Figure 1

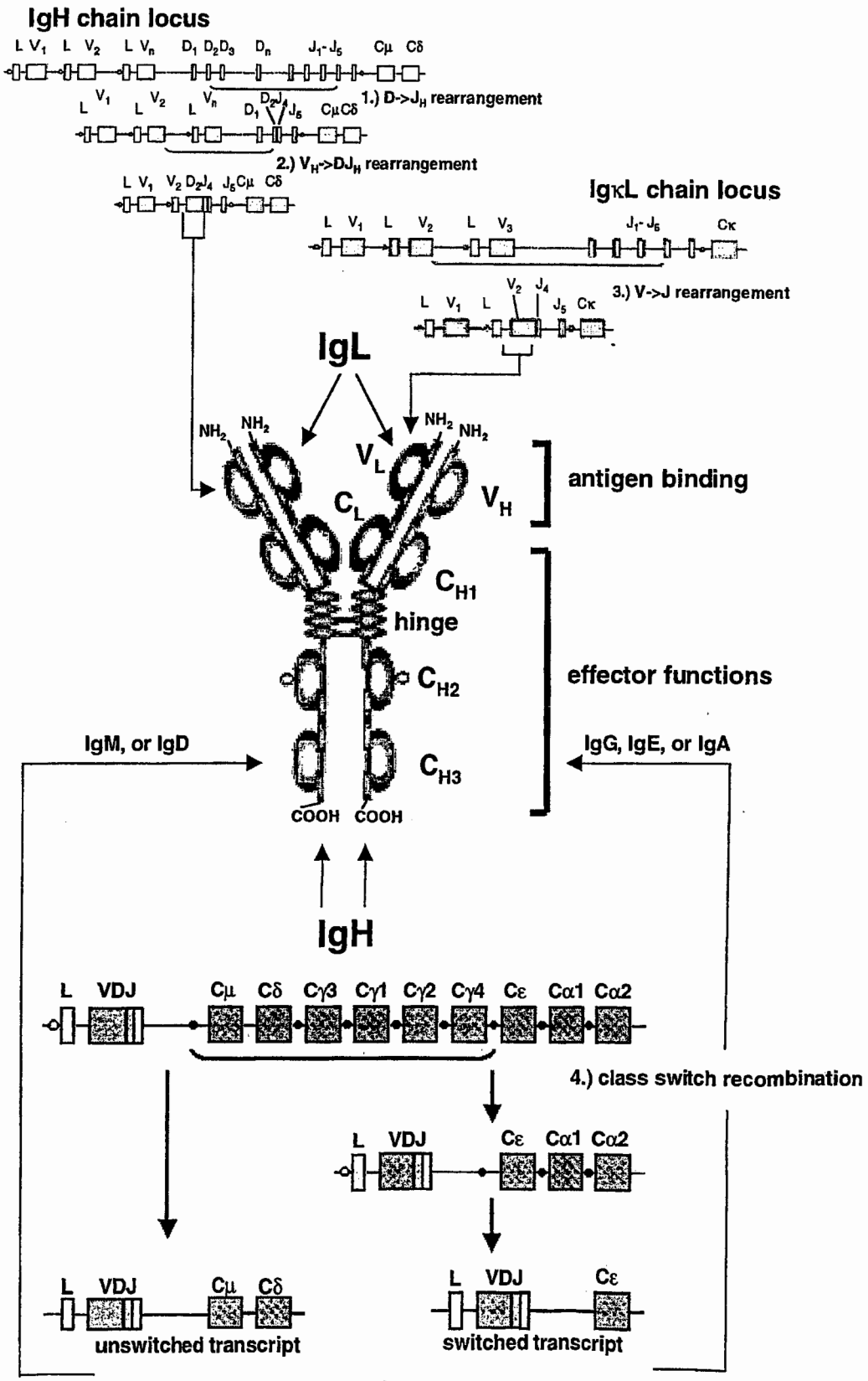

IgH chain locus
1.) D->JH rearrangement
2.) VH->DJH rearrangement
IgκL chain locus
3.) V->J rearrangement
IgL
antigen binding
effector functions
hinge
IgM, or IgD
IgG, IgE, or IgA
COOH
IgH
4.) class switch recombination
unswitched transcript
switched transcript

Figure 2

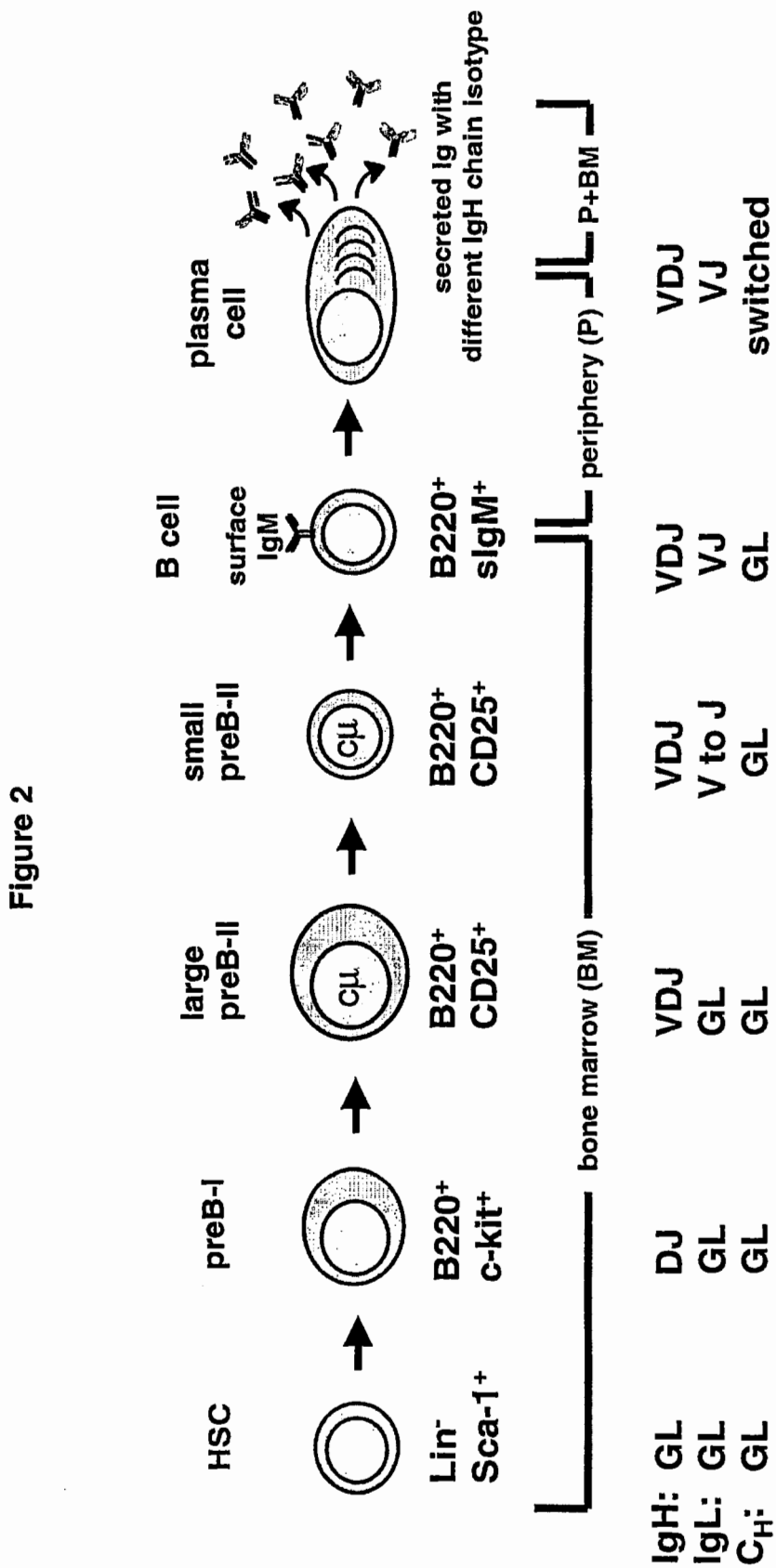

HSC
pre B-I
large preB-II
small preB-II
B cell
surface IgM
plasma cell
secreted Ig with different IgH chain isotype
Lin⁻ Sca-1⁺
B220⁺ c-kit⁺
B220⁺ CD25⁺
B220⁺ CD25⁺
B220⁺ sIgM⁺
cμ
cμ
bone marrow (BM)
periphery (P)
P+BM
IgH: GL DJ VDJ VDJ VDJ VDJ
IgL: GL GL GL V to J VJ VJ
C_H: GL GL GL GL GL switched

Figure 3

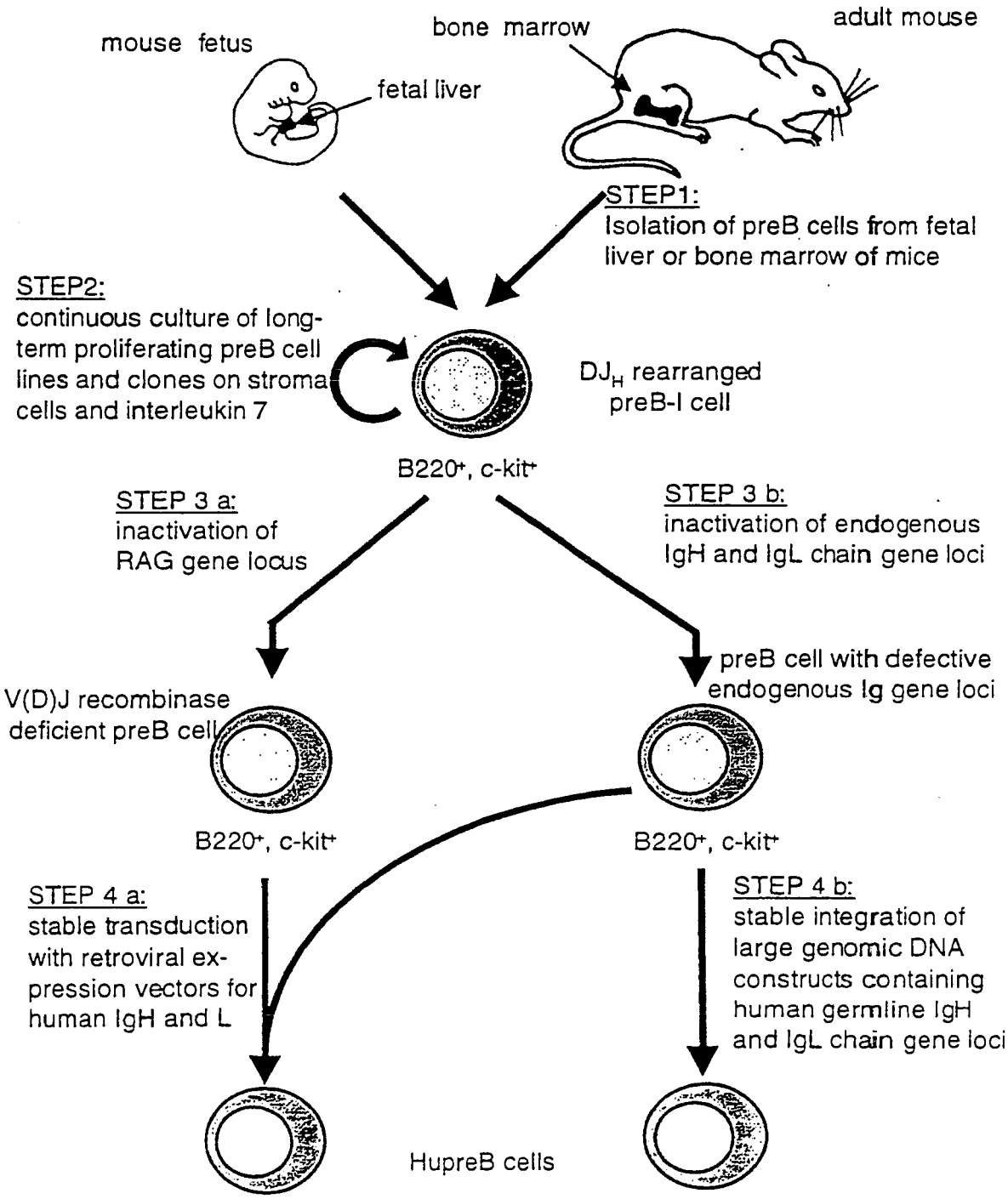

mouse fetus
fetal liver
bone marrow
adult mouse
STEP1:
Isolation of preB cells from fetal liver or bone marrow of mice
STEP2:
continuous culture of long-term proliferating preB cell lines and clones on stroma cells and interleukin 7
DJH rearranged preB-I cell
B220+, c-kit+
STEP 3 a:
inactivation of RAG gene locus
STEP 3 b:
inactivation of endogenous IgH and IgL chain gene loci
V(D)J recombinase deficient preB cell
preB cell with defective endogenous Ig gene loci
B220+, c-kit+
B220+, c-kit+
STEP 4 a:
stable transduction with retroviral expression vectors for human IgH and L
STEP 4 b:
stable integration of large genomic DNA constructs containing human germline IgH and IgL chain gene loci
HupreB cells
With the potential to express human antibodies from stably transduced retroviral expression constructs upon differentiation of HupreBs either in tissue culture in vitro, or upon transplantation in vivo.
With the potential to rearrange the germline human IgH and L chain gene loci and to express human antibodies therefrom upon differentiation of HupreBs either in tissue culture in vitro, or upon transplantation in vivo.

Figure 4

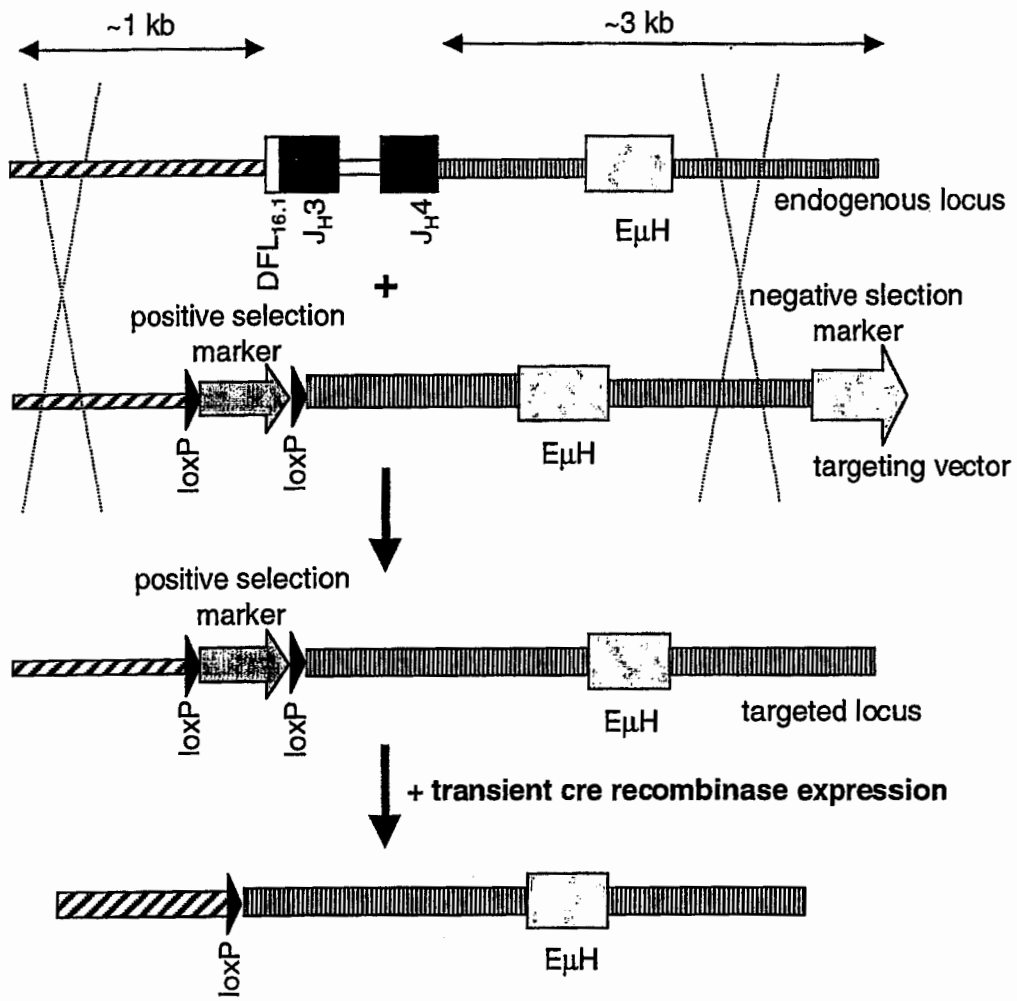

~1 kb
~3 kb
DFL16.1
JH3
JH4
EμH
endogenous locus
+
positive selection marker
negative slection marker
loxP
loxP
EμH
targeting vector
positive selection marker
loxP
loxP
EμH
targeted locus
+ transient cre recombinase expression
loxP
EμH
targeted locus after loxP mediated deletion of the selection marker

Figure 5a

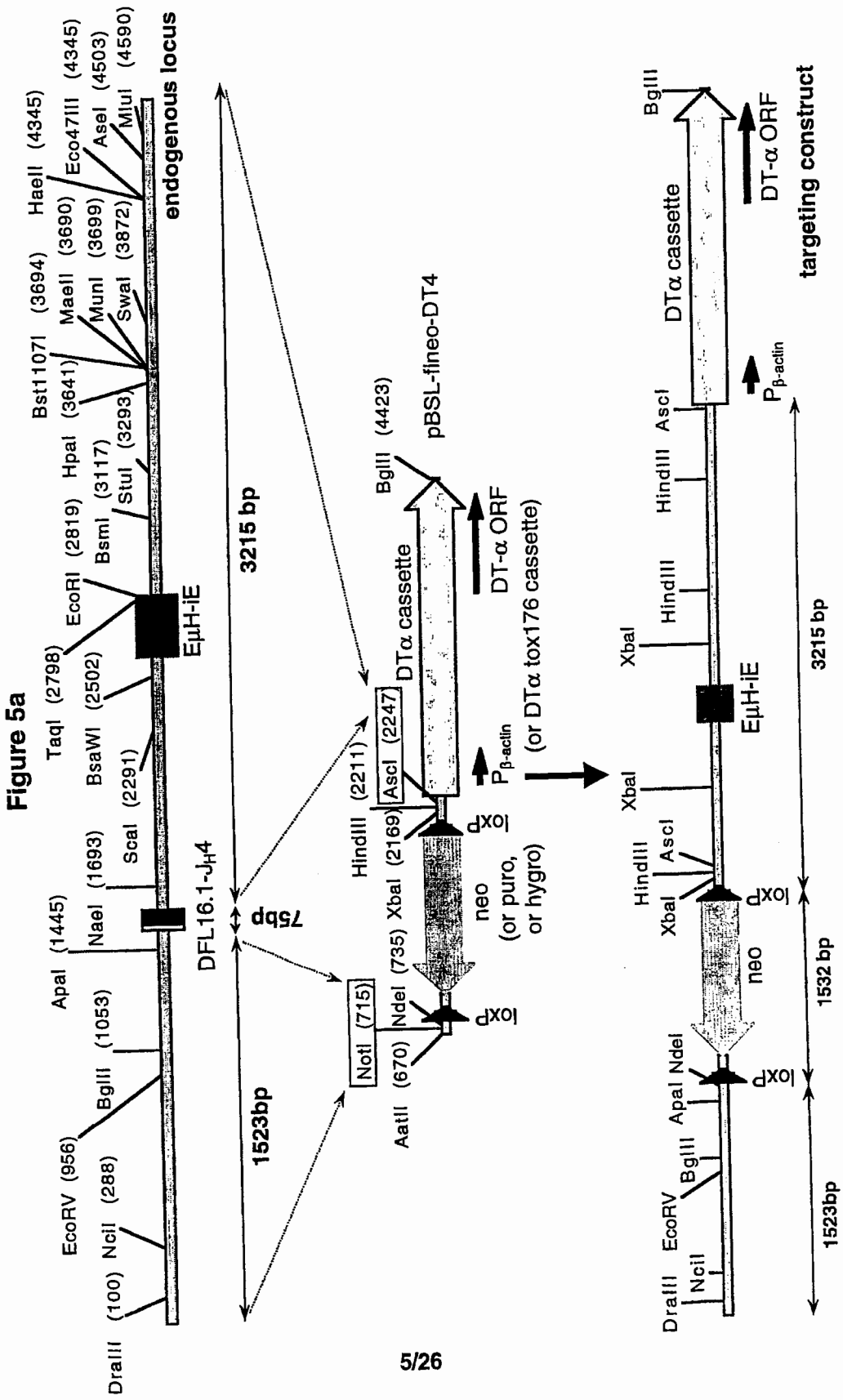

endogenous locus
DraIII (100)
NciI (288)
EcoRV (956)
BglII (1053)
ApaI (1445)
NaeI (1693)
ScaI (2291)
BsaWI (2502)
TaqI (2798)
EcoRI (2819)
BsmI (3117)
StuI (3293)
Bst1107I (3641)
HpaI (3694)
MaeII (3690)
MunI (3699)
SwaI (3872)
Eco47III (4345)
HaeII (4345)
AseI (4503)
MluI (4590)
EμH-iE
DFL16.1-JH4
75bp
3215 bp
1523bp
AatII (670)
NotI (715)
NdeI (735)
XbaI (2169)
HindIII (2211)
AscI (2247)
DTα cassette
BglII (4423)
pBSL-fineo-DT4
DT-α ORF
Pβ-actin
loxP
neo
(or puro, or hygro)
(or DTα tox176 cassette)
targeting construct
XbaI
HindIII
AscI
1532 bp
1523bp

Figure 5b

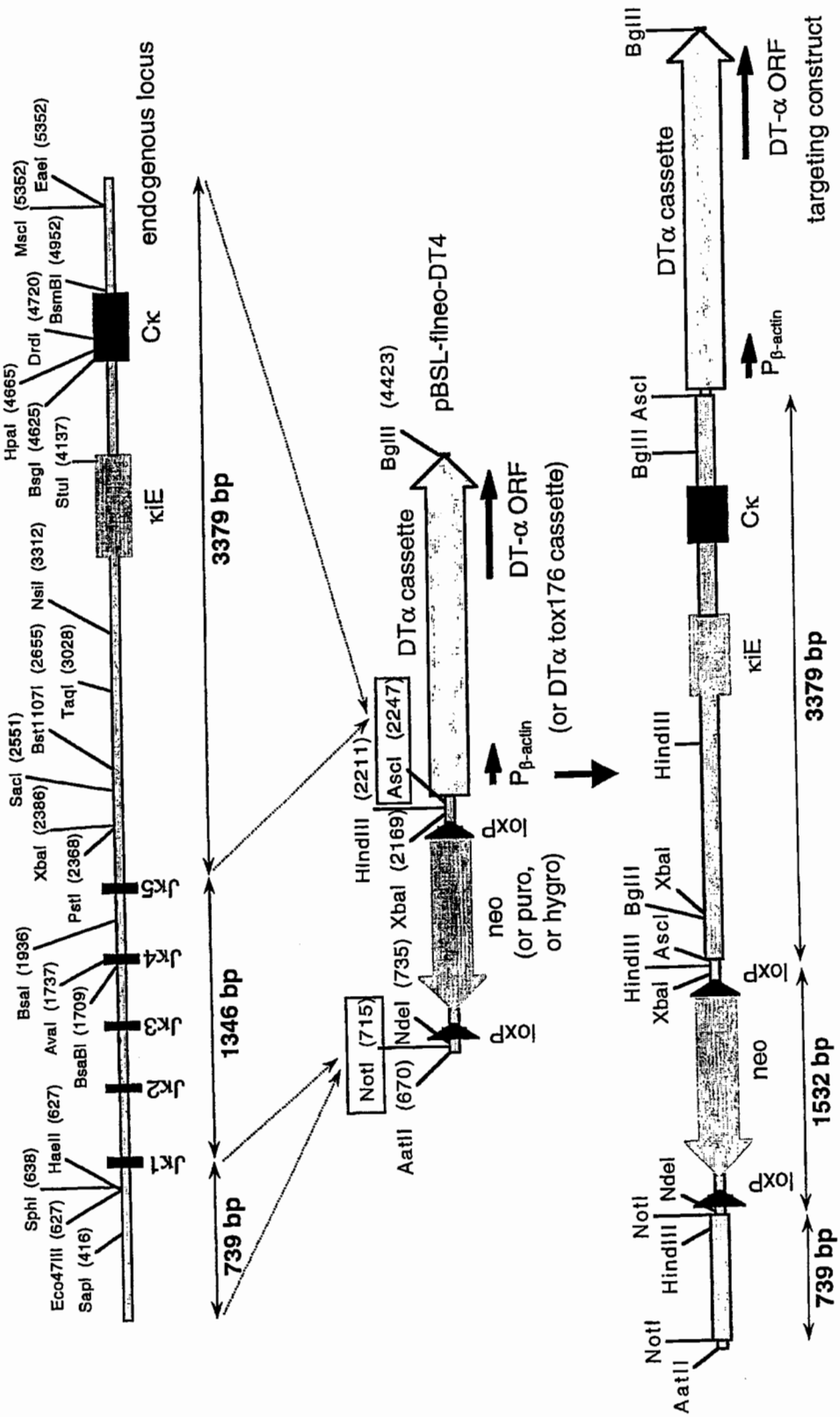

endogenous locus
Cκ
κiE
Jκ5
Jκ4
Jκ3
Jκ2
Jκ1
SphI (638)
Eco47III (627)
SapI (416)
HaeII (627)
BsaBI (1709)
AvaI (1737)
BsaI (1936)
PstI (2368)
XbaI (2386)
SacI (2551)
Bst1107I (2655)
TaqI (3028)
NsiI (3312)
StuI (4137)
BsgI (4625)
HpaI (4665)
DrdI (4720)
BsmBI (4952)
MscI (5352)
EaeI (5352)
3379 bp
1346 bp
739 bp
pBSL-flneo-DT4
BglII (4423)
DTα cassette
DT-α ORF
(or DTα tox176 cassette)
AscI (2247)
HindIII (2211)
XbaI (2169)
XbaI (735)
NdeI (715)
NotI (670)
AatII
loxP
neo
(or puro,
or hygro)
Pβ-actin
targeting construct
BglII
BglII AscI
HindIII
BglII
XbaI
AscI
HindIII
XbaI
NdeI
NotI
HindIII
NotI
AatII
1532 bp
739 bp

Figure 5c

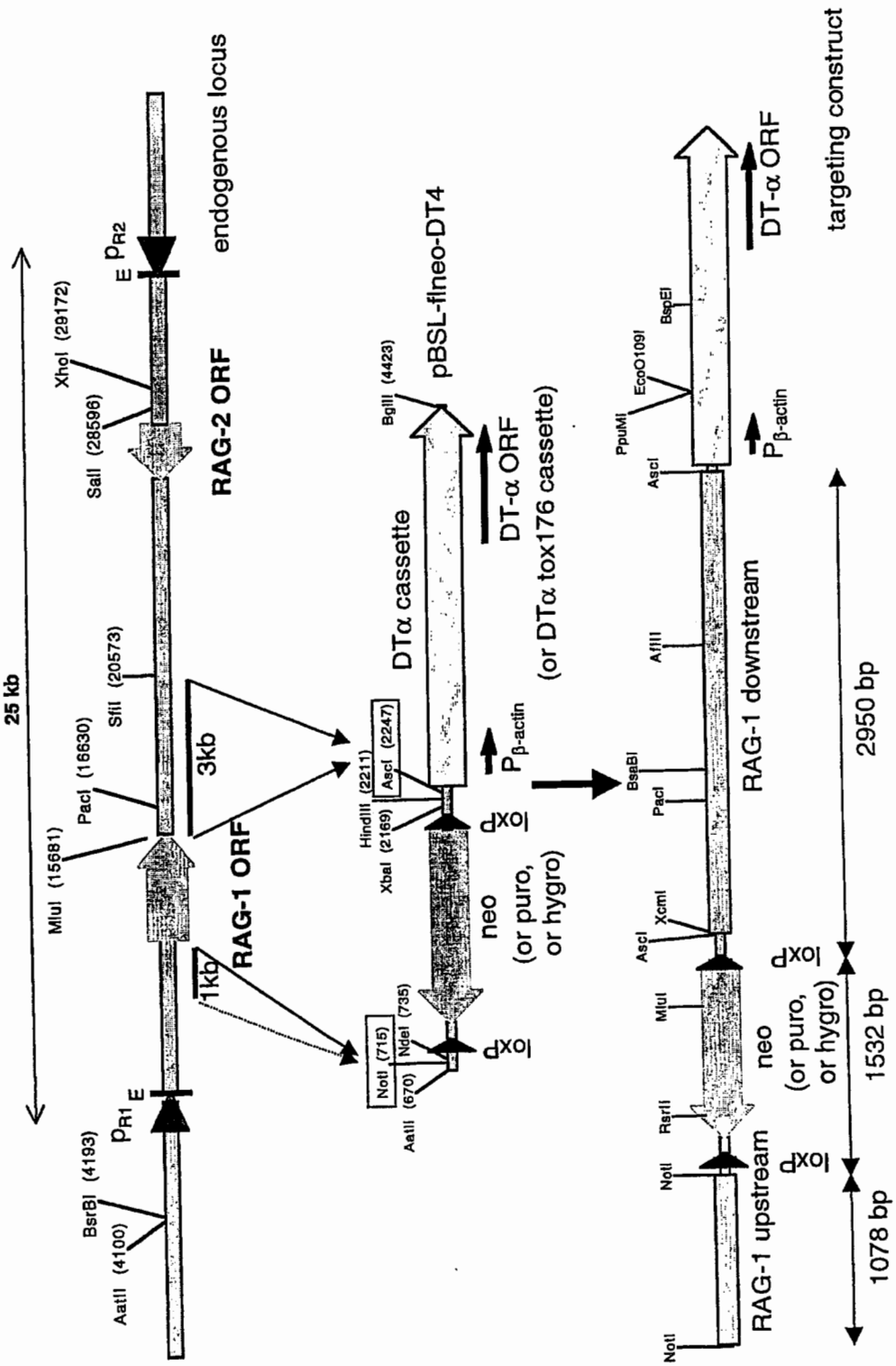
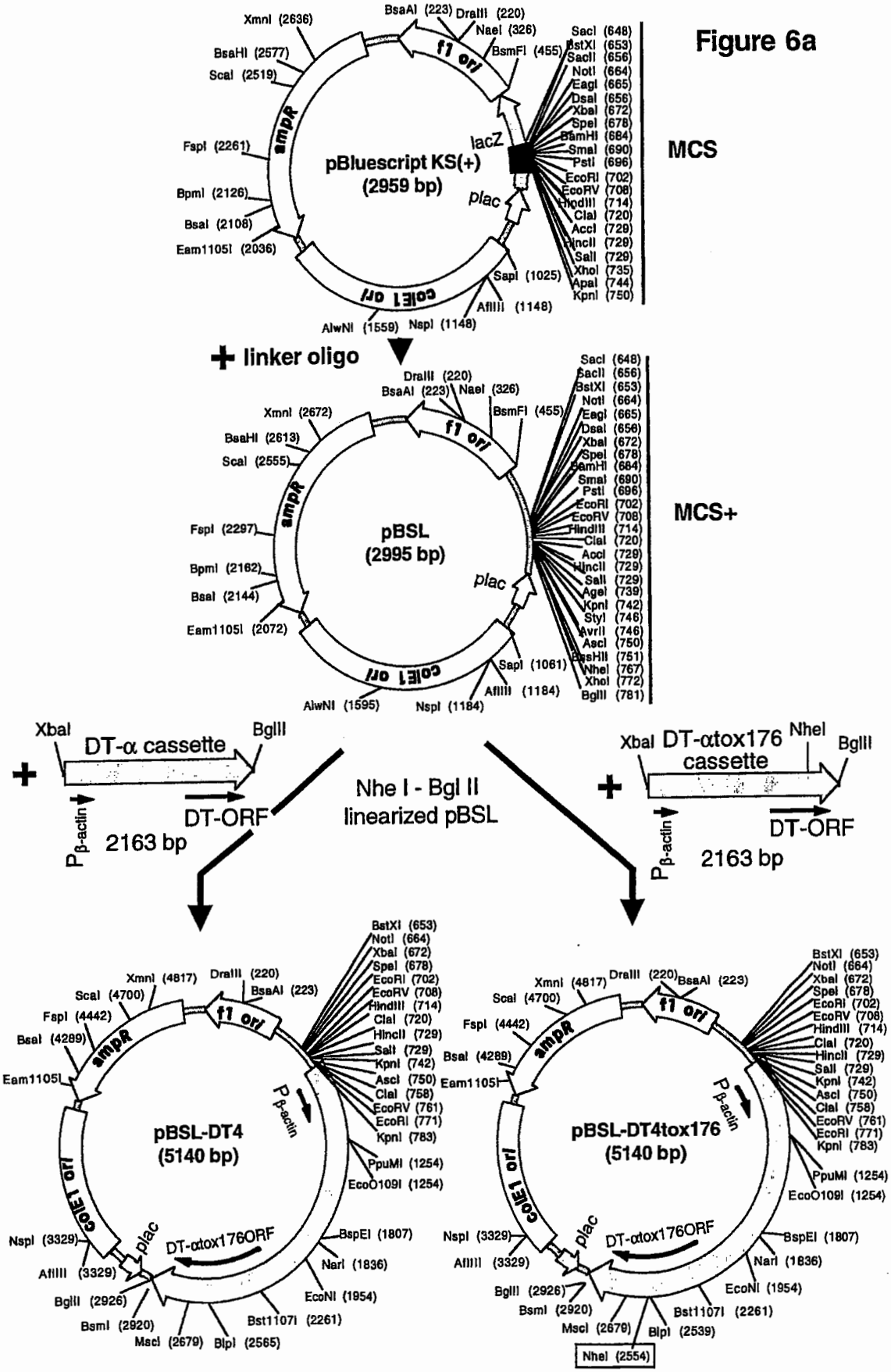

endogenous locus
RAG-2 ORF
RAG-1 ORF
25 kb
3kb
1kb
pBSL-flneo-DT4
DTα cassette
DT-α ORF
(or DTα tox176 cassette)
neo
(or puro, or hygro)
loxP
targeting construct
RAG-1 upstream
RAG-1 downstream
2950 bp
1532 bp
1078 bp
XhoI (28172)
SalI (28596)
SfiI (20573)
PacI (16630)
MluI (15681)
BsrBI (4193)
AatII (4100)
BglII (4423)
HindIII (2211)
AscI (2247)
XbaI (2169)
NotI (715)
NdeI (735)
AatII (670)
PpuMI
EcoO109I
BspEI
AflII
BsaBI
PacI
XcmI
AscI
MluI
RsrII
NotI

Figure 6a


pBluescript KS(+)
(2959 bp)
MCS
+ linker oligo
pBSL
(2995 bp)
MCS+
Xbal
DT-α cassette
BglII
DT-ORF
Pβ-actin
2163 bp
Nhe I - Bgl II
linearized pBSL
Xbal DT-αtox176 cassette NheI BglII
pBSL-DT4
(5140 bp)
pBSL-DT4tox176
(5140 bp)
DT-αtox176ORF
ampR
f1 ori
lacZ
plac
ColE1 ori

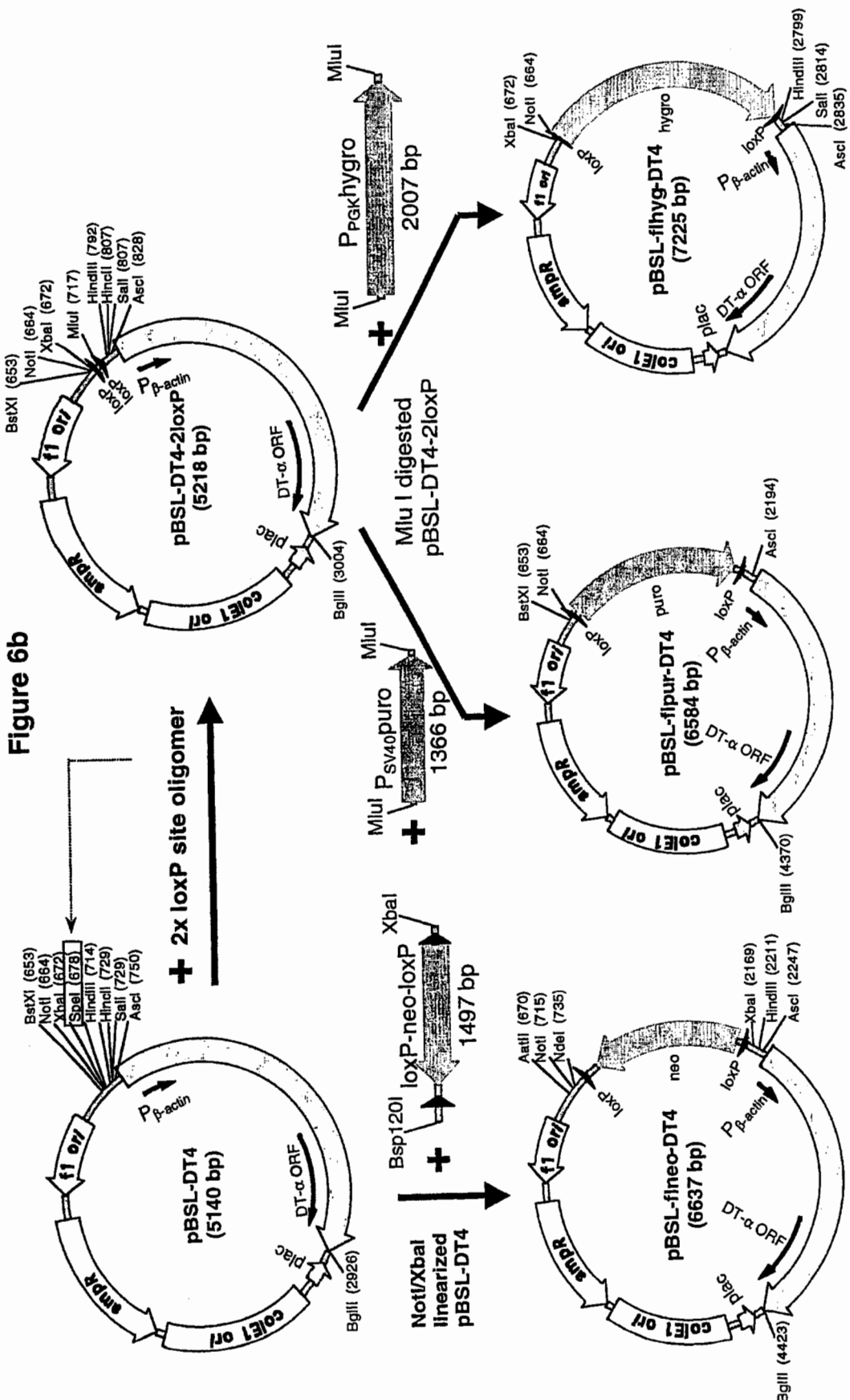

pBSL-DT4
(5140 bp)
pBSL-DT4-2loxP
(5218 bp)
pBSL-flhyg-DT4
(7225 bp)
pBSL-flpur-DT4
(6584 bp)
pBSL-fineo-DT4
(6637 bp)
2x loxP site oligomer
MluI PPGKhygro
2007 bp
Mlu I digested
pBSL-DT4-2loxP
MluI PSV40puro
1366 bp
Bsp120I loxP-neo-loxP
1497 bp
XbaI
NotI/XbaI
linearized
pBSL-DT4
DT-α ORF
hygro
puro
neo
ampR
ColE1 ori
f1 ori
loxP
plac
BglII (2926)
BglII (3004)
BglII (4370)
BglII (4423)

Figure 6b

Figure 7a

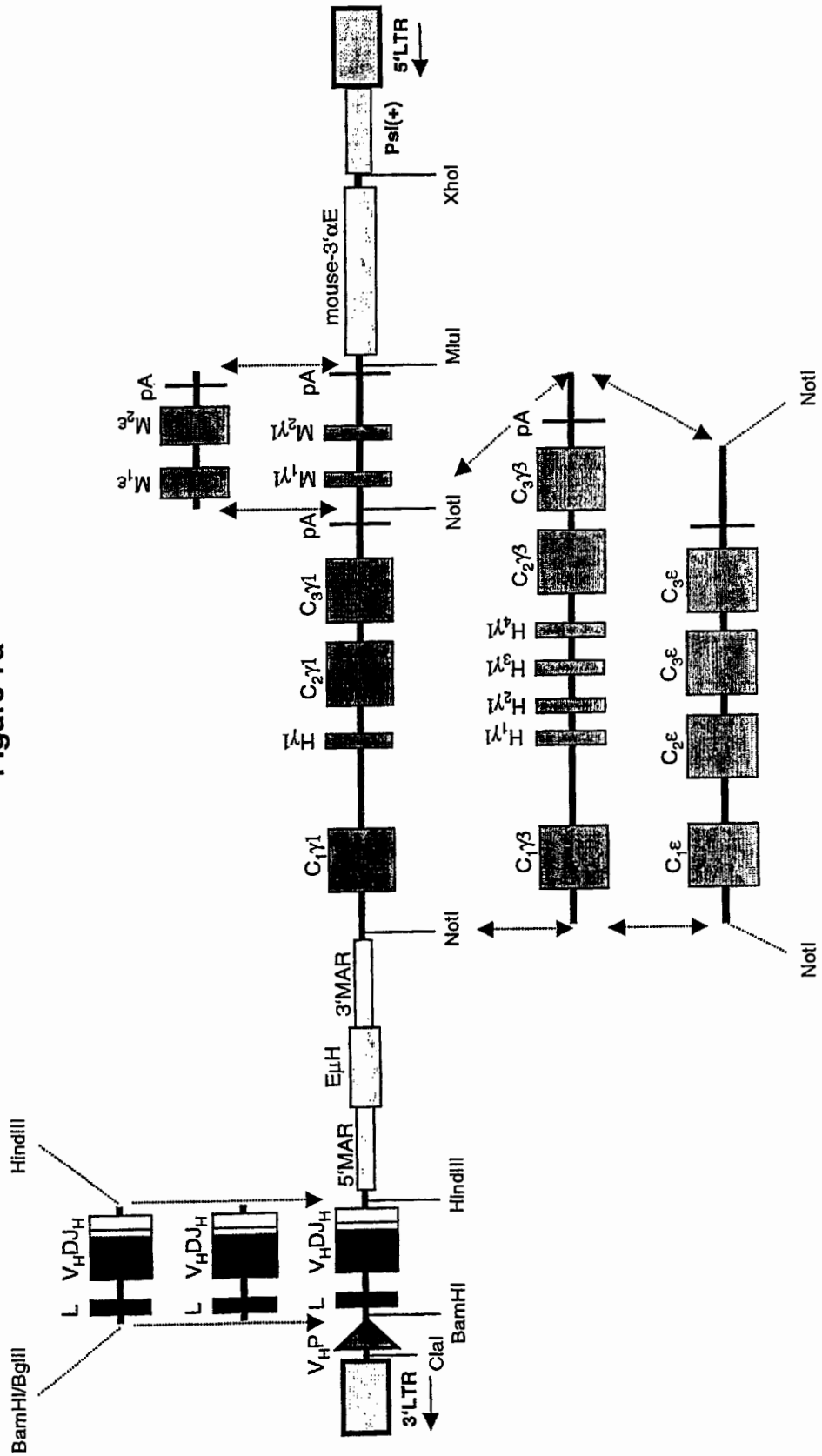

5'LTR
PstI(+)
XhoI
mouse-3'αE
MluI
pA
$M_2\varepsilon$
$M_1\varepsilon$
$M_2\gamma1$
$M_1\gamma1$
NotI
$C_3\gamma1$
$C_2\gamma1$
$H\gamma1$
$C_1\gamma1$
3'MAR
EμH
5'MAR
HindIII
V_HDJ_H
L
V_HP
BamHI
ClaI
3'LTR
BamHI/BglII
$C_3\gamma3$
$C_2\gamma3$
$H_4\gamma1$
$H_3\gamma1$
$H_2\gamma1$
$H_1\gamma1$
$C_1\gamma3$
$C_3\varepsilon$
$C_2\varepsilon$
$C_1\varepsilon$

Figure 7b

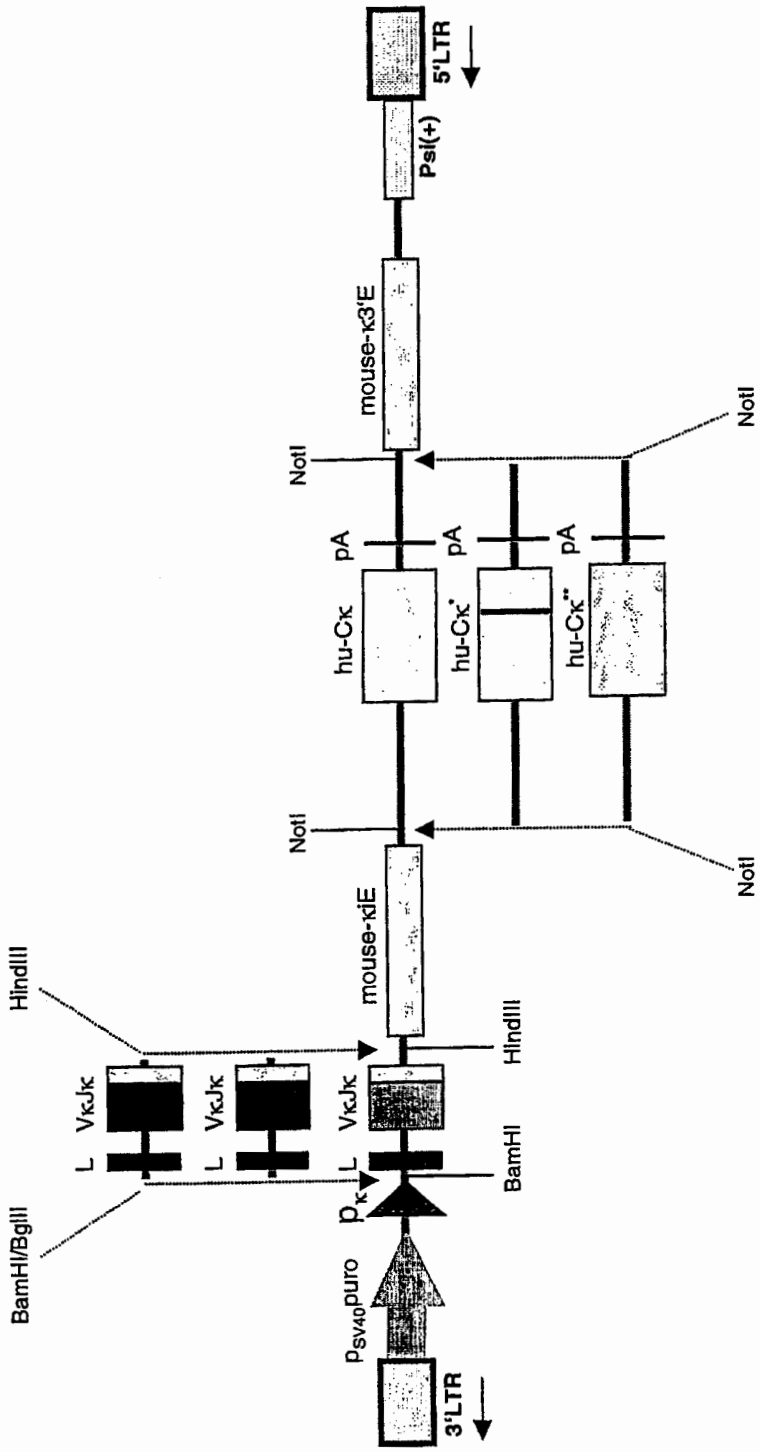

5'LTR
PsiI(+)
mouse-κ3'E
NotI
NotI
pA
pA
pA
hu-Cκ
hu-Cκ'
hu-Cκ''
NotI
NotI
mouse-κdE
HindIII
HindIII
VκJκ
VκJκ
VκJκ
L
L
L
BamHI
BamHI/BglII
pκ
pSV40puro
3'LTR

Figure 8

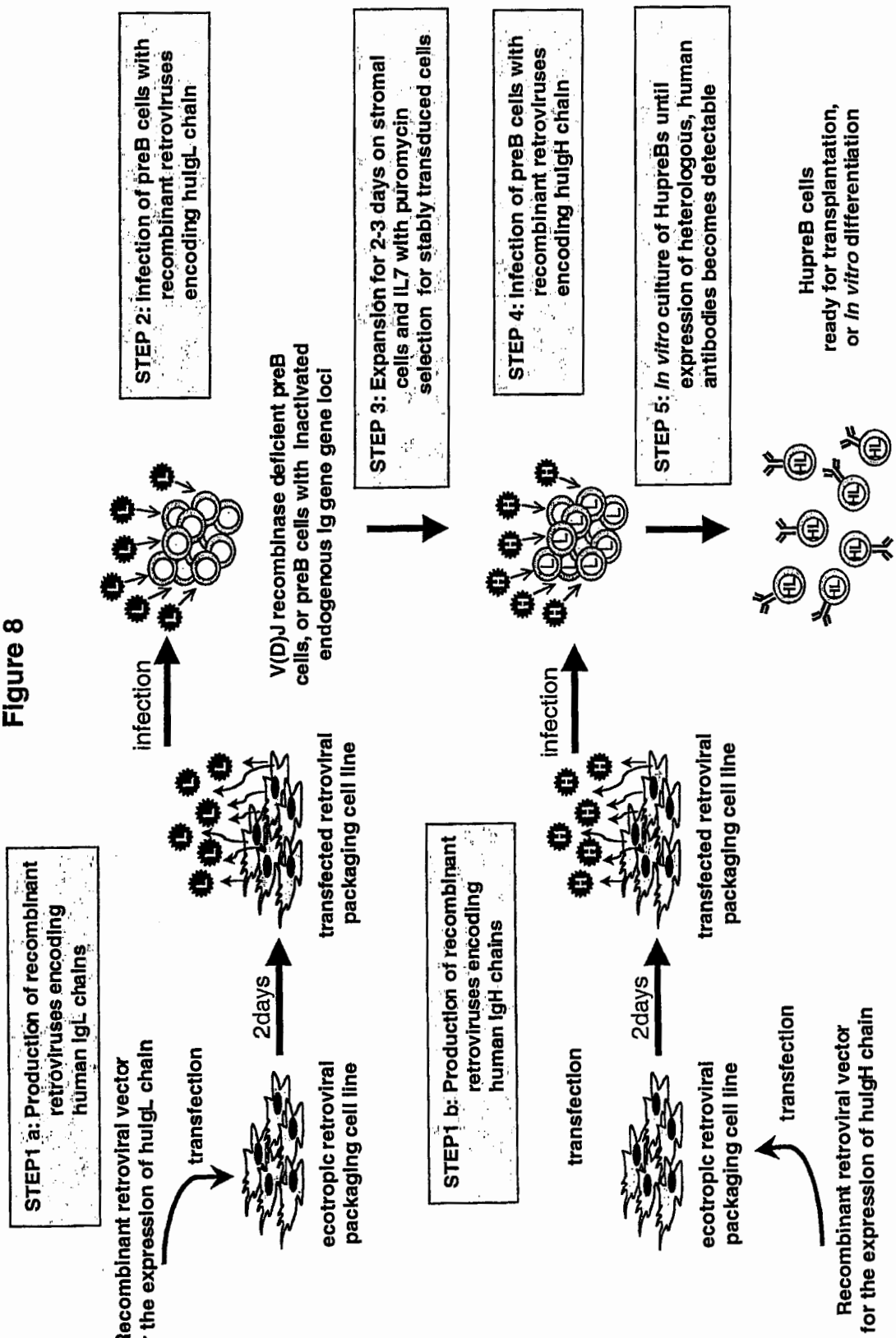

STEP1 a: Production of recombinant retroviruses encoding human IgL chains
Recombinant retroviral vector for the expression of hulgL chain
transfection
ecotropic retroviral packaging cell line
2days
transfected retroviral packaging cell line
infection
V(D)J recombinase deficient preB cells, or preB cells with inactivated endogenous Ig gene gene loci
STEP 2: infection of preB cells with recombinant retroviruses encoding hulgL chain
STEP 3: Expansion for 2-3 days on stromal cells and IL7 with puromycin selection for stably transduced cells
STEP 4: infection of preB cells with recombinant retroviruses encoding hulgH chain
STEP 5: In vitro culture of HupreBs until expression of heterologous, human antibodies becomes detectable
HupreB cells ready for transplantation, or In vitro differentiation
STEP1 b: Production of recombinant retroviruses encoding human IgH chains
Recombinant retroviral vector for the expression of hulgH chain
transfection
ecotropic retroviral packaging cell line
2days
transfected retroviral packaging cell line
infection

Figure 9

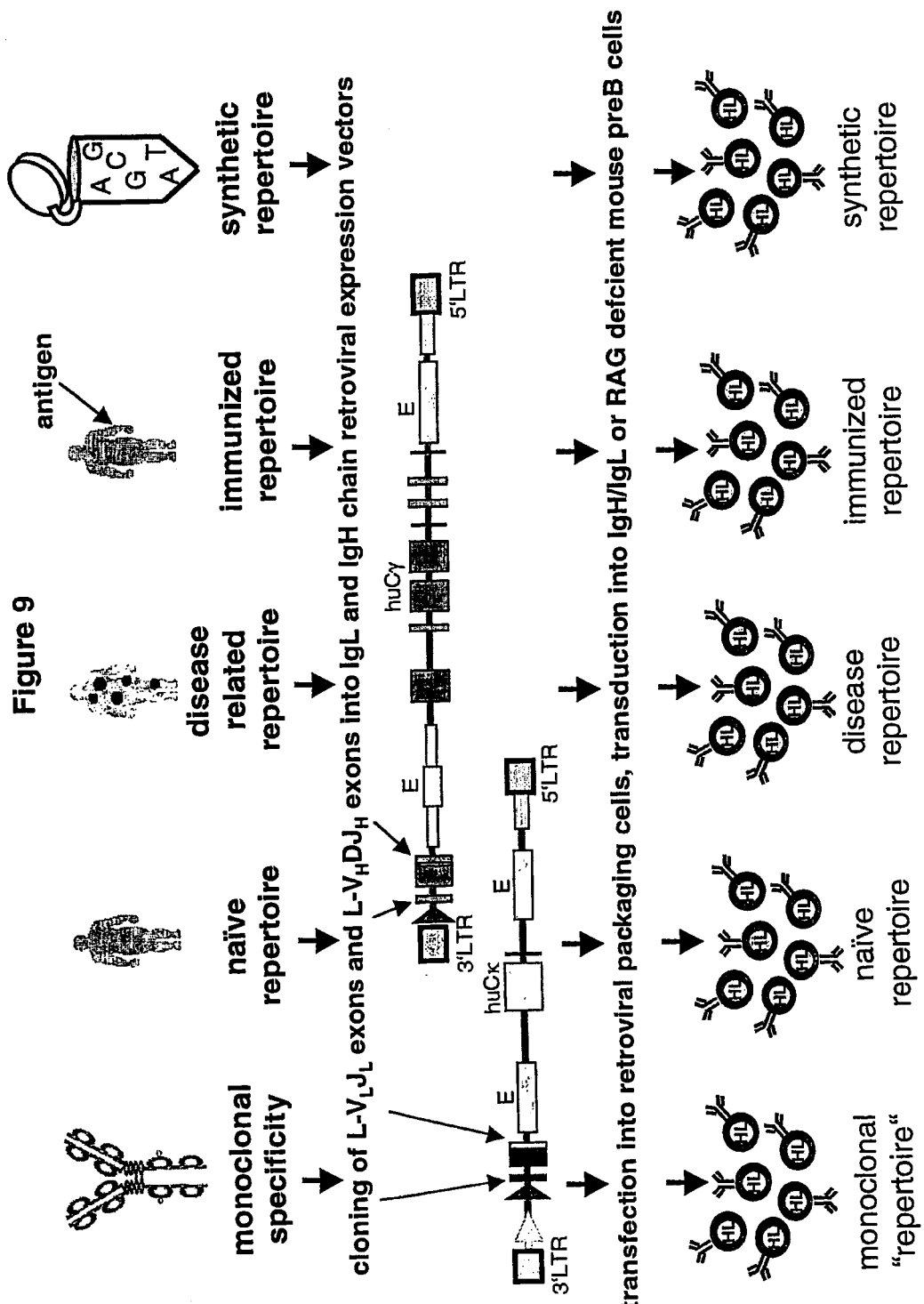

monoclonal specificity
naïve repertoire
disease related repertoire
antigen
immunized repertoire
synthetic repertoire
cloning of L-V_LJ_L exons and L-V_HDJ_H exons into IgL and IgH chain retroviral expression vectors
E
huCκ
3'LTR
5'LTR
huCγ
transfection into retroviral packaging cells, transduction into IgH/IgL or RAG defcient mouse preB cells
monoclonal "repertoire"
naïve repertoire
disease repertoire
immunized repertoire
synthetic repertoire

Figure 10

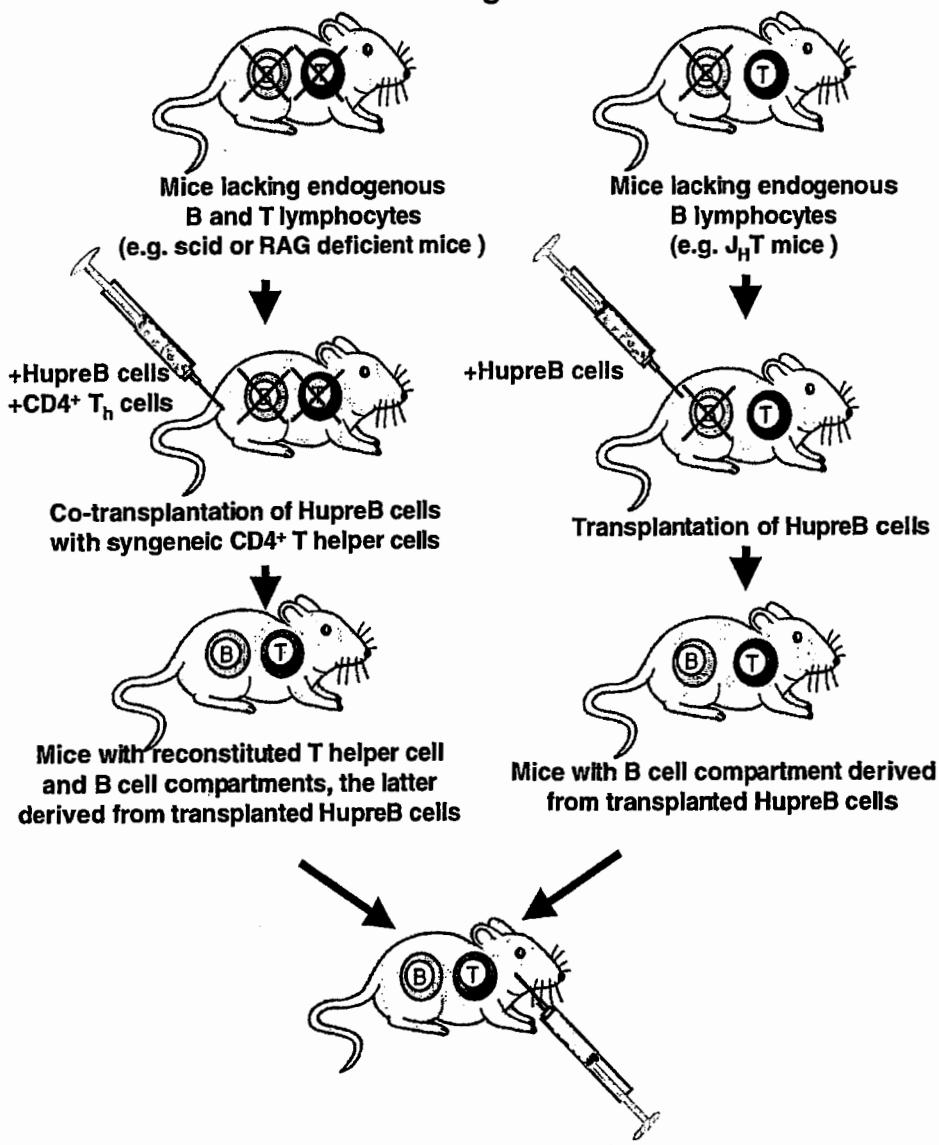

Mice lacking endogenous B and T lymphocytes (e.g. scid or RAG deficient mice)
Mice lacking endogenous B lymphocytes (e.g. J_HT mice)
+HupreB cells +CD4+ T_h cells
+HupreB cells
Co-transplantation of HupreB cells with syngeneic CD4+ T helper cells
Transplantation of HupreB cells
Mice with reconstituted T helper cell and B cell compartments, the latter derived from transplanted HupreB cells
Mice with B cell compartment derived from transplanted HupreB cells
Immunization protocol with desired antigen and adjuvant
Humoral immune response involving the production of exclusively heterologous, human antibodies
Isolation of splenic B cells and generation of hybridoma clones for the generation of antigen specific monoclonal human antibodies

Figure 11a

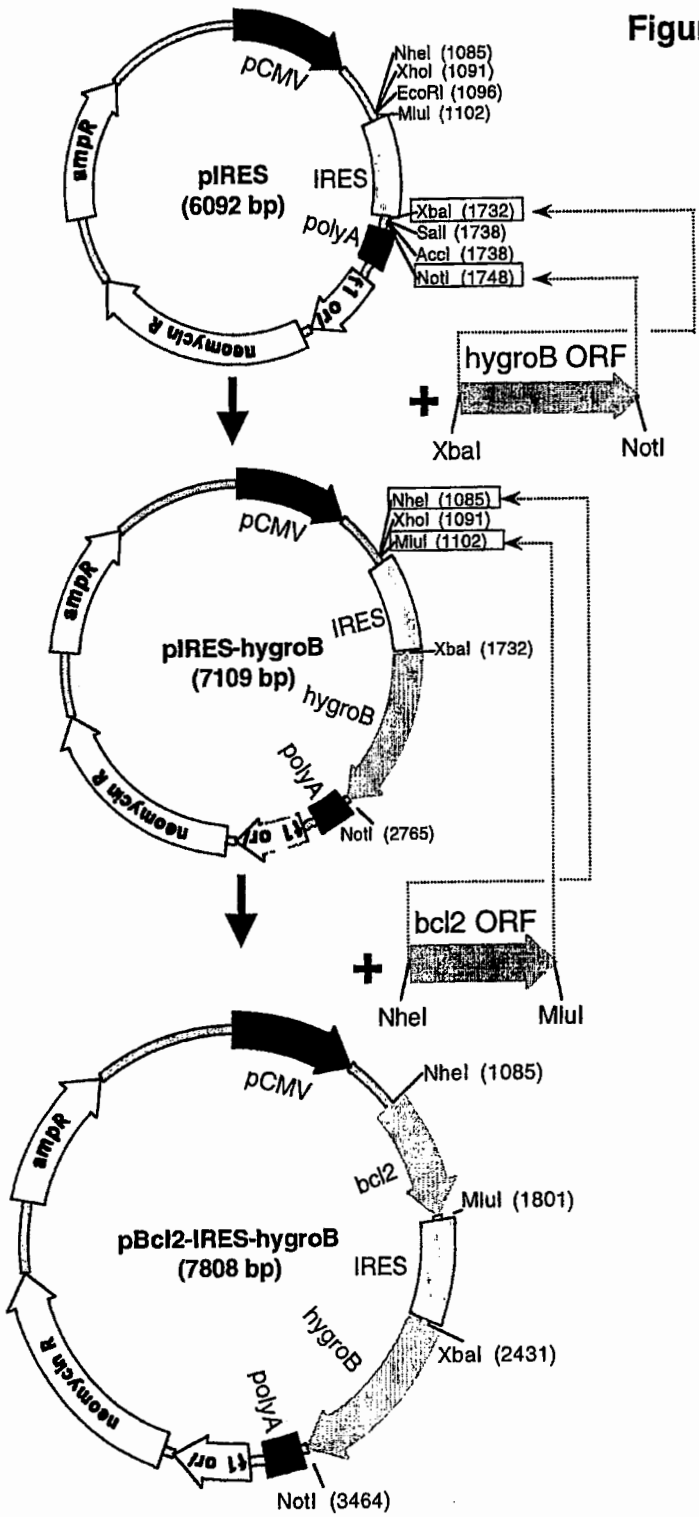

pCMV
NheI (1085)
XhoI (1091)
EcoRI (1096)
MluI (1102)
ampR
pIRES
(6092 bp)
IRES
polyA
XbaI (1732)
SalI (1738)
AccI (1738)
NotI (1748)
f1 ori
neomycin R
hygroB ORF
XbaI
NotI
pCMV
NheI (1085)
XhoI (1091)
MluI (1102)
ampR
pIRES-hygroB
(7109 bp)
IRES
XbaI (1732)
hygroB
polyA
neomycin R
f1 ori
NotI (2765)
bcl2 ORF
NheI
MluI
pCMV
NheI (1085)
ampR
bcl2
MluI (1801)
pBcl2-IRES-hygroB
(7808 bp)
IRES
hygroB
XbaI (2431)
polyA
neomycin R
f1 ori
NotI (3464)

Figure 11b

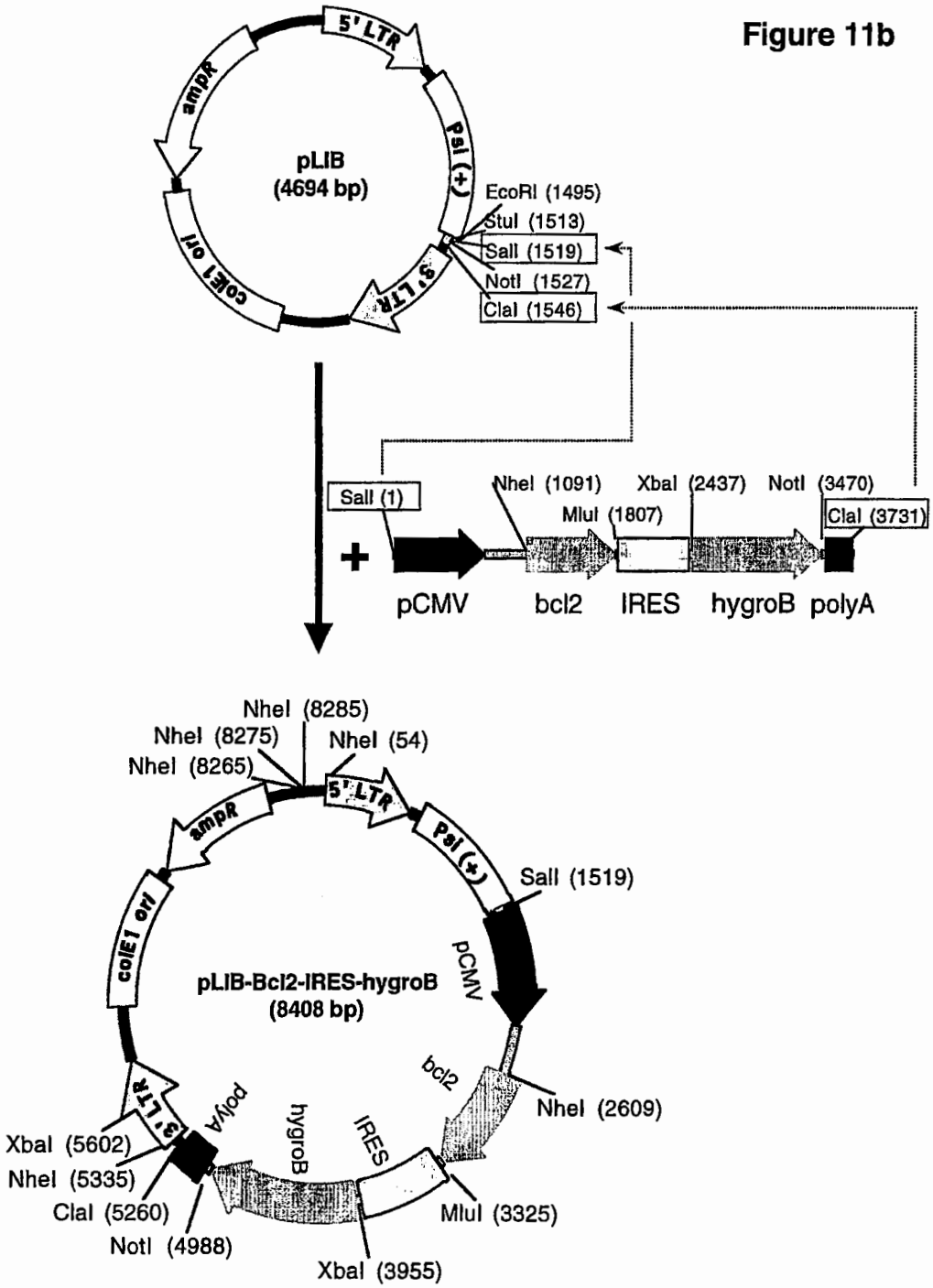

5' LTR
ampR
Psi (+)
pLIB
(4694 bp)
ColE1 ori
3' LTR
EcoRI (1495)
StuI (1513)
SalI (1519)
NotI (1527)
ClaI (1546)
SalI (1)
NheI (1091)
XbaI (2437)
NotI (3470)
MluI (1807)
ClaI (3731)
pCMV
bcl2
IRES
hygroB
polyA
NheI (8285)
NheI (8275)
NheI (54)
NheI (8265)
pLIB-Bcl2-IRES-hygroB
(8408 bp)
SalI (1519)
NheI (2609)
MluI (3325)
XbaI (3955)
NotI (4988)
ClaI (5260)
NheI (5335)
XbaI (5602)

Figure12a

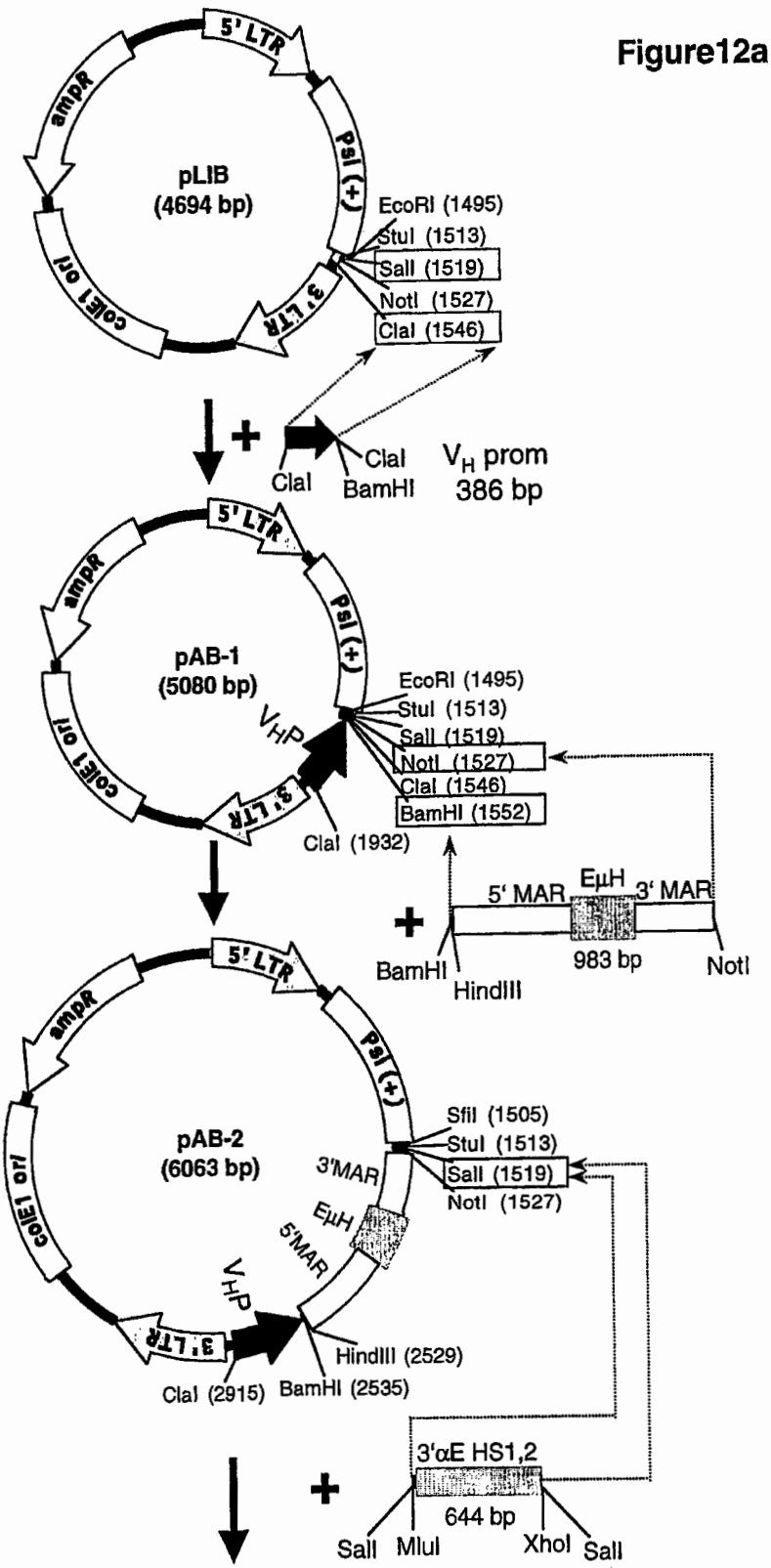

5' LTR
ampR
Psi (+)
pLIB
(4694 bp)
ColE1 ori
3' LTR
EcoRI (1495)
StuI (1513)
SalI (1519)
NotI (1527)
ClaI (1546)
ClaI
BamHI
V_H prom
386 bp
pAB-1
(5080 bp)
VHP
BamHI (1552)
ClaI (1932)
5' MAR
EμH
3' MAR
983 bp
BamHI
HindIII
NotI
pAB-2
(6063 bp)
SfiI (1505)
3'MAR
5'MAR
HindIII (2529)
ClaI (2915)
BamHI (2535)
3'αE HS1,2
644 bp
SalI
MluI
XhoI
SalI

Figure 12b

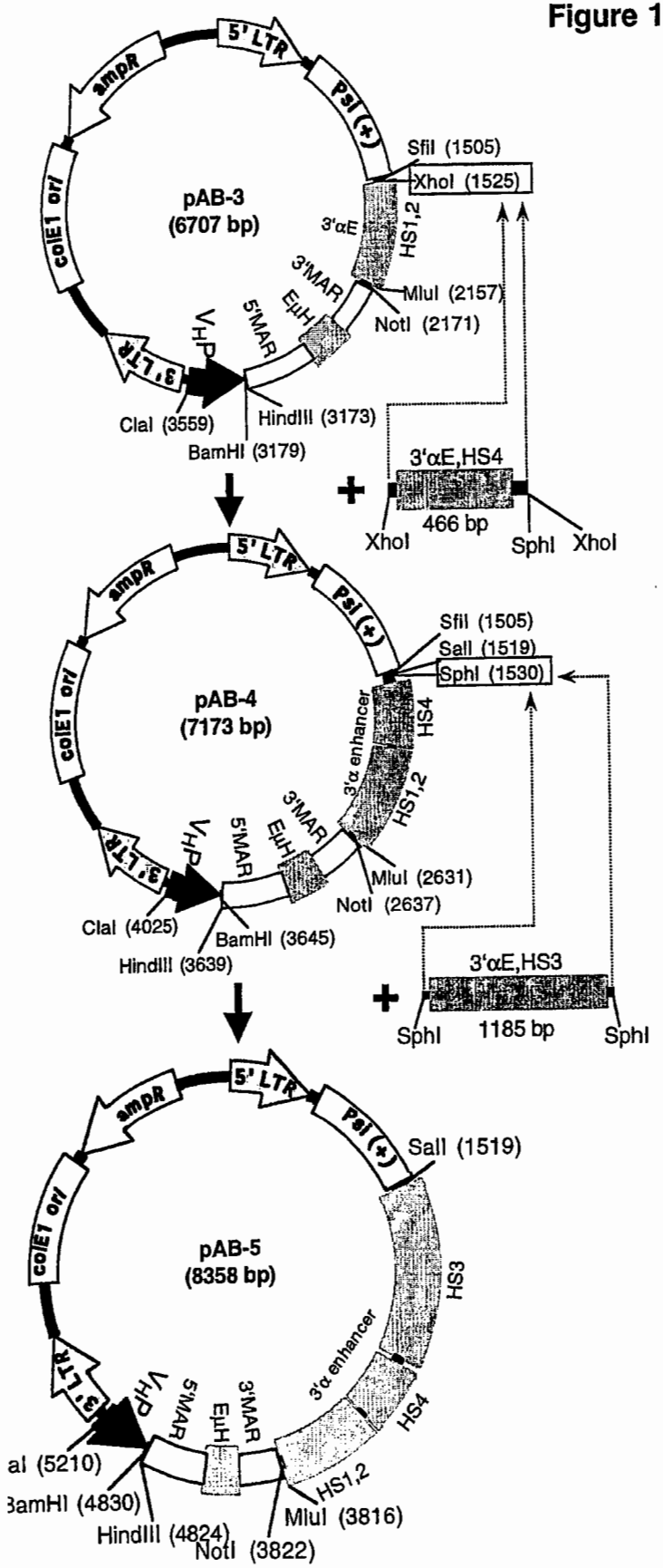

pAB-3
(6707 bp)
5' LTR
ampR
Psi (+)
colE1 ori
3' LTR
VHP
5'MAR
EμH
3'MAR
3'αE
HS1,2
SfiI (1505)
XhoI (1525)
MluI (2157)
NotI (2171)
ClaI (3559)
HindIII (3173)
BamHI (3179)
3'αE,HS4
466 bp
XhoI
SphI
XhoI
pAB-4
(7173 bp)
SfiI (1505)
SalI (1519)
SphI (1530)
HS4
3'α enhancer
HS1,2
MluI (2631)
NotI (2637)
ClaI (4025)
BamHI (3645)
HindIII (3639)
3'αE,HS3
SphI
1185 bp
SphI
pAB-5
(8358 bp)
SalI (1519)
HS3
3'α enhancer
HS4
HS1,2
MluI (3816)
NotI (3822)
HindIII (4824)
BamHI (4830)
al (5210)

Figure 12c

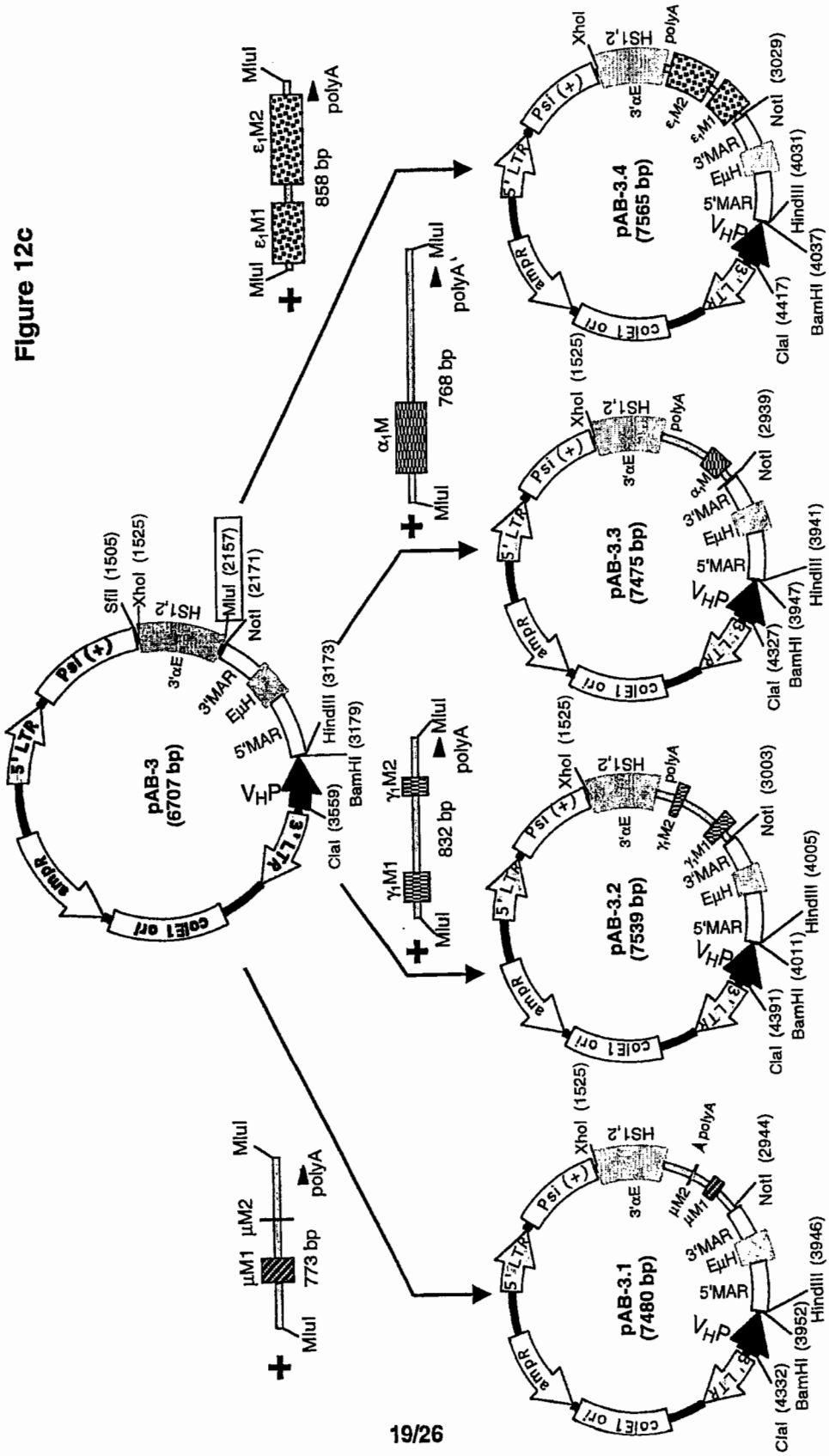

pAB-3
(6707 bp)
pAB-3.4
(7565 bp)
pAB-3.3
(7475 bp)
pAB-3.2
(7539 bp)
pAB-3.1
(7480 bp)
768 bp
832 bp
773 bp
858 bp
polyA
MluI
ClaI (3559)
BamHI (3179)
HindIII (3173)
NotI (2171)
MluI (2157)
SfiI (1505)
XhoI (1525)
ClaI (4417)
BamHI (4037)
HindIII (4031)
NotI (3029)
ClaI (4327)
BamHI (3947)
HindIII (3941)
NotI (2939)
ClaI (4391)
BamHI (4011)
HindIII (4005)
NotI (3003)
ClaI (4332)
BamHI (3952)
HindIII (3946)
NotI (2944)
5'LTR
Psi (+)
HS1,2
3'αE
3'MAR
EμH
5'MAR
VHP
3'LTR
ampR
colE1 ori

Figure 12d

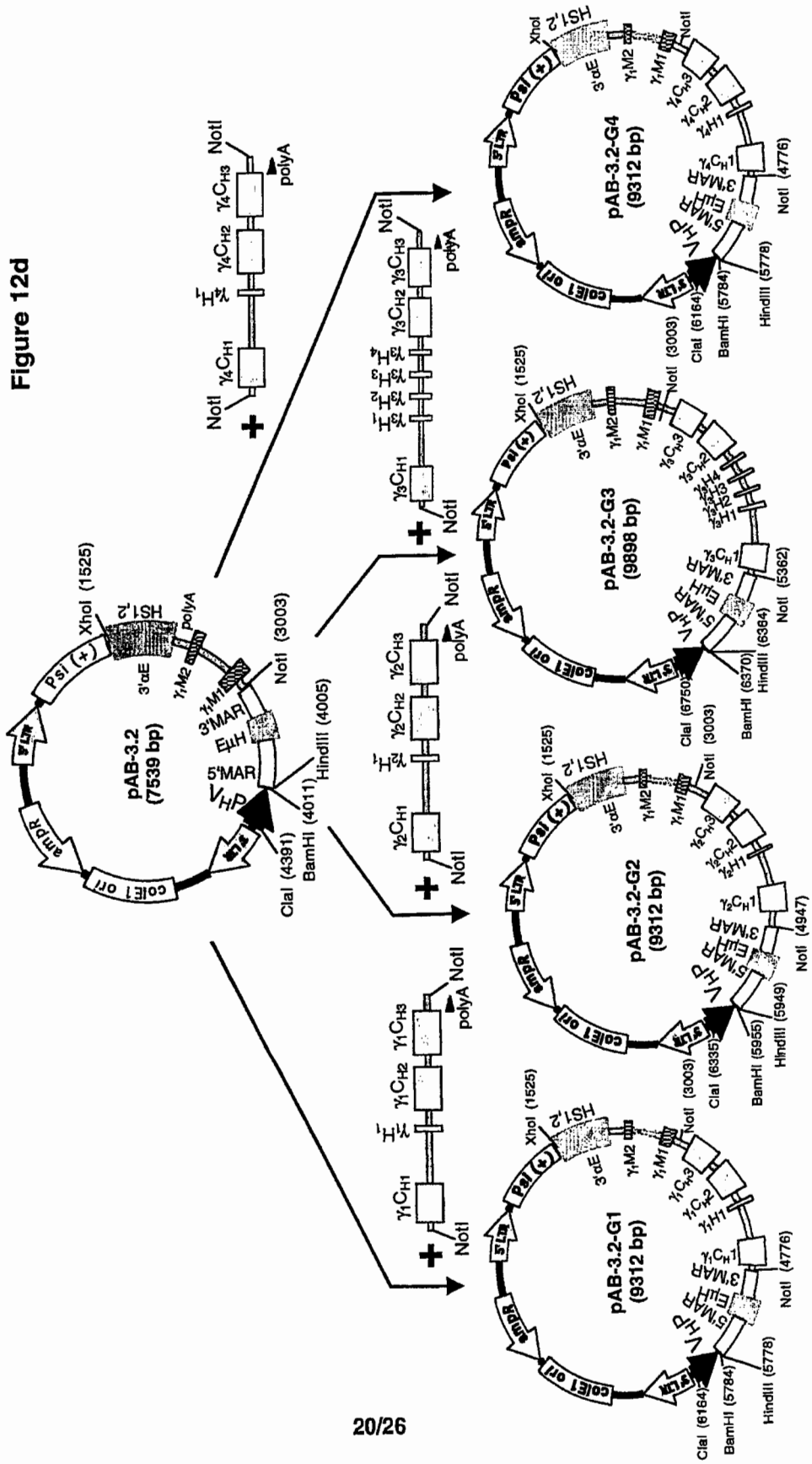

pAB-3.2
(7539 bp)
pAB-3.2-G4
(9312 bp)
pAB-3.2-G3
(9898 bp)
pAB-3.2-G2
(9312 bp)
pAB-3.2-G1
(9312 bp)
HS1,2
3'αE
3'MAR
5'MAR
VHP
colE1 ori
ampR
Psi (+)
pA
polyA
XhoI (1525)
NotI (3003)
HindIII (4005)
ClaI (4391)
BamHI (4011)
NotI (4776)
HindIII (5778)
BamHI (5784)
ClaI (6164)
NotI (5362)
HindIII (6364)
BamHI (6370)
ClaI (6750)
NotI (4947)
HindIII (5949)
BamHI (5955)
ClaI (6335)

Figure 12e

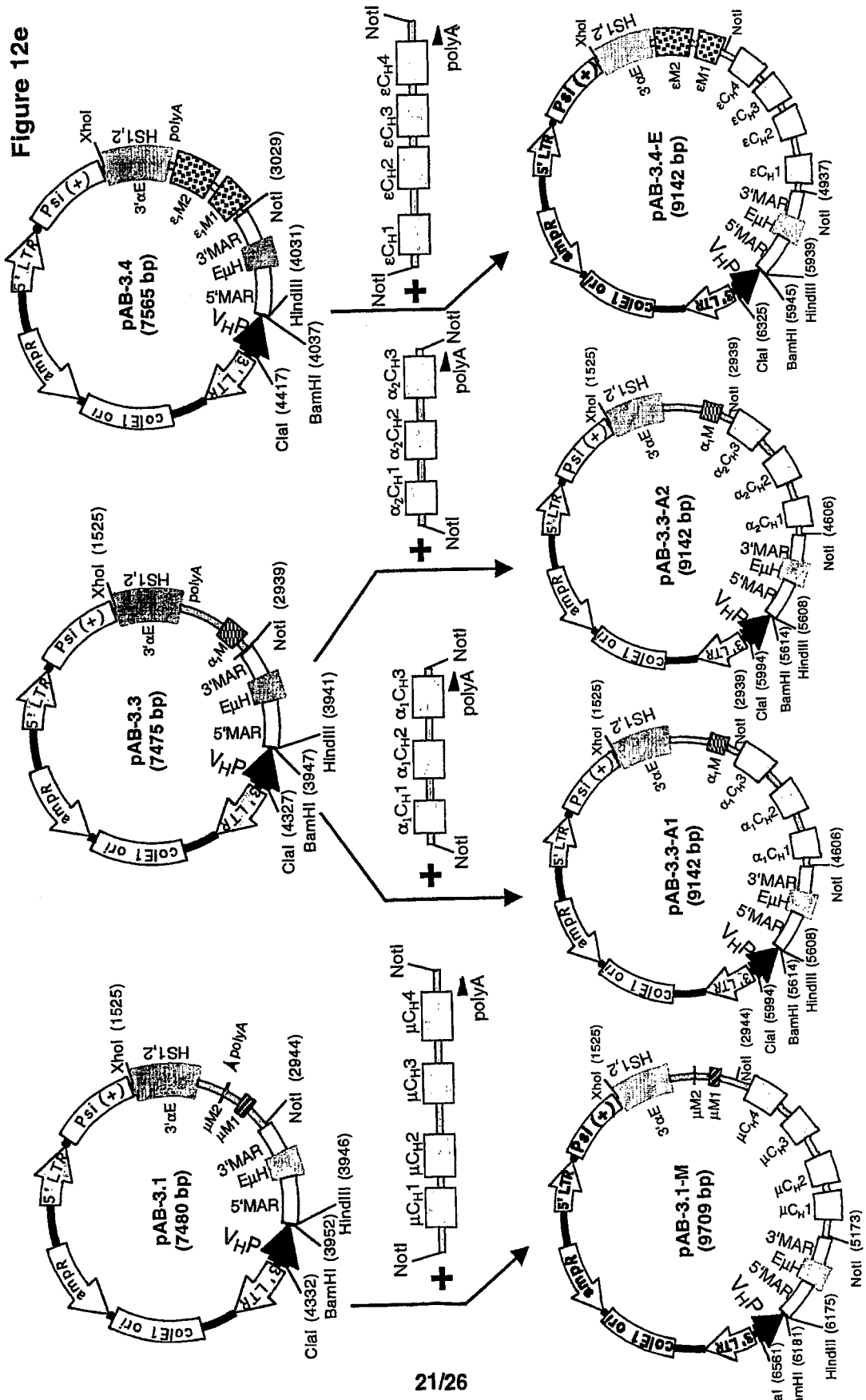

pAB-3.4 (7565 bp)
pAB-3.3 (7475 bp)
pAB-3.1 (7480 bp)
pAB-3.4-E (9142 bp)
pAB-3.3-A2 (9142 bp)
pAB-3.3-A1 (9142 bp)
pAB-3.1-M (9709 bp)
XhoI (1525)
NotI (3029)
HindIII (4031)
BamHI (4037)
ClaI (4417)
NotI (2939)
HindIII (3941)
BamHI (3947)
ClaI (4327)
NotI (2944)
HindIII (3946)
BamHI (3952)
ClaI (4332)
NotI (4937)
HindIII (5939)
BamHI (5945)
ClaI (6325)
NotI (4606)
HindIII (5608)
BamHI (5614)
ClaI (5994)
NotI (5173)
HindIII (6175)
BamHI (6181)
ClaI (6561)
NotI
polyA
HS1,2
3'αE
Psi (+)
5' LTR
3' LTR
ampR
ColE1 ori
3'MAR
EμH
5'MAR
VHP
εM1
εM2
αM
μM1
μM2
εCH1
εCH2
εCH3
εCH4
α1CH1
α1CH2
α1CH3
α2CH1
α2CH2
α2CH3
μCH1
μCH2
μCH3
μCH4

Figure13a

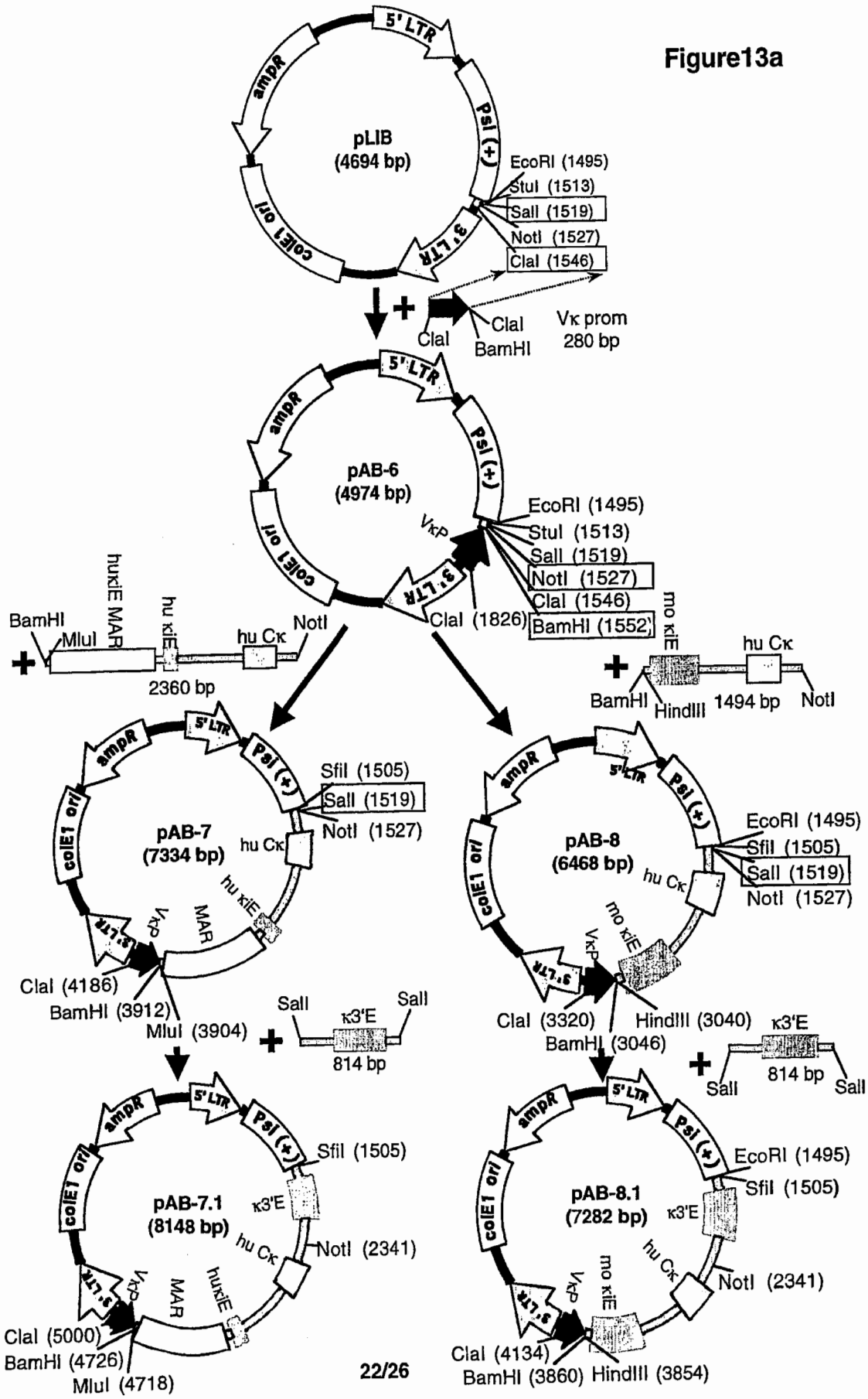

pLIB
(4694 bp)
5' LTR
ampR
Psi (+)
ColE1 ori
3' LTR
EcoRI (1495)
StuI (1513)
SalI (1519)
NotI (1527)
ClaI (1546)
ClaI
BamHI
Vκ prom
280 bp
pAB-6
(4974 bp)
Vκp
ClaI (1826)
BamHI (1552)
BamHI
MluI
huκiE MAR
huκiE
huCκ
NotI
2360 bp
moκiE
BamHI
HindIII
1494 bp
pAB-7
(7334 bp)
SfiI (1505)
SalI (1519)
NotI (1527)
MAR
ClaI (4186)
BamHI (3912)
MluI (3904)
SalI
κ3'E
814 bp
pAB-8
(6468 bp)
ClaI (3320)
HindIII (3040)
BamHI (3046)
pAB-7.1
(8148 bp)
NotI (2341)
ClaI (5000)
BamHI (4726)
MluI (4718)
pAB-8.1
(7282 bp)
ClaI (4134)
BamHI (3860)
HindIII (3854)

Figure 13b

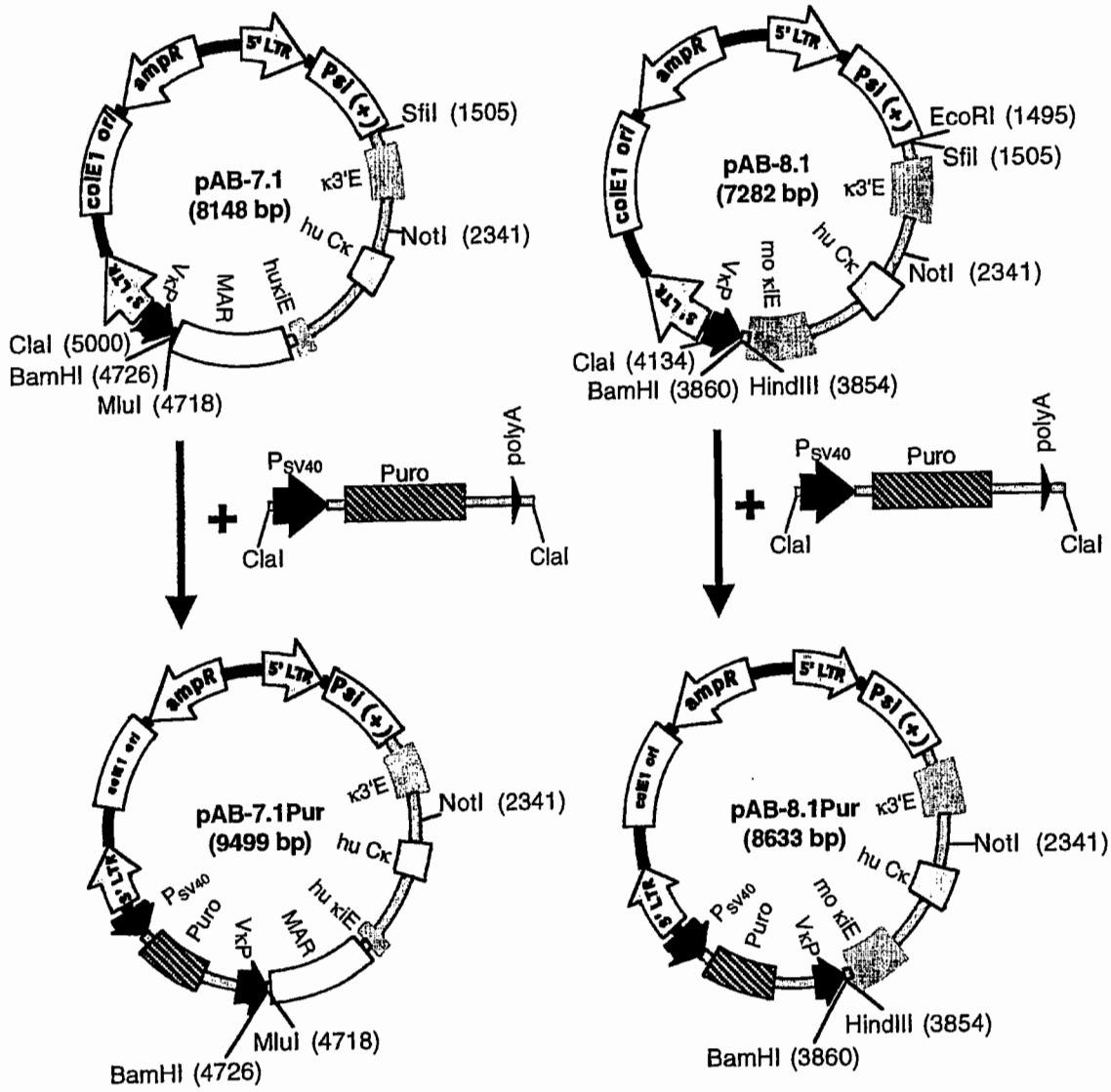

pAB-7.1
(8148 bp)
ampR
5' LTR
Psi (+)
colE1 ori
κ3'E
hu Cκ
huκiE
MAR
VκP
3' LTR
SfiI (1505)
NotI (2341)
ClaI (5000)
BamHI (4726)
MluI (4718)
pAB-8.1
(7282 bp)
EcoRI (1495)
SfiI (1505)
NotI (2341)
mo κiE
ClaI (4134)
BamHI (3860)
HindIII (3854)
PSV40
Puro
polyA
ClaI
pAB-7.1Pur
(9499 bp)
pAB-8.1Pur
(8633 bp)

Figure 14a

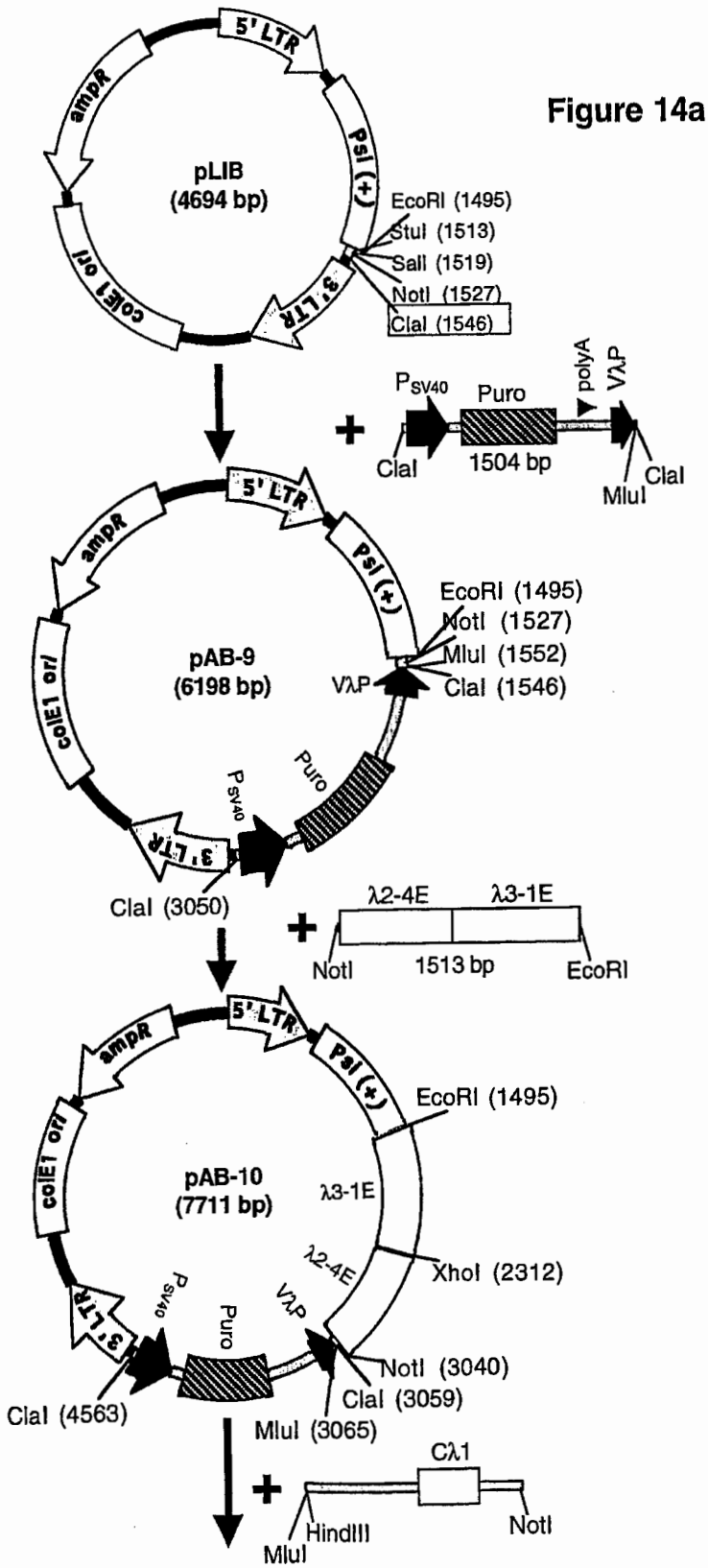
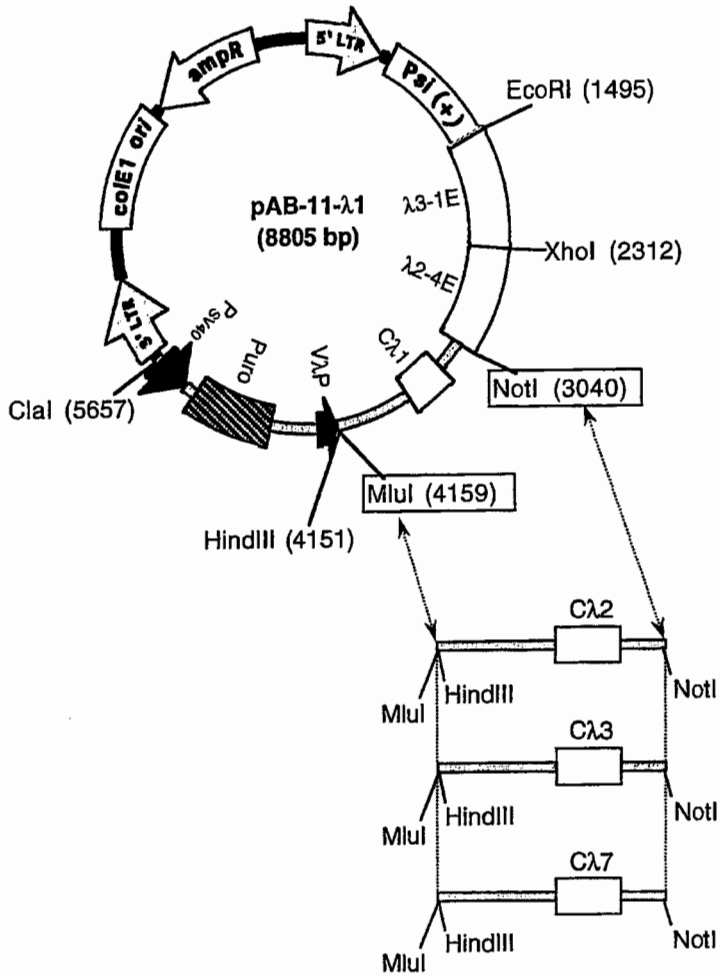
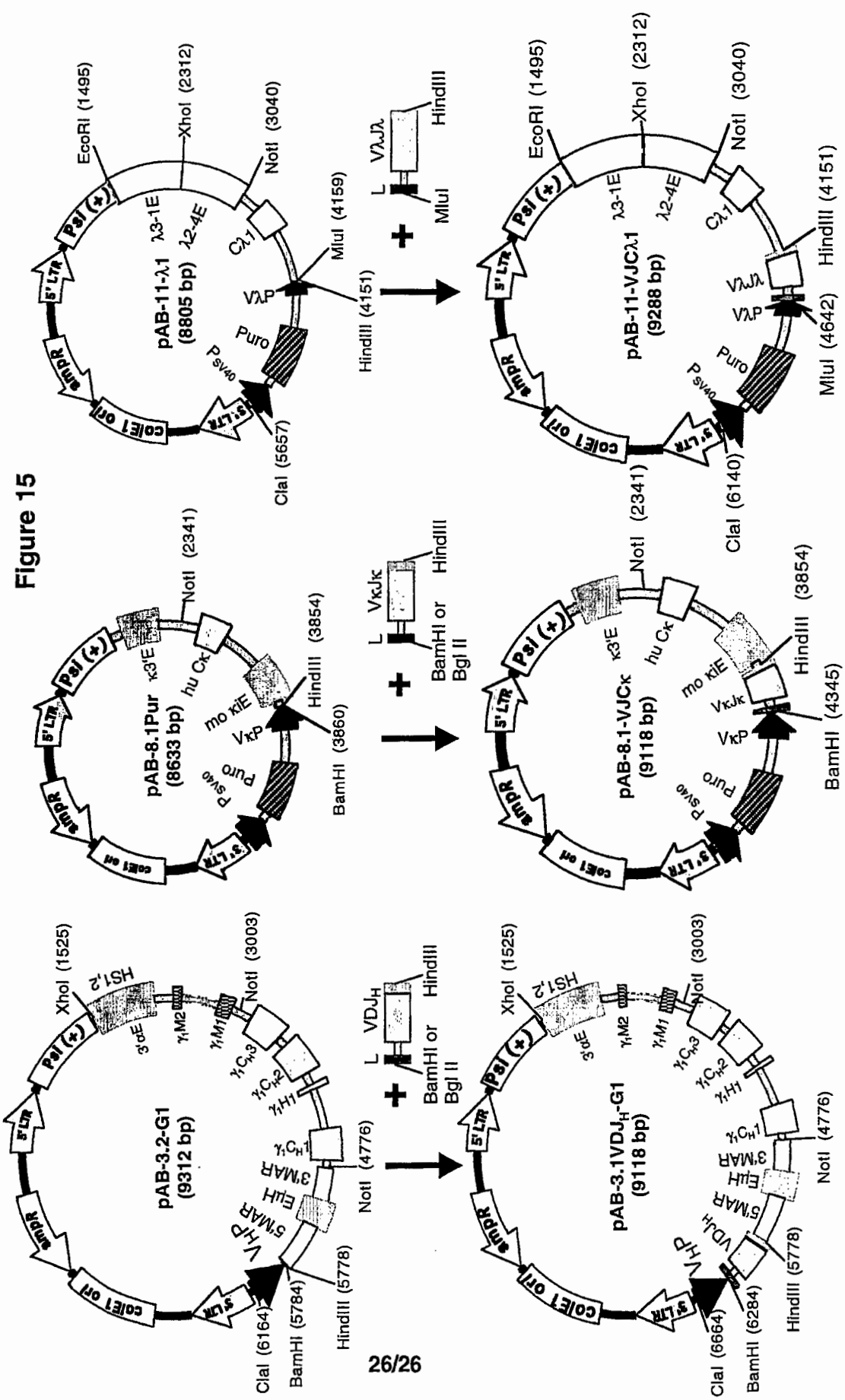

pLIB
(4694 bp)
5' LTR
ampR
Psi (+)
3' LTR
colE1 ori
EcoRI (1495)
StuI (1513)
SalI (1519)
NotI (1527)
ClaI (1546)
PSV40
Puro
polyA
VλP
ClaI
1504 bp
MluI
ClaI
pAB-9
(6198 bp)
EcoRI (1495)
NotI (1527)
MluI (1552)
ClaI (1546)
VλP
ClaI (3050)
λ2-4E
λ3-1E
NotI
1513 bp
EcoRI
pAB-10
(7711 bp)
EcoRI (1495)
λ3-1E
λ2-4E
XhoI (2312)
NotI (3040)
ClaI (3059)
MluI (3065)
ClaI (4563)
Cλ1
HindIII
NotI
MluI

Figure 14b


pAB-11-λ1
(8805 bp)
ampR
5' LTR
Psi (+)
EcoRI (1495)
λ3-1E
XhoI (2312)
λ2-4E
Cλ1
NotI (3040)
VλP
Puro
PSV40
3' LTR
ClaI (5657)
MluI (4159)
HindIII (4151)
Cλ2
MluI
HindIII
NotI
Cλ3
MluI
HindIII
NotI
Cλ7
MluI
HindIII
NotI


pAB-11-λ1
(8805 bp)
EcoRI (1495)
XhoI (2312)
NotI (3040)
MluI (4159)
HindIII (4151)
ClaI (5657)
pAB-11-VJCλ1
(9288 bp)
MluI (4642)
ClaI (6140)
pAB-8.1Pur
(8633 bp)
NotI (2341)
HindIII (3854)
BamHI (3860)
pAB-8.1-VJCκ
(9118 bp)
BamHI (4345)
pAB-3.2-G1
(9312 bp)
XhoI (1525)
NotI (3003)
NotI (4776)
HindIII (5778)
ClaI (6164)
BamHI (5784)
pAB-3.1VDJH-G1
(9118 bp)
ClaI (6664)
BamHI (6284)
HindIII
BamHI or
Bgl II
Figure 15