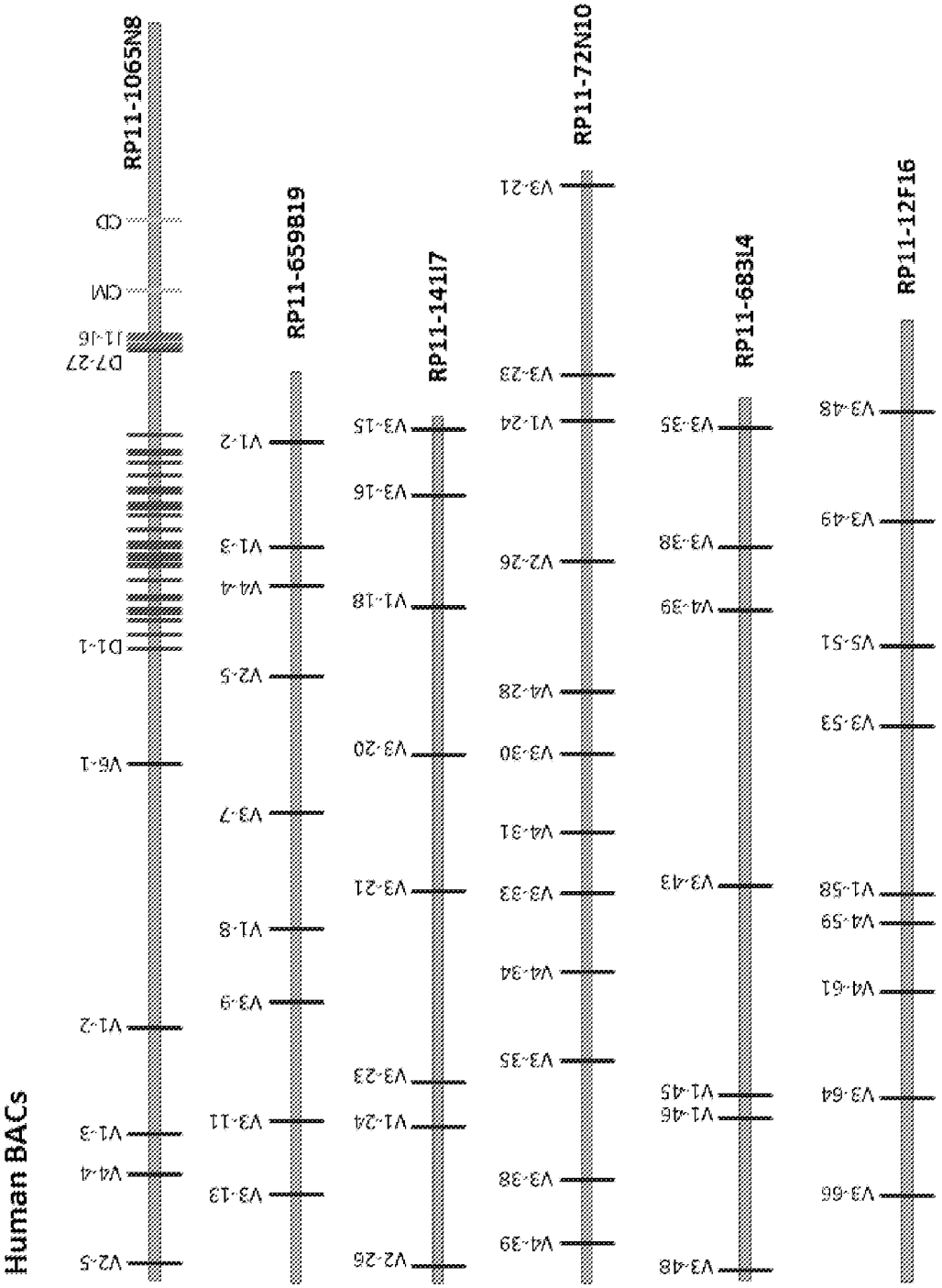

Human BACs
RP11-1065N8
CD
CM
J1-J6
D7-27
D1-1
V6-1
V1-2
V1-3
V4-4
V2-5
RP11-659B19
V1-2
V1-3
V4-4
V2-5
V3-7
V1-8
V3-9
V3-11
V3-13
RP11-1411I7
V3-15
V3-16
V1-18
V3-20
V3-21
V3-23
V1-24
V2-26
RP11-72N10
V3-21
V3-23
V1-24
V2-26
V4-28
V3-30
V4-31
V3-33
V4-34
V3-35
V3-38
V4-39
RP11-68314
V3-35
V3-38
V4-39
V3-43
V1-45
V1-46
V3-48
RP11-12F16
V3-48
V3-49
V5-51
V3-53
V1-58
V4-59
V4-61
V3-64
V3-66

Figure 1

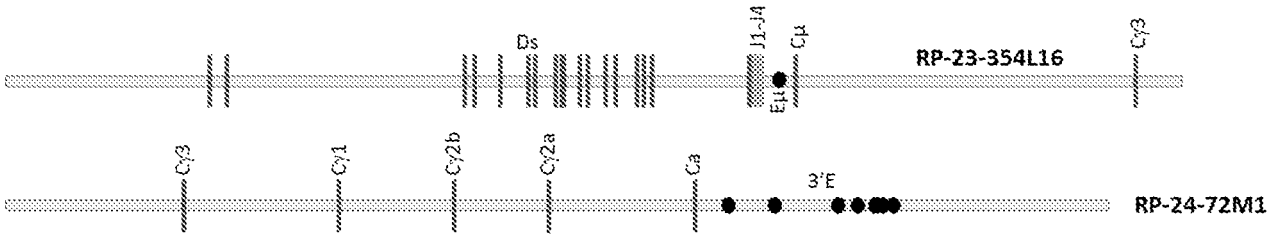


Figure 2

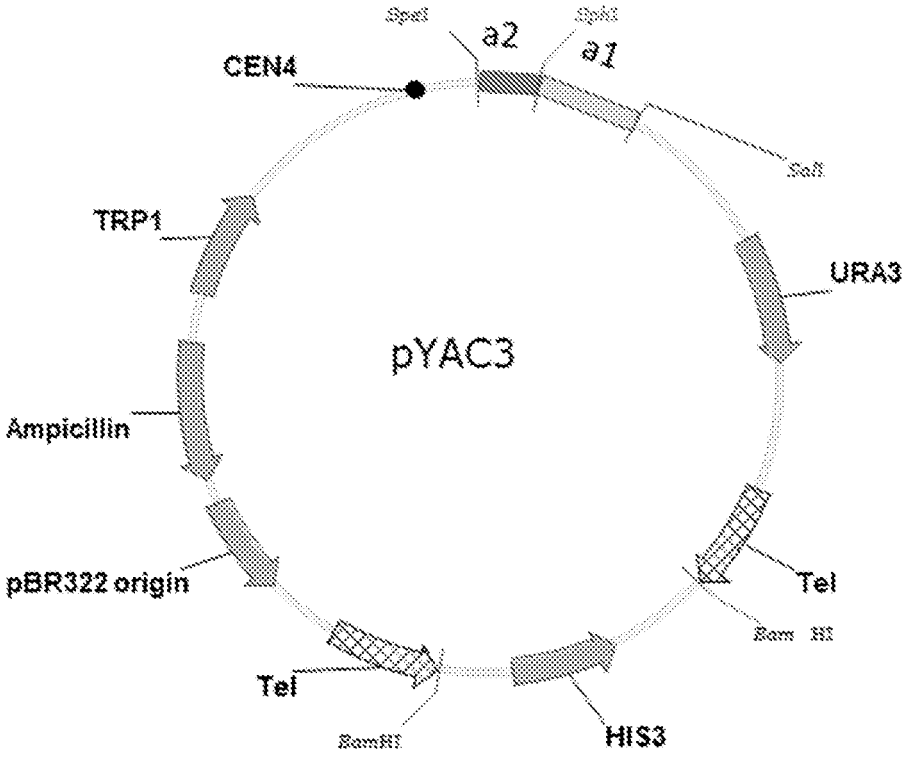


Figure 3

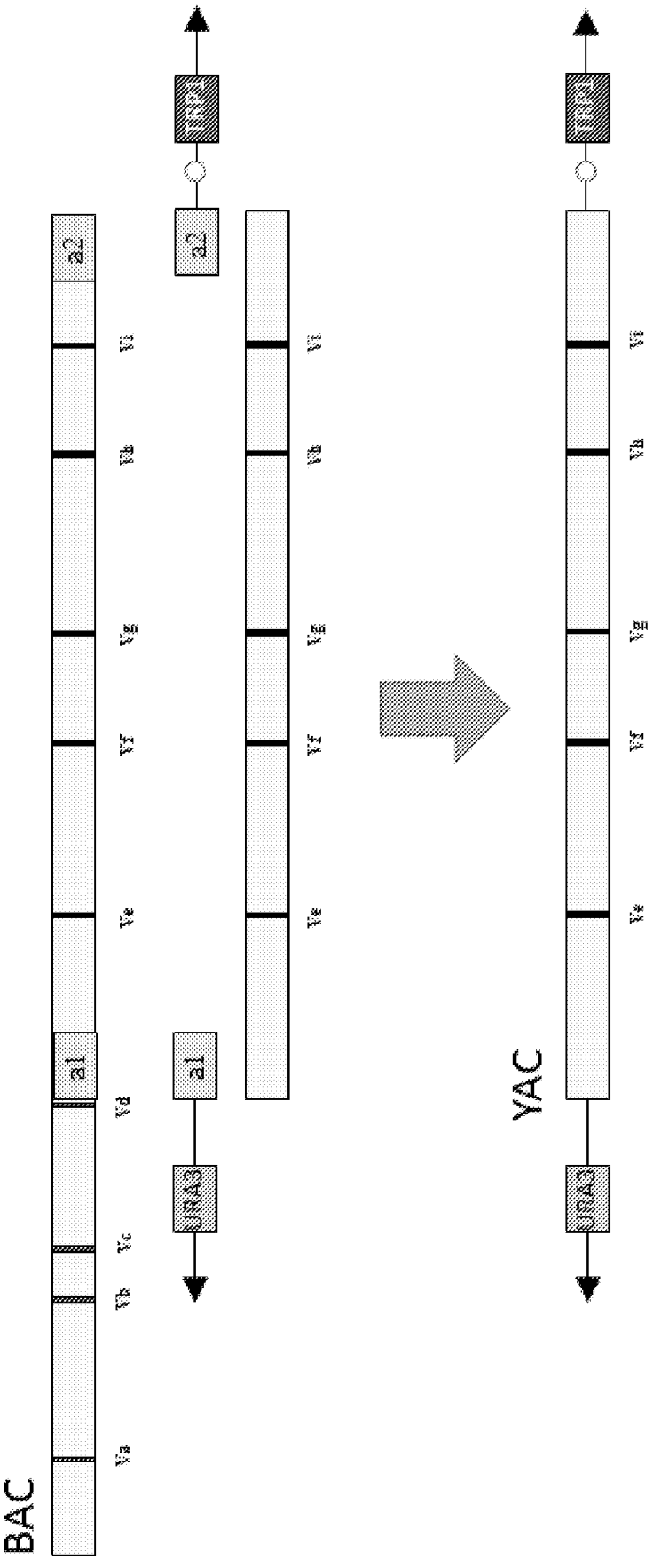


Figure 4

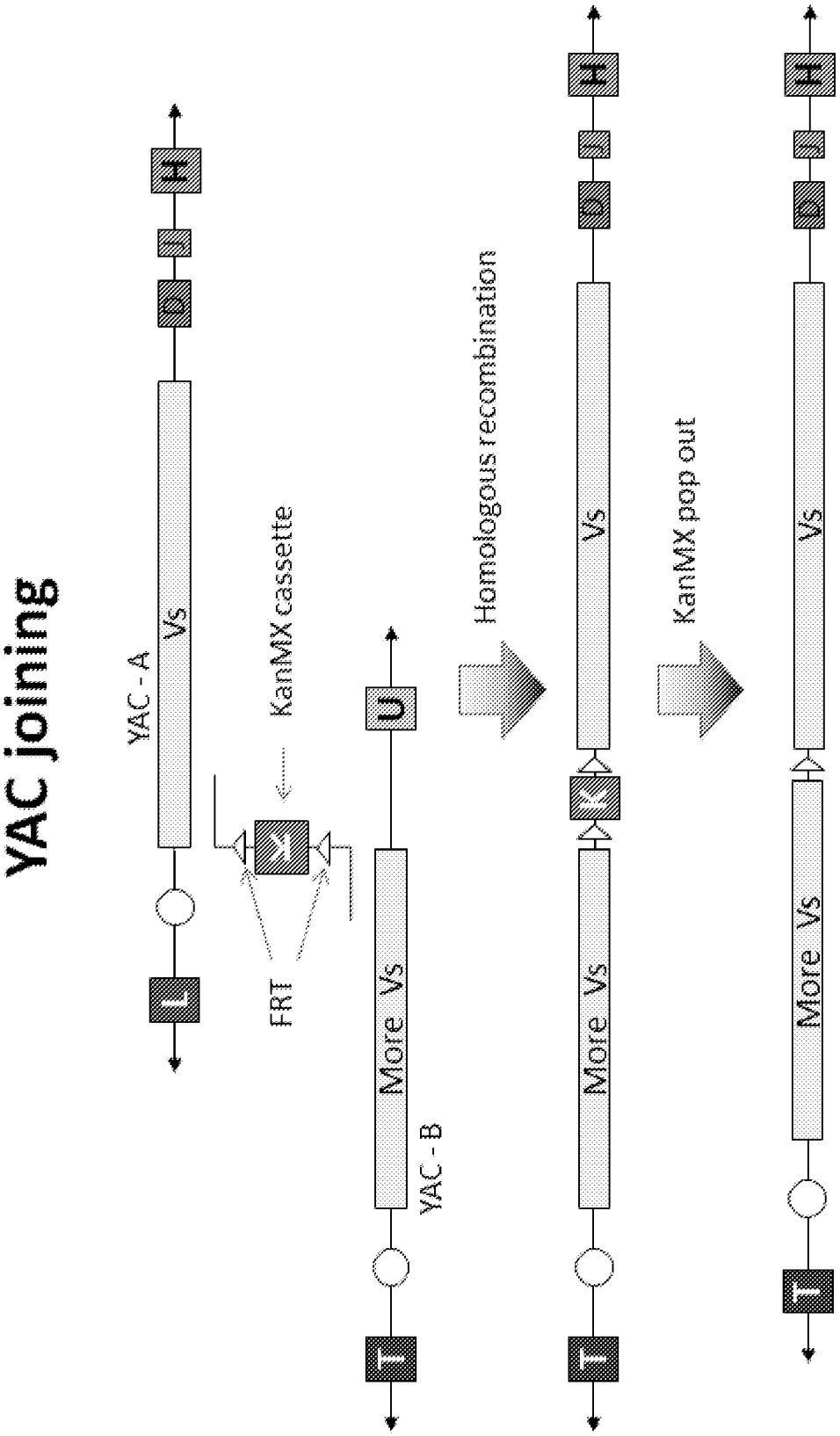

YAC joining
YAC - A
Vs
H
D
U
L
KanMX cassette
K
FRT
More Vs
YAC - B
U
T
Homologous recombination
Vs
H
D
U
K
More Vs
T
KanMX pop out
Vs
H
D
U
More Vs
T

Figure 5

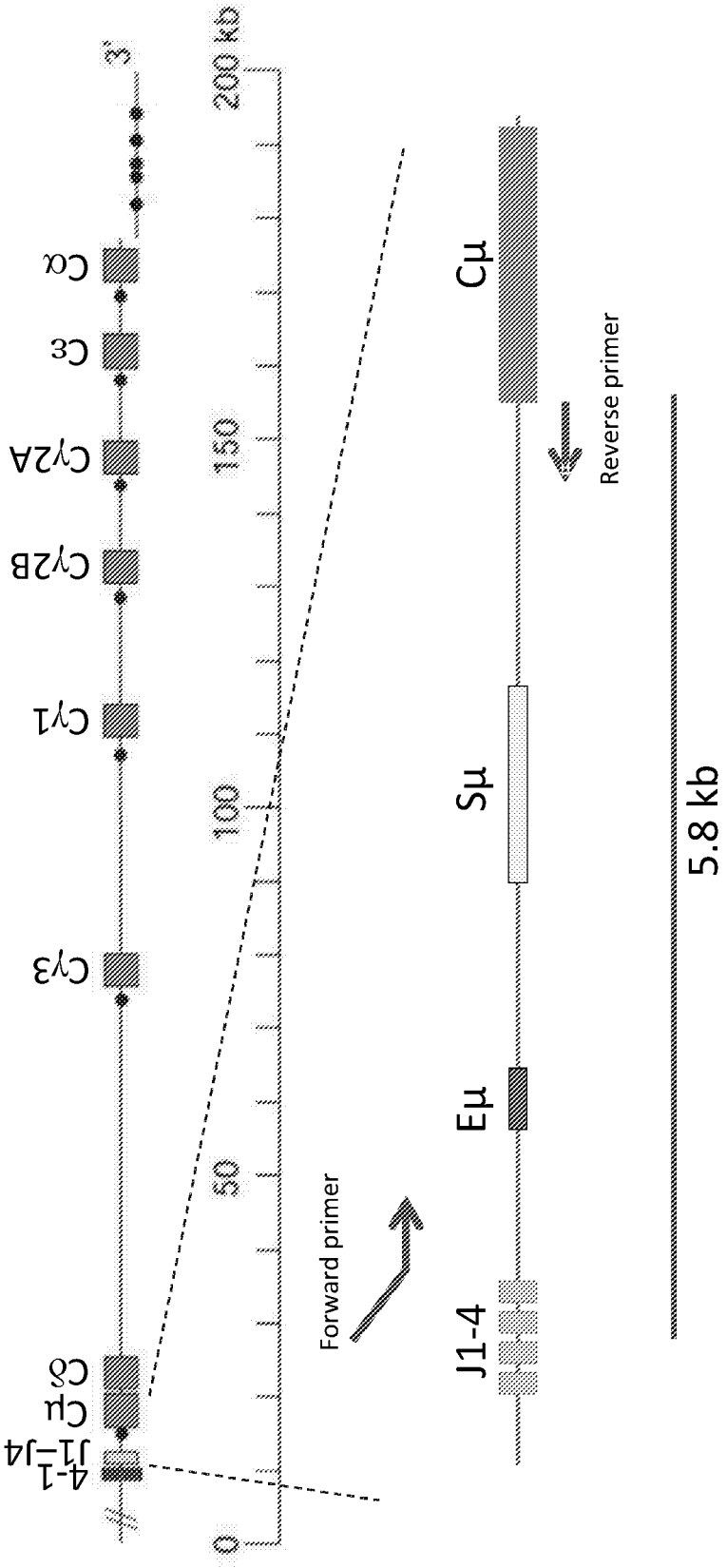

0
50
100
150
200 kb
3'
Cα
Cε
Cγ2A
Cγ2B
Cγ1
Cγ3
Cδ
Cμ
Jl–J4
4-1
Cμ
Sμ
Eμ
J1-4
Forward primer
Reverse primer
5.8 kb

Figure 6

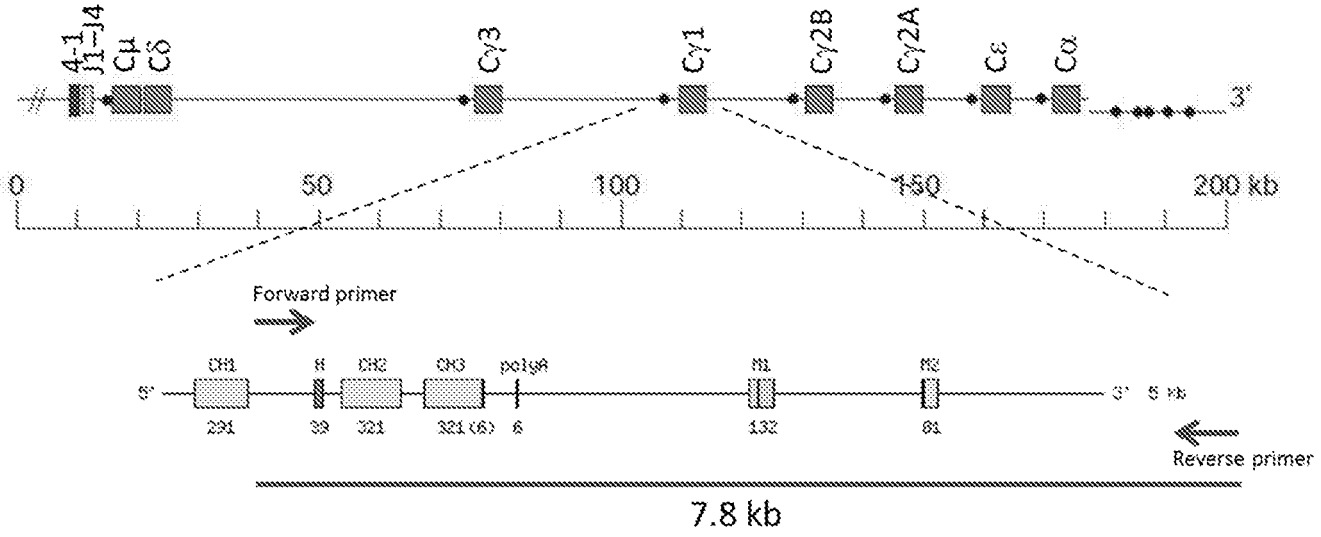


Figure 7

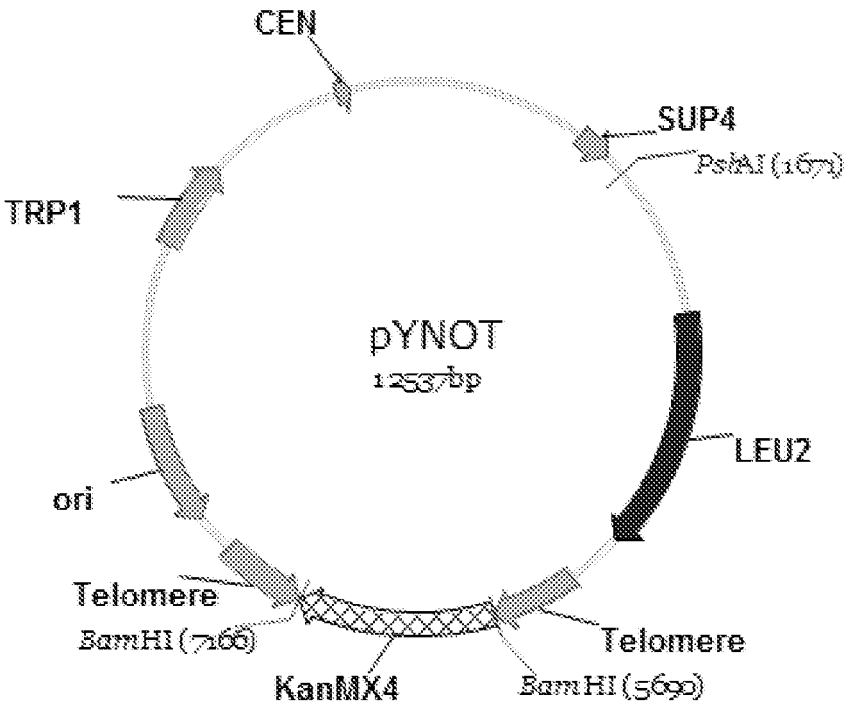


Figure 8

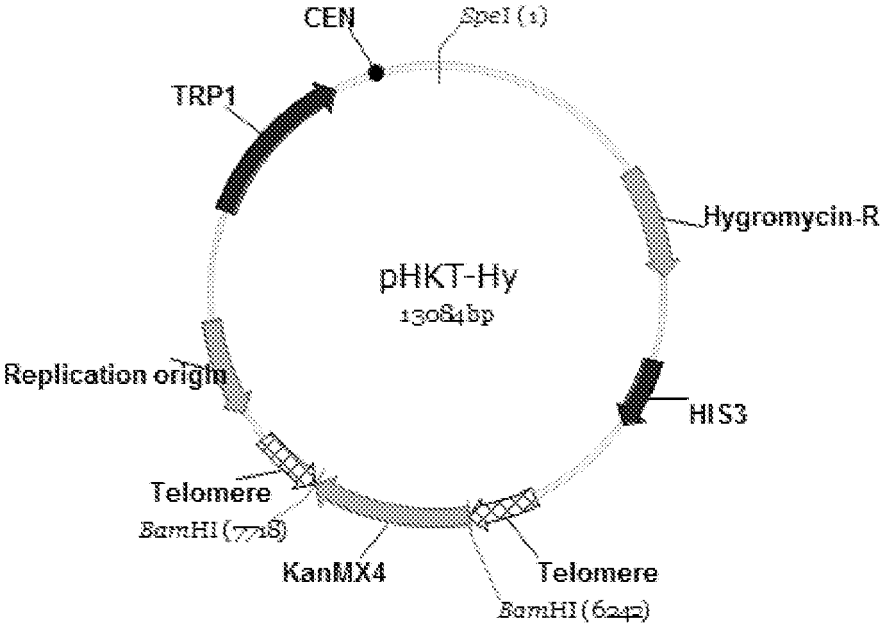

CEN
SpeI (1)
TRP1
Hygromycin-R
pHKT-Hy
13084bp
Replication origin
HIS3
Telomere
BamHI (7718)
KanMX4
Telomere
BamHI (6242)

Figure 9

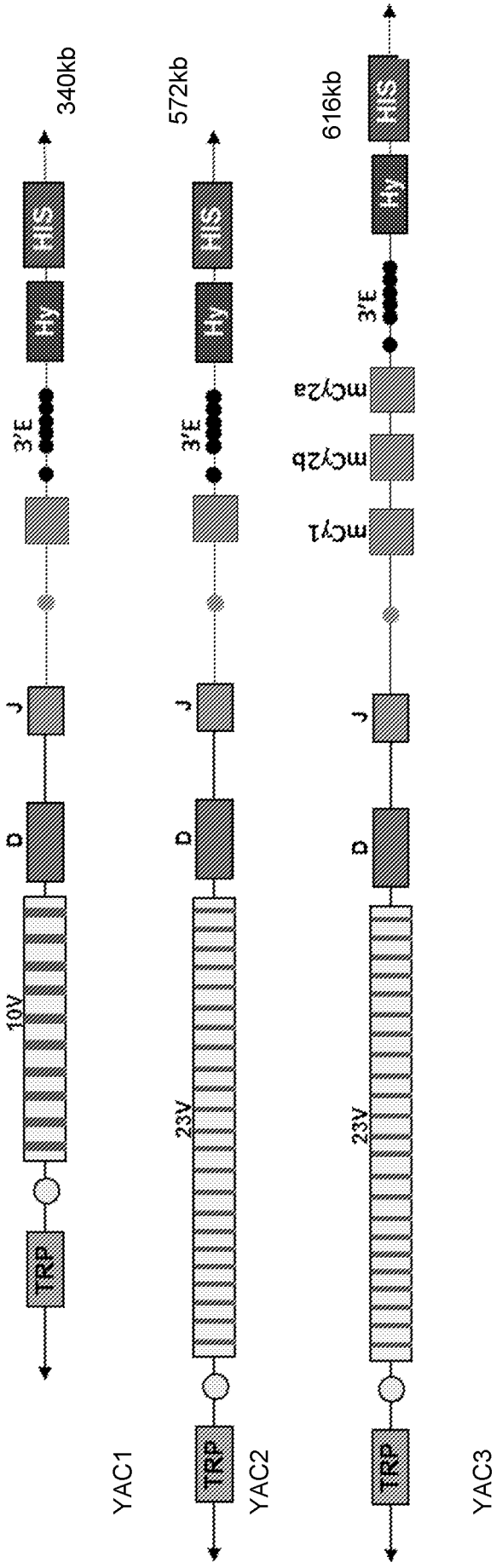

340kb
572kb
616kb
HIS
Hγ
3'E
mcγ2a
mcγ2b
mcγ1
J
D
10V
23V
TRP
YAC1
YAC2
YAC3

Figure 10

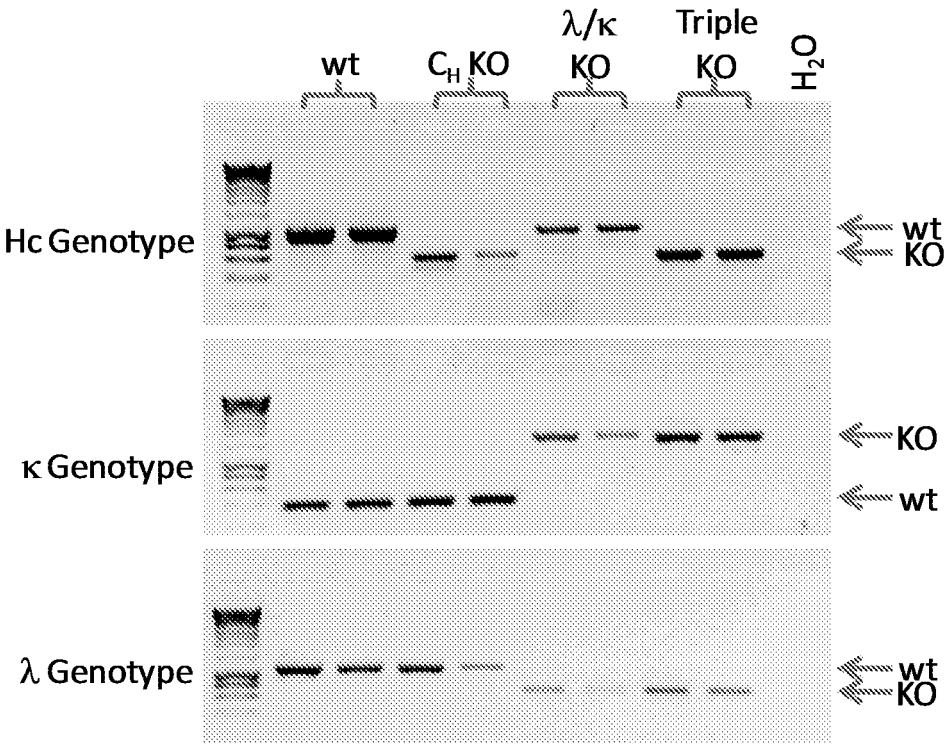
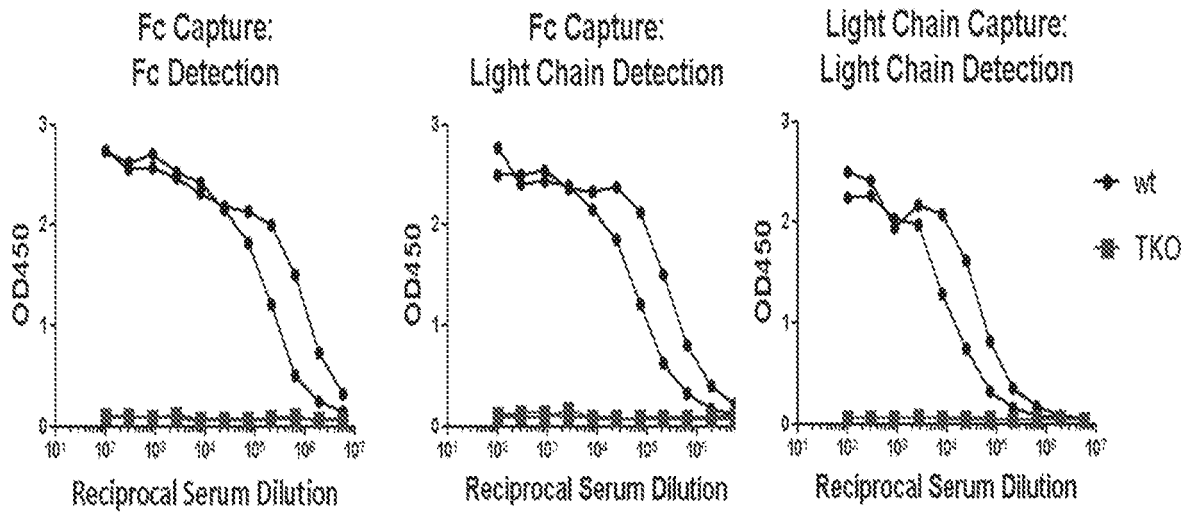

wt
C_H KO
λ/κ KO
Triple KO
H_2O
Hc Genotype
wt
KO
κ Genotype
KO
wt
λ Genotype
wt
KO

Figure 11

a b c



TKO has no mouse heavy chain in serum

TKO has no mouse heavy chain-light chain complexes in serum

TKO has no mouse light chains in serum

Figure 12

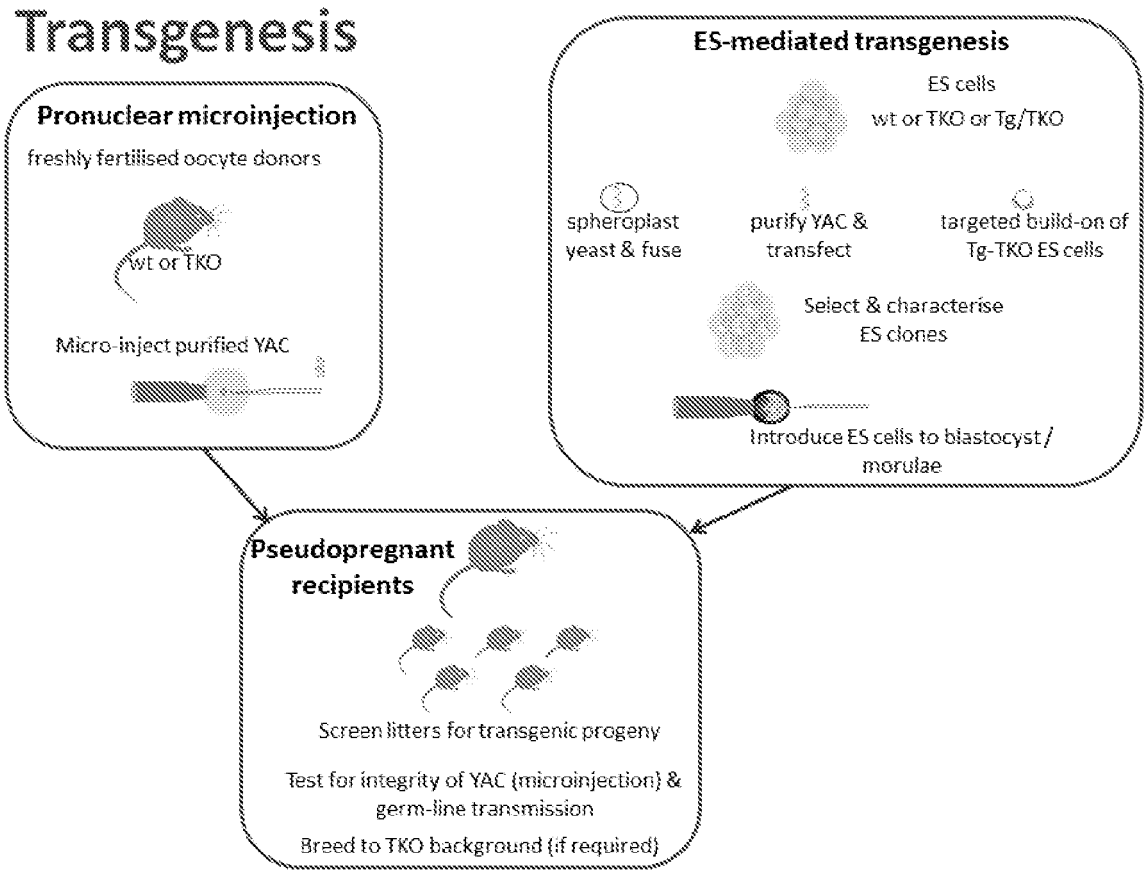
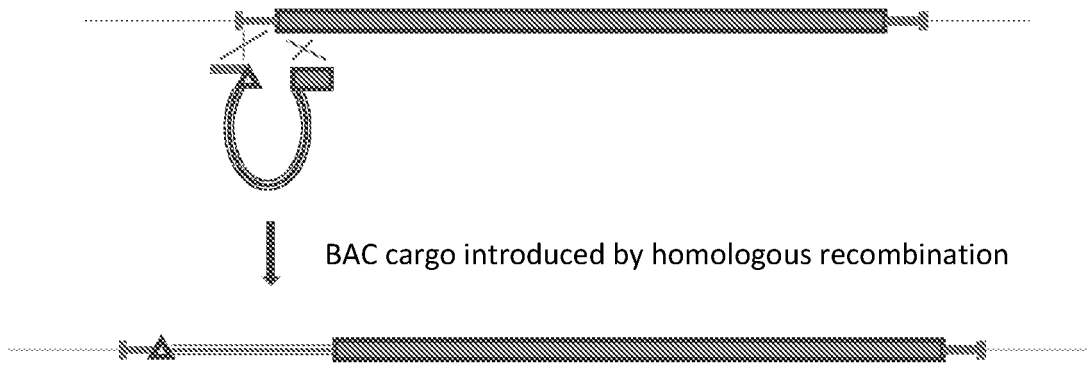


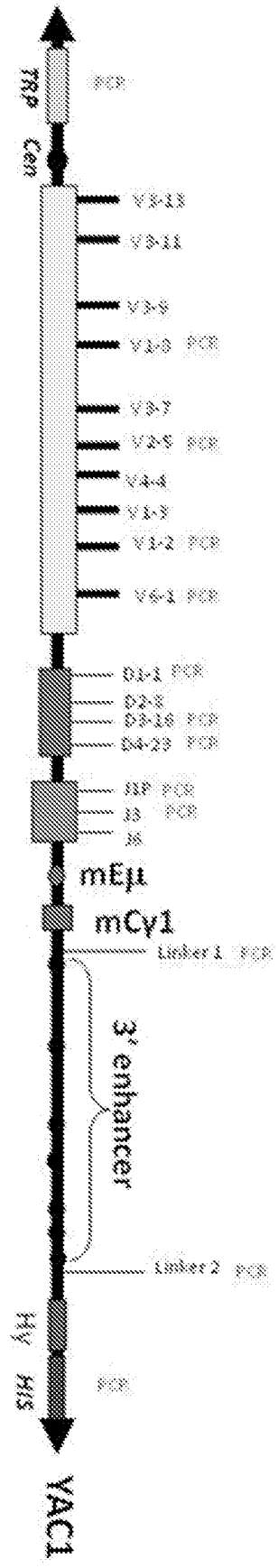
Figure 13



YAC arm
Floxed selectable marker
sequence introduced on YAC
sequence introduced on BAC

Figure 14

a


TRP
Cen
PCR
V3-13
V3-11
V3-9
V1-8 PCR
V3-7
V2-5 PCR
V4-4
V3-3
V1-2 PCR
V6-1 PCR
D1-1 PCR
D2-8
D3-16 PCR
D4-23 PCR
JH PCR
J3 PCR
J6
mEμ
mCγ1
Linker 1 PCR
3' enhancer
Linker 2 PCR
HY
HIS
PCR
YAC1

b

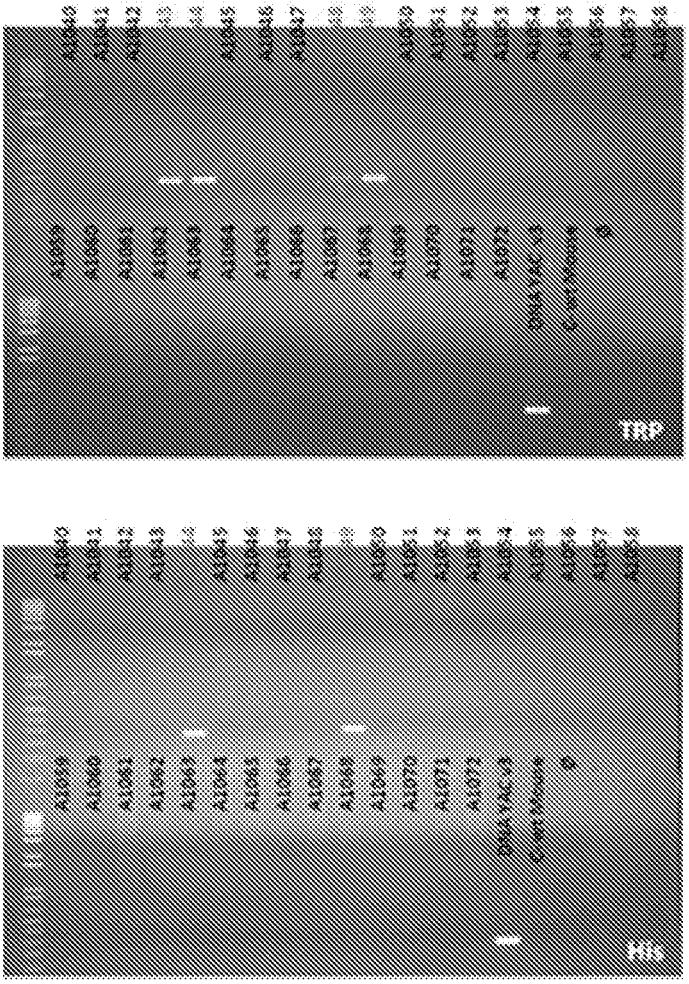
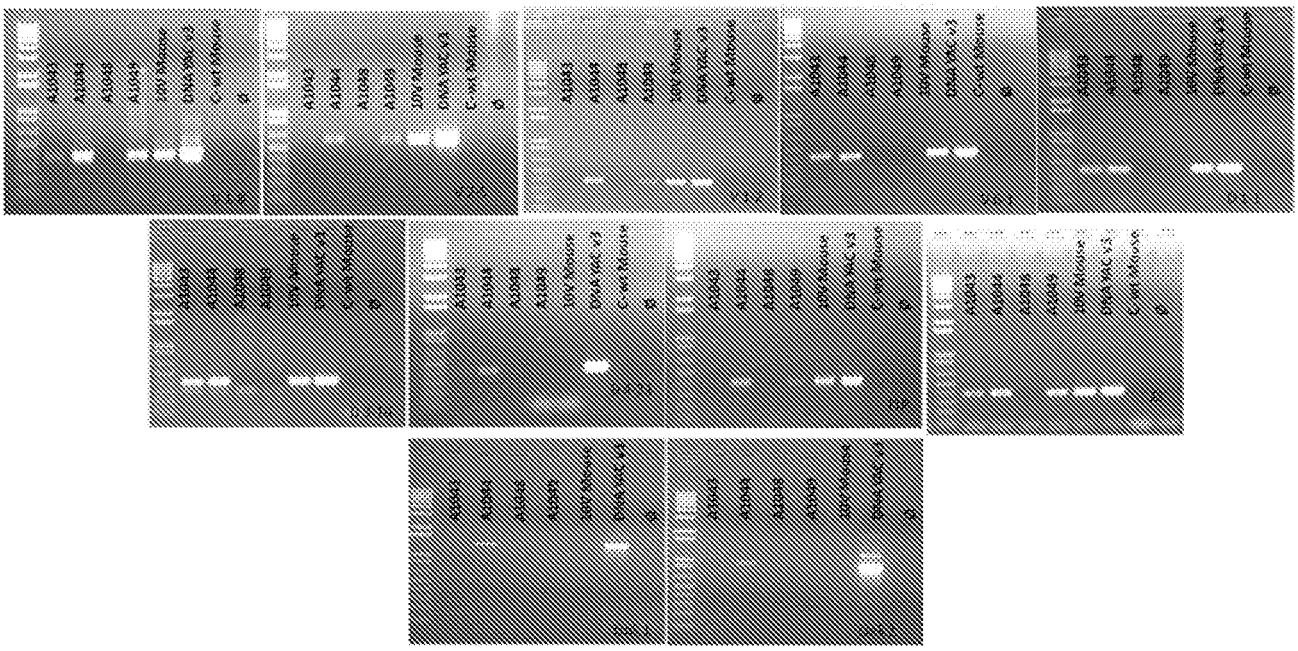

TRP
HIS

Figure 15



b cont.

YAC1 Mice	Trp 1	V 3-13	V 1-8	V 2-5	V 1-2	V 6-1	D1-1	D3-16	D4-23	J1P	J3	Linker 1 PCR2	Linker 2 PCR3	His 3
A1043	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓	✗	✗	✗
A1044	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A1048	✓	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
A1049	✓	✓	✓	✓	✓	✗	✗	✗	✗	✓	✓	✗	✗	✓
A1085	✗	✗			✗	✓	✓				✗	✗	✗	✗

Positive PCR : ✓ Negative PCR : ✗

c

Figure 15 cont.

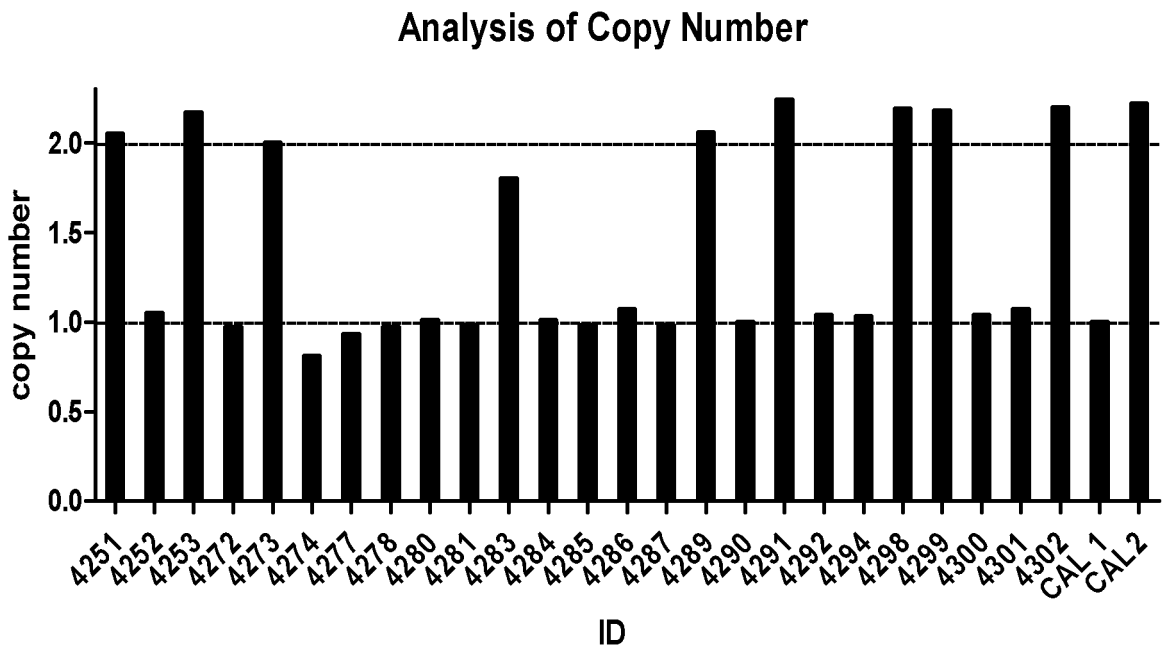


Figure 16

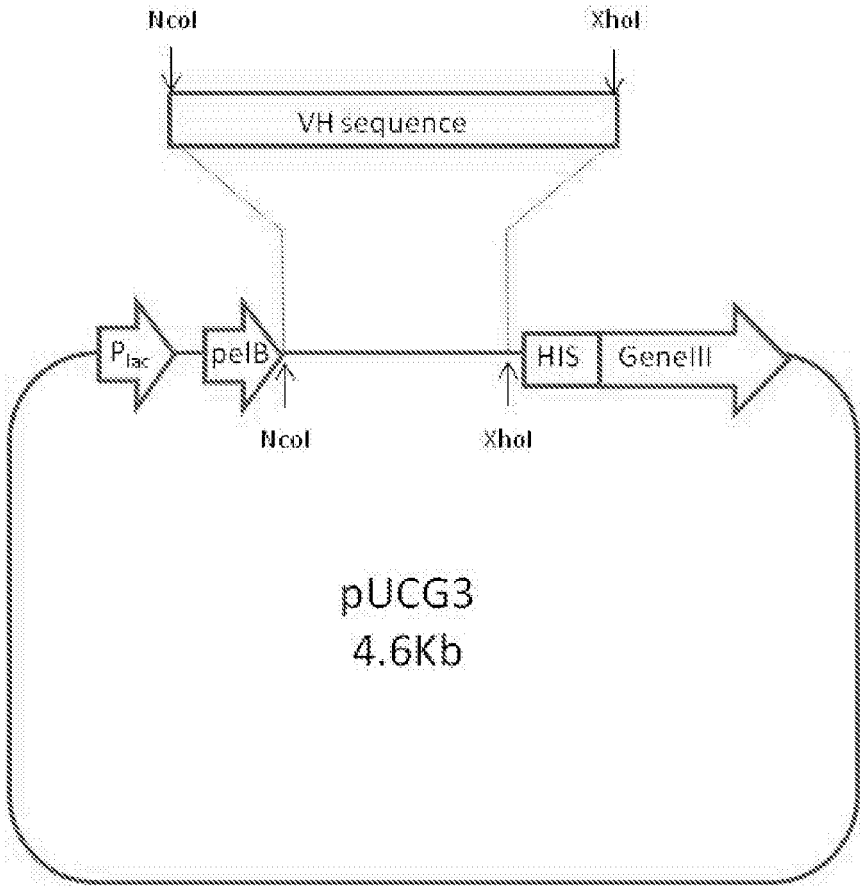


Figure 17

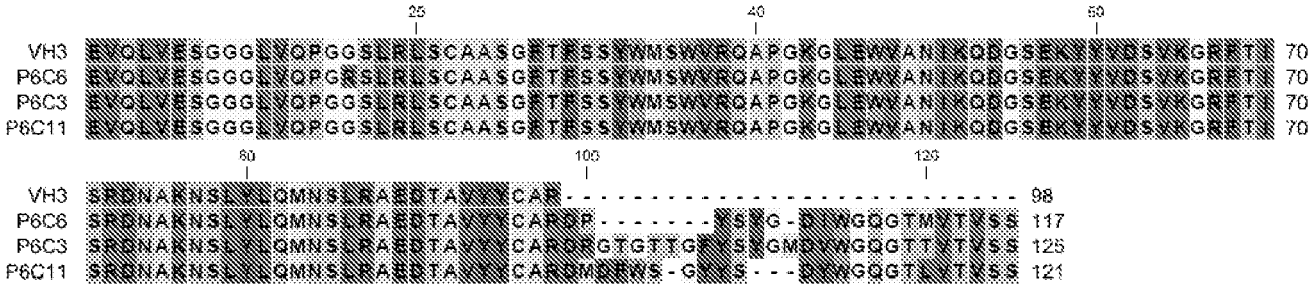
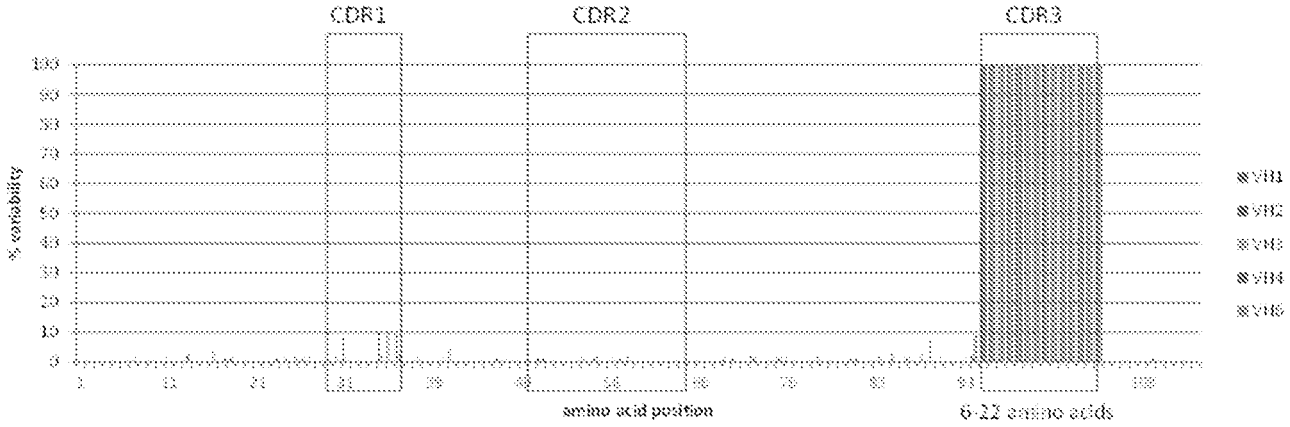


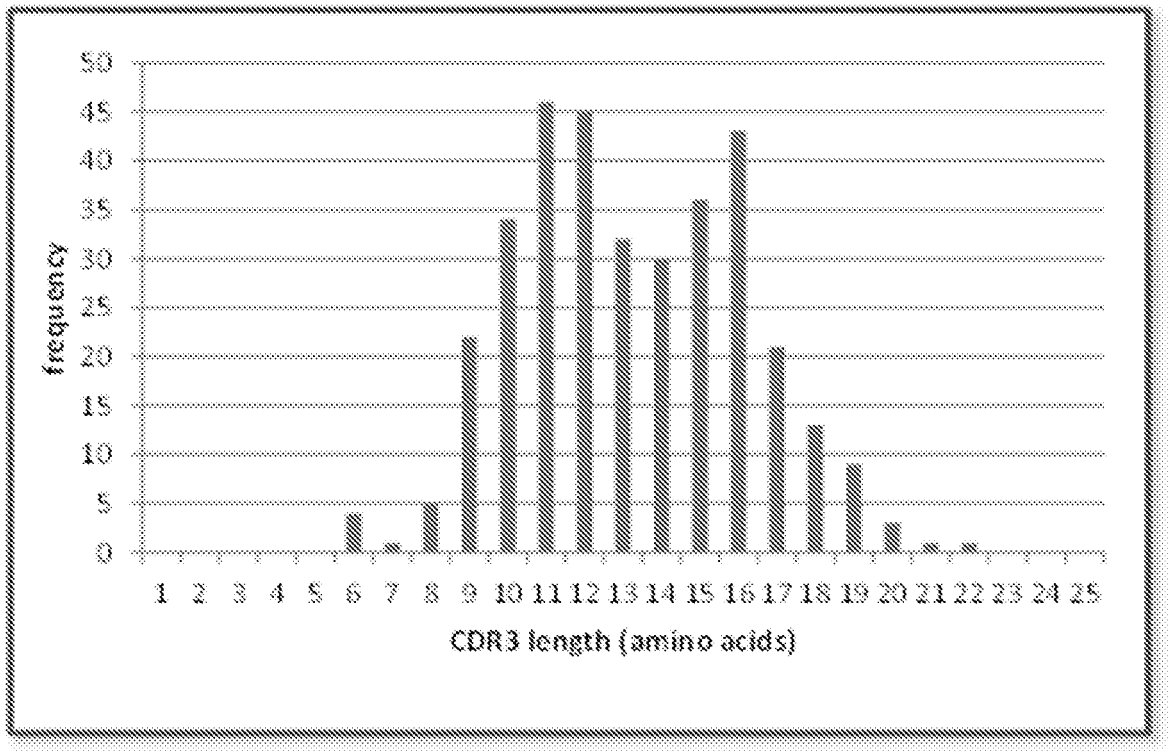
Figure 18



	VH1	VH2	VH3	VH4	VH6	Total
# of clones	69	64	77	68	57	335

a.

Figure 19



b.

Figure 19 cont.

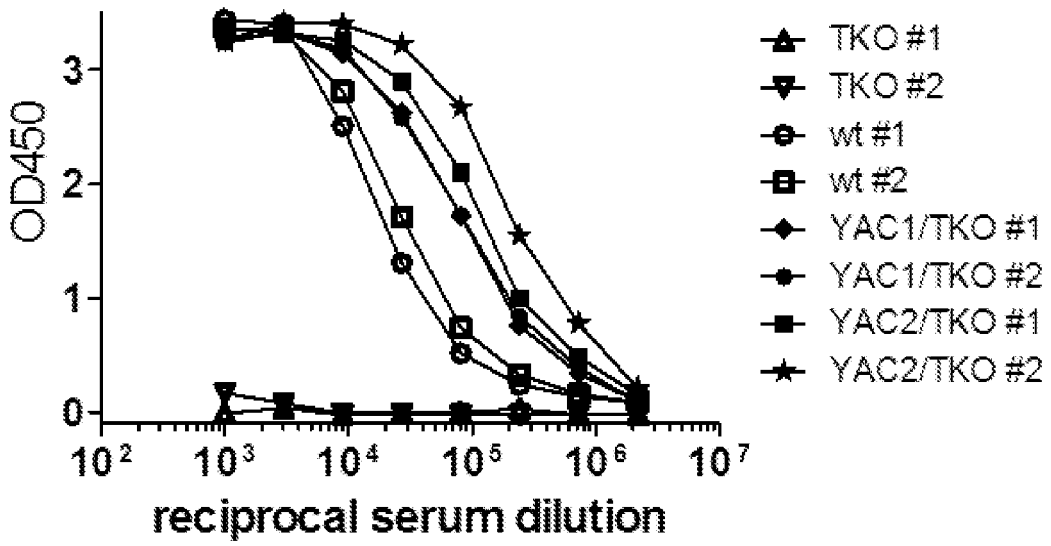


Figure 20

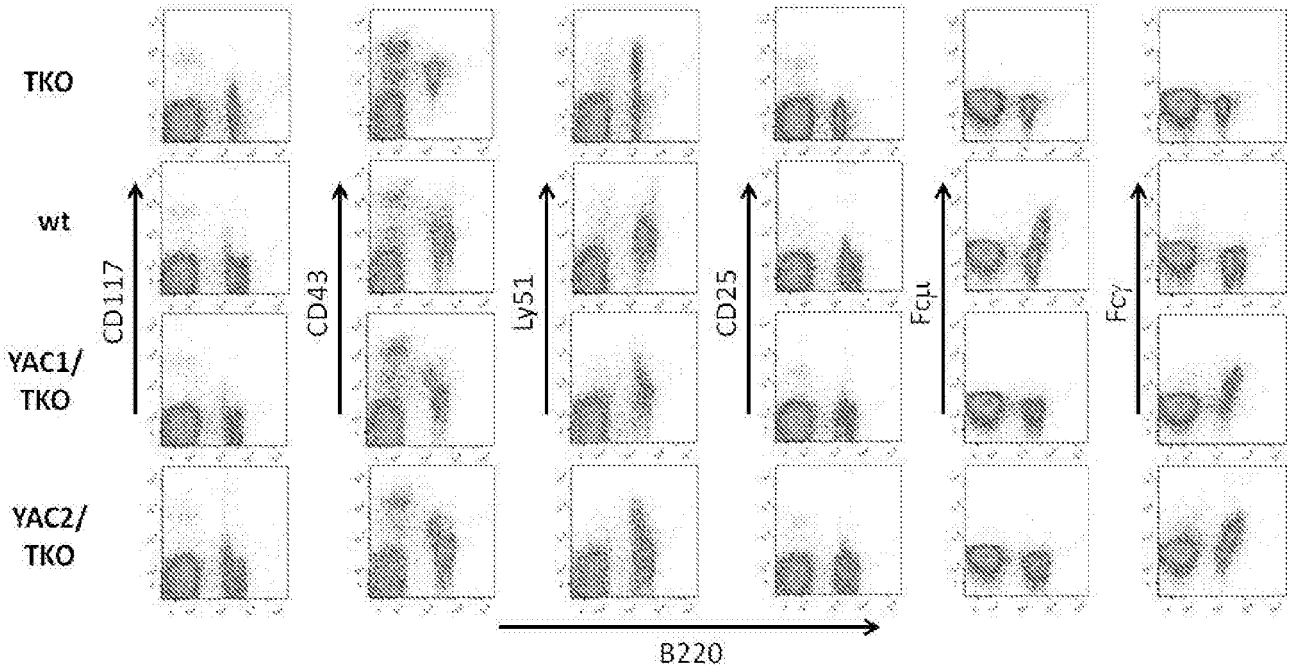
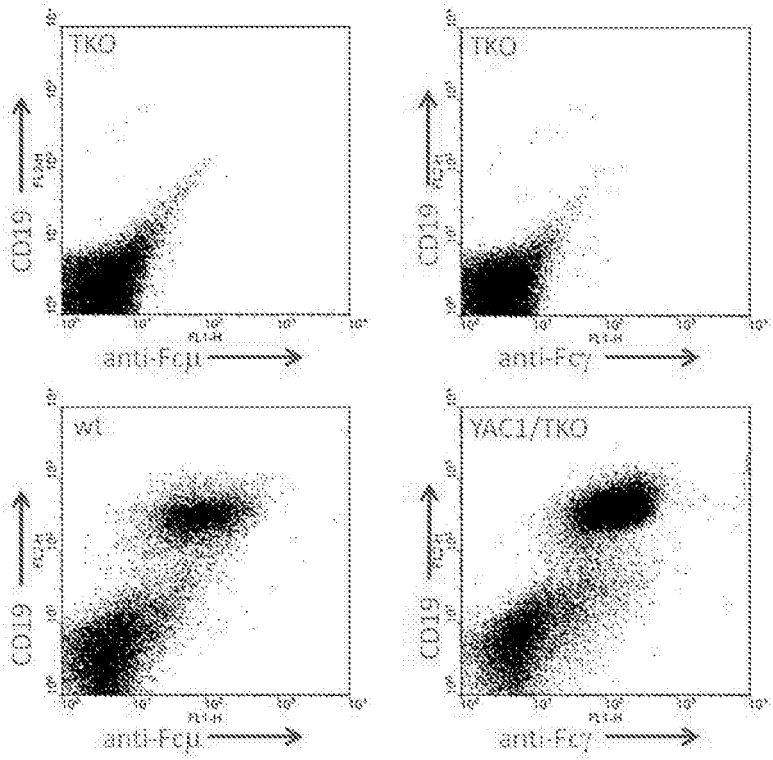
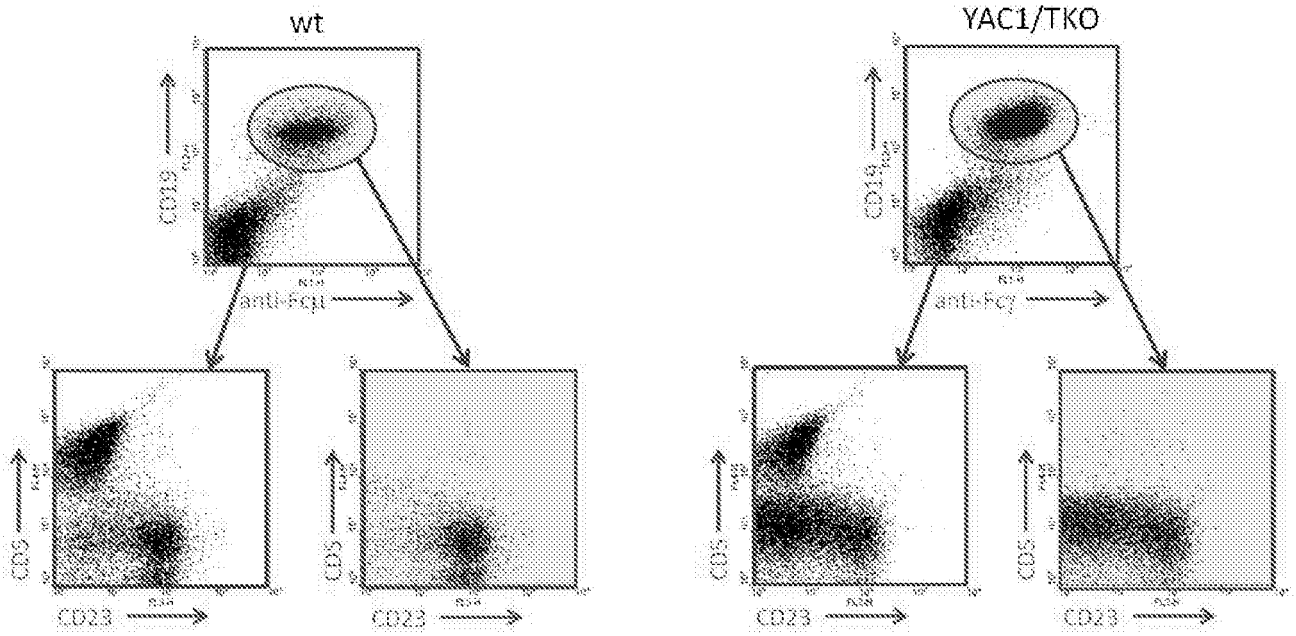


Figure 21

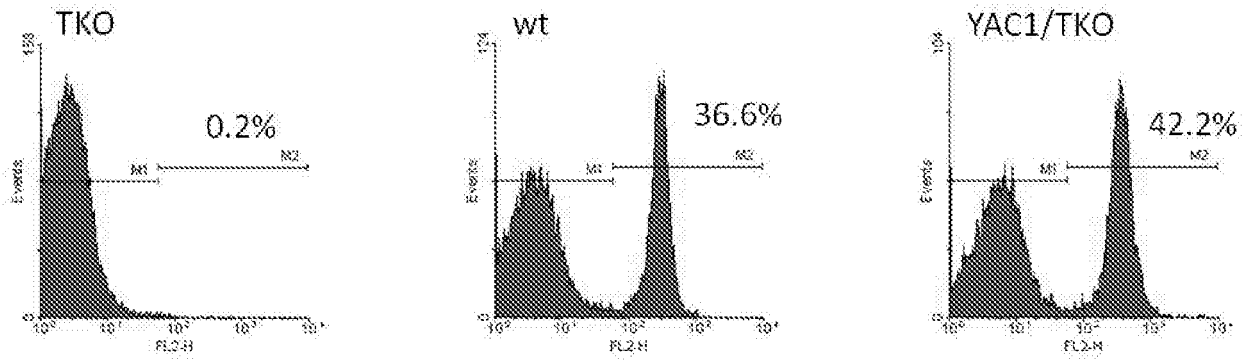


a

Figure 22



b



c.

Figure 22 cont.

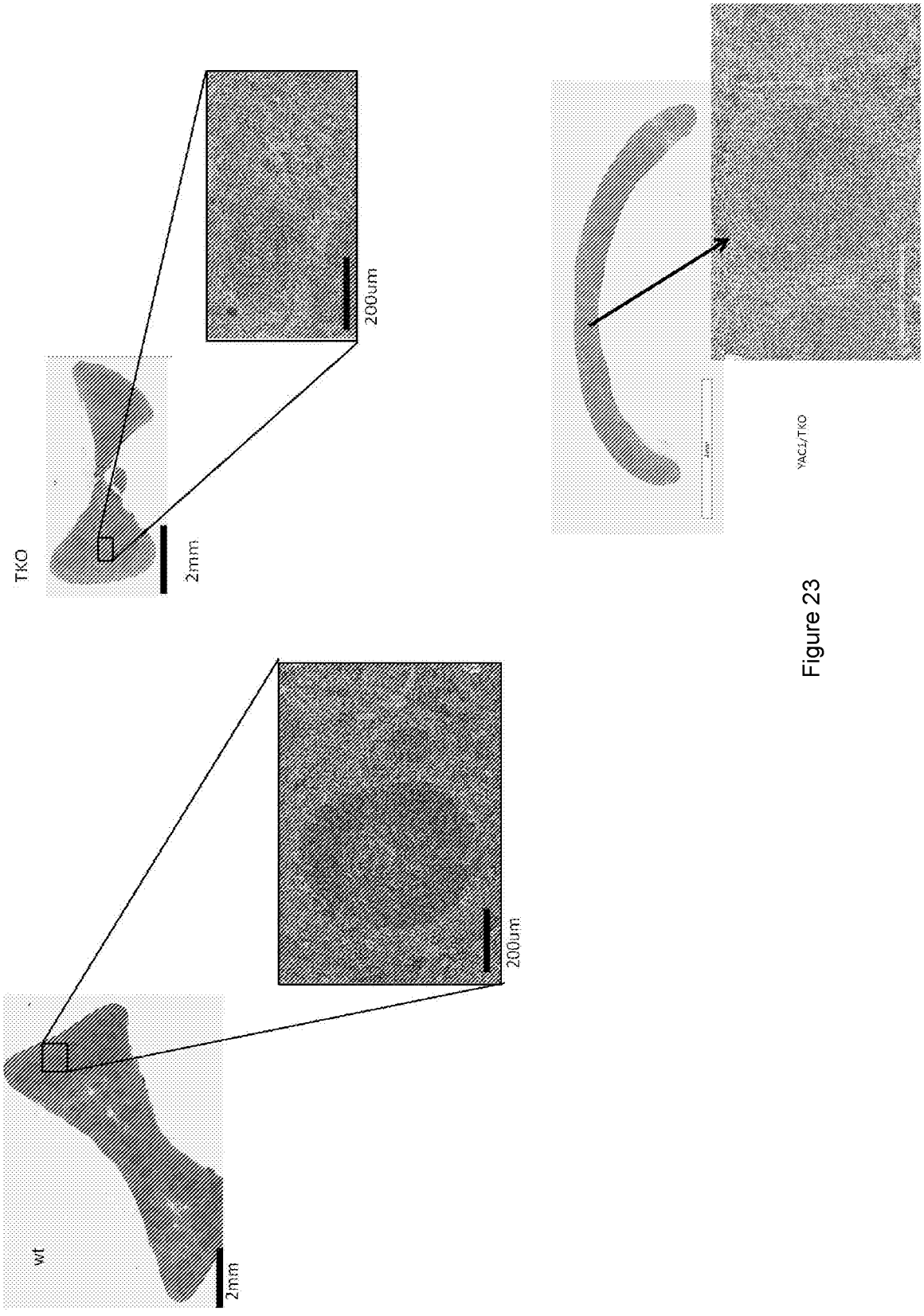


Figure 23

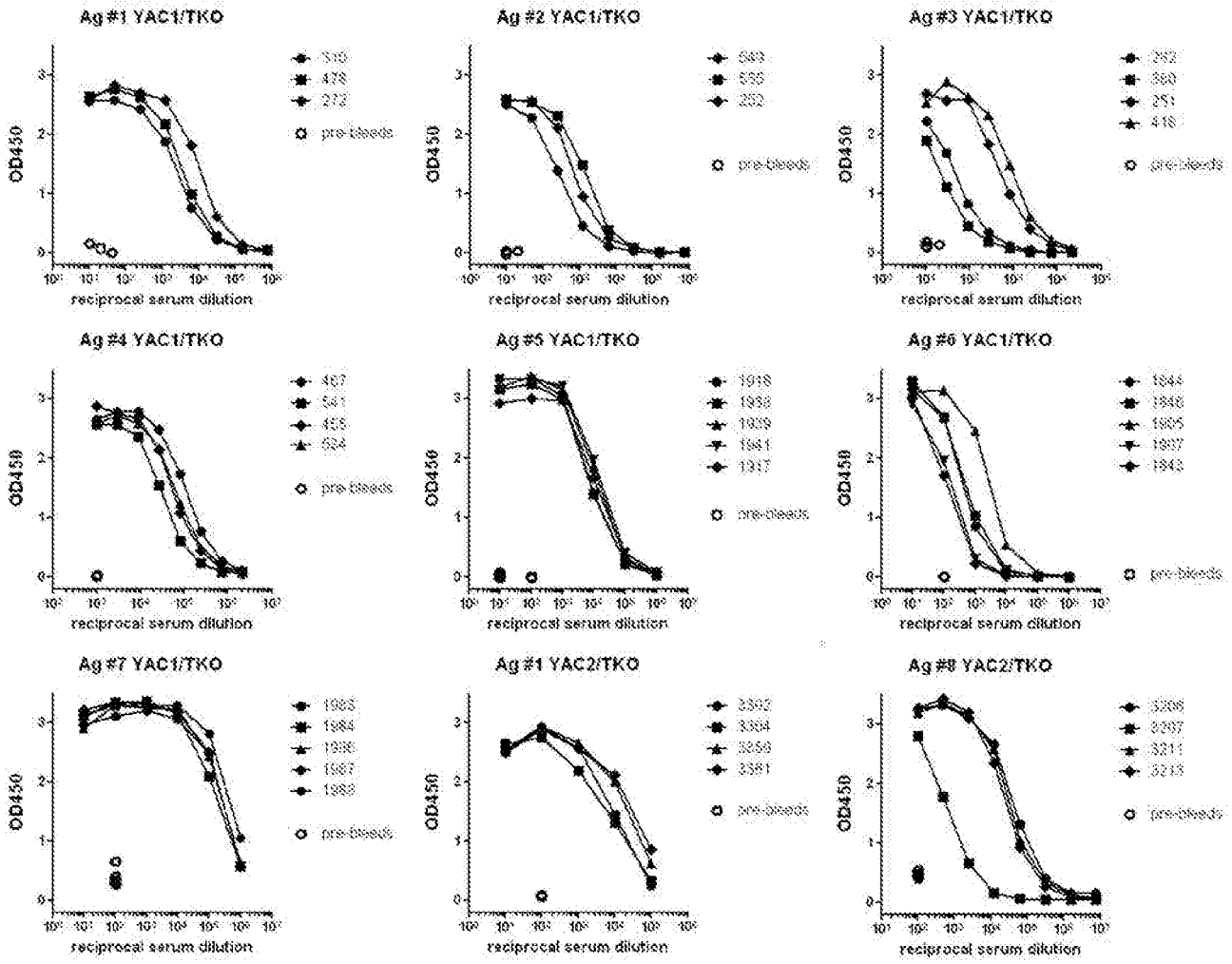
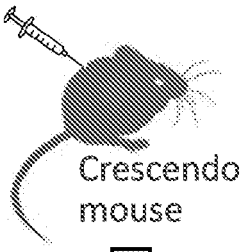

Ag #1 YAC1/TKO
Ag #2 YAC1/TKO
Ag #3 YAC1/TKO
Ag #4 YAC1/TKO
Ag #5 YAC1/TKO
Ag #6 YAC1/TKO
Ag #7 YAC1/TKO
Ag #1 YAC2/TKO
Ag #8 YAC2/TKO
OD450
reciprocal serum dilution
pre-bleeds

Figure 24

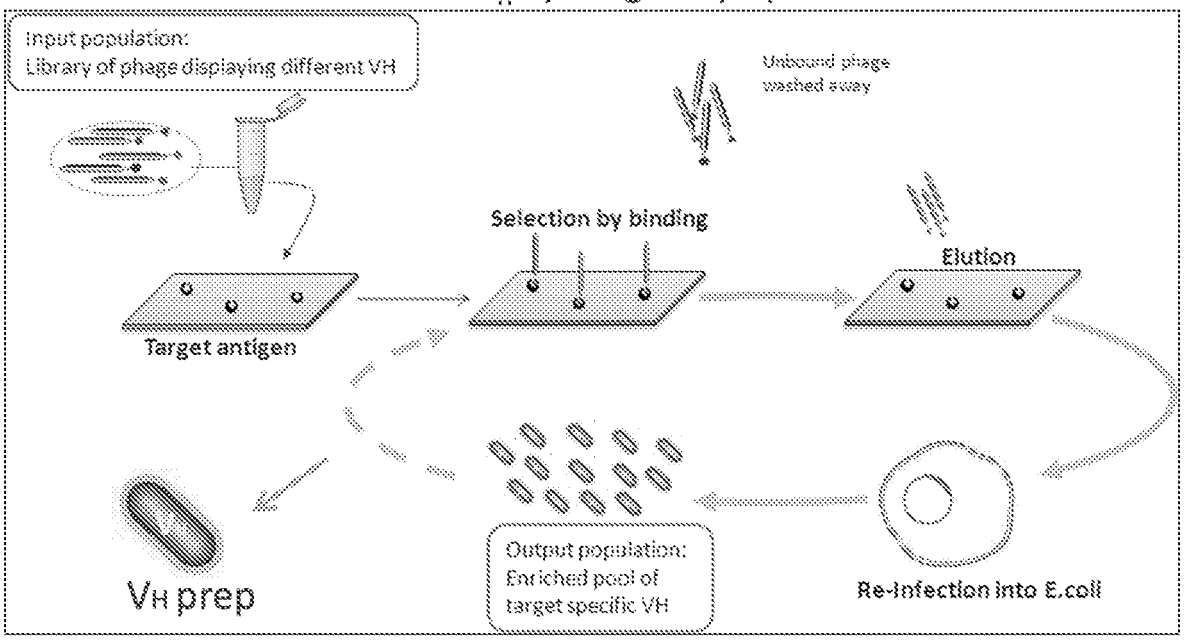


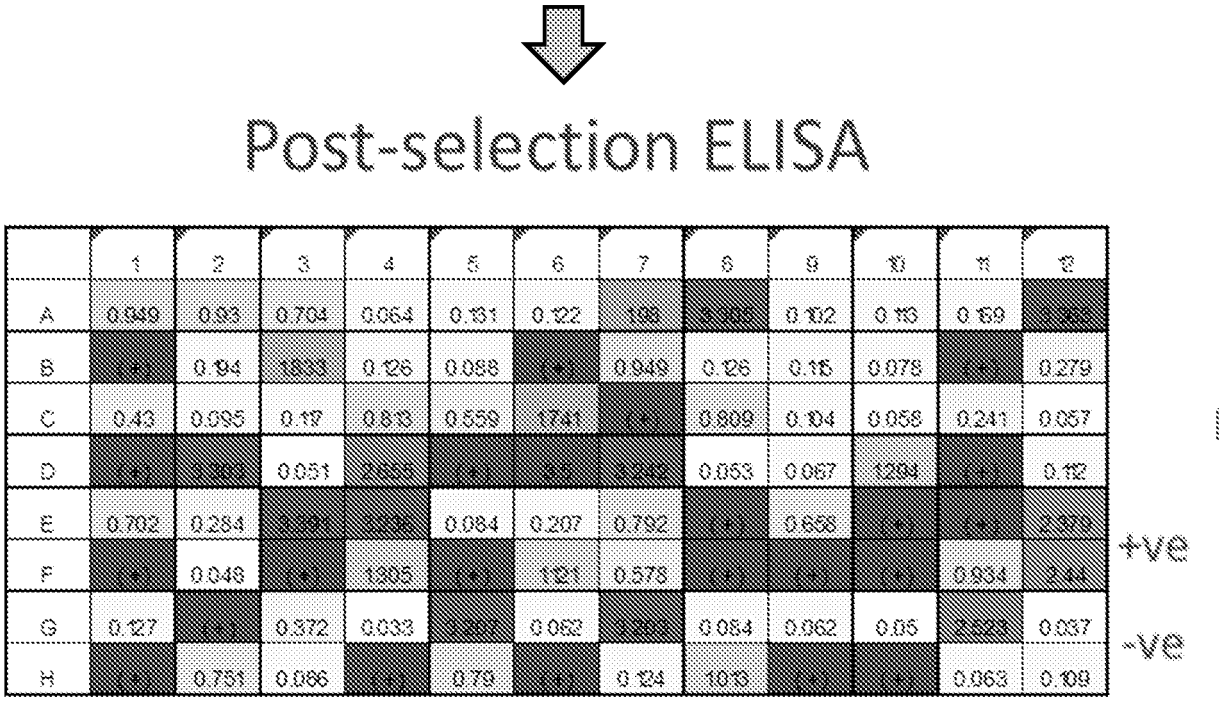
Extract mRNA from spleen and lymph nodes

RT-PCR V_H for each germline family

Clone V_H into phage display vector

Isolate V_H by Phage Display





	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.03	0.704	0.064	0.131	0.122	1.03	2.165	0.102	0.113	0.159	2.62
B	2.1	0.194	1.833	0.126	0.088	2.1	0.949	0.126	0.115	0.078	2.1	0.279
C	0.43	0.035	0.17	0.613	0.559	1.741	2.1	0.809	0.104	0.058	0.241	0.057
D	2.1	1.903	0.051	2.035	2.1	2.1	1.242	0.053	0.067	1.204	2.1	0.112
E	0.702	0.284	1.784	2.035	0.084	0.207	0.792	2.1	0.658	2.1	2.1	1.379
F	2.1	0.048	2.1	1.205	2.1	1.121	0.573	2.1	2.1	2.1	0.934	2.1
G	0.127	2.1	0.372	0.033	1.207	0.062	1.283	0.054	0.062	0.05	2.023	0.037
H	2.1	0.751	0.066	2.1	0.79	2.1	0.124	1.013	2.1	2.1	0.063	0.109

Figure 25

Pre-Selection Library

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.065	0.061	0.059	0.053	0.064	0.053	0.087	0.052	0.057	0.063	0.057	0.065
B	0.076	0.071	0.072	0.078	0.098	0.102	0.081	0.072	0.078	0.082	0.303	0.083
C	0.056	0.054	0.053	0.064	0.065	0.07	0.07	0.054	0.055	0.059	0.064	0.061
D	0.048	0.058	0.052	0.056	0.056	0.05	0.047	0.051	0.049	0.062	0.049	0.085
E	0.056	0.054	0.05	0.046	0.051	0.102	0.047	0.048	0.048	0.049	0.049	1.078
F	0.067	0.048	0.05	0.049	0.051	0.119	0.072	0.053	0.05	0.049	0.051	2.611
G	0.041	0.041	0.042	0.047	0.048	0.061	0.046	0.055	0.039	0.036	0.032	0.031
H	0.076	0.056	0.053	0.05	0.055	0.055	0.05	0.053	0.049	0.049	0.061	0.051

+ve

-ve

After one round of Selection Library

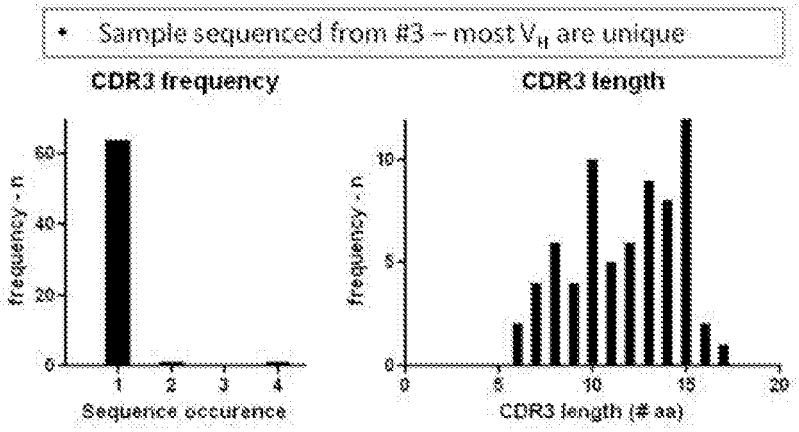
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.349	0.136	0.133	0.842	0.185	0.225	0.169	0.312	0.169	0.175	0.158	0.296
B	0.593	0.261	0.587	0.238	0.338	0.188	0.239	0.801	0.52	0.09	0.357	0.475
C	0.208	0.248	0.066	0.173	0.088	0.208	0.324	0.075	0.166	0.244	0.053	0.12
D	0.115	0.808	0.226	0.219	0.07	0.272	0.361	0.334	0.084	0.328	0.153	0.481
E	1.069	0.881	0.111	0.504	0.119	0.28	0.067	0.221	0.153	0.436	0.056	1.42
F	0.122	0.321	0.402	0.085	0.377	0.351	0.296	0.136	0.129	0.056	0.572	1.946
G	0.337	0.173	0.374	0.146	0.802	0.508	0.075	0.244	0.052	0.066	0.193	0.04
H	0.34	0.174	0.148	0.36	0.088	0.183	0.191	0.182	0.101	0.122	0.383	0.153

+ve

-ve

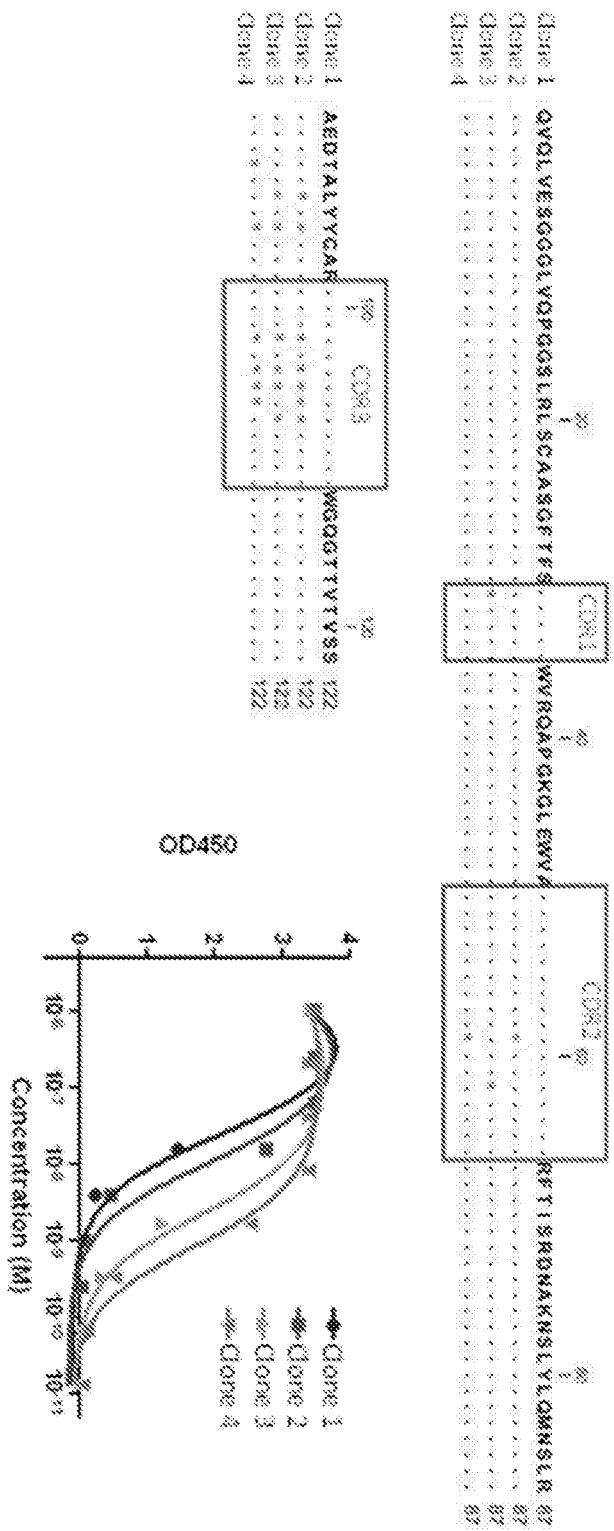
Figure 26

Mouse	Library Size
#1	$2.8x10^8$
#2	$2.2x10^8$
#3	$1.3x10^8$
#4	7×10^8
Total	1.3×10^9



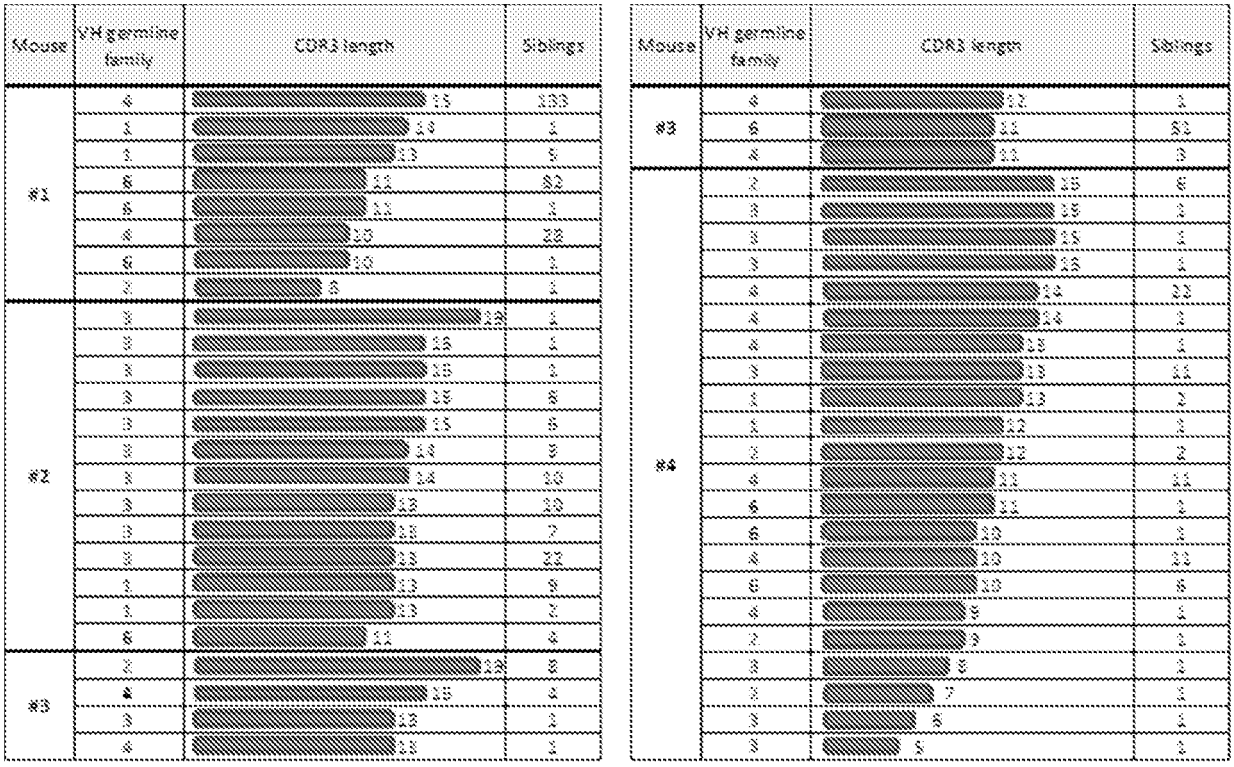
a.

Figure 27



b.

Figure 27 cont.



c.

Figure 27 cont.

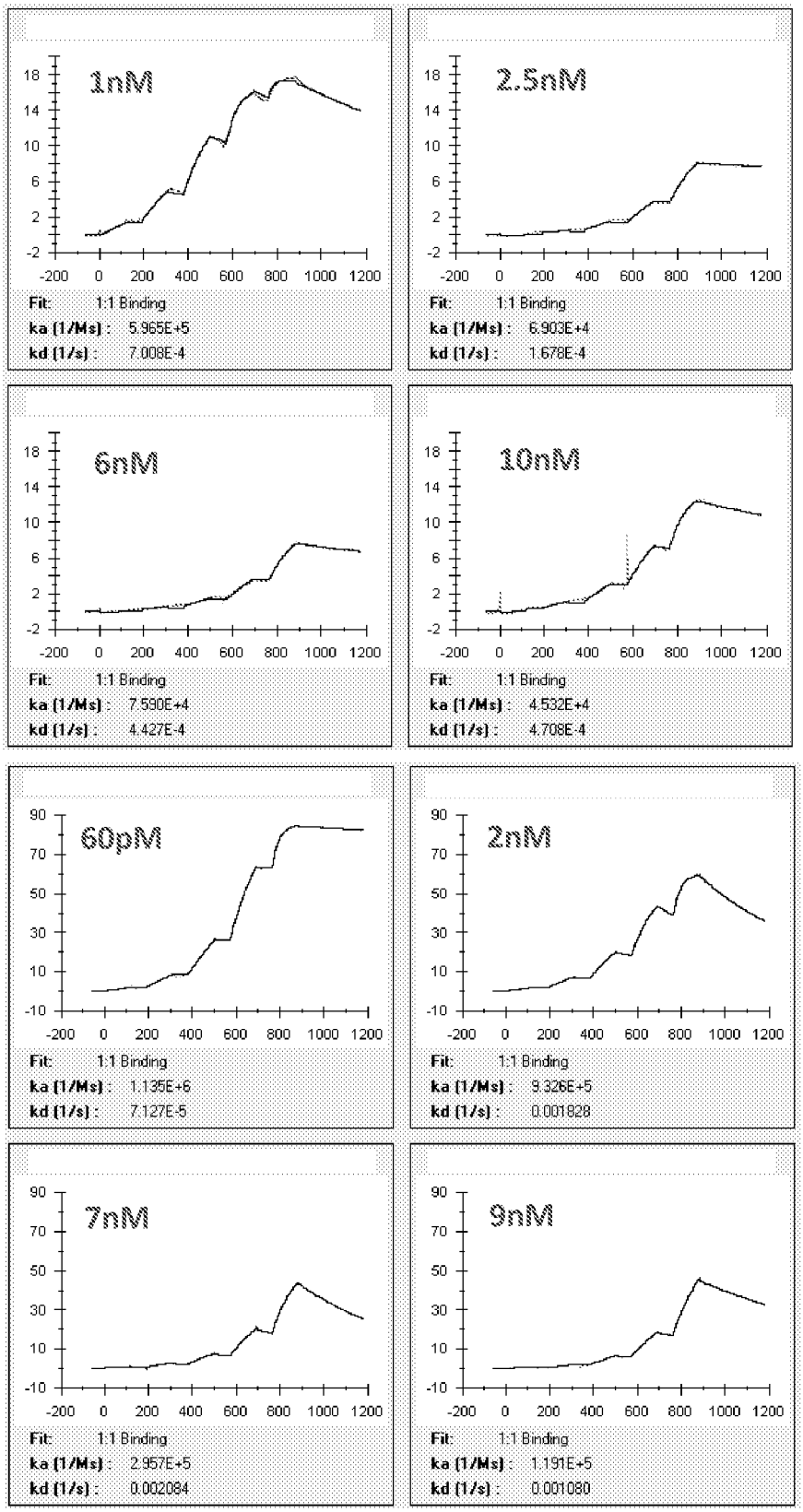

1nM
Fit: 1:1 Binding
ka (1/Ms) : 5.965E+5
kd (1/s) : 7.008E-4
2.5nM
Fit: 1:1 Binding
ka (1/Ms) : 6.903E+4
kd (1/s) : 1.678E-4
6nM
Fit: 1:1 Binding
ka (1/Ms) : 7.590E+4
kd (1/s) : 4.427E-4
10nM
Fit: 1:1 Binding
ka (1/Ms) : 4.532E+4
kd (1/s) : 4.708E-4
60pM
Fit: 1:1 Binding
ka (1/Ms) : 1.135E+6
kd (1/s) : 7.127E-5
2nM
Fit: 1:1 Binding
ka (1/Ms) : 9.326E+5
kd (1/s) : 0.001828
7nM
Fit: 1:1 Binding
ka (1/Ms) : 2.957E+5
kd (1/s) : 0.002084
9nM
Fit: 1:1 Binding
ka (1/Ms) : 1.191E+5
kd (1/s) : 0.001080

Figure 28

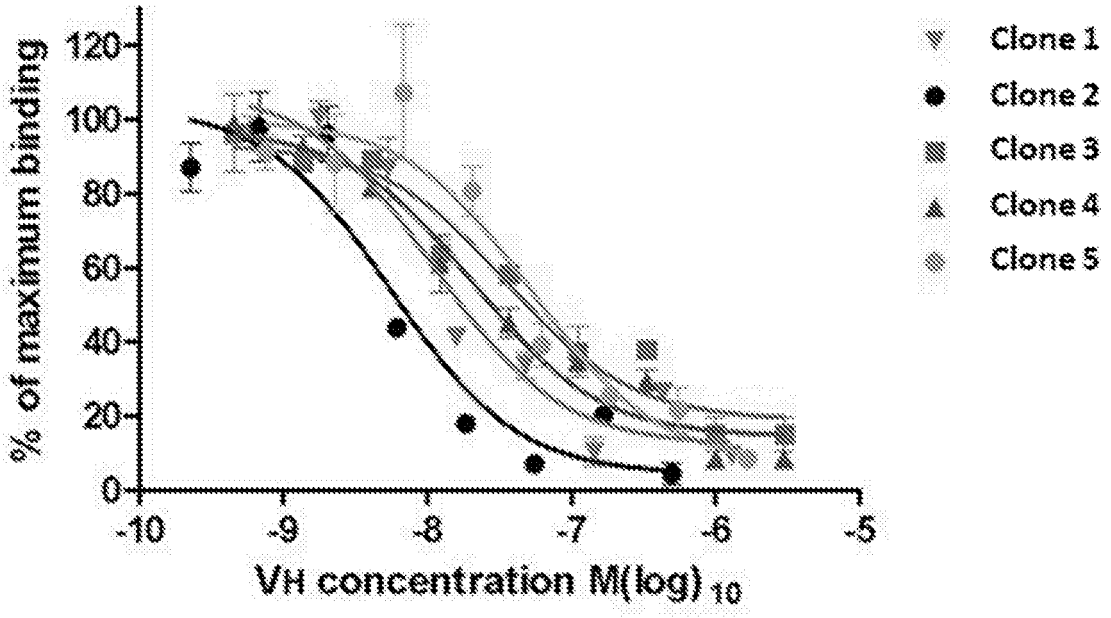
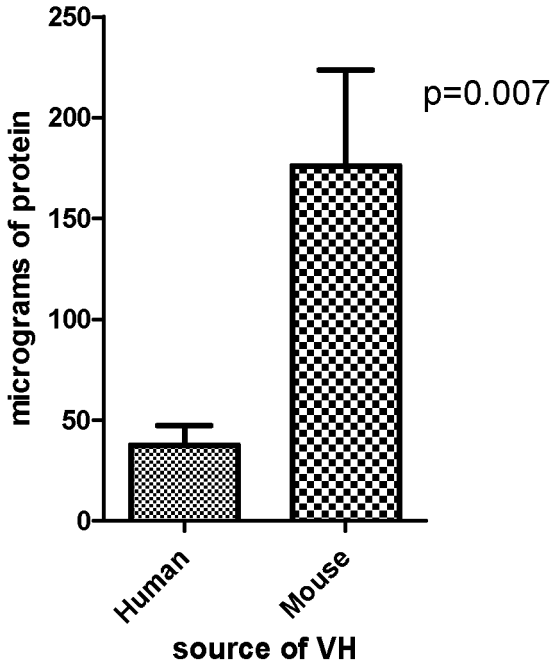
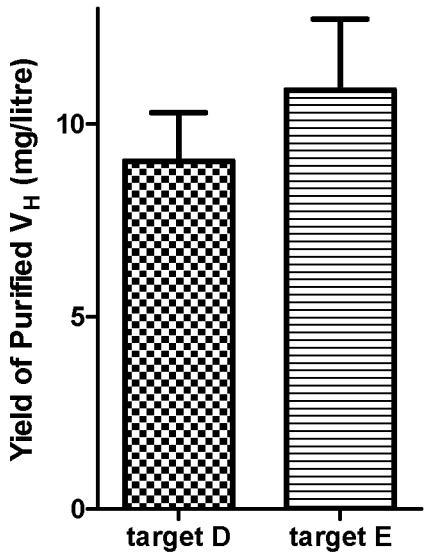

% of maximum binding
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
VH concentration M(log)10
Clone 1
Clone 2
Clone 3
Clone 4
Clone 5

Figure 29


250
200
150
100
50
0
micrograms of protein
p=0.007
Human
Mouse
source of VH

a.


10
5
0
Yield of Purified V_H (mg/litre)
target D
target E

b.

Figure 30

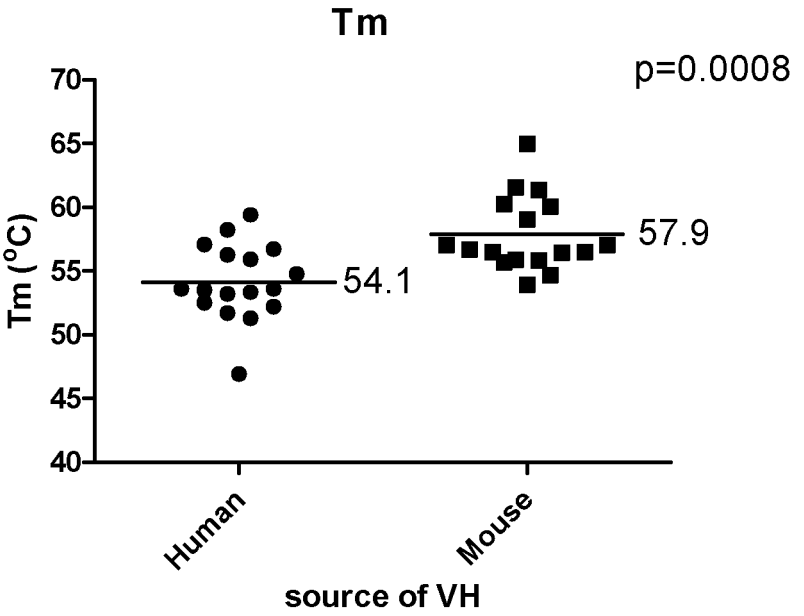


Figure 31

a

b

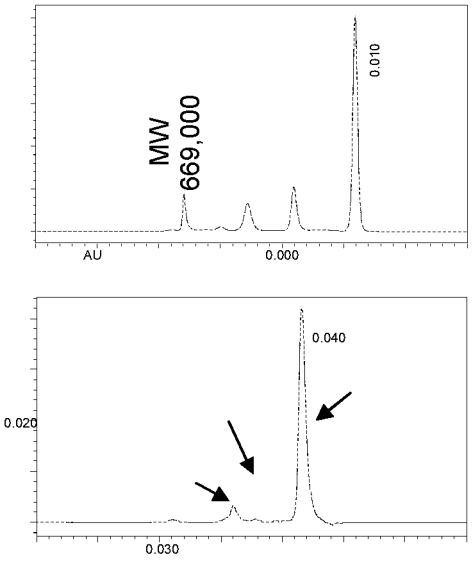


Figure 32

c

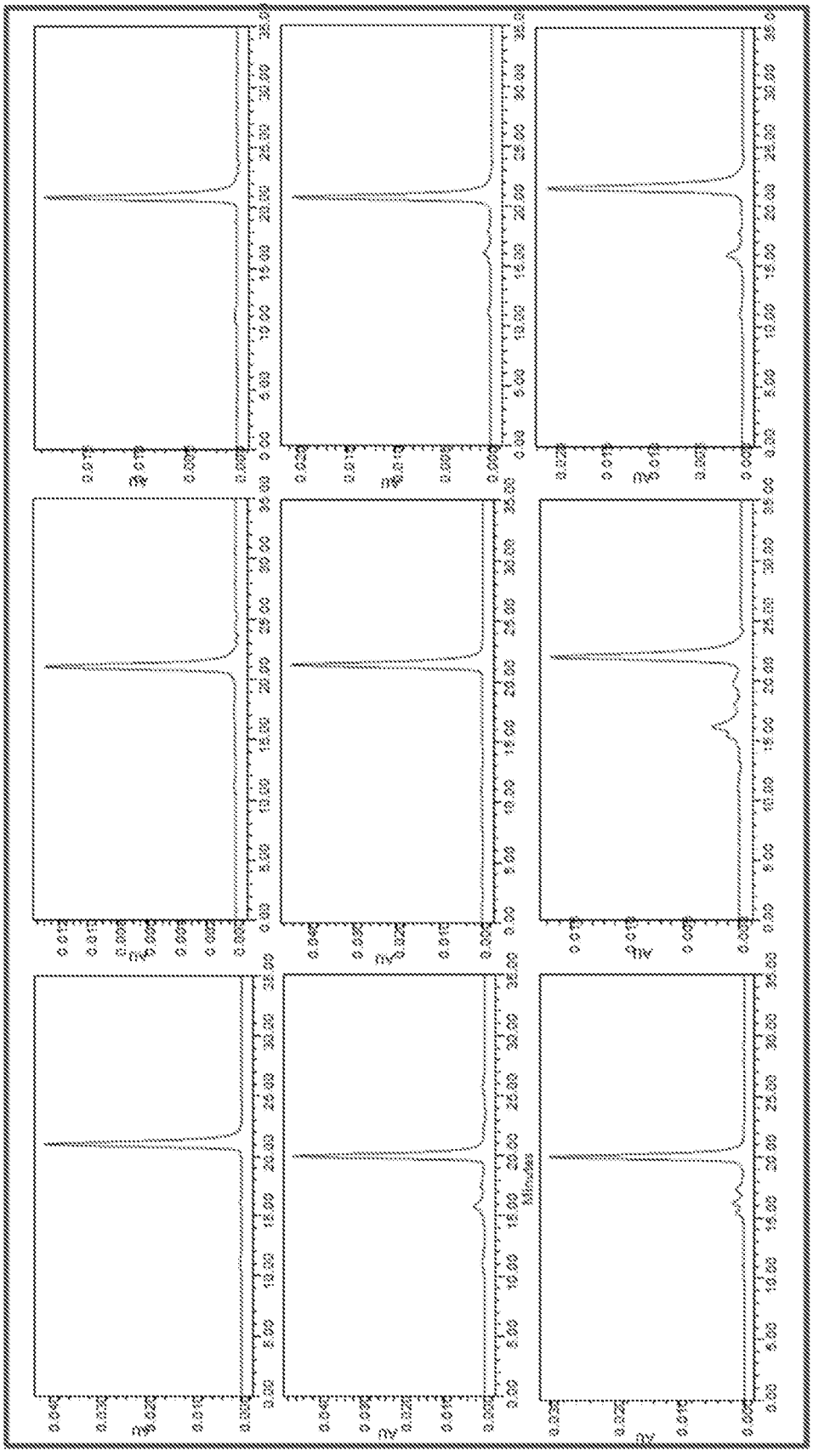


Figure 32 cont.

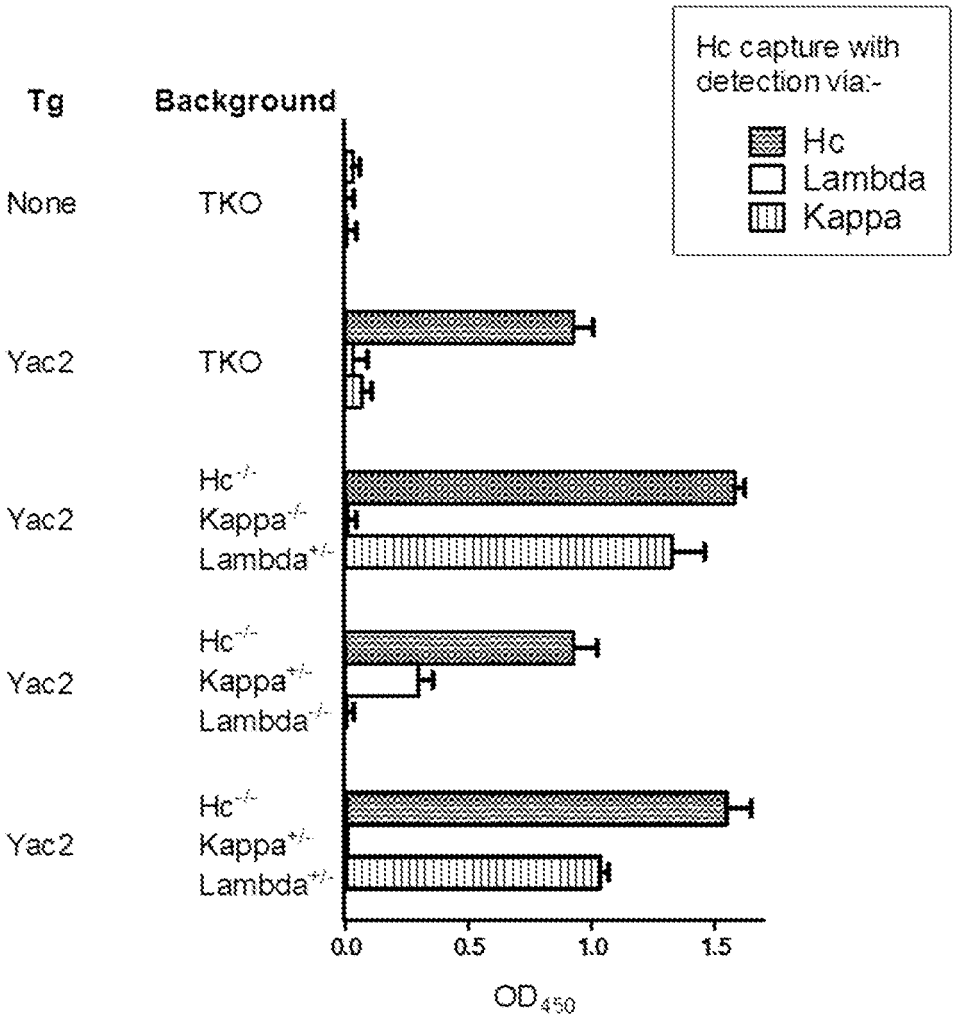

Hc capture with detection via:-
Hc
Lambda
Kappa
Tg
Background
None
TKO
Yac2
TKO
Yac2
Hc-/-
Kappa-/-
Lambda+/-
Yac2
Hc-/-
Kappa+/-
Lambda-/-
Yac2
Hc-/-
Kappa+/-
Lambda+/-
0.0
0.5
1.0
1.5
OD450

Figure 33

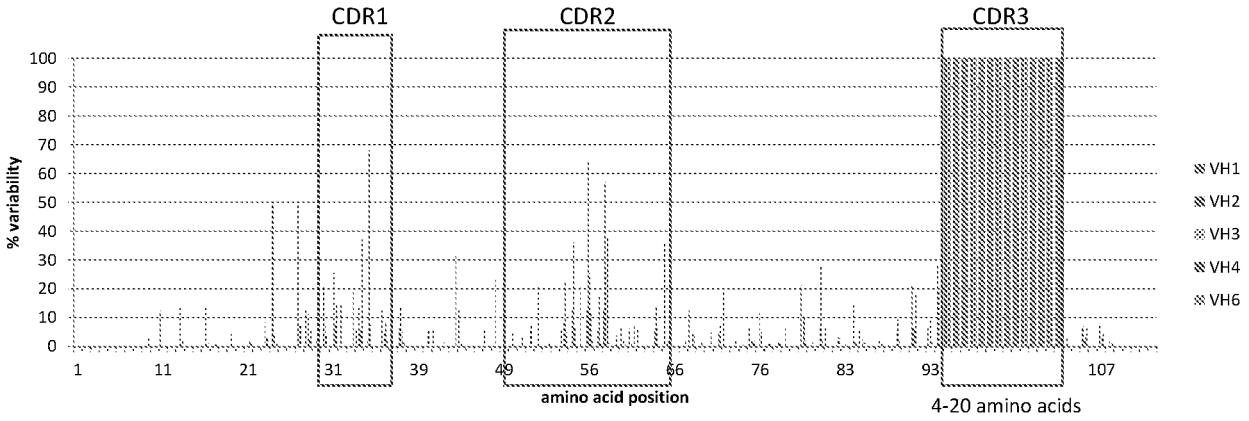

CDR1
CDR2
CDR3
100
90
80
70
60
50
40
30
20
10
0
% variability
1
11
21
31
39
49
56
66
76
83
93
107
amino acid position
4-20 amino acids
VH1
VH2
VH3
VH4
VH6

Figure 34

i.)

VH	Library size
VH1	1.32×10^{10}
VH2	4.7×10^{9}
VH3	1.34×10^{10}
VH4	2.6×10^{9}
VH6	4.2×10^{8}
TOTAL	3.81×10^{10}

ii.)

VH family	# clones analysed	number of times sequence occurs							
		1 x	2 x	3 x	4 x	5 x	6 x	7 x	8 x	9 x
VH1	51	49	1	0	0	0	0	0	0	0
VH2	59	41	1	1	1	0	0	0	0	1
VH3	82	74	4	0	0	0	0	0	0	0
VH4	73	67	3	0	0	0	0	0	0	0
VH6	70	61	2	0	0	1	0	0	0	0

iii.)

Figure 35