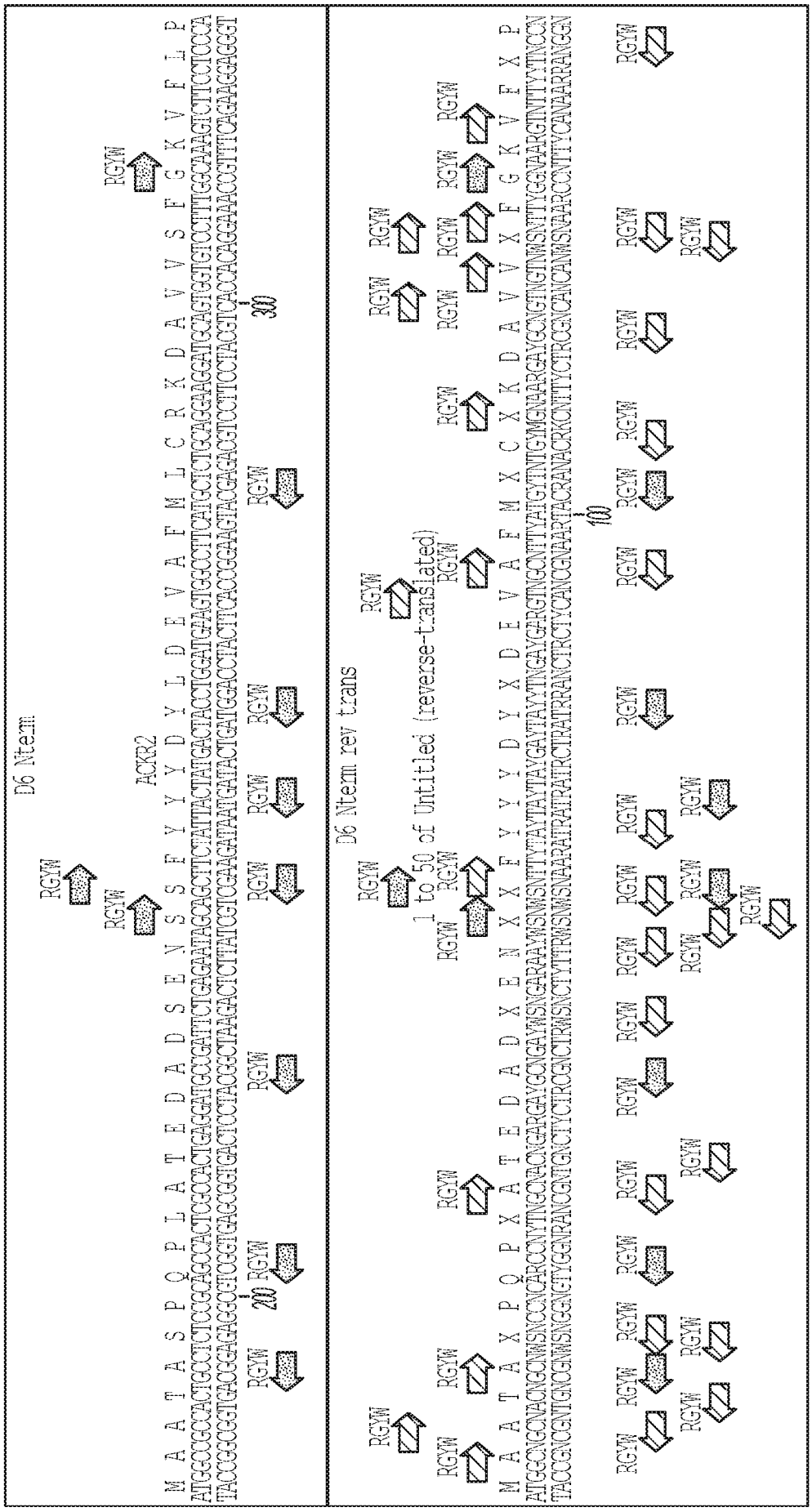
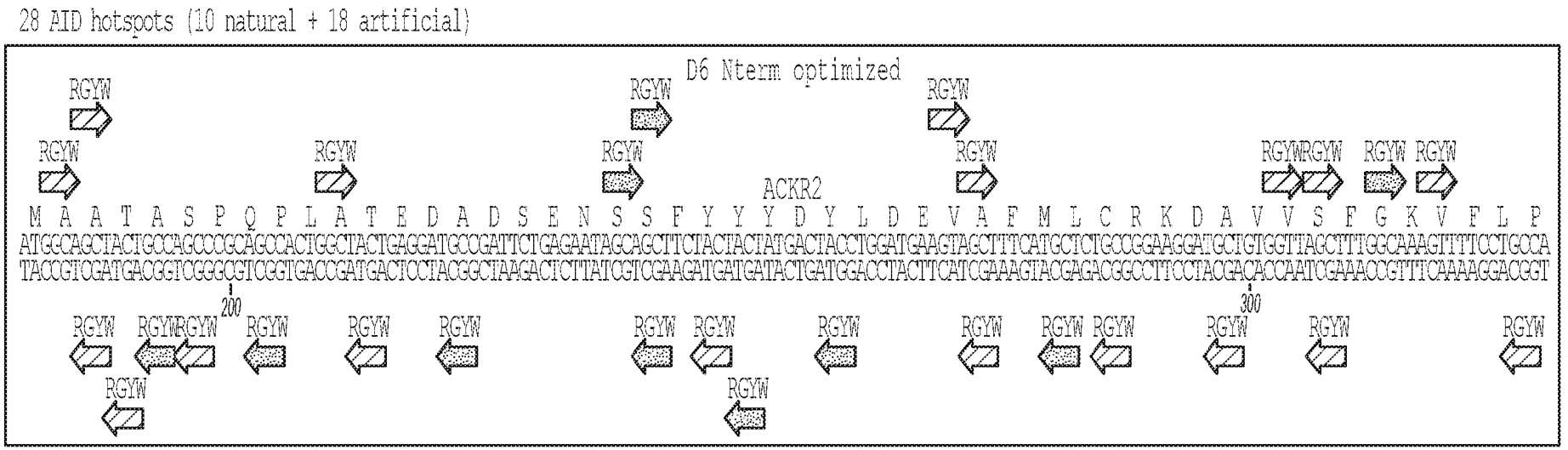

D6 Ntem
ACKR2
D6 Ntem rev trans
1 to 50 of Untitled (reverse-translated)
RGYW

FIG. 1A

FIG. 1A


28 AID hotspots (10 natural + 18 artificial)
D6 Nterm optimized
ACKR2
RGYW
RGYWRGYW
M A A T A S P Q P L A T E D A D S E N S S F Y Y Y D Y L D E V A F M L C R K D A V V S F G K V F L P
ATGGCAGCTACTGCCAGCCCCGCAGCCACTGGCTACTGAGGATGCCGATTCTGAGAATAGCAGCTTCTACTACTATGACTACCTGGATGAAGTAGCTTTCATGCTCTGCCGGAAGGATGCTGTGGTTAGCTTTGGCAAAGTTTTCCTGCCA
TACCGTCGATGACGGTCGGGCGTCGGTGACCGATGACTCCTACGGCTAAGACTCTTATCGTCGAAGATGATGATACTGATGGACCTACTTCATCGAAAGTACGAGACGGCCTTCCTACGACACCAATCGAAACCGTTTCAAAAGGACGGT
200
300

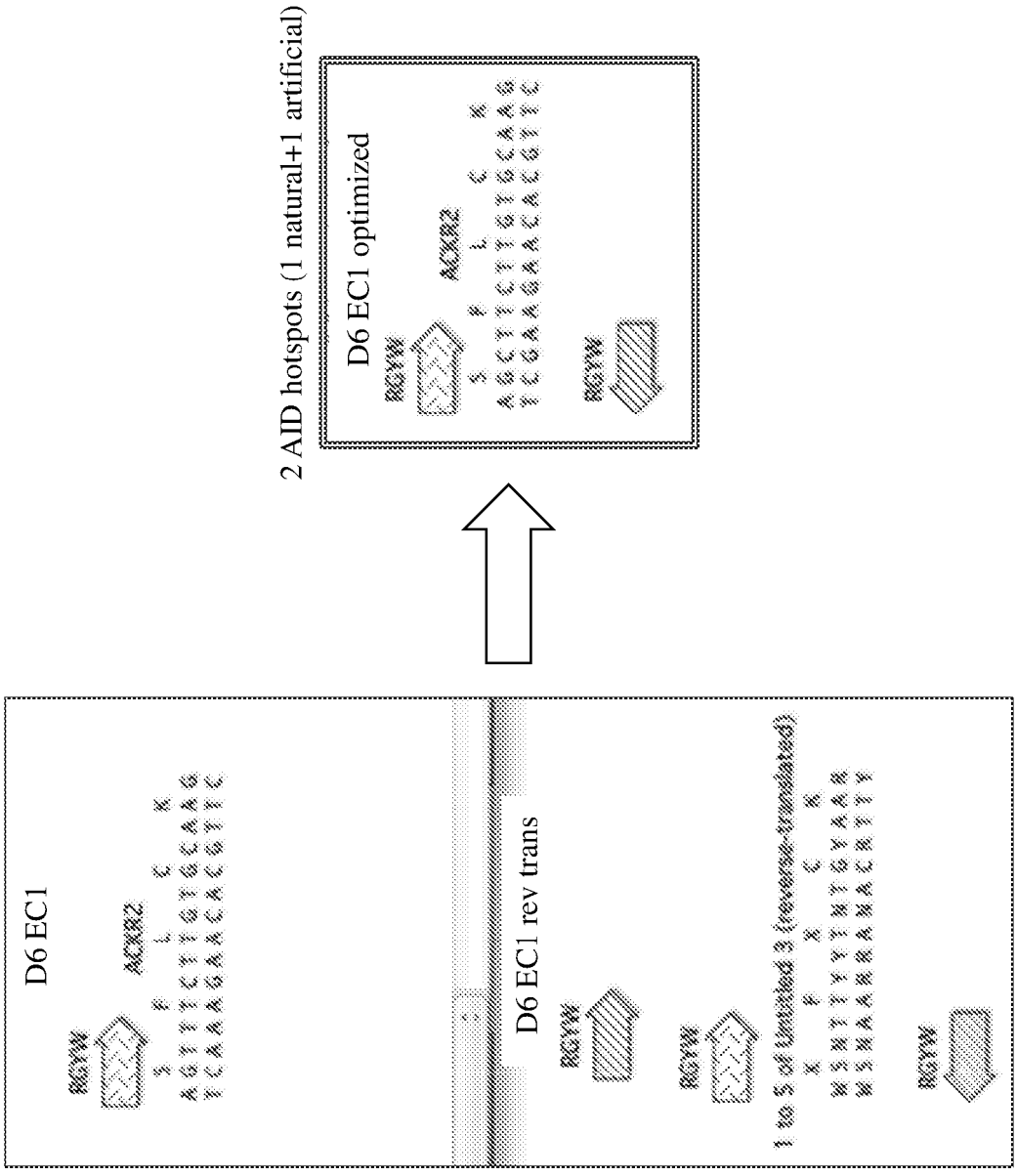
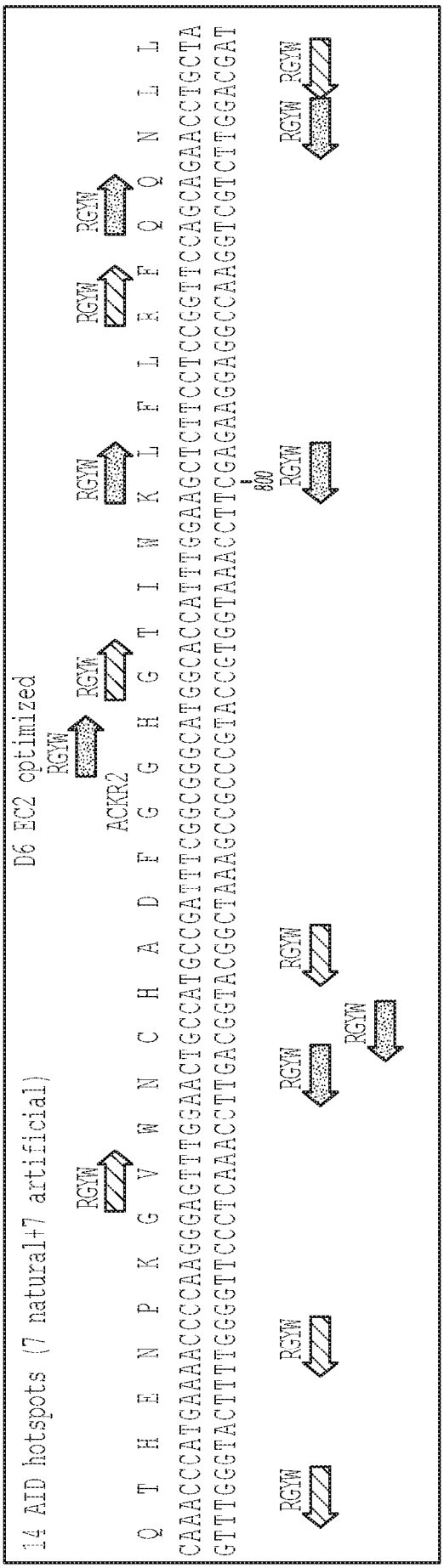

D6 EC1
RGYW
ACWR2
D6 EC1 rev trans
RGYW
RGYW
RGYW
1 to 5 of Untitled 3 (reverse-translated)
2 AID hotspots (1 natural+1 artificial)
D6 EC1 optimized
RGYW
ACWR2
RGYW

Figure 1B

FIG 1C


14 AID hotspots (7 natural+7 artificial)
D6 EC2 optimized
ACKR2
RGYW
Q T H E N P K G V W N C H A D F G G H G T I W K L F L E E F Q Q N L L
CAAACCCATGAAAACCCCAAGGGAGTTTGGAACTGCCATGCCGATTTCGGCGGGCATGGCACCATTTGGAAGCTCTTCCTCCGGTTCCAGCAGAACCTGCTA
GTTTGGGTACTTTTGGGGTTCCCTCAAACCTTGACGGTACGGCTAAAGCCGCCCGTACCGTGGTAAACCTTCGAGAAGGAGGCCAAGGTCGTCTTGGACGAT
800

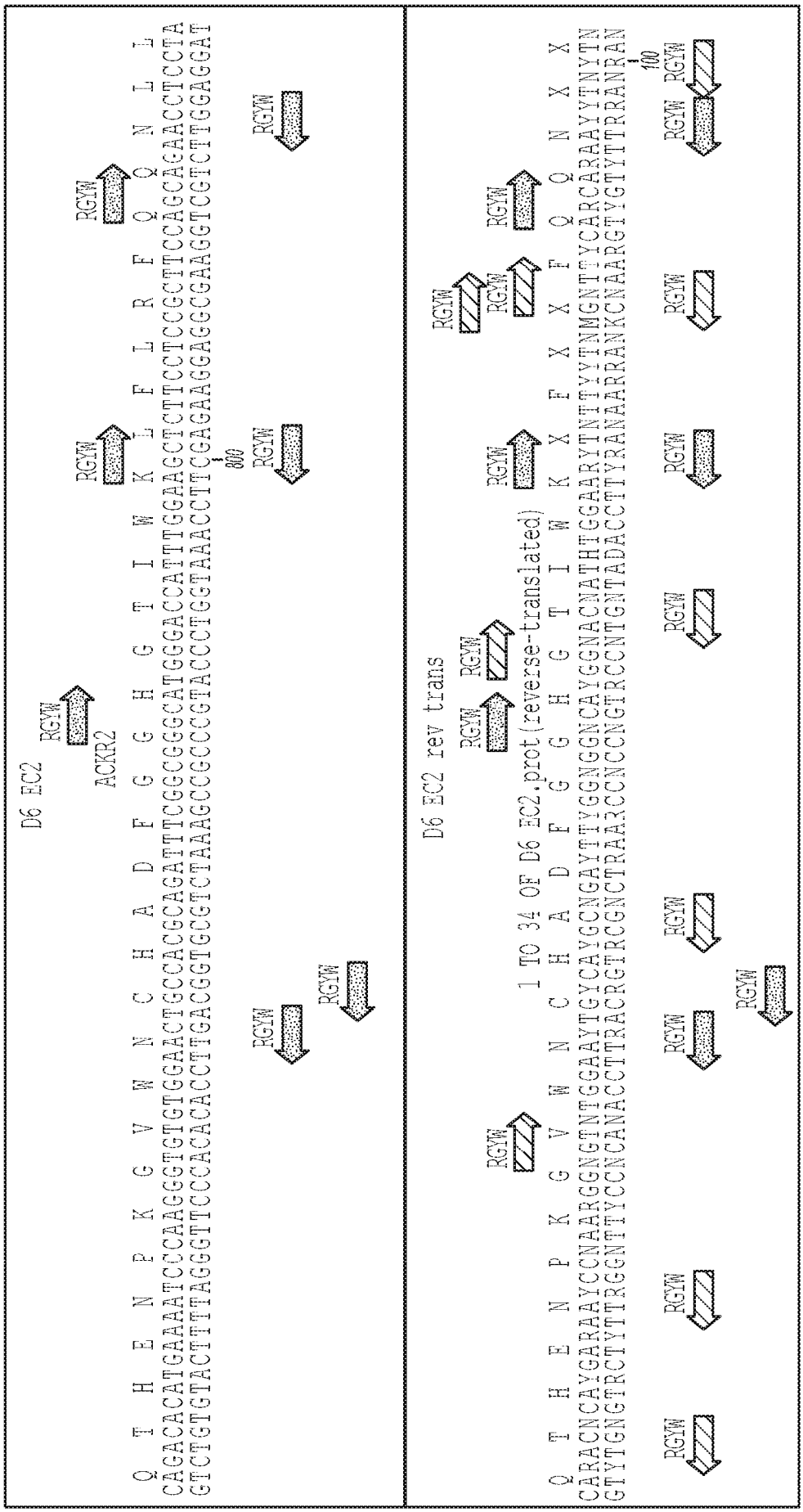

D6 EC2
ACKR2
RGYW
Q T H E N P K G V W N C H A D F G G H G T I W K L F L R F Q Q N L L
CAGACACATGAAAATCCCAAGGGTGTGTGGAACTTGCCACGCAGATTTCGGGCGGGCATGGGACCATTTGGAAGCTCTTCCTCCGCTTCCAGCAGCAACCTCCTA
GTCTGTGTACTTTTAGGGTTCCCACACACCTTGACGGTGCGTCTAAAGCCGCCCGTACCCTGGTAAACCTTCGAGAAGGCGAAGGCGTCGTTCTTGGAGGAT
1
800
D6 EC2 rev trans
1 TO 34 OF D6 EC2.prot(reverse-translated)
Q T H E N P K G V W N C H A D F G G H G T I W K X F X X F Q Q N X X
CARACNCAYGARAAYCCNAARGGNGTNTGGAAYTGYCAYGCNGAYTTYGGNGGNCAYGGNACNATHTGGAARYTNTTYYTNMGNTTYCARCARAAYYTNYTN
GTYTGNGTRCTYTTRGGNTTYCCNCANACCTTRACRGTRCGNCTRAARCCNCCNGTRCCNTGNTADACCTTYRANAARRANKCNAARGTYGTYTTRRANRAN
1
100

FIG. 1C

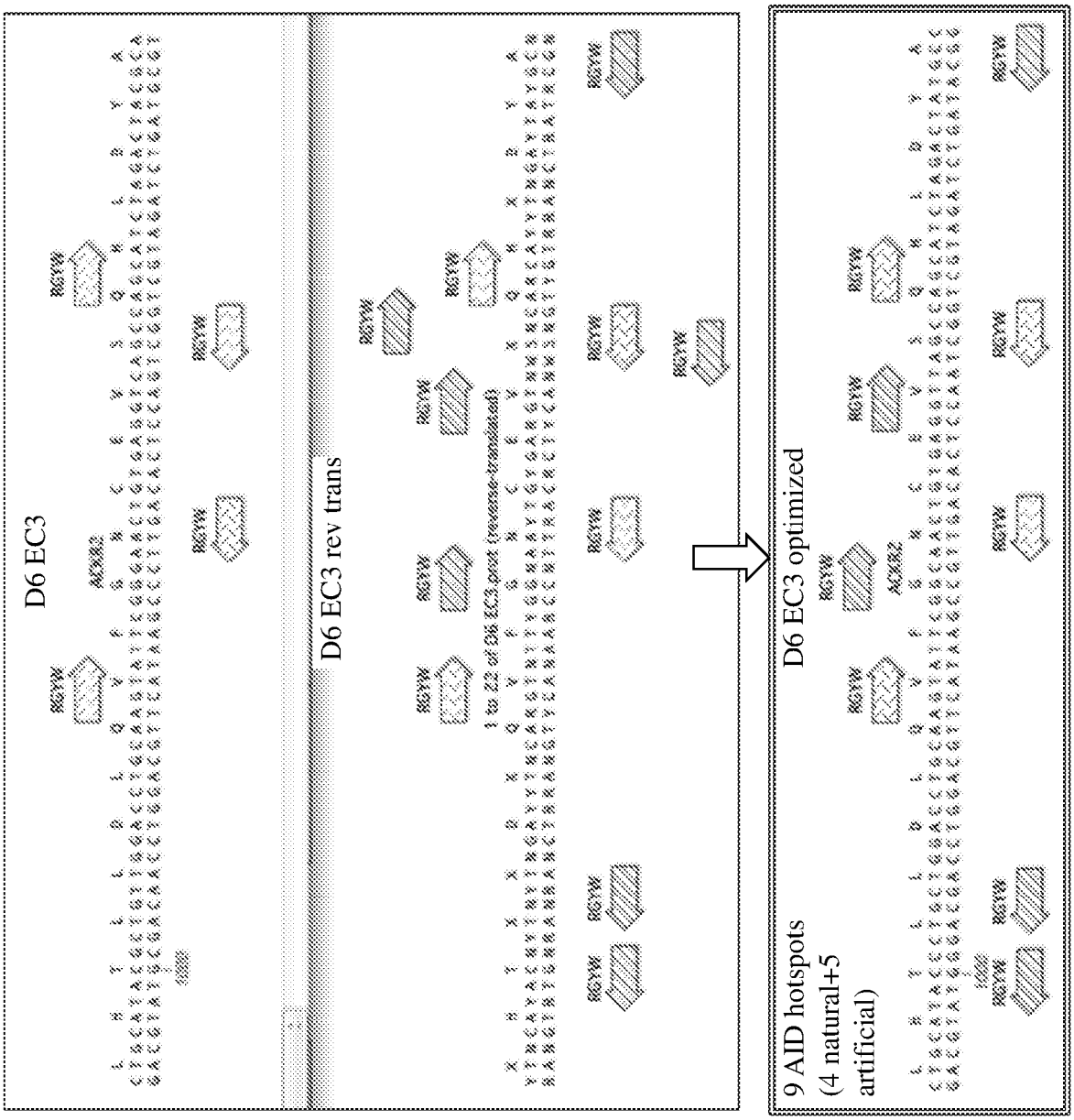

D6 EC3
D6 EC3 rev trans
D6 EC3 optimized
9 AID hotspots
(4 natural+5
artificial)
AGRR2
RGYW

Figure 1D

D_H sequence name	5' RSS (SEQ ID NO)	3' RSS (SEQ ID NO)
global RSS consensus	***ACAAAAACC***NNNNNNNNNNNNN***CACAGTG*** (51)	***CACAGTG***NNNNNNNNNNNNN***ACAAAAACC*** (52)
human D_H consensus	**GGWTWYTGW**NNNNNNNNNNNNN**CACTGTG** (53)	**CACAGTG**NNNNNNNNNNNNN**BCMRMAACY** (54)
mouse D_H consensus	**GATTTTTGT**NNNNNNNNNNNNN**TACTGTG** (55)	**CACAGTG**NNNNNNNNNNNNN**ACAAAAACC** (56)
optimized RSS	**GGATTTTGT**NNNNNNNNNNNNN**CACTGTG** (57)	**CACAGTG**NNNNNNNNNNNNN**TCAAAAACC** (58)
1-1 opt	**GGATTTTGT**ACAGCCCCGAGT**CACTGTG** (59)	**CACAGTG**AGAAAAACTGTG**TCAAAAACC** (60)
1-7 opt	**GGATTTTGT**ACAGCCCCGAGT**CACTGTG** (61)	**CACAGTG**AGAAAAGCTTCG**TCAAAAACC** (62)
1-14 ORF opt	**GGATTTTGT**ACAGCCCCGAGT**CACTGTG** (63)	**CACAGTG**AGAATAGCTACG**TCAAAAACC** (64)
1-20 opt	**GGATTTTGT**ACAGCCCCGAGT**CACTGTG** (65)	**CACAGTG**AGAAAAACTGTG**TCAAAAACC** (66)
1-26 opt	**GGATTTTGT**ACAGCCCCGAGT**CACTGTG** (67)	**CACAGTG**AGAAAAGCTATG**TCAAAAACC** (68)
2-2*02 opt	**GGATTTTGT**GGGGGCTCGTGT**CACTGTG** (69)	**CACAGTG**ACACAGCCCCATT**CAAAAACC** (70)
2-8*01 opt	**GGATTTTGT**GGGGGCTCGTGT**CACTGTG** (71)	**CACAGTG**ACACAGCCCCATT**CAAAAACC** (72)
2-15 opt	**GGATTTTGT**GGGGGCTCGTGT**CACTGTG** (73)	**CACAGTG**ACACAGACCCATT**CAAAAACC** (74)
2-21*02 opt	**GGATTTTGT**GGGGGTTCGTGT**CACTGTG** (75)	**CACAGTG**ACACAACCCCATT**CAAAAACC** (76)
3-3*01 opt	**GGATTTTGT**TGAGGTCTGTGT**CACTGTG** (77)	**CACAGTG**TCACAGAGTCCAT**CAAAAACC** (78)
3-9 opt	**GGATTTTGT**TGAGGTCTGTGT**CACTGTG** (79)	**CACAGTG**TCACAGAGTCCAT**CAAAAACC** (80)
3-10*01 opt	**GGATTTTGT**TGAGGTCTGTGT**CACTGTG** (81)	**CACAGTG**TCACAGAGTCCAT**CAAAAACC** (82)
3-16*02 opt	**GGATTTTGT**TGAGGTCTGTGT**CACTGTG** (83)	**CACAGTG**TCACACGGTCCAT**CAAAAACC** (84)
3-22 opt	**GGATTTTGT**TGAGGTCTGTGT**CACTGTG** (85)	**CACAGTG**TCACAGAGTCCAT**CAAAAACC** (86)
4-4 opt	**GGATTTTGT**GAAGGGTCCTCC**CACTGTG** (87)	**CACAGTG**ATGAACCCAGCAT**CAAAAACC** (88)
4-11 ORF opt	**GGATTTTGT**GAAGGGCCCTCC**CACTGTG** (89)	**CACAGTG**ATGAACCCAGTG**TCAAAAACC** (90)
4-17 opt	**GGATTTTGT**GAAGGCCCCTCC**CACTGTG** (91)	**CACAGTG**ATGAAACTAGCA**TCAAAAACC** (92)
4-23 ORF opt	**GGATTTTGT**GAAGGGCCCTCC**CACTGTG** (93)	**CACAGTG**ATGAAACCAGCA**TCAAAAACC** (94)
5-5 opt	**GGATTTTGT**CAGGGGGTGTCA**CACTGTG** (95)	**CACAGTG**GTGCTGCCCATAT**CAAAAACC** (96)
5-12 opt	**GGATTTTGT**CAGGCGATGTCA**CACTGTG** (97)	**CACAGTG**GTGCCGCCCATAT**CAAAAACC** (98)
5-18 opt	**GGATTTTGT**CAGGGGGTGTCA**CACTGTG** (99)	**CACAGTG**GTGCTGCCCATA**TCAAAAACC** (100)
5-24 ORF opt	**GGATTTTGT**CAGGGGGTGCCA**CACTGTG** (101)	**CACAGTG**GTGCCGCCCATA**TCAAAAACC** (102)
6-6 opt	**GGATTTTGT**AGGTGTCTGTGT**CACTGTG** (103)	**CACAGTG**ACACTCGCCAGG**TCAAAAACC** (104)
6-13 opt	**GGATTTTGT**AGGTGTCTGTGT**CACTGTG** (105)	**CACAGTG**ACACTCACCCAG**TCAAAAACC** (106)
6-19 opt	**GGATTTTGT**AGCTGTCTGTAT**CACTGTG** (107)	**CACAGTG**ACACTCGCCAGG**TCAAAAACC** (108)
6-25 (not optimized)	**GGTTTCTGA**AGCTGTCTGTGTCACAGTC (109)	CACAATGACACTGGGCAGGACAGAAACC (110)
7-27 (not optimized)	GGGTTTGGCTGAGCTGAGAAC**CACTGTG** (111)	**CACAGTG**ATTGGCAGCTCT***CAAAAACC*** (112)

Figure 2

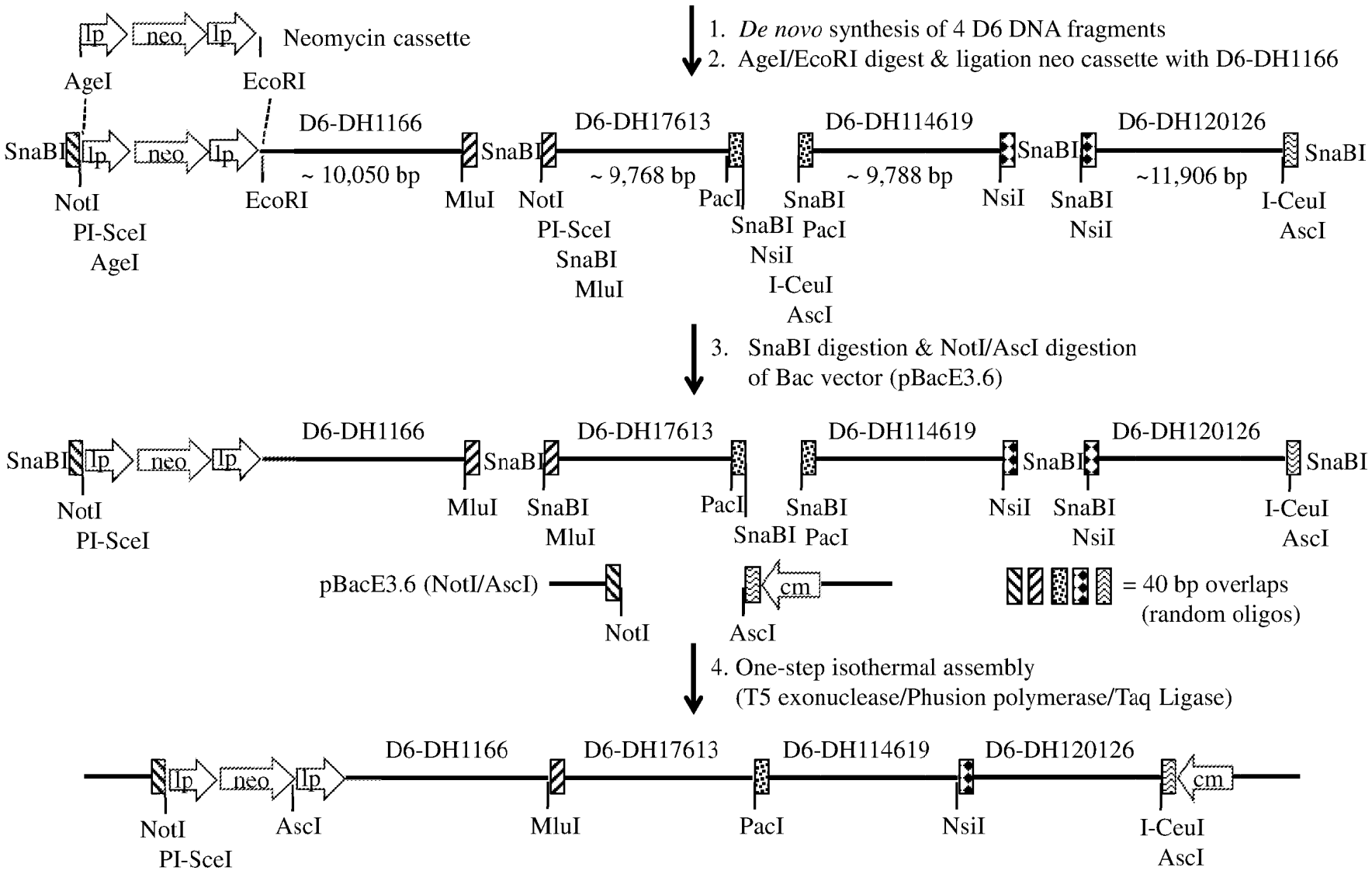

Ip
neo
Ip
Neomycin cassette
1. De novo synthesis of 4 D6 DNA fragments
2. AgeI/EcoRI digest & ligation neo cassette with D6-DH1166
AgeI
EcoRI
SnaBI
D6-DH1166
~ 10,050 bp
MluI
SnaBI
D6-DH17613
~ 9,768 bp
PacI
D6-DH114619
~ 9,788 bp
NsiI
SnaBI
D6-DH120126
~11,906 bp
SnaBI
NotI
PI-SceI
AgeI
EcoRI
NotI
PI-SceI
SnaBI
MluI
SnaBI
NsiI
I-CeuI
AscI
SnaBI
PacI
SnaBI
NsiI
SnaBI
I-CeuI
AscI
3. SnaBI digestion & NotI/AscI digestion
of Bac vector (pBacE3.6)
SnaBI
D6-DH1166
MluI
SnaBI
D6-DH17613
PacI
SnaBI
D6-DH114619
NsiI
SnaBI
D6-DH120126
SnaBI
NotI
PI-SceI
MluI
SnaBI
PacI
SnaBI
NsiI
I-CeuI
AscI
pBacE3.6 (NotI/AscI)
NotI
cm
AscI
= 40 bp overlaps
(random oligos)
4. One-step isothermal assembly
(T5 exonuclease/Phusion polymerase/Taq Ligase)
D6-DH1166
D6-DH17613
D6-DH114619
D6-DH120126
NotI
PI-SceI
AscI
MluI
PacI
NsiI
I-CeuI
AscI

Figure 3A

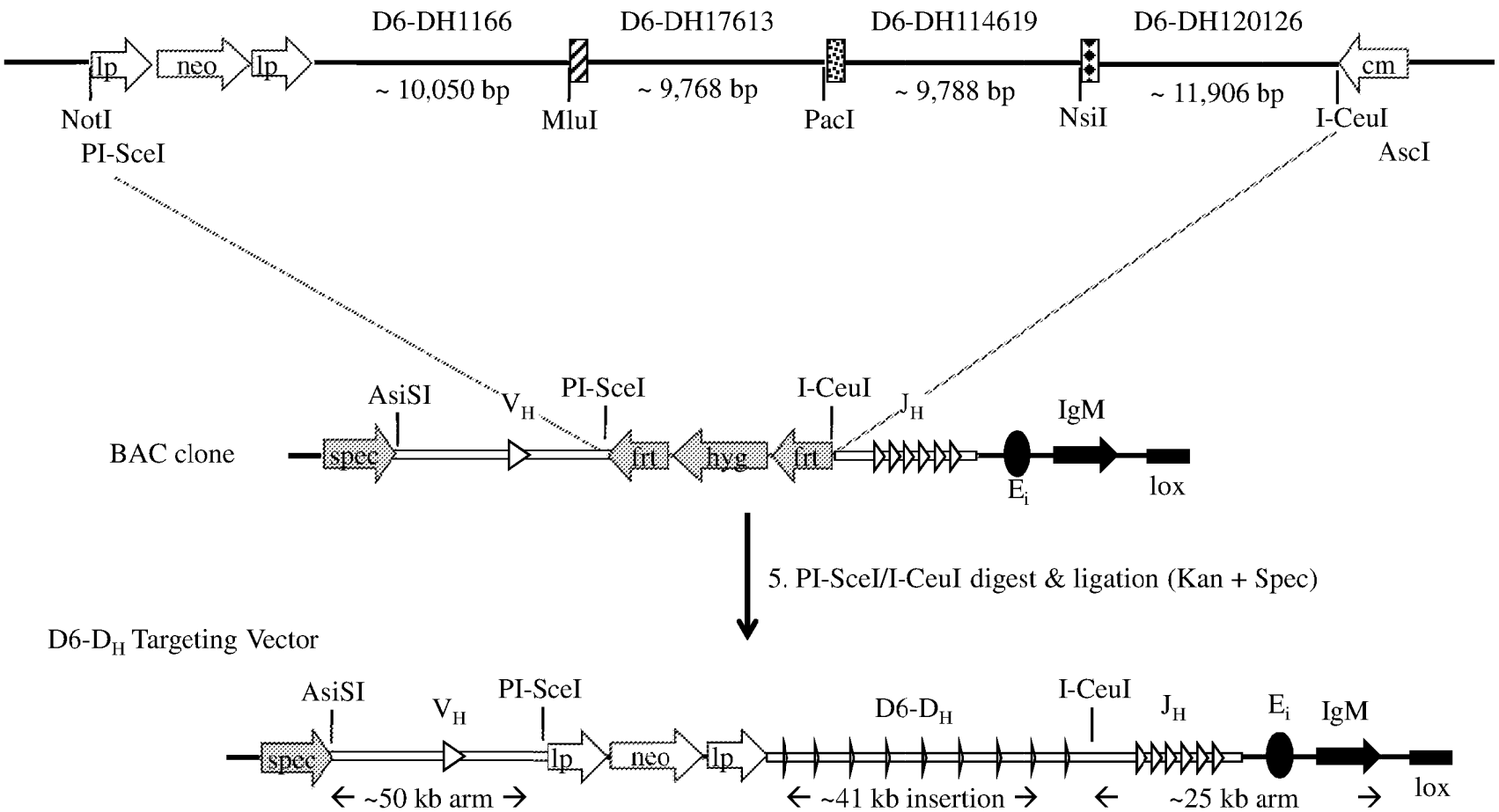

D6-DH1166
D6-DH17613
D6-DH114619
D6-DH120126
lp
neo
lp
cm
~ 10,050 bp
~ 9,768 bp
~ 9,788 bp
~ 11,906 bp
NotI
PI-SceI
MluI
PacI
NsiI
I-CeuI
AscI
AsiSI
V_H
PI-SceI
I-CeuI
J_H
IgM
BAC clone
spec
frt
hyg
frt
E_i
lox
5. PI-SceI/I-CeuI digest & ligation (Kan + Spec)
D6-D_H Targeting Vector
AsiSI
V_H
PI-SceI
D6-D_H
I-CeuI
J_H
E_i
IgM
spec
lp
neo
lp
lox
← ~50 kb arm →
← ~41 kb insertion →
← ~25 kb arm →

Figure 3B

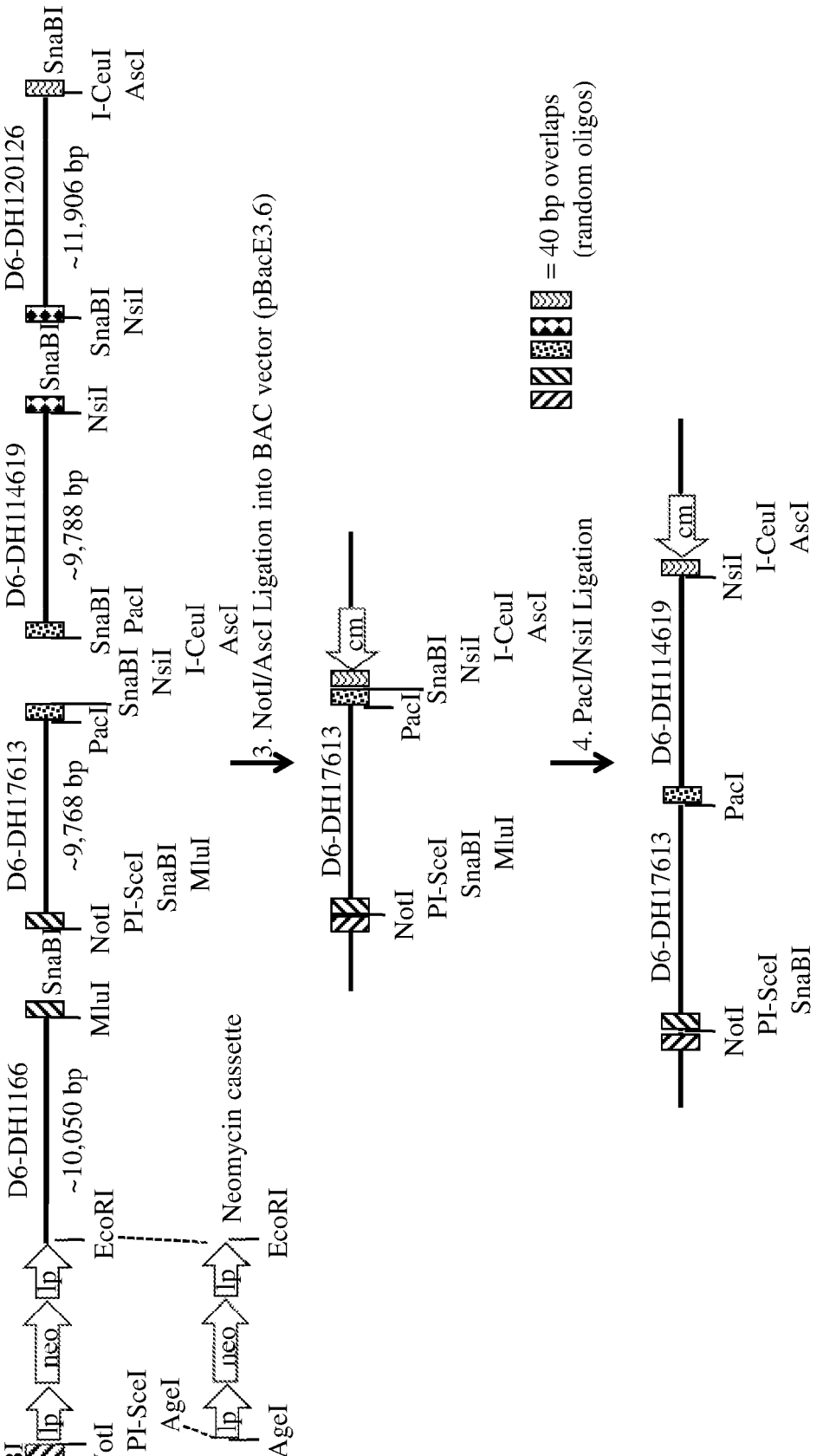

1. De novo synthesis of 4 D6-DH DNA fragments
2. AgeI/EcoRI ligation of neo cassette with D6-DH1166
3. NotI/AscI Ligation into BAC vector (pBacE3.6)
4. PacI/NsiI Ligation
D6-DH1166
D6-DH17613
D6-DH114619
D6-DH120126
SnaBI
Ip
neo
~10,050 bp
~9,768 bp
~9,788 bp
~11,906 bp
NotI
PI-SceI
AgeI
EcoRI
MluI
PacI
NsiI
I-CeuI
AscI
Neomycin cassette
cm
= 40 bp overlaps
(random oligos)

Figure 4A

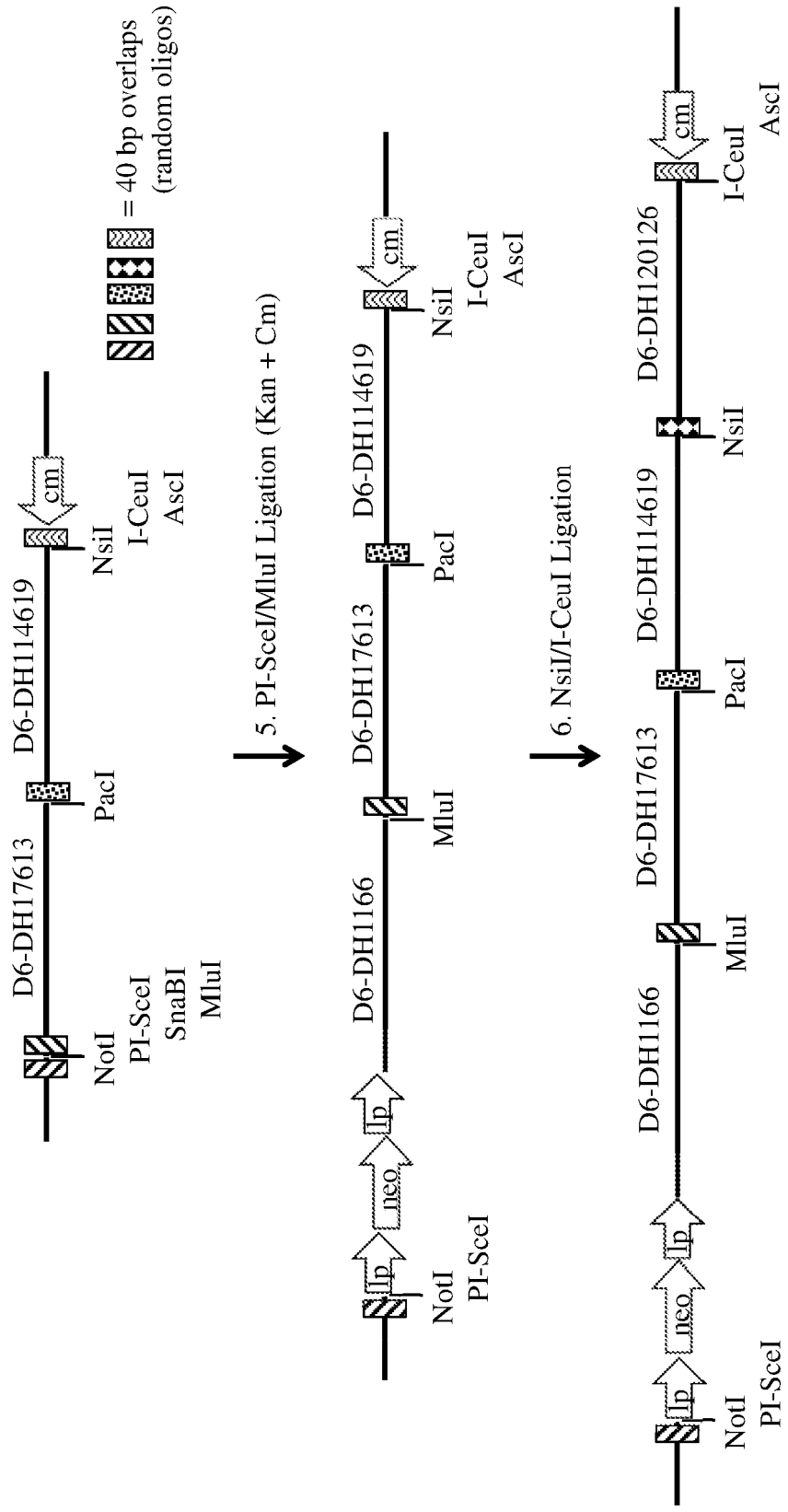

D6-DH17613
D6-DH114619
cm
NotI
PI-SceI
SnaBI
MluI
PacI
NsiI
I-CeuI
AscI
= 40 bp overlaps
(random oligos)
5. PI-SceI/MluI Ligation (Kan + Cm)
D6-DH1166
lp
neo
rp
D6-DH17613
D6-DH114619
cm
NotI
PI-SceI
MluI
PacI
NsiI
I-CeuI
AscI
6. NsiI/I-CeuI Ligation
D6-DH120126

Figure 4B

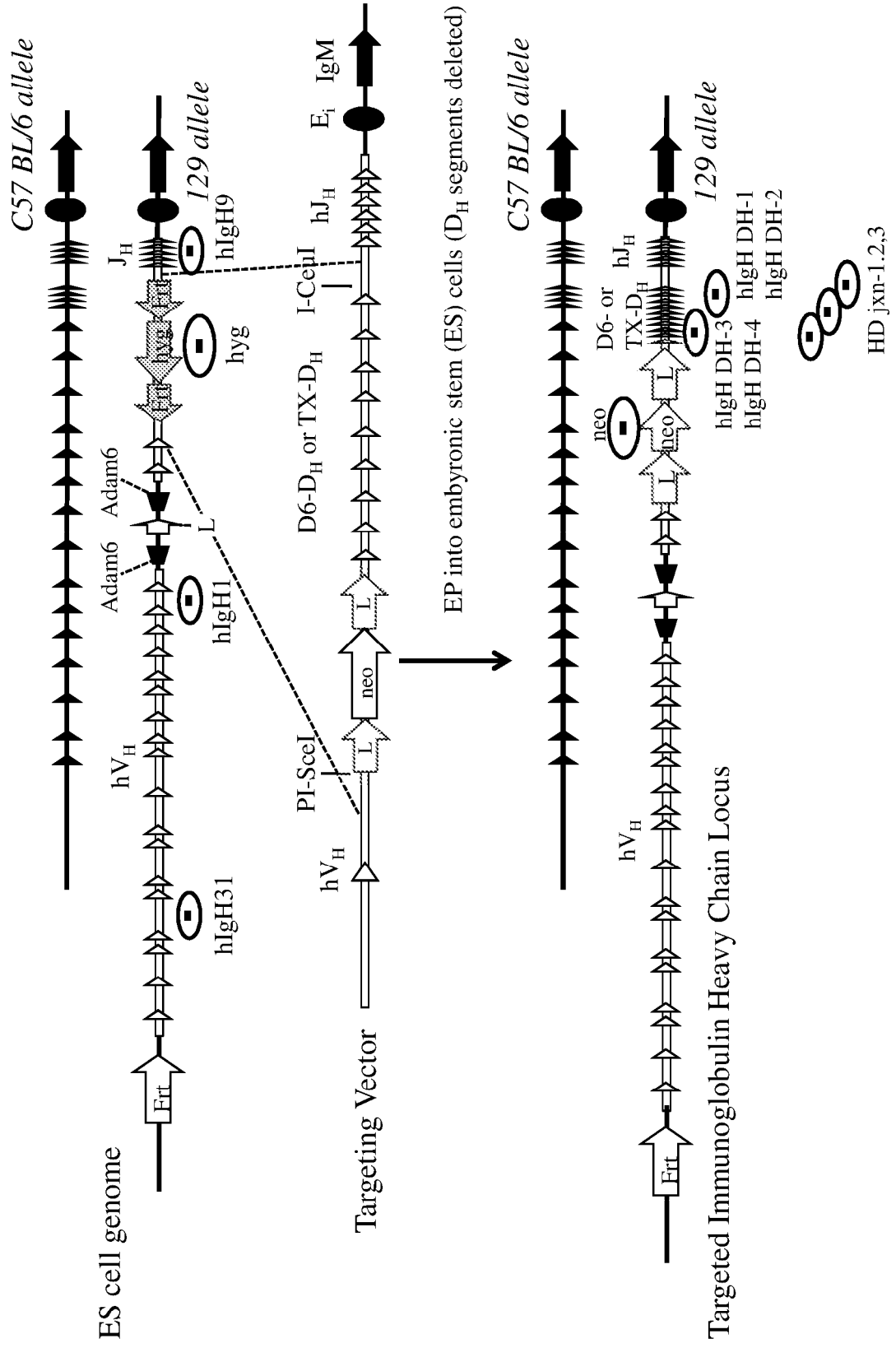

C57 BL/6 allele
129 allele
ES cell genome
Targeting Vector
EP into embryonic stem (ES) cells (D$_H$ segments deleted)
C57 BL/6 allele
129 allele
Targeted Immunoglobulin Heavy Chain Locus
Ert
hygr
hyg
neo
L
hV$_H$
J$_H$
Adam6
hIgH1
hIgH31
hIgH9
PI-SceI
D6-D$_H$ or TX-D$_H$
I-CeuI
hJ$_H$
E$_i$
IgM
D6- or TX-D$_H$
hIgH DH-3
hIgH DH-4
hIgH DH-1
hIgH DH-2
HD jxn-1,2,3

Figure 5

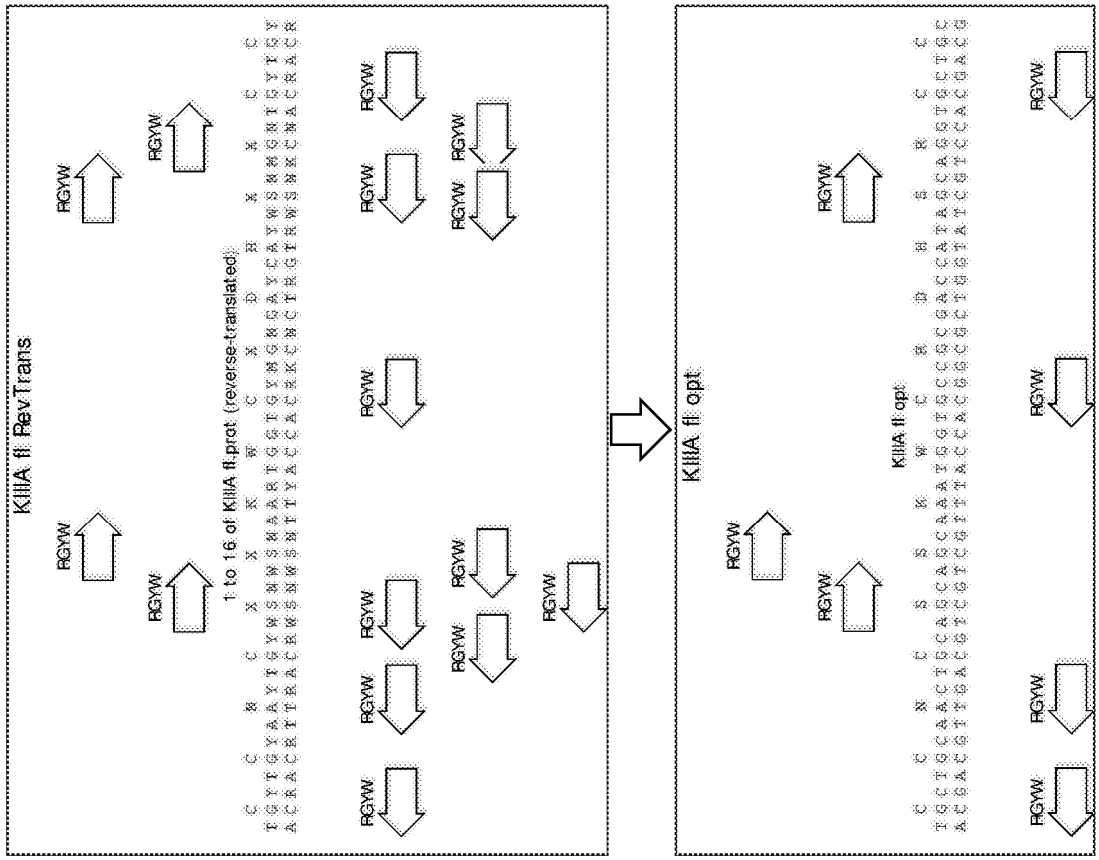

KillA fl: RevTrans
1: to 1.6 of KillA fl.prot (reverse-translated)
RGYW
KillA fl: opt

Figure 6A

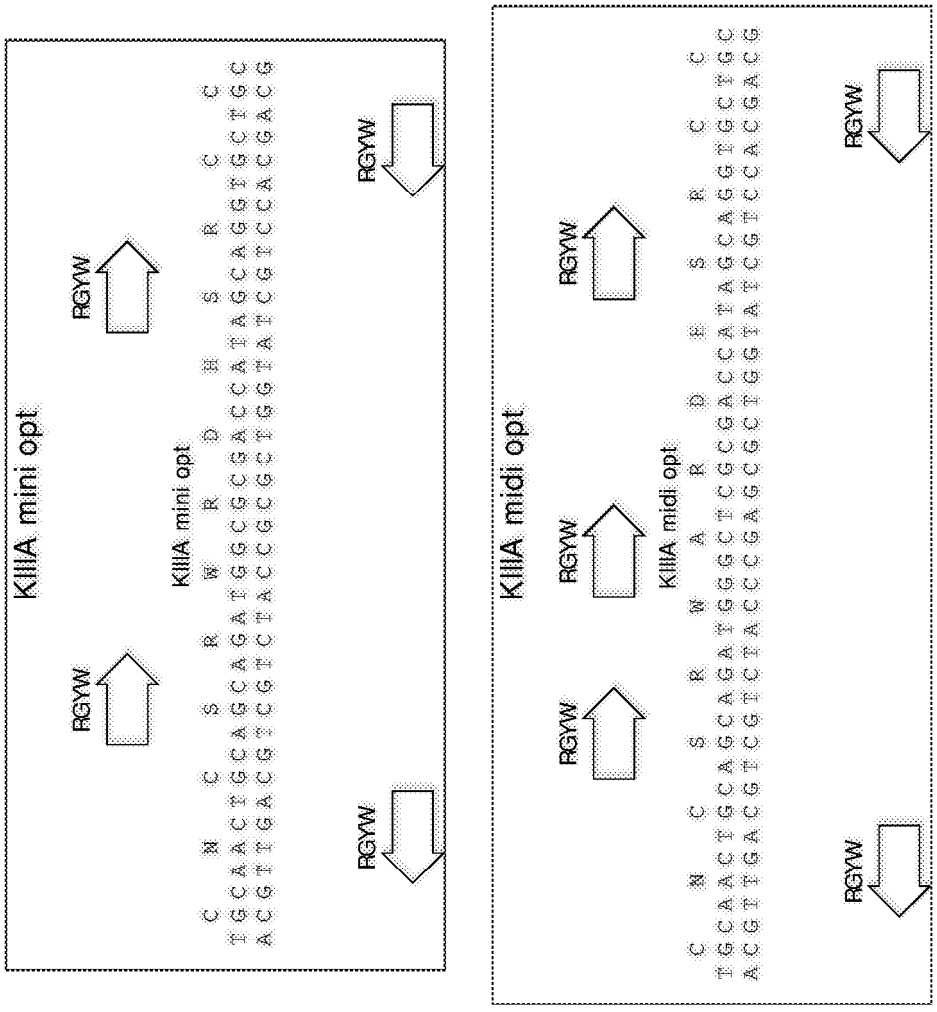

KIIIA mini opt
RGYW
RGYW
RGYW
RGYW
KIIIA mini opt
KIIIA midi opt
RGYW
RGYW
RGYW
RGYW
KIIIA midi opt

Figure 6B

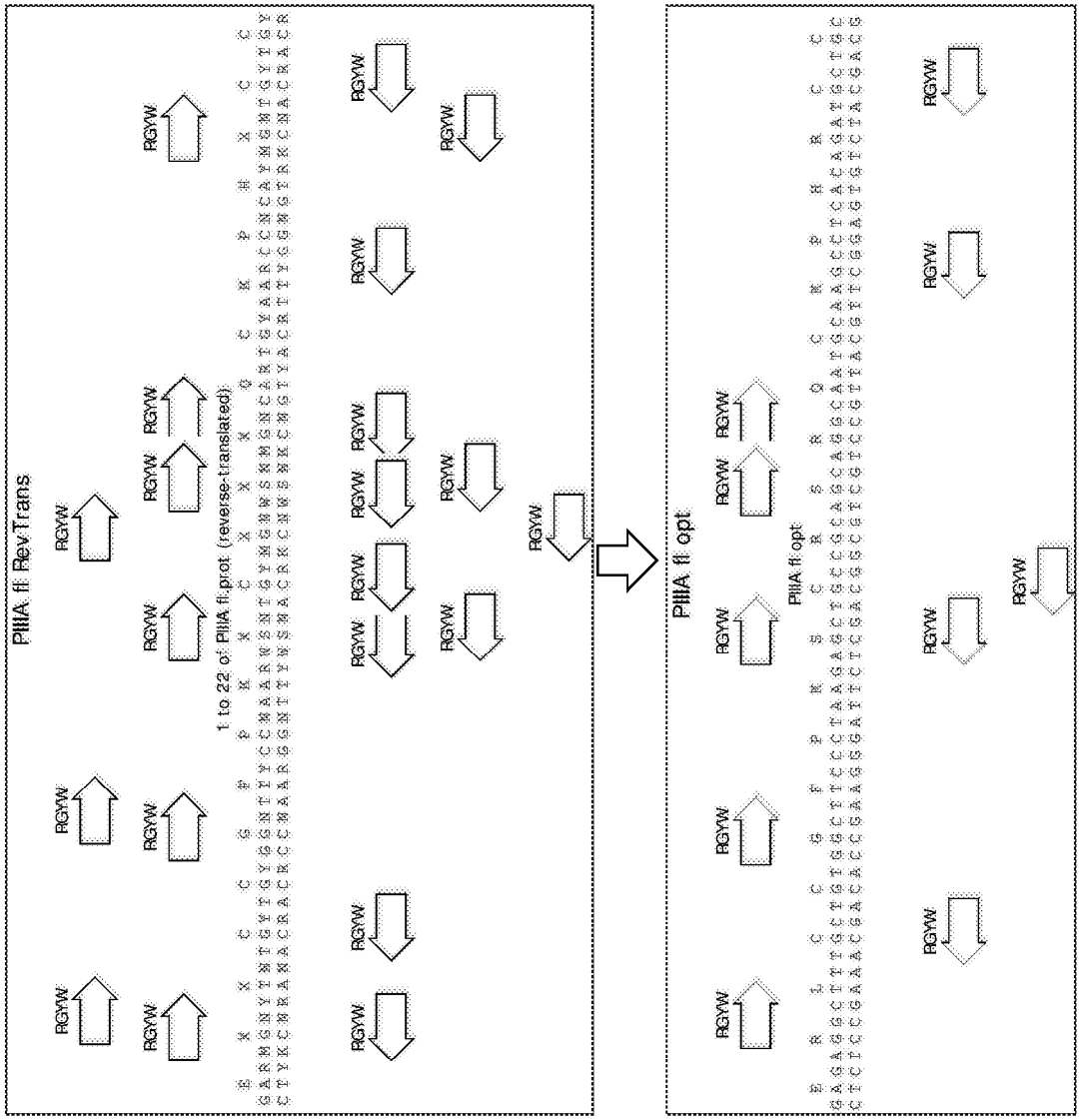

PllIA fl RevTrans
RGYW
1 to 22 of: PllIA fl.prot (reverse-translated)
PllIA fl opt
PllIA fl opt

Figure 6C

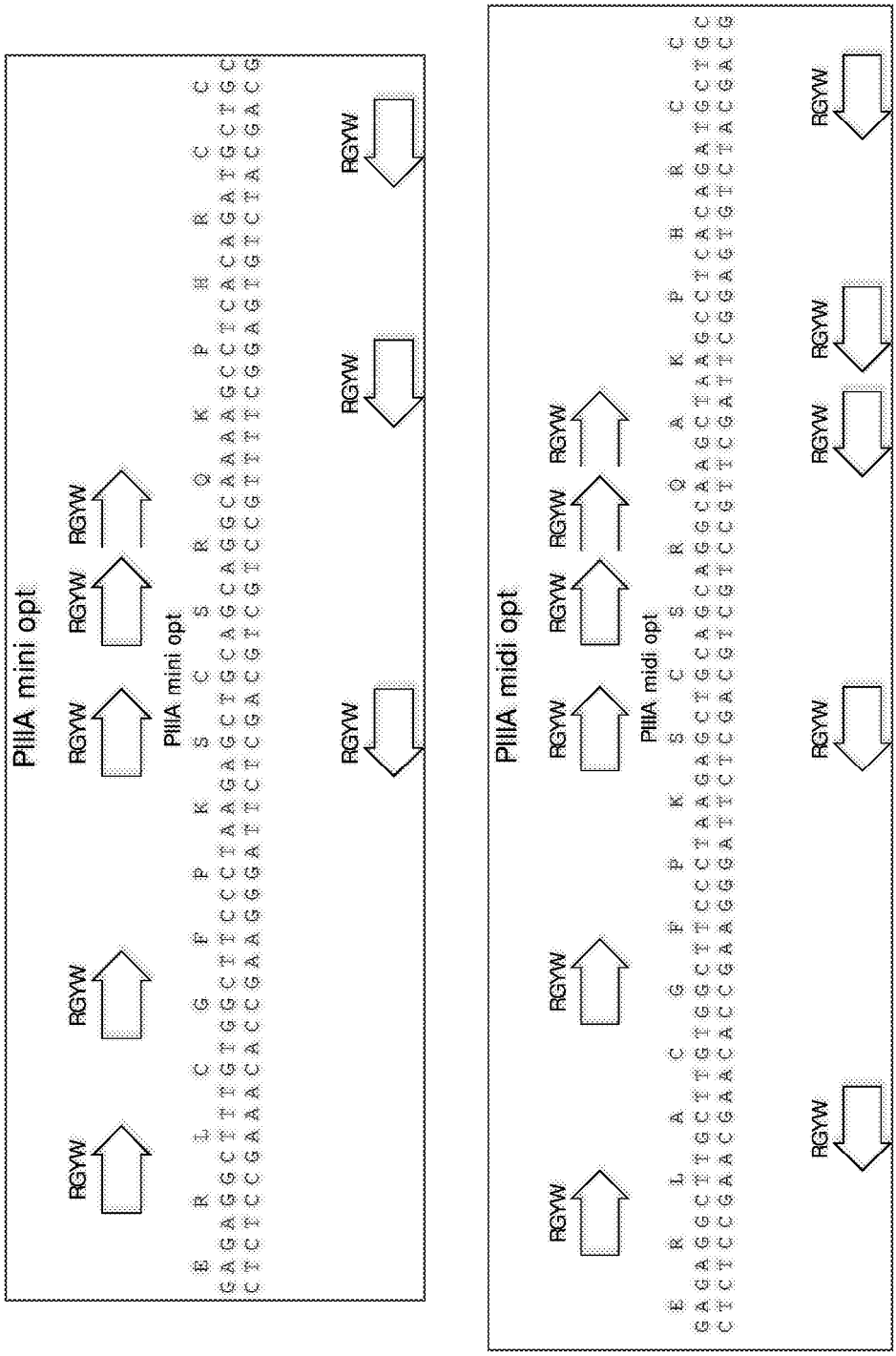


Figure 6D

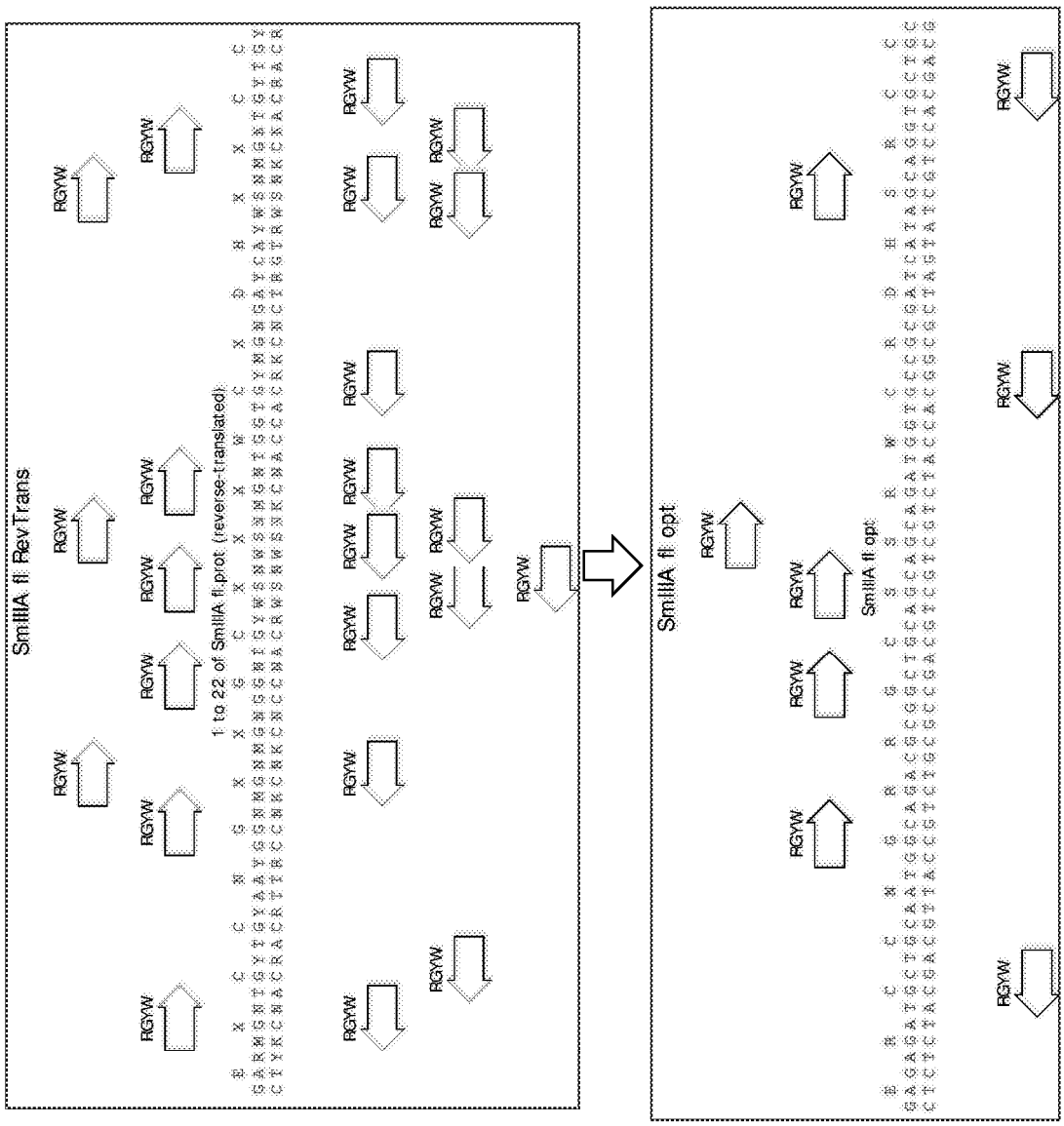

SmIIIA fl: Rev Trans
1 to 22 of SmIIIA fl:prot (reverse-translated)
RGYW
SmIIIA fl: opt
SmIIIA fl: opt

Figure 6E

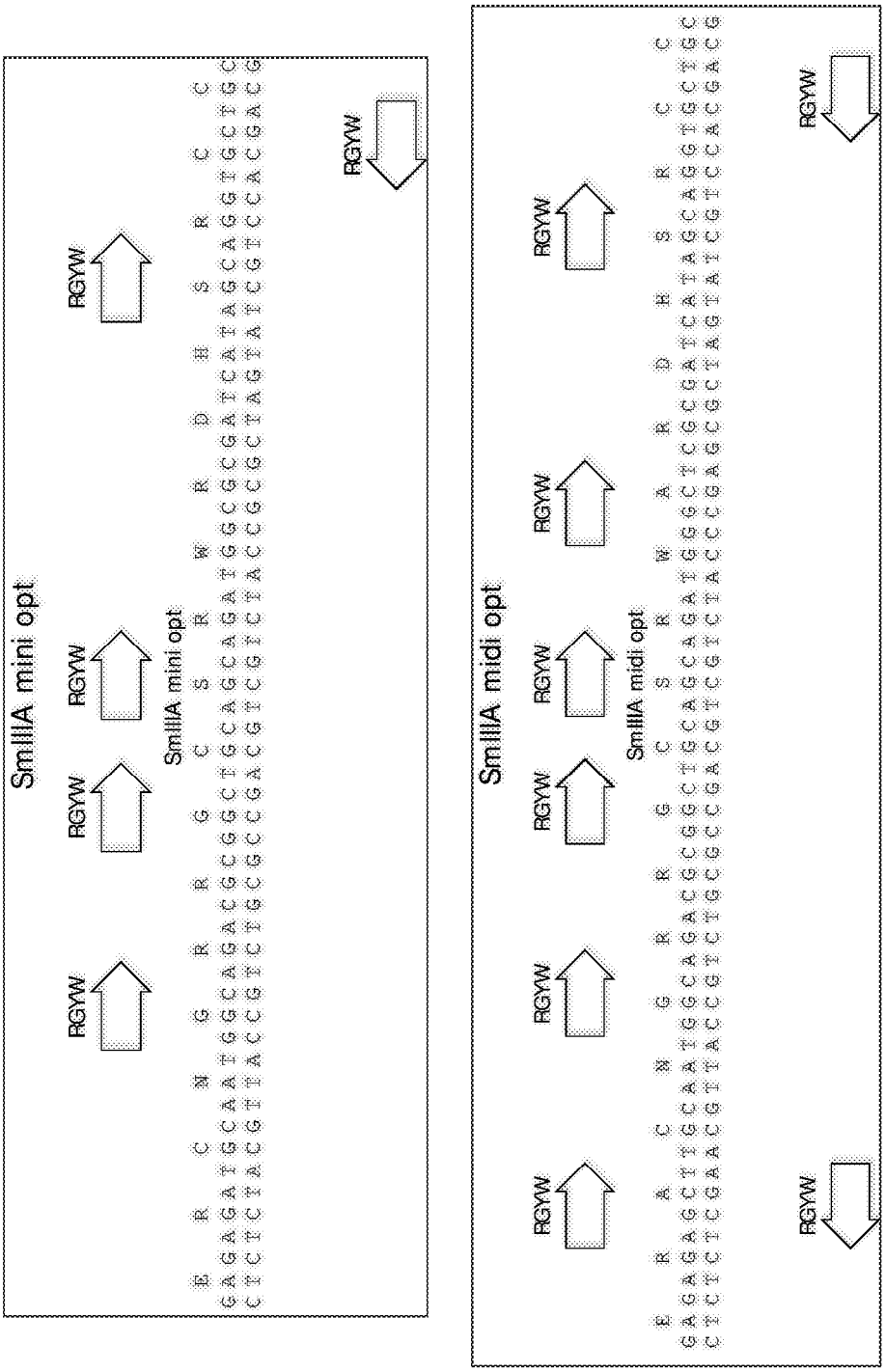

SmIIIA mini opt
RGYW
RGYW
RGYW
RGYW
RGYW
SmIIIA midi opt
RGYW
RGYW
RGYW
RGYW
RGYW
RGYW

Figure 6F

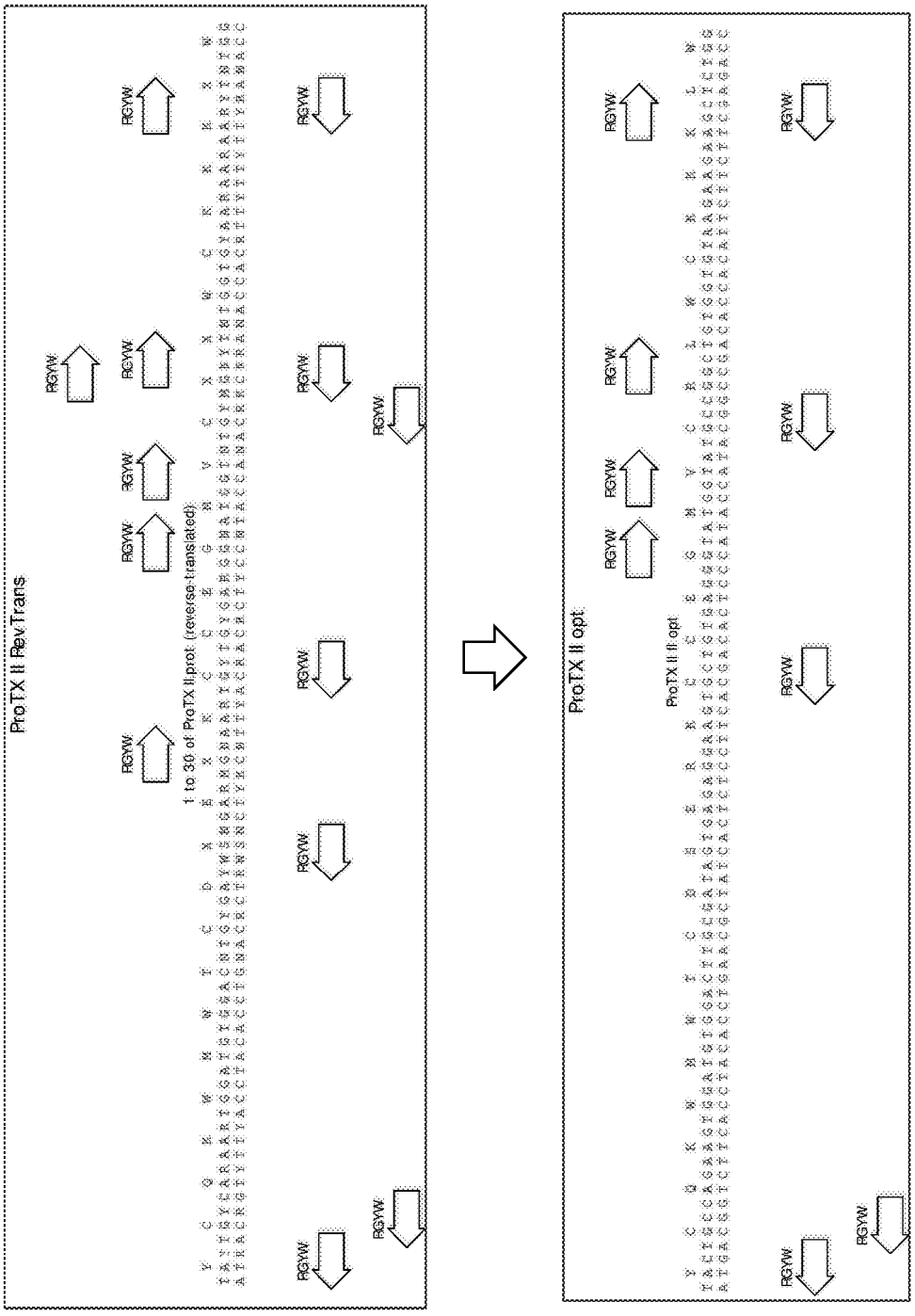

ProTX II Rev.Trans
1 to 30 of ProTX II.prot (reverse-translated)
RGYW
ProTX II opt.
ProTX II opt

Figure 6G

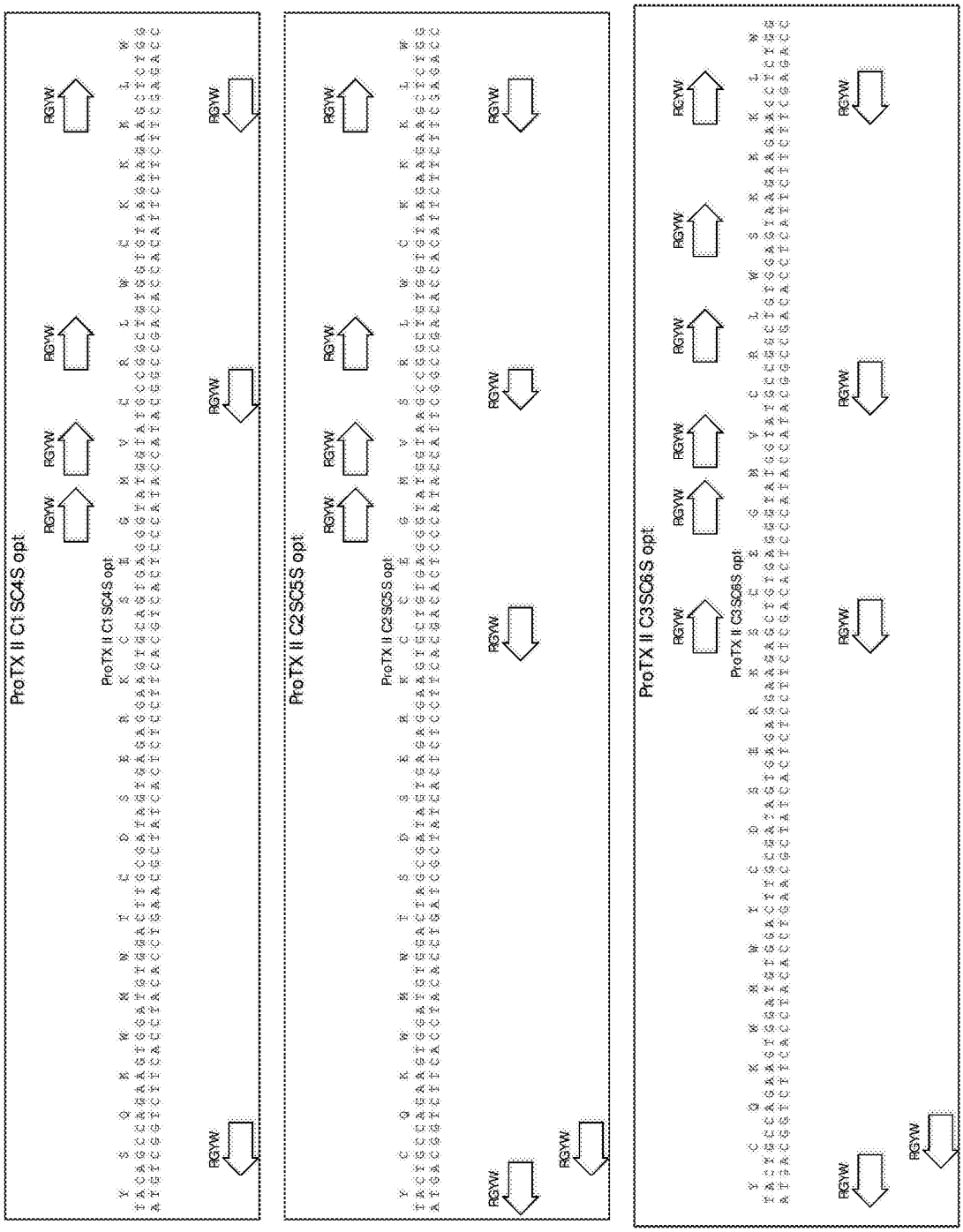

ProTX II C1SC4S opt
RGYW
ProTX II C2SC5S opt
RGYW
ProTX II C3SC6S opt
RGYW

Figure 6H

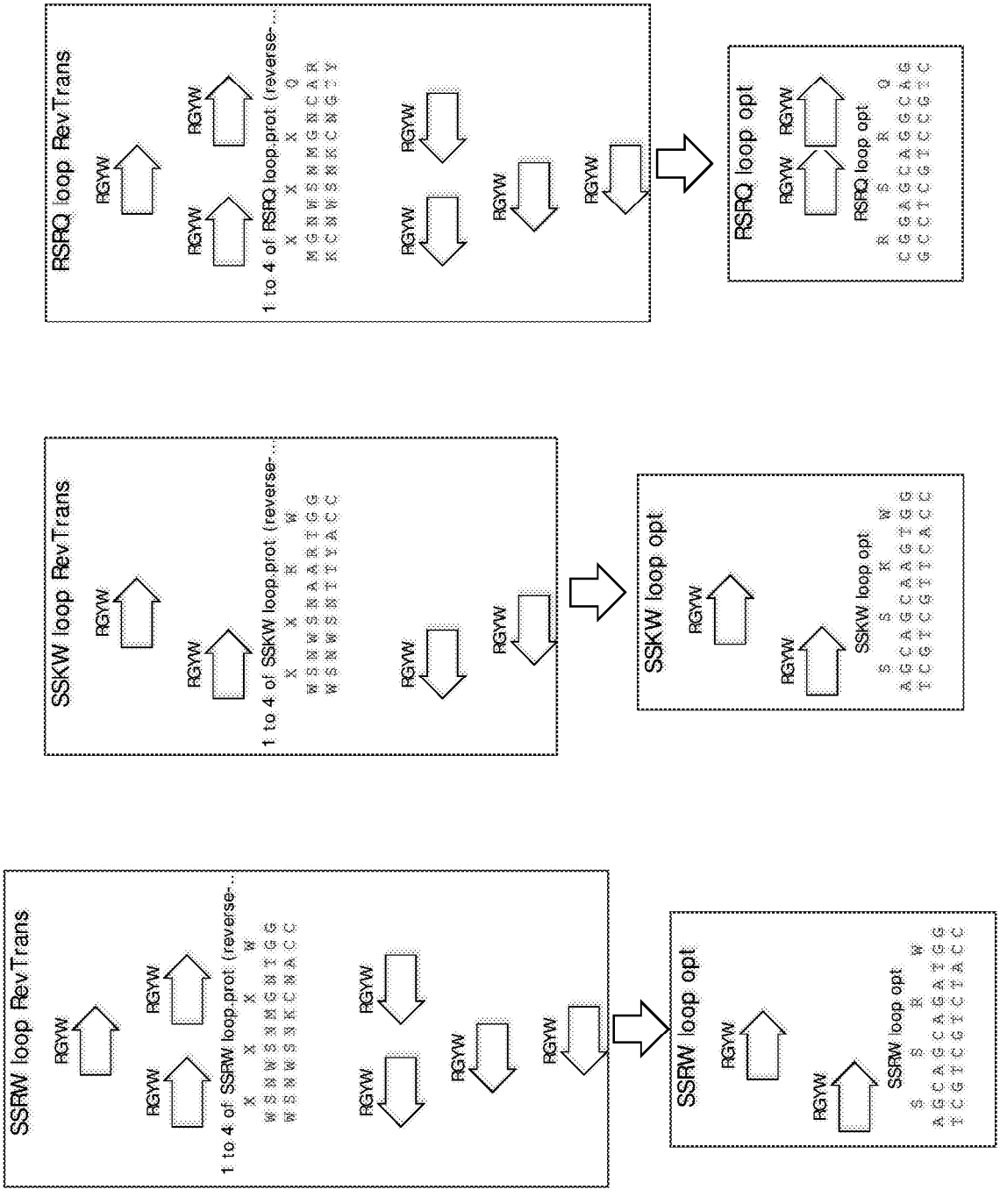

SSRW loop RevTrans
RGYW
RGYW
RGYW
RGYW
RGYW
RGYW
RGYW
SSRW loop opt
RGYW
RGYW
SSRW loop opt
SSKW loop RevTrans
RGYW
RGYW
RGYW
RGYW
RGYW
SSKW loop opt
RGYW
RGYW
SSKW loop opt
RSRQ loop RevTrans
RGYW
RGYW
RGYW
RGYW
RGYW
RGYW
RGYW
RSRQ loop opt
RGYW
RGYW
RSRQ loop opt

Figure 6I

KIIIA or SmIIIA mini/midi in D_H3 segments

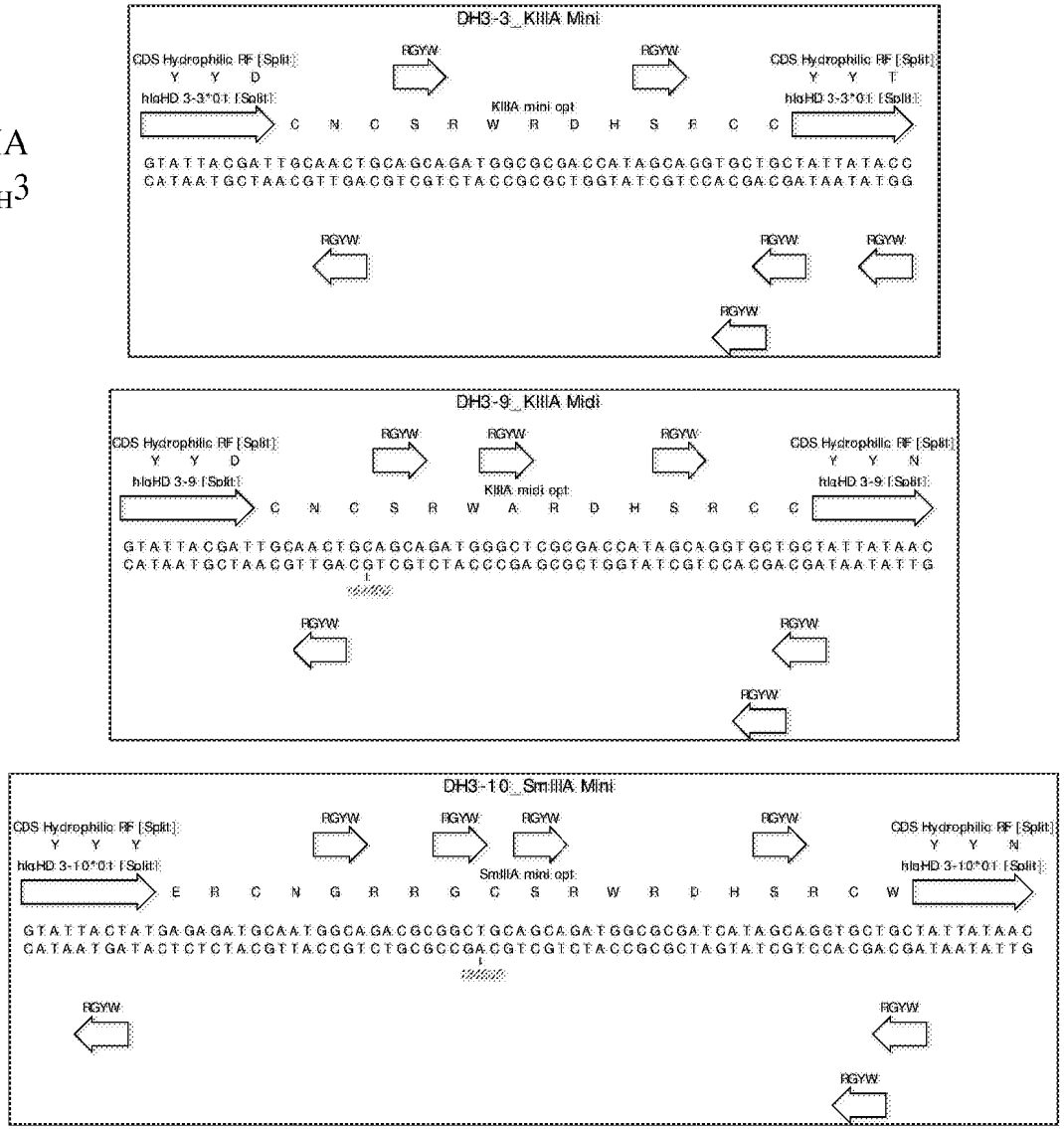


Figure 6J

SmIIIA or PIIIA mini/midi in D_H3 or D_H1 segments

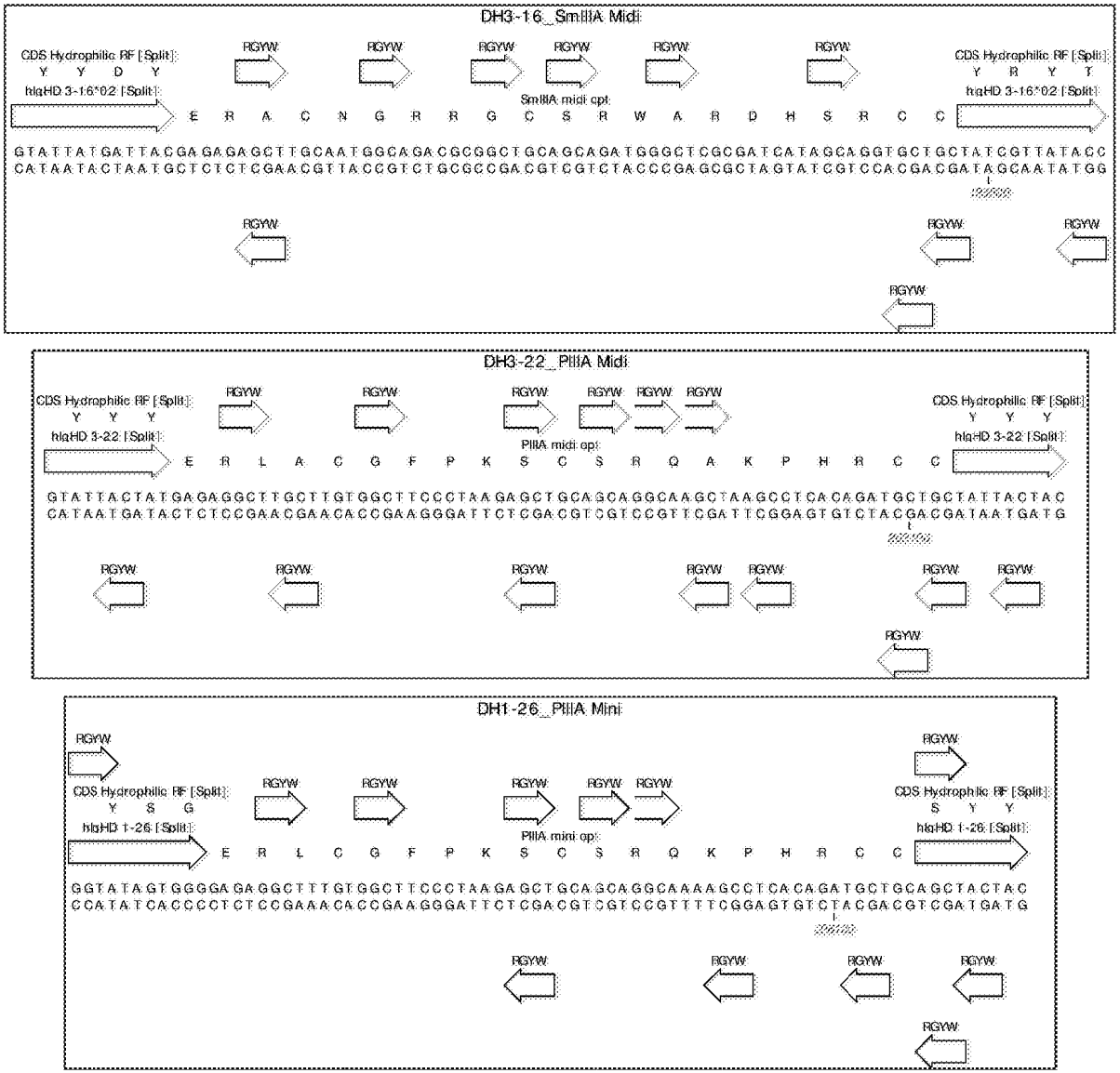


Figure 6K

SSRW or RSRQ loops in D_H2 segments

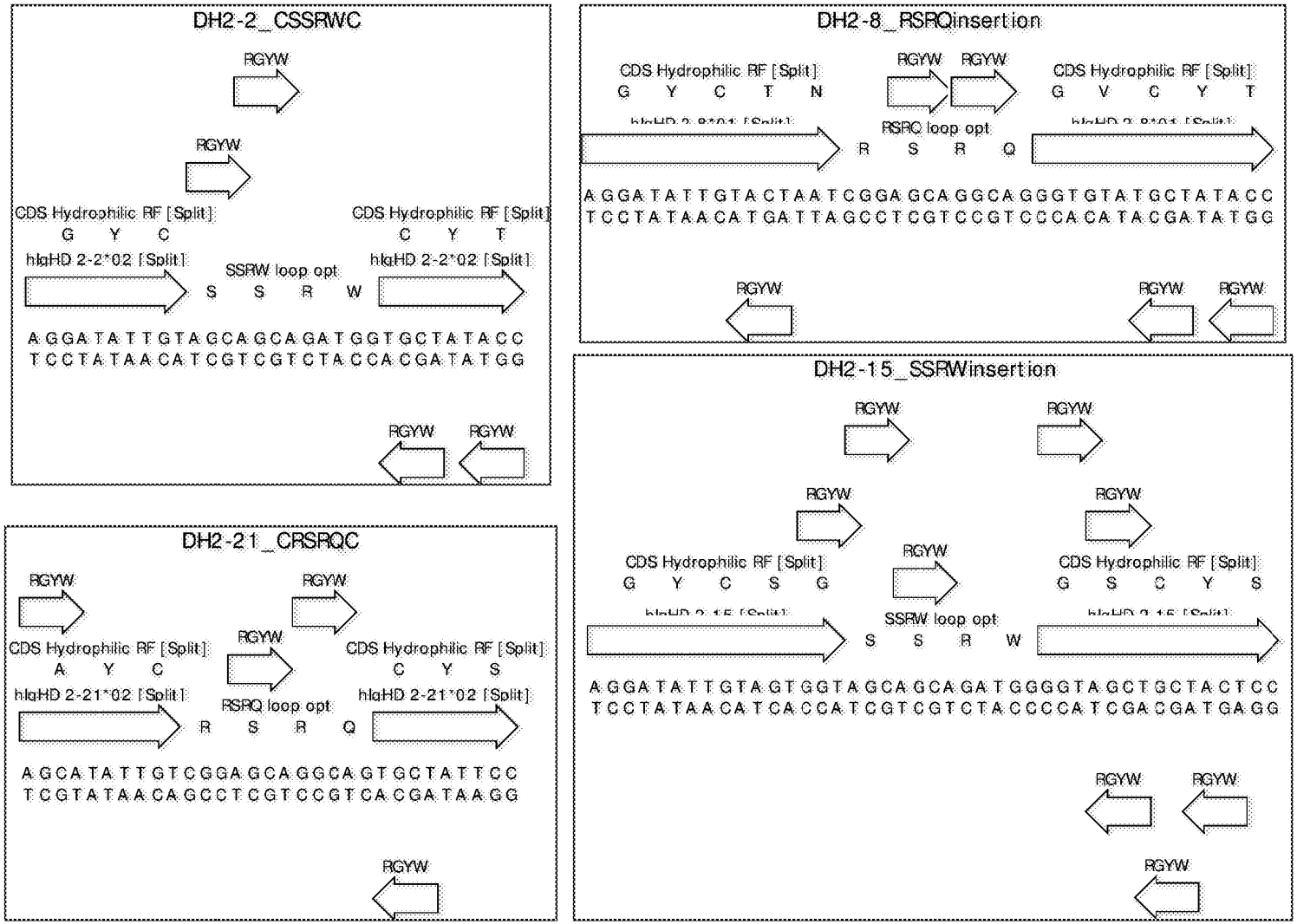


Figure 6L

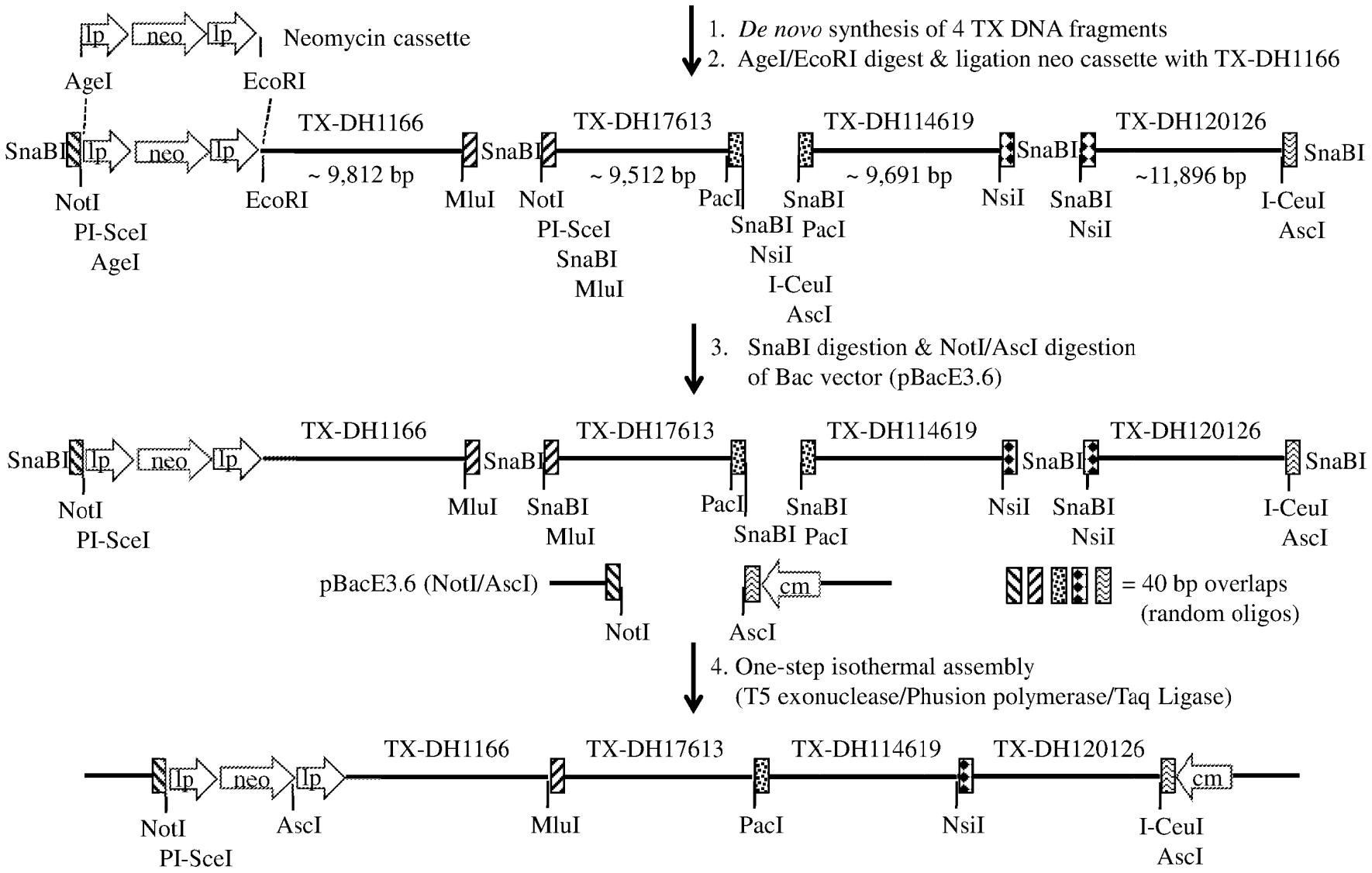

lp
neo
lp
Neomycin cassette
AgeI
EcoRI
1. De novo synthesis of 4 TX DNA fragments
2. AgeI/EcoRI digest & ligation neo cassette with TX-DH1166
SnaBI
TX-DH1166
~ 9,812 bp
MluI
SnaBI
TX-DH17613
~ 9,512 bp
PacI
TX-DH114619
~ 9,691 bp
NsiI
SnaBI
TX-DH120126
~11,896 bp
SnaBI
NotI
PI-SceI
AgeI
EcoRI
NotI
PI-SceI
SnaBI
MluI
SnaBI
NsiI
I-CeuI
AscI
SnaBI
PacI
SnaBI
NsiI
I-CeuI
AscI
3. SnaBI digestion & NotI/AscI digestion
of Bac vector (pBacE3.6)
pBacE3.6 (NotI/AscI)
NotI
cm
AscI
= 40 bp overlaps
(random oligos)
4. One-step isothermal assembly
(T5 exonuclease/Phusion polymerase/Taq Ligase)
NotI
PI-SceI
AscI
MluI
PacI
NsiI
I-CeuI
AscI

Figure 7A

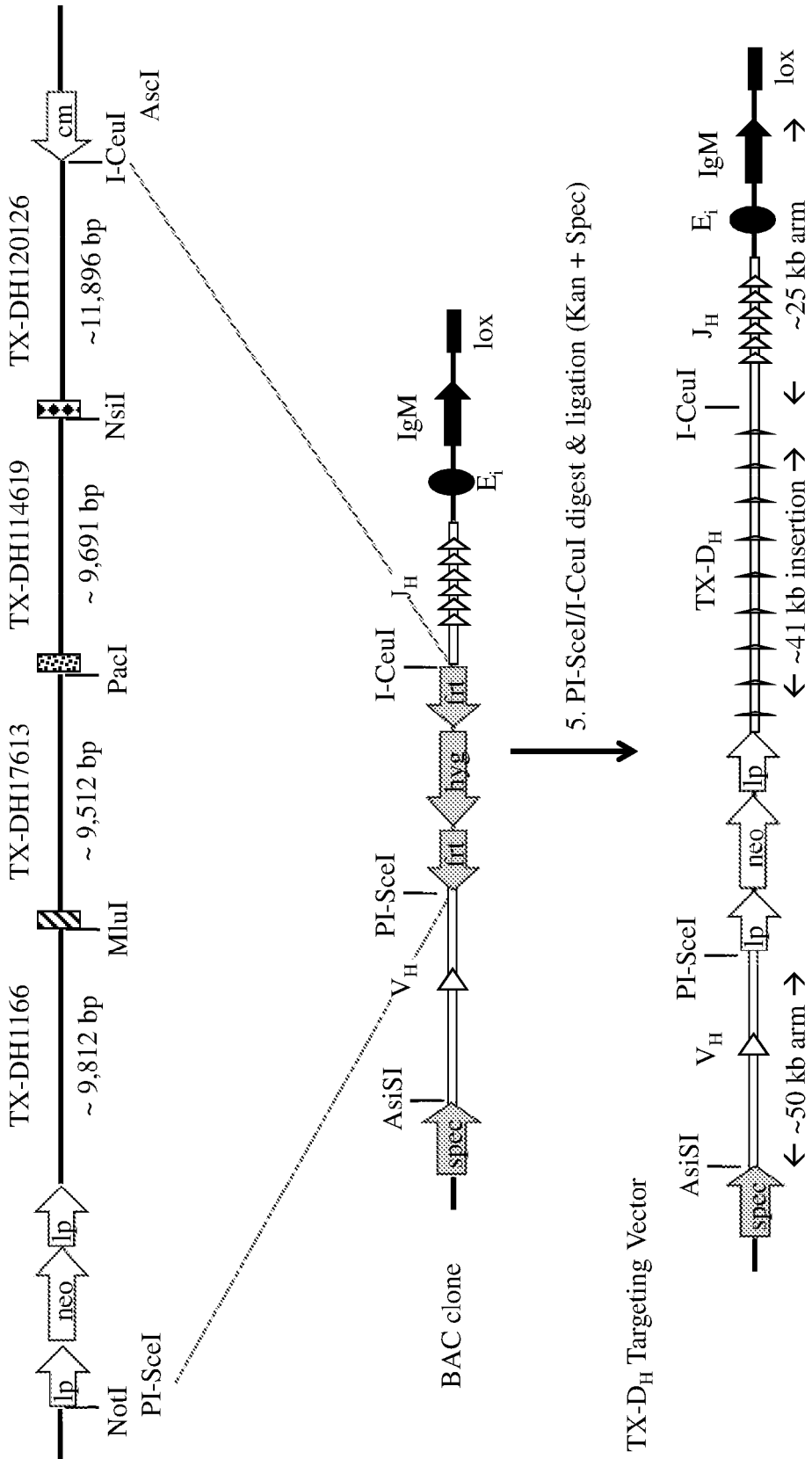

TX-DH1166
TX-DH17613
TX-DH114619
TX-DH120126
~ 9,812 bp
~ 9,512 bp
~ 9,691 bp
~11,896 bp
lp
neo
lp
cm
NotI
MluI
PacI
NsiI
I-CeuI
AscI
PI-SceI
BAC clone
AsiSI
spec
PI-SceI
V_H
frt
hyg
frt
I-CeuI
J_H
IgM
E_i
lox
5. PI-SceI/I-CeuI digest & ligation (Kan + Spec)
TX-D_H Targeting Vector
AsiSI
spec
V_H
PI-SceI
lp
neo
lp
TX-D_H
I-CeuI
J_H
E_i
IgM
lox
← ~50 kb arm →
← ~41 kb insertion →
← ~25 kb arm →

Figure 7B

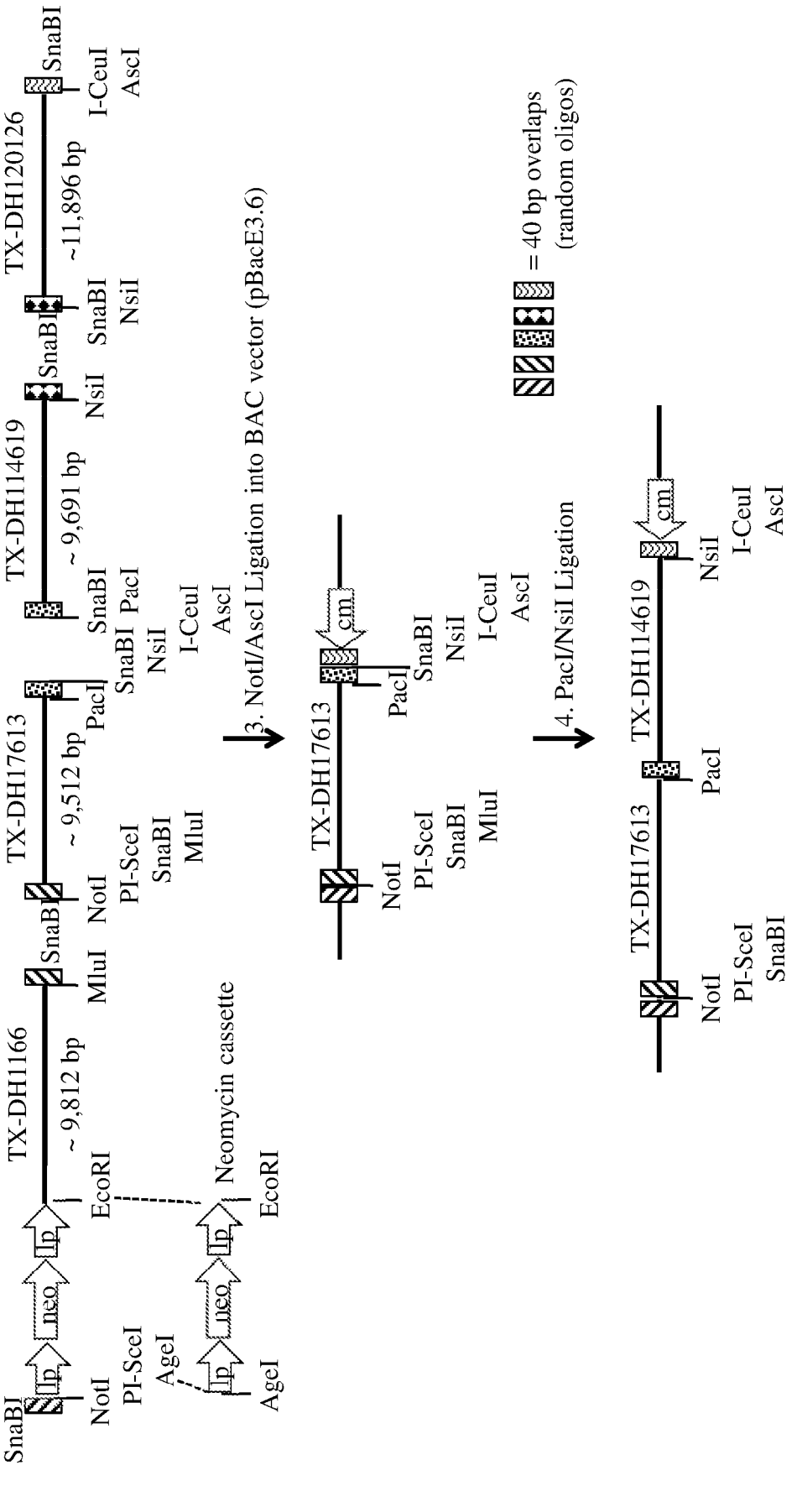

1. De novo synthesis of 4 TX-DH DNA fragments
2. AgeI/EcoRI ligation of neo cassette with TX-DH1166
TX-DH1166
SnaBI
Ip
neo
Ip
~ 9,812 bp
SnaBI
MluI
NotI
PI-SceI
AgeI
Ip
neo
Ip
EcoRI
Neomycin cassette
AgeI
EcoRI
TX-DH17613
~ 9,512 bp
PacI
NotI
PI-SceI
SnaBI
MluI
SnaBI
PacI
NsiI
I-CeuI
AscI
3. NotI/AscI Ligation into BAC vector (pBacE3.6)
TX-DH114619
~ 9,691 bp
SnaBI
NsiI
TX-DH120126
SnaBI
~11,896 bp
I-CeuI
SnaBI
NsiI
AscI
TX-DH17613
cm
PacI
NotI
PI-SceI
SnaBI
MluI
SnaBI
NsiI
I-CeuI
AscI
4. PacI/NsiI Ligation
= 40 bp overlaps
(random oligos)
TX-DH17613
TX-DH114619
cm
NotI
PI-SceI
SnaBI
MluI
PacI
NsiI
I-CeuI
AscI

Figure 8A

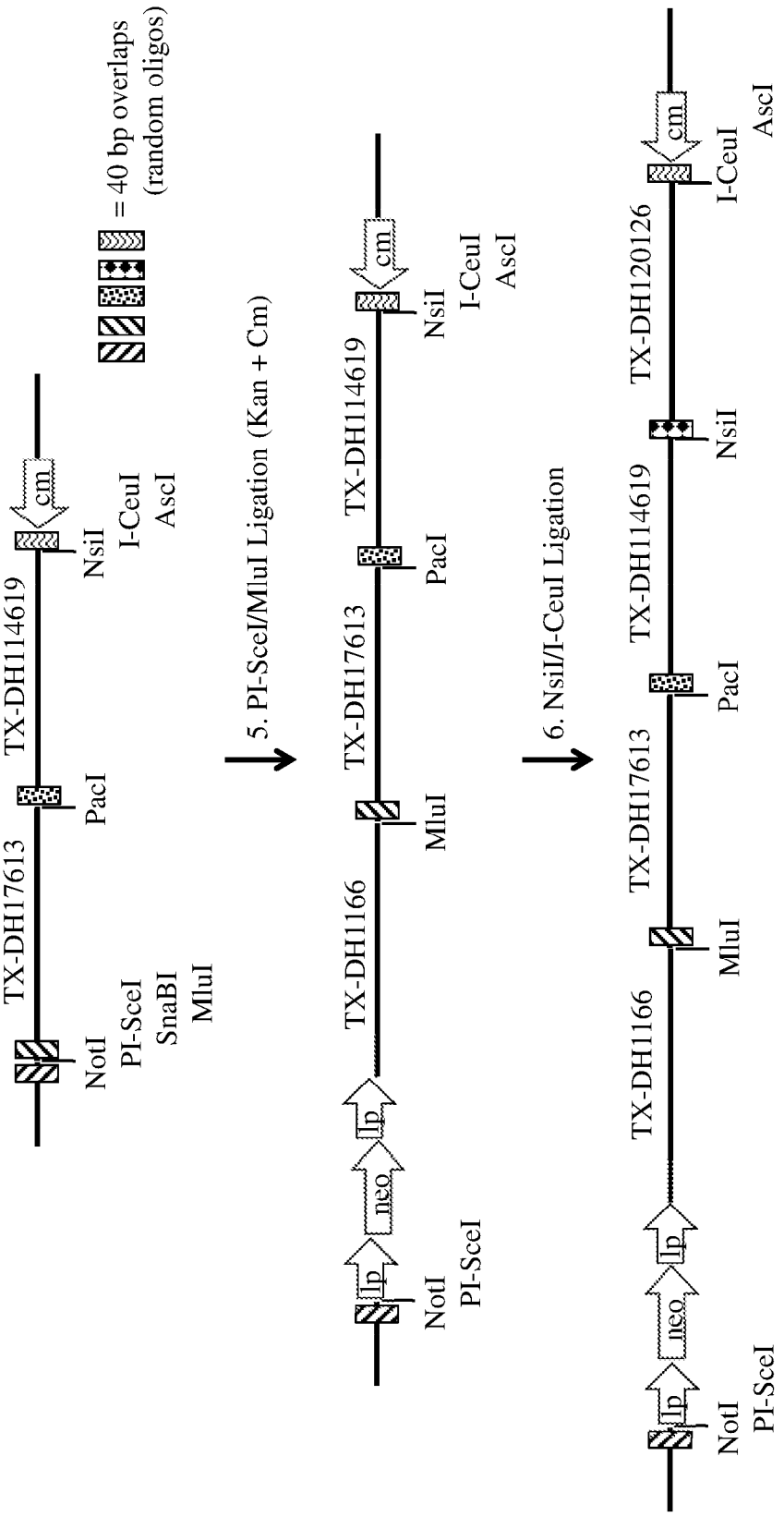

TX-DH17613
TX-DH114619
cm
NotI
PacI
NsiI
PI-SceI
I-CeuI
SnaBI
AscI
MluI
= 40 bp overlaps
(random oligos)
5. PI-SceI/MluI Ligation (Kan + Cm)
TX-DH1166
TX-DH17613
TX-DH114619
lp
neo
lp
cm
NotI
PI-SceI
MluI
PacI
NsiI
I-CeuI
AscI
6. NsiI/I-CeuI Ligation
TX-DH1166
TX-DH17613
TX-DH114619
TX-DH120126
lp
neo
lp
cm
NotI
PI-SceI
MluI
PacI
NsiI
I-CeuI
AscI

Figure 8B

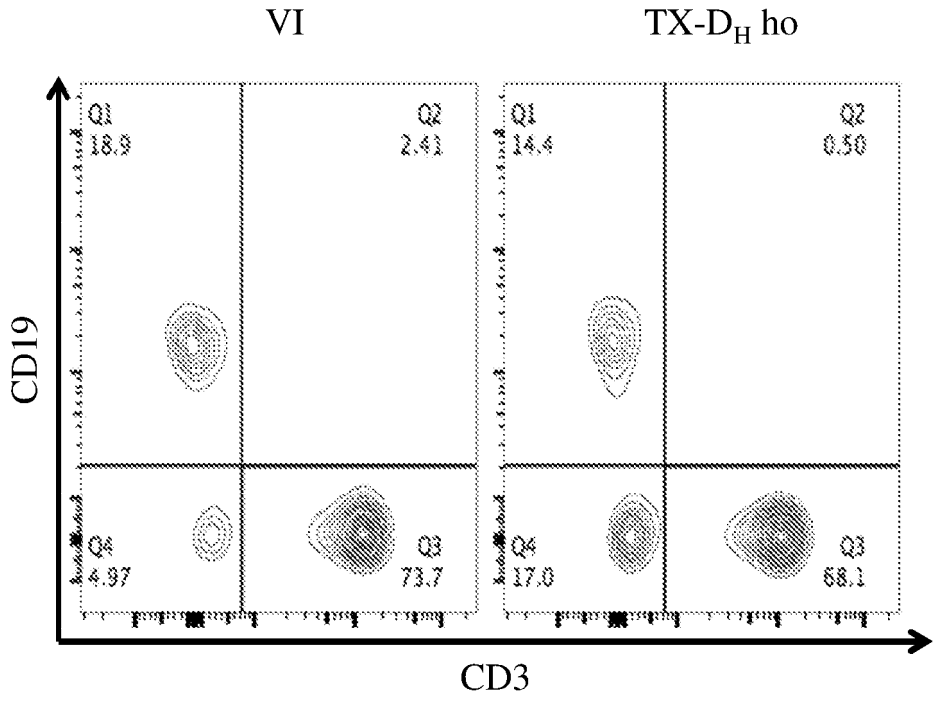


Figure 9A

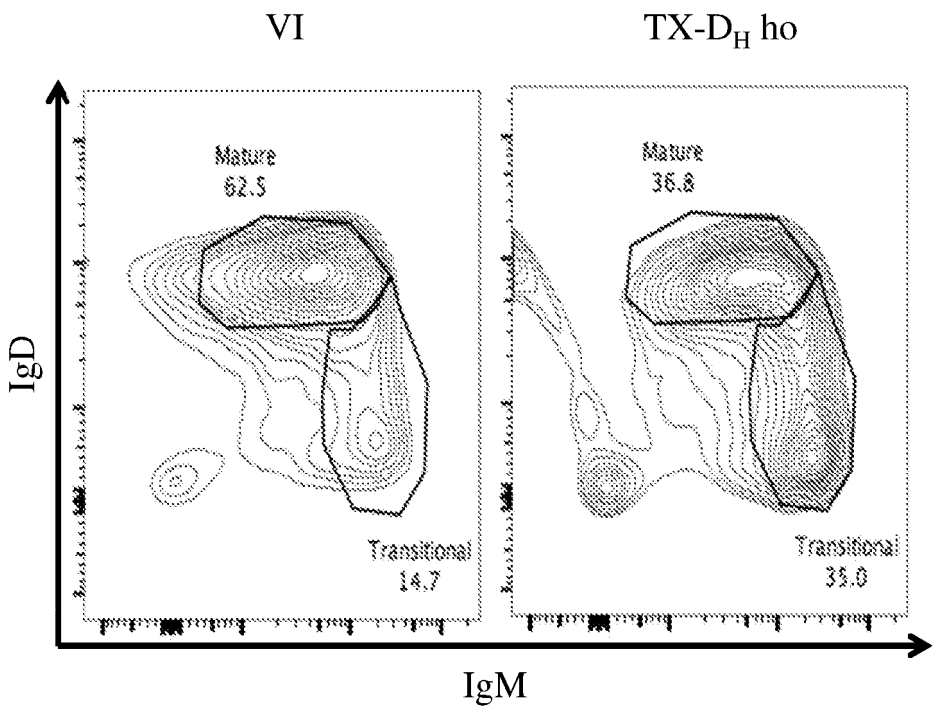


Figure 9B

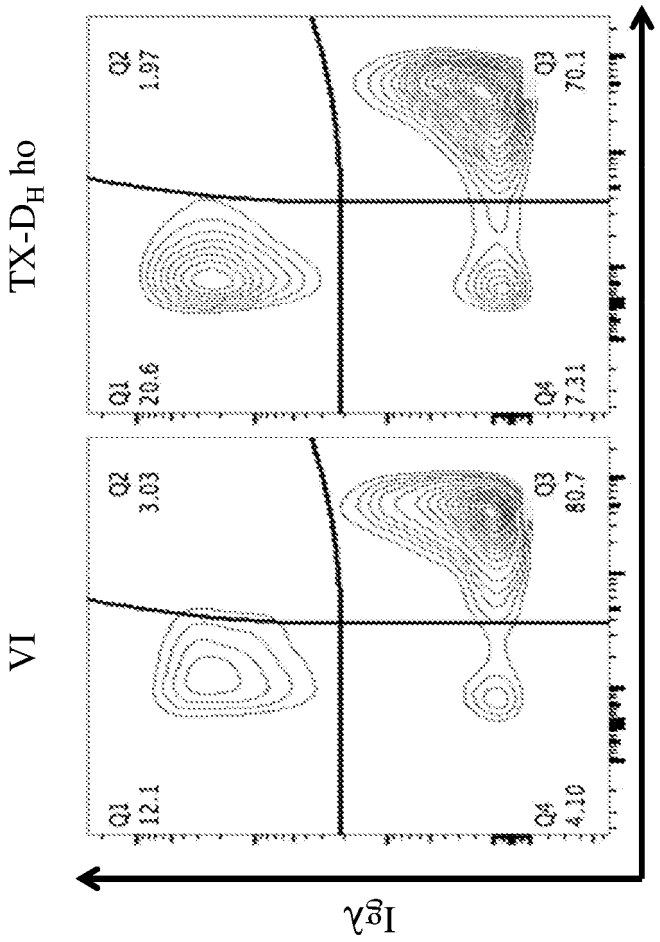

VI
TX-D$_H$ ho
Q1
12.1
Q2
3.03
Q3
80.7
Q4
4.10
Q1
20.6
Q2
1.97
Q3
70.1
Q4
7.31
Igλ
Igκ

Figure 9C

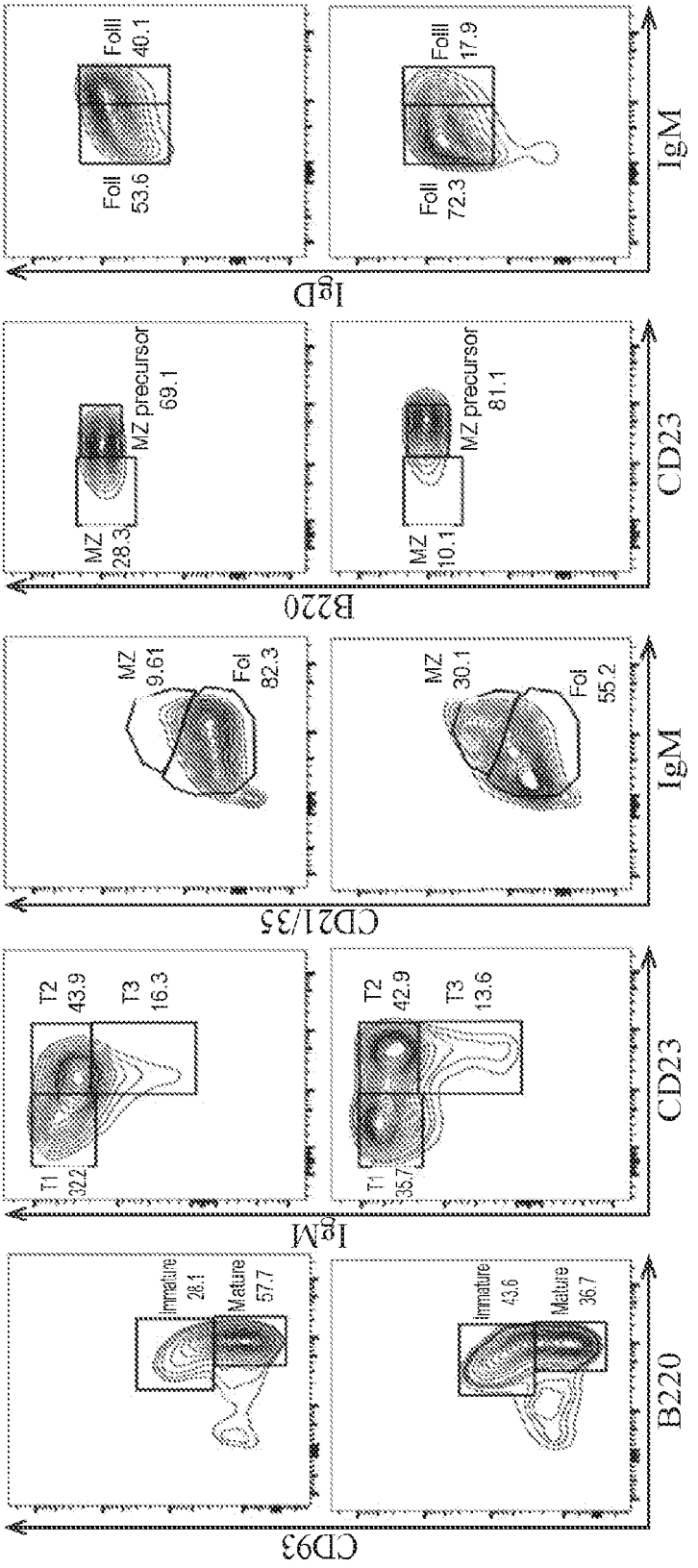

CD93
B220
Immature
26.1
Mature
57.7
Immature
43.6
Mature
36.7
IgM
CD23
T1
32.2
T2
43.9
T3
16.3
T1
35.7
T2
42.9
T3
13.6
CD21/35
IgM
MZ
9.61
Fol
82.3
MZ
30.1
Fol
55.2
B220
CD23
MZ
28.3
MZ precursor
69.1
MZ
10.1
MZ precursor
81.1
IgD
IgM
Foll
53.6
FolII
40.1
Foll
72.3
FolII
17.9

Figure 9D

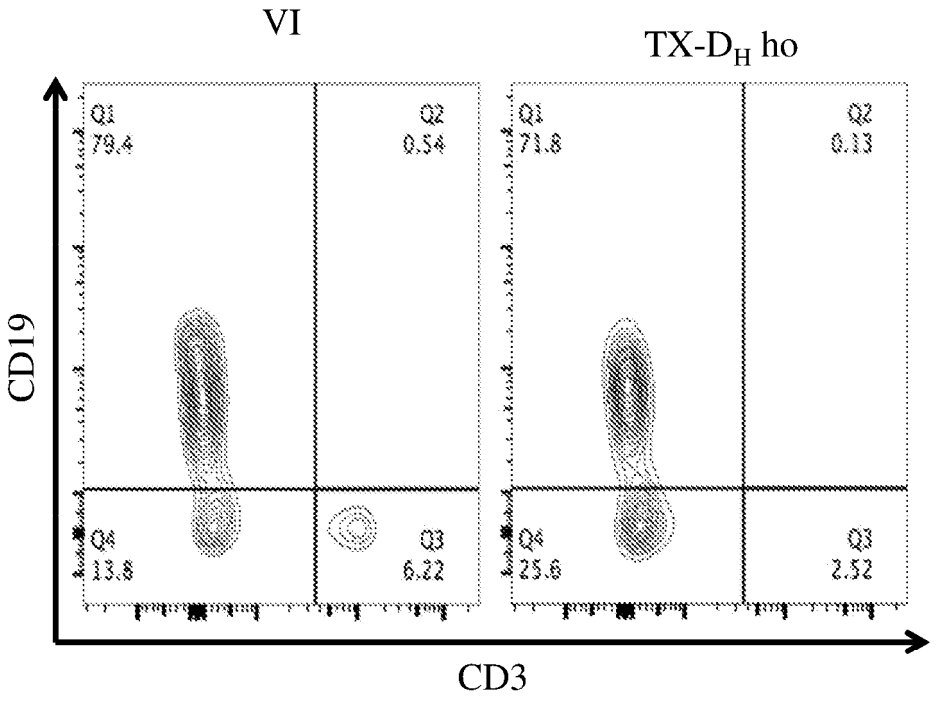


Figure 10A

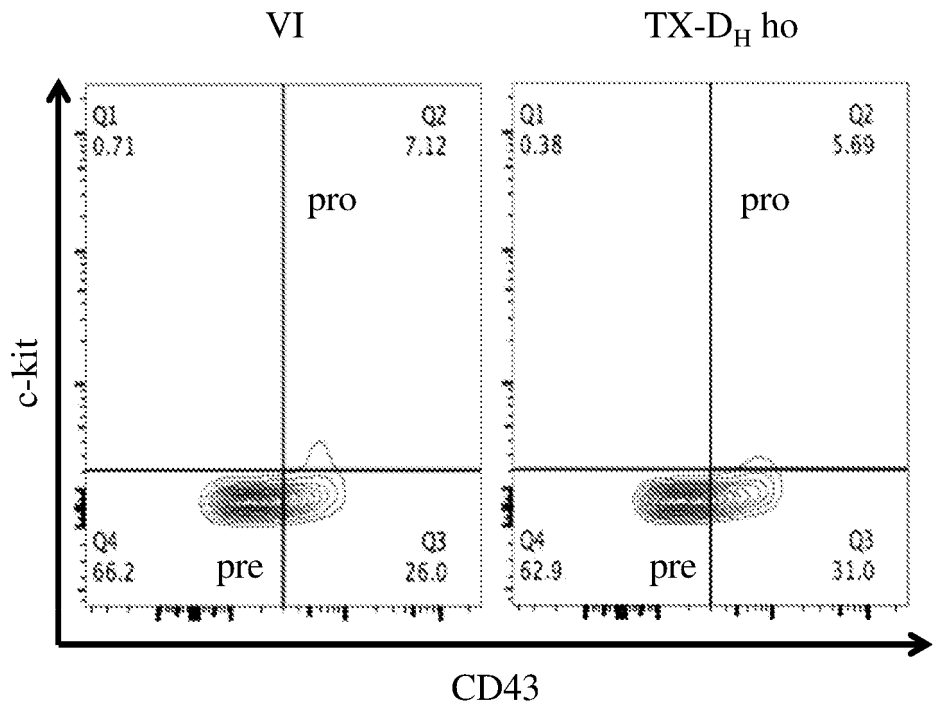


Figure 10B

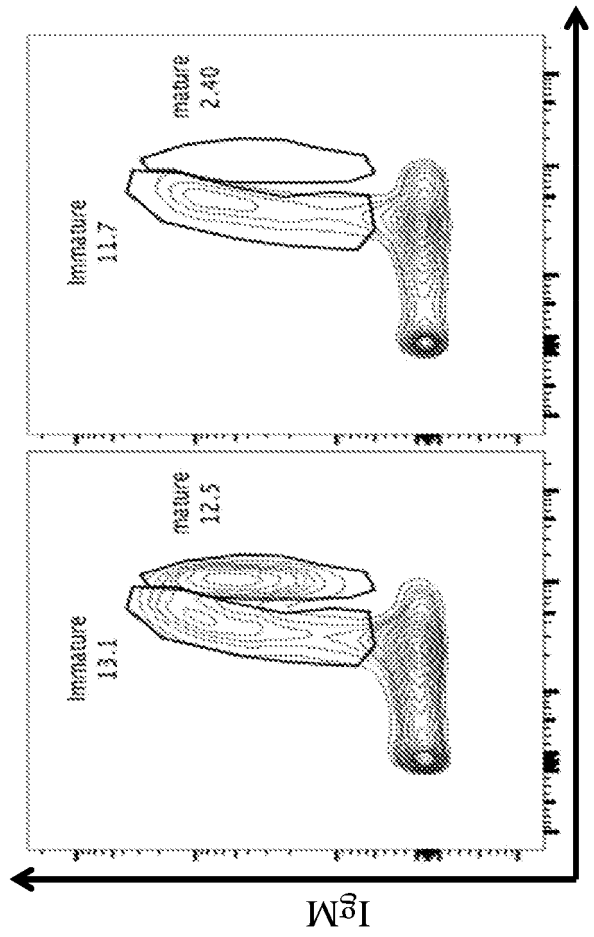

VI
TX-D$_H$ ho
immature
13.1
mature
12.5
immature
11.7
mature
2.40
IgM
B220

Figure 10C

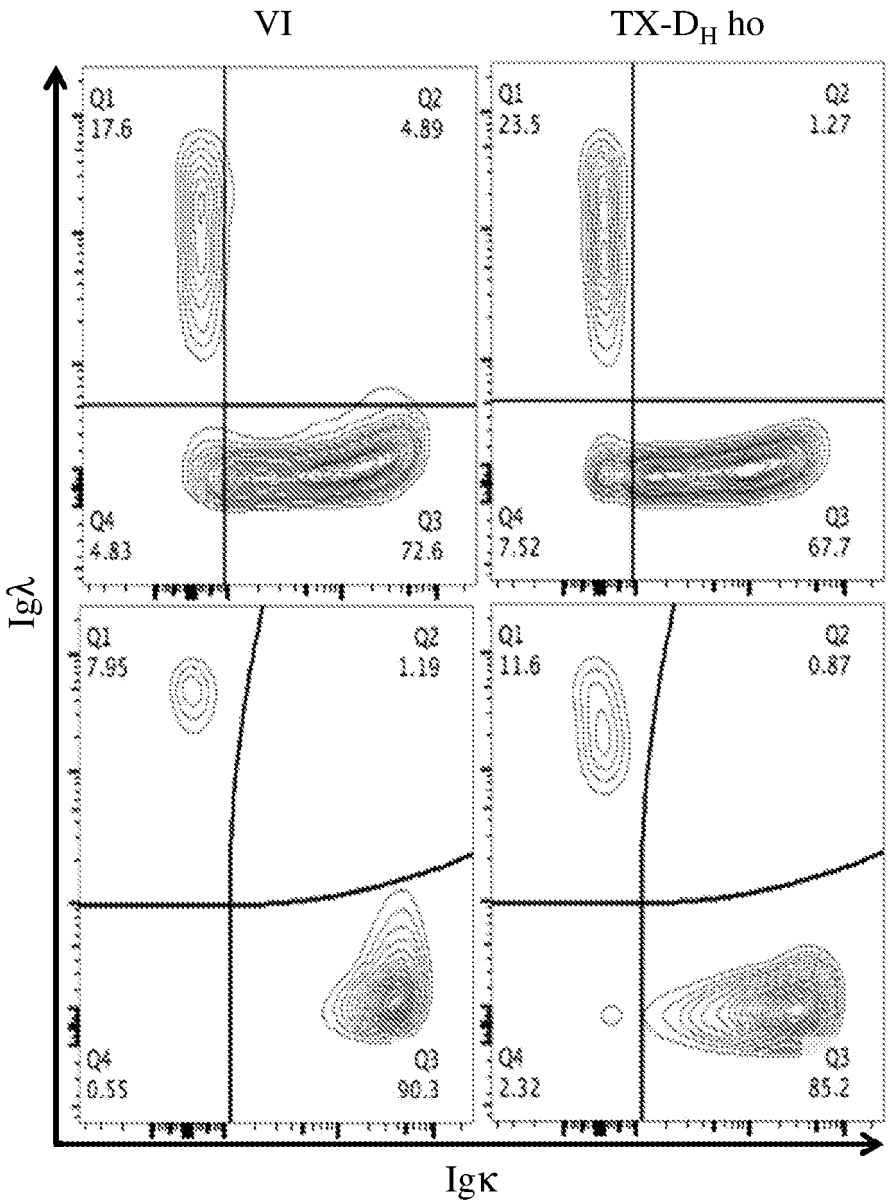

VI
TX-D$_H$ ho
Igλ
Igκ
Q1
17.6
Q2
4.89
Q4
4.83
Q3
72.6
Q1
23.5
Q2
1.27
Q4
7.52
Q3
67.7
Q1
7.95
Q2
1.19
Q4
0.55
Q3
90.3
Q1
11.6
Q2
0.87
Q4
2.32
Q3
85.2

Figure 10D

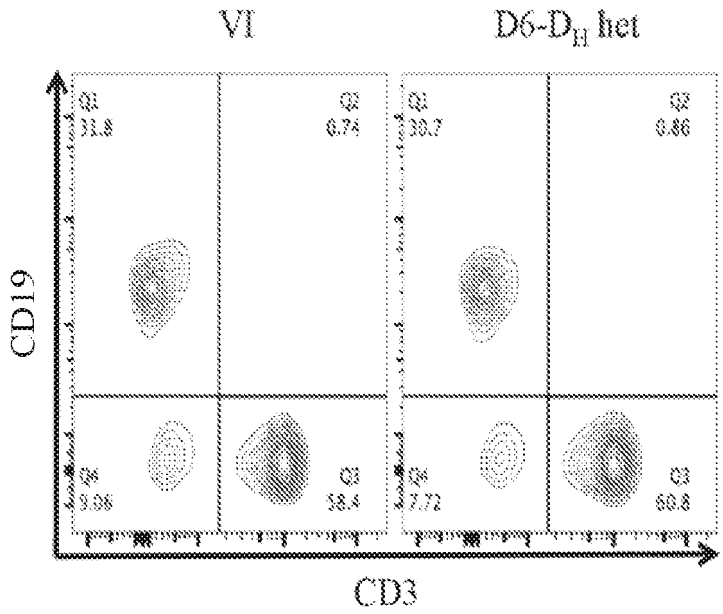


Figure 11A

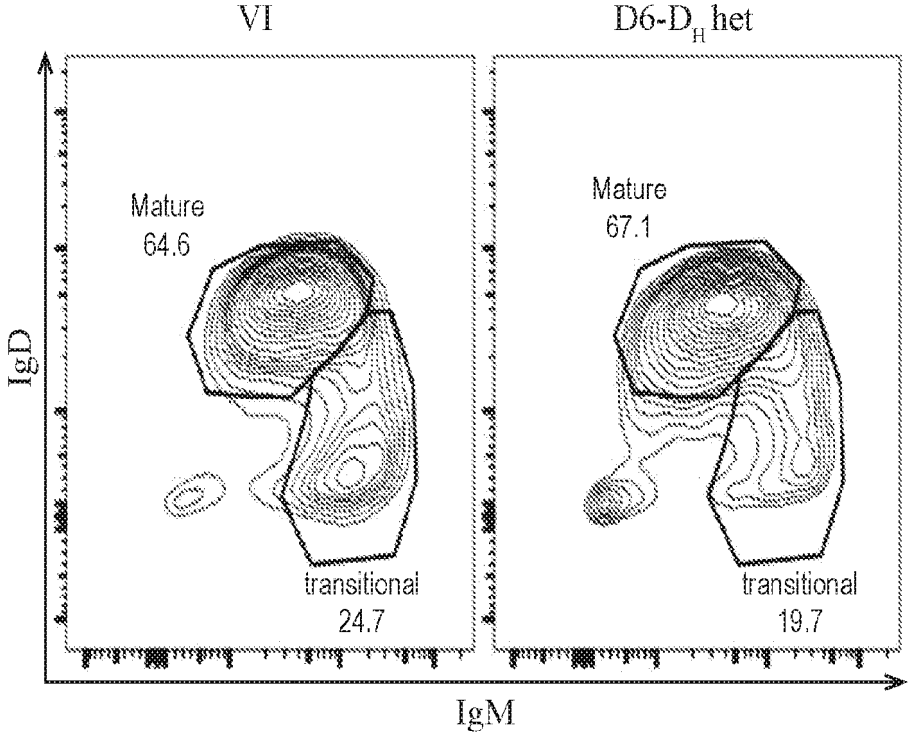


Figure 11B

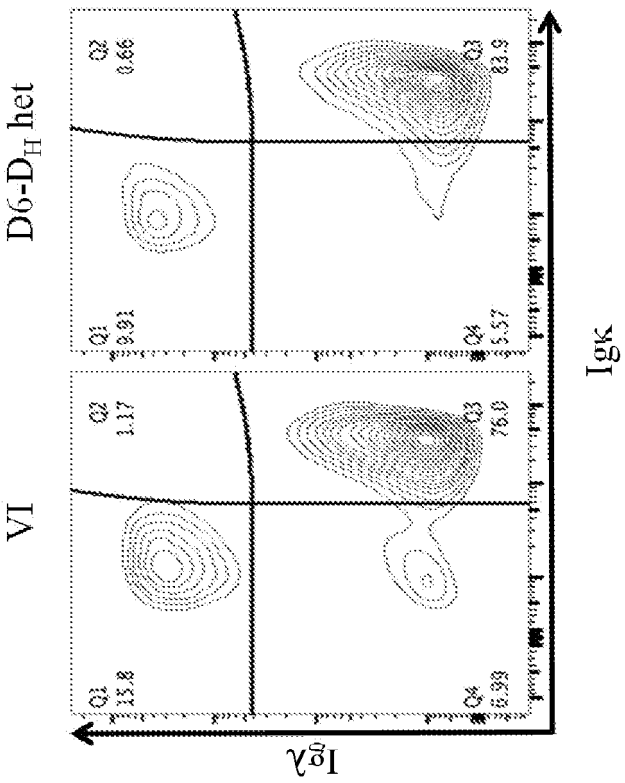

VI
D6-D$_H$ het
Q1
0.91
Q2
0.66
Q3
83.9
Q4
5.57
Q1
13.8
Q2
1.17
Q3
76.0
Q4
8.98
Igλ
Igκ

Figure 11C

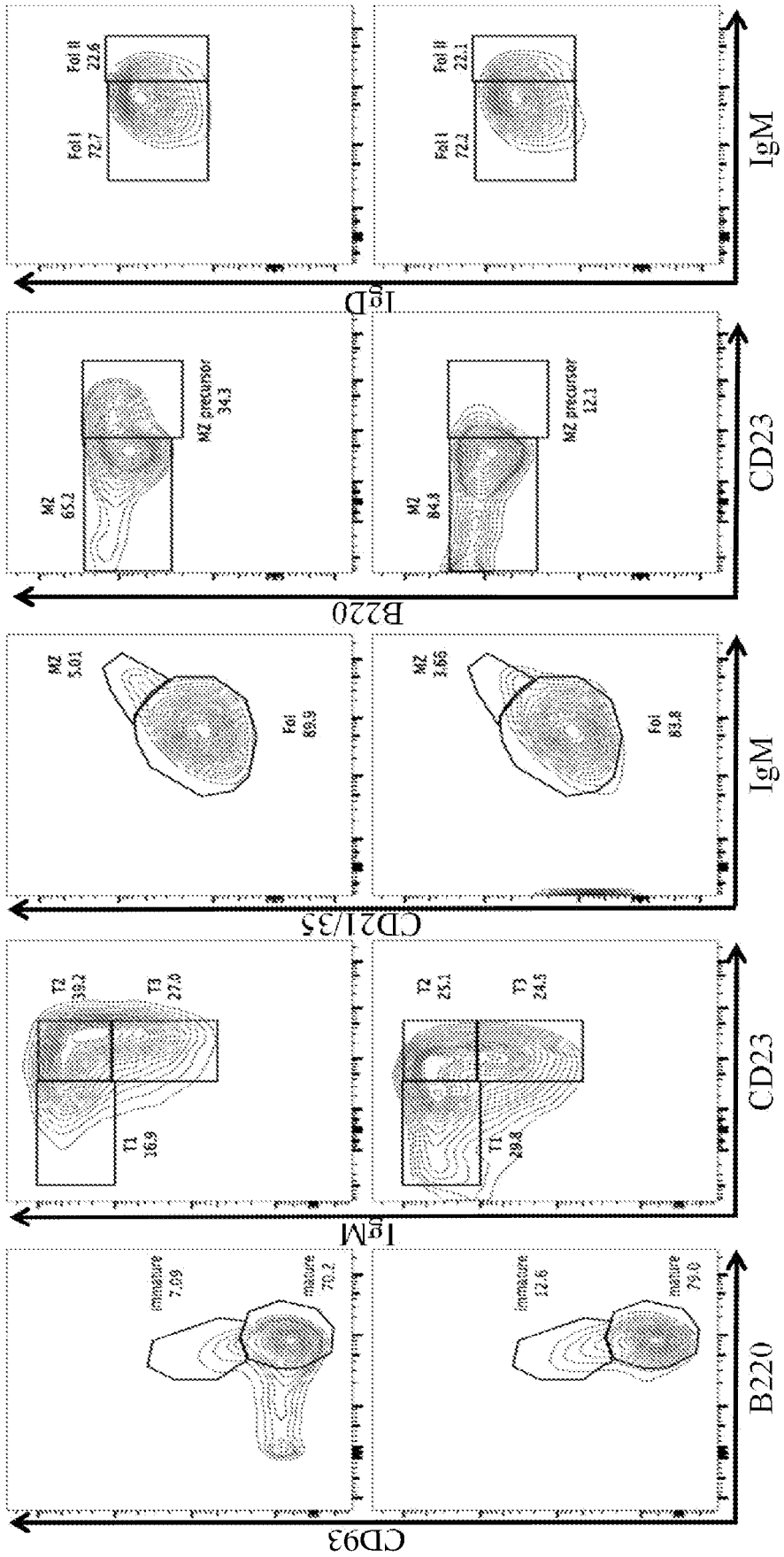

CD93
B220
immature
7.09
mature
70.2
immature
12.6
mature
79.0
IgM
CD23
T2
39.2
T3
27.0
T1
16.9
T2
25.1
T3
24.3
T1
28.8
CD21/35
IgM
MZ
5.01
Fol
69.9
MZ
3.66
Fol
83.8
B220
CD23
MZ
65.2
MZ precursor
34.3
MZ
84.8
MZ precursor
12.3
IgD
IgM
Fol II
22.6
Fol I
72.7
Fol II
22.1
Fol I
72.2

Figure 11D

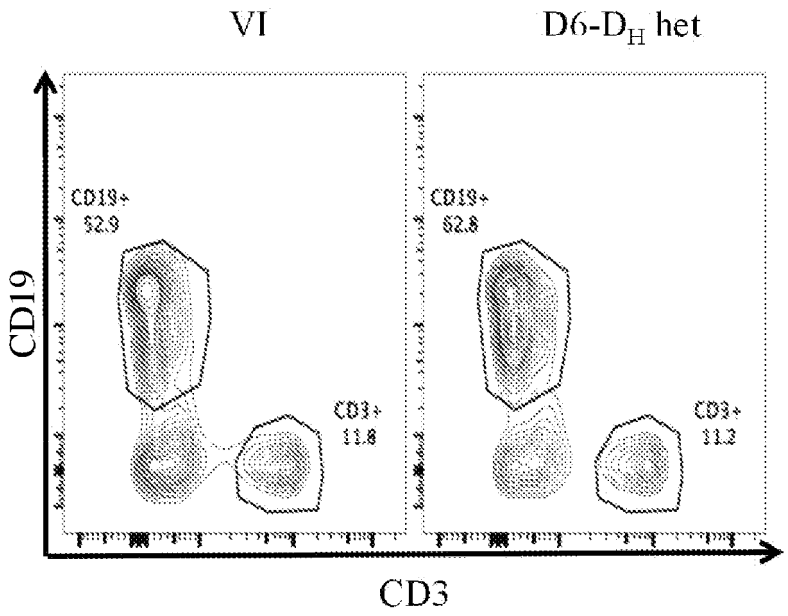


Figure 12A

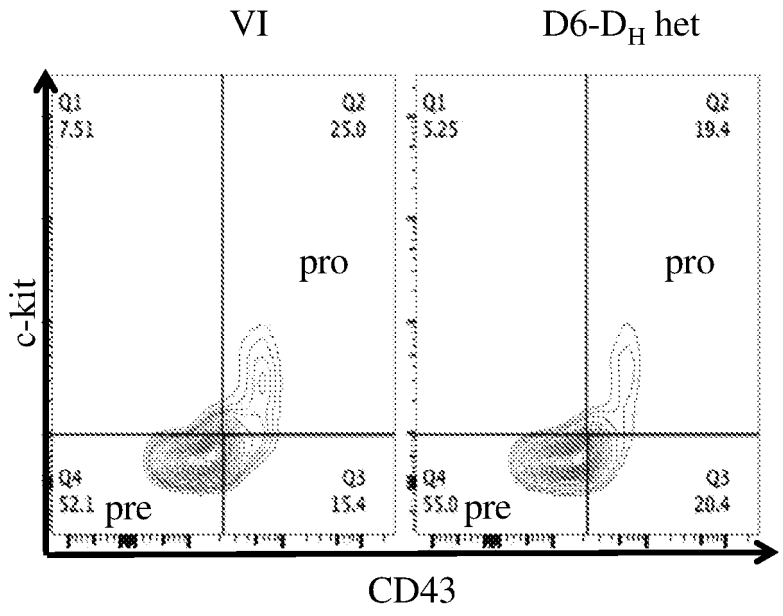


Figure 12B

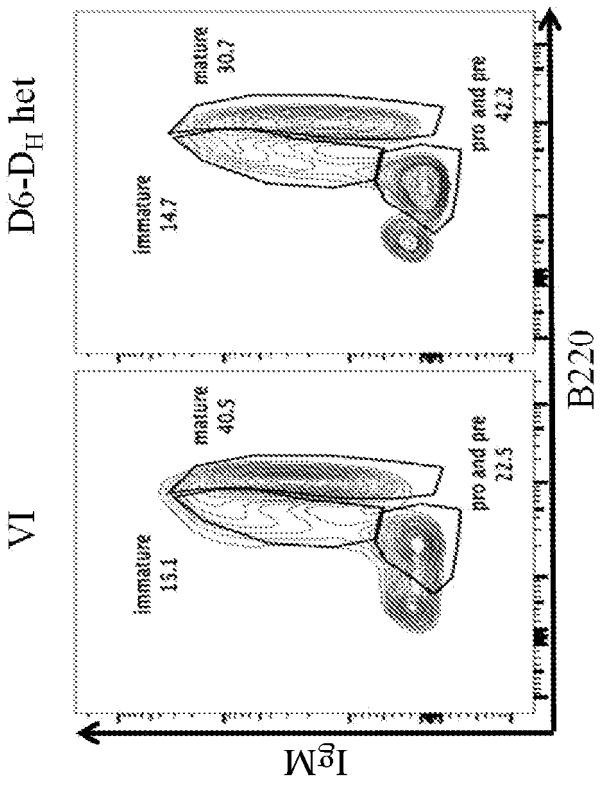

VI
D6-D$_H$ het
immature
13.1
mature
40.5
pro and pre
22.5
immature
14.7
mature
30.7
pro and pre
42.2
IgM
B220

Figure 12C

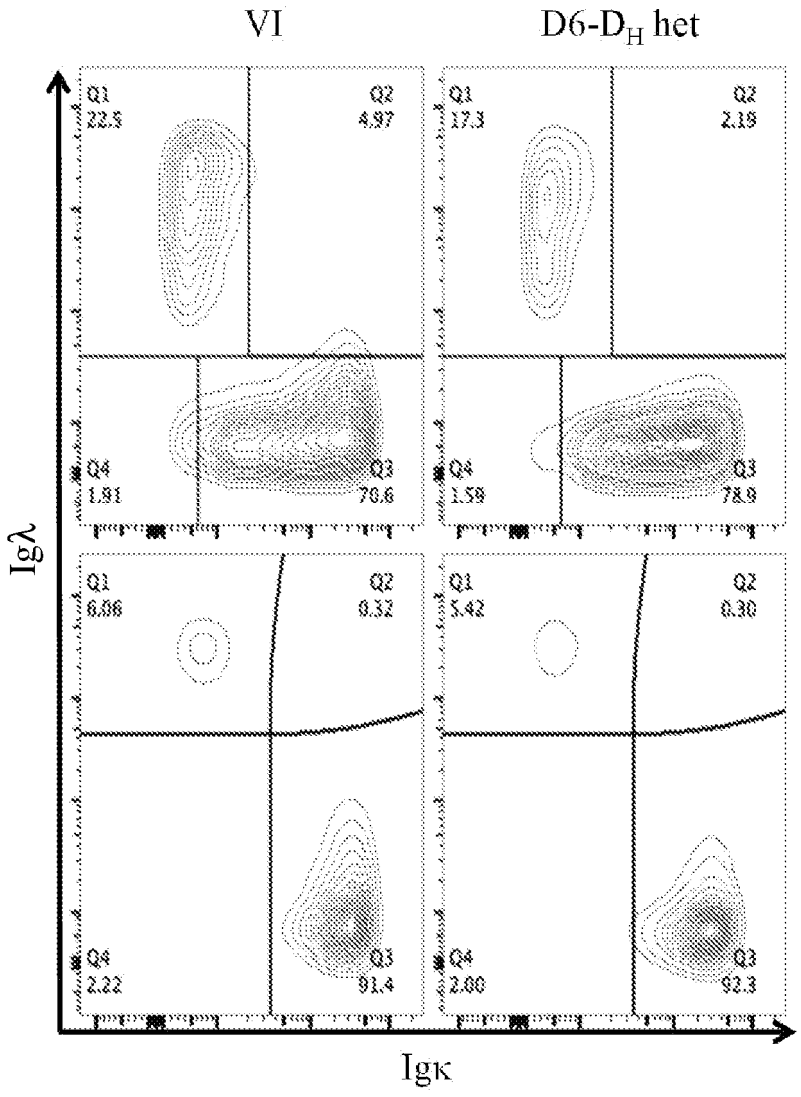

VI
D6-D$_H$ het
Q1
22.5
Q2
4.97
Q4
1.91
Q3
70.6
Q1
17.3
Q2
2.18
Q4
1.59
Q3
78.9
Q1
6.08
Q2
0.32
Q4
2.22
Q3
91.4
Q1
5.42
Q2
0.30
Q4
2.00
Q3
92.3
Igλ
Igκ

Figure 12D

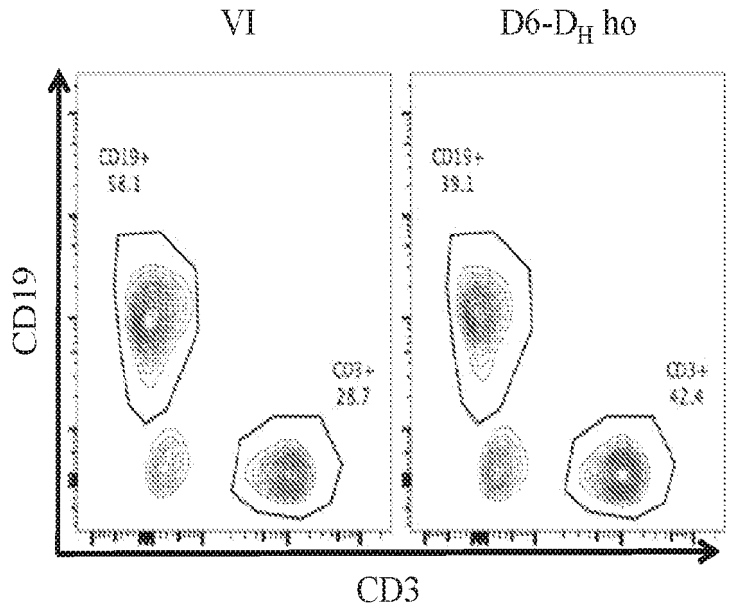


Figure 13A

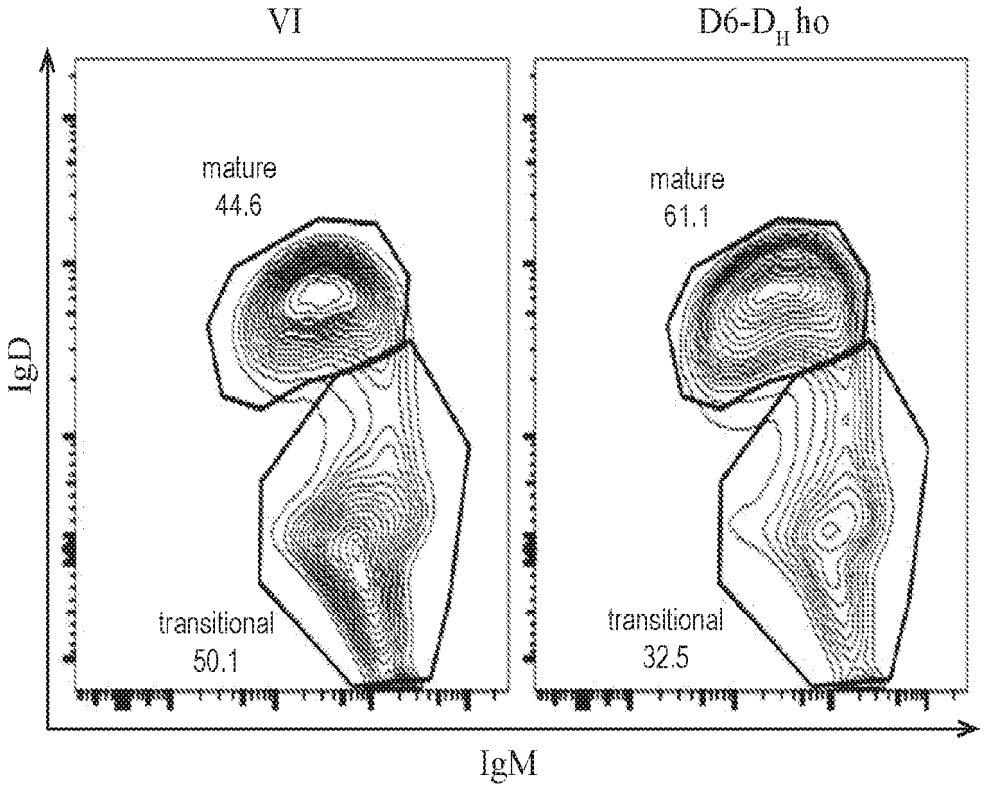


Figure 13B

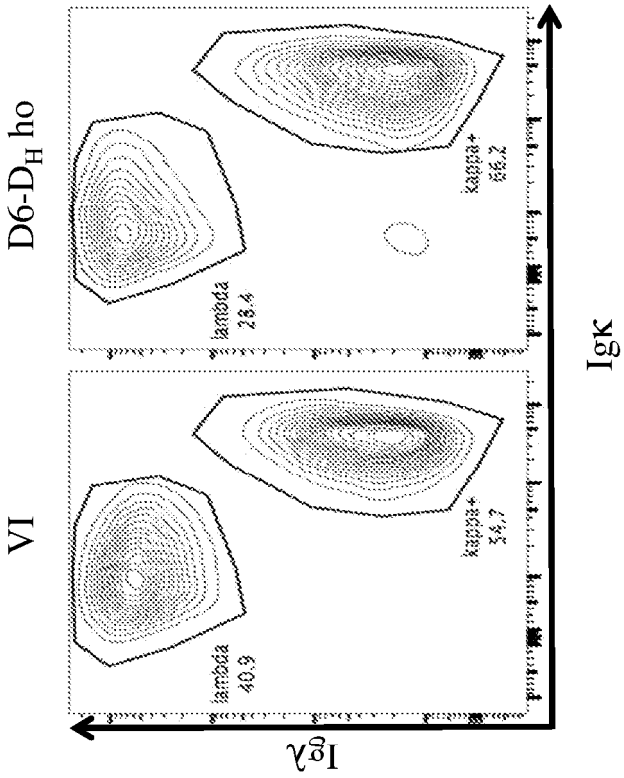


Figure 13C

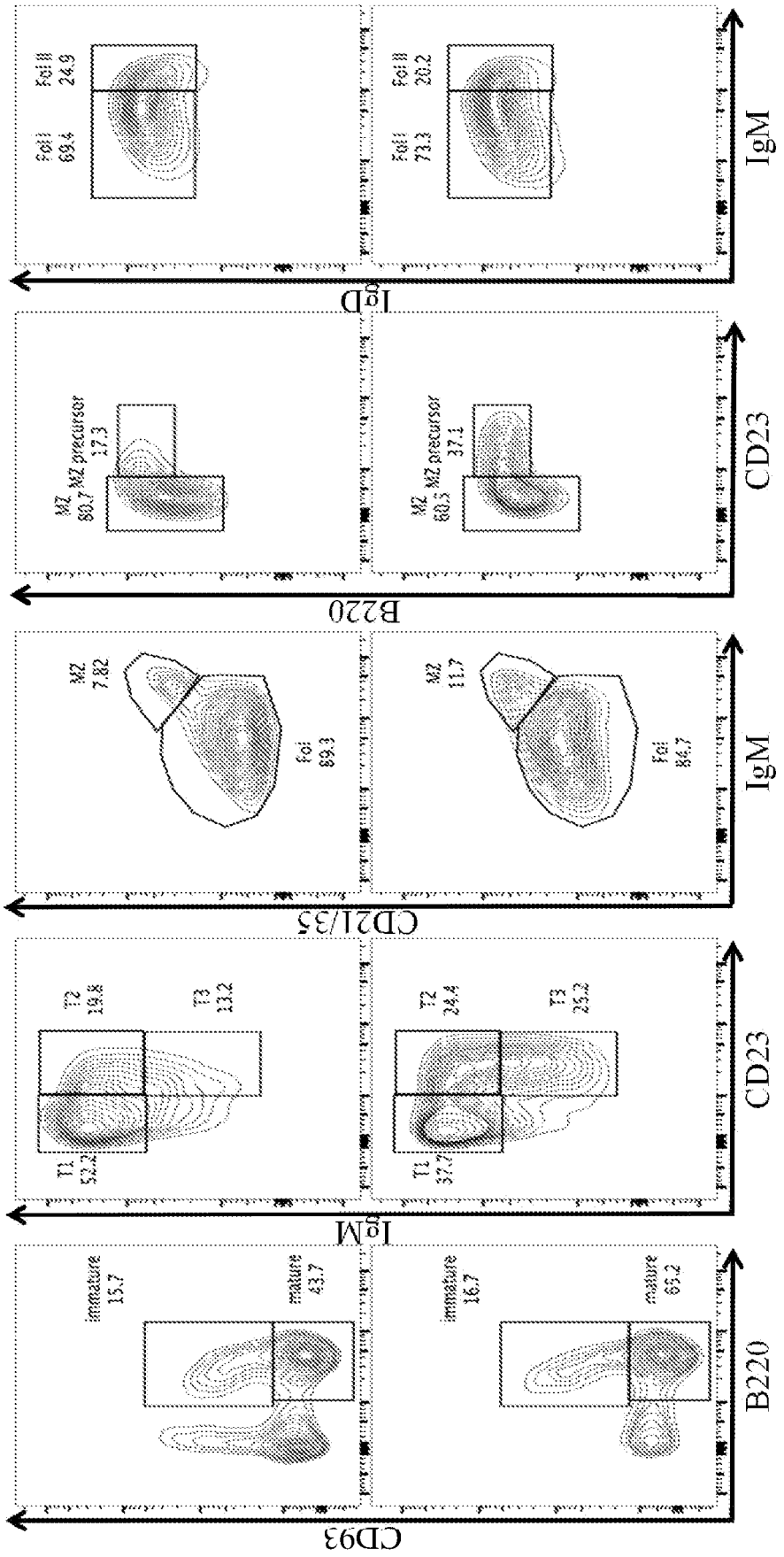

CD93
B220
immature
15.7
mature
43.7
immature
16.7
mature
65.2
IgM
CD23
T1
52.2
T2
19.8
T3
13.2
T1
37.7
T2
24.4
T3
25.2
CD21/35
IgM
MZ
7.82
Fol
89.3
MZ
11.7
Fol
84.7
B220
CD23
MZ
80.7
MZ precursor
17.3
MZ
60.5
MZ precursor
37.1
IgD
IgM
Fol I
69.4
Fol II
24.9
Fol I
73.3
Fol II
20.2

Figure 13D

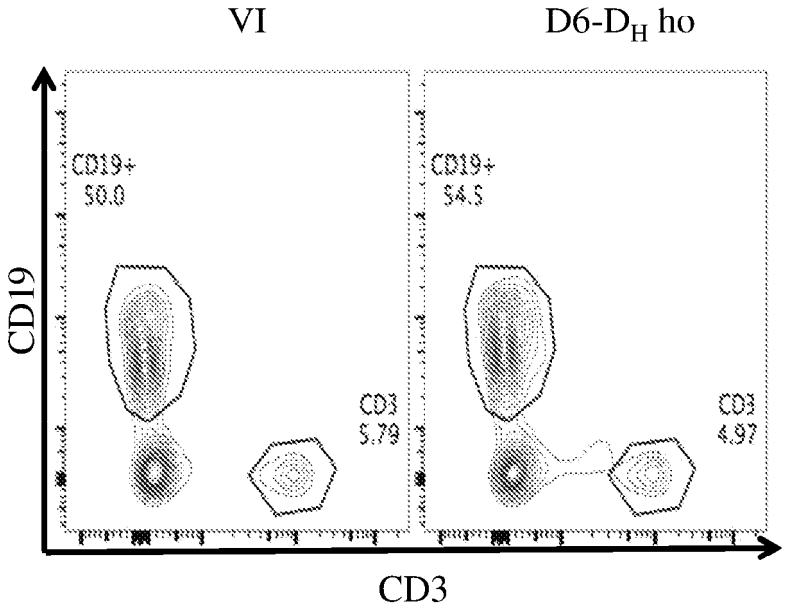


Figure 14A

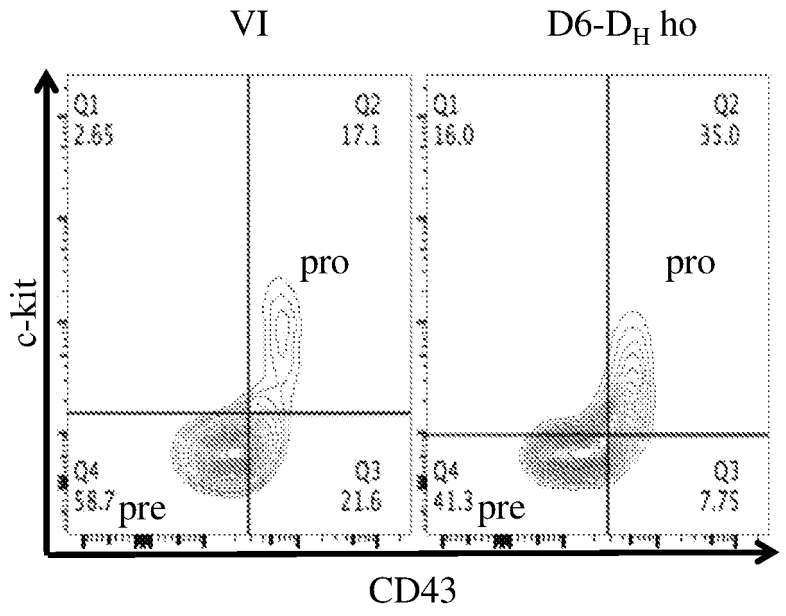


Figure 14B

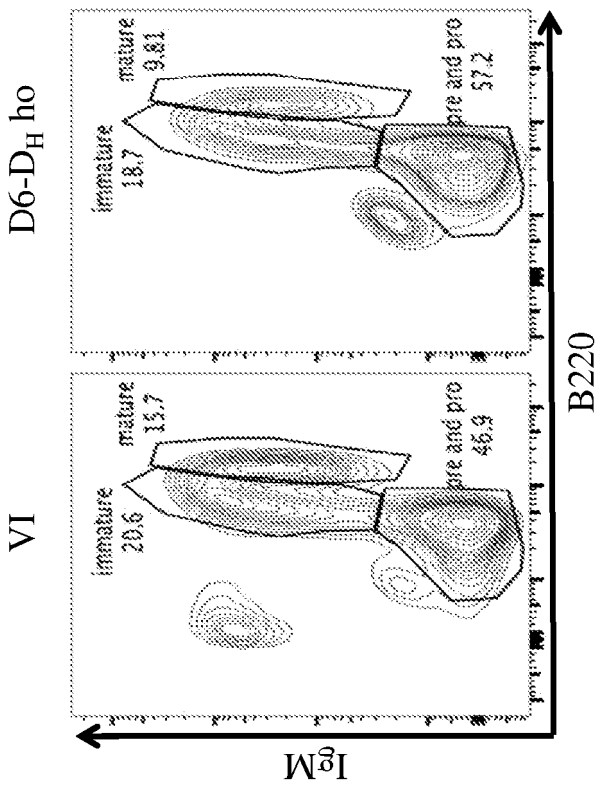

VI
D6-D$_H$ ho
immature
20.6
mature
15.7
pre and pro
46.9
immature
18.7
mature
9.81
pre and pro
57.2
IgM
B220

Figure 14C

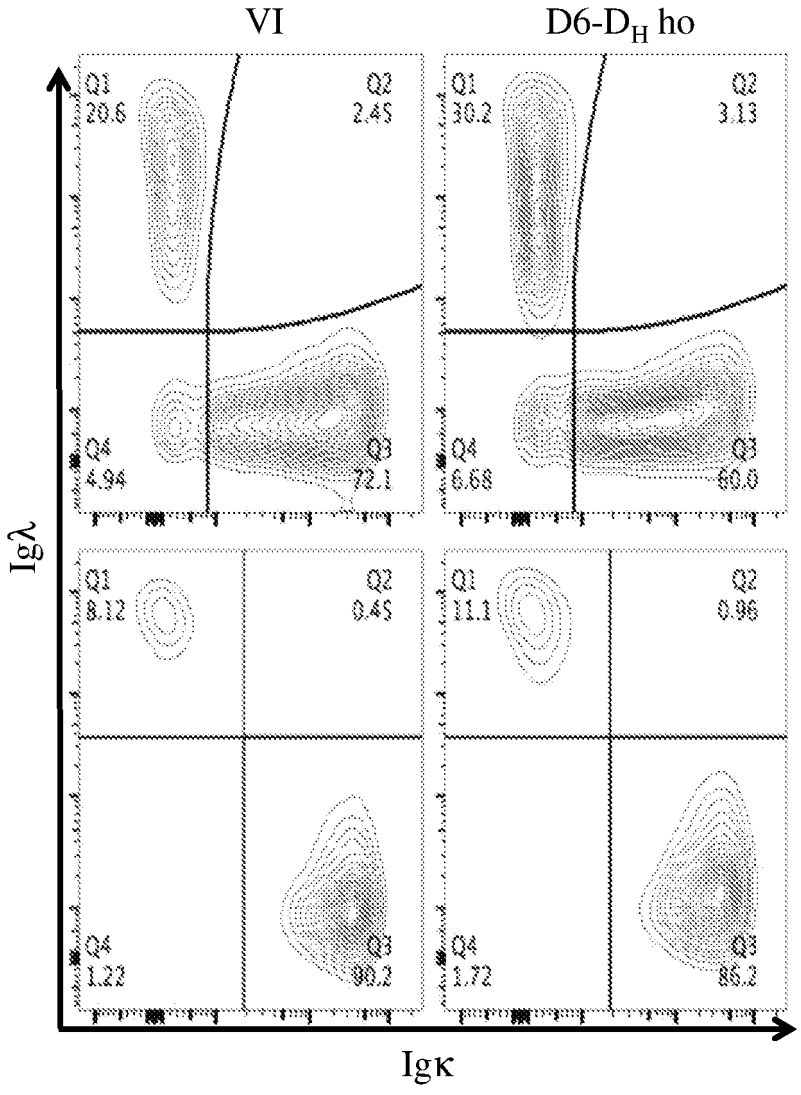

VI
D6-D$_H$ ho
Q1
20.6
Q2
2.45
Q4
4.94
Q3
72.1
Q1
30.2
Q2
3.13
Q4
6.68
Q3
60.0
Q1
8.12
Q2
0.45
Q4
1.22
Q3
90.2
Q1
11.1
Q2
0.96
Q4
1.72
Q3
86.2
Igλ
Igκ

Figure 14D

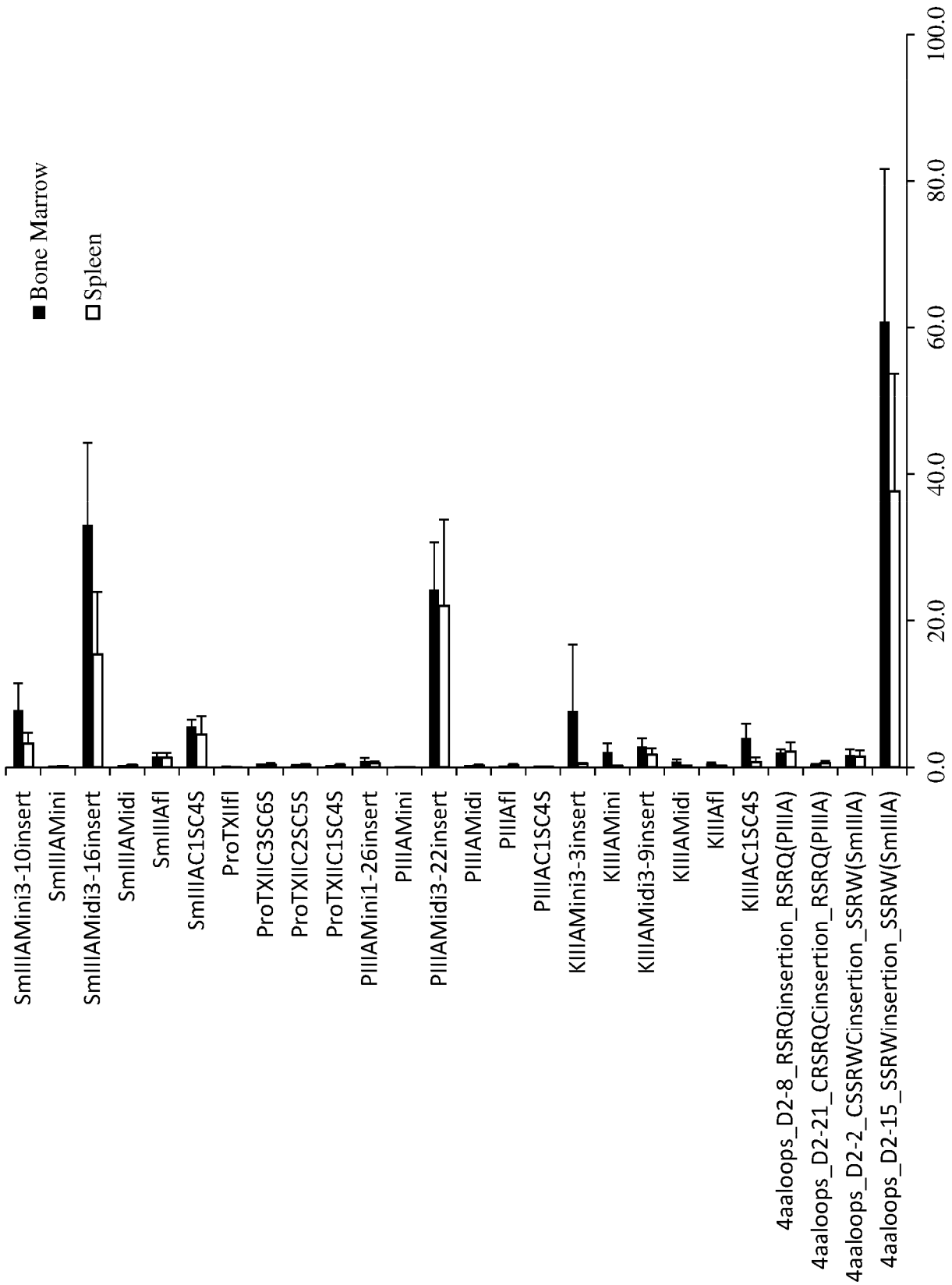

Bone Marrow
Spleen
SmIIIAMini3-10insert
SmIIIAMini
SmIIIAMidi3-16insert
SmIIIAMidi
SmIIIAfl
SmIIIAC1SC4S
ProTXIIfl
ProTXIIC3SC6S
ProTXIIC2SC5S
ProTXIIC1SC4S
PIIIAMini1-26insert
PIIIAMini
PIIIAMidi3-22insert
PIIIAMidi
PIIIAfl
PIIIAC1SC4S
KIIIAMini3-3insert
KIIIAMini
KIIIAMidi3-9insert
KIIIAMidi
KIIIAfl
KIIIAC1SC4S
4aaloops_D2-8_RSRQinsertion_RSRQ(PIIIA)
4aaloops_D2-21_CRSRQCinsertion_RSRQ(PIIIA)
4aaloops_D2-2_CSSRWCinsertion_SSRW(SmIIIA)
4aaloops_D2-15_SSRWinsertion_SSRW(SmIIIA)
0.0
20.0
40.0
60.0
80.0
100.0
%Sequences

Figure 15

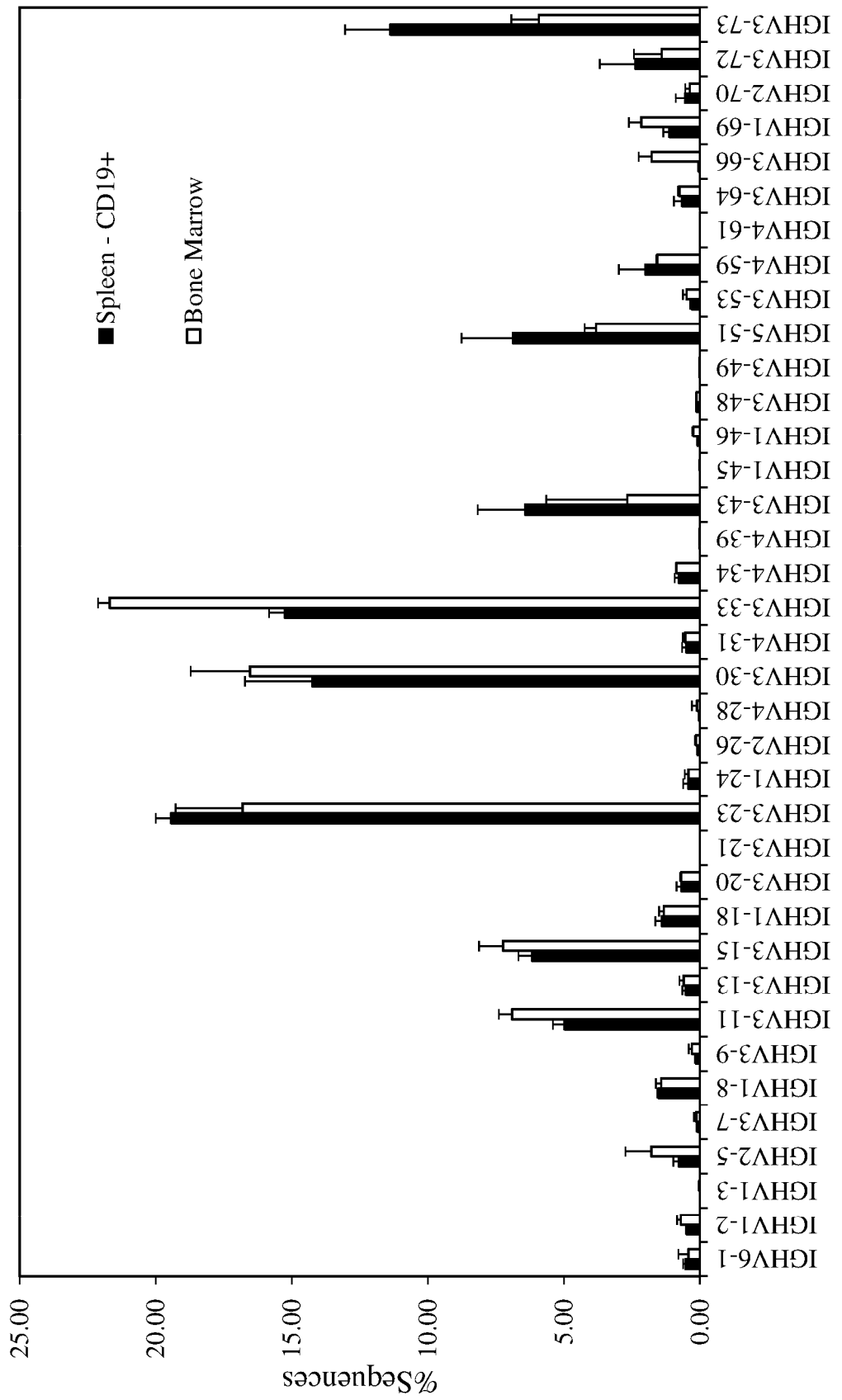

%Sequences
0.00
5.00
10.00
15.00
20.00
25.00
Spleen - CD19+
Bone Marrow
IGHV6-1
IGHV1-2
IGHV1-3
IGHV2-5
IGHV3-7
IGHV1-8
IGHV3-9
IGHV3-11
IGHV3-13
IGHV3-15
IGHV1-18
IGHV3-20
IGHV3-21
IGHV3-23
IGHV1-24
IGHV2-26
IGHV4-28
IGHV3-30
IGHV4-31
IGHV3-33
IGHV4-34
IGHV4-39
IGHV3-43
IGHV1-45
IGHV1-46
IGHV3-48
IGHV3-49
IGHV5-51
IGHV3-53
IGHV4-59
IGHV4-61
IGHV3-64
IGHV3-66
IGHV1-69
IGHV2-70
IGHV3-72
IGHV3-73

Figure 16

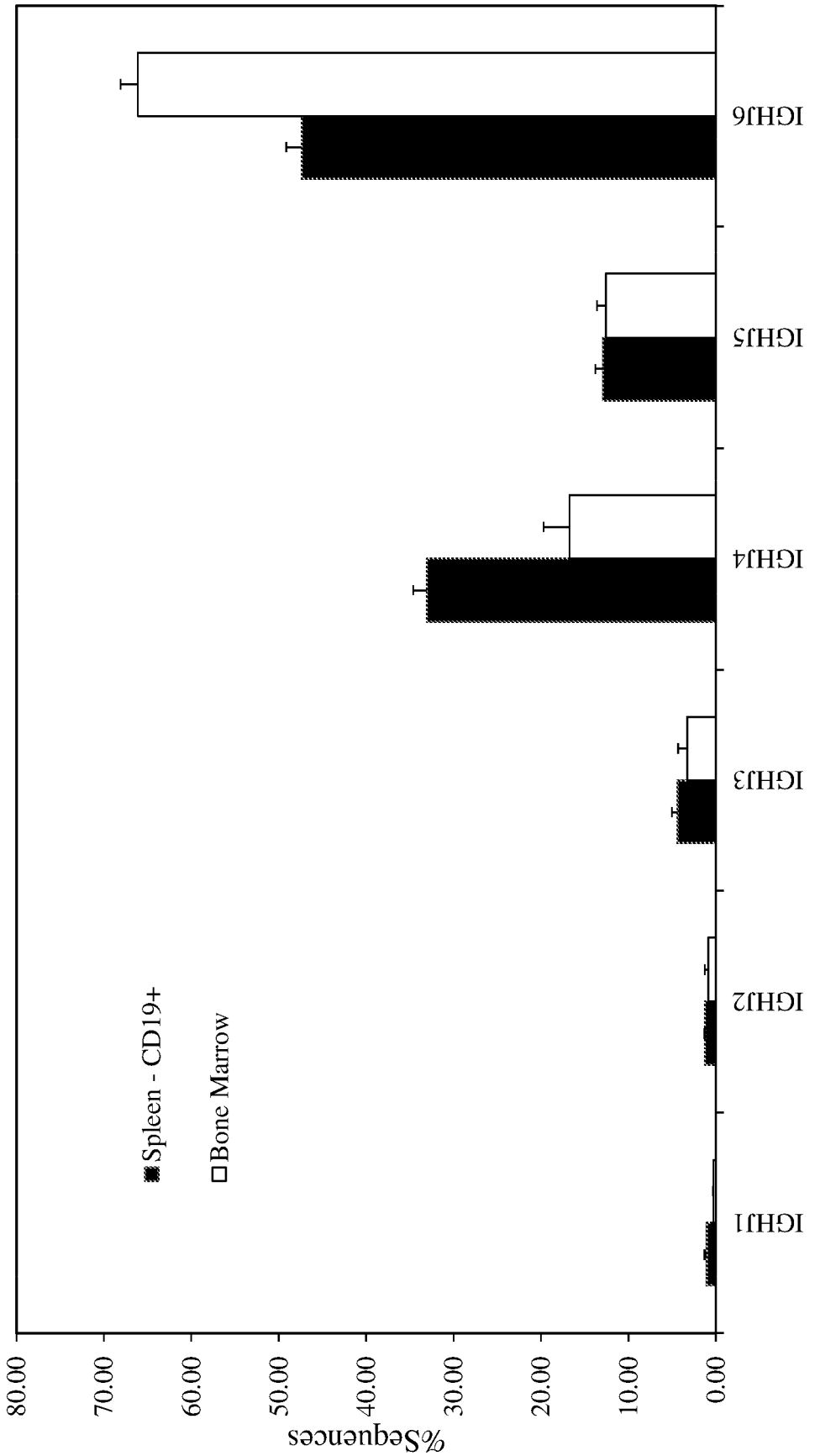

80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
%Sequences
Spleen - CD19+
Bone Marrow
IGHJ1
IGHJ2
IGHJ3
IGHJ4
IGHJ5
IGHJ6

Figure 17

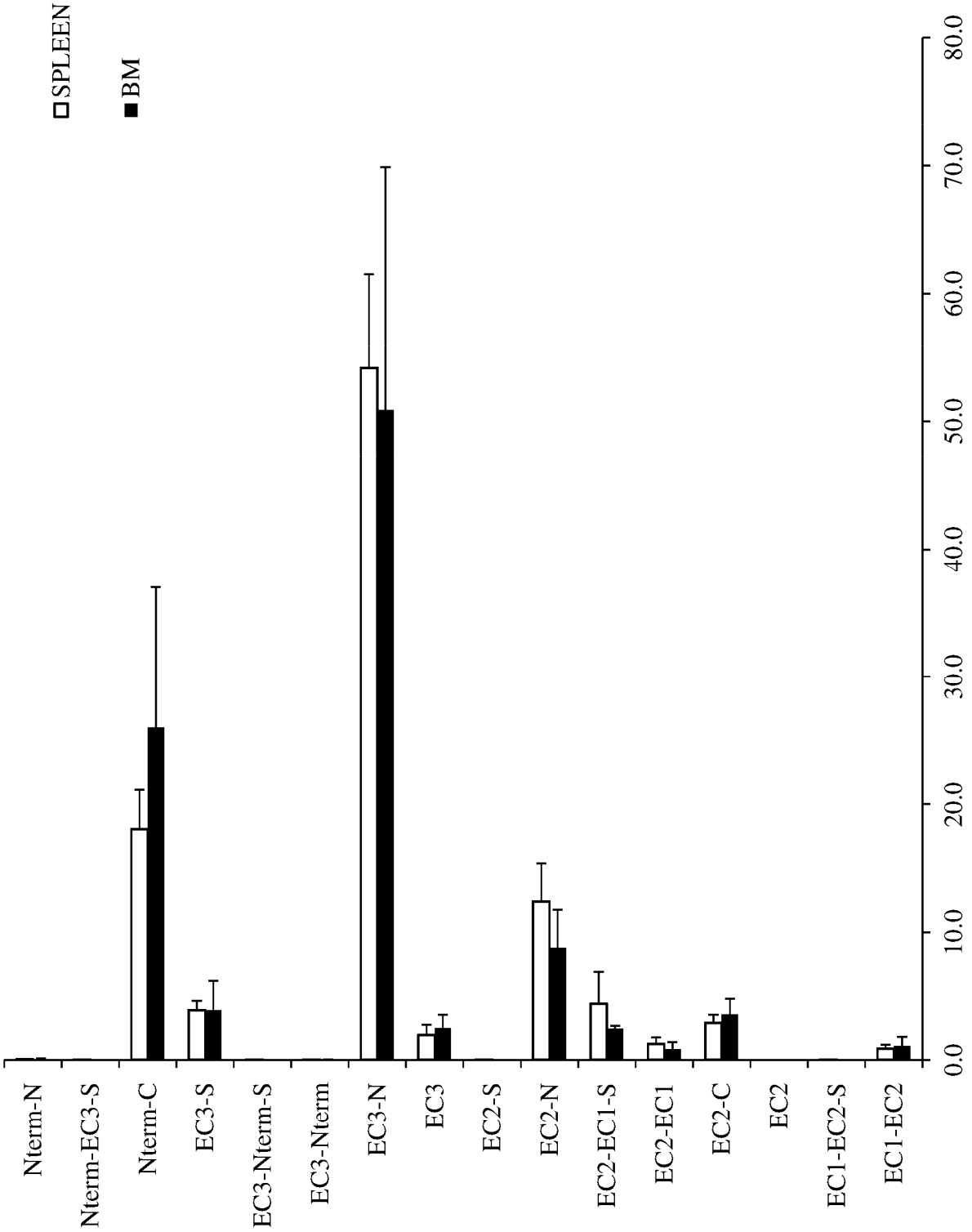

SPLEEN
BM
Nterm-N
Nterm-EC3-S
Nterm-C
EC3-S
EC3-Nterm-S
EC3-Nterm
EC3-N
EC3
EC2-S
EC2-N
EC2-EC1-S
EC2-EC1
EC2-C
EC2
EC1-EC2-S
EC1-EC2
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
%Sequences

Figure 18

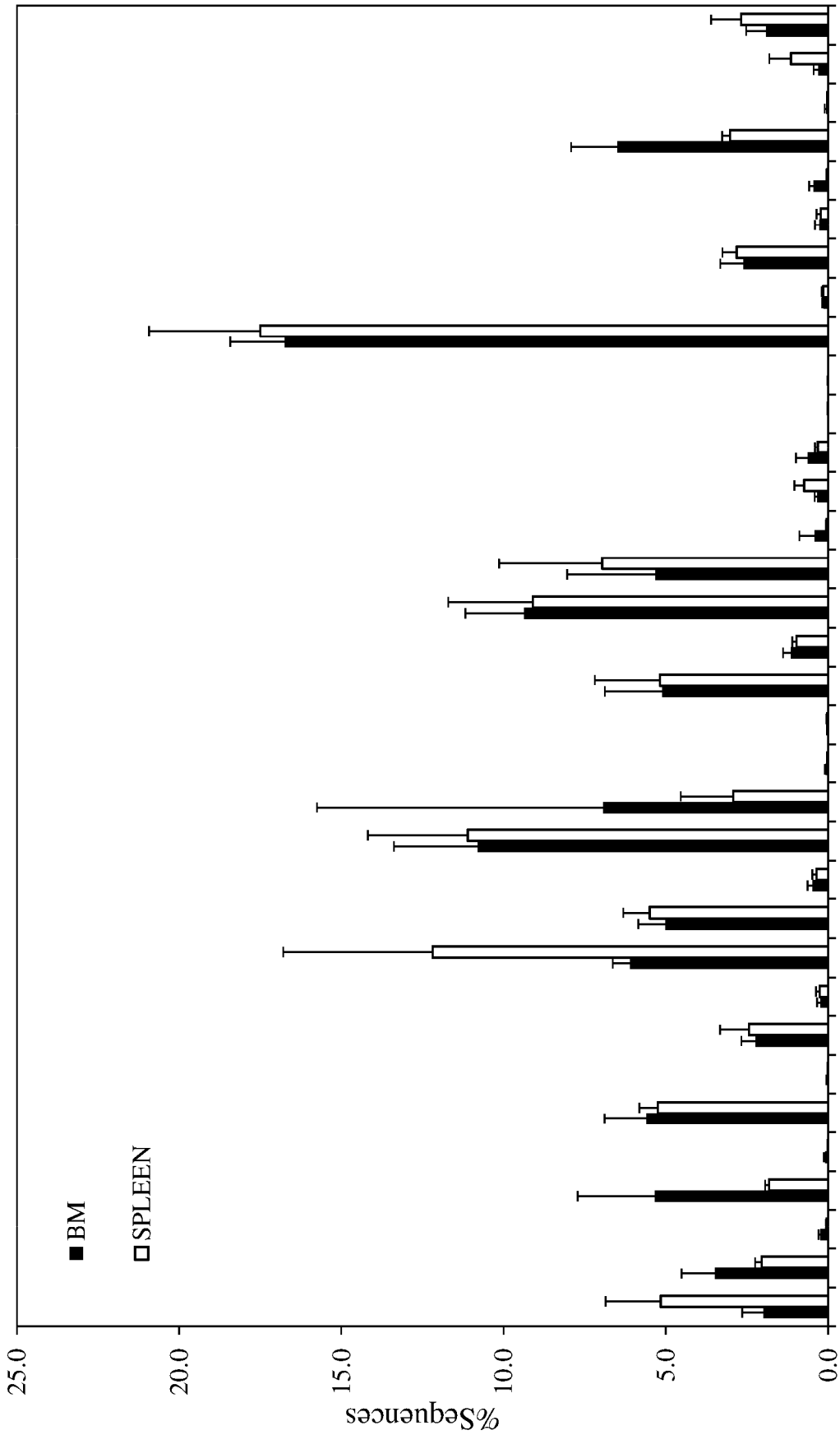

%Sequences
0.0
5.0
10.0
15.0
20.0
25.0
BM
SPLEEN
IGHV6-1
IGHV1-2
IGHV1-3
IGHV2-5
IGHV3-7
IGHV1-8
IGHV3-9
IGHV3-11
IGHV3-13
IGHV3-15
IGHV1-18
IGHV3-20
IGHV3-23
IGHV1-24
IGHV2-26
IGHV4-28
IGHV3-30
IGHV4-31
IGHV3-33
IGHV4-34
IGHV4-39
IGHV3-43
IGHV1-46
IGHV3-48
IGHV3-49
IGHV5-51
IGHV3-53
IGHV4-59
IGHV3-64
IGHV3-66
IGHV1-69
IGHV2-70
IGHV3-72
IGHV3-73

Figure 19

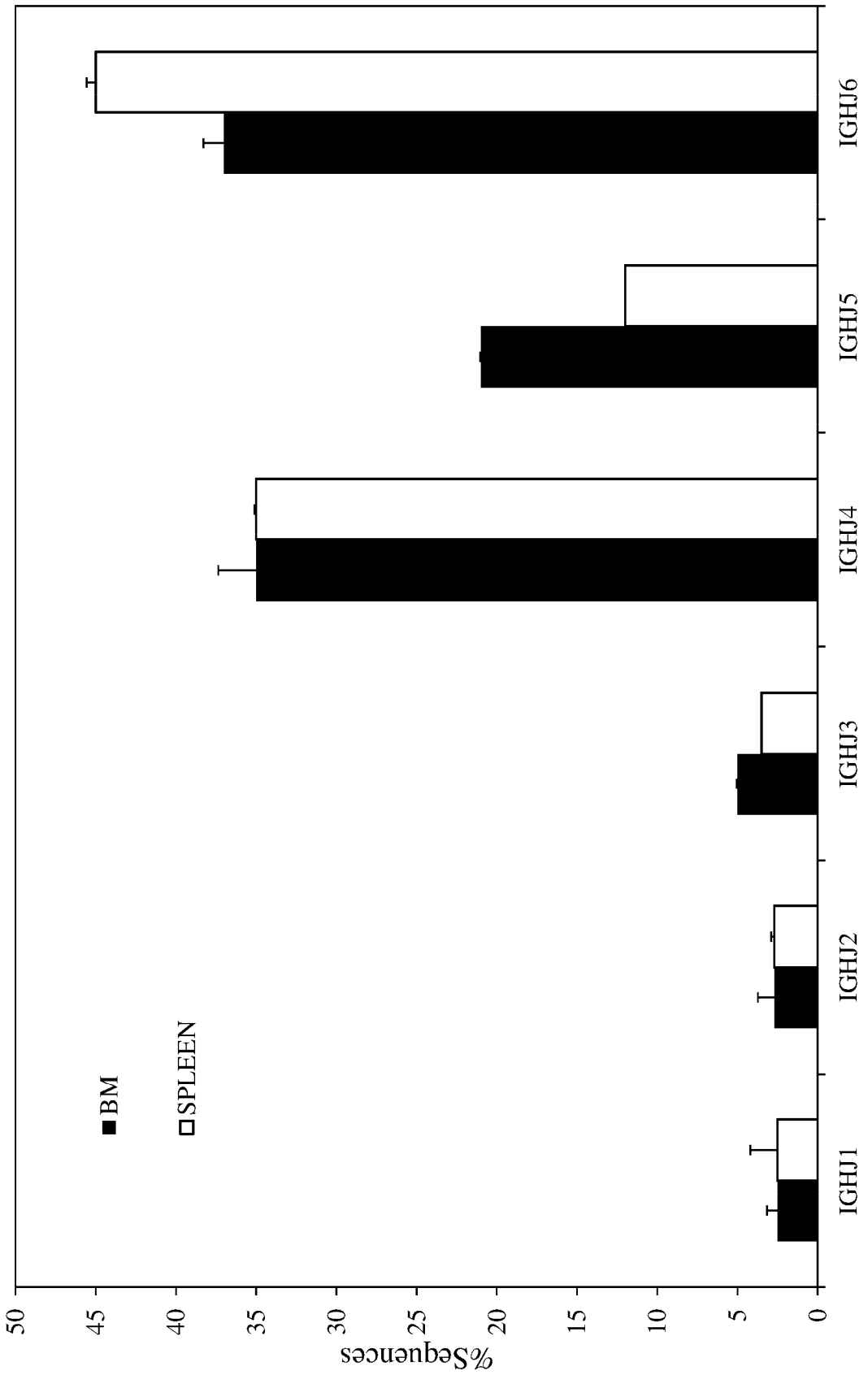

50
45
40
35
30
25
20
15
10
5
0
%Sequences
BM
SPLEEN
IGHJ1
IGHJ2
IGHJ3
IGHJ4
IGHJ5
IGHJ6

Figure 20

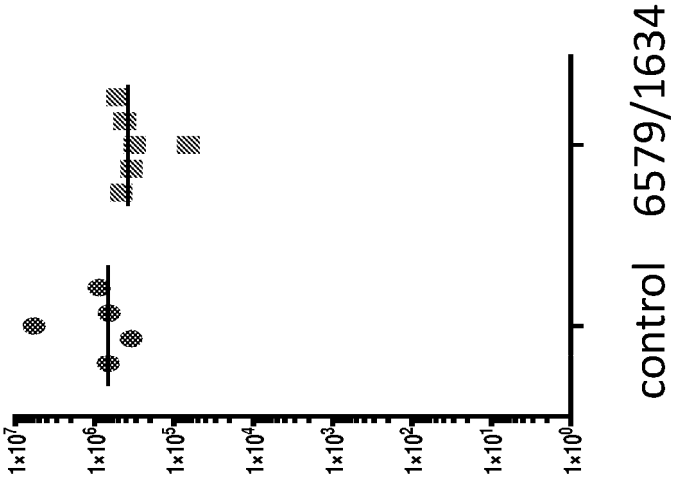


Figure 21