Fig. 1:

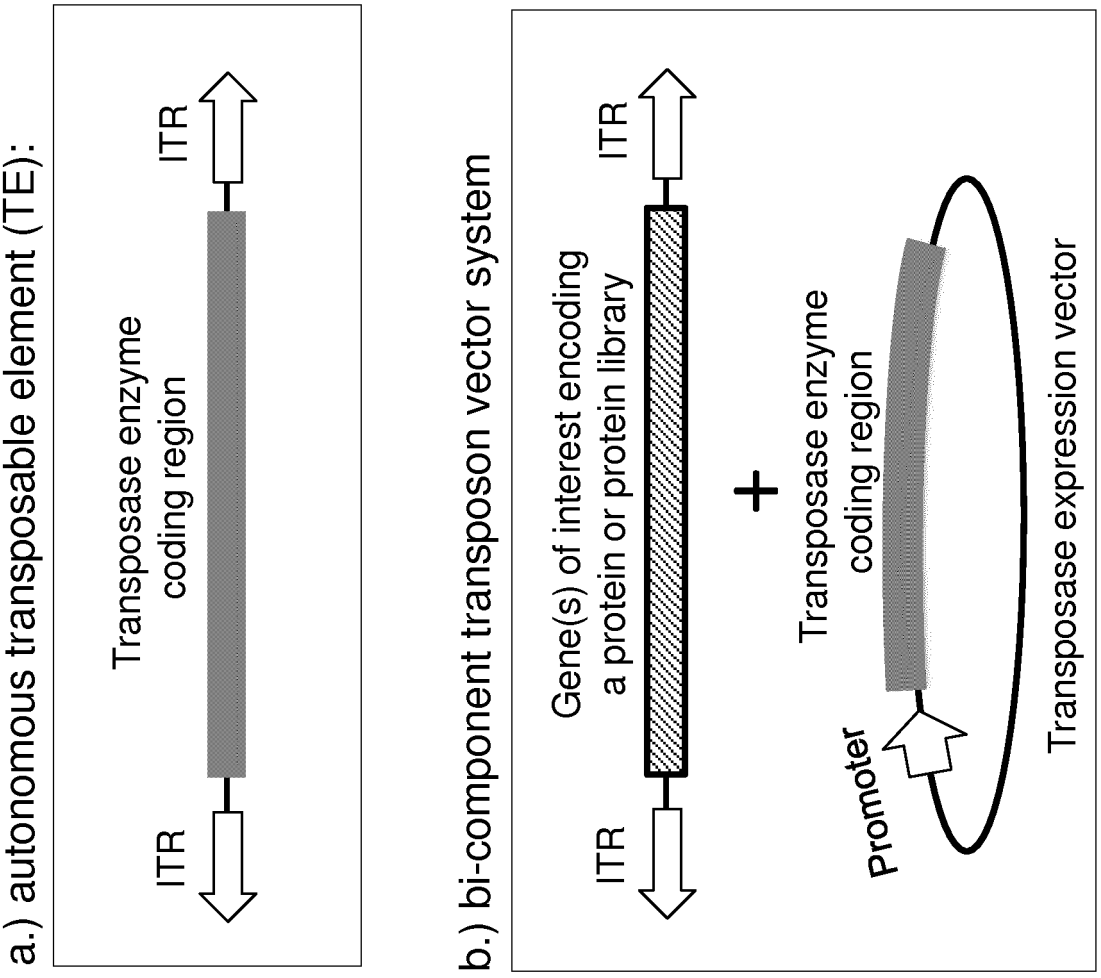

a.) autonomous transposable element (TE):
ITR
Transposase enzyme coding region
ITR
b.) bi-component transposon vector system
ITR
Gene(s) of interest encoding a protein or protein library
ITR
+
Promoter
Transposase enzyme coding region
Transposase expression vector

Fig. 2:

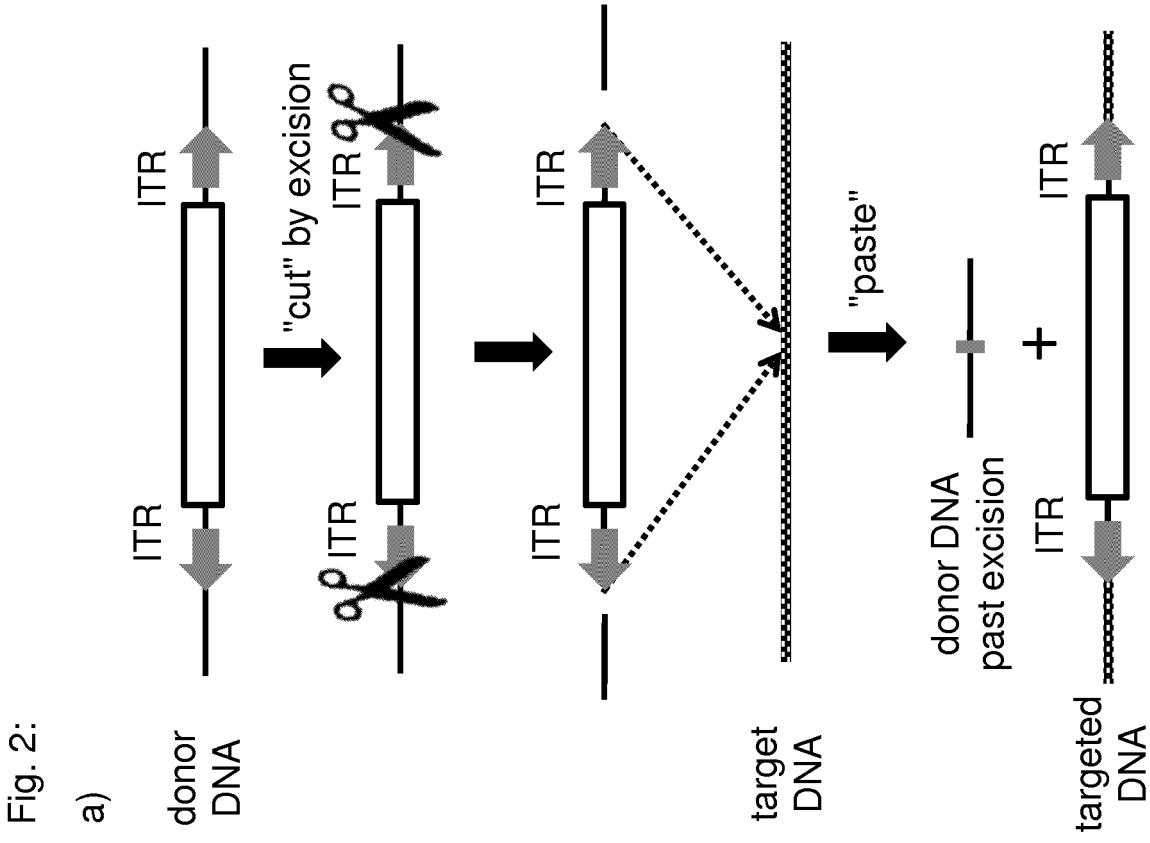

a)
donor DNA
ITR
ITR
"cut" by excision
ITR
ITR
ITR
ITR
target DNA
"paste"
donor DNA past excision
ITR
targeted DNA
b)
donor DNA
ITR
ITR
"copy" by replication
ITR
ITR
target DNA
"paste"
donor DNA
ITR
ITR
+
targeted DNA
ITR
ITR

Fig. 3:

Transposon Family (*Transposon*)	Species origin	Host cell range
PiggyBac	*Trichoplusia ni*	Mouse , Human, Pig
Tcl-mariner/ *(Sleeping Beauty)*	*salmonid*	Zebrafish, Xenopus, Mouse, Human
(*Frog Prince*)	*R. pipiens*	Human, Hamster, Xenopus, Zebrafish
(Himar1)	*H. irritans*	Human
(Passport)	*P. platessa*	Human, Monkey, Hamster, Turkey. Chicken, Pig
(Minos)	*D. hydei*	Human, Mouse
hAT (Tol1, Tol2)	*O. latipe*	Zebrafish, Xenopus, Mouse, Human, Chicken
Ac/Ds	*Z. mays*	Zebrafish, Human
PIF, Harbinger, Harbinger3-DR	*D. rerio*	Zebrafish, Human
(*Hsmar1*)	*H. sapiens*	Human, Zebrafish

Fig. 4

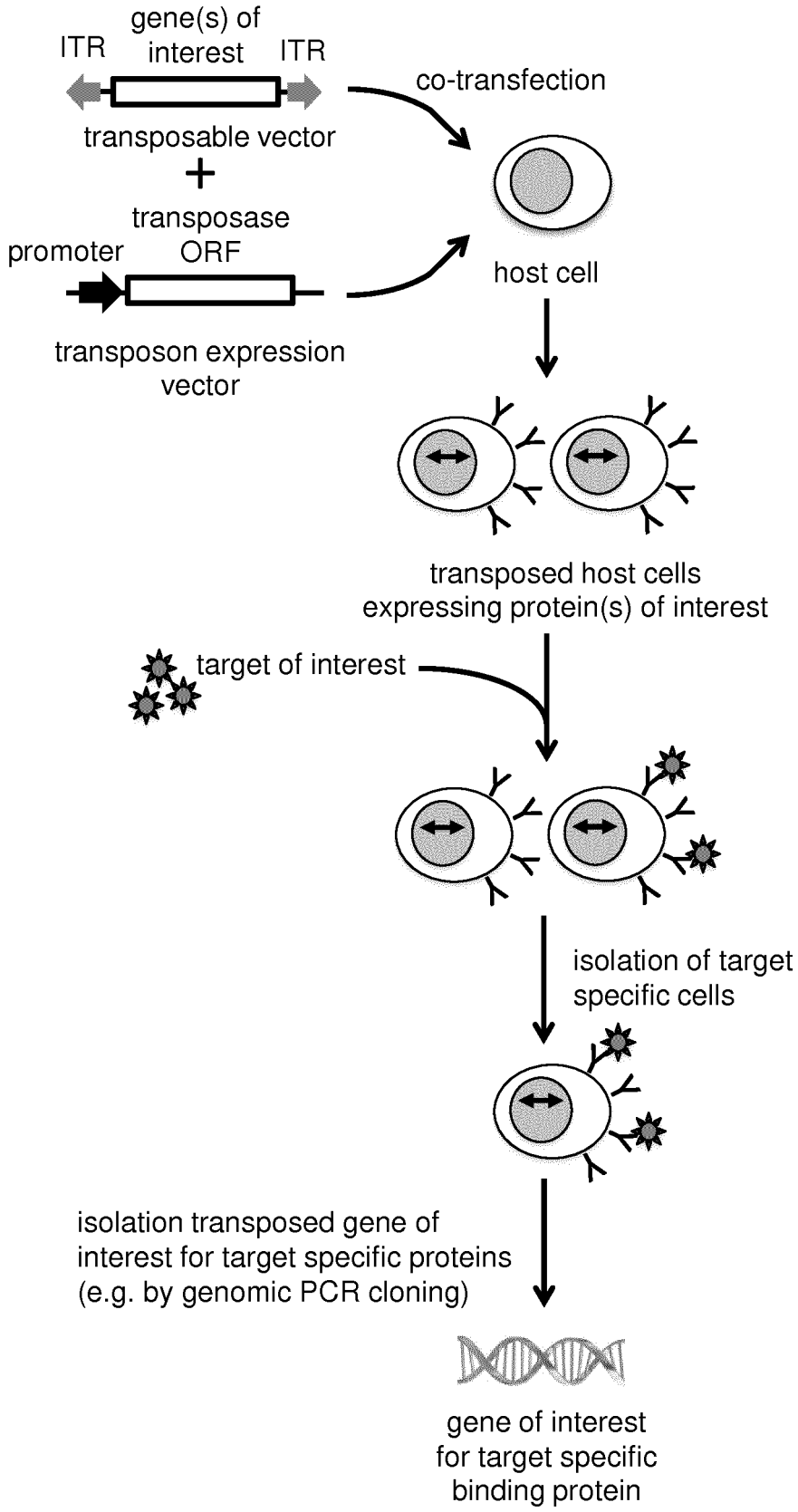

gene(s) of interest
ITR
ITR
co-transfection
transposable vector
+
transposase
promoter
ORF
host cell
transposon expression vector
transposed host cells expressing protein(s) of interest
target of interest
isolation of target specific cells
isolation transposed gene of interest for target specific proteins (e.g. by genomic PCR cloning)
gene of interest for target specific binding protein

Fig. 5 a)

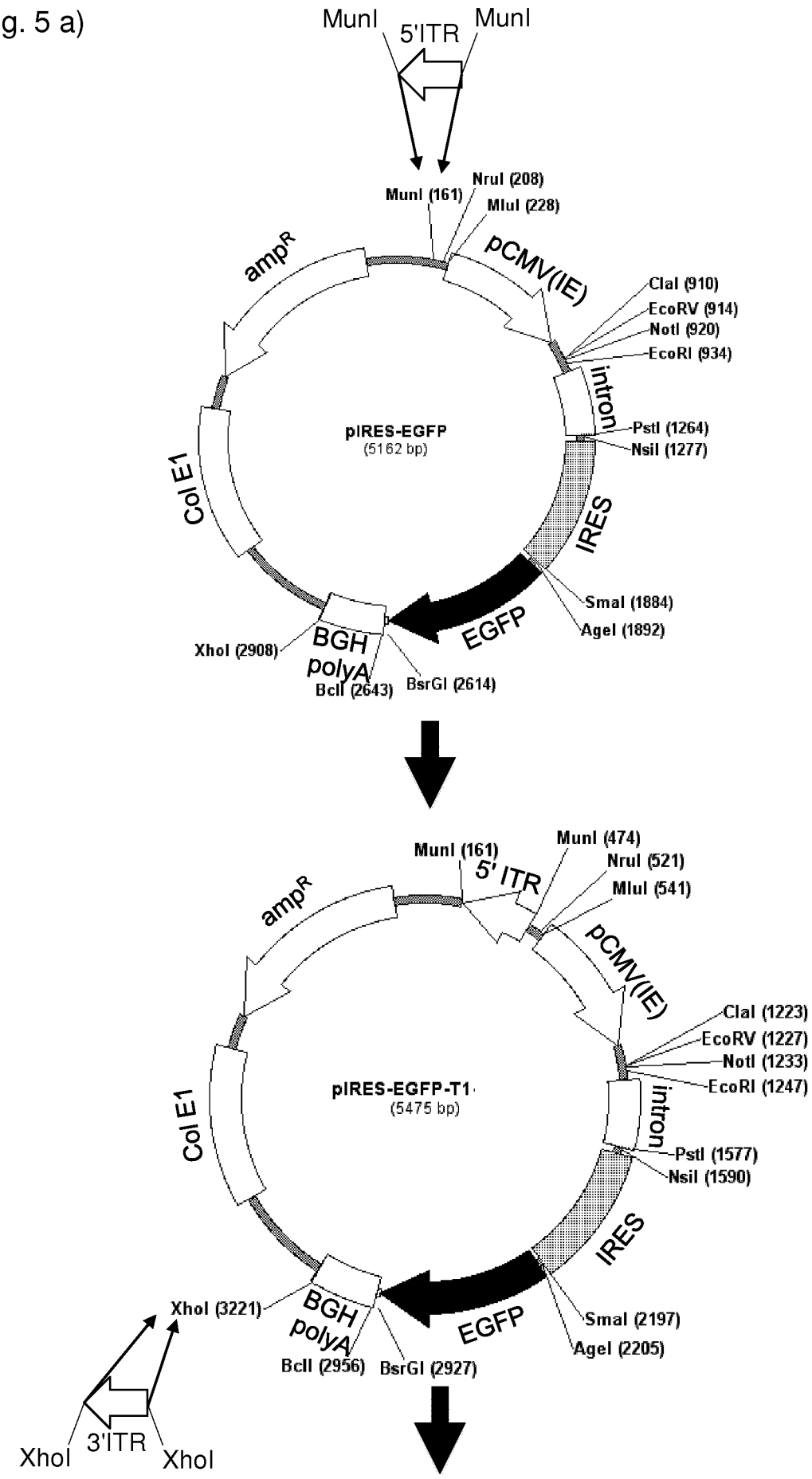

MunI
5'ITR
MunI
MunI (161)
NruI (208)
MluI (228)
pCMV(IE)
ClaI (910)
EcoRV (914)
NotI (920)
EcoRI (934)
intron
PstI (1264)
NsiI (1277)
IRES
SmaI (1884)
AgeI (1892)
EGFP
BGH polyA
BclI (2643)
BsrGI (2614)
XhoI (2908)
ampR
ColE1
pIRES-EGFP
(5162 bp)
MunI (161)
MunI (474)
NruI (521)
MluI (541)
5' ITR
pCMV(IE)
ClaI (1223)
EcoRV (1227)
NotI (1233)
EcoRI (1247)
intron
PstI (1577)
NsiI (1590)
IRES
SmaI (2197)
AgeI (2205)
EGFP
BGH polyA
BclI (2956)
BsrGI (2927)
XhoI (3221)
ampR
ColE1
pIRES-EGFP-T1
(5475 bp)
XhoI
3'ITR
XhoI

Fig. 5 b)

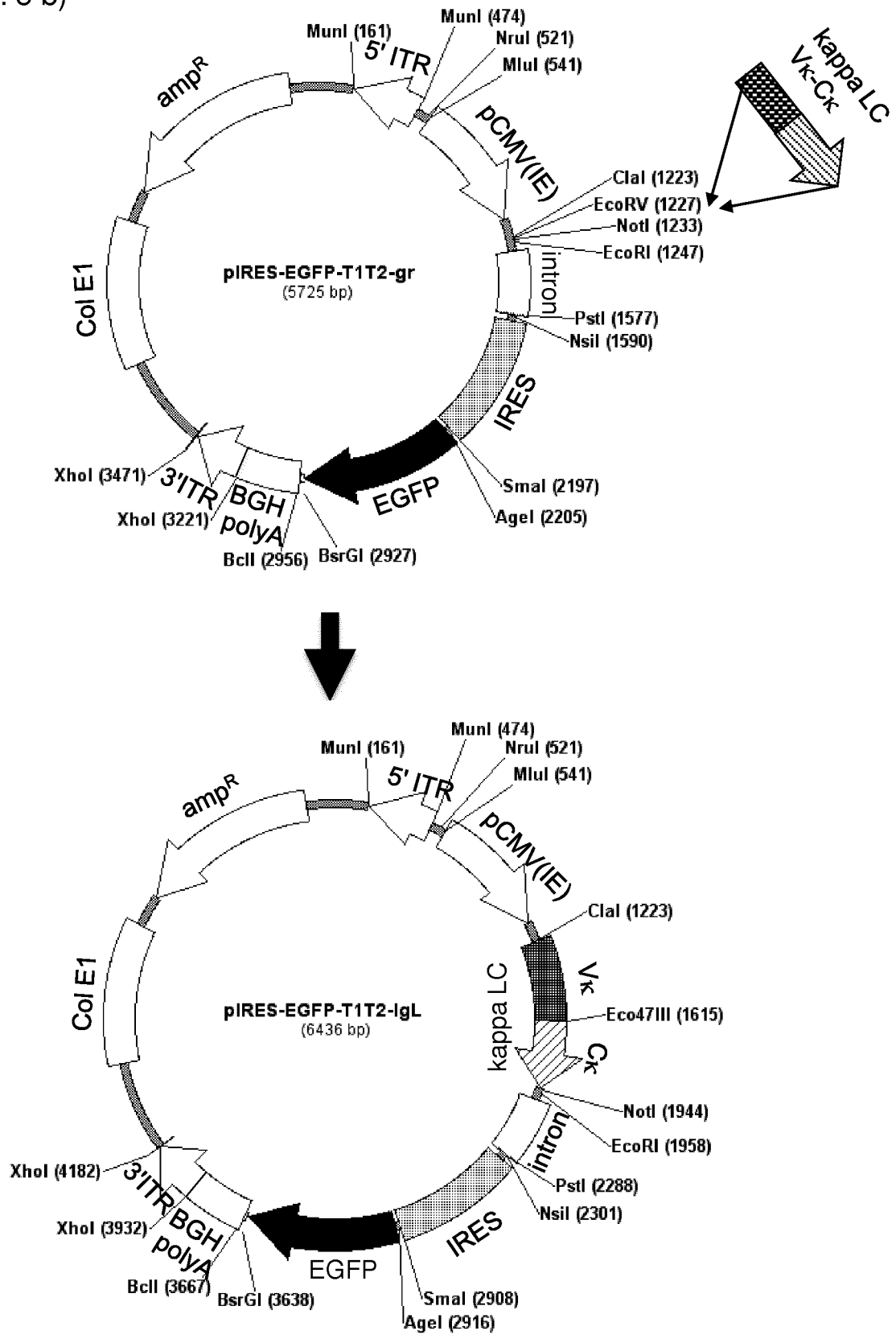

MunI (161)
MunI (474)
NruI (521)
MluI (541)
5' ITR
ampR
pCMV(IE)
ClaI (1223)
EcoRV (1227)
NotI (1233)
EcoRI (1247)
intron
PstI (1577)
NsiI (1590)
IRES
SmaI (2197)
AgeI (2205)
EGFP
BsrGI (2927)
BclI (2956)
XhoI (3221)
BGH polyA
3'ITR
XhoI (3471)
Col E1
pIRES-EGFP-T1T2-gr
(5725 bp)
kappa LC
Vκ-Cκ
MunI (161)
MunI (474)
NruI (521)
MluI (541)
5' ITR
ampR
pCMV(IE)
ClaI (1223)
Vκ
Eco47III (1615)
Cκ
kappa LC
NotI (1944)
EcoRI (1958)
intron
PstI (2288)
NsiI (2301)
IRES
SmaI (2908)
AgeI (2916)
EGFP
BsrGI (3638)
BclI (3667)
XhoI (3932)
BGH polyA
3'ITR
XhoI (4182)
Col E1
pIRES-EGFP-T1T2-IgL
(6436 bp)

Fig. 6

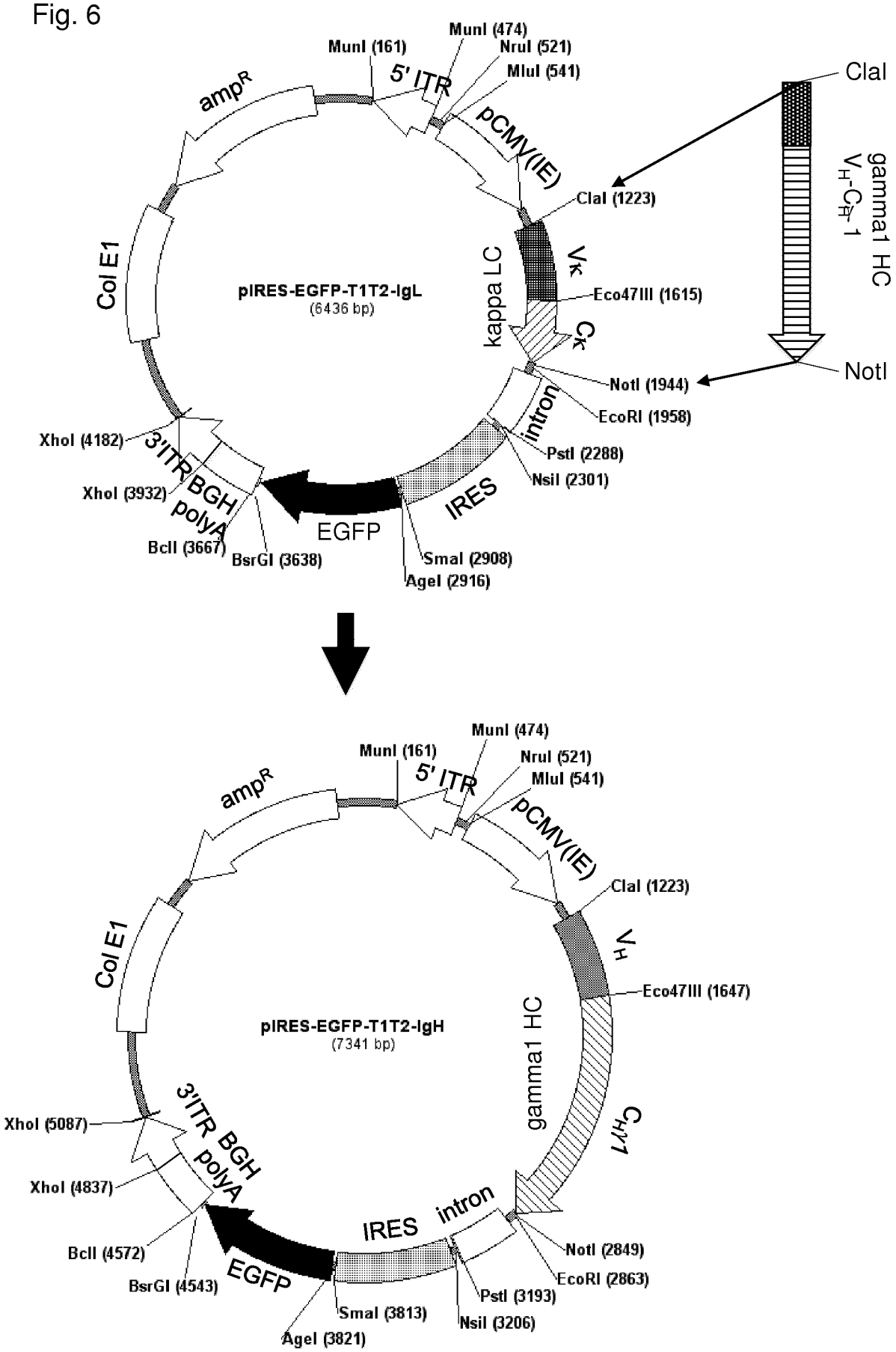

MunI (161)
MunI (474)
NruI (521)
MluI (541)
5' ITR
pCMV(IE)
ClaI (1223)
ClaI
gamma1 HC
V_H-C_H-1
NotI
NotI (1944)
EcoRI (1958)
intron
PstI (2288)
NsiI (2301)
IRES
SmaI (2908)
AgeI (2916)
EGFP
BsrGI (3638)
BclI (3667)
3'ITR BGH polyA
XhoI (3932)
XhoI (4182)
ampR
Col E1
pIRES-EGFP-T1T2-IgL
(6436 bp)
kappa LC
V_K
C_K
Eco47III (1615)
MunI (161)
MunI (474)
NruI (521)
MluI (541)
5' ITR
pCMV(IE)
ClaI (1223)
V_H
Eco47III (1647)
gamma1 HC
C_{H1}
intron
NotI (2849)
EcoRI (2863)
PstI (3193)
NsiI (3206)
IRES
SmaI (3813)
AgeI (3821)
EGFP
BsrGI (4543)
BclI (4572)
3'ITR BGH polyA
XhoI (4837)
XhoI (5087)
ampR
Col E1
pIRES-EGFP-T1T2-IgH
(7341 bp)

Fig. 7

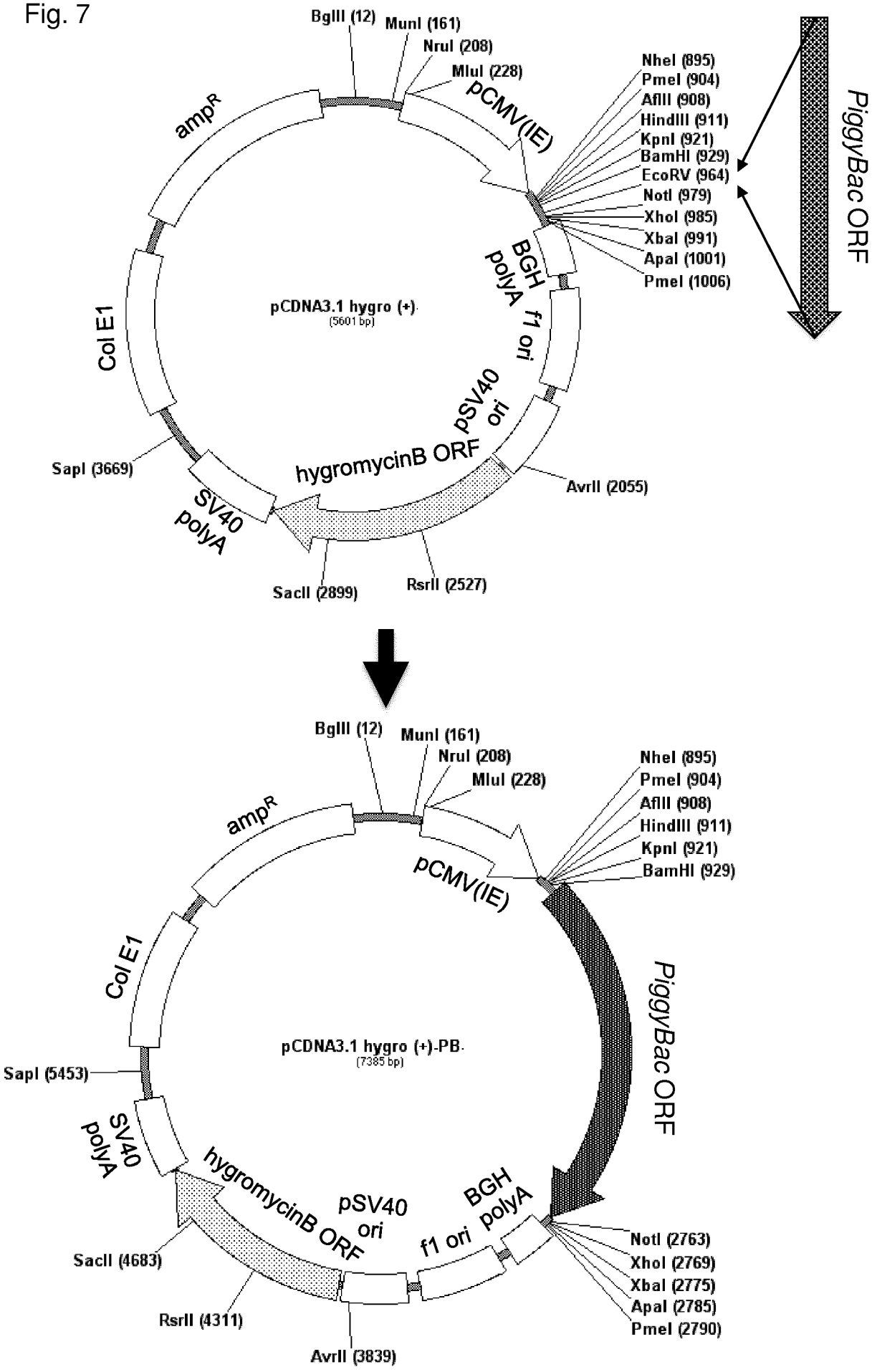

BglII (12)
MunI (161)
NruI (208)
MluI (228)
pCMV(IE)
NheI (895)
PmeI (904)
AflII (908)
HindIII (911)
KpnI (921)
BamHI (929)
EcoRV (964)
NotI (979)
XhoI (985)
XbaI (991)
ApaI (1001)
PmeI (1006)
PiggyBac ORF
ampR
BGH polyA
f1 ori
pCDNA3.1 hygro (+)
(5601 bp)
Col E1
pSV40 ori
hygromycinB ORF
SapI (3669)
AvrII (2055)
SV40 polyA
SacII (2899)
RsrII (2527)
BglII (12)
MunI (161)
NruI (208)
MluI (228)
NheI (895)
PmeI (904)
AflII (908)
HindIII (911)
KpnI (921)
BamHI (929)
pCMV(IE)
ampR
Col E1
PiggyBac ORF
pCDNA3.1 hygro (+)-PB
(7385 bp)
SapI (5453)
SV40 polyA
hygromycinB ORF
pSV40 ori
BGH polyA
f1 ori
SacII (4683)
NotI (2763)
XhoI (2769)
XbaI (2775)
ApaI (2785)
PmeI (2790)
RsrII (4311)
AvrII (3839)

Fig. 8

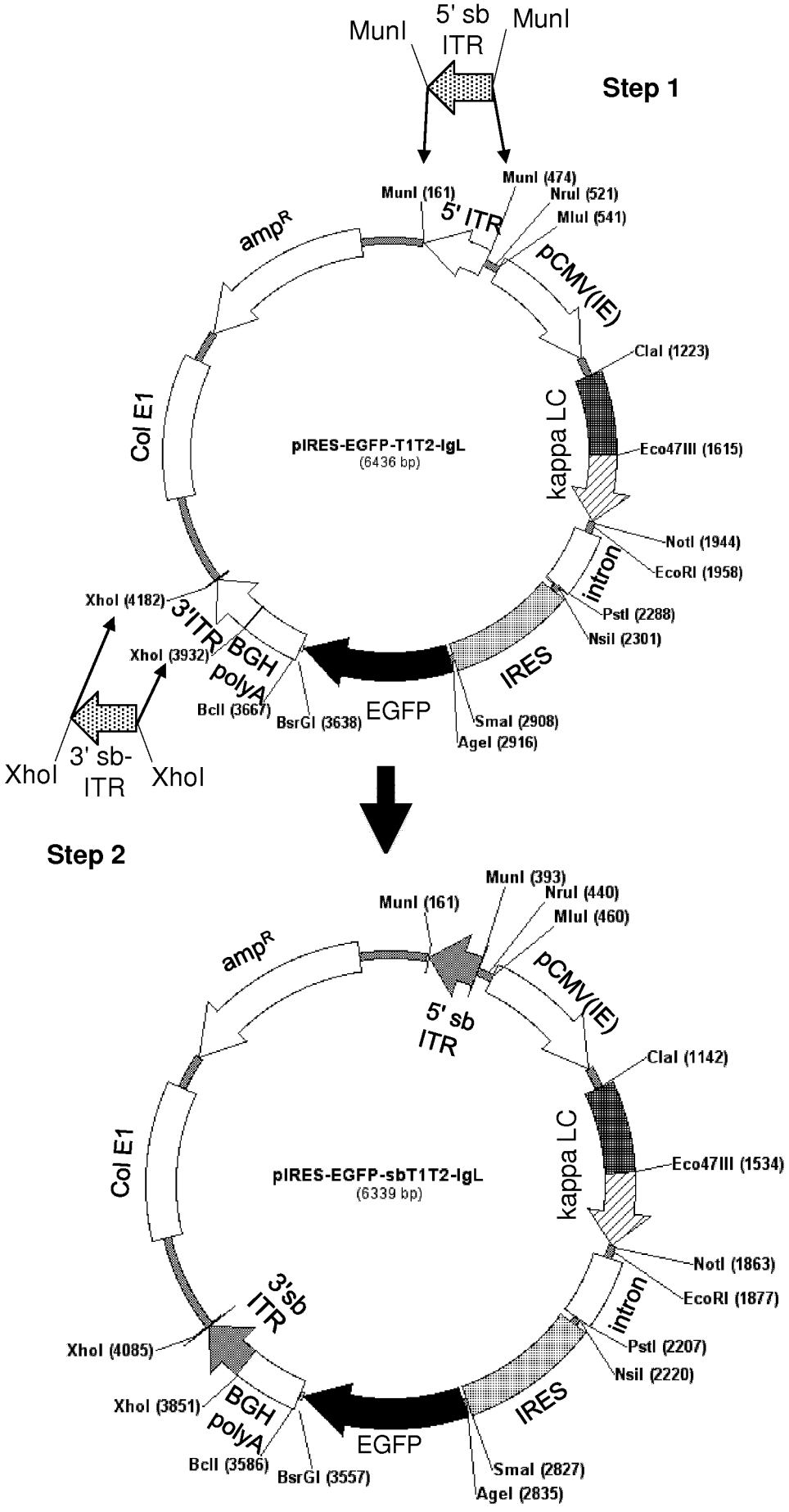

MunI
5' sb ITR
MunI
Step 1
MunI (161)
MunI (474)
NruI (521)
MluI (541)
5' ITR
ampR
pCMV(IE)
ClaI (1223)
Col E1
pIRES-EGFP-T1T2-IgL
(6436 bp)
kappa LC
Eco47III (1615)
NotI (1944)
EcoRI (1958)
intron
PstI (2288)
NsiI (2301)
XhoI (4182)
3'ITR
XhoI (3932)
BGH polyA
IRES
BclI (3667)
BsrGI (3638)
EGFP
SmaI (2908)
AgeI (2916)
XhoI
3' sb-ITR
XhoI
Step 2
MunI (393)
NruI (440)
MluI (460)
MunI (161)
ampR
pCMV(IE)
5' sb ITR
ClaI (1142)
Col E1
pIRES-EGFP-sbT1T2-IgL
(6339 bp)
kappa LC
Eco47III (1534)
NotI (1863)
EcoRI (1877)
3'sb ITR
intron
PstI (2207)
NsiI (2220)
XhoI (4085)
XhoI (3851)
BGH polyA
IRES
EGFP
BclI (3586)
BsrGI (3557)
SmaI (2827)
AgeI (2835)

Fig. 9

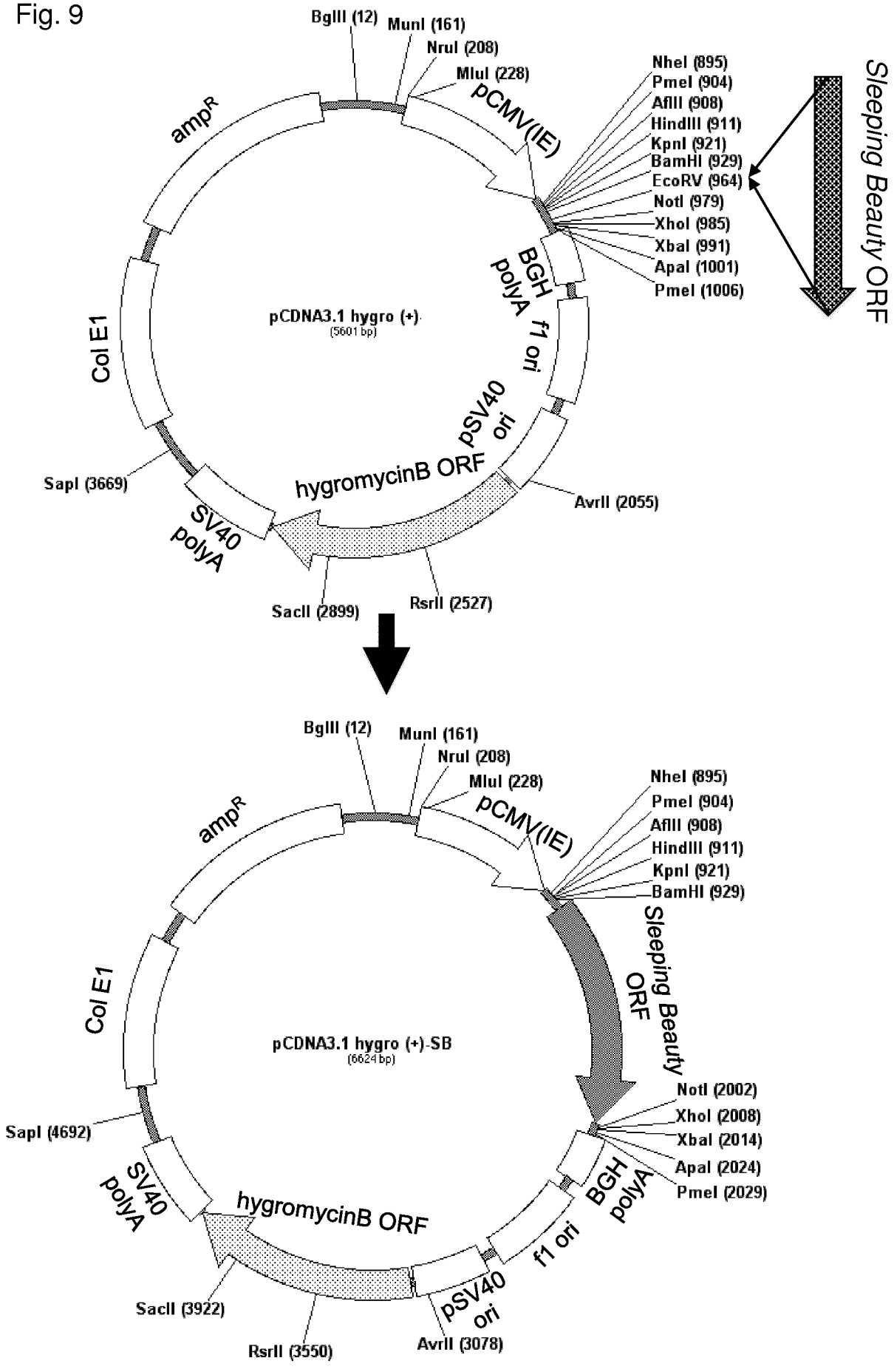
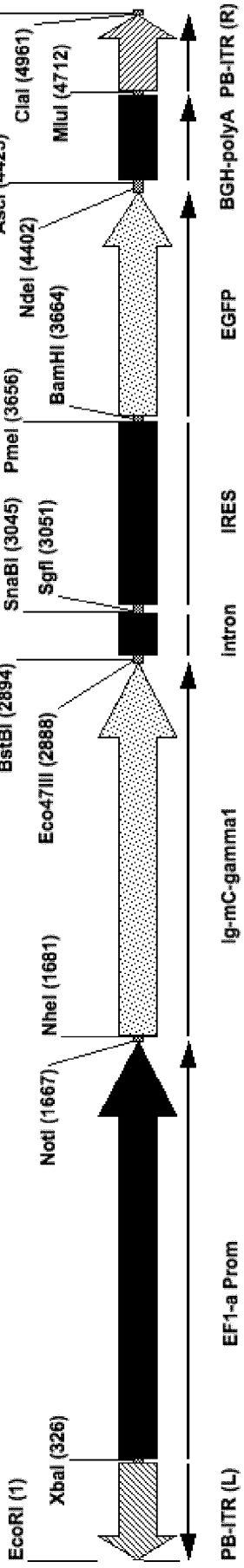
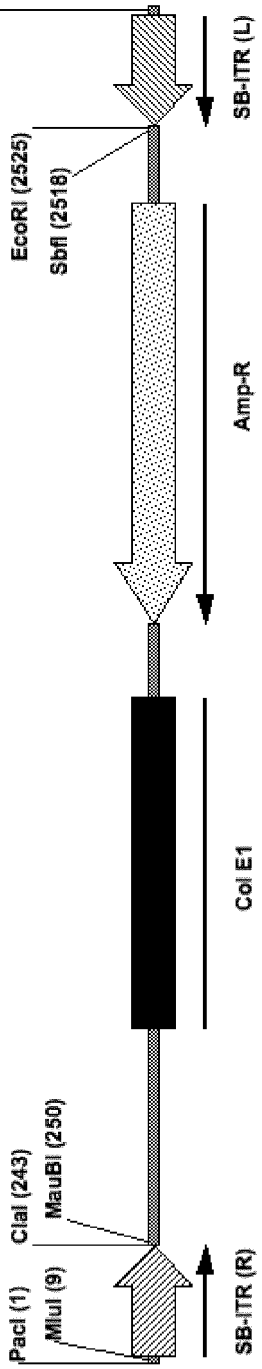

BglII (12)
MunI (161)
NruI (208)
MluI (228)
pCMV(IE)
NheI (895)
PmeI (904)
AflII (908)
HindIII (911)
KpnI (921)
BamHI (929)
EcoRV (964)
NotI (979)
XhoI (985)
XbaI (991)
ApaI (1001)
PmeI (1006)
Sleeping Beauty ORF
ampR
BGH polyA
f1 ori
pSV40 ori
Col E1
pCDNA3.1 hygro (+)
(5601 bp)
hygromycinB ORF
SapI (3669)
SV40 polyA
AvrII (2055)
SacII (2899)
RsrII (2527)
BglII (12)
MunI (161)
NruI (208)
MluI (228)
pCMV(IE)
NheI (895)
PmeI (904)
AflII (908)
HindIII (911)
KpnI (921)
BamHI (929)
Sleeping Beauty ORF
ampR
Col E1
pCDNA3.1 hygro (+)-SB
(6624 bp)
NotI (2002)
XhoI (2008)
XbaI (2014)
ApaI (2024)
PmeI (2029)
SapI (4692)
SV40 polyA
hygromycinB ORF
BGH polyA
f1 ori
pSV40 ori
SacII (3922)
RsrII (3550)
AvrII (3078)

Fig. 10:

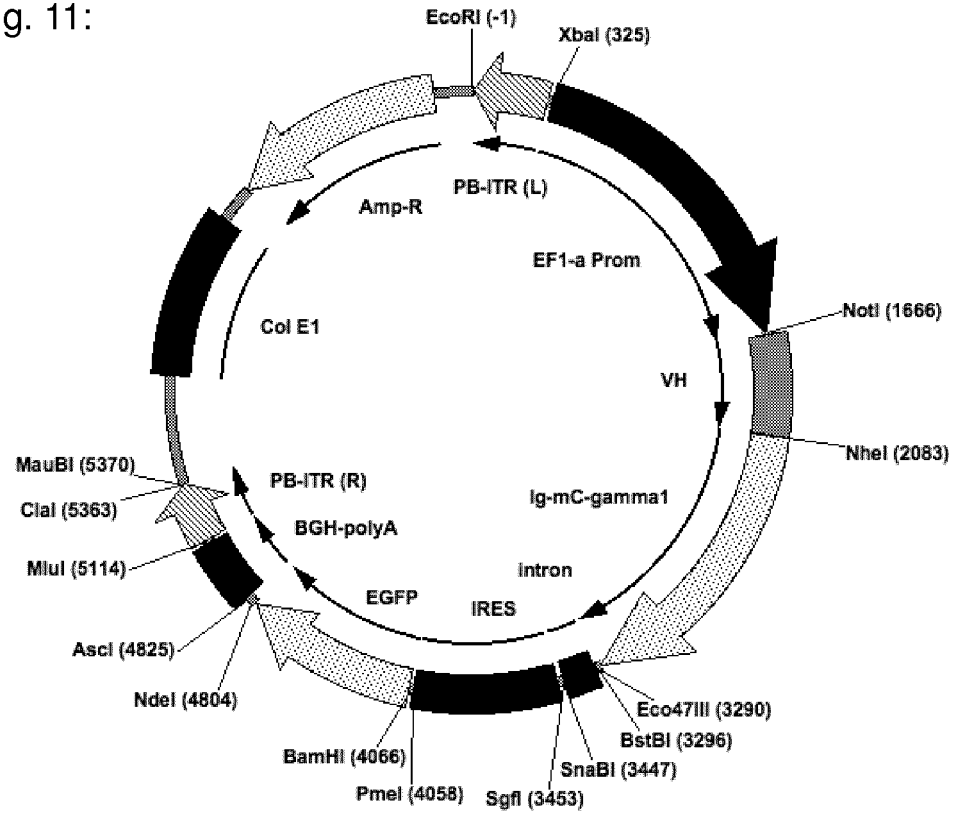


Functional elements and restriction enzyme sites in 4975 bp long DNA fragment Seq-ID20

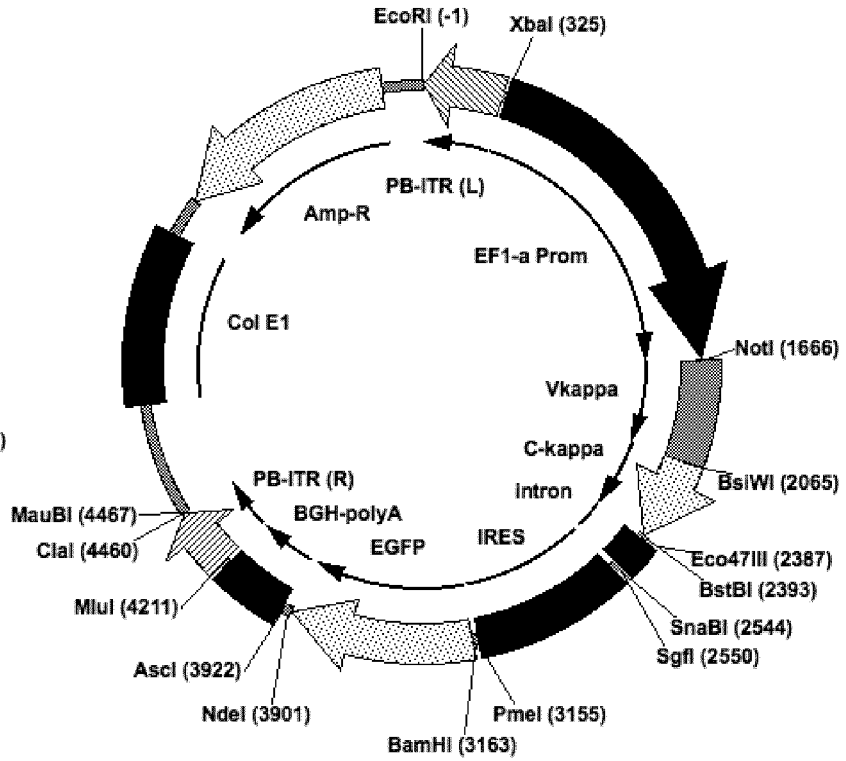


Functional elements and restriction enzyme sites in 2774 bp long DNA fragment Seq-ID21

Fig. 11:



Transposable mIg-gamma1 HC vector



Transposable Ig-kappa LC vector

No	construct name	ITRs from:	VH of:
1	pPB-EGFP-HC-Ac10	*PiggyBac*	αCD30-Ac10
2	pPB-EGFP-HC-hBU12	*PiggyBac*	αCD19-hBU12
3	pSB-EGFP-HC-Ac10	*SleepingBeauty*	αCD30-Ac10
4	pSB-EGFP-HC-hBU12	*SleepingBeauty*	αCD19-hBU12

No	construct name	ITRs from:	VL of:
1	pPB-EGFP-LC-Ac10	*PiggyBac*	αCD30-Ac10
2	pPB-EGFP-LC-hBU12	*PiggyBac*	αCD19-hBU12
3	pSB-EGFP-LC-Ac10	*SleepingBeauty*	αCD30-Ac10
4	pSB-EGFP-LC-hBU12	*SleepingBeauty*	αCD19-hBU12

Fig. 12:

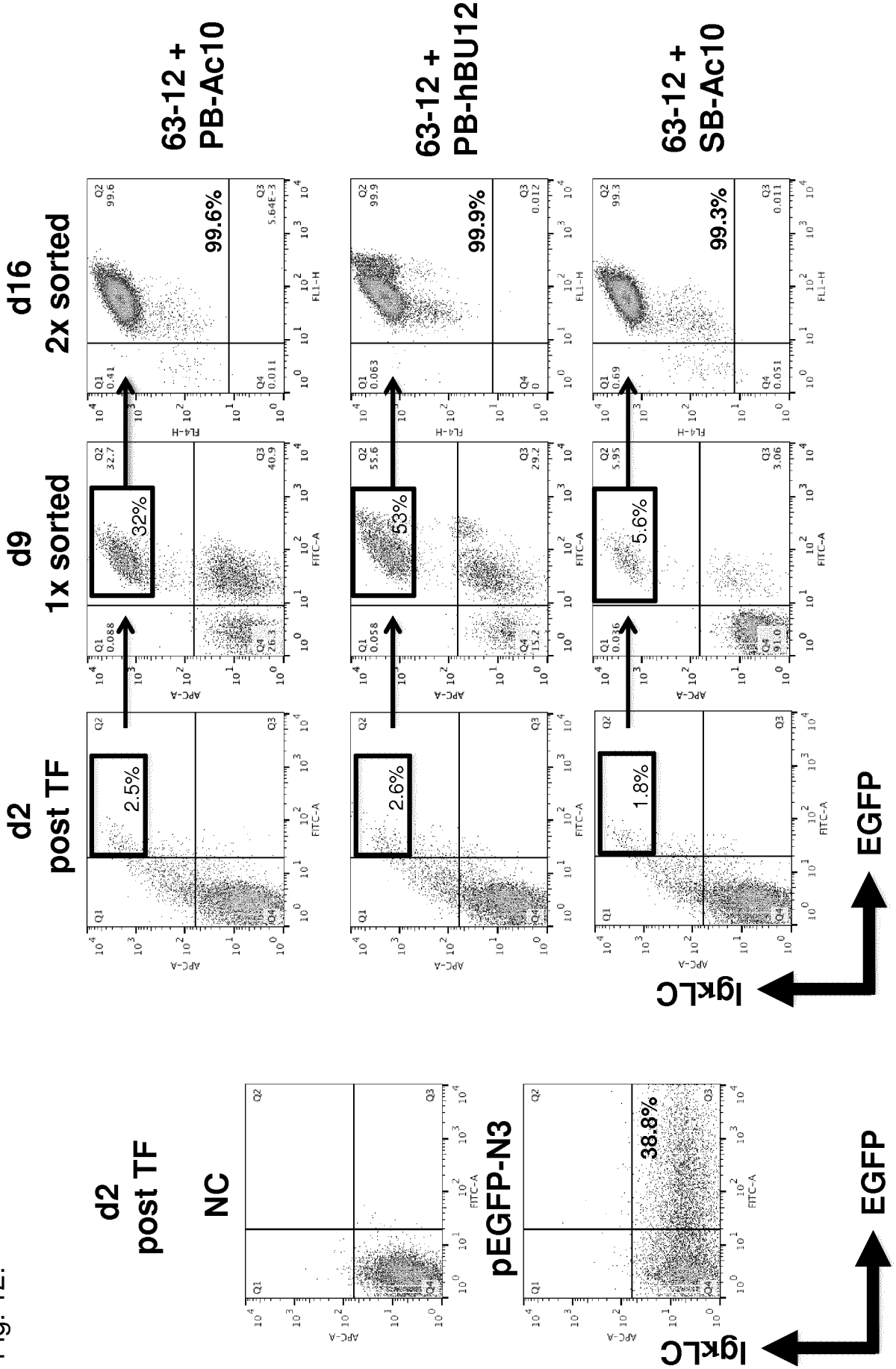

d2
post TF
NC
pEGFP-N3
38.8%
IgκLC
EGFP
d2
post TF
d9
1x sorted
d16
2x sorted
63-12 +
PB-Ac10
2.5%
32%
99.6%
63-12 +
PB-hBU12
2.6%
53%
99.9%
63-12 +
SB-Ac10
1.8%
5.6%
99.3%
IgκLC
EGFP

Fig. 13:

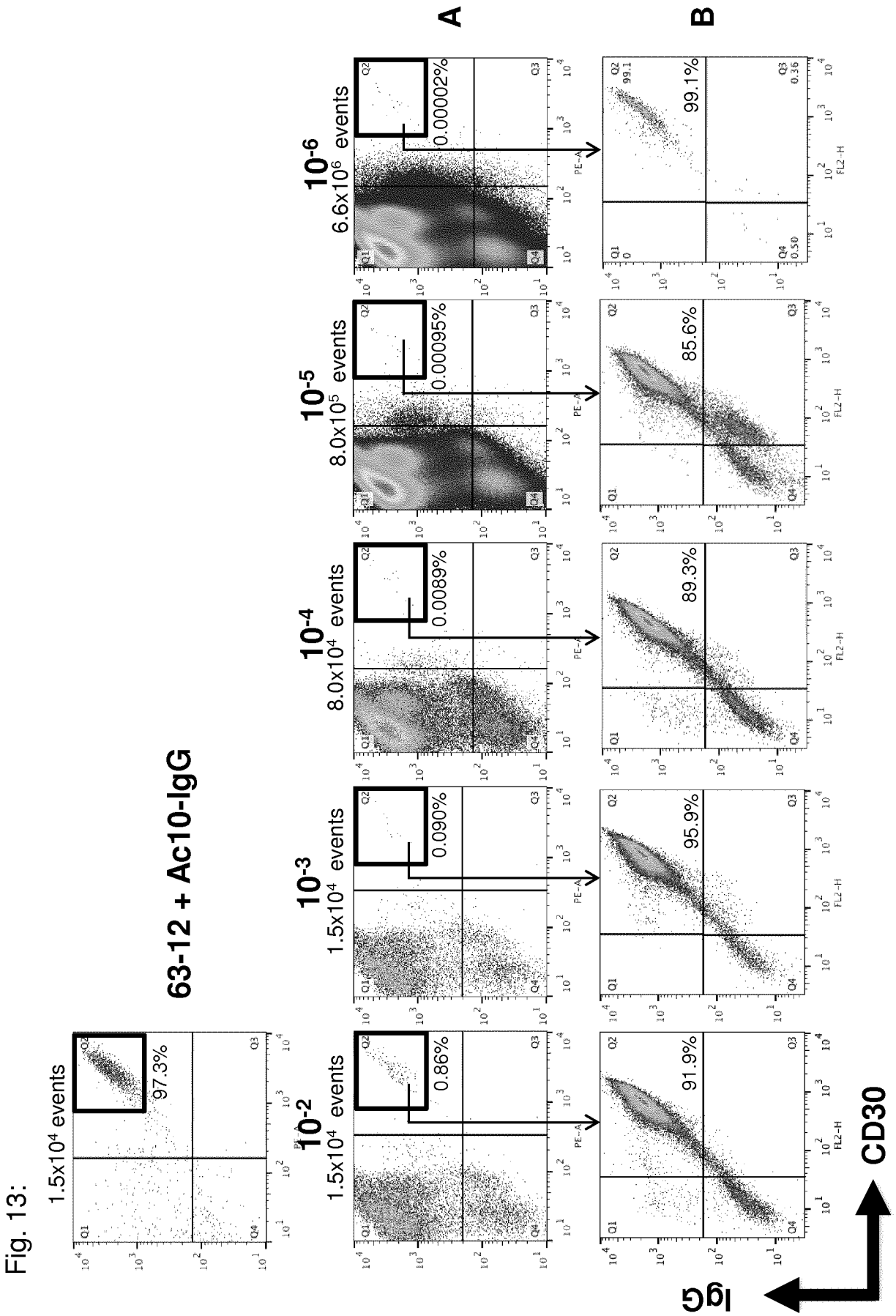

63-12 + Ac10-IgG
A
B
1.5x10⁴ events
97.3%
10-2
1.5x10⁴ events
0.86%
91.9%
10-3
1.5x10⁴ events
0.090%
95.9%
10-4
8.0x10⁴ events
0.0089%
89.3%
10-5
8.0x10⁵ events
0.00095%
85.6%
10-6
6.6x10⁶ events
0.00002%
99.1%
IgG
CD30

Fig. 14:

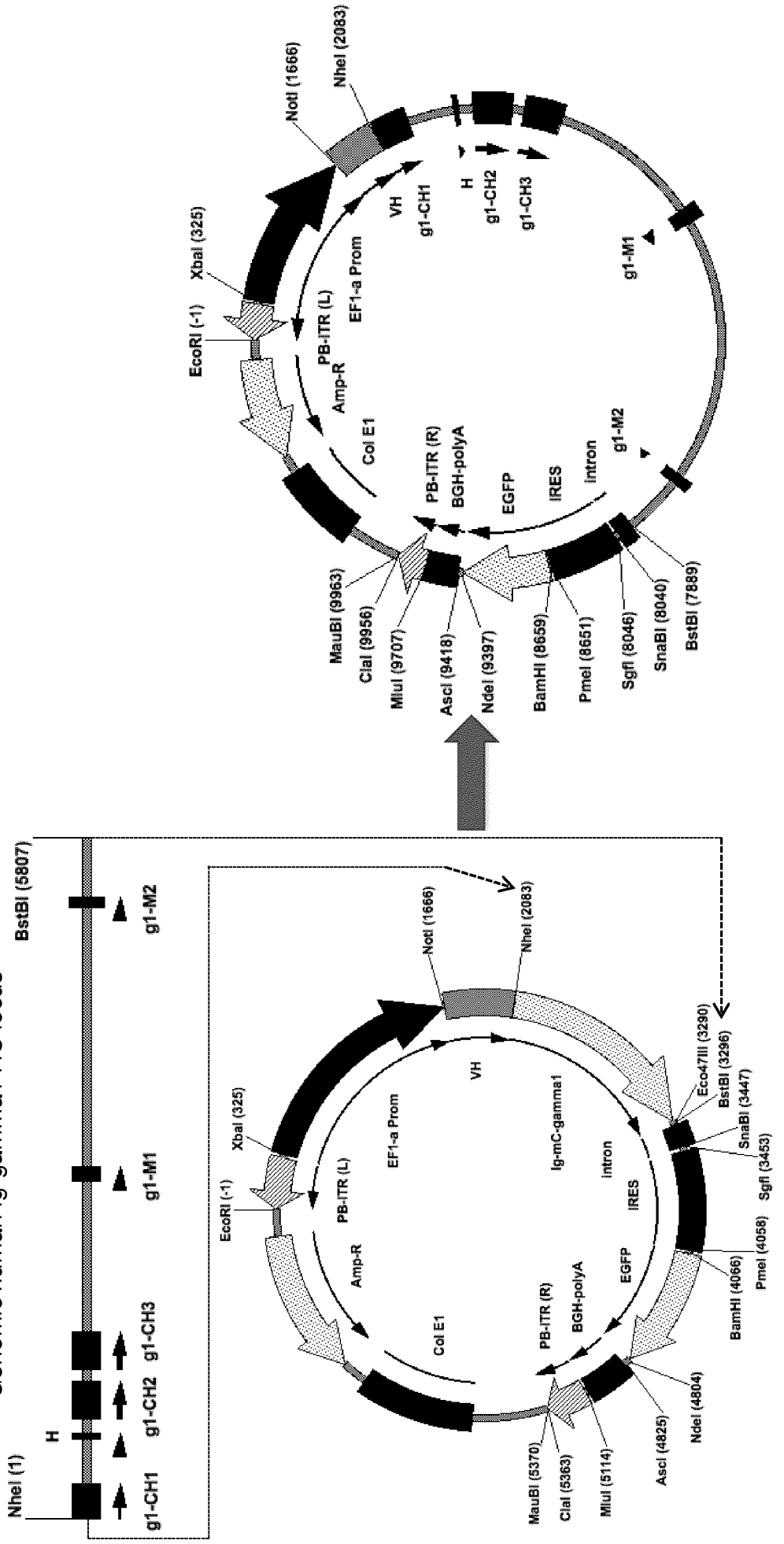

Genomic human Ig-gamma1 HC locus
NheI (1)
H
g1-CH1
g1-CH2
g1-CH3
BstBI (5807)
g1-M2
g1-M1
NotI (1666)
NheI (2083)
XbaI (325)
EcoRI (-1)
PB-ITR (L)
EF1-a Prom
VH
g1-CH1
H
g1-CH2
g1-CH3
g1-M1
Amp-R
Col E1
PB-ITR (R)
BGH-polyA
EGFP
IRES
Intron
g1-M2
MauBI (9963)
ClaI (9956)
MluI (9707)
AscI (9418)
NdeI (9397)
BamHI (8659)
PmeI (8651)
SgfI (8046)
SnaBI (8040)
BstBI (7889)
Eco47III (3290)
BstBI (3296)
SnaBI (3447)
SgfI (3453)
PmeI (4058)
BamHI (4066)
NdeI (4804)
AscI (4825)
MluI (5114)
ClaI (5363)
MauBI (5370)
Ig-mC-gamma1

Fig. 15

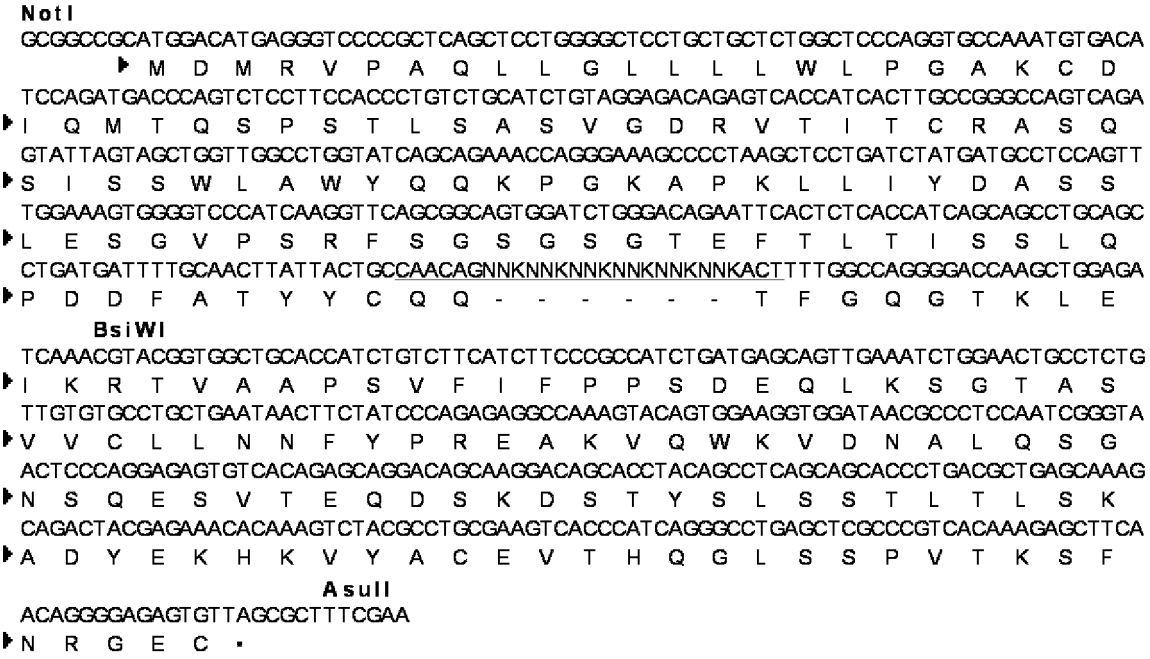

NotI
GCGGCCGCATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCAAATGTGACA
M D M R V P A Q L L G L L L L W L P G A K C D
TCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCCAGTCAGA
I Q M T Q S P S T L S A S V G D R V T I T C R A S Q
GTATTAGTAGCTGGTTGGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGATGCCTCCAGTT
S I S S W L A W Y Q Q K P G K A P K L L I Y D A S S
TGGAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACCATCAGCAGCCTGCAGC
L E S G V P S R F S G S G S G T E F T L T I S S L Q
CTGATGATTTTGCAACTTATTACTGCCAACAGNNKNNKNNKNNKNNKACTTTTGGCCAGGGGACCAAGCTGGAGA
P D D F A T Y Y C Q Q - - - - - - T F G Q G T K L E
BsiWI
TCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTG
I K R T V A A P S V F I F P P S D E Q L K S G T A S
TTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTA
V V C L L N N F Y P R E A K V Q W K V D N A L Q S G
ACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAG
N S Q E S V T E Q D S K D S T Y S L S S T L T L S K
CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA
A D Y E K H K V Y A C E V T H Q G L S S P V T K S F
AsuII
ACAGGGGAGAGTGTTAGCGCTTTCGAA
N R G E C ▪

Fig. 16

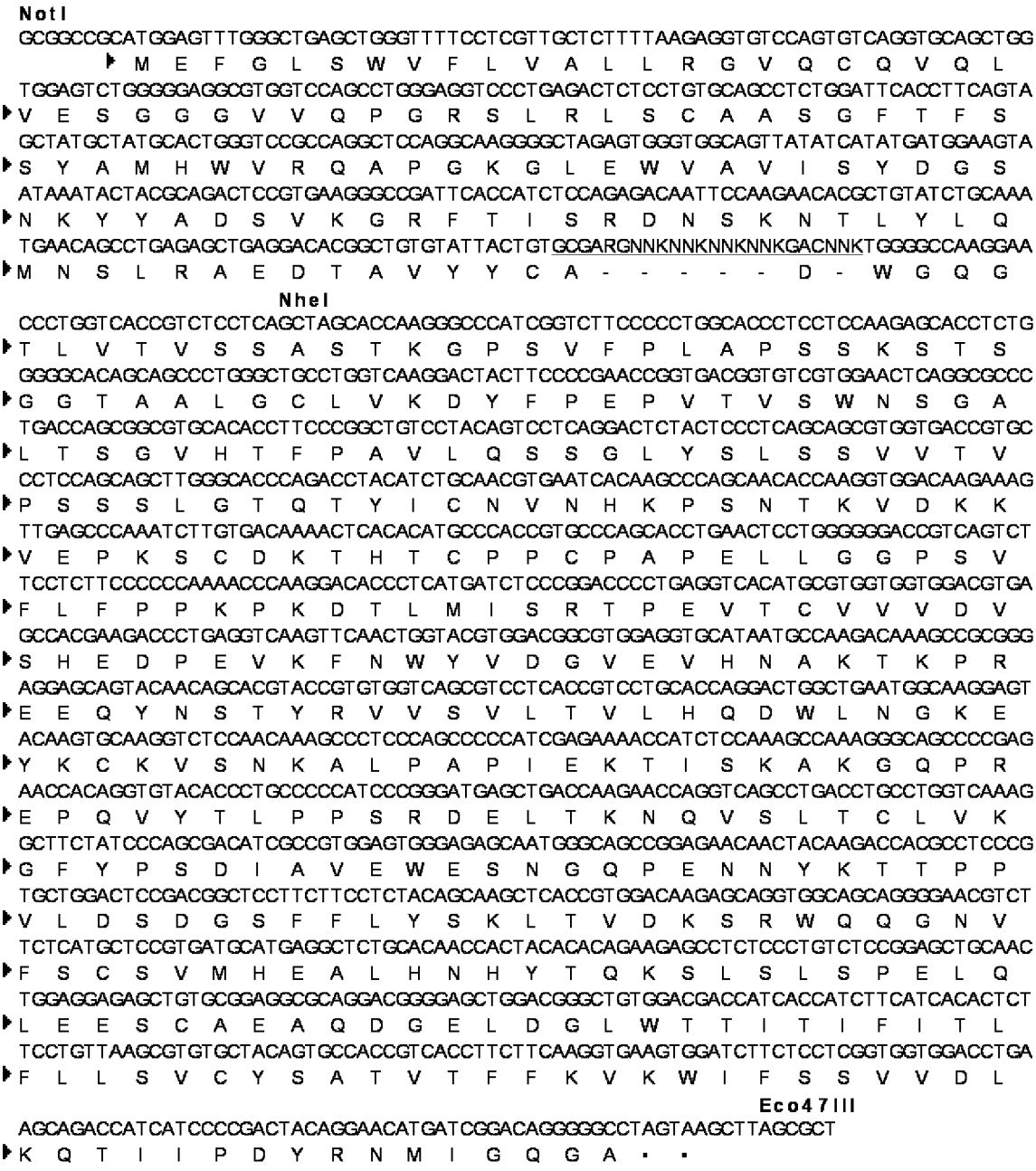


Fig. 17

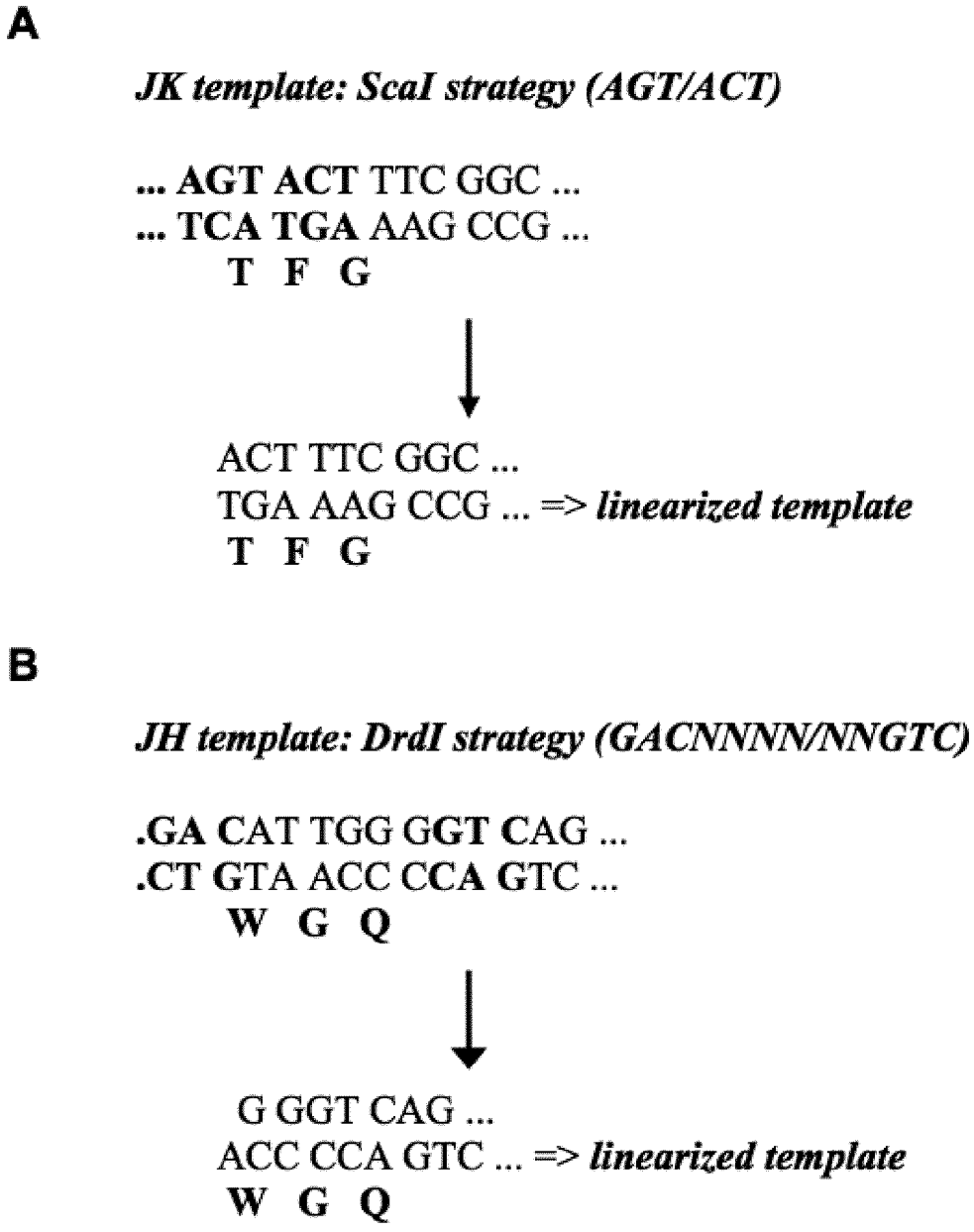
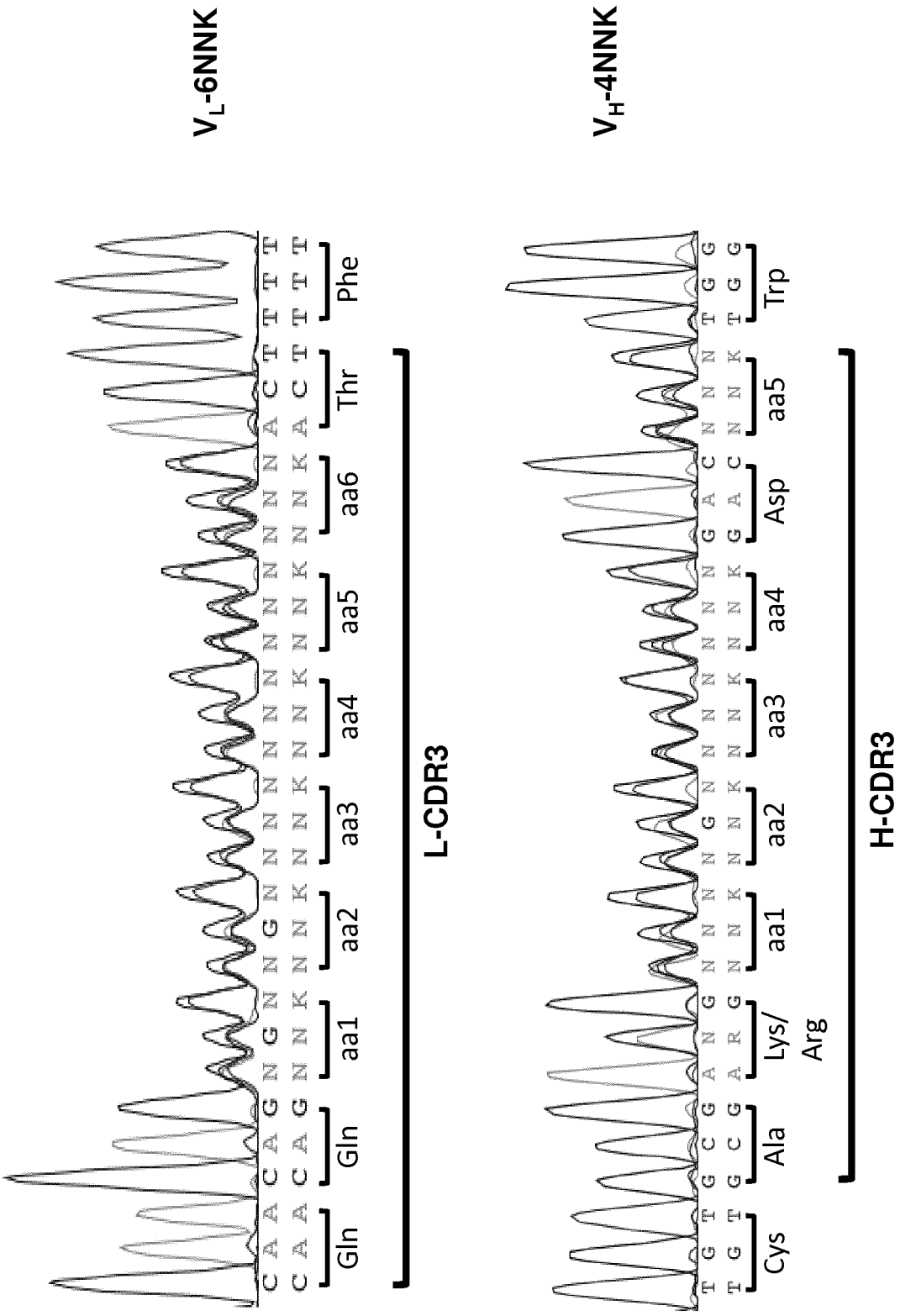

A
JK template: ScaI strategy (AGT/ACT)
... AGT ACT TTC GGC ...
... TCA TGA AAG CCG ...
T F G
ACT TTC GGC ...
TGA AAG CCG ... => linearized template
T F G
B
JH template: DrdI strategy (GACNNNN/NNGTC)
.GA CAT TGG GGT CAG ...
.CT GTA ACC CCA GTC ...
W G Q
G GGT CAG ...
ACC CCA GTC ... => linearized template
W G Q

Fig. 18:


A
V_L-6NNK
C A A C A G G N N G N N N N N N N N N N N N N N N A C T T T T
C A A C A G N N N K N N N K N N N K N N N K N N N K A C T T T T
Gln Gln aa1 aa2 aa3 aa4 aa5 aa6 Thr Phe
L-CDR3
B
V_H-4NNK
T G T G C G A N G N N N G N N N K N N N K N N N K G A C N N N K T G G G
T G T G C G A R G N N K N N N K N N N K N N N K G A C N N N K T G G G
Cys Ala Lys/ Arg aa1 aa2 aa3 aa4 Asp aa5 Trp
H-CDR3