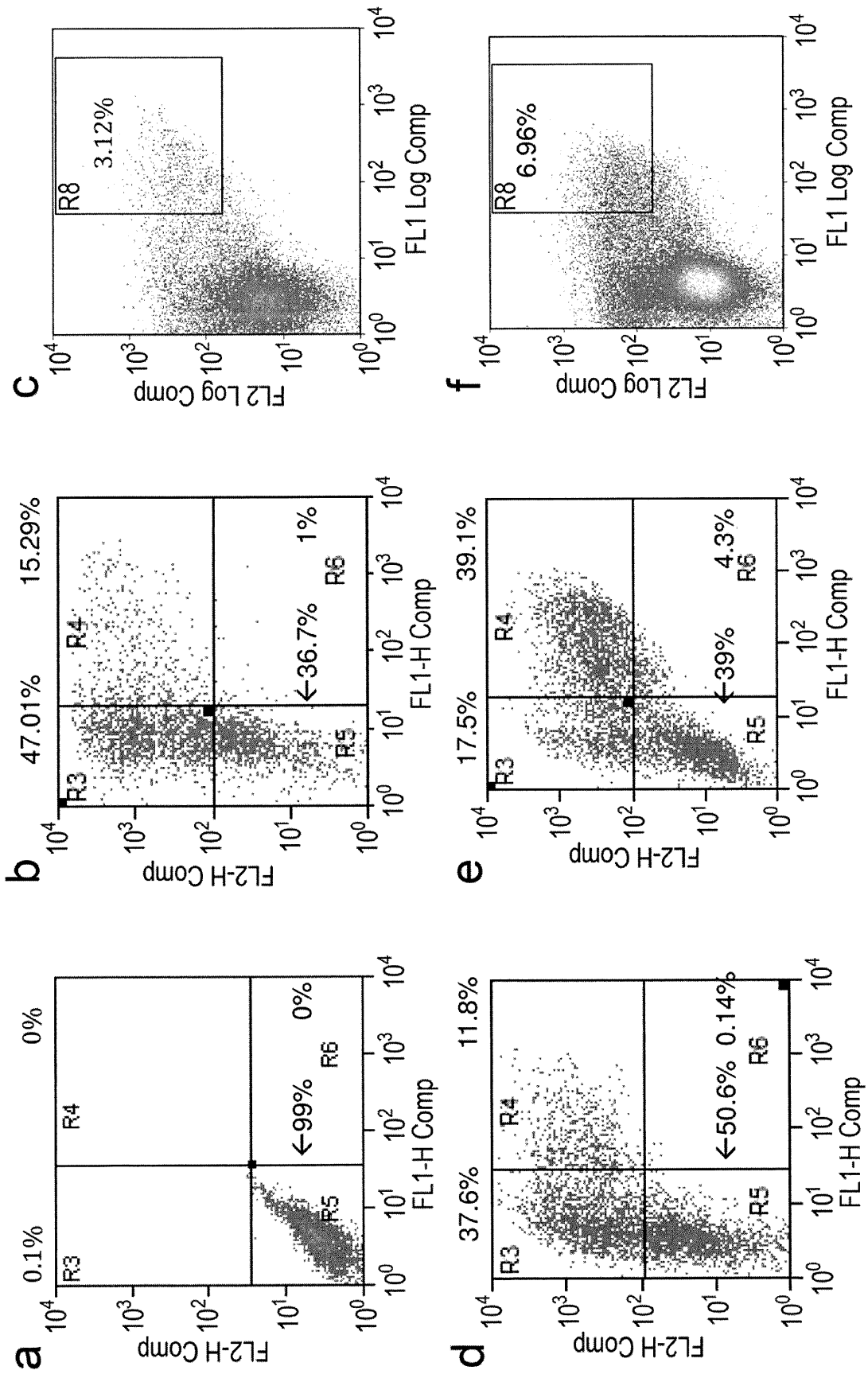

a
b
c
d
e
f
FL2-H Comp
FL1-H Comp
FL2 Log Comp
FL1 Log Comp
R3
R4
R5
R6
R8
0.1%
0%
99%
0%
47.01%
15.29%
36.7%
1%
3.12%
37.6%
11.8%
50.6%
0.14%
17.5%
39.1%
39%
4.3%
6.96%

Fig. 9

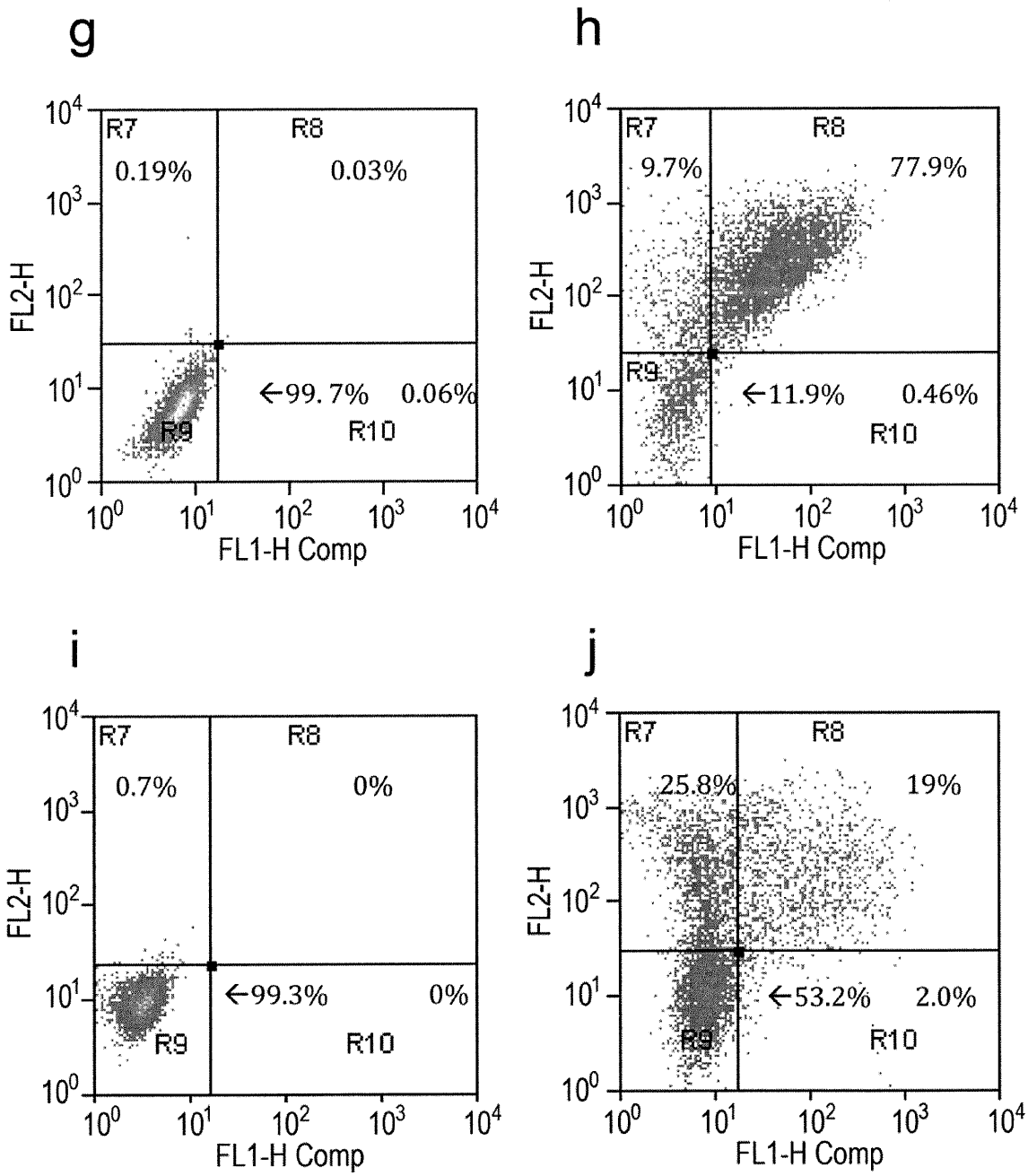



Fig. 9
(continued)

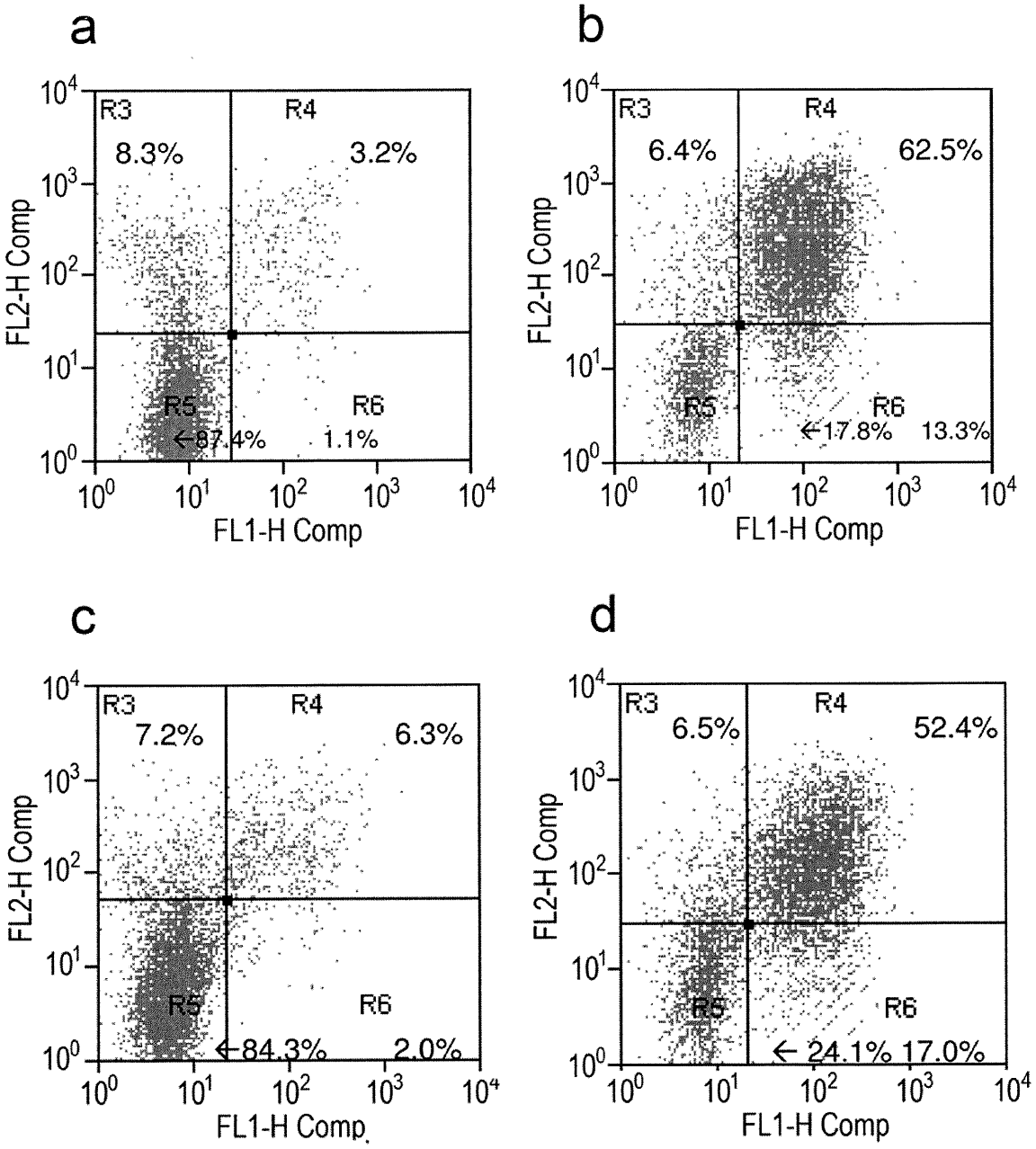

a
b
c
d
R3
R4
R5
R6
8.3%
3.2%
87.4%
1.1%
6.4%
62.5%
17.8%
13.3%
7.2%
6.3%
84.3%
2.0%
6.5%
52.4%
24.1%
17.0%
FL2-H Comp
FL1-H Comp
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$

Fig. 10

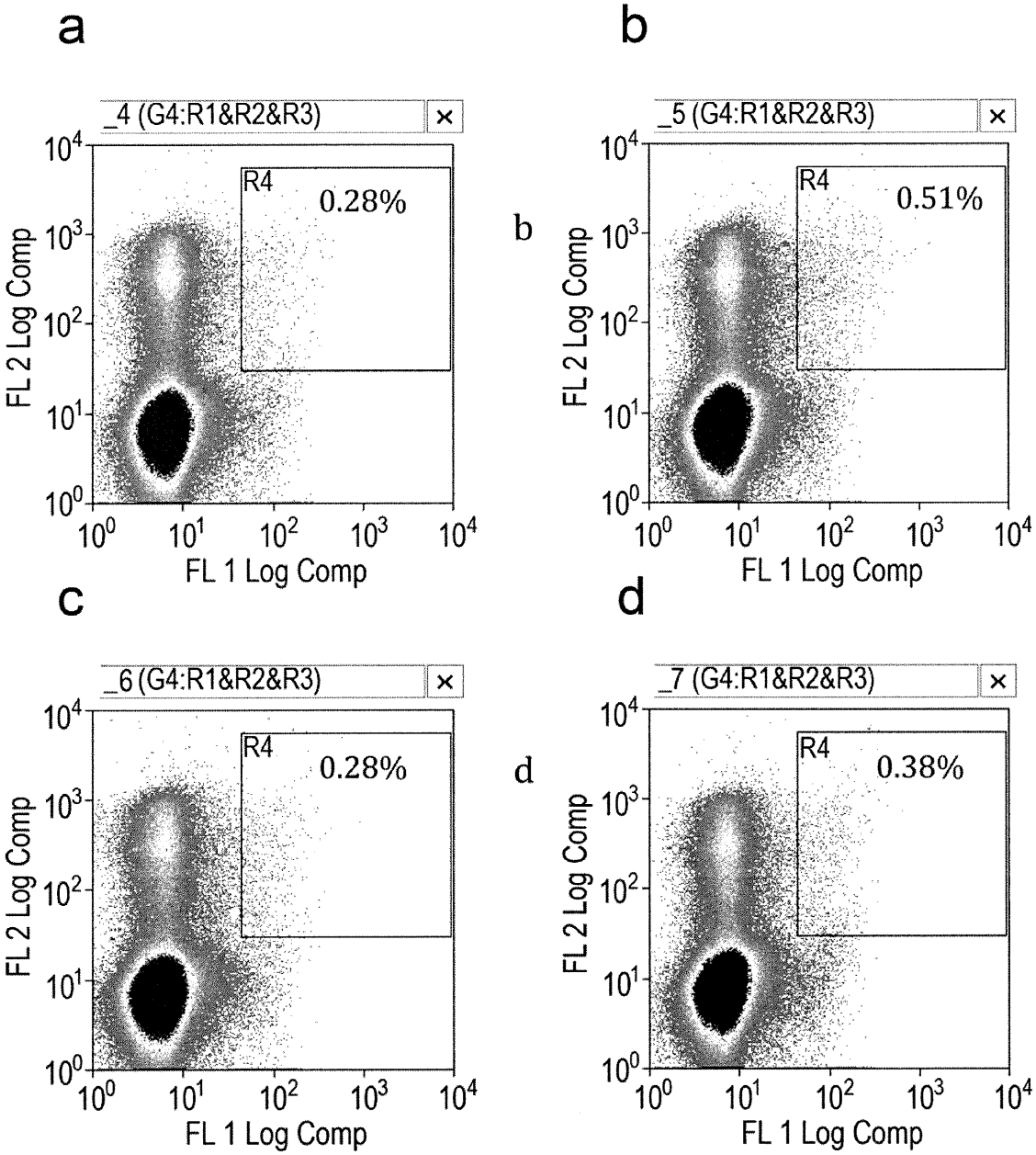

a
_4 (G4:R1&R2&R3)
R4
0.28%
FL 2 Log Comp
FL 1 Log Comp
b
b
_5 (G4:R1&R2&R3)
R4
0.51%
FL 2 Log Comp
FL 1 Log Comp
c
_6 (G4:R1&R2&R3)
R4
0.28%
FL 2 Log Comp
FL 1 Log Comp
d
d
_7 (G4:R1&R2&R3)
R4
0.38%
FL 2 Log Comp
FL 1 Log Comp

Fig. 11

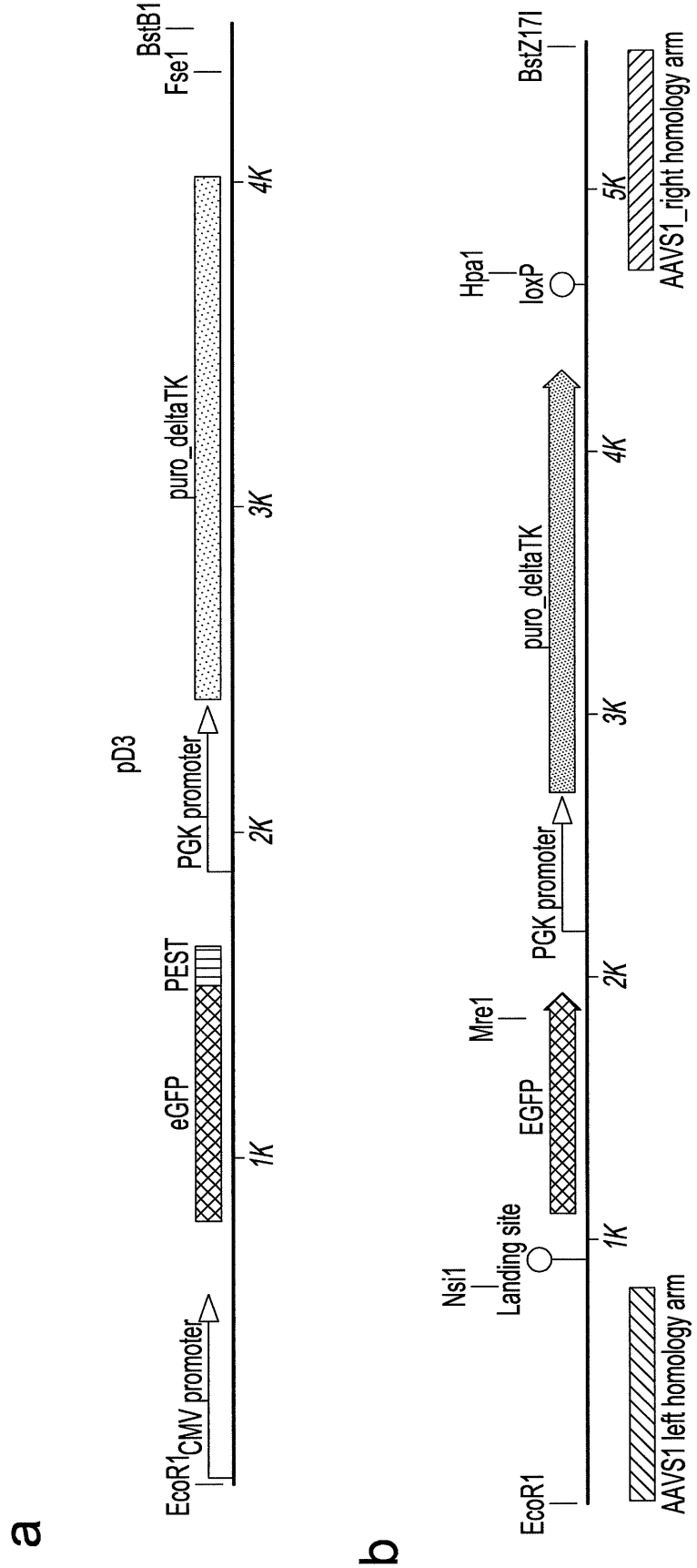

a
EcoR1
CMV promoter
eGFP
PEST
1K
pD3
PGK promoter
2K
puro_deltaTK
3K
Fse1
BstB1
4K
b
EcoR1
AAVS1 left homology arm
Nsi1
Landing site
EGFP
Mre1
1K
PGK promoter
2K
puro_deltaTK
3K
Hpa1
loxP
4K
BstZ17I
5K
AAVS1_right homology arm

Fig. 12

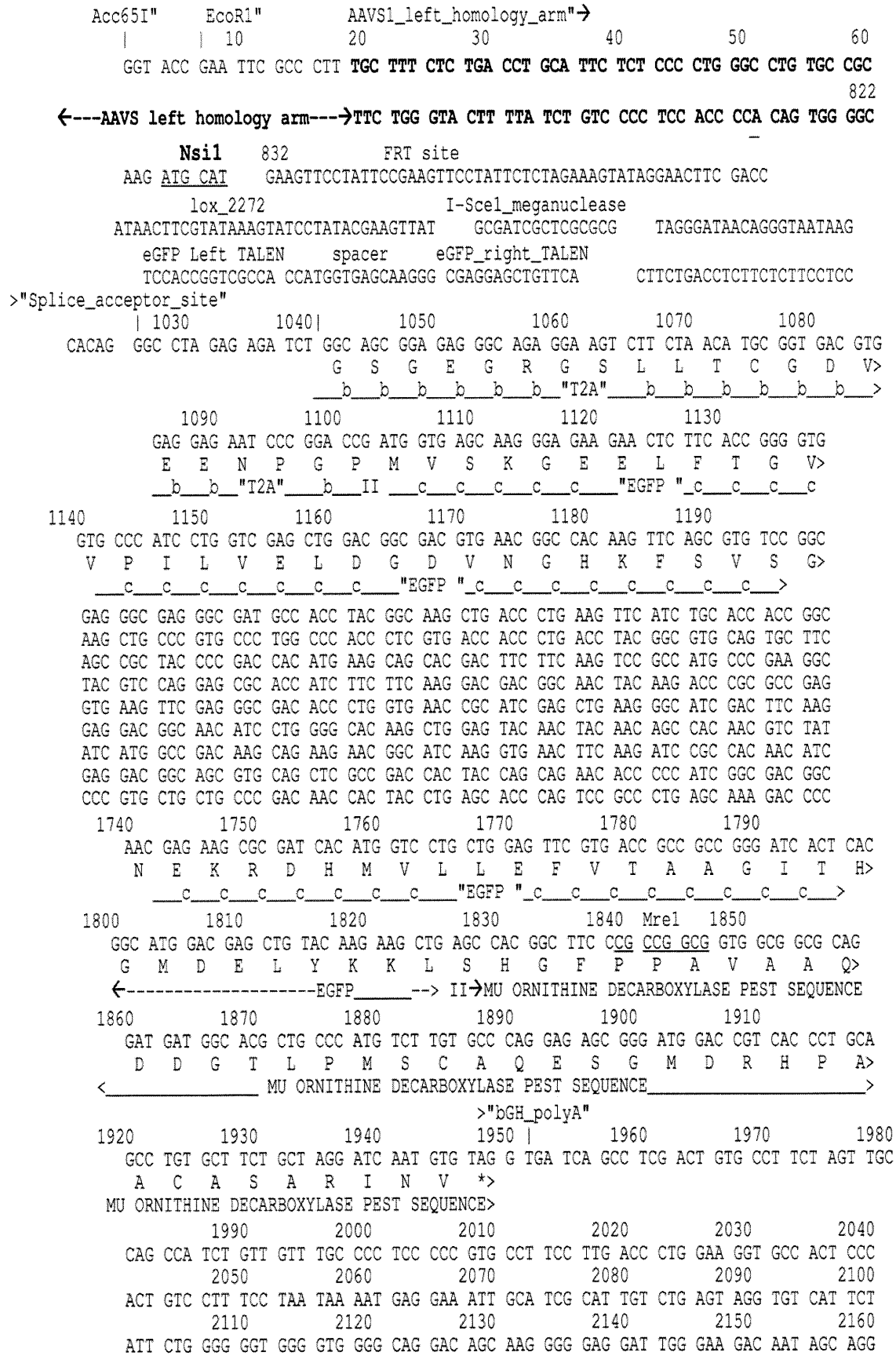

Acc65I"
EcoR1"
AAVS1_left_homology_arm"→
GGT ACC GAA TTC GCC CTT TGC TTT CTC TGA CCT GCA TTC TCT CCC CTG GGC CTG TGC CGC
822
←---AAVS left homology arm--->TTC TGG GTA CTT TTA TCT GTC CCC TCC ACC CCA CAG TGG GGC
Nsi1
832
FRT site
AAG ATG CAT
GAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC GACC
lox_2272
I-SceI_meganuclease
ATAACTTCGTATAAAGTATCCTATACGAAGTTAT
GCGATCGCTCGCGCG
TAGGGATAACAGGGTAATAAG
eGFP Left TALEN
spacer
eGFP_right_TALEN
TCCACCGGTCGCCA CCATGGTGAGCAAGGG CGAGGAGCTGTTCA
CTTCTGACCTCTTCTCTTCCTCC
>"Splice_acceptor_site"
CACAG GGC CTA GAG AGA TCT GGC AGC GGA GAG GGC AGA GGA AGT CTT CTA ACA TGC GGT GAC GTG
G S G E G R G S L L T C G D V>
"T2A"
GAG GAG AAT CCC GGA CCG ATG GTG AGC AAG GGA GAA GAA CTC TTC ACC GGG GTG
E E N P G P M V S K G E E L F T G V>
"T2A"
II
"EGFP "
GTG CCC ATC CTG GTC GAG CTG GAC GGC GAC GTG AAC GGC CAC AAG TTC AGC GTG TCC GGC
V P I L V E L D G D V N G H K F S V S G>
"EGFP "
GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC ACC ACC GGC
AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC CTG ACC TAC GGC GTG CAG TGC TTC
AGC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC GCC ATG CCC GAA GGC
TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG
GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC ATC GAC TTC AAG
GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC AAC TAC AAC AGC CAC AAC GTC TAT
ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC
GAG GAC GGC AGC GTG CAG CTC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC
CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC ACC CAG TCC GCC CTG AGC AAA GAC CCC
AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CAC
N E K R D H M V L L E F V T A A G I T H>
"EGFP "
GGC ATG GAC GAG CTG TAC AAG AAG CTG AGC CAC GGC TTC CCG CCG GCG GTG GCG GCG CAG
G M D E L Y K K L S H G F P P A V A A Q>
←----------EGFP_____--> II→MU ORNITHINE DECARBOXYLASE PEST SEQUENCE
GAT GAT GGC ACG CTG CCC ATG TCT TGT GCC CAG GAG AGC GGG ATG GAC CGT CAC CCT GCA
D D G T L P M S C A Q E S G M D R H P A>
<_____ MU ORNITHINE DECARBOXYLASE PEST SEQUENCE_____>
>"bGH_polyA"
GCC TGT GCT TCT GCT AGG ATC AAT GTG TAG G TGA TCA GCC TCG ACT GTG CCT TCT AGT TGC
A C A S A R I N V *>
MU ORNITHINE DECARBOXYLASE PEST SEQUENCE>
CAG CCA TCT GTT GTT TGC CCC TCC CCC GTG CCT TCC TTG ACC CTG GAA GGT GCC ACT CCC
ACT GTC CTT TCC TAA TAA AAT GAG GAA ATT GCA TCG CAT TGT CTG AGT AGG TGT CAT TCT
ATT CTG GGG GGT GGG GTG GGG CAG GAC AGC AAG GGG GAG GAT TGG GAA GAC AAT AGC AGG

Fig. 13

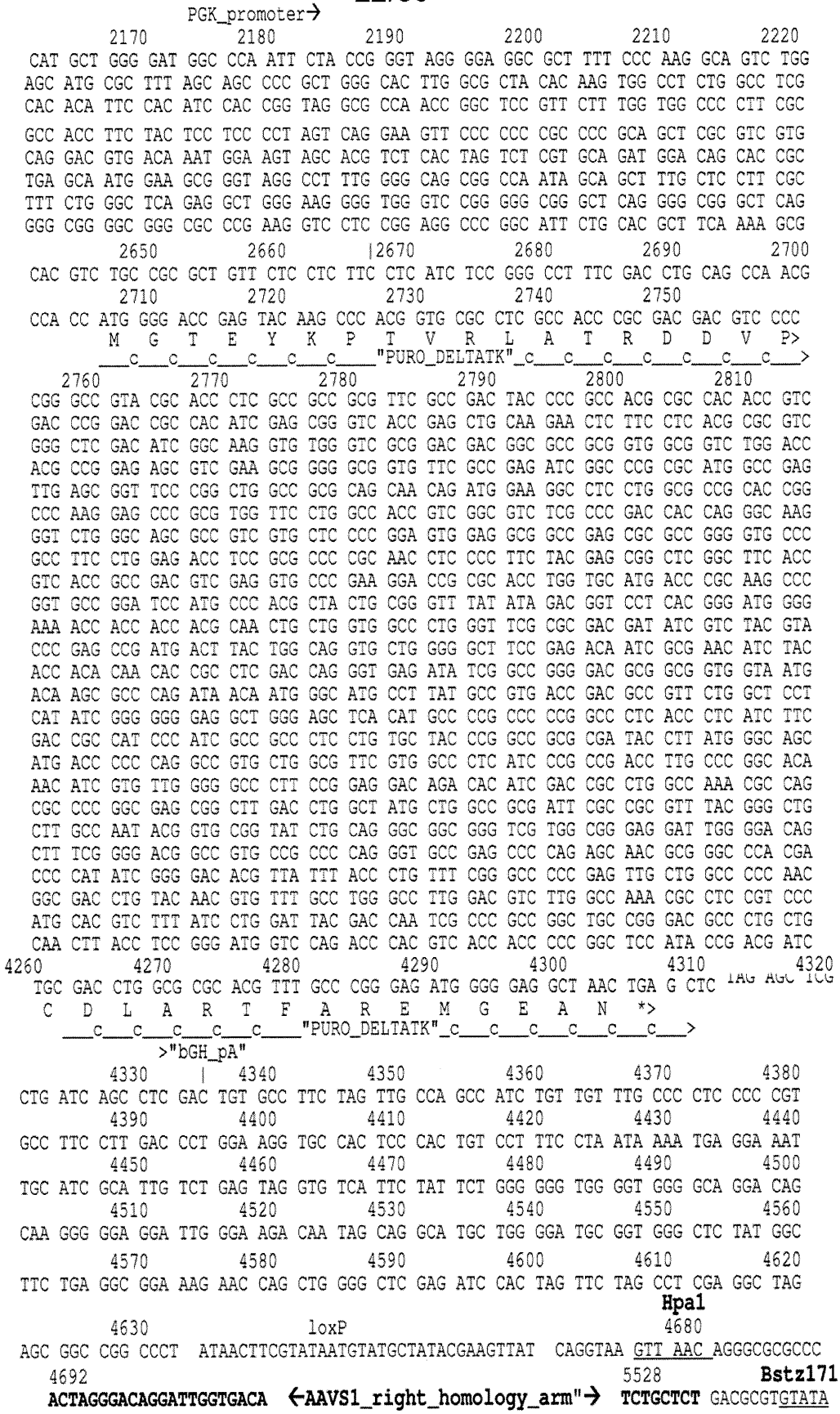



Fig. 13 (continued)

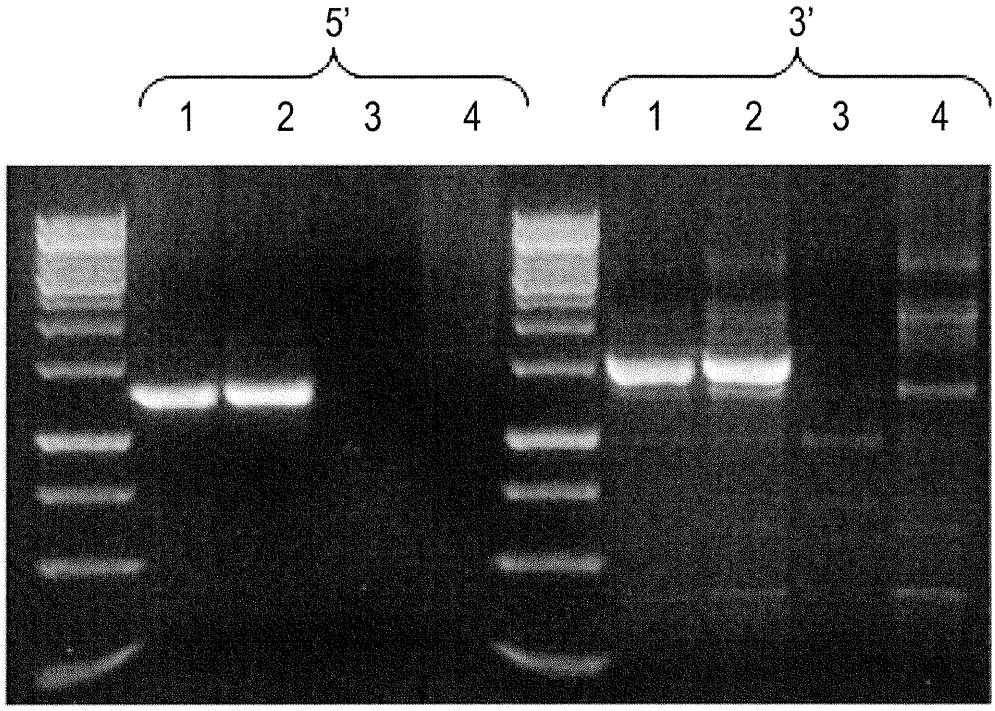



Fig. 14

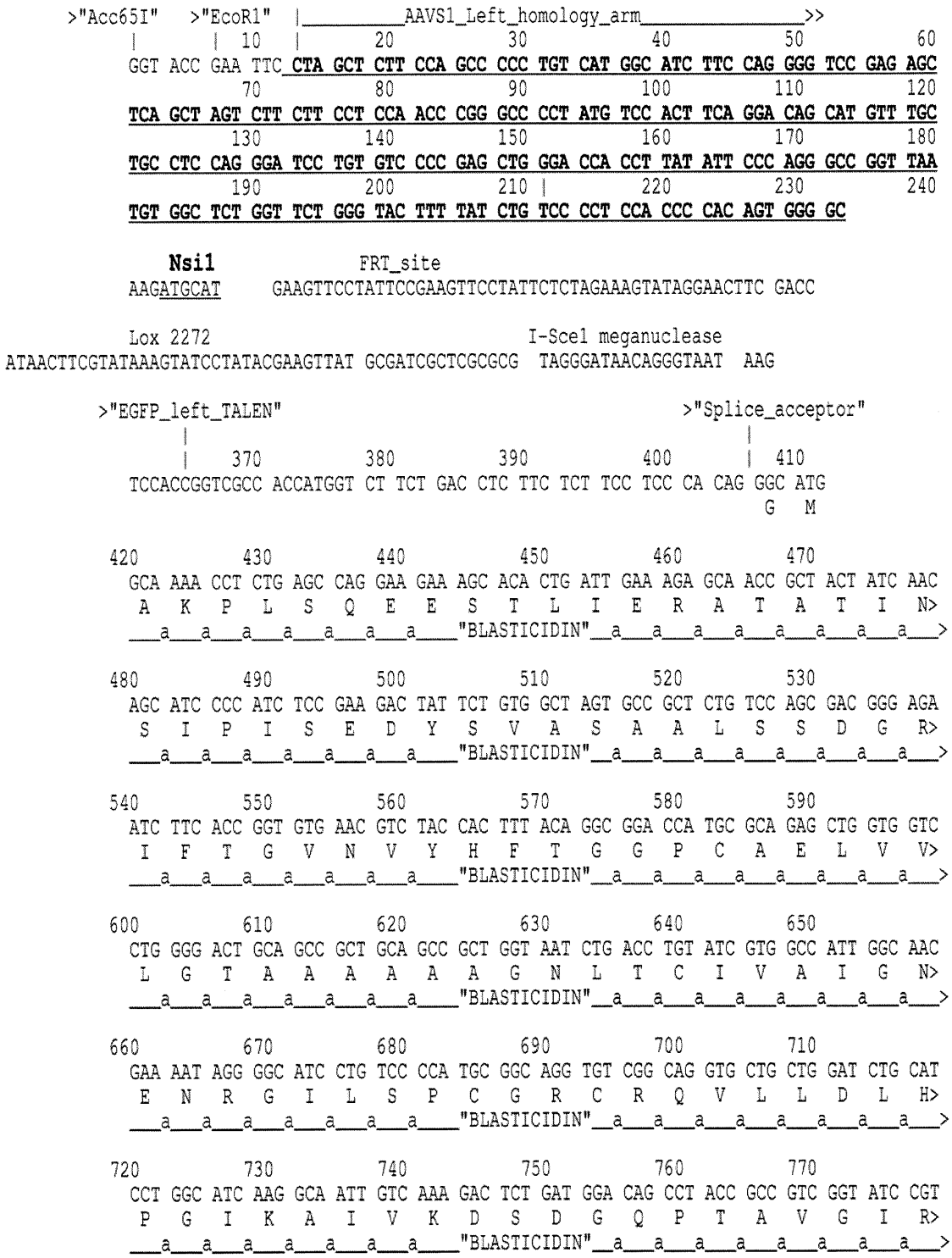

>"Acc65I"
>"EcoR1"
AAVS1_Left_homology_arm
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TCA GCT AGT CTT CTT CCT CCA ACC CGG GCC CCT ATG TCC ACT TCA GGA CAG CAT GTT TGC
TGC CTC CAG GGA TCC TGT GTC CCC GAG CTG GGA CCA CCT TAT ATT CCC AGG GCC GGT TAA
TGT GGC TCT GGT TCT GGG TAC TTT TAT CTG TCC CCT CCA CCC CAC AGT GGG GC
Nsi1
AAGATGCAT
FRT_site
GAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC GACC
Lox 2272
ATAACTTCGTATAAAGTATCCTATACGAAGTTAT
I-SceI meganuclease
GCGATCGCTCGCGCG TAGGGATAACAGGGTAAT AAG
>"EGFP_left_TALEN"
>"Splice_acceptor"
TCCACCGGTCGCC ACCATGGT CT TCT GAC CTC TTC TCT TCC TCC CA CAG GGC ATG
G M
GCA AAA CCT CTG AGC CAG GAA GAA AGC ACA CTG ATT GAA AGA GCA ACC GCT ACT ATC AAC
A K P L S Q E E S T L I E R A T A T I N>
"BLASTICIDIN"
AGC ATC CCC ATC TCC GAA GAC TAT TCT GTG GCT AGT GCC GCT CTG TCC AGC GAC GGG AGA
S I P I S E D Y S V A S A A L S S D G R>
"BLASTICIDIN"
ATC TTC ACC GGT GTG AAC GTC TAC CAC TTT ACA GGC GGA CCA TGC GCA GAG CTG GTG GTC
I F T G V N V Y H F T G G P C A E L V V>
"BLASTICIDIN"
CTG GGG ACT GCA GCC GCT GCA GCC GCT GGT AAT CTG ACC TGT ATC GTG GCC ATT GGC AAC
L G T A A A A A A G N L T C I V A I G N>
"BLASTICIDIN"
GAA AAT AGG GGC ATC CTG TCC CCA TGC GGC AGG TGT CGG CAG GTG CTG CTG GAT CTG CAT
E N R G I L S P C G R C R Q V L L D L H>
"BLASTICIDIN"
CCT GGC ATC AAG GCA ATT GTC AAA GAC TCT GAT GGA CAG CCT ACC GCC GTC GGT ATC CGT
P G I K A I V K D S D G Q P T A V G I R>
"BLASTICIDIN"

Fig. 15

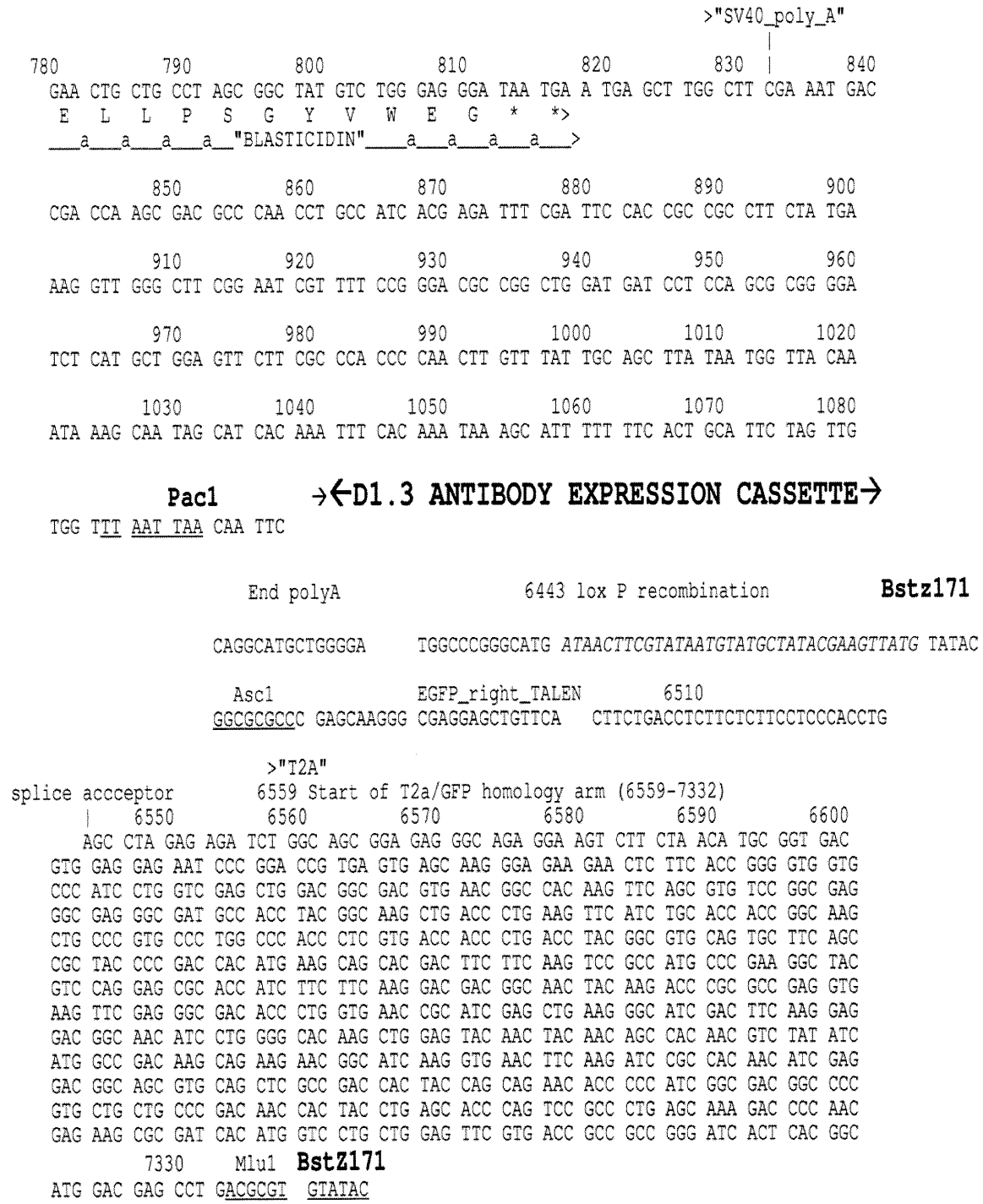



Fig. 15 (continued)

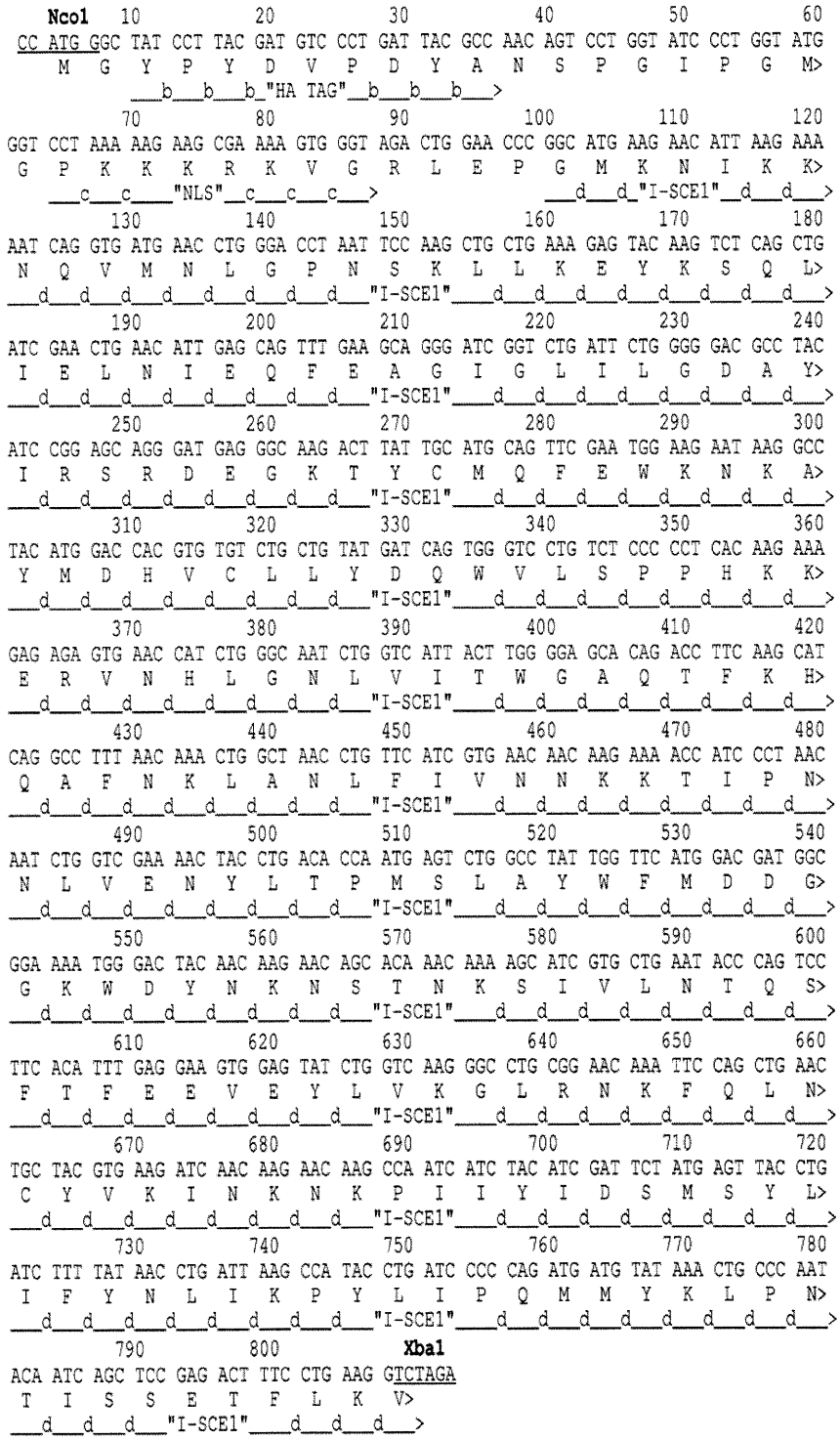
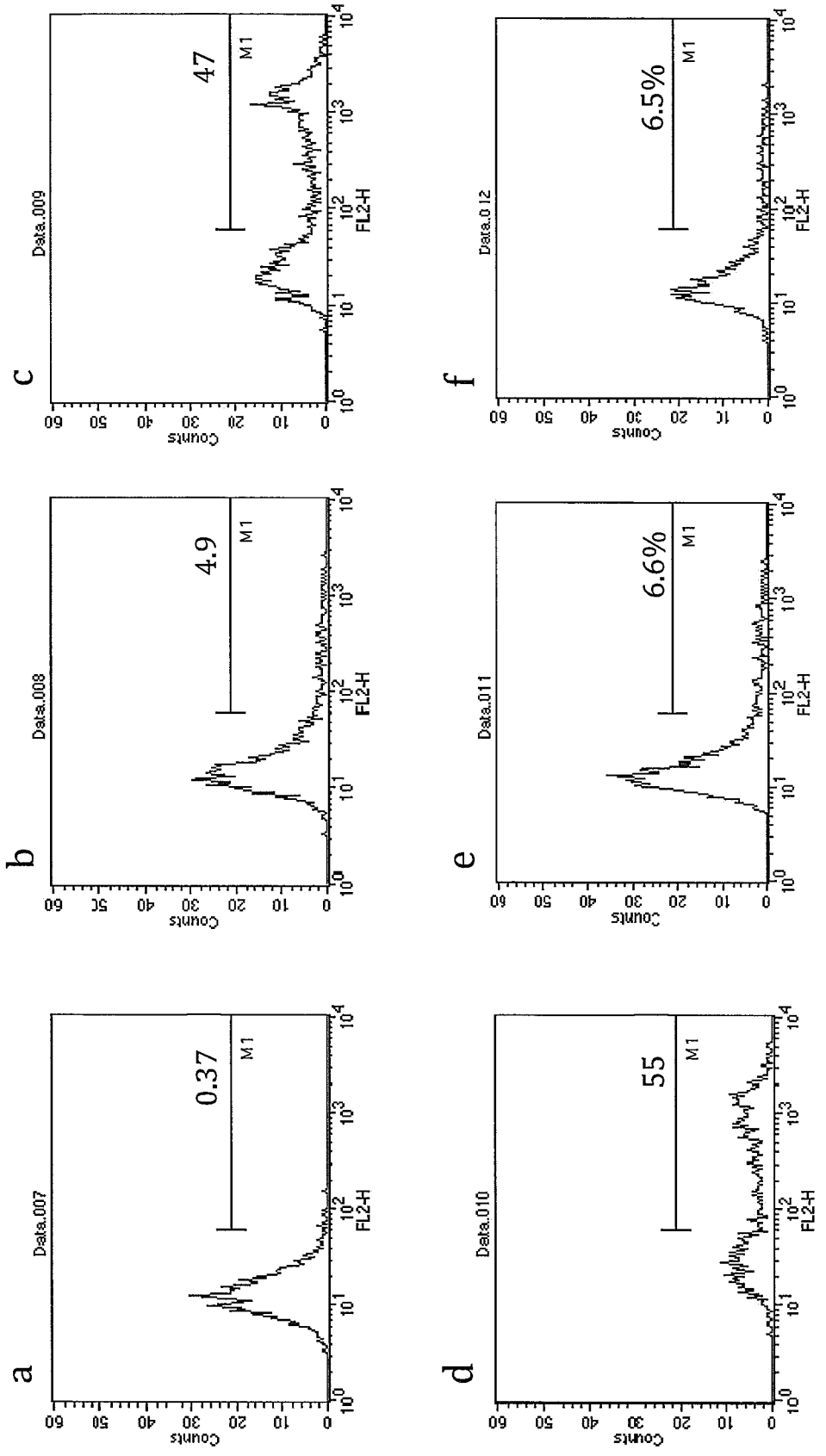

NcoI
10 20 30 40 50 60
CC ATG GGC TAT CCT TAC GAT GTC CCT GAT TAC GCC AAC AGT CCT GGT ATC CCT GGT ATG
M G Y P Y D V P D Y A N S P G I P G M>
___b___b___b_"HA TAG"__b___b___b___>
70 80 90 100 110 120
GGT CCT AAA AAG AAG CGA AAA GTG GGT AGA CTG GAA CCC GGC ATG AAG AAC ATT AAG AAA
G P K K K R K V G R L E P G M K N I K K>
___c___c____"NLS"__c___c___c___> ___d___d_"I-SCE1"__d___d___>
130 140 150 160 170 180
AAT CAG GTG ATG AAC CTG GGA CCT AAT TCC AAG CTG CTG AAA GAG TAC AAG TCT CAG CTG
N Q V M N L G P N S K L L K E Y K S Q L>
___d___d___d___d___d___d___d___d___"I-SCE1"____d___d___d___d___d___d___d___d___>
190 200 210 220 230 240
ATC GAA CTG AAC ATT GAG CAG TTT GAA GCA GGG ATC GGT CTG ATT CTG GGG GAC GCC TAC
I E L N I E Q F E A G I G L I L G D A Y>
___d___d___d___d___d___d___d___d___"I-SCE1"____d___d___d___d___d___d___d___d___>
250 260 270 280 290 300
ATC CGG AGC AGG GAT GAG GGC AAG ACT TAT TGC ATG CAG TTC GAA TGG AAG AAT AAG GCC
I R S R D E G K T Y C M Q F E W K N K A>
___d___d___d___d___d___d___d___d___"I-SCE1"____d___d___d___d___d___d___d___d___>
310 320 330 340 350 360
TAC ATG GAC CAC GTG TGT CTG CTG TAT GAT CAG TGG GTC CTG TCT CCC CCT CAC AAG AAA
Y M D H V C L L Y D Q W V L S P P H K K>
___d___d___d___d___d___d___d___d___"I-SCE1"____d___d___d___d___d___d___d___d___>
370 380 390 400 410 420
GAG AGA GTG AAC CAT CTG GGC AAT CTG GTC ATT ACT TGG GGA GCA CAG ACC TTC AAG CAT
E R V N H L G N L V I T W G A Q T F K H>
___d___d___d___d___d___d___d___d___"I-SCE1"____d___d___d___d___d___d___d___d___>
430 440 450 460 470 480
CAG GCC TTT AAC AAA CTG GCT AAC CTG TTC ATC GTG AAC AAC AAG AAA ACC ATC CCT AAC
Q A F N K L A N L F I V N N K K T I P N>
___d___d___d___d___d___d___d___d___"I-SCE1"____d___d___d___d___d___d___d___d___>
490 500 510 520 530 540
AAT CTG GTC GAA AAC TAC CTG ACA CCA ATG AGT CTG GCC TAT TGG TTC ATG GAC GAT GGC
N L V E N Y L T P M S L A Y W F M D D G>
___d___d___d___d___d___d___d___d___"I-SCE1"____d___d___d___d___d___d___d___d___>
550 560 570 580 590 600
GGA AAA TGG GAC TAC AAC AAG AAC AGC ACA AAC AAA AGC ATC GTG CTG AAT ACC CAG TCC
G K W D Y N K N S T N K S I V L N T Q S>
___d___d___d___d___d___d___d___d___"I-SCE1"____d___d___d___d___d___d___d___d___>
610 620 630 640 650 660
TTC ACA TTT GAG GAA GTG GAG TAT CTG GTC AAG GGC CTG CGG AAC AAA TTC CAG CTG AAC
F T F E E V E Y L V K G L R N K F Q L N>
___d___d___d___d___d___d___d___d___"I-SCE1"____d___d___d___d___d___d___d___d___>
670 680 690 700 710 720
TGC TAC GTG AAG ATC AAC AAG AAC AAG CCA ATC ATC TAC ATC GAT TCT ATG AGT TAC CTG
C Y V K I N K N K P I I Y I D S M S Y L>
___d___d___d___d___d___d___d___d___"I-SCE1"____d___d___d___d___d___d___d___d___>
730 740 750 760 770 780
ATC TTT TAT AAC CTG ATT AAG CCA TAC CTG ATC CCC CAG ATG ATG TAT AAA CTG CCC AAT
I F Y N L I K P Y L I P Q M M Y K L P N>
___d___d___d___d___d___d___d___d___"I-SCE1"____d___d___d___d___d___d___d___d___>
790 800 XbaI
ACA ATC AGC TCC GAG ACT TTC CTG AAG GTCTAGA
T I S S E T F L K V>
___d___d___d___"I-SCE1"____d___d___d___>

Fig. 16

Figure 17


a
Data.007
0.37
M1
Counts
FL2-H
b
Data.008
4.9
M1
Counts
FL2-H
c
Data.009
47
M1
Counts
FL2-H
d
Data.010
55
M1
Counts
FL2-H
e
Data.011
6.6%
M1
Counts
FL2-H
f
Data.012
6.5%
M1
Counts
FL2-H

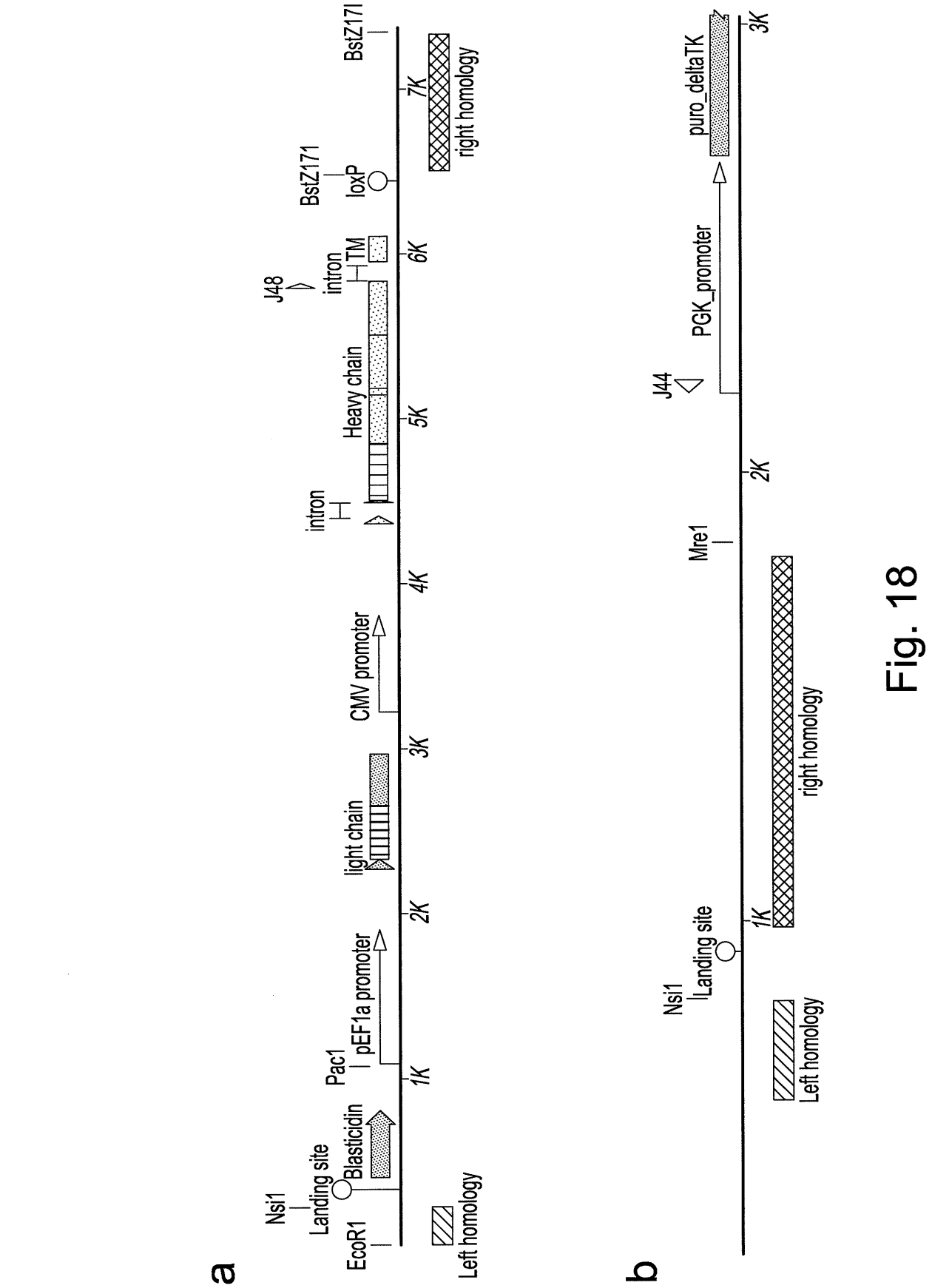

a
Nsi1
Landing site
EcoR1
Blasticidin
Pac1
pEF1a promoter
light chain
CMV promoter
intron
Heavy chain
J48
intron
TM
BstZ171
loxP
BstZ17I
Left homology
right homology
1K
2K
3K
4K
5K
6K
7K
b
Nsi1
Landing site
Left homology
Mre1
right homology
J44
PGK_promoter
puro_deltaTK
1K
2K
3K

Fig. 18

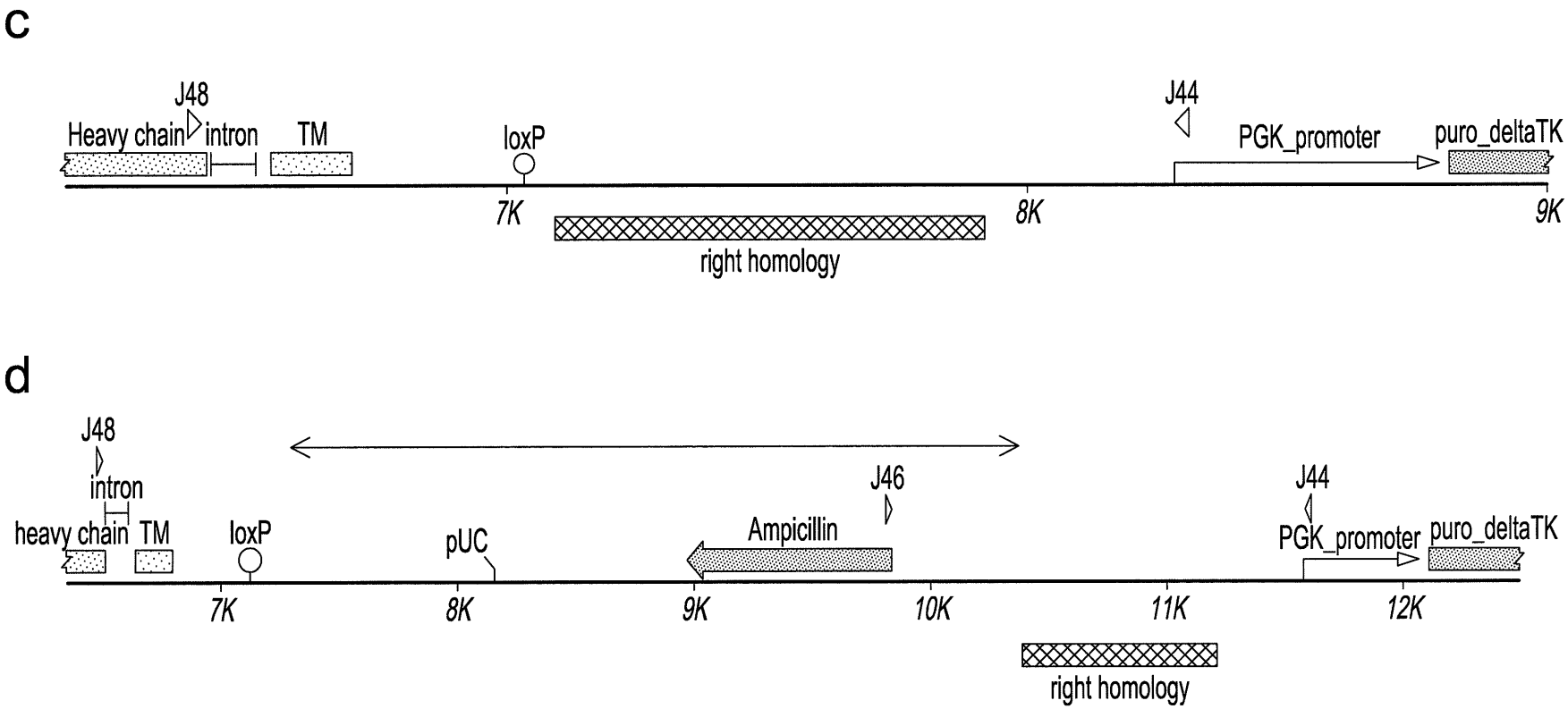





Fig. 18
(continued)

e

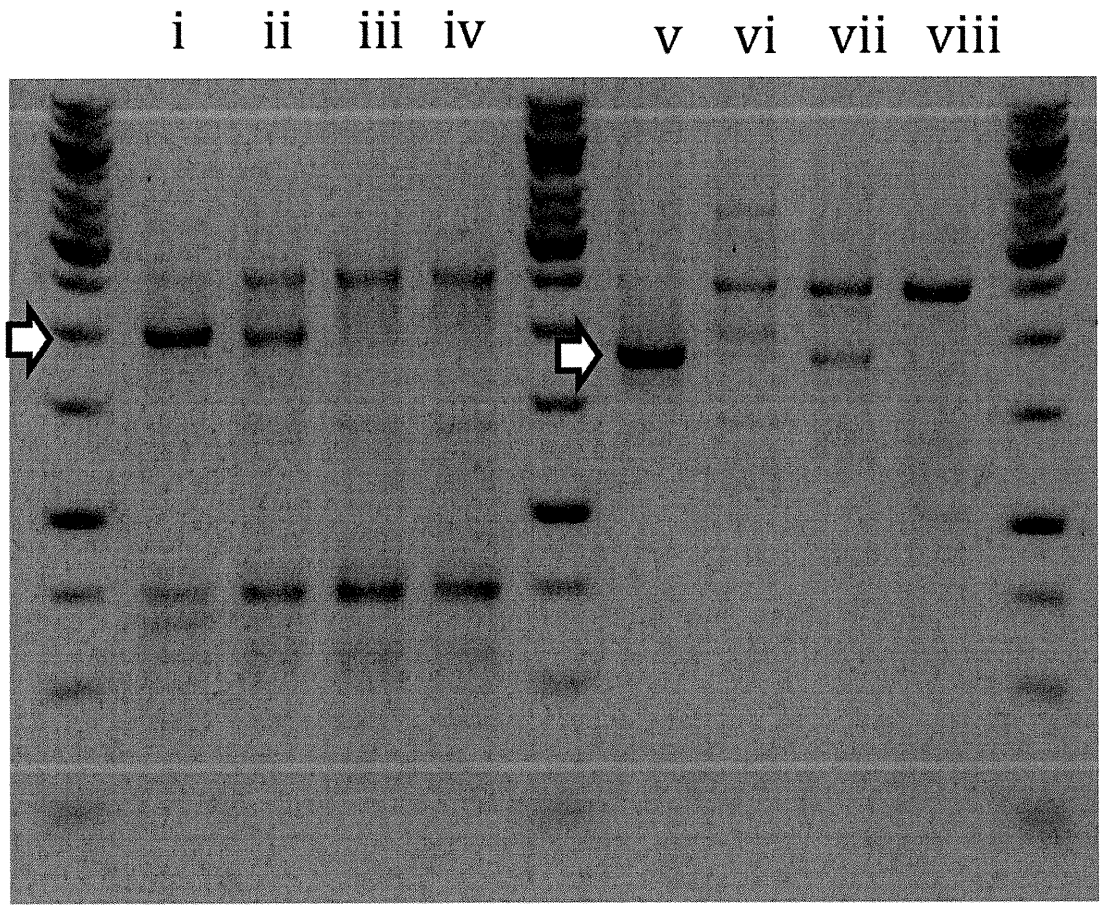
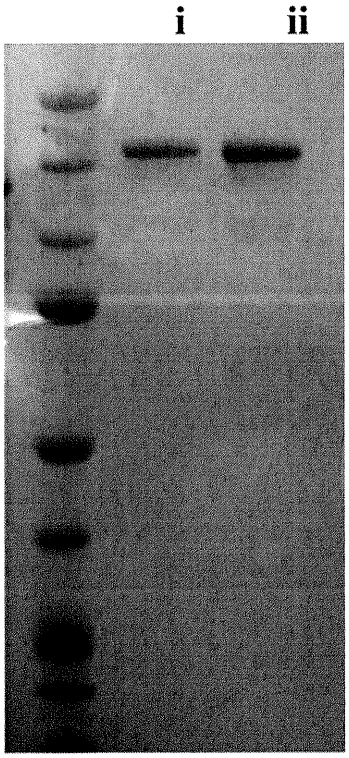



Fig. 18
(continued)

a


i
ii

b

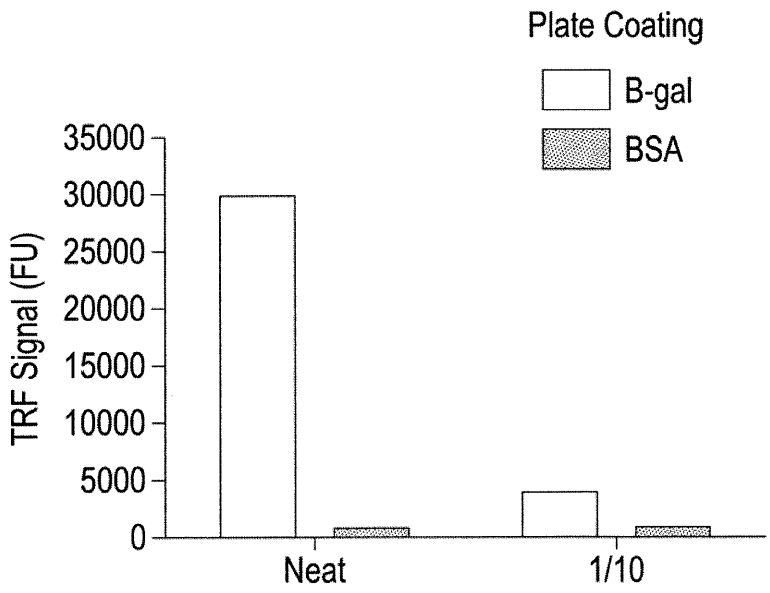

Plate Coating
B-gal
BSA
35000
30000
25000
20000
15000
10000
5000
0
TRF Signal (FU)
Neat
1/10

Fig. 19

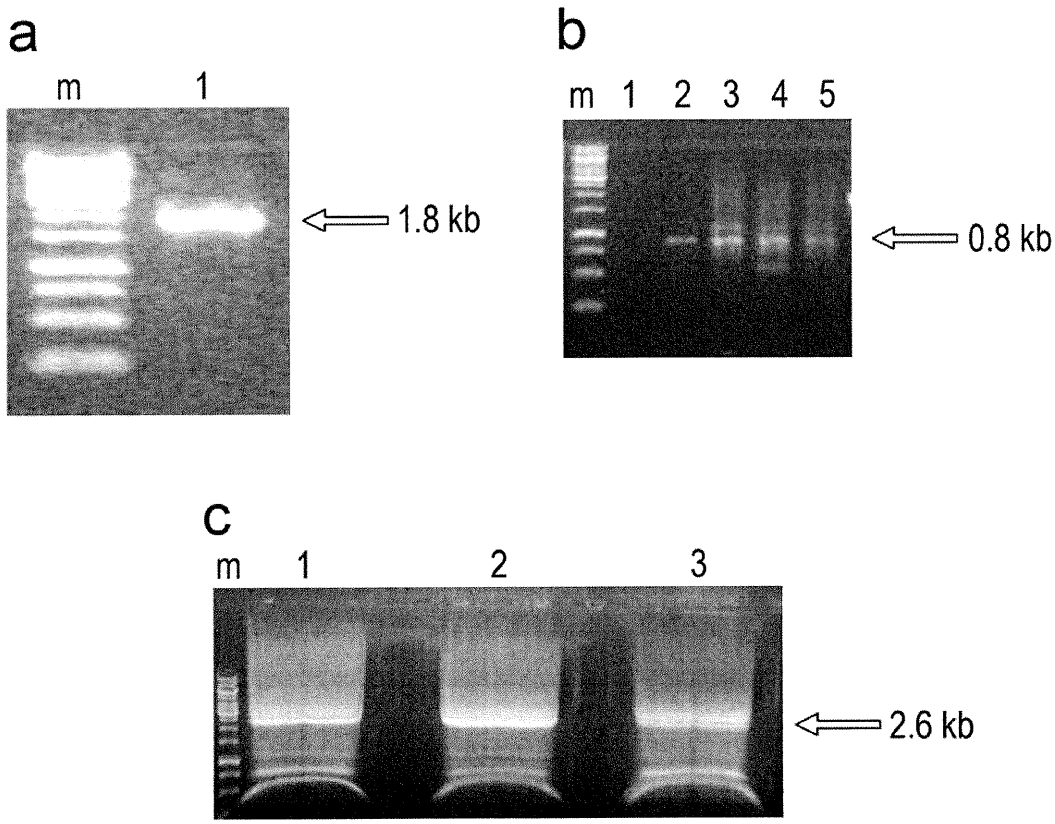



Fig. 20

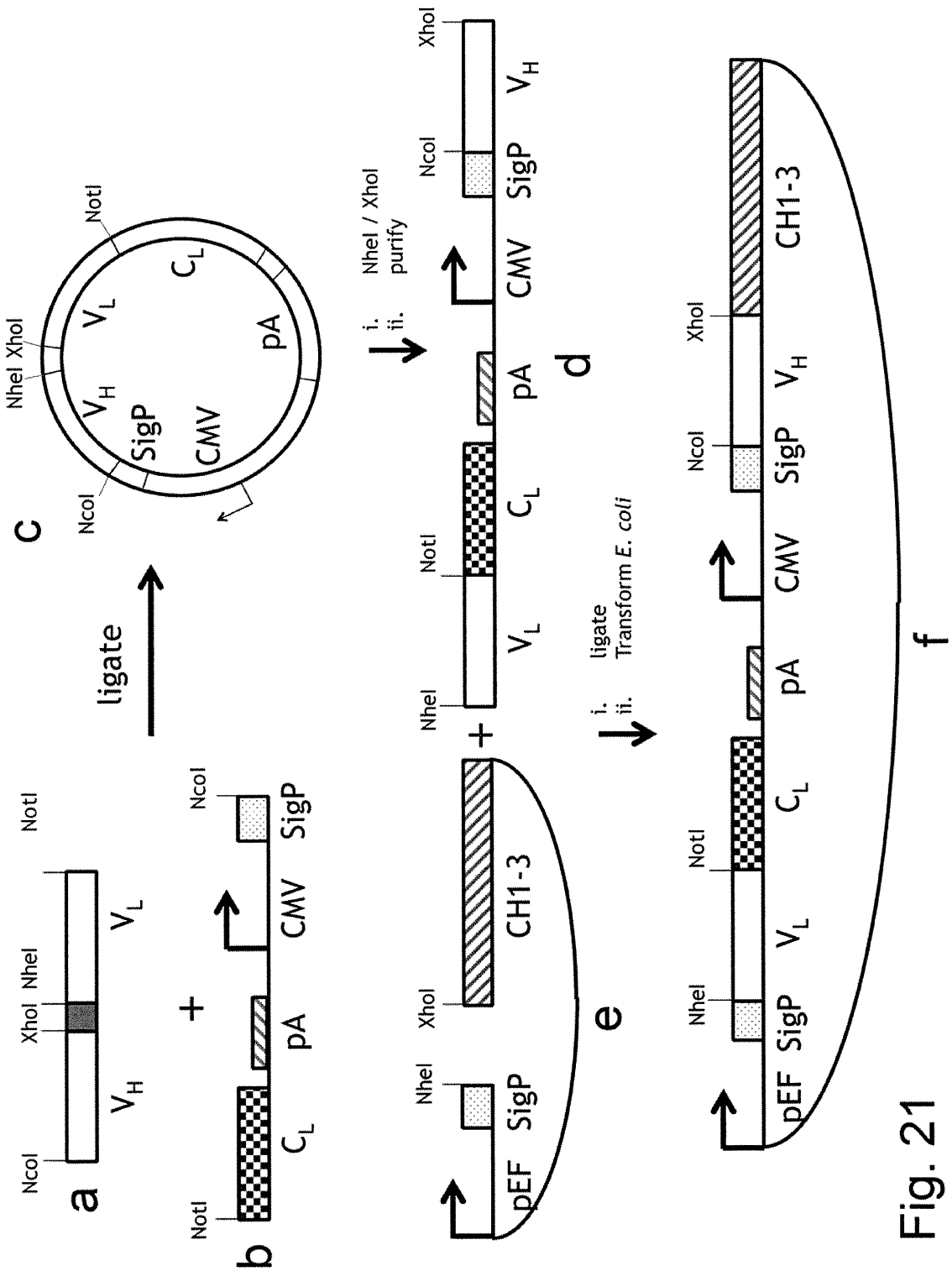
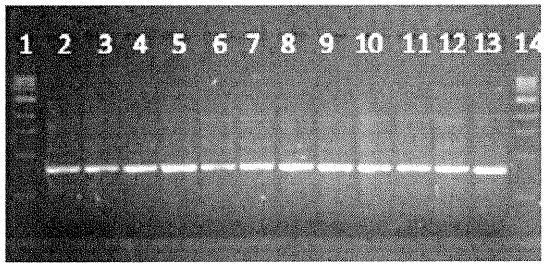

a
NcoI
NheI
XhoI
NotI
$V_H$
$V_L$
b
NotI
$C_L$
pA
CMV
SigP
NcoI
+
ligate
c
NcoI
NheI
XhoI
NotI
$V_H$
$V_L$
SigP
CMV
$C_L$
pA
d
i. NheI / XhoI
ii. purify
NheI
NotI
$V_L$
$C_L$
pA
CMV
SigP
NcoI
XhoI
$V_H$
+
e
NheI
XhoI
pEF
SigP
CH1-3
i. ligate
ii. Transform *E. coli*
f
NheI
NotI
pEF
SigP
$V_L$
$C_L$
pA
CMV
SigP
NcoI
XhoI
$V_H$
CH1-3

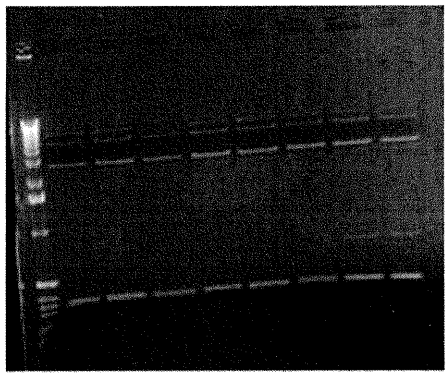
Fig. 21

a



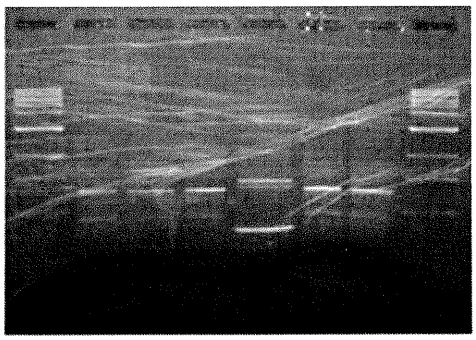

⇐ scFv (~780 bp)

b



⇐ DNA product d (Fig. 21) - 2.7 kb

c



⇐ DNA product d (Fig. 21) 1.4 kb

Fig. 22

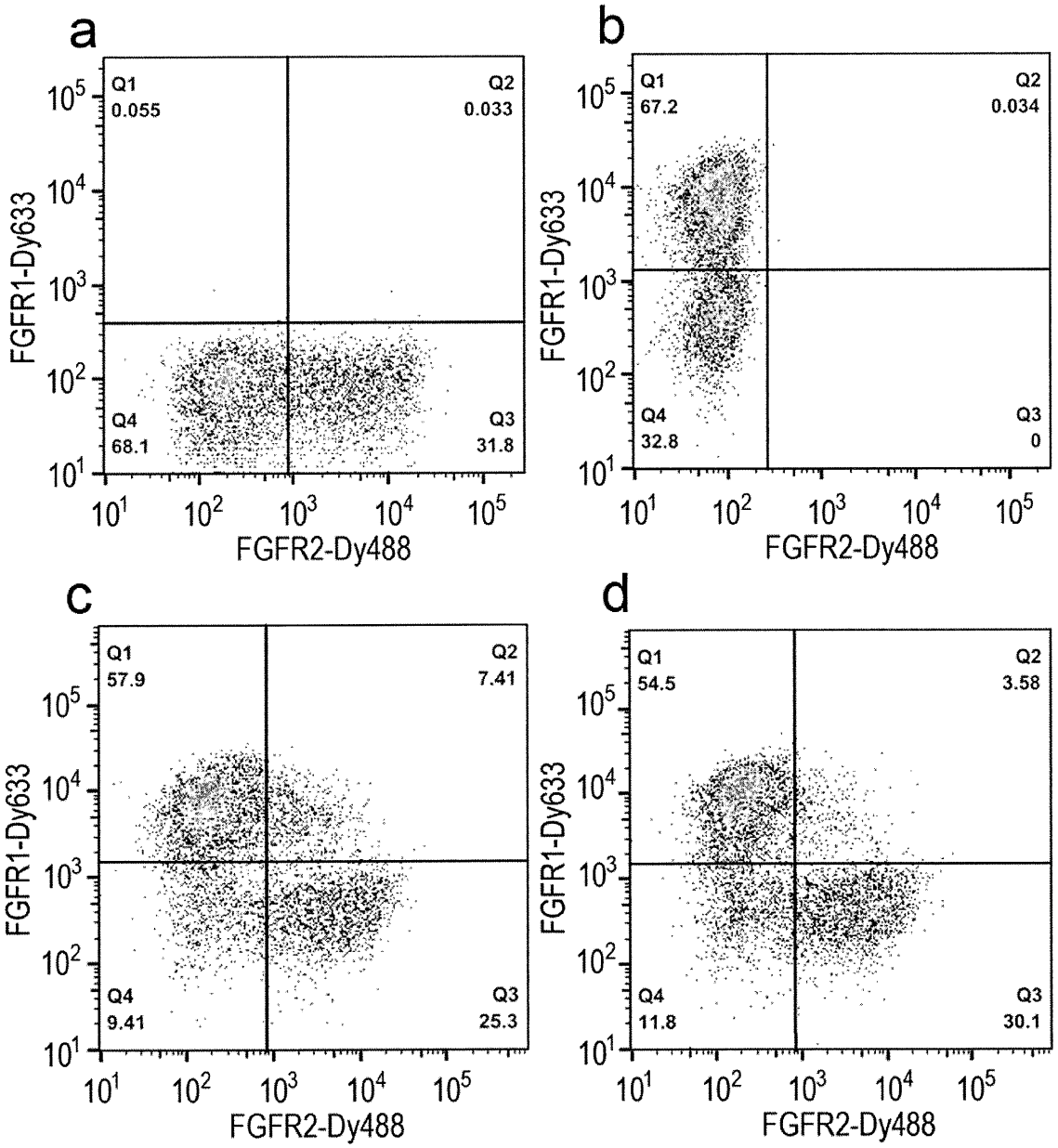

a
Q1
0.055
Q2
0.033
Q4
68.1
Q3
31.8
FGFR1-Dy633
FGFR2-Dy488
b
Q1
67.2
Q2
0.034
Q4
32.8
Q3
0
FGFR1-Dy633
FGFR2-Dy488
c
Q1
57.9
Q2
7.41
Q4
9.41
Q3
25.3
FGFR1-Dy633
FGFR2-Dy488
d
Q1
54.5
Q2
3.58
Q4
11.8
Q3
30.1
FGFR1-Dy633
FGFR2-Dy488

Fig. 23

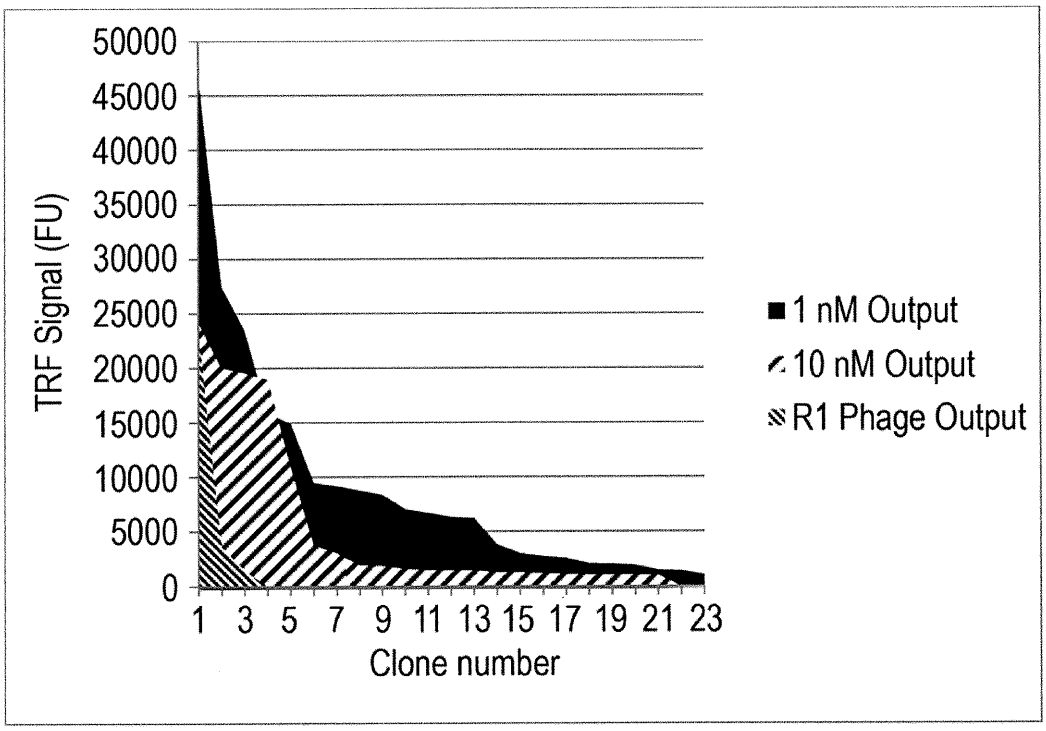
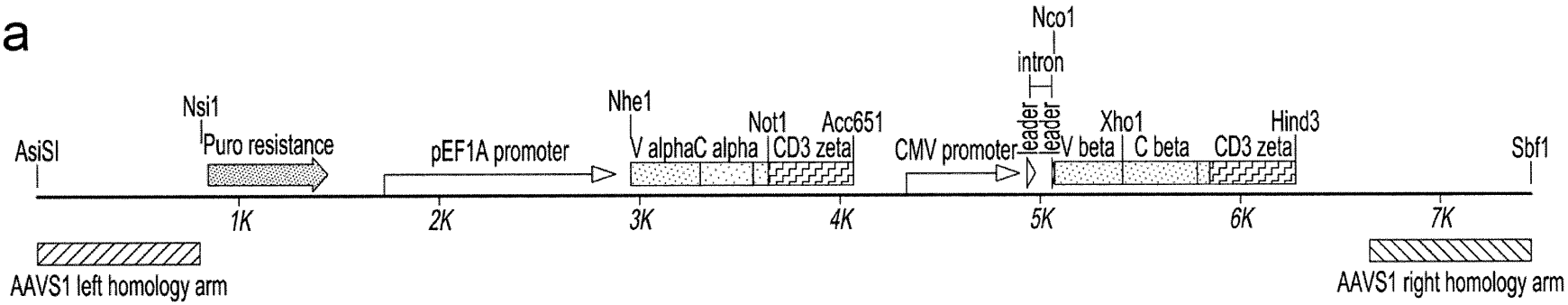

50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
TRF Signal (FU)
1 3 5 7 9 11 13 15 17 19 21 23
Clone number
1 nM Output
10 nM Output
R1 Phage Output

Fig. 24

a






b

```
                          Nsi1    Splice_acceptor
                           |  820     |  830         840         | 850         860
A TGC ATC TTC TGA CCT CTT CTC TTC CTC CCA CAG GGC ATG ACC GAG TAC AAG CCC ACG GTG CGC CTC
                                                   M   T   E   Y   K   P   T   V   R   L >
                                                      ___" PURO RESISTANCE_____>
```

Fig. 25

C

gctagcaagcaggaagtgactcagatcccagccgctctgagcgtgcctgagggagaaaac
**Nhe1** K Q E V T Q I P A A L S V P E G E N
ctggtcctgaattgcagtttcaccgactcagccatctataacctgcagtggtttcgccag
L V L N C S F T D S A I Y N L Q W F R Q
gatccaggcaagggactgacctccctgctgctgattcagagctcccagagggaacagaca
D P G K G L T S L L L I Q S S Q R E Q T
tctggcagactgaatgctagtctggacaaatctagtggacggtctaccctgtacatcgca
S G R L N A S L D K S S G R S T L Y I A
gccagccagcctggagattccgcaacatatctgtgcgccgtgcgcccacttacaggcgga
A S Q P G D S A T Y L C A V R P L T G G
agctacattcccaccttcgggcgaggtacaagcctgatcgtgcacccagacatccagaat
S Y I P T F G R G T S L I V H P D I Q N
ccggagcccgccgtataccagctgaaggaccccagaagccaggacagcaccctgtgcctg
P E P A V Y Q L K D P R S Q D S T L C L
ttcaccgacttcgacagccagatcaacgtgcccaagacaatggaaagcggcaccttcatc
F T D F D S Q I N V P K T M E S G T F I
accgacaagaccgtgctggacatgaaggctatggacagcaagagcaacggcgccattgcc
T D K T V L D M K A M D S K S N G A I A
tggtccaaccagaccagcttcacatgccaggacatcttcaaagagacaaacgccacctac
W S N Q T S F T C Q D I F K E T N A T Y
cccagcagcgacgtgccctgtgatgccaccctgaccgagaagtccttcgagacagacatg
P S S D V P C D A T L T E K S F E T D M
Aacctgaacttccagaacctgtcc<u>gcggccgc</u>aggcctgctggatcccaagctgtgctac
**Not1**
N L N F Q N L S A A A G L L D P K L C Y
ctgctggacgggatcctgttcatctacggtgtgatcctgactgccctgttcctgcgagtc
L L D G I L F I Y G V I L T A L F L R V
aaattttctcggagtgccgacgctcctgcataccagcaggggcagaaccagctgtataac
K F S R S A D A P A Y Q Q G Q N Q L Y N
gagctgaatctgggtcggagggaggaatatgacgtgctggataagagacgcggcagggat
E L N L G R R E E Y D V L D K R R G R D
ccagaaatgggggcaagccccagcgacggaaaaaccctcaggagggactgtataatgaa
P E M G G K P Q R R K N P Q E G L Y N E
ctgcagaaggacaaaatggccgaggcttactctgaaattgggatgaagggcgagaggaga
L Q K D K M A E A Y S E I G M K G E R R
cgcggcaaaggacacgatggcctgtaccagggactgagcactgctaccaaggacacatat
R G K G H D G L Y Q G L S T A T K D T Y
gatgctctgcatatgcaggcactgcccctagataataa<u>ggtacc</u>
D A L H M Q A L P P R - - **Acc65I**

Fig. 25

d

ccatggccaacgctggagtgactcagacccctaagttccaggtcctgaaaactgggcag
**Nco** M A N A G V T Q T P K F Q V L K T G Q
agtatgaccctgcagtgcgcacaggacatgaatcacgagtacatgtcatggtatcggcag
S M T L Q C A Q D M N H E Y M S W Y R Q
gatccagggatgggtctgaggctgatccattacagcgtgggcgctggaactaccgaccag
D P G M G L R L I H Y S V G A G T T D Q
ggcgaggtgcccaacggatataatgtctcaagaagcaccacagaagatttcccactgcga
G E V P N G Y N V S R S T T E D F P L R
ctgctgagcgccgctcctagccagacatccgtgtacttttgcgccagctccaatgtcggg
L L S A A P S Q T S V Y F C A S S N V G
aacaccggcgagctgttctttggggaaggttcccgcctgacagtgctcgaggacctgaga
N T G E L F F G E G S R L T V L E D L R
**Xho1**
aacgtgacccccccccaaggtgtccctgttcgagcctagcaaggccgagatcgccaacaag
N V T P P K V S L F E P S K A E I A N K
cagaaagccaccctcgtgtgcctggccagaggcttcttccccgaccacgtggaactgtct
Q K A T L V C L A R G F F P D H V E L S
tggtgggtcaacggcaaagaggtgcacagcggcgtgtccaccgatccccaggcctacaaa
W W V N G K E V H S G V S T D P Q A Y K
gagagcaactacagctactgcctgagcagcagactgcgggtgtccgccaccttctggcac
E S N Y S Y C L S S R L R V S A T F W H
aacccccggaaccacttcagatgccaggtgcagtttcacggcctgagcgaagaggacaag
N P R N H F R C Q V Q F H G L S E E D K
tggcccgagggcagccctaagcccgtgacccagaatatctctgccgaagcctggggcaga
W P E G S P K P V T Q N I S A E A W G R
gccgactgtggcattaccagcgccagctaccagcagggcgtgctgtctgccaccatcctg
A D C G I T S A S Y Q Q G V L S A T I L
tacgaggtcgcgagcggactgctggacccaaagctgtgctacctgctggatgggatcctg
Y E V A S G L L D P K L C Y L L D G I L
ttcatctacggtgtgattctgacagccctgttcctgcgagtcaagttcagccggagcgcc
F I Y G V I L T A L F L R V K F S R S A
gacgcaccagcataccagcaggggcagaatcagctgtataacgagctgaatctgggtcgg
D A P A Y Q Q G Q N Q L Y N E L N L G R
agggaggaatacgacgtgctggataagagacgcggcagggatcccgaaatgggcggaaag
R E E Y D V L D K R R G R D P E M G G K
cctcagcgacggaaaaacccacaggagggactgtacaatgaactgcagaaggacaaaatg
P Q R R K N P Q E G L Y N E L Q K D K M
gctgaggcatattctgaaatcggcatgaagggagagaggagacgcggcaaaggacacgat
A E A Y S E I G M K G E R R R G K G H D
gggctgtaccagggtctgagtacagccactaaggacacctatgatgccctgcatatgcag
G L Y Q G L S T A T K D T Y D A L H M Q
gctctgccacccagataataaaagctt
A L P P R - - **Hind 3**

Fig. 25 (continued)

e

gctagccagaaagaggtggaacagaacagcggccctctgagcgtgccagaaggcgctatc
Nhe1 Q K E V E Q N S G P L S V P E G A I
gccagcctgaactgcacctacagctttctgggcagccagagcttcttctggtacagacag
A S L N C T Y S F L G S Q S F F W Y R Q
tacagcggcaagagccccgagctgatcatgttcacctacagagagggcgacaaagaggac
Y S G K S P E L I M F T Y R E G D K E D
ggcagattcaccgcccagctgaacaaggccagccagcacgtgtccctgctgatcagagac
G R F T A Q L N K A S Q H V S L L I R D
agccagcctagcgacagcgccacctacctgtgcgccgtgaatgatggcggcagactgacc
S Q P S D S A T Y L C A V N D G G R L T
tttggcgacggcaccaccctgaccgtgaagcctgacatccagaatccggagcccgccgta
F G D G T T L T V K P D I Q N P E P A V
taccagctgaaggaccccagaagccaggacagcaccctgtgcctgttcaccgacttcgac
Y Q L K D P R S Q D S T L C L F T D F D
agccagatcaacgtgcccaagacaatggaaagcggcaccttcatcaccgacaagaccgtg
S Q I N V P K T M E S G T F I T D K T V
ctggacatgaaggctatggacagcaagagcaacggcgccattgcctggtccaaccagacc
L D M K A M D S K S N G A I A W S N Q T
agcttcacatgccaggacatcttcaaagagacaaacgccacctaccccagcagcgacgtg
S F T C Q D I F K E T N A T Y P S S D V
ccctgtgatgccaccctgaccgagaagtccttcgagacagacatgaacctgaacttccag
P C D A T L T E K S F E T D M N L N F Q
aacctgtccgcggccgc
N L S Not1

f

Nco1
ccatggccagccagaccatccatcagtggcctgccaccctggtgcagcctgtgggatct
M A S Q T I H Q W P A T L V Q P V G S
cctctgagcctggaatgcaccgtggaaggcaccagcaaccccaacctgtactggtacaga
P L S L E C T V E G T S N P N L Y W Y R
caggccgctggcagaggcccccagctgctgttttactggggccccttggccagatcagc
Q A A G R G P Q L L F Y W G P F G Q I S
agcgaggtgccccagaacctgagcgccagcagaccccaggaccggcagtttatcctgagc
S E V P Q N L S A S R P Q D R Q F I L S
agcaagaagctgctgctgagcgacagcggcttctacctgtgcgcttggagcgagacaggc
S K K L L L S D S G F Y L C A W S E T G
ctgggcatgggcggatggcagtttggcgagggcagcagactgacagtgctcgag
L G M G G W Q F G E G S R L T V L E
Xho1

Fig. 25 (continued)

g

```
5′ C ACG CGG CAC GCG GGT GAA NNS NNS CCT WCN ATG TAA GGG TGG AAG CCC GCTC
   C ACG CGG CAC GCG GGT GAA NNS NNS CCT WGN ATG TAA GGG TGG AAG CCC GCTC 5′
     C   A   V   R   P   L   X   X   G  S/T  Y   I   P   T   F   G   R
        ------------------------CDR3"-------------------------
```

h

```
5′ G TAC TTT TGC GCC AGC TCC NNS STC GGG NNS ACC GGC GAG CTG TTC TTTG
   C ATG AAA ACG CGG TCG AGG NNS SAG CCC NNS TGG CCG CTC GAC AAG AAAC 5′
     Y   F   C   A   S   S   N  V/L  G   N   T   G   E   L   F   F
                            --------------CDR3---------------
```

N=A,C,G or T
S= C OR G
W= A or T

Fig. 25 (continued)

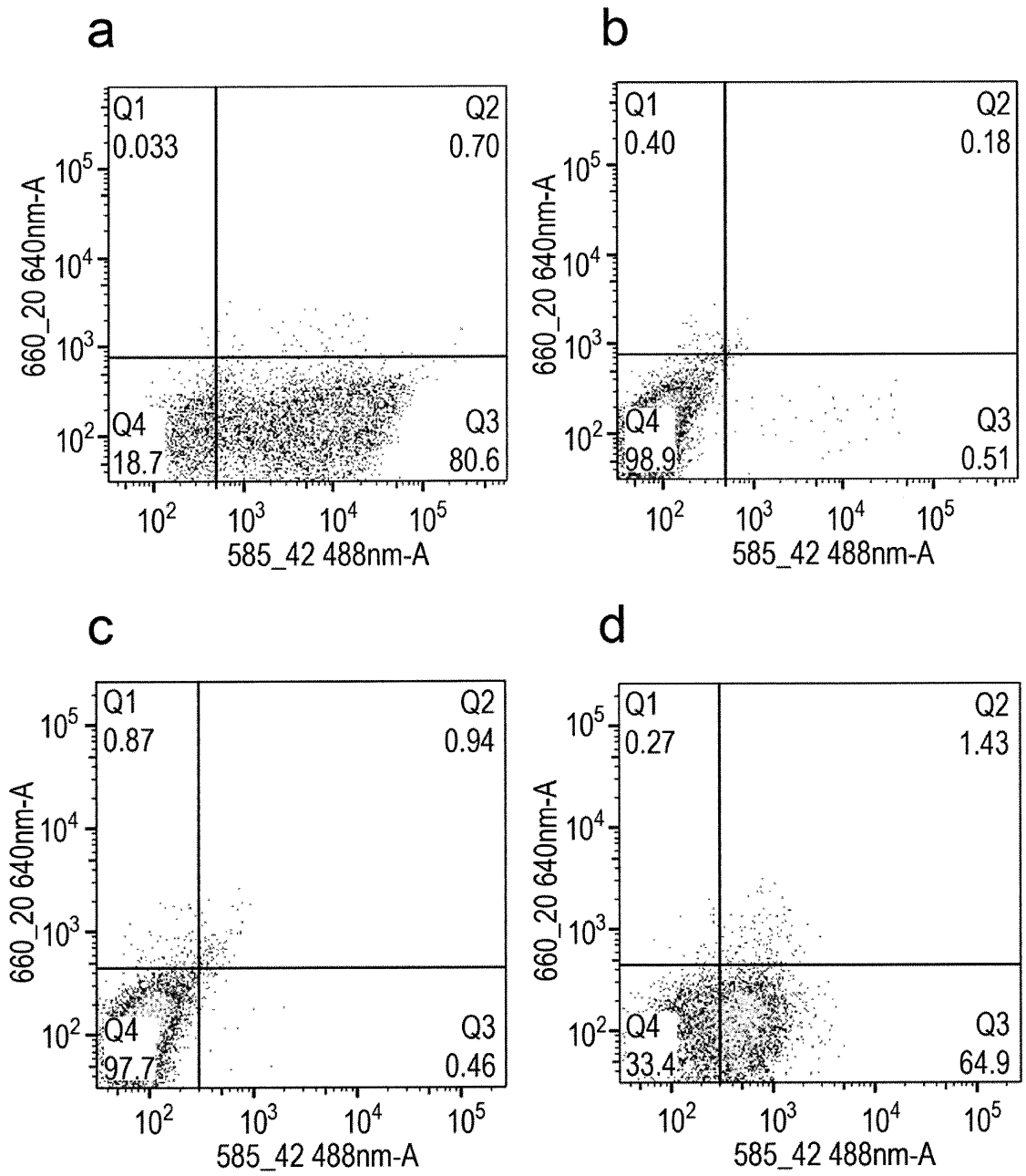

a
Q1
0.033
Q2
0.70
Q4
18.7
Q3
80.6
660_20 640nm-A
585_42 488nm-A
b
Q1
0.40
Q2
0.18
Q4
98.9
Q3
0.51
660_20 640nm-A
585_42 488nm-A
c
Q1
0.87
Q2
0.94
Q4
97.7
Q3
0.46
660_20 640nm-A
585_42 488nm-A
d
Q1
0.27
Q2
1.43
Q4
33.4
Q3
64.9
660_20 640nm-A
585_42 488nm-A

Fig. 26

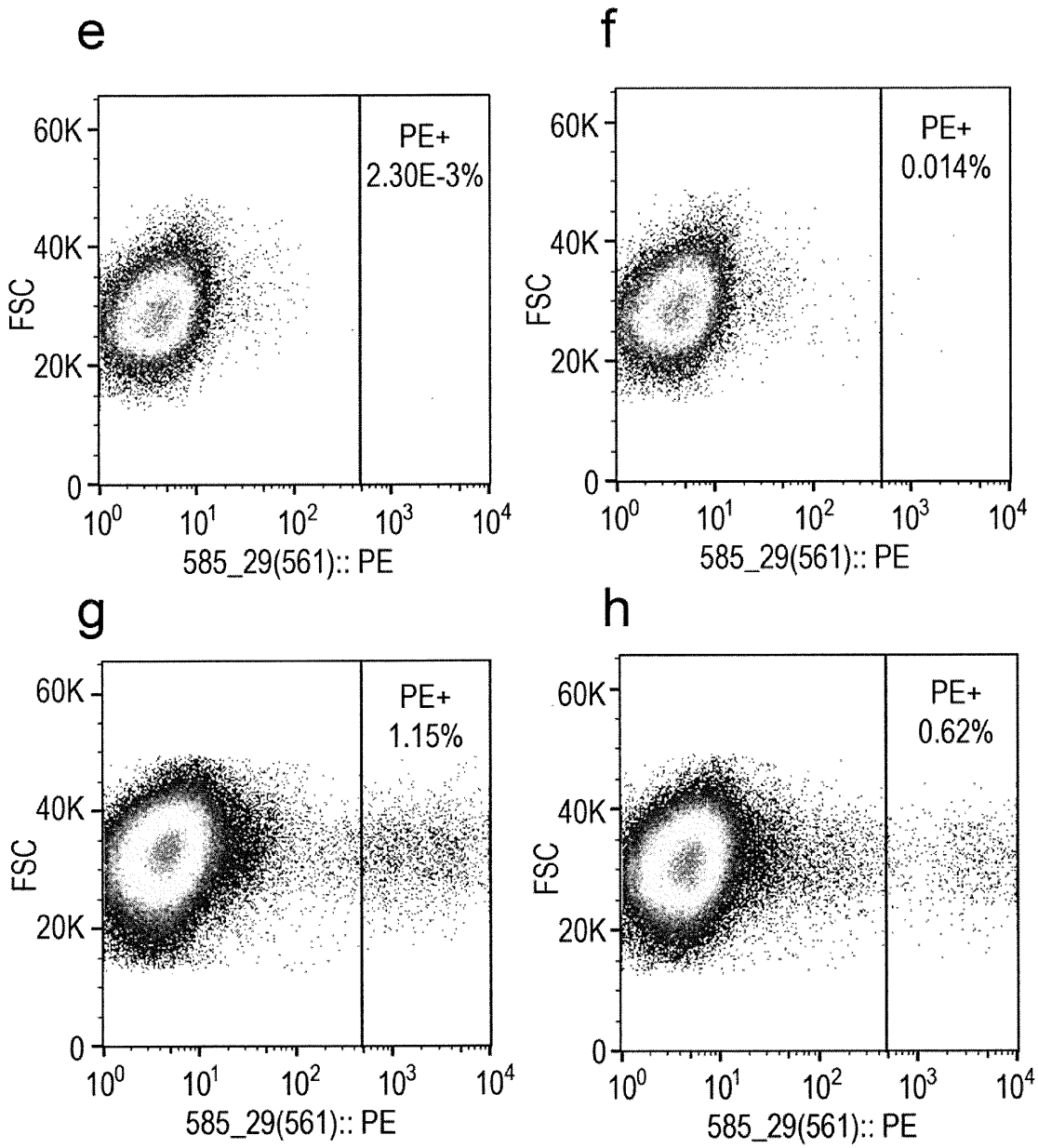



Fig. 26
(continued)

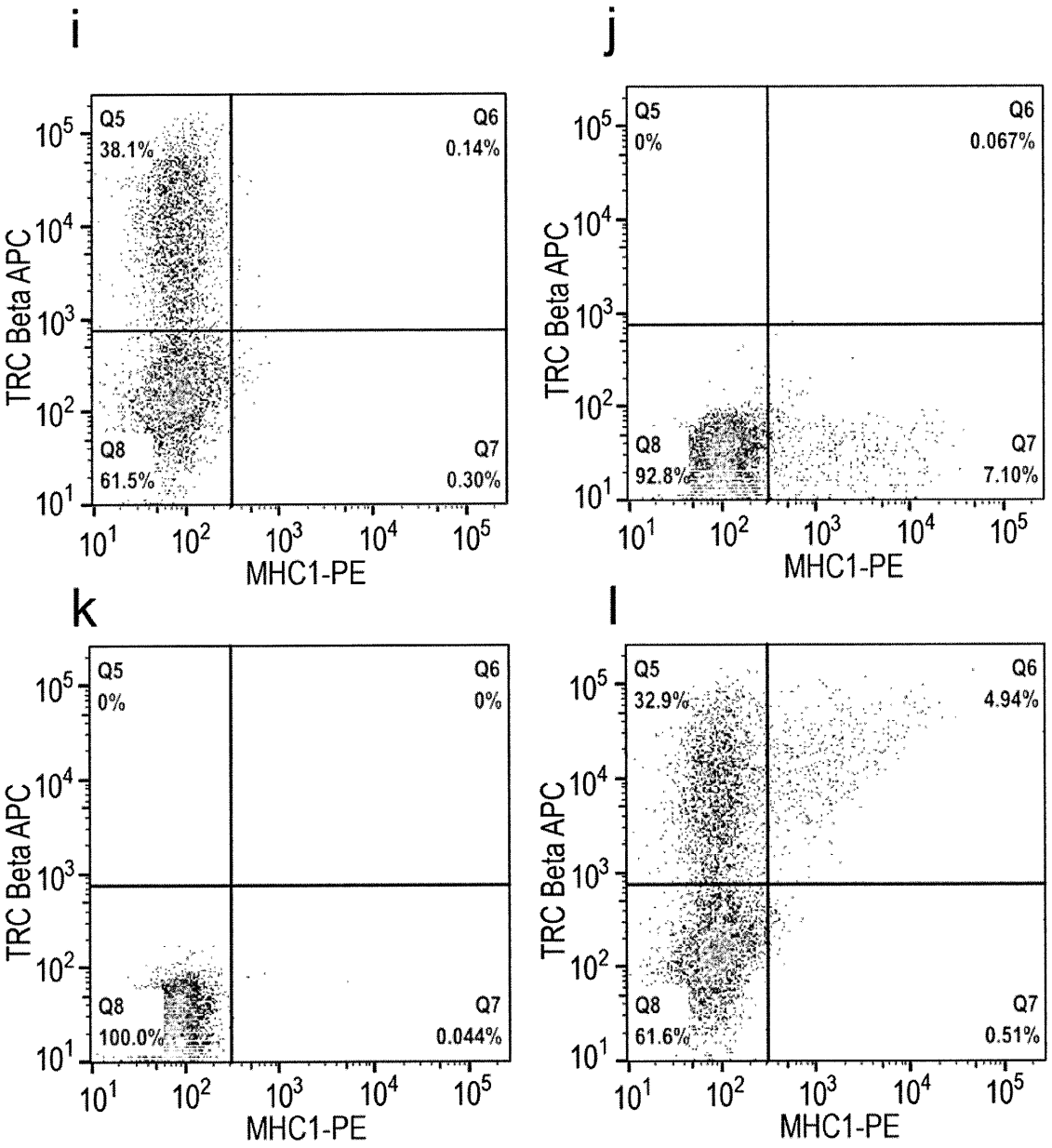



Fig. 26
(continued)

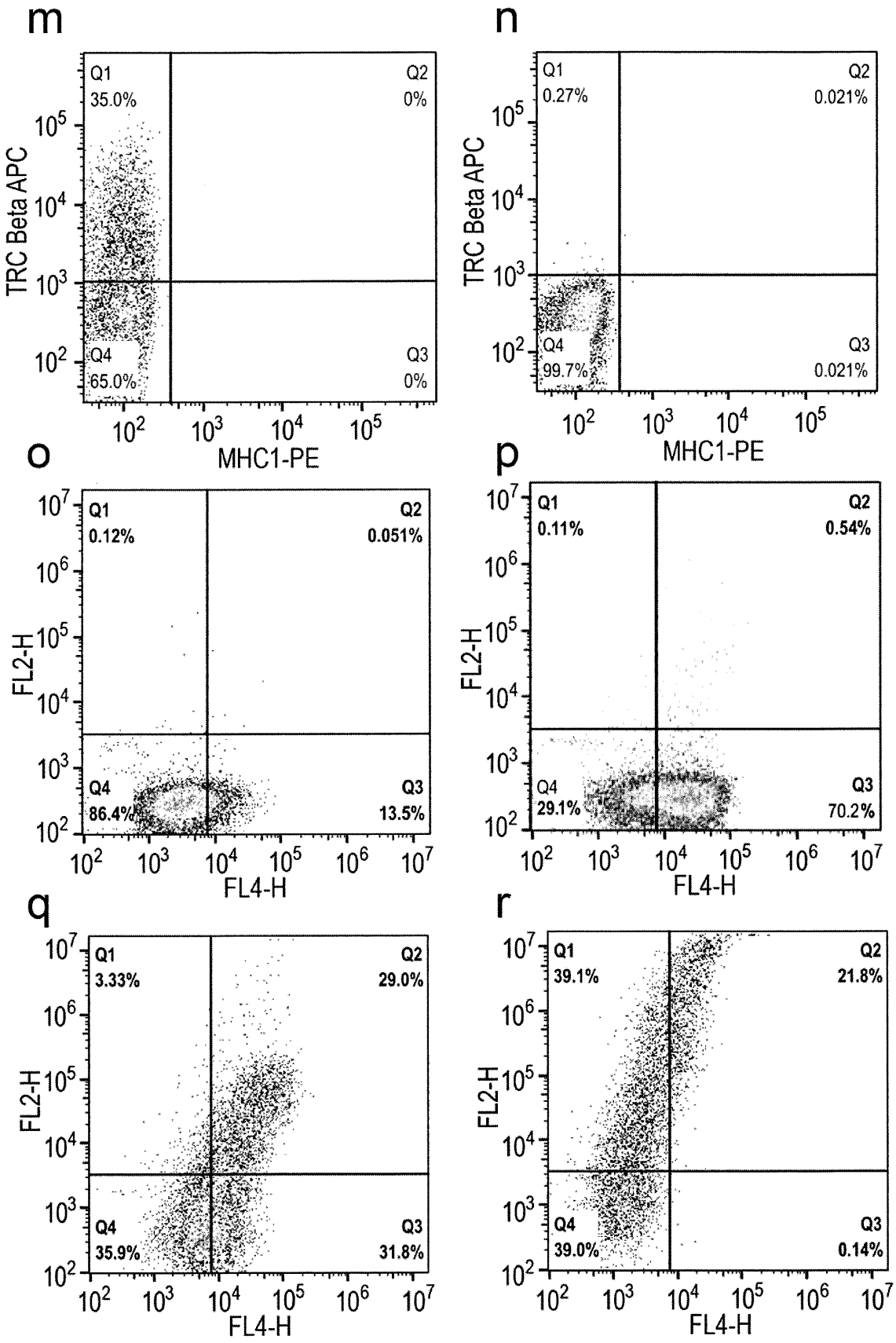



Fig. 26 (continued)

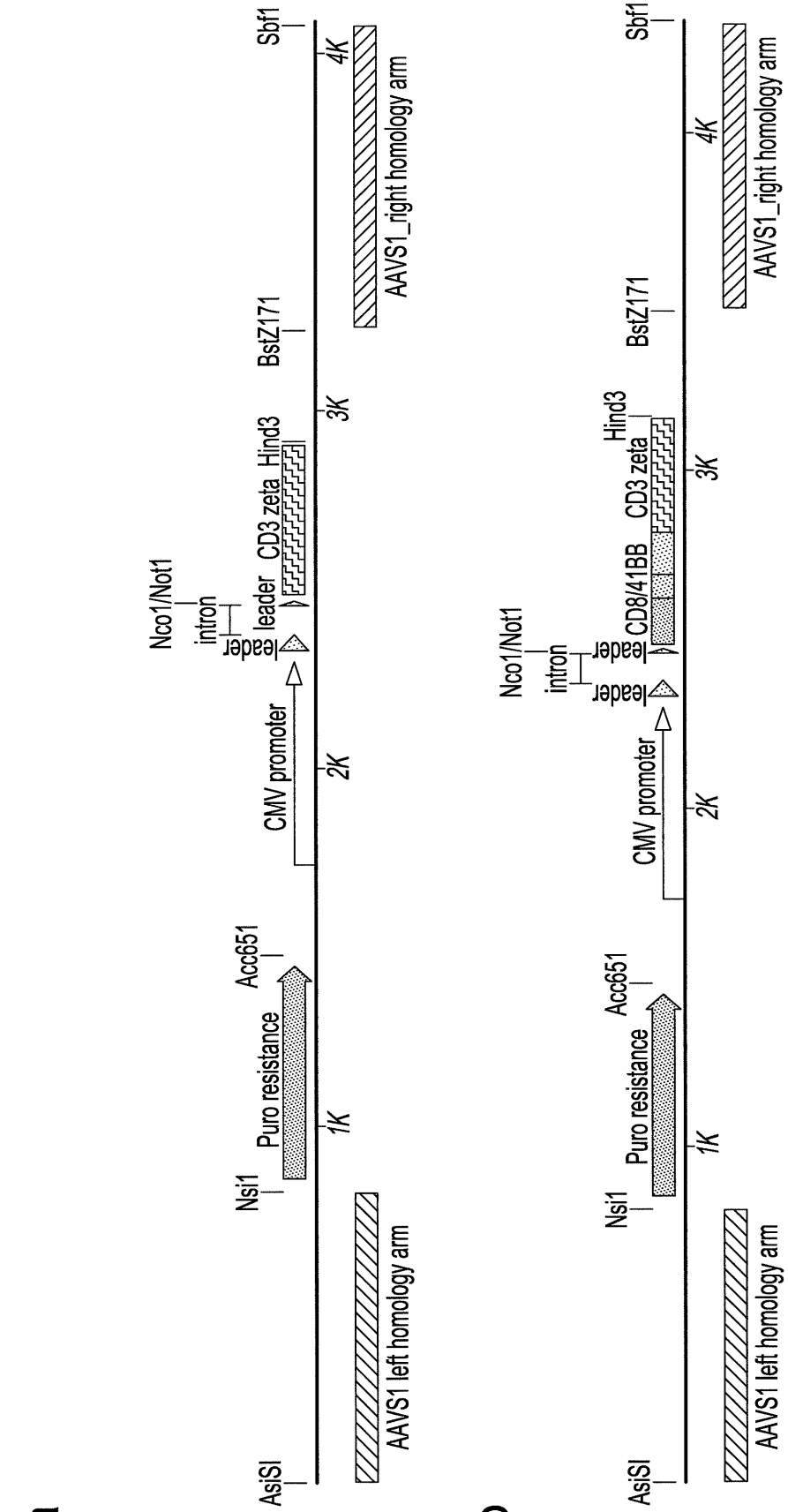

a
AsiSI
AAVS1 left homology arm
Nsi1
Puro resistance
Acc651
1K
CMV promoter
2K
Nco1/Not1
intron
leader
leader
CD3 zeta
Hind3
3K
BstZ171
Sbf1
4K
AAVS1_right homology arm
b
AsiSI
AAVS1 left homology arm
Nsi1
Puro resistance
Acc651
1K
CMV promoter
2K
Nco1/Not1
intron
leader
leader
CD8/41BB
CD3 zeta
Hind3
3K
BstZ171
Sbf1
4K
AAVS1_right homology arm

Fig. 27

C

Leader **Nco1** **Not1**

ggcgccatggcccaggtcgcggccgcaagcggactgctggacccaaagctgtgctacctg
G A M A S G L L D P K L C Y L
ctggatgggatcctgttcatctacggtgtgattctgacagccctgttcctgcgagtcaag
L D G I L F I Y G V I L T A L F L R V K
ttcagccggagcgccgacgcaccagcataccagcaggggcagaatcagctgtataacgag
F S R S A D A P A Y Q Q G Q N Q L Y N E
ctgaatctgggtcggagggaggaatacgacgtgctggataagagacgcggcagggatccc
L N L G R R E E Y D V L D K R R G R D P
gaaatgggcggaaagcctcagcgacggaaaaacccacaggagggactgtacaatgaactg
E M G G K P Q R R K N P Q E G L Y N E L
cagaaggacaaaatggctgaggcatattctgaaatcggcatgaagggagagaggagacgc
Q K D K M A E A Y S E I G M K G E R R R
ggcaaaggacacgatgggctgtaccagggtctgagtacagccactaaggacacctatgat
G K G H D G L Y Q G L S T A T K D T Y D
**Hind3**
gccctgcatatgcaggctctgccacccagataataaaagctt
A L H M Q A L P P R - -

Fig. 27 (continued)

d

Leader **Nco1** **Not1** I---CD8 hinge/Transmembrane→

gccatggcc caggtcgcggccgca acaacaaccccagcccccagacctcctaccccct

A M A T T T P A P R P P T P

gcccctacaattgccagccagcctctgagcctgaggcccgaggcttgtagaccagctgct

A P T I A S Q P L S L R P E A C R P A A

ggcggagccgtgcacaccagaggactggatttcgcctgcgacatctacatctgggccccct

G G A V H T R G L D F A C D I Y I W A P

←---CD8 hinge transmembrane---II 4-1BB→

ctggccggcacatgtggcgtgctgctgctgagcctcgtgatcaccctgtactgc aagcgg

L A G T C G V L L L S L V I T L Y C K R

ggcagaaagaaactgctgtacatctttaagcagcccttcatgcggcccgtgcagaccacc

G R K K L L Y I F K Q P F M R P V Q T T

←-- 4-1BB--I

caggaagaggacggctgctcctgcagattccccgaggaagaagaaggc ggctgcgagctg

Q E E D G C S C R F P E E E E G G C E L

I---CD3ζ→

agagtgaagttcagcagatccgccgacgcccctgcctacaagcagggccagaaccagctg

R V K F S R S A D A P A Y K Q G Q N Q L

tacaacgagctgaacctgggcagacgggaagagtacgacgtgctggacaagcggagaggc

Y N E L N L G R R E E Y D V L D K R R G

cgggacccagagatgggcggaaagcccagaagaaagaacccccaggaaggcctgtataac

R D P E M G G K P R R K N P Q E G L Y N

gaactgcagaaagacaaaatggccgaggcctacagcgagatcggaatgaagggcgagcgg

E L Q K D K M A E A Y S E I G M K G E R

agaagaggcaaggggcacgatggcctgtaccagggcctgagcaccgccaccaaggacacc

R R G K G H D G L Y Q G L S T A T K D T

tatgacgccctgcacatgcaggccctgcccctagataataaaagctt

Y D A L H M Q A L P P R - - **Hind 3**

Fig. 27 (continued)

e

**Nco1**

g**ccatgg**ccgaagtgaaactgcaggagtctggacccggcctggtggccccatctcagtct
A M A E V K L Q E S G P G L V A P S Q S
ctgagcgtgacctgtaccgtgtccggcgtgtccctgcctgactatggcgtgtcctggatc
L S V T C T V S G V S L P D Y G V S W I
agacagcccccagaaagggcctggaatggctgggagtgatctggggcagcgaaaccacc
R Q P P R K G L E W L G V I W G S E T T
tactacaacagcgccctgaagtcccggctgaccatcatcaaggacaactccaagagccag
Y Y N S A L K S R L T I I K D N S K S Q
gtgttcctgaagatgaacagcctgcagaccgacgacaccgccatctactactgcgccaag
V F L K M N S L Q T D D T A I Y Y C A K
cactactactacggcggcagctacgctatggactactggggccagggcacctcggtcacc
H Y Y Y G G S Y A M D Y W G Q G T S V T
gtctcgagtggtggaggcggttcaggcggaggtggctctggcggtggcgctagcgacatc
V S S G G G G S G G G G S G G G A S D I
cagatgacccagaccaccagcagcctgagcgccagcctgggcgatagagtgaccatcagc
Q M T Q T T S S L S A S L G D R V T I S
tgcagagccagccaggacatcagcaagtacctgaactggtatcagcagaaacccgacggc
C R A S Q D I S K Y L N W Y Q Q K P D G
accgtgaagctgctgatctaccacaccagcagactgcacagcggcgtgcccagcagattt
T V K L L I Y H T S R L H S G V P S R F
tccggctctggcagcggcaccgactacagcctgaccatctccaacctggaacaggaagat
S G S G S G T D Y S L T I S N L E Q E D
atcgctacctacttctgtcagcaaggcaacaccctgccctacaccttcggcggagggacc
I A T Y F C Q Q G N T L P Y T F G G G T
aagctggagatcaaacgtaccgcggccgca
K L E I K R T A A A
**Not1**

Fig. 27 (continued)

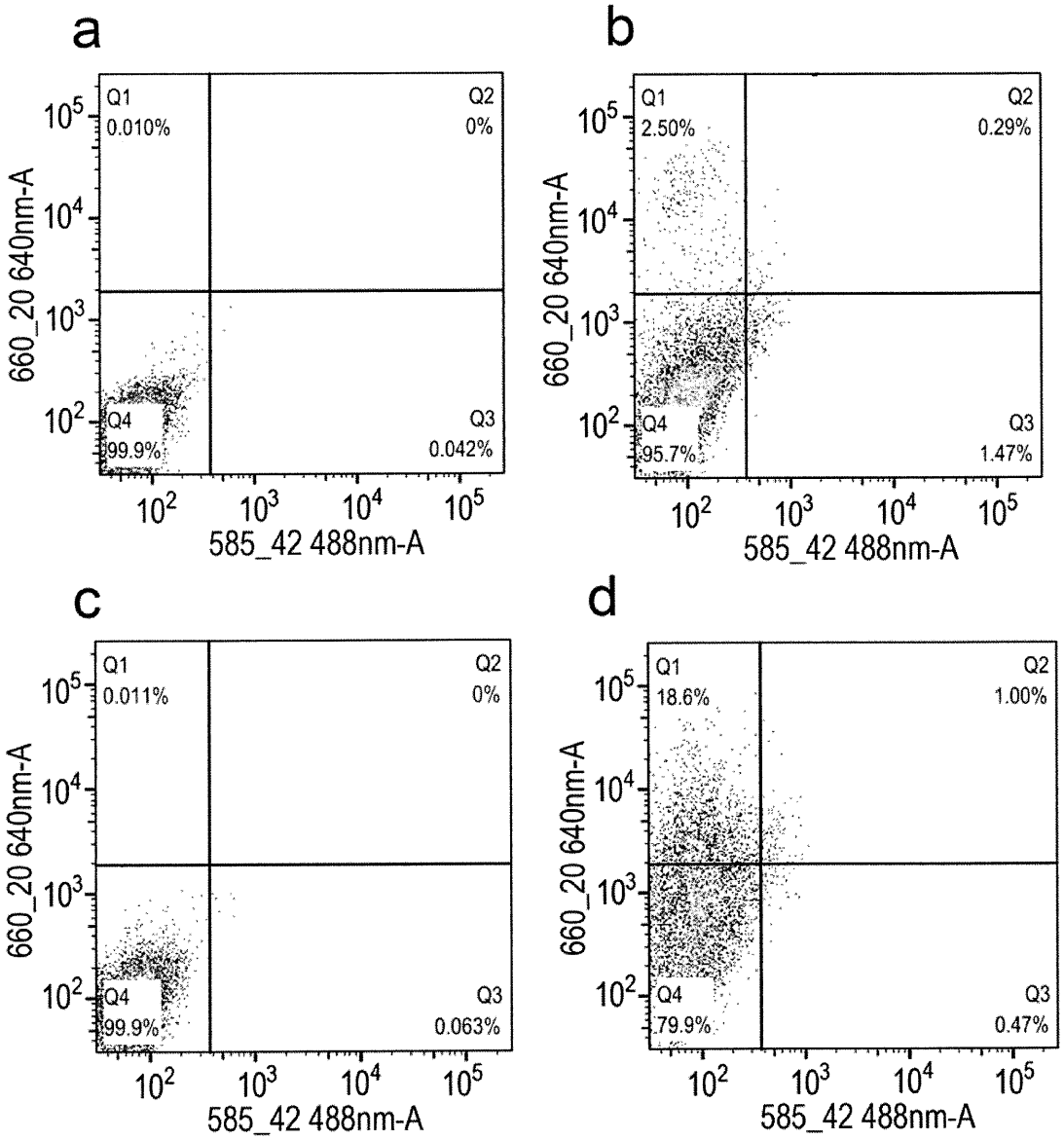

a
Q1
0.010%
Q2
0%
Q4
99.9%
Q3
0.042%
660_20 640nm-A
585_42 488nm-A
b
Q1
2.50%
Q2
0.29%
Q4
95.7%
Q3
1.47%
660_20 640nm-A
585_42 488nm-A
c
Q1
0.011%
Q2
0%
Q4
99.9%
Q3
0.063%
660_20 640nm-A
585_42 488nm-A
d
Q1
18.6%
Q2
1.00%
Q4
79.9%
Q3
0.47%
660_20 640nm-A
585_42 488nm-A

Fig. 28

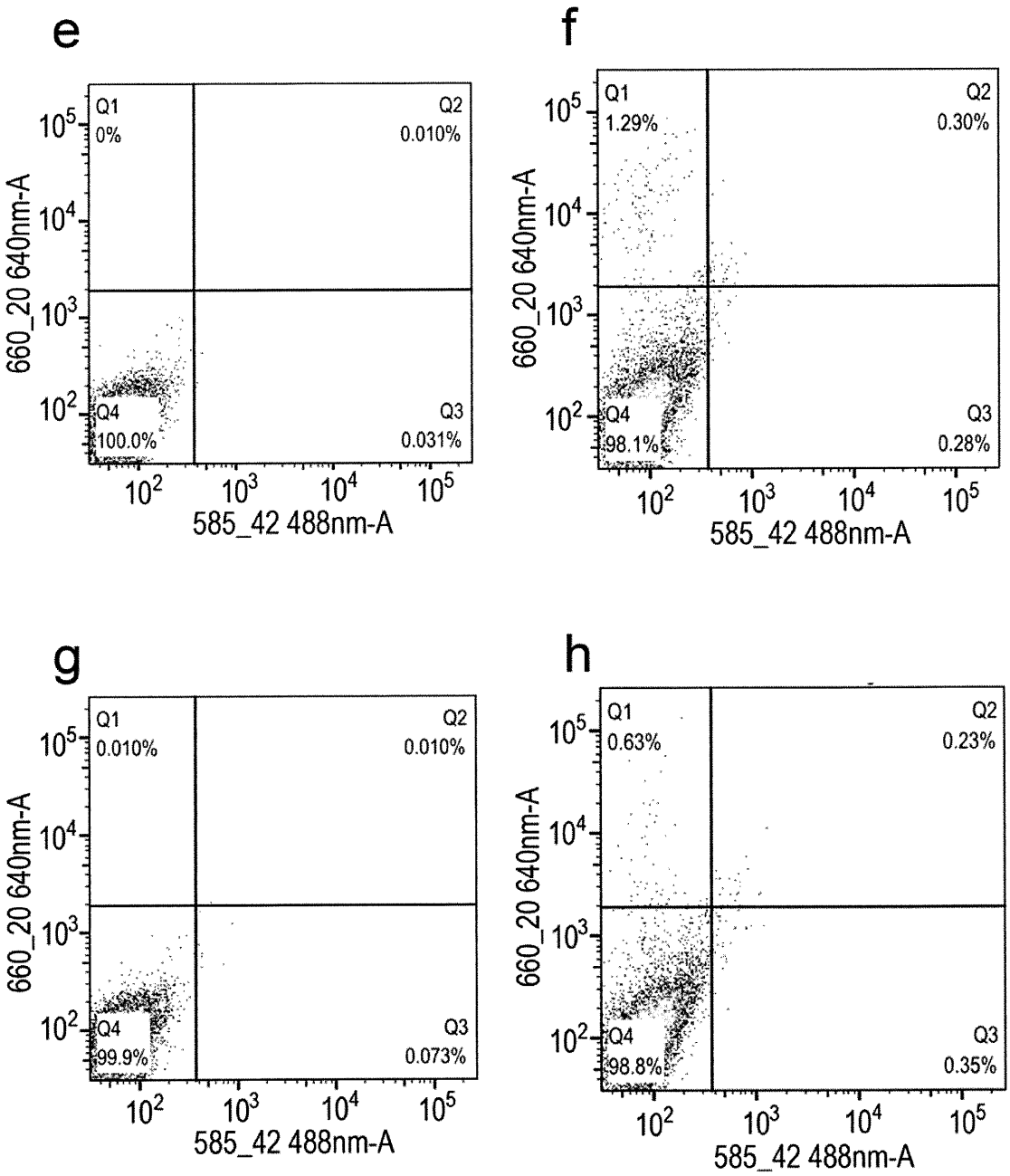



Fig. 28
(continued)

a

Nco1

gccatggctgctacaggcgtgcgggctgtgcccggcaatgagaacagcctggaaatcgag
A M A A T G V R A V P G N E N S L E I E
gaactggccagattcgccgtggacgagcacaacaagaaagagaacgccctgctggaattc
E L A R F A V D E H N K K E N A L L E F
gtgcgggtcgtgaaggccaaagagcagtggagcgaggccgacaacgactggcacaccatg
V R V V K A K E Q **W S E A D N D W H** T M
tactacctgaccctggaagccaaggacggcggcaagaagaagctgtacgaggccaaagtg
Y Y L T L E A K D G G K K K L Y E A K V
tgggtcaagctggacctggaaacctggcagcacttcaacttcaaagagctccaggaattc
W V K **L D L E T W Q H F** N F K E L Q E F
aagcccgtgggcgacgctgcggccgcg
K P V G D A A A A

Not1

b

Nco1

gccatggctgctacaggcgtgcgggctgtgcccggcaatgagaacagcctggaaatcgag
A M A A T G V R A V P G N E N S L E I E
gaactggccagattcgccgtggacgagcacaacaagaaagagaacgccctgctggaattc
E L A R F A V D E H N K K E N A L L E F
gtgcgggtcgtgaaggccaaagagcaggaacagcccatcggcgagcaccccgtgaacgac
V R V V K A K E Q **E Q P I G E H P V N D**
accatgtactacctgaccctggaagccaaggacggcggcaagaagaagctgtacgaggcc
T M Y Y L T L E A K D G G K K K L Y E A
aaagtgtgggtcaagcggtggctgcggttcaccgagatctacaacttcaaagagctccag
K V W V K **R W L R F T E I Y** N F K E L Q
gaattcaagcccgtgggcgacgctgcggccgcg
E F K P V G D A A A A

Not1

Fig. 29

C

**Adhiron_mut1**

5′ CAGGGTCAGGTAGTACATGGTSNNSNNSNN(SNN)$_n$ CTGCTCTTTGGCCTTCACGAC

**Adhiron_mut2**

CTGGAGCTCTTTGAAGTTSNNSNNSNN(SNN)$_n$ CTTGACCCACACTTTGGC

GTC GTG AAG GCC AAA GAG CAG (NNS)$_n$ NNS NNS NNS ACC ATG TAC TAC CTG ACC CTG
CAG CAC TTC CGG TTT CTC GTC (NNS)$_n$ NNS NNS NNS TGG TAC ATG ATG GAC TGG GAC
V V K A K E Q X X X X T M Y Y L T L

GCC AAA GTG TGG GTC AAG (NNS)$_n$ NNS NNS NNS AAC TTC AAA GAG CTC CAG
CGG TTT CAC ACC CAG TTC (NNS)$_n$ NNS NNS NNS TTG AAG TTT CTC GAG GTC
A K V W V K X X X X N F K E L Q

Fig. 29 (continued)

d

**Nco1**

gccatggccggtgtgtgccccaagatcttgaaaaagtgccgccgtgacagcgattgt
A M A G V C P K I L K K C R R D S D C
cccggcgcctgcatctgccgcggcaatggctattgcggagcggccgca
P G A C I C R G N G Y C G **Not 1**

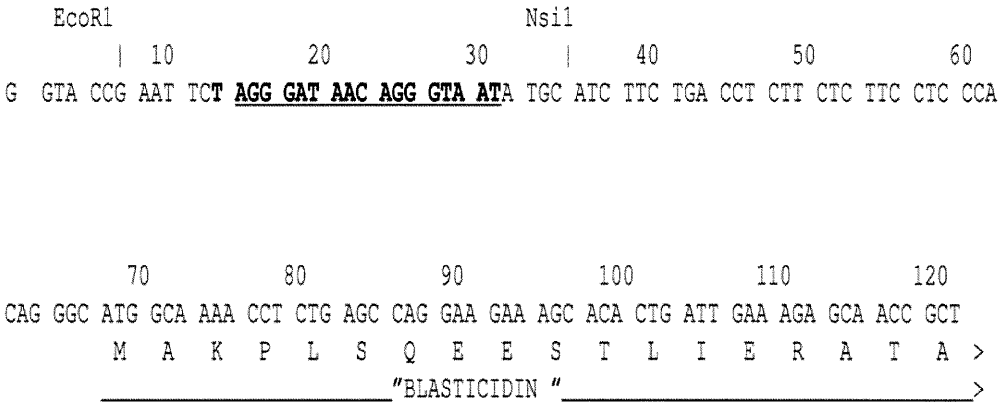
e

GGT GTG TGC VNS VNS VNS VNS VNS VNS VNS VNS VNS VNS TGC CGC CGT
CCA CAC ACG BNS BNS BNS BNS BNS BNS BNS BNS BNS BNS ACG GCG GCA
G V C X X X X X X X X X X C R R

GAC AGC GAT TGT CCC GGC GCC TGC ATC TGC CGC GGC AAT GGC TAT TGC GGA
CTG TCG CTA ACA GGG CCG CGG ACG TAG ACG GCG CCG TTA CCG ATA ACG CCT
D S D C P G A C I C R G N G Y C G

Fig. 29
(continued)

a


EcoR1
Nsi1
10 20 30 40 50 60
G GTA CCG AAT TCT AGG GAT AAC AGG GTA ATA TGC ATC TTC TGA CCT CTT CTC TTC CTC CCA
70 80 90 100 110 120
CAG GGC ATG GCA AAA CCT CTG AGC CAG GAA GAA AGC ACA CTG ATT GAA AGA GCA ACC GCT
M A K P L S Q E E S T L I E R A T A >
"BLASTICIDIN ">

b

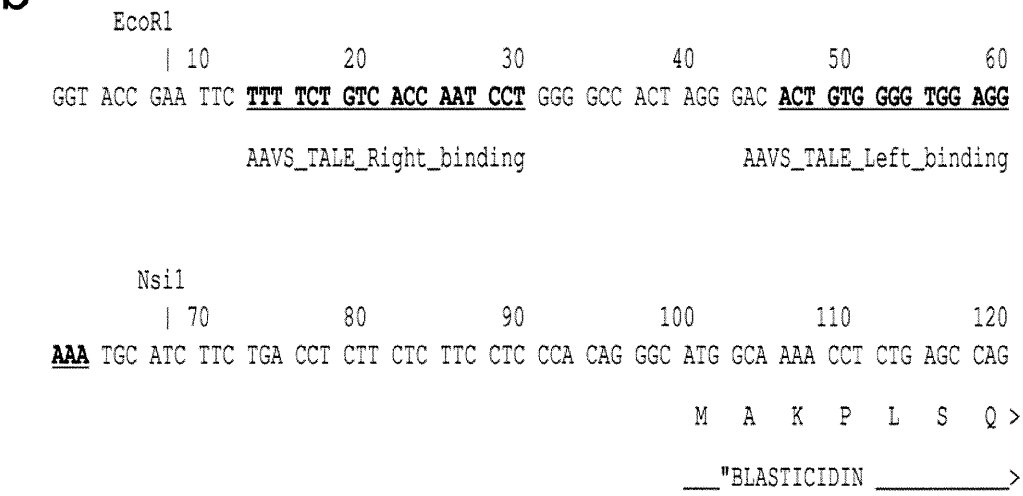

EcoR1
10 20 30 40 50 60
GGT ACC GAA TTC TTT TCT GTC ACC AAT CCT GGG GCC ACT AGG GAC ACT GTG GGG TGG AGG
AAVS_TALE_Right_binding
AAVS_TALE_Left_binding
Nsi1
70 80 90 100 110 120
AAA TGC ATC TTC TGA CCT CTT CTC TTC CTC CCA CAG GGC ATG GCA AAA CCT CTG AGC CAG
M A K P L S Q >
"BLASTICIDIN >

Fig. 30

a

Primer J60>

GCGATCGCGCTGATTGGCTTCTTTTCCTCCCGCCGTGTGTGAAAACACAAATGGCGTGTTTT
GGTTGGCGTAAGGCGCCTGTCAGTTAACGGCAGCCGGAGTGCGCAGCCGCCGGCAGCCTCGC
TCTGCCCACTGGGTGGGGCGGGAGGTAGGTGGGGTGAGGCGAGCTGGACGTGCGGGCGCGGT
CGGCCTCTGGCGGGGCGGGGGAGGGGAGGGAGGGTCAGCGAAAGTAGCTCGCGCGCGAGCGG
CCGCCCACCCTCCCCTTCCTCTGGGGGAGTCGTTTTACCCGCCGCCGGCCGGGCCTCGTCGT
CTGATTGGCTCTCGGGGCCCAGAAAACTGGCCCTTGCCATTGGCTCGTGTTCGTGCAAGTTG
AGTCCATCCGCCGGCCAGCGGGGGCGGCGAGGAGGCGCTCCCAGGTTCCGGCCCTCCCCTCG
GCCCCGCGCCGCAGAGTCTGGCCGCGCGCCCCTGCGCAACGTGGCAGGAAGCGCGCGCTGGG
GGCGGGGACGGGCAGTAGGGCTGAGCGGCTGCGGGGCGGGTGCAAGCACGTTTCCGACTTGA
GTTGCCTCAAGAGGGGCGTGCTGAGCCAGACCTCCATCGCGCACTCCGGGGAGTGGAGGGAA
GGAGCGAGGGCTCAGTTGGGCTGTTTTGGAGGCAGGAAGCACTTGCTCTCCCAAAGTCGCTC
TGAGTTGTTATCAGTAAGGGAGCTGCAGTGGAGTAGGCGGGGAGAAGGCCGCACCCTTCTCC
GGAGGGGGGAGGGGAGTGTTGCAATACCTTTCTGGGAGTTCTCTGCTGCCTCCTGGCTTCTG
AGGACCGCCCTGGGCCTGGGAGAATCCCTTCCCCCTCTTCCCTCGTGATCTGCAACTCCAGT
CTTTCTAGAATGCATTAAGGGATCTGTAGGGCGCAGTAGTCCAGGGTTTCCTTGATGATGTC
< 2706  Nsi1  2709→

ATACTTATCCTGTCCCTTTTTTTTCCACAGCTCGCGGTTGAGGACAAACTCTTCGCGGTCTT
TCCAGTGGGGATCGACGGTATCGTAGAGTCGAGGCCGCTCTAGGAATTCACGCCGCCACC
←Primer 2710

ATG ACC GAG
 M  T  E
  Puromycin→

b

BstZ171  Primer J61→

GTATACGGGAATTGAACAGGTGTAAAATTGGAGGGACAAGACTTCCCACAGATTTTCGGTTT
TGTCGGGAAGTTTTTTAATAGGGGCAAATAAGGAAAATGGGAGGATAGGTAGTCATCTGGGG
TTTTATGCAGCAAAACTACAGGTTATTATTGCTTGTGATCCGCCTCGGAGTATTTTCCATCG
AGGTAGATTAAAGACATGCTCACCCGAGTTTTATACTCTCCTGCTTGAGATCCTTACTACAG
TATGAAATTACAGTGTCGCGAGTTAGACTATGTAAGCAGAATTTTAATCATTTTTAAAGAGC
CCAGTACTTCATATCCATTTCTCCCGCTCCTTCTGCAGCCTTATCAAAAGGTATTTTAGAAC
ACTCATTTTAGCCCCATTTTCATTTATTATACTGGCTTATCCAACCCCTAGACAGAGCATTG
GCATTTTCCCTTTCCTGATCTTAGAAGTCTGATGACTCATGAAACCAGACAGATTAGTTACA
TACACCACAAATCGAGGCTGTAGCTGGGGCCTCAACACTGCAGTTCTTTTATAACTCCTTAG
TACACTTTTTGTTGATCCTTTGCCTTGATCCTTAATTTTCAGTGTCTATCACCTCTCCCGTC
AGGTGGTGTTCCACATTTGGGCCTATTCTCAGTCCAGGGAGTTTTACAACAATAGATGTATT
GAGAATCCAACCTCCTGCAGG
←Primer J62 Sbf1

Fig. 31