

Three basic vector backbones

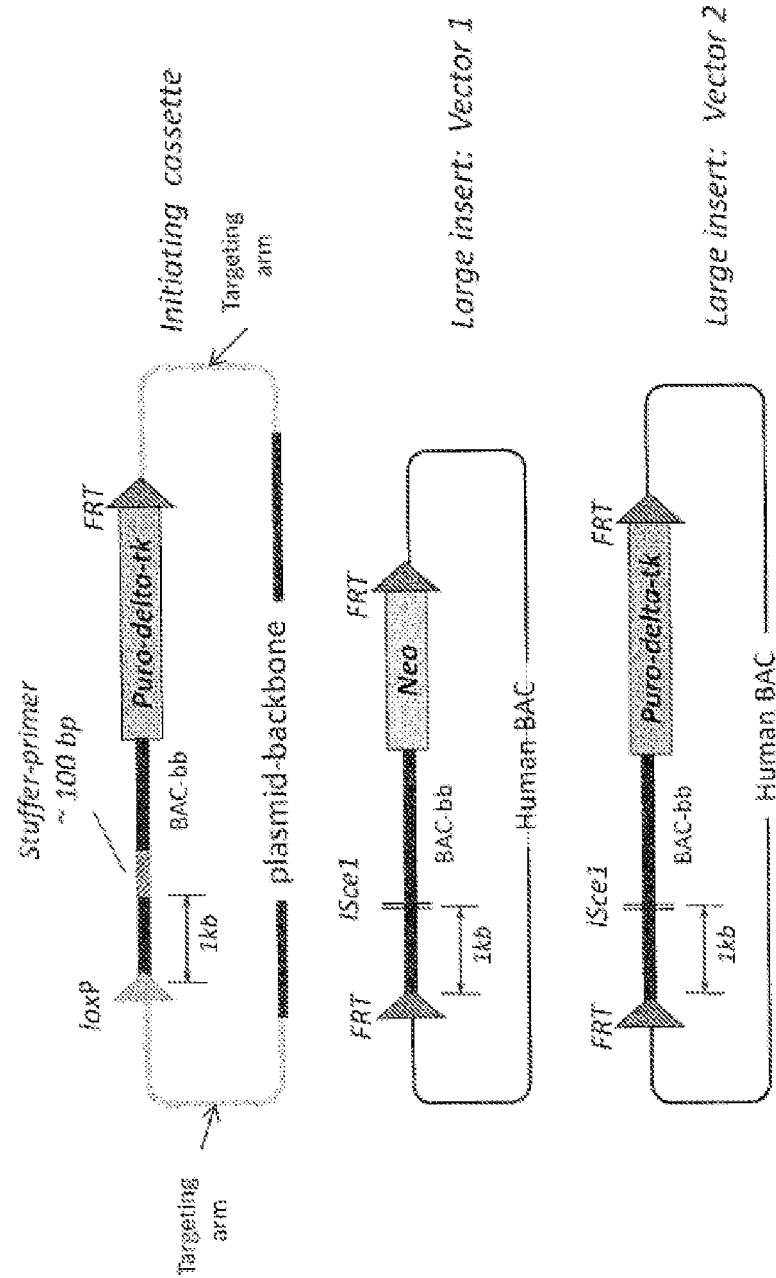
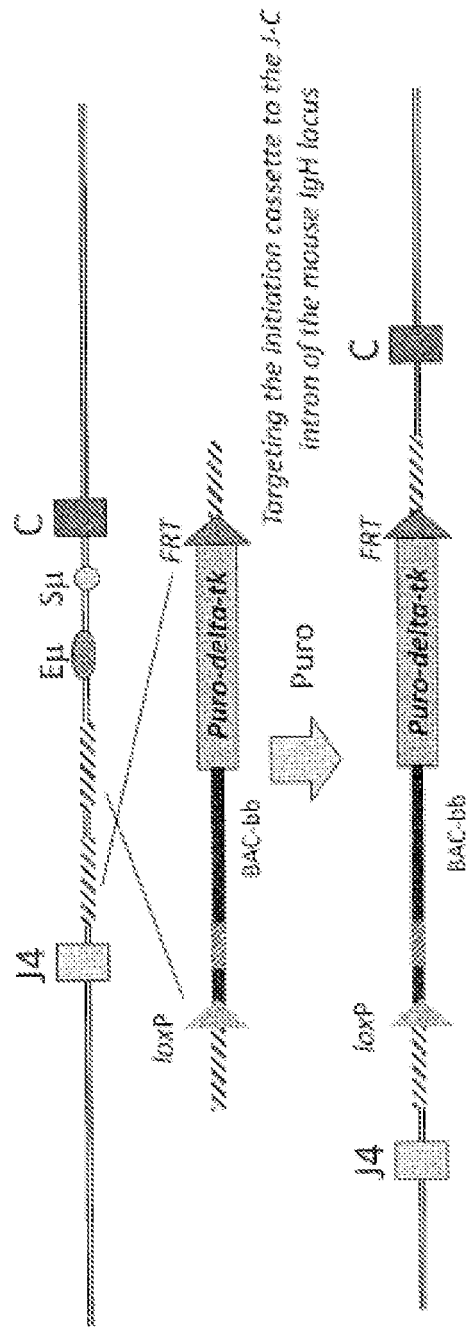


Fig. 1

Entry / Initiation vector: Step 1



Validation:

1. Standard gene-targeting LR-PCR/Southern
2. Make chimaeras & breed in parallel with next step

Fig. 2

Addition vector: Step 2

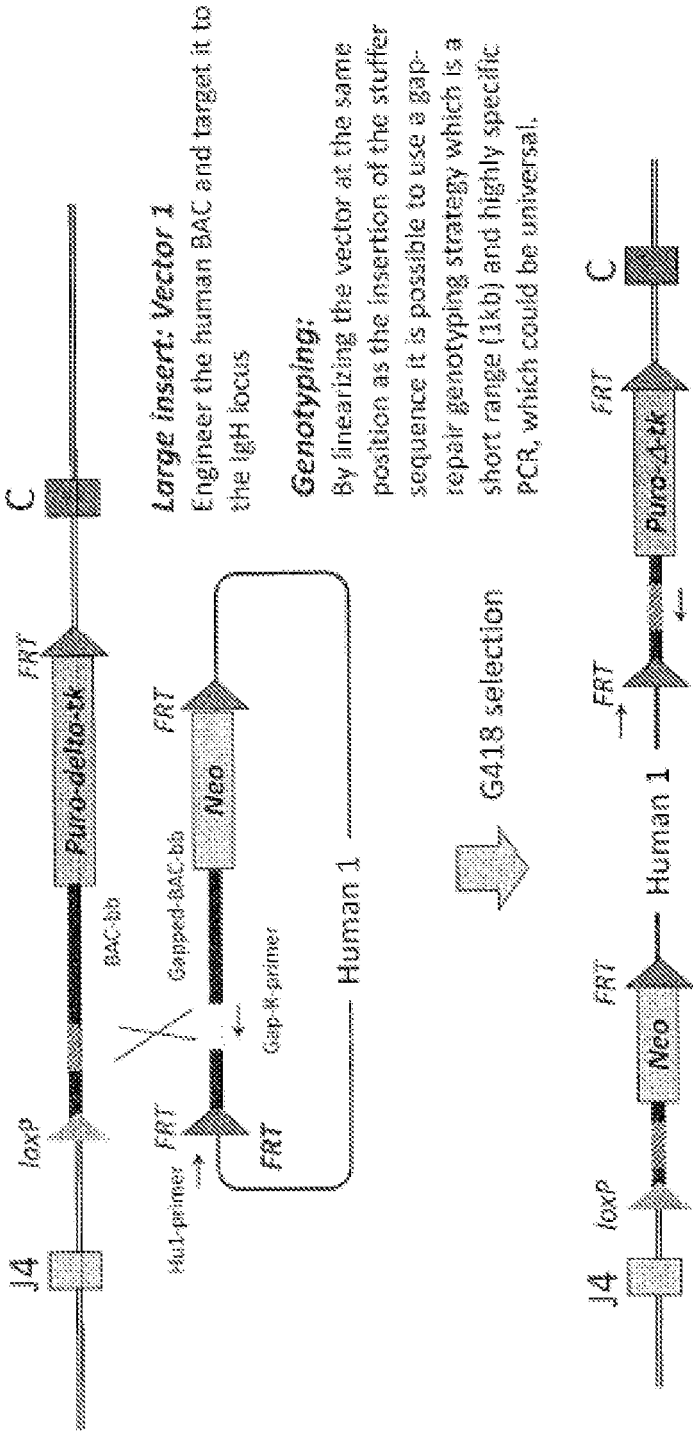


Fig. 3

Addition vector: Step 2

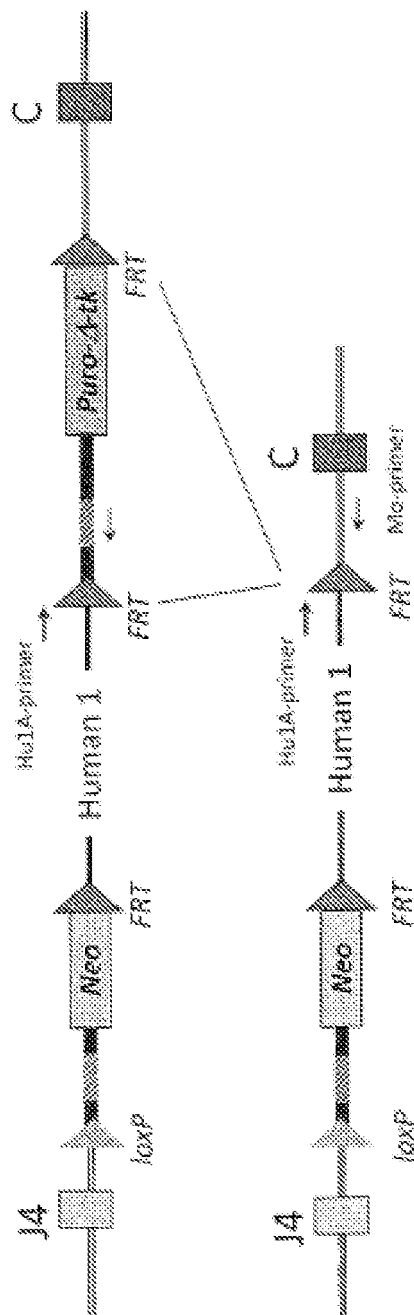


Fig. 4

Addition vector: Step 3A

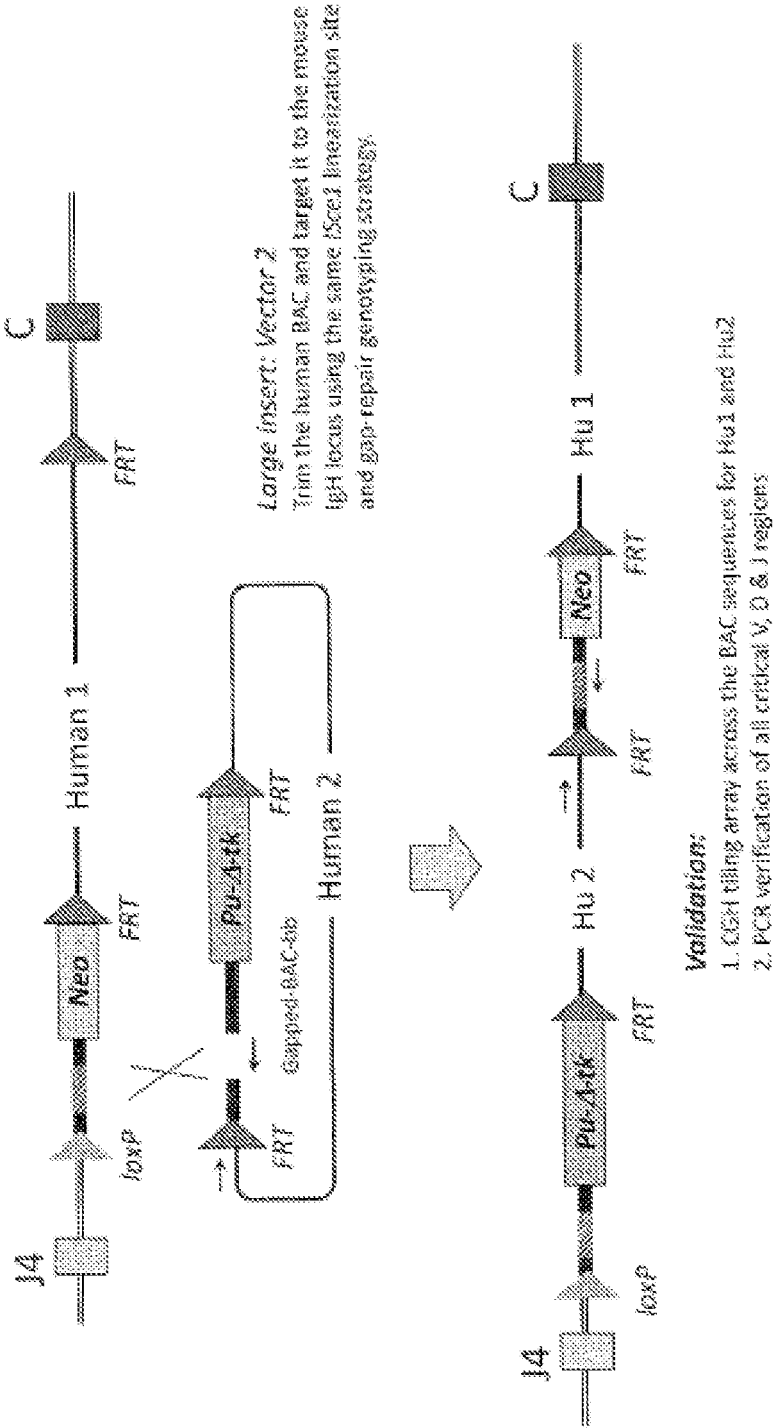


Fig. 5

Addition vector: Step 3B

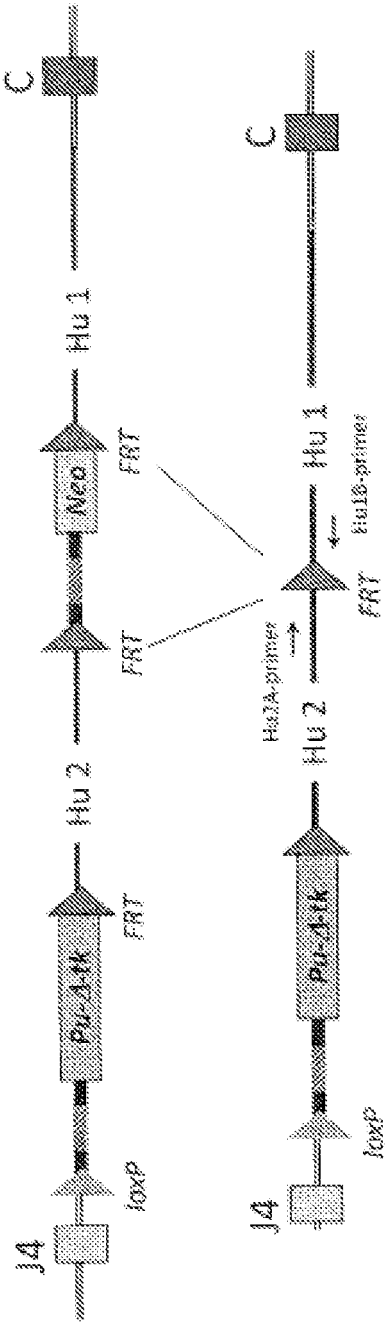
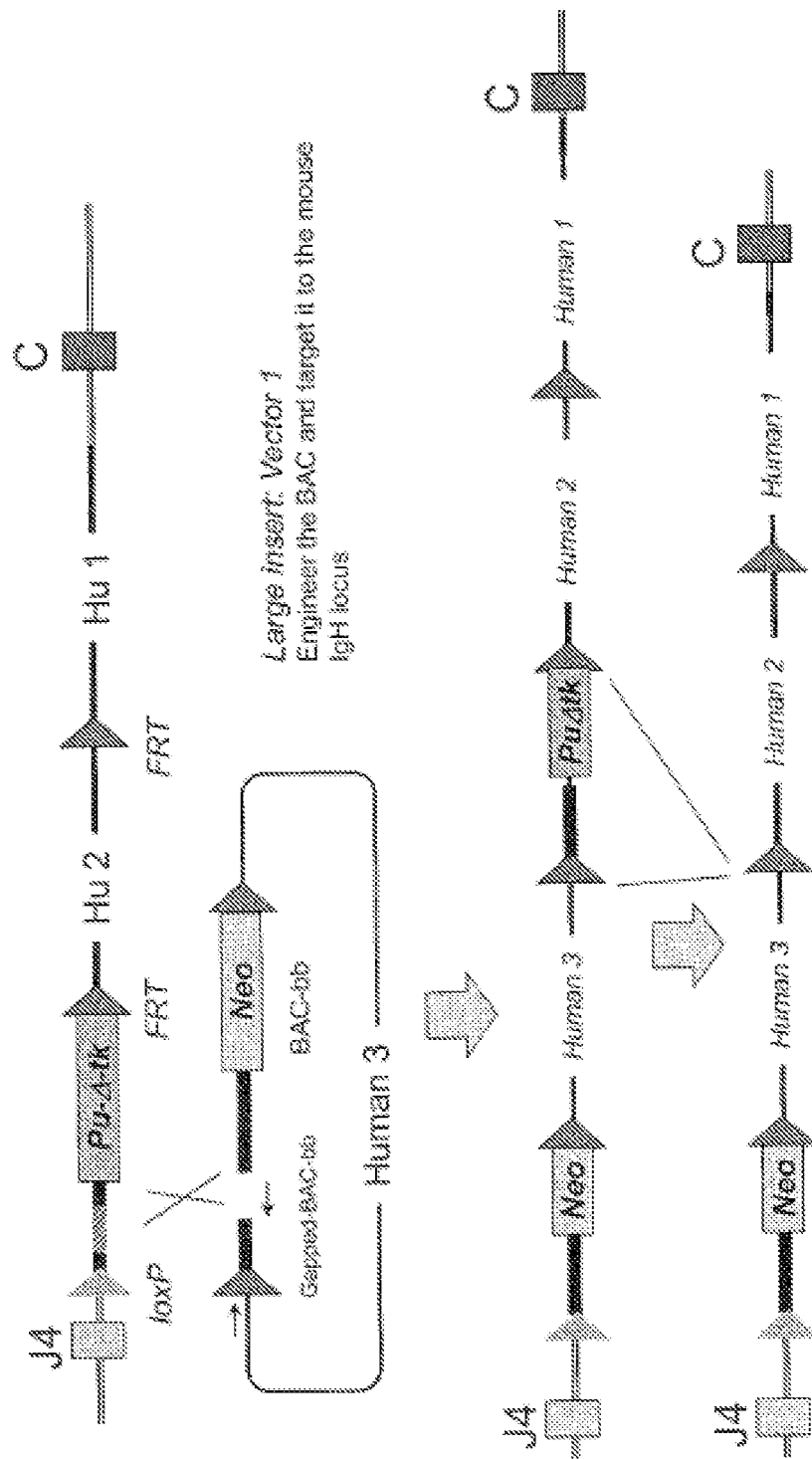


Fig. 6

Addition vector: Step 4



2

A humanized mouse *IgH* locus

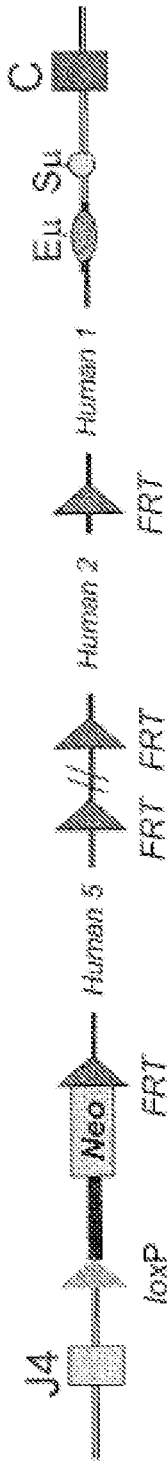


Fig. 8

BAC landing pad inserted at the IgH locus

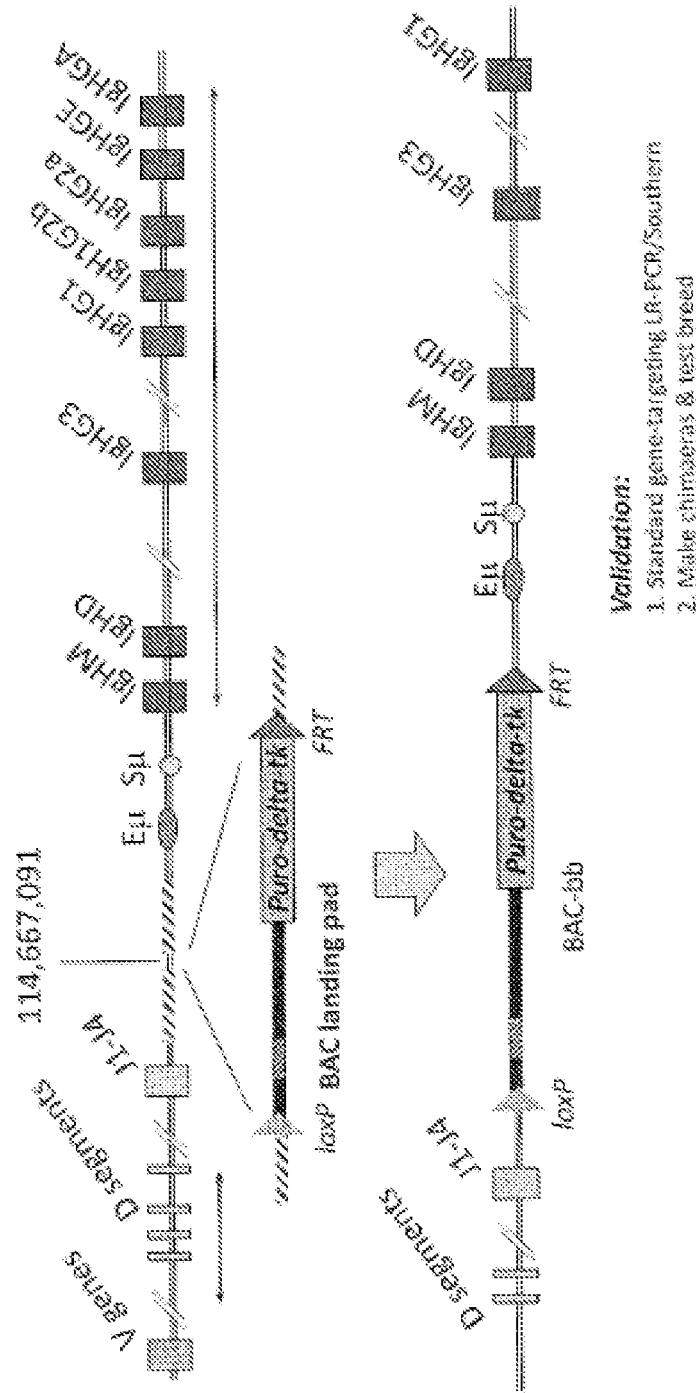
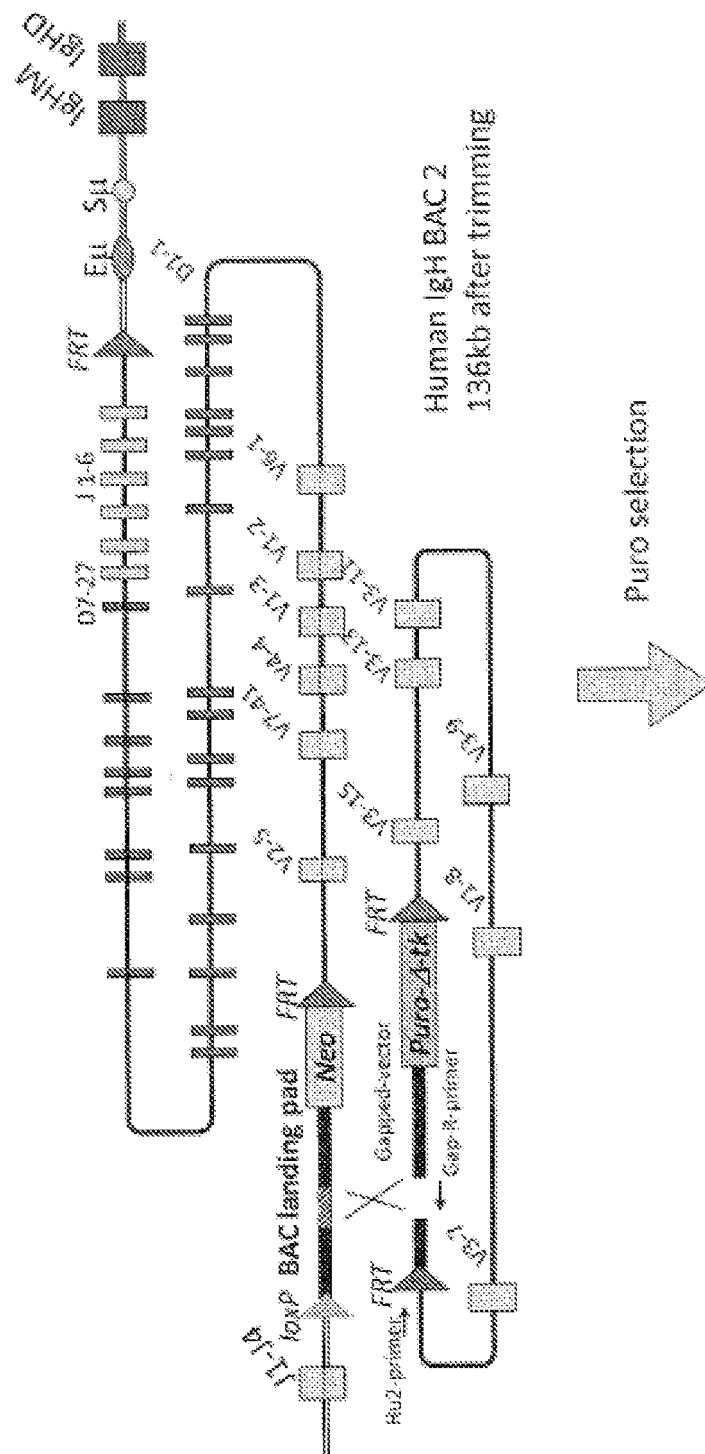


Fig. 9

2nd BAC insertion – Step 3



21.

BAC Landing Pad at Igk Locus

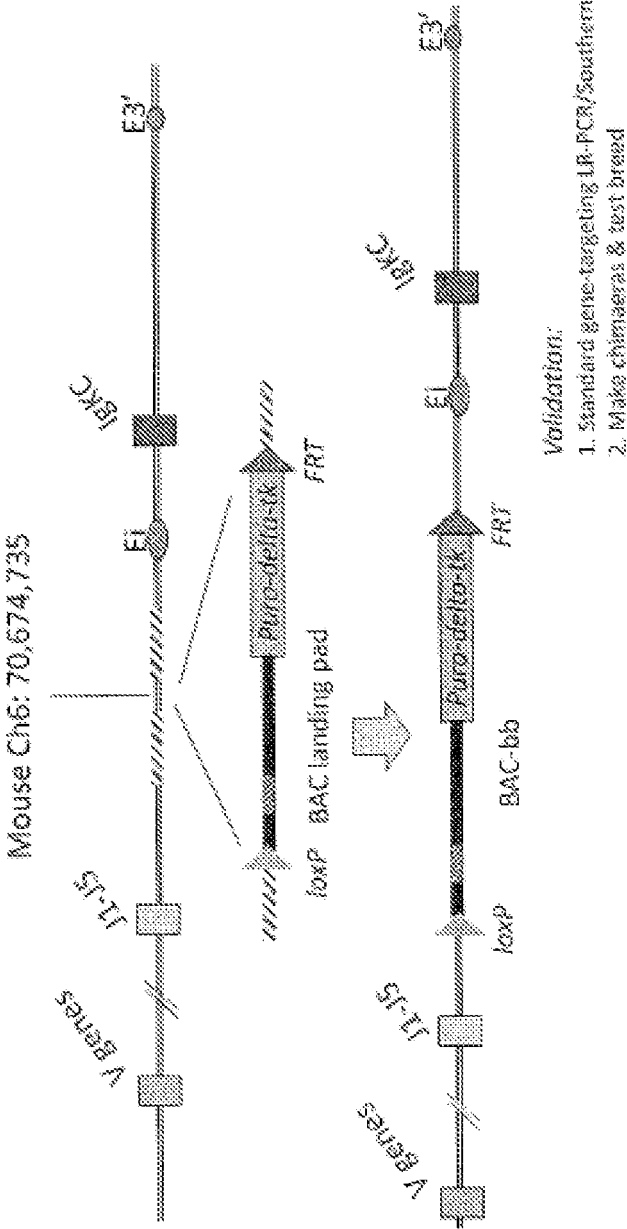
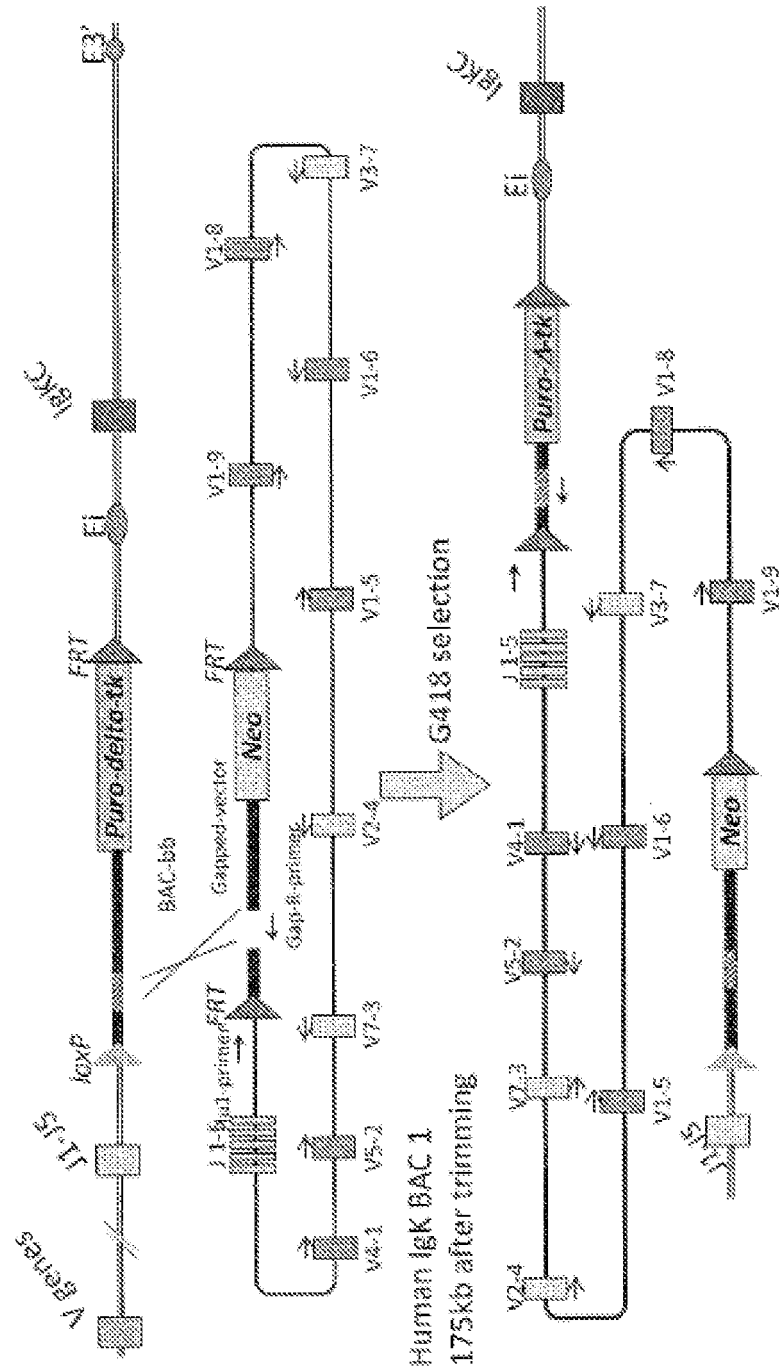


Fig. 14

1st BAC Insertion – Step 1



2nd BAC Insertion – Step 3

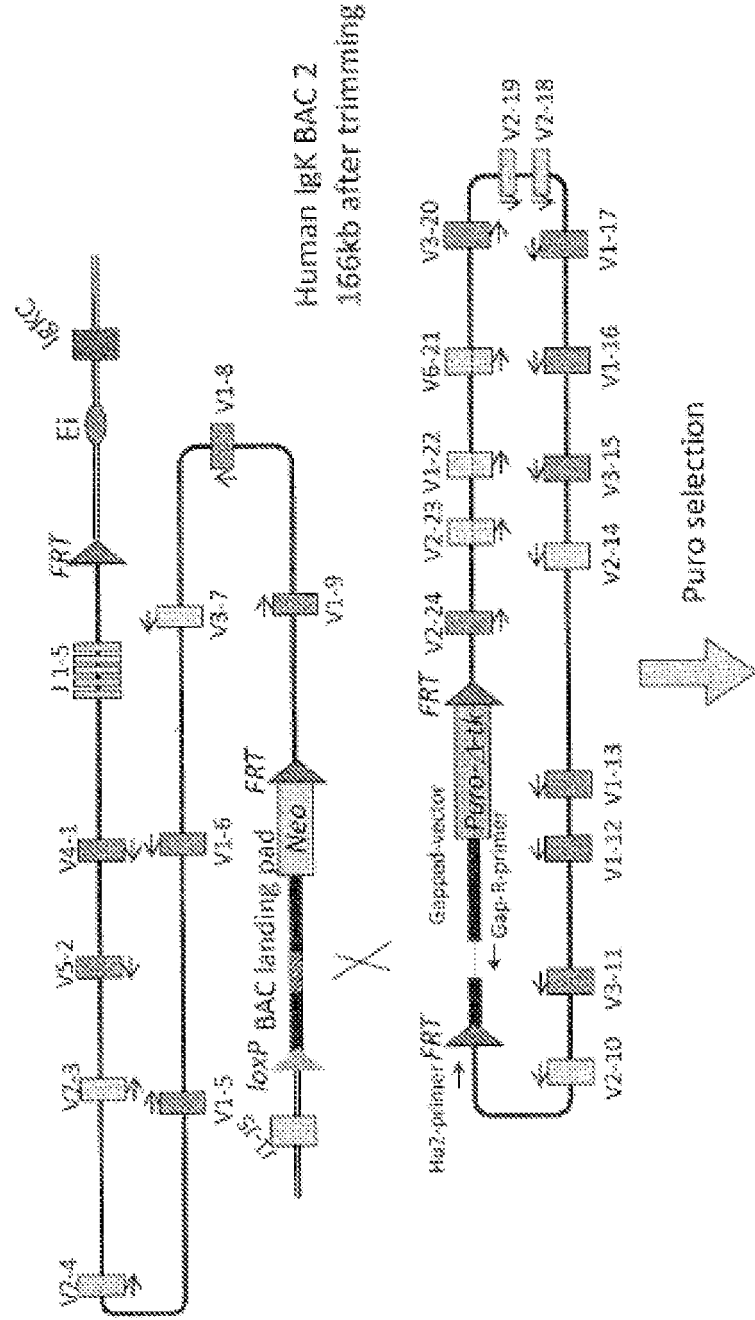


Fig. 17

Rapid Testing of ES Cells with Humanized
IgH and IgK Loci

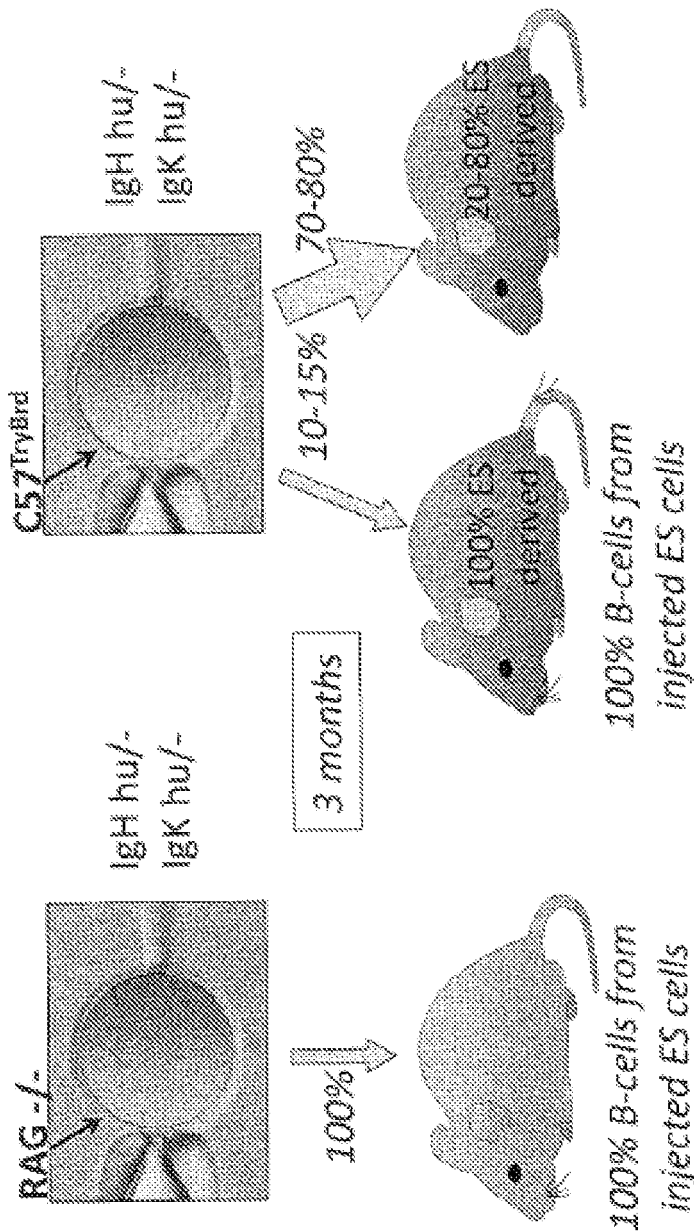


Fig. 19

Antibody Generation in Chimaeric Mice
with Humanized IgH and IgK Loci

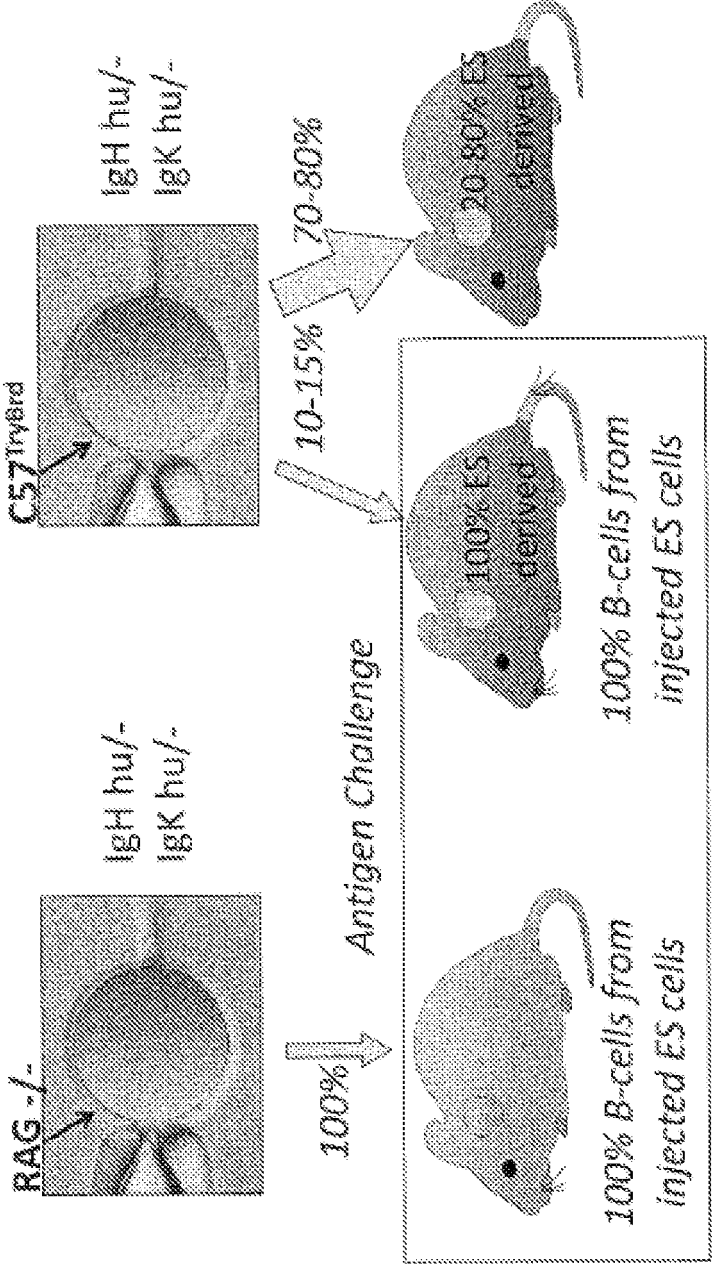


Fig. 20

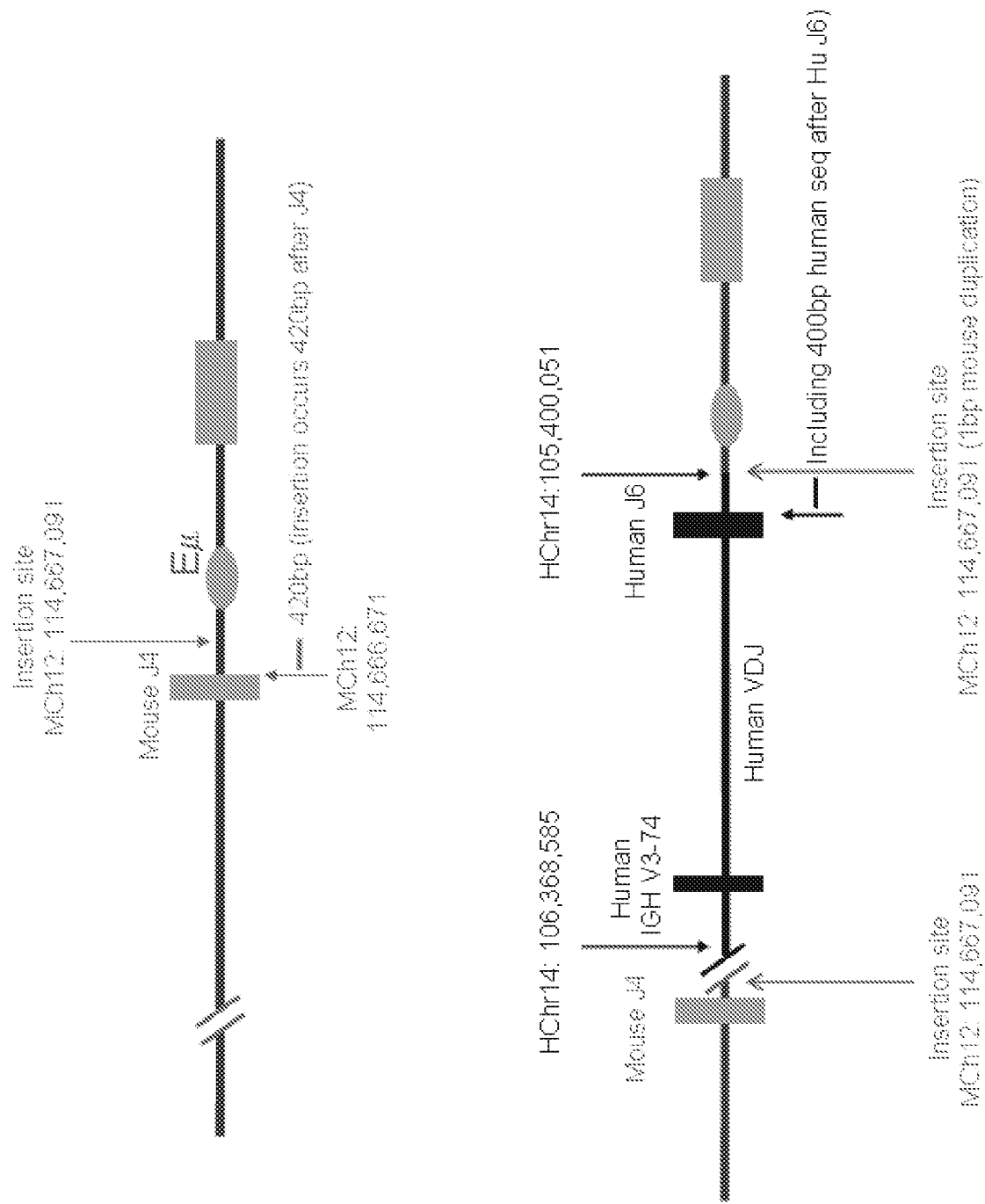


Fig. 21

Targeting of the Landing Pad

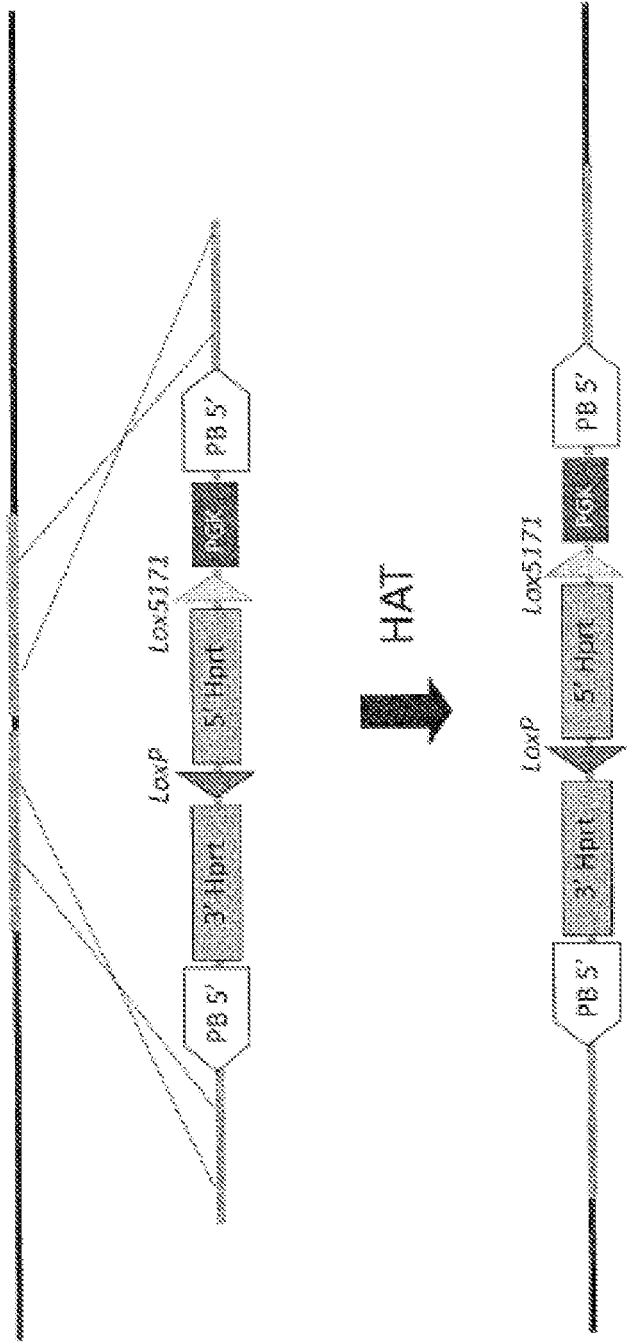


Fig. 22

Insertion of the 1st BAC

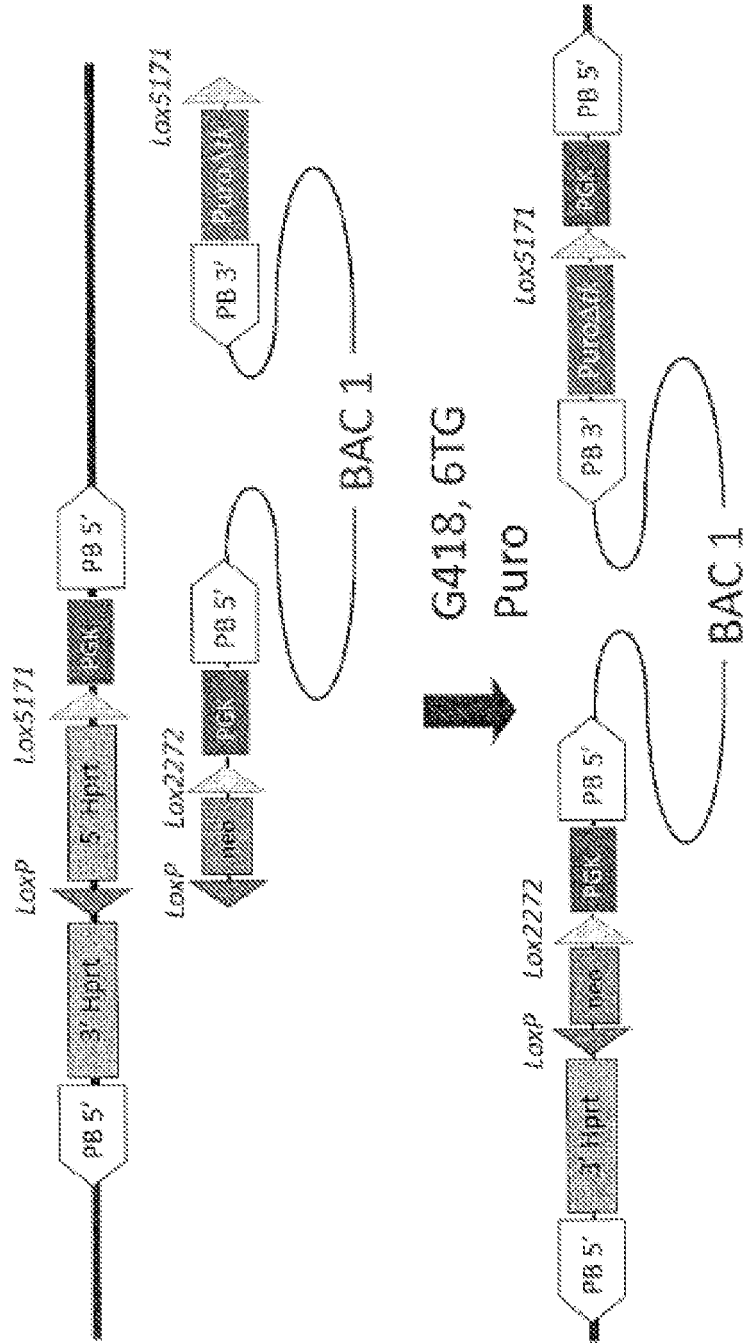


Fig. 23

Curing of the 3' Modification

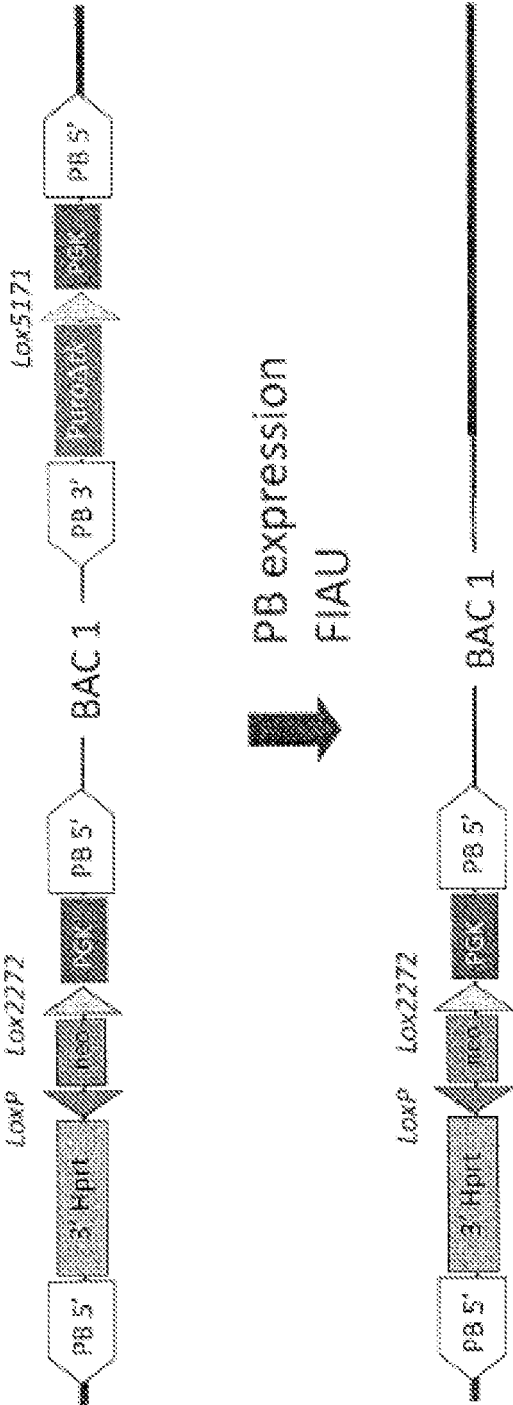


Fig. 24

Insertion of the 2nd BAC

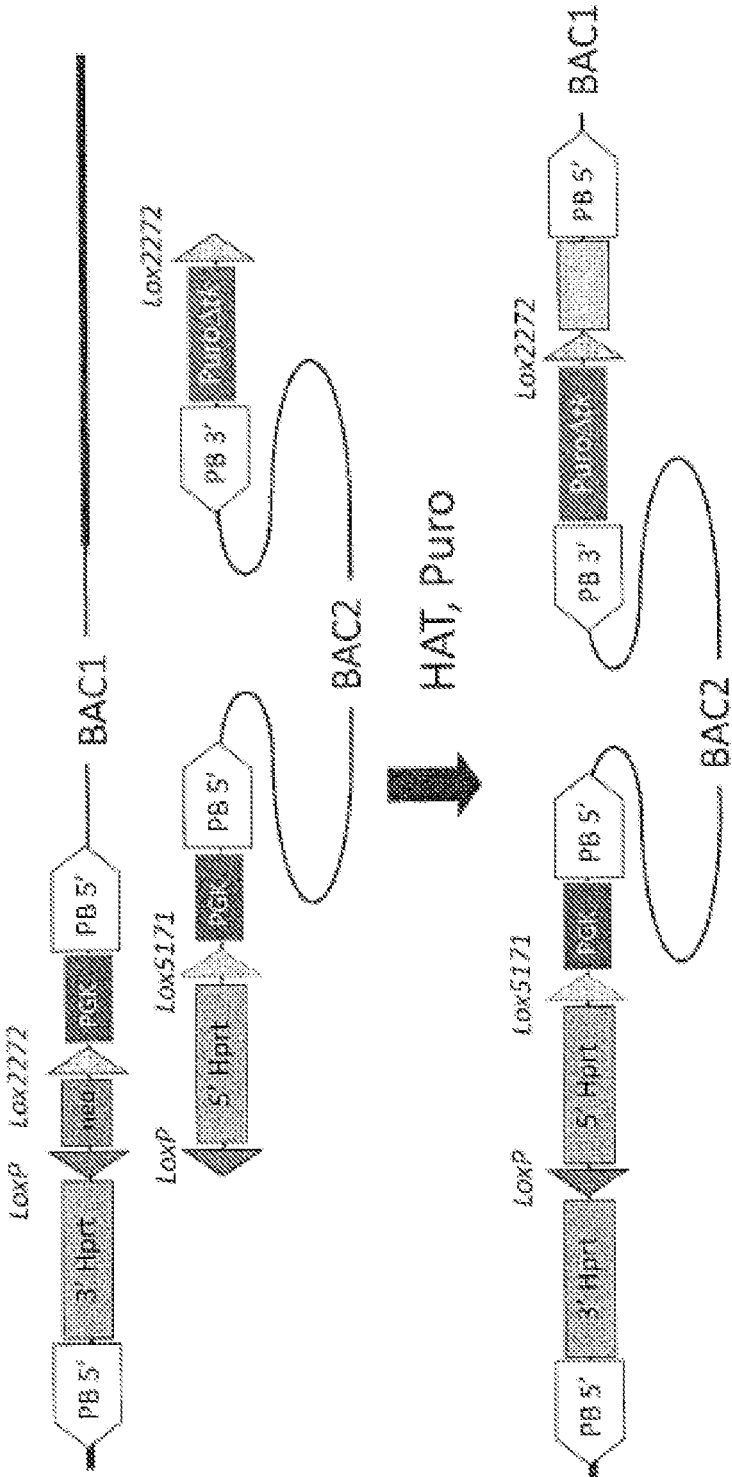


Fig. 25

Removing the 3' Modification

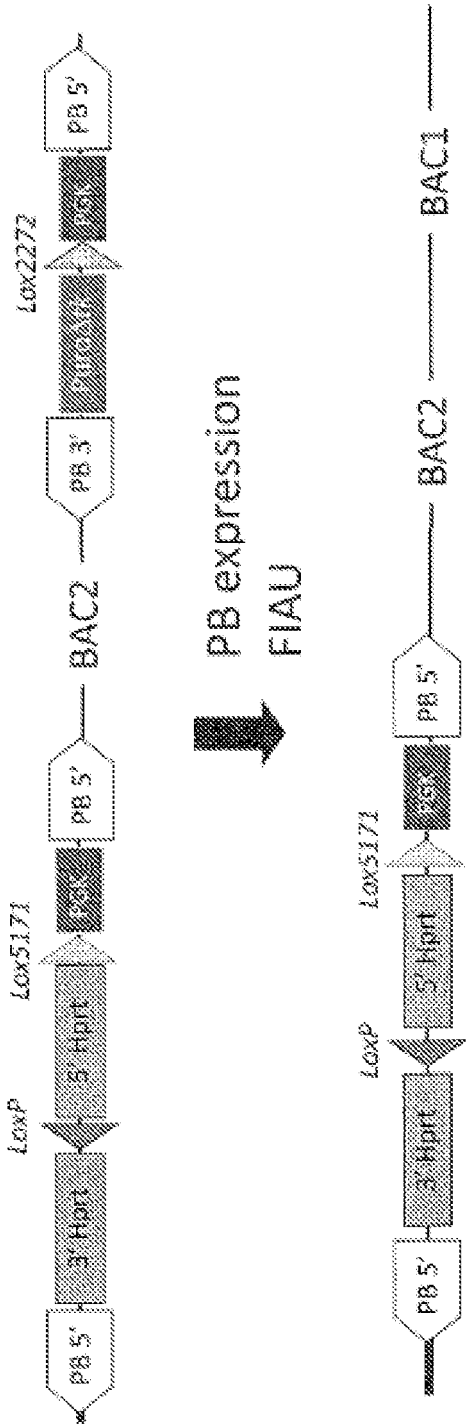


Fig. 26

Targeting the “Flip-Over” Vector

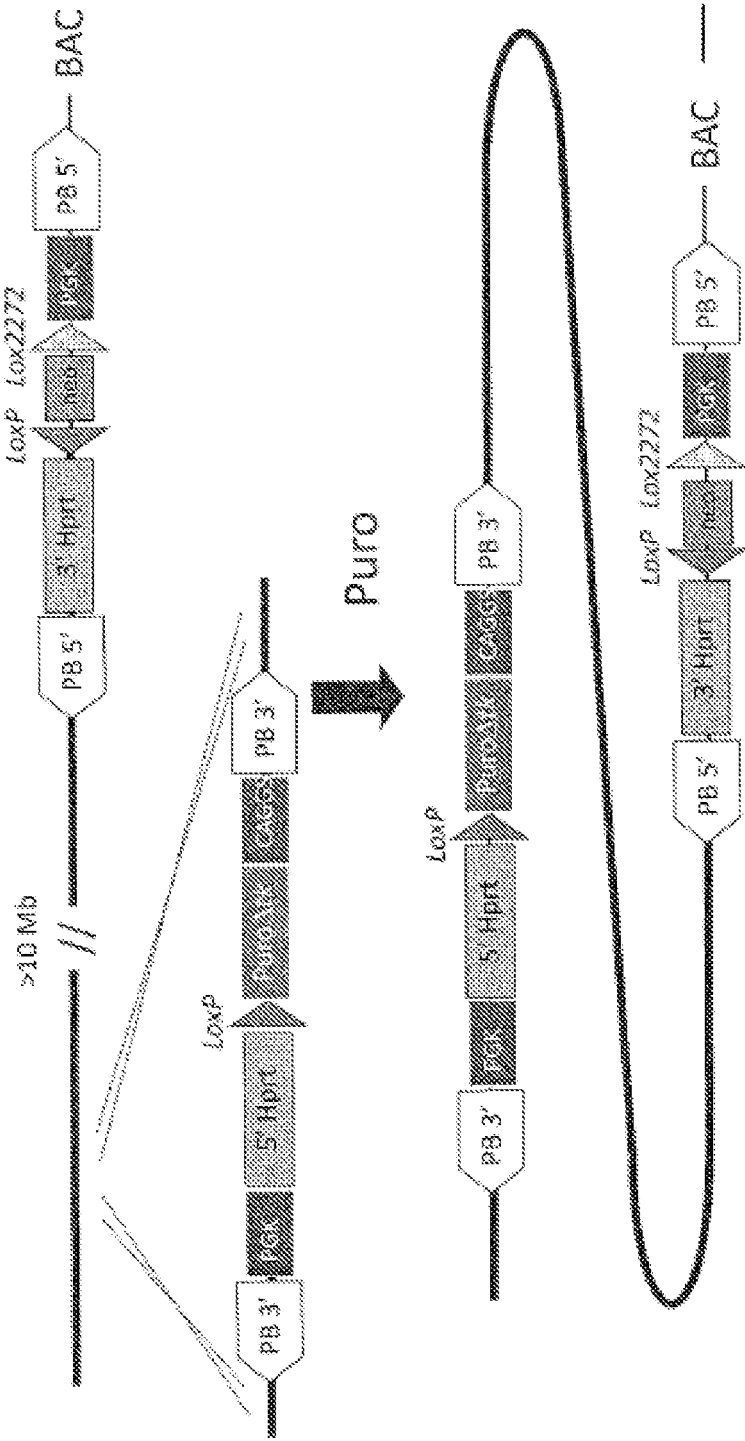


Fig. 27

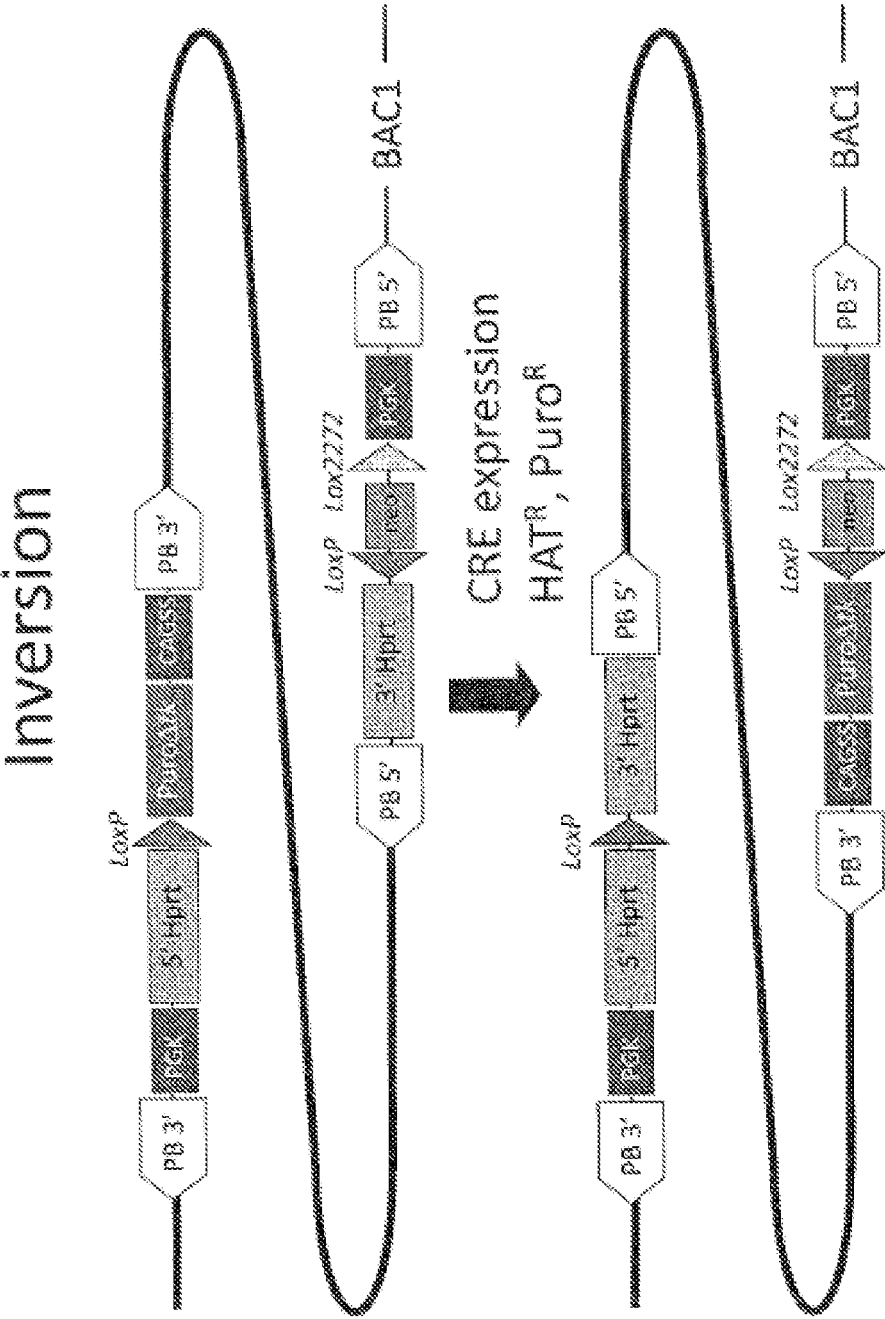


Fig. 28

Removing All Modifications

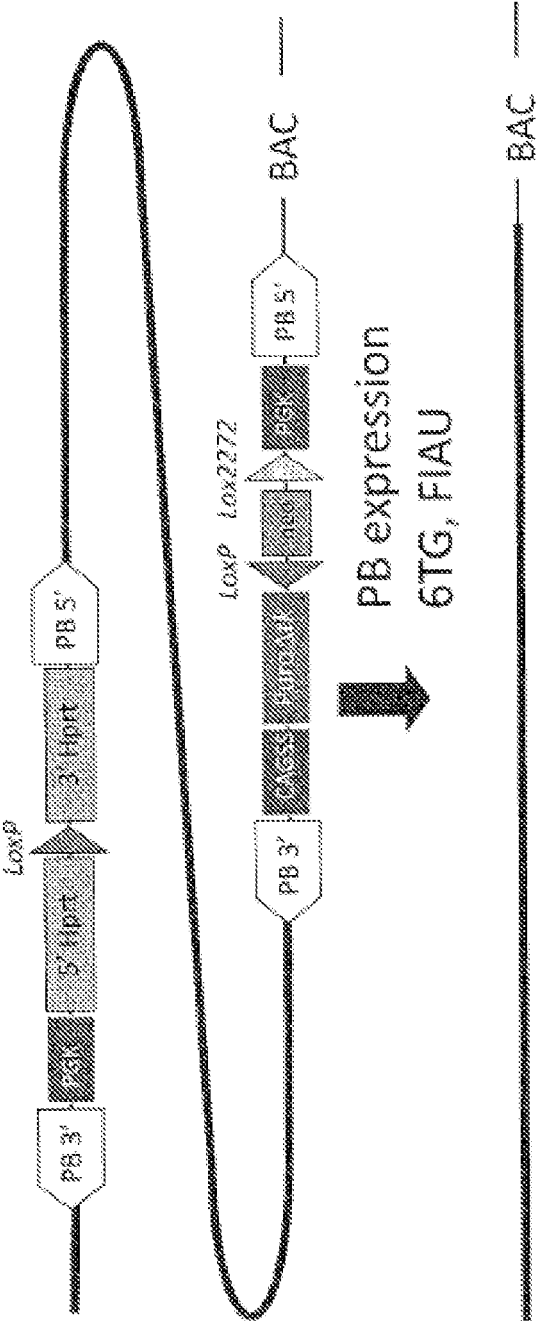
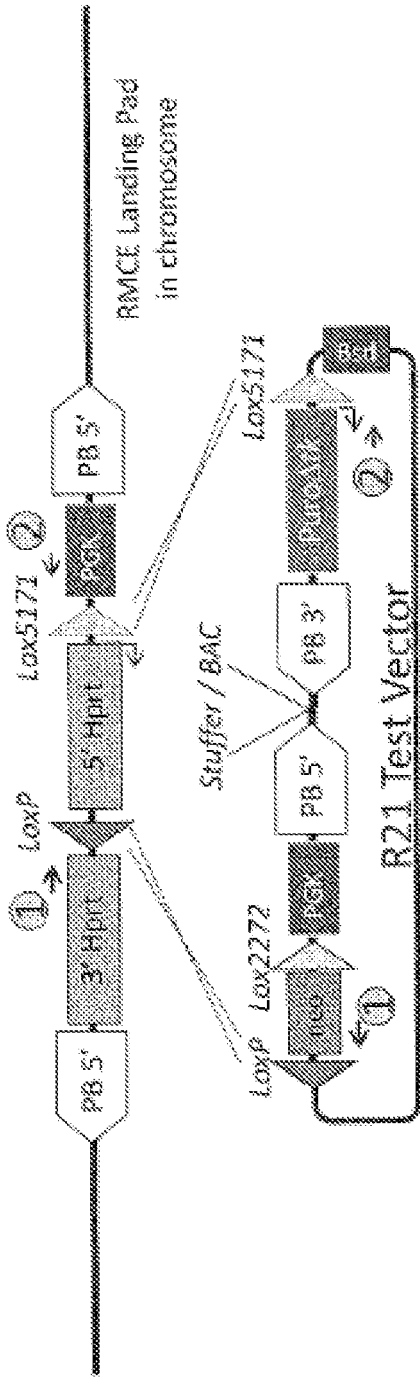


Fig. 29

Sequential RMCE – Integration into Landing Pad



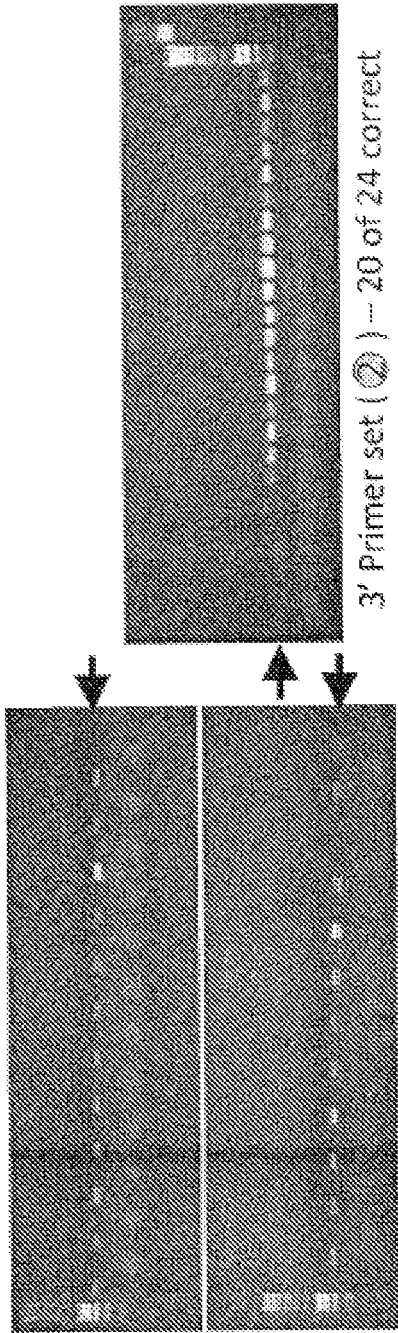
R21 transfected w/ Cre-expression Cassette

Selection:	Puro	Puro + 6TG	G418	G418 + 6TG
# Colonies:	239	220	360	198

Fig. 30

Confirmation of Successful Insertion into Landing Pad

Colonies : 6-TG and Puro - Resistant



5' Primer set (①) – 23 of 24 correct

3' Primer set (②) – 20 of 24 correct

Summary:

Colony #	Puro + 6TG		Puro		G418 + 6TG		G418	
	220		239		198		360	
Junction PCR	5'	3'	5'	3'	5'	3'	5'	3'
	23/24	20/24	22/24	19/24	22/24	22/24	11/24	11/24

Fig. 31

PCR Confirmation of 3' End Curing

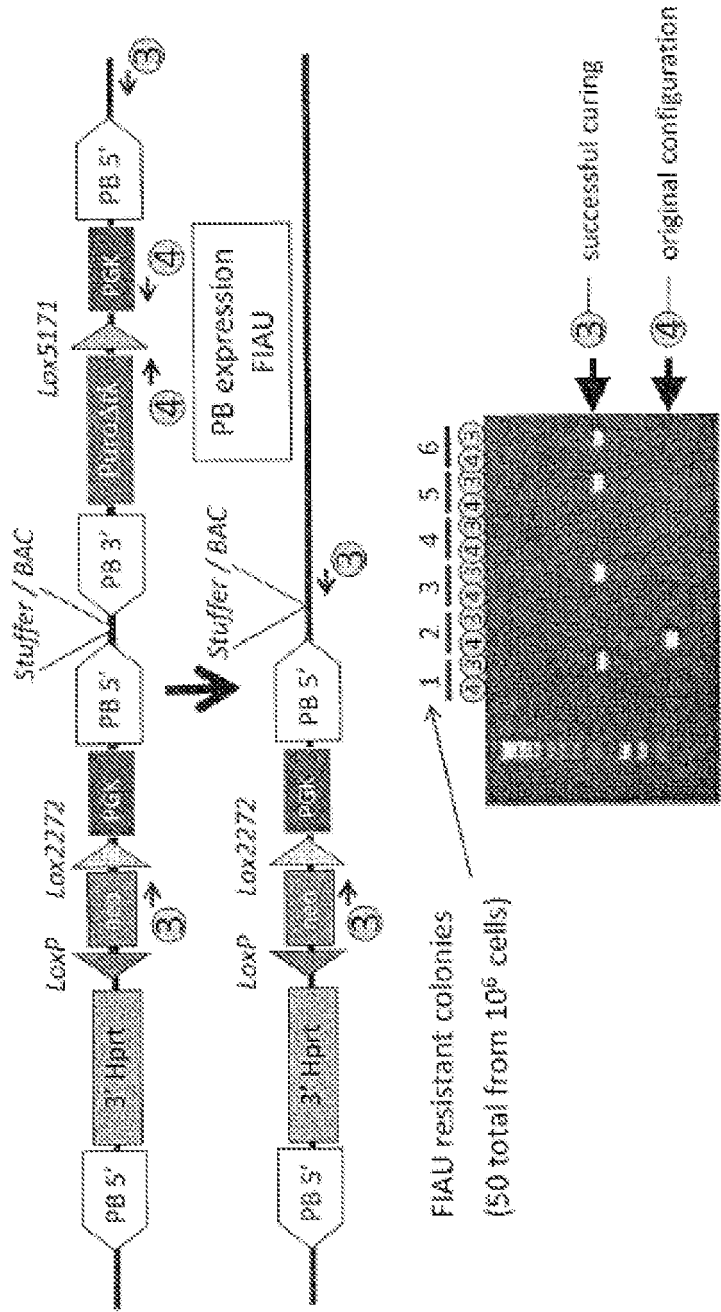


Fig. 32

Insertion of BAC#1 – PCR Diagnostics

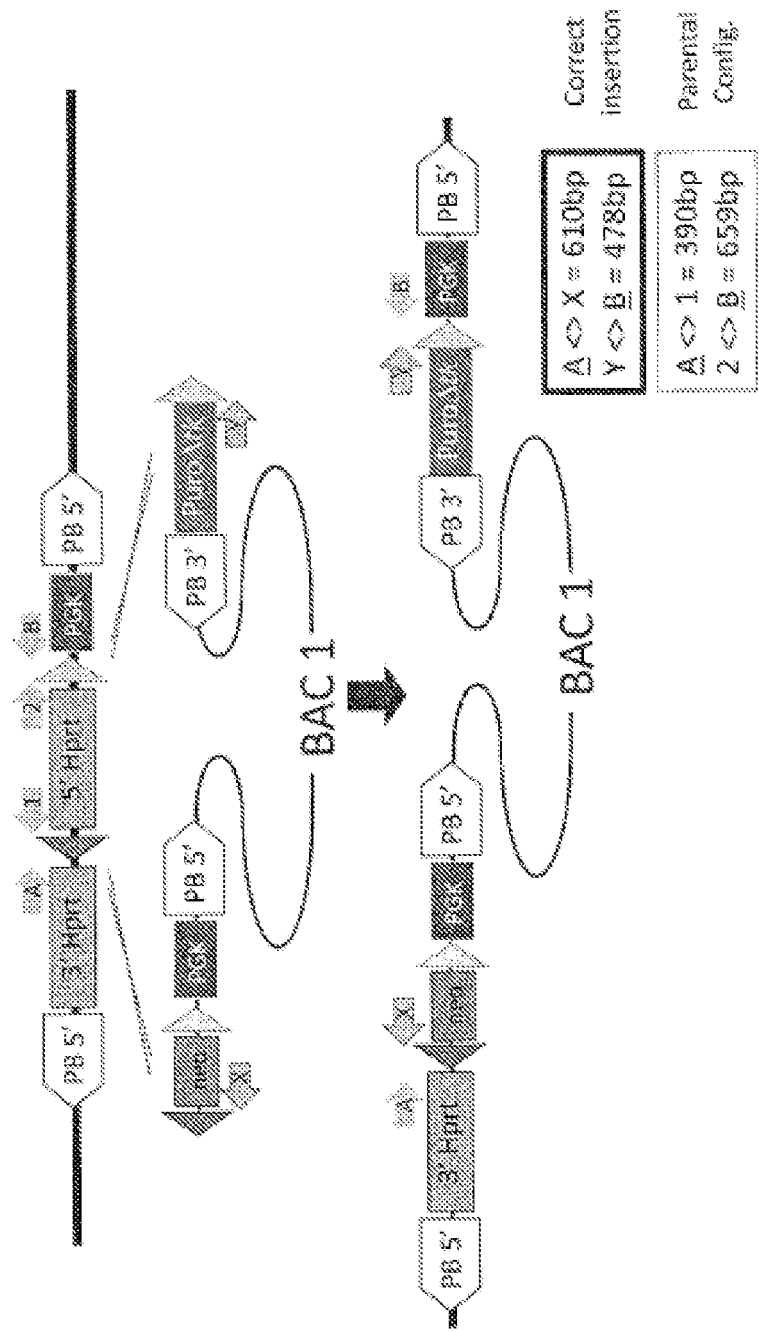
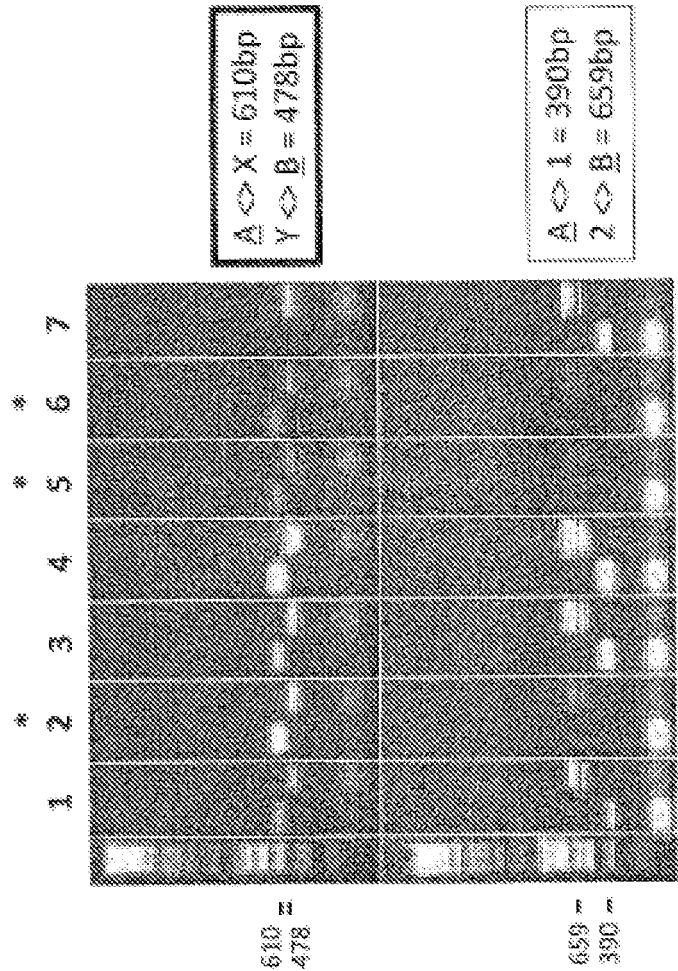


Fig. 33

Insertion of BAC#1 – PCR Diagnostics



*Clones # 2, 5 and 6 have BAC#1 inserted
Clones # 1, 3, 4 and 7 likely have BAC#1
inserted, but the samples are mixed with
parental cell lines.

Fig. 34

35/75

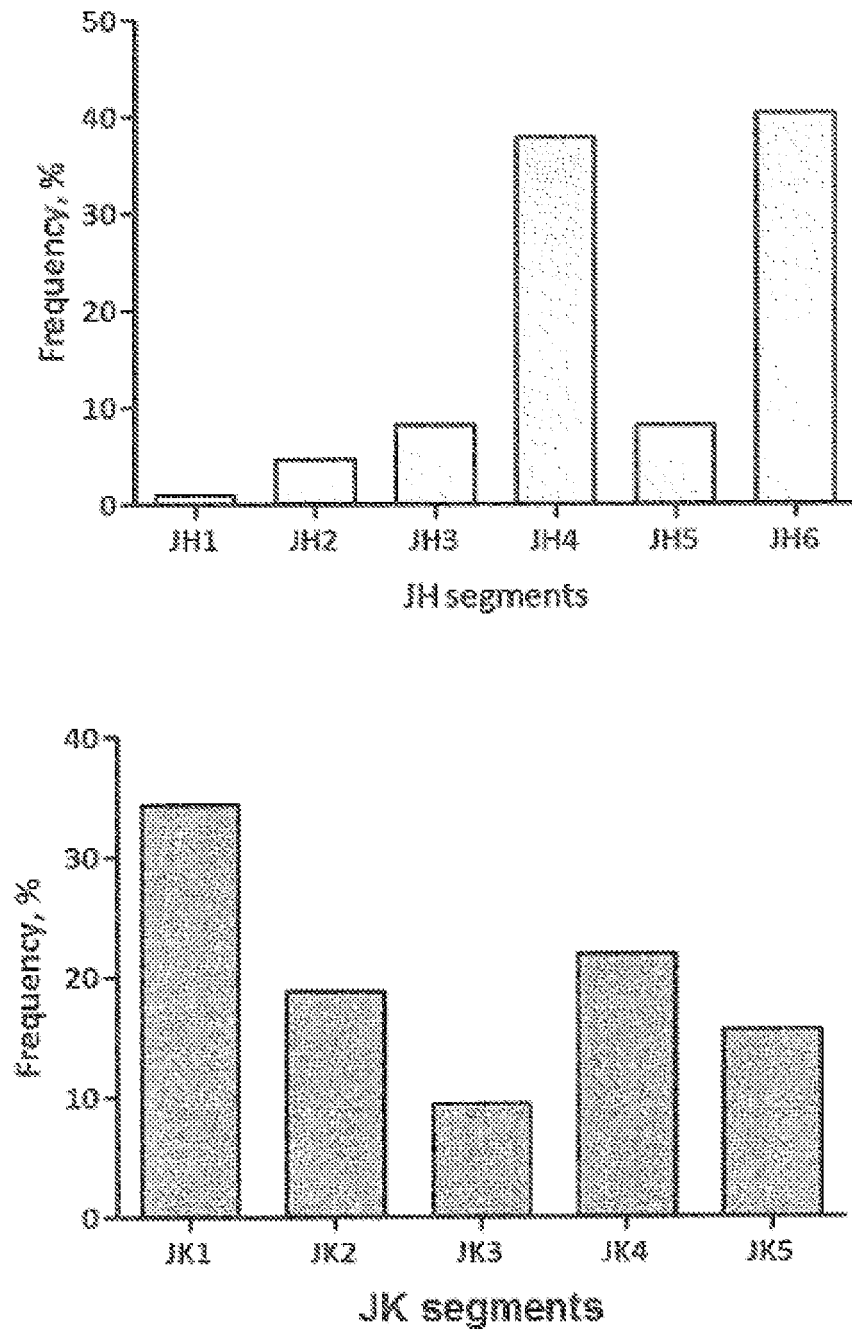


Fig. 35

36/75

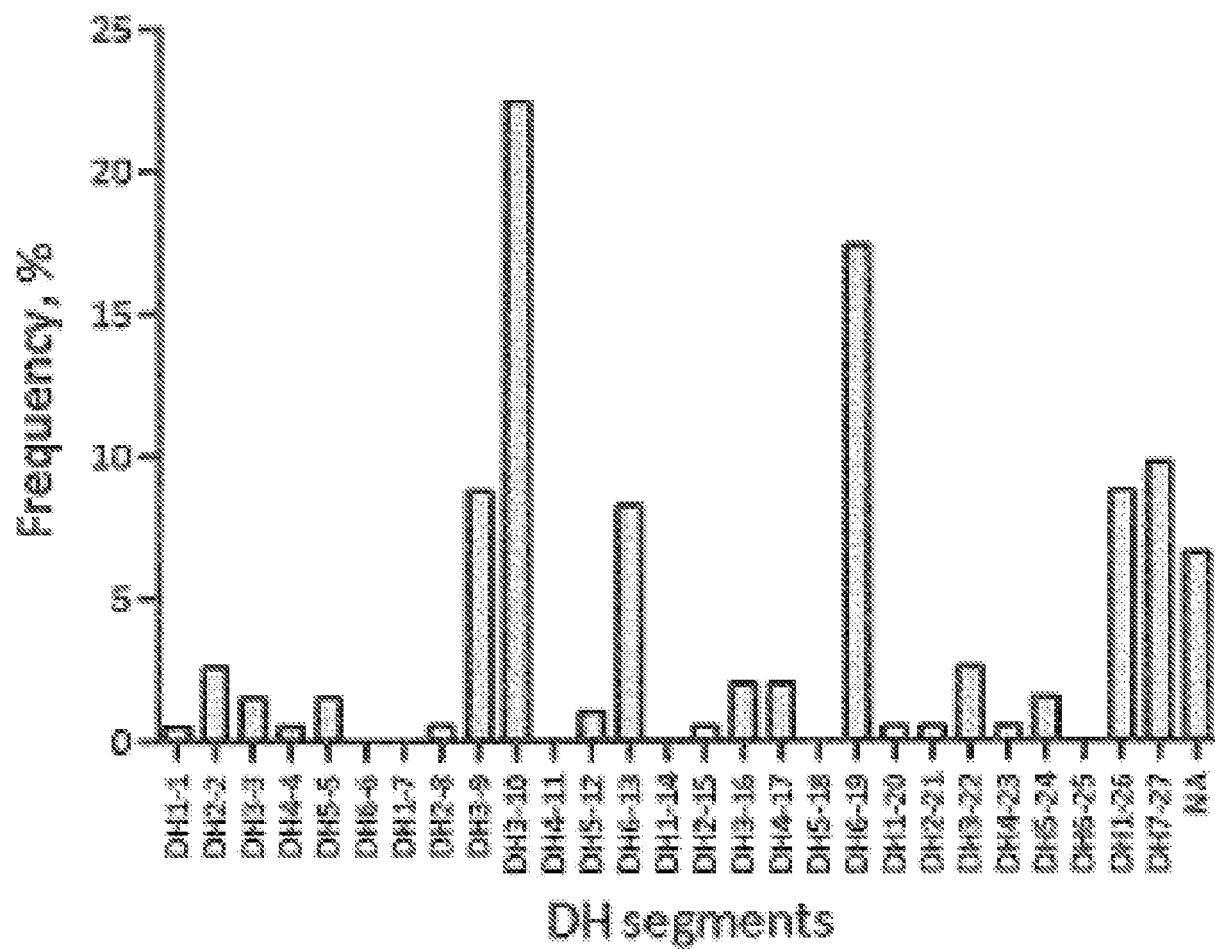


Fig. 36

37/75

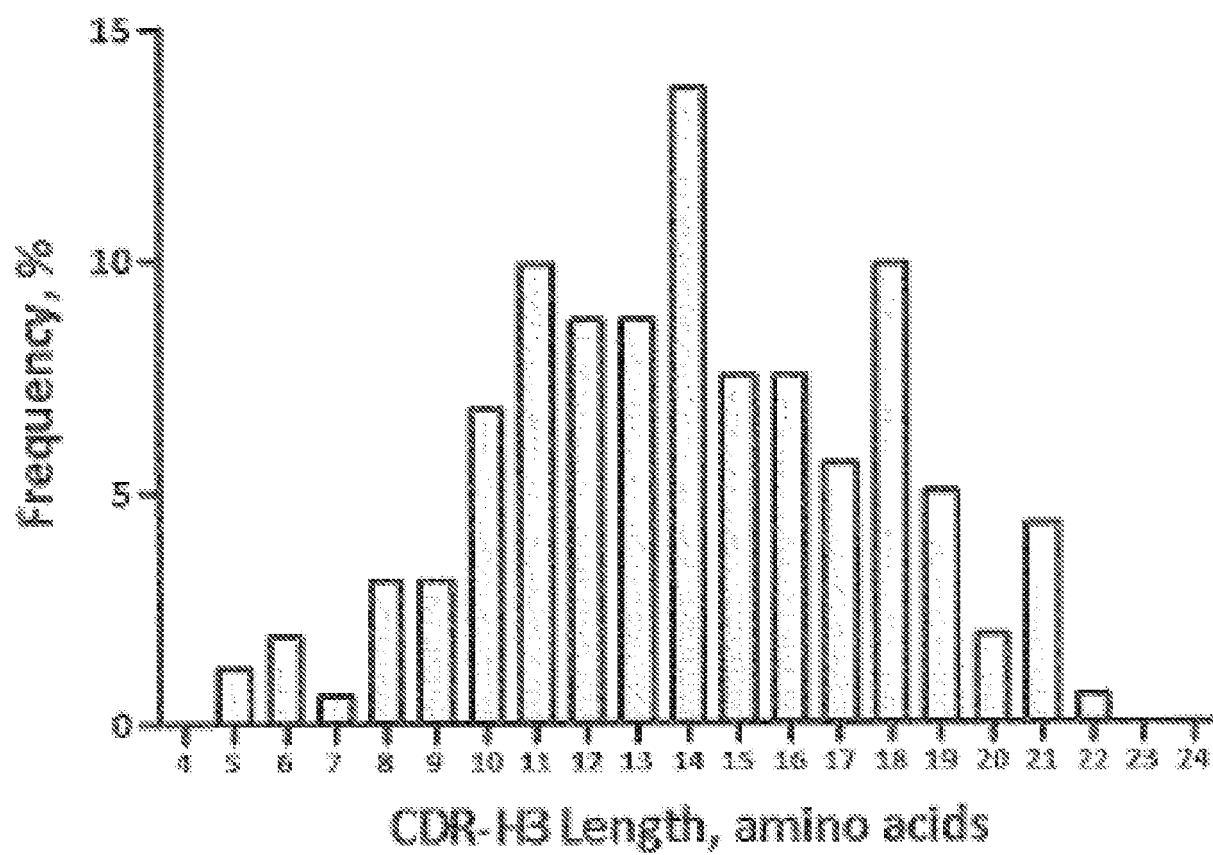


Fig. 37

38/75

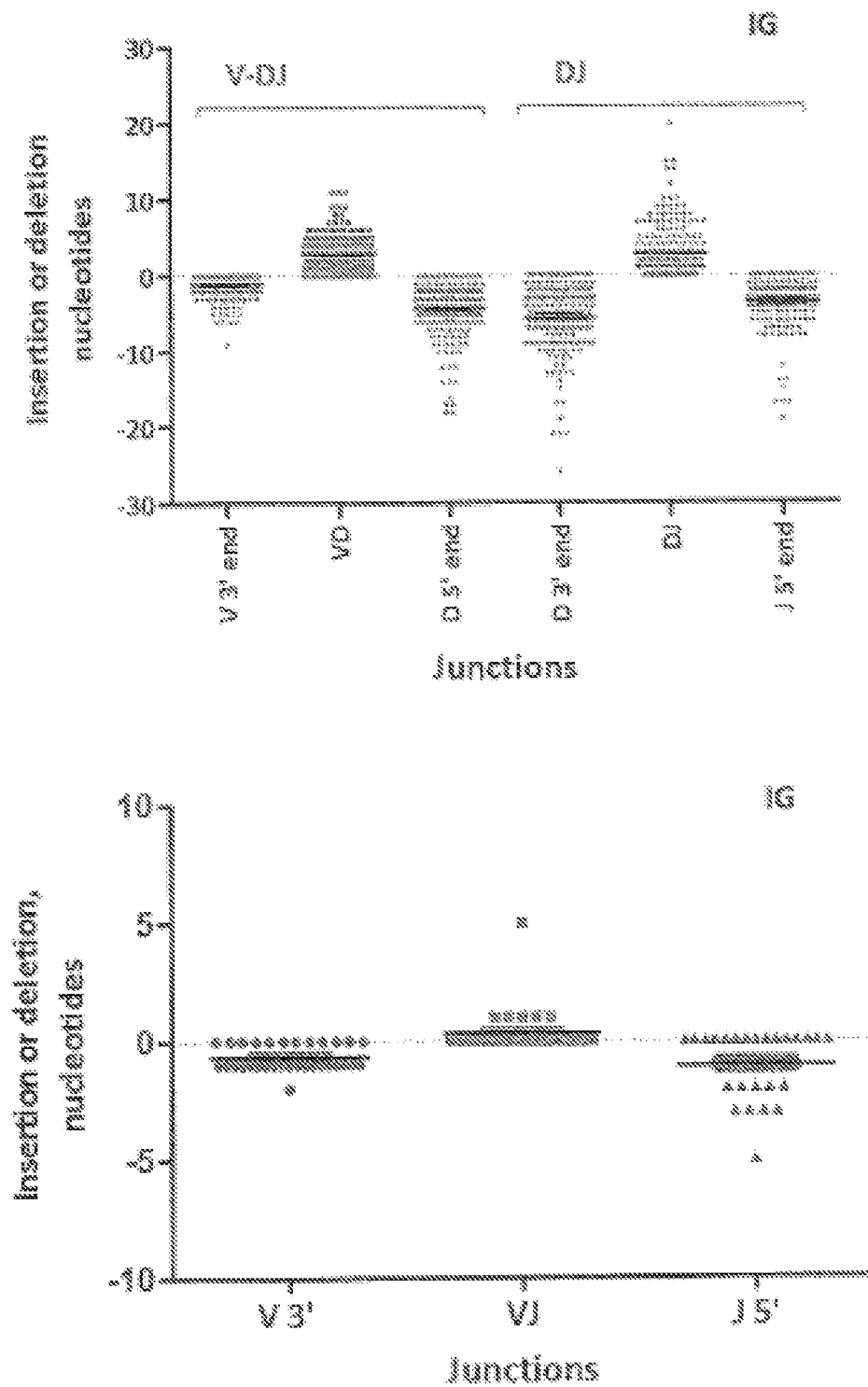


Fig. 38

Distribution of JH Usage Within Each VHS

	JH1	JH2	JH3	JH4	JH5	JH6
V2-5		1	5	5	2	1
V4-4		1	1	1		8
V1-3	1	6	6	49	13	40
V1-2	1			1		1
V6-1		1	4	18	1	29
Total	2	9	16	74	16	79

The data includes 196 independent sequences.

Fig. 39

Nucleotide Gain or Loss at VJ Joints Generates IGH Variants

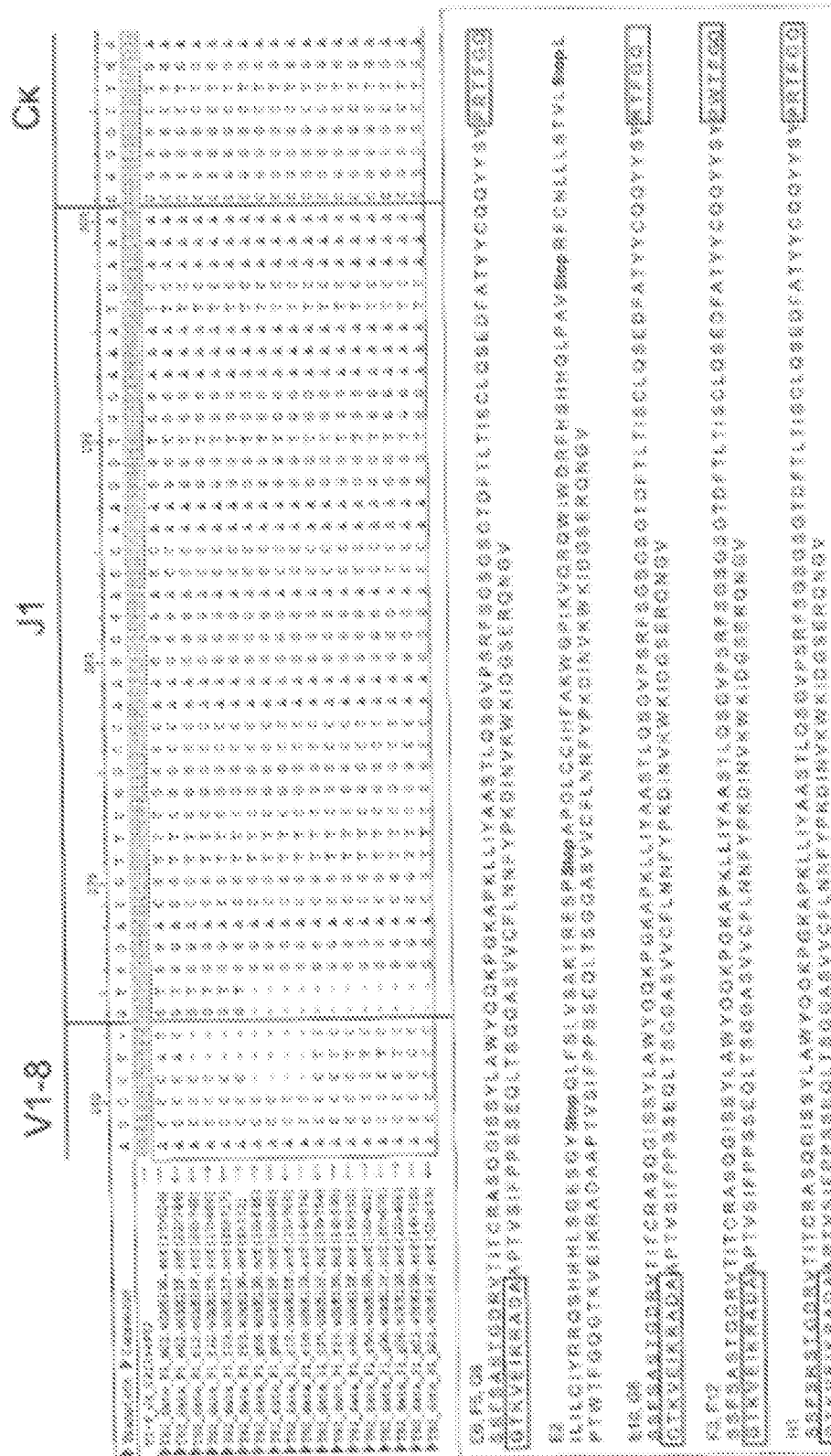


Fig. 41

Joint Diversity Produces Functional CDS

VT₆J1_CK
VAGWPGIERNQOKPLSSWWSIRRLVWSPKVSQHQSAAYVIGNSLSPSAAC
SLWHLGLITANSIVLLTFGGQTKVEIKRAAAPTVSIFPPSSSEQLTSGGASVVCFL
CFLNNFYPKDINVKWKIDGSEKQNGV
K12
SSWLAWYQOKPKAPKLLIYKASSLEGGVPSRFSQSSQTEFTLTISSLQPDQ
PATYCCQYNSYSTFGGQTKVEIKRAAAPTVSIFPPSSSEQLTSGGASVVCFL
NNFYPKDINVKWKIDGSEKQNGV
VT₆J4_CK
AGWPGIERNQOKPLSSWWSIRRLVWSPKVSQHQSAAYVIGNSLSPSAACG
SLWHLGLITANSIVLLTFGGQTKVEIKRAAAPTVSIFPPSSSEQLTSGGASVVCFL
CFLNNFYPKDINVKWKIDGSEKQNGV
E1
SSWLAWYQOKPKAPKLLIYKASSLEGGVPSRFSQSSQTEFTLTISSLQPDQ
PATYCCQYNSYSTFGGQTKVEIKRAAAPTVSIFPPSSSEQLTSGGASVVCFL
NNFYPKDINVKWKIDGSEKQNGV
E2
SSWLAWYQOKPKAPKLLIYKASSLEGGVPSRFSQSSQTEFTLTISSLQPDQ
PATYCCQYNSYSTFGGQTKVEIKRAAAPTVSIFPPSSSEQLTSGGASVVCFL
NNFYPKDINVKWKIDGSEKQNGV
E3
SSWLAWYQOKPKAPKLLIYKASSLEGGVPSRFSQSSQTEFTLTISSLQPDQ
PATYCCQYNSYSTFGGQTKVEIKRAAAPTVSIFPPSSSEQLTSGGASVVCFL
NNFYPKDINVKWKIDGSEKQNGV

Fig. 43

44/75

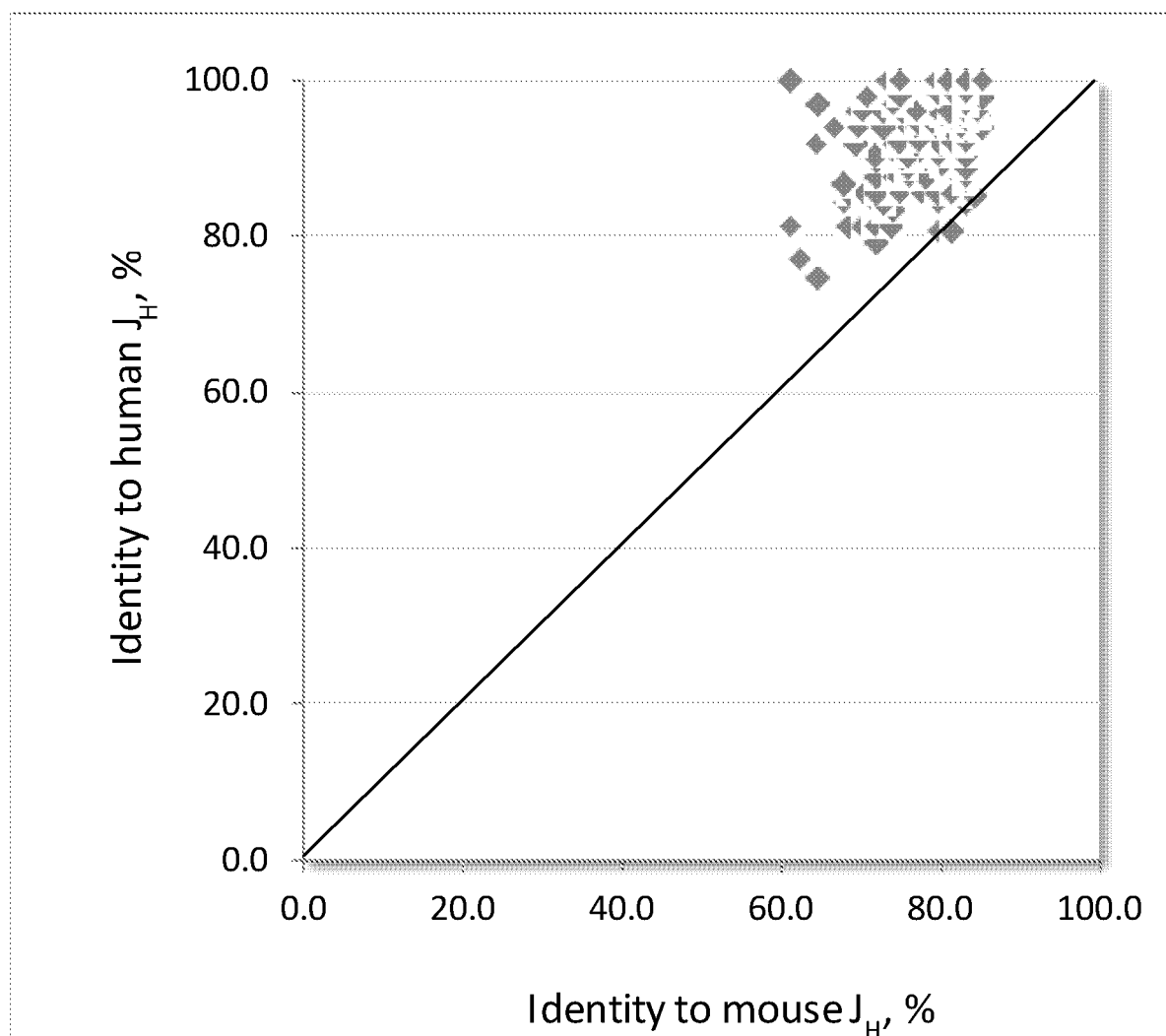
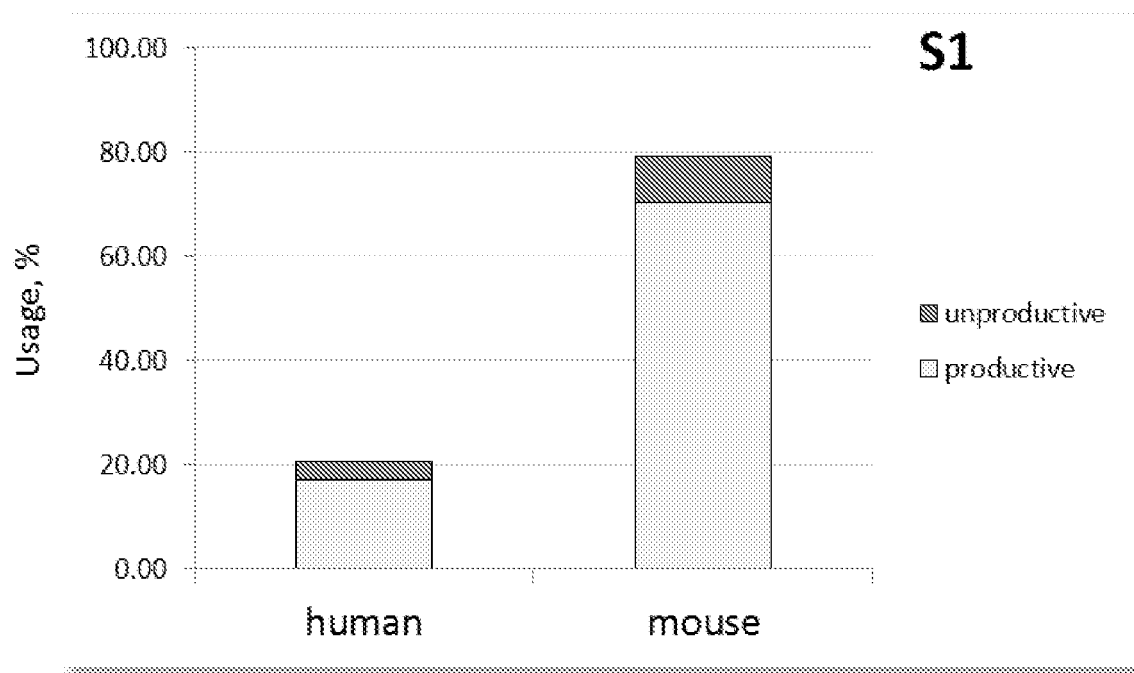


Fig. 44

45/75

**Fig. 45**

46/75

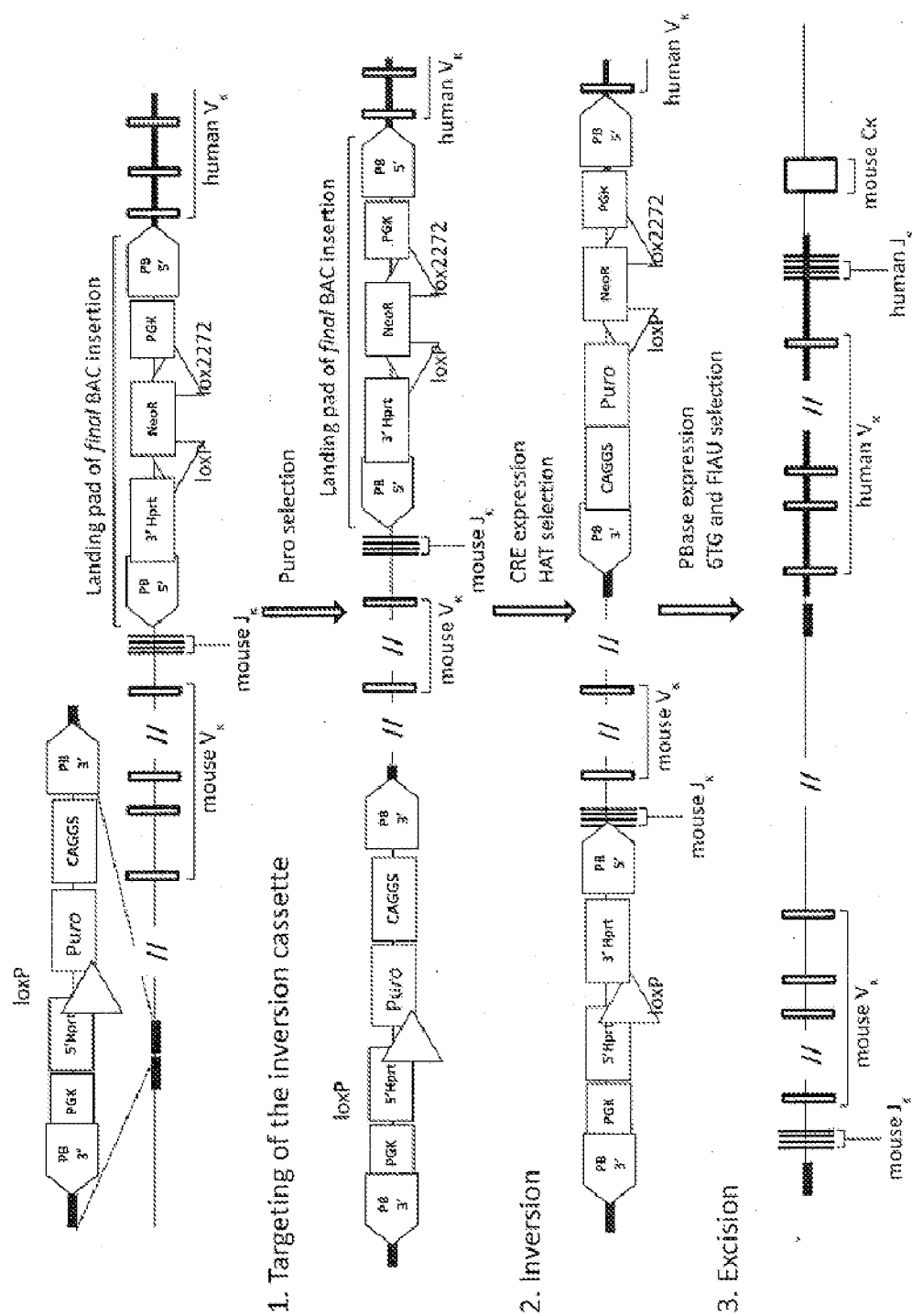


Fig. 46

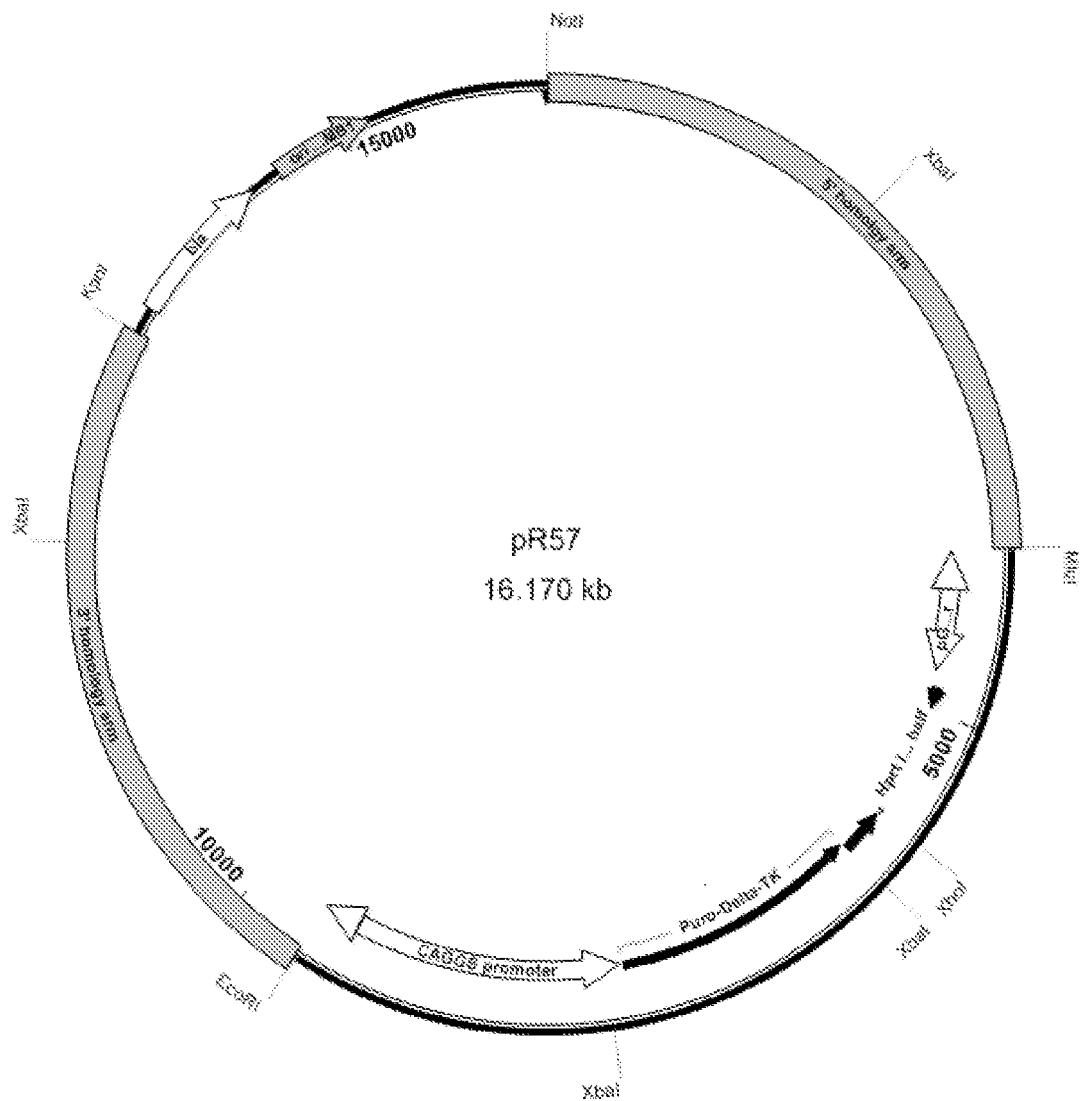


Fig. 47

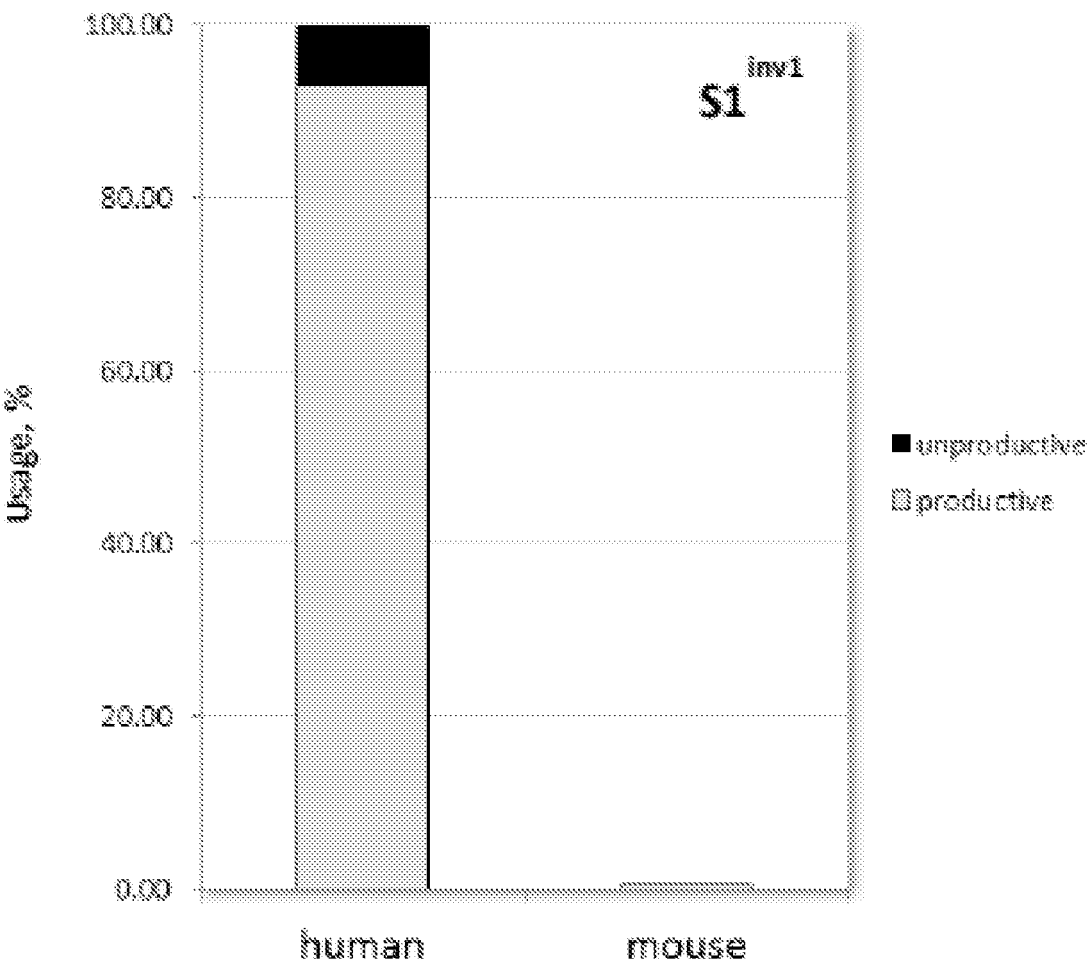


Fig. 48

49/75

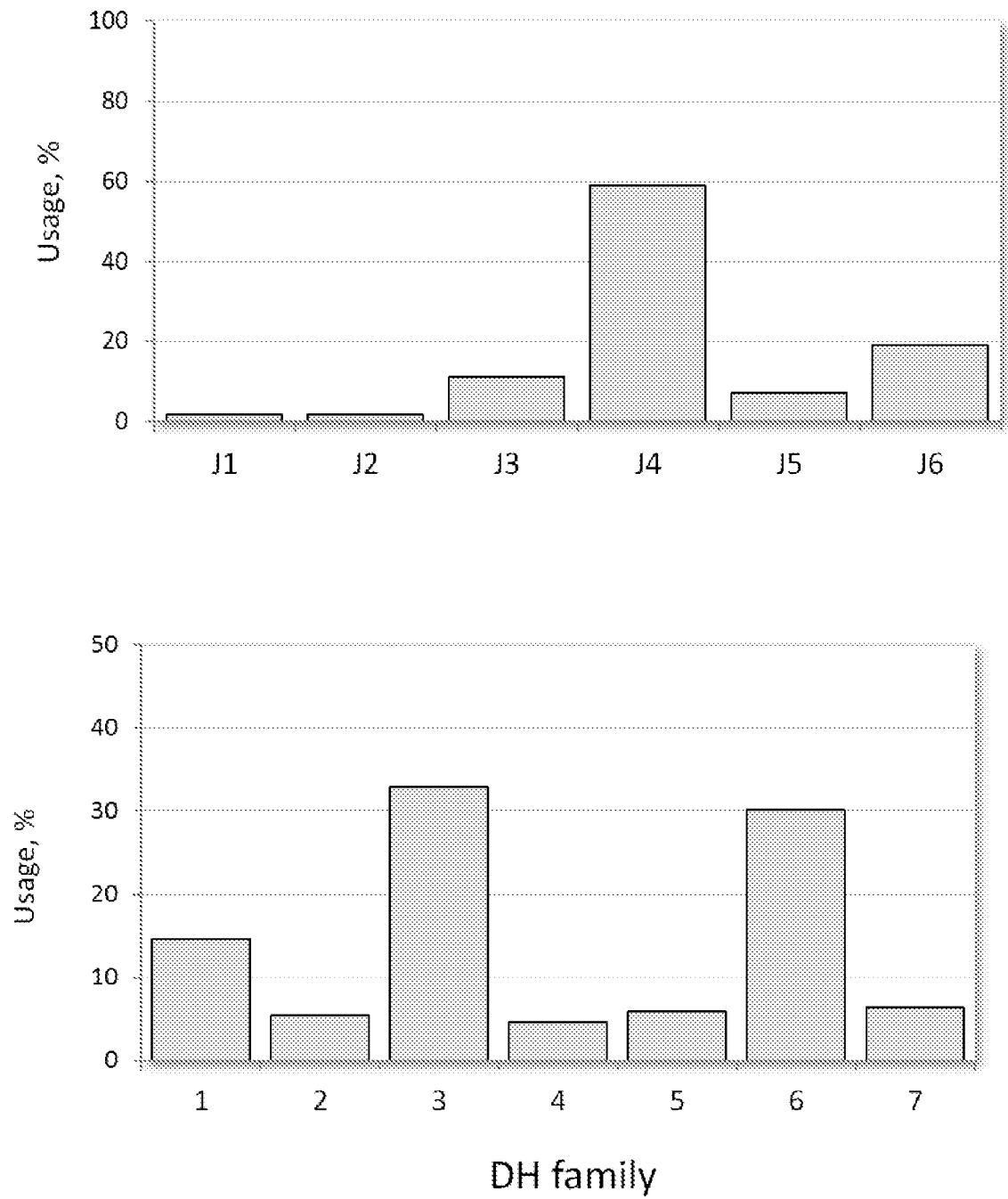


Fig. 49

50/75

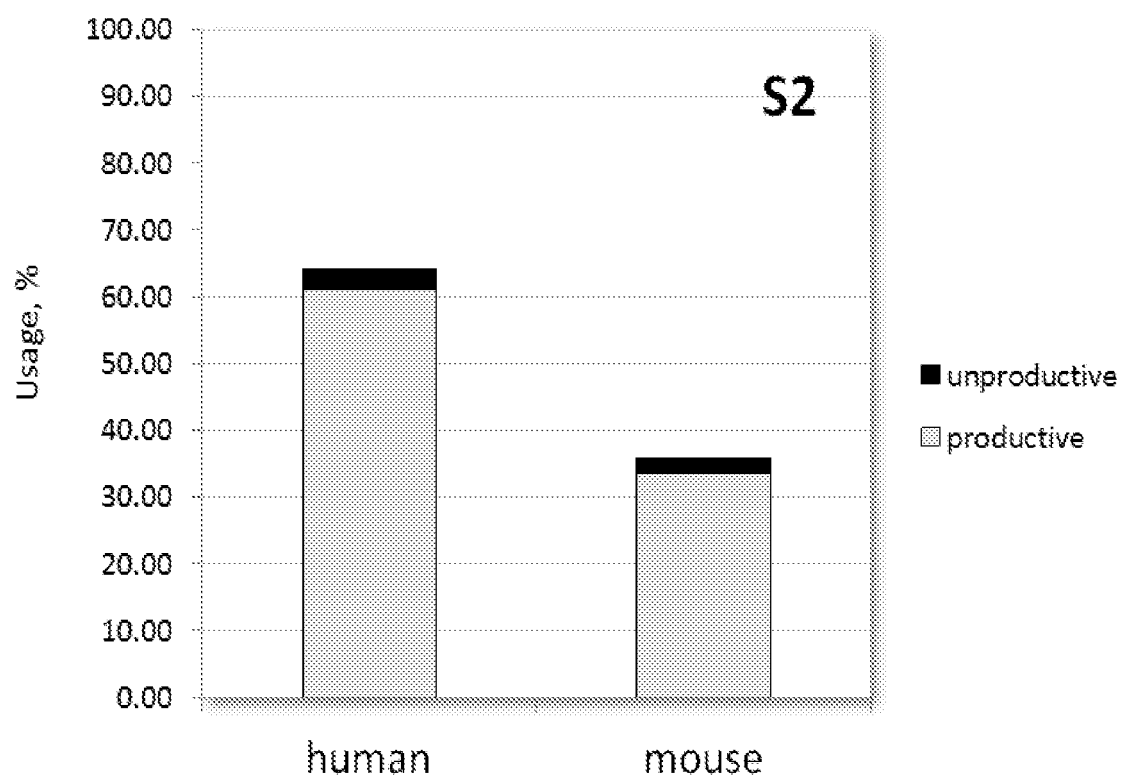


Fig. 50

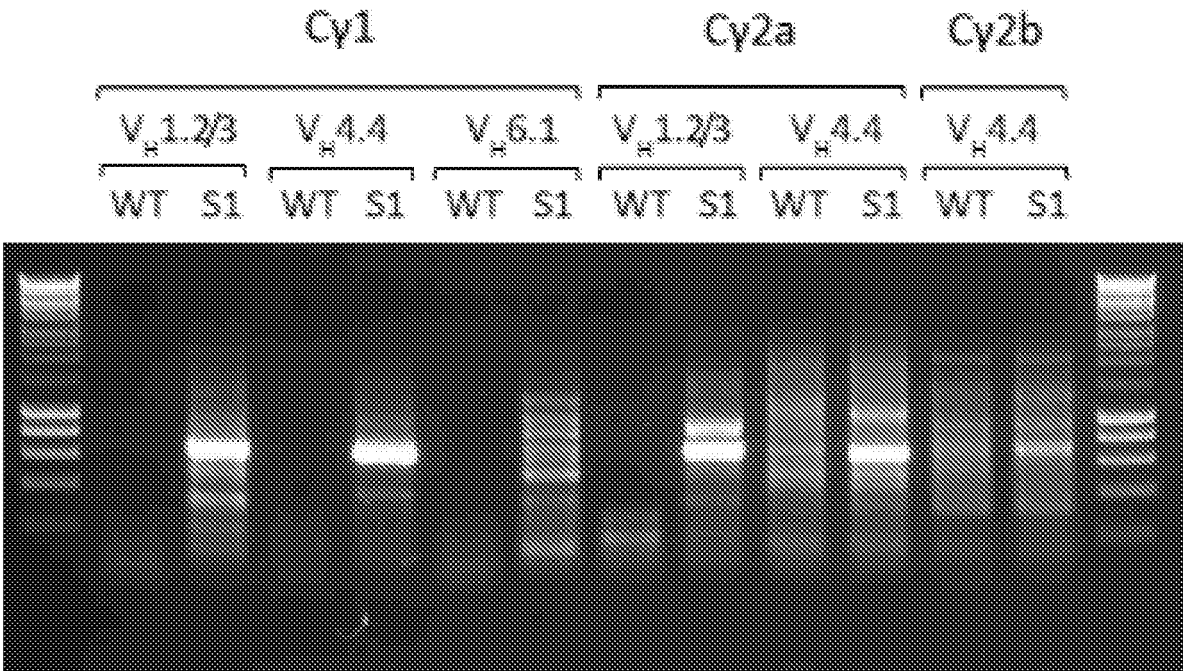


Fig. 51

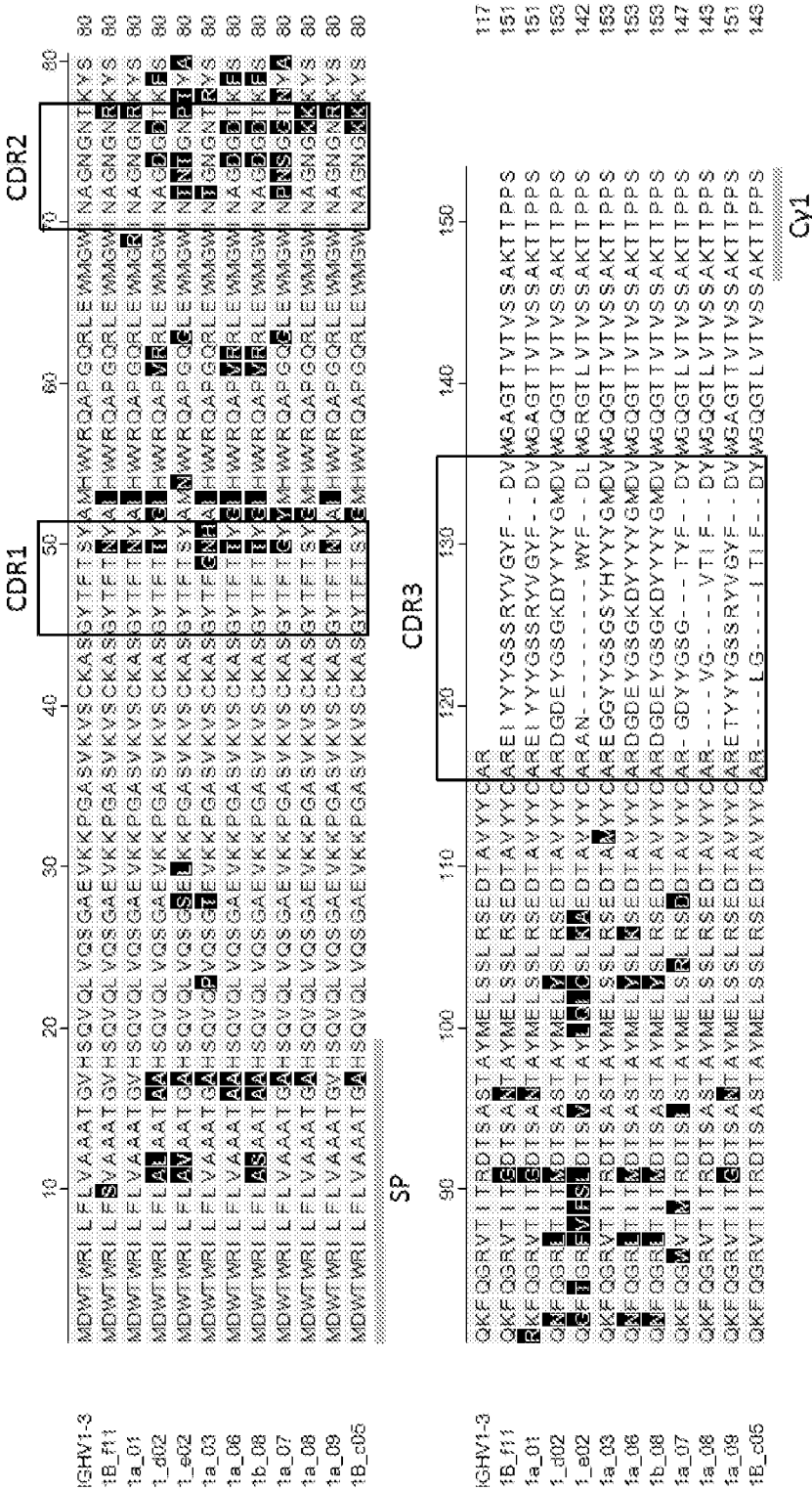


Fig. 52

53/75

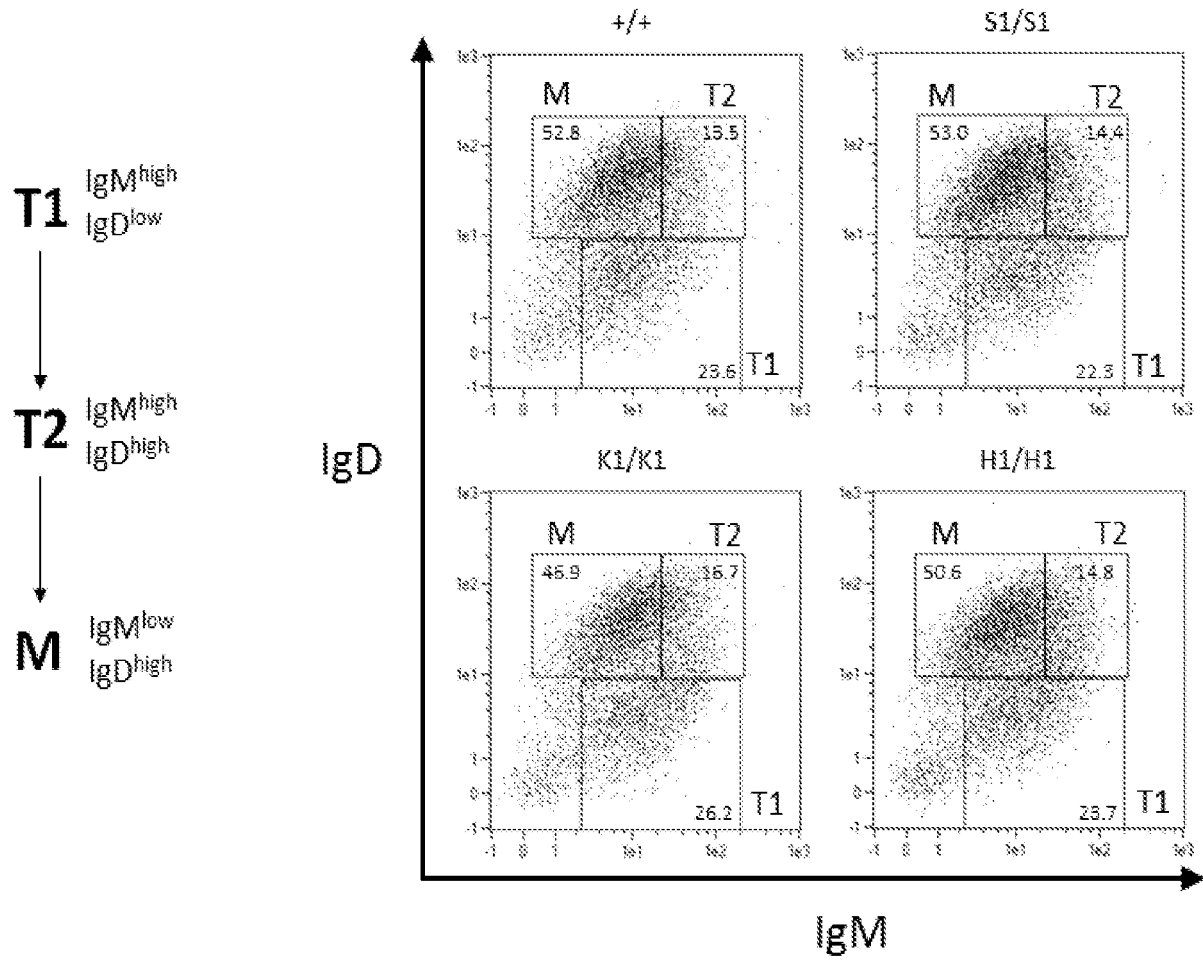


Fig. 53

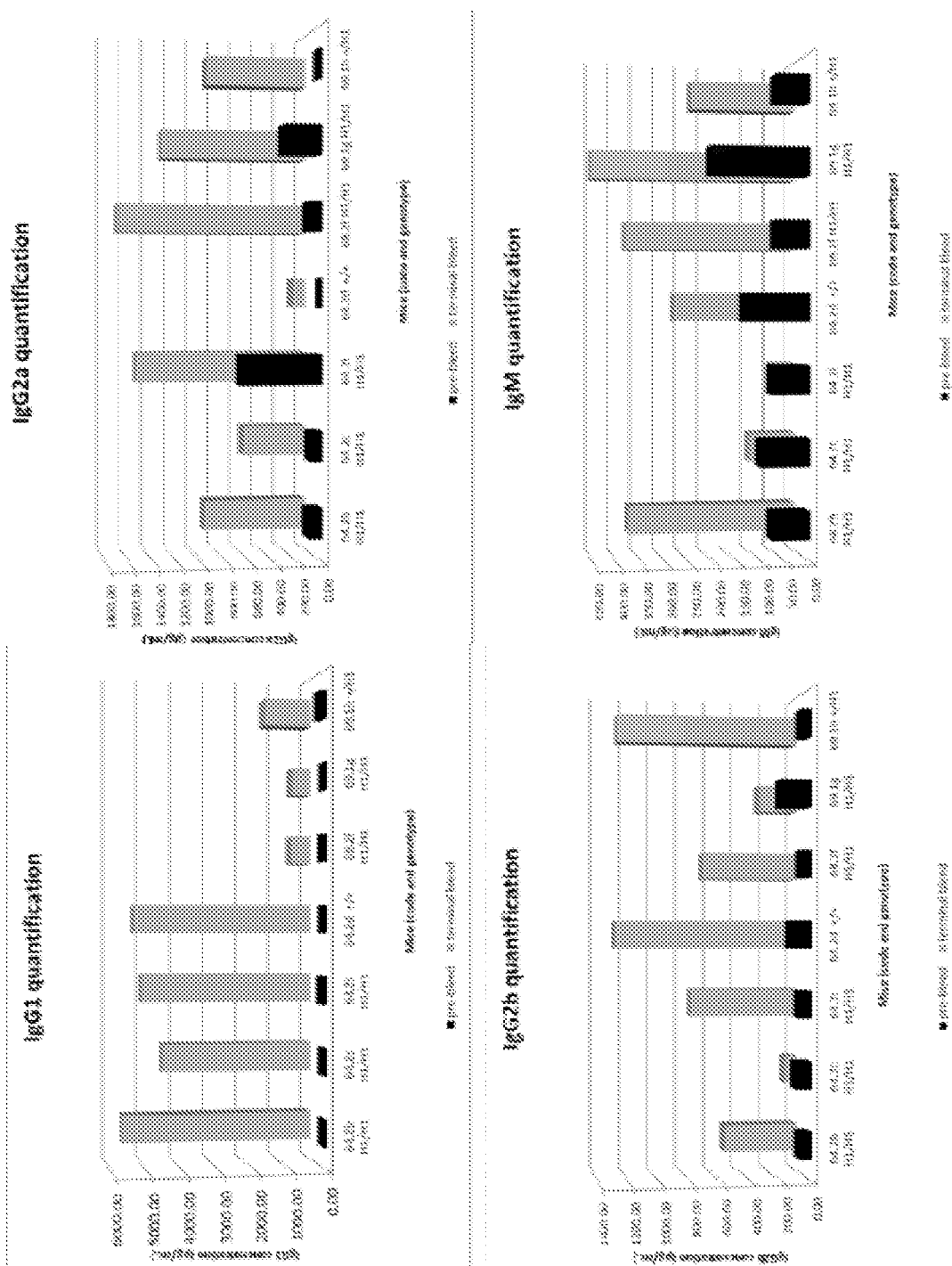


Fig. 54A

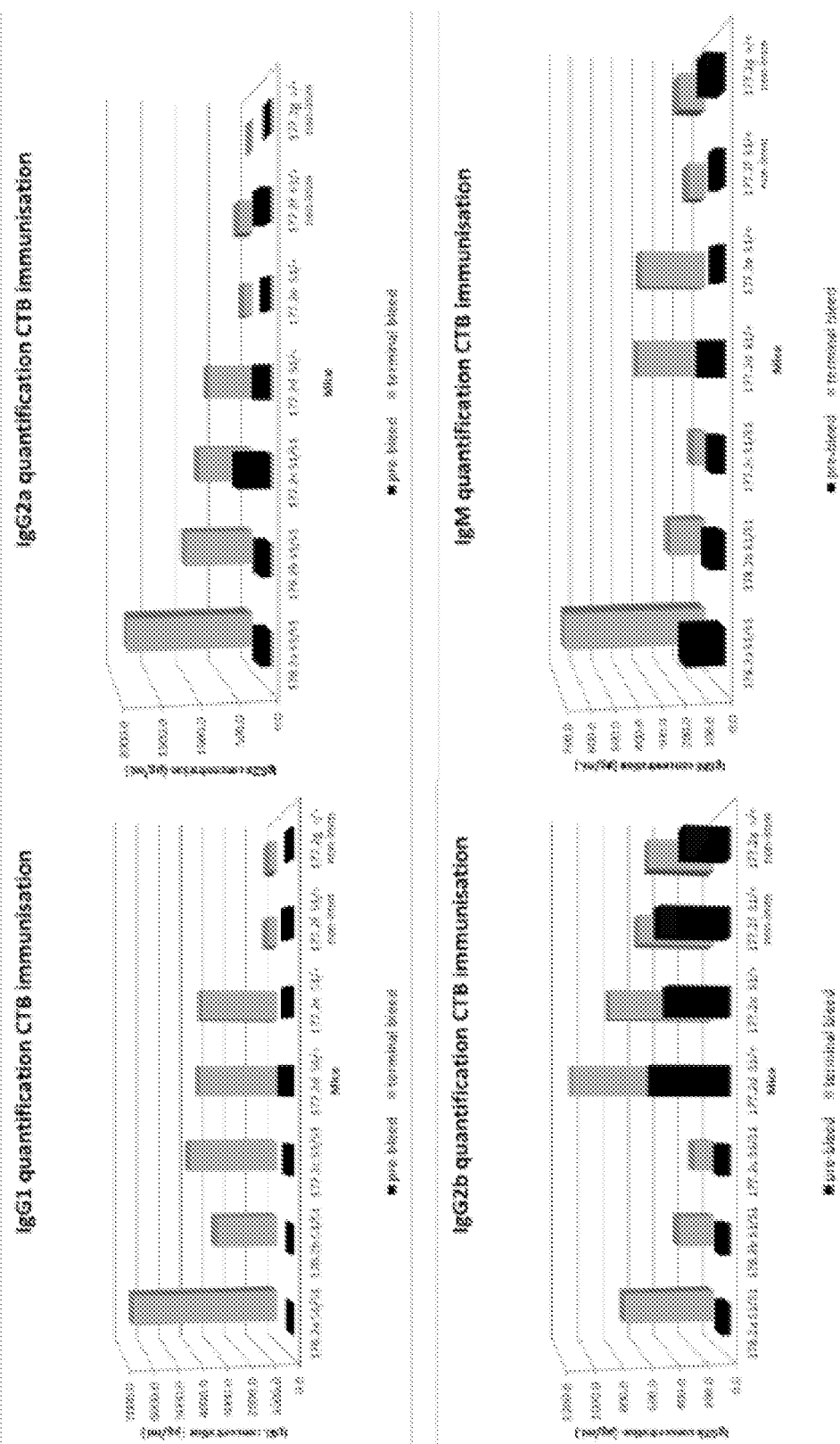


Fig. 54B

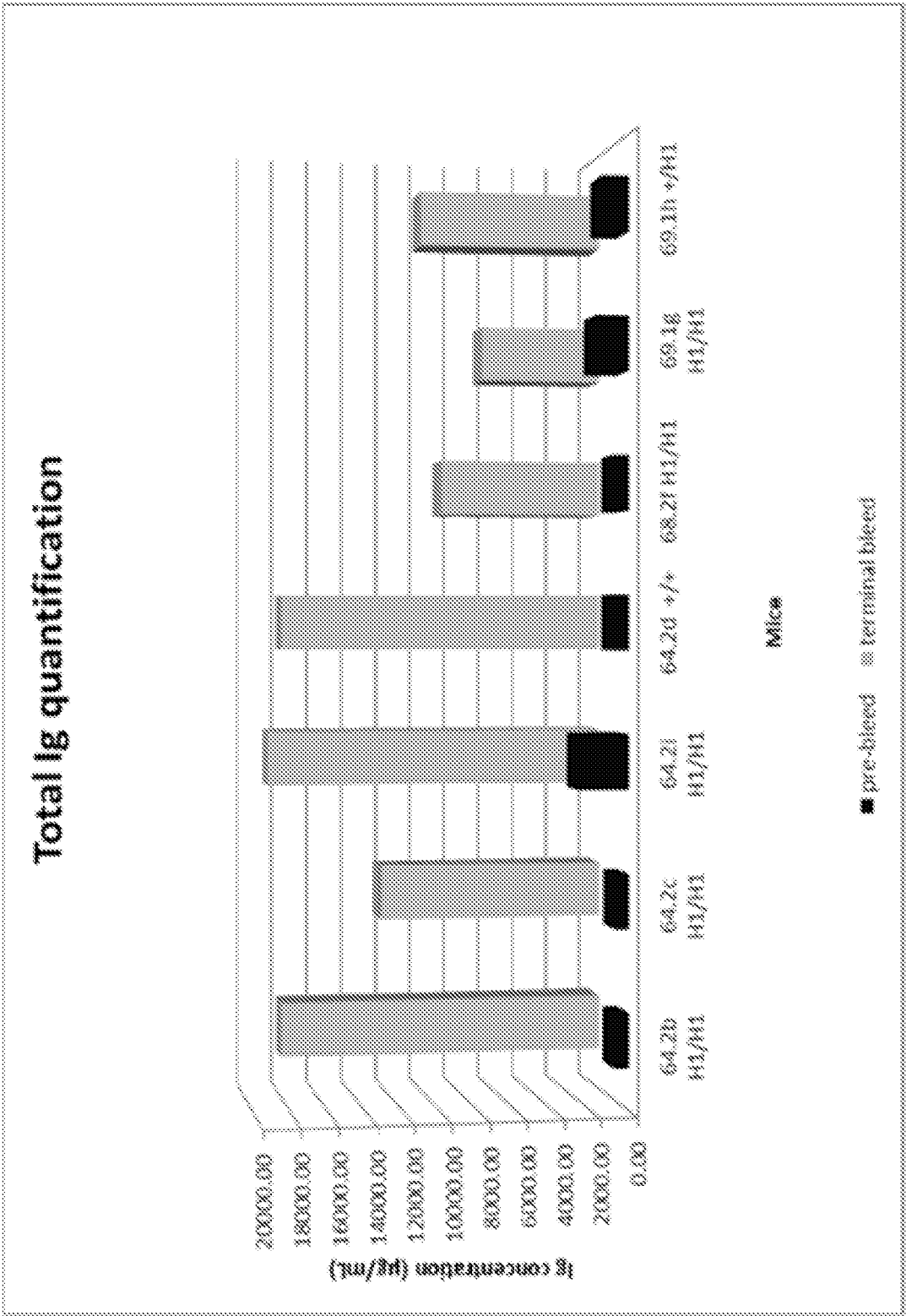


Fig. 55A

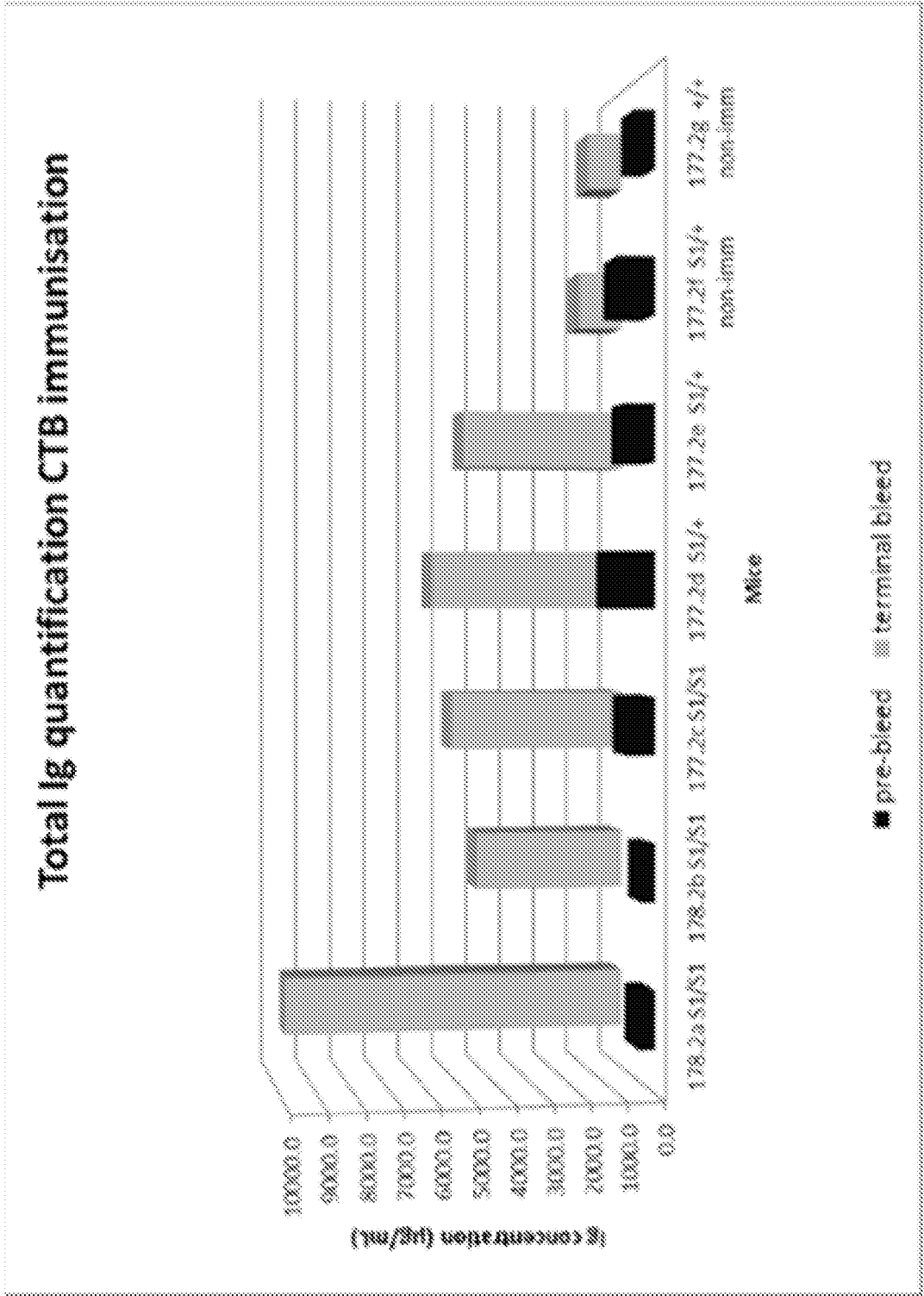
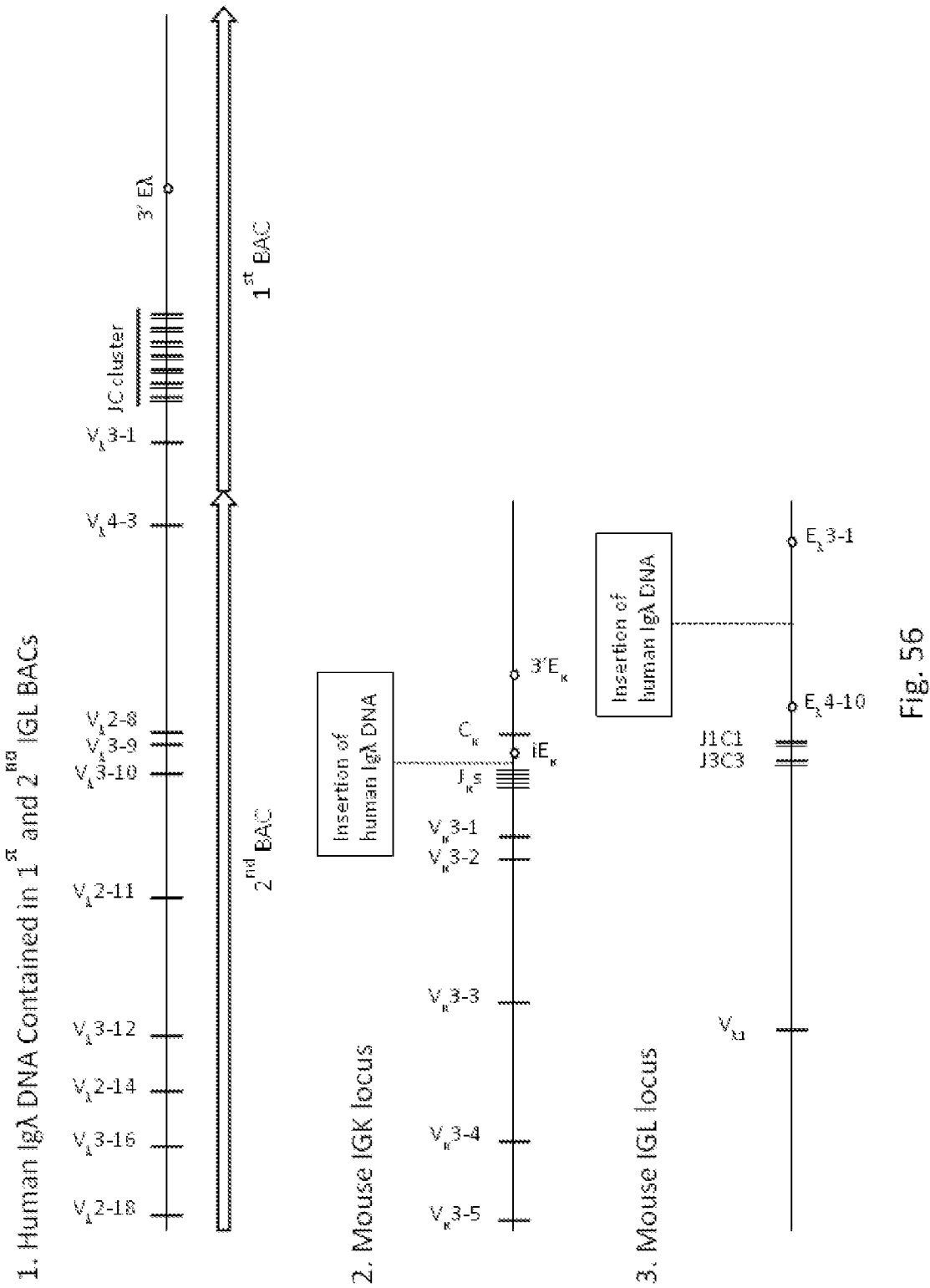


Fig. 55B



59/75

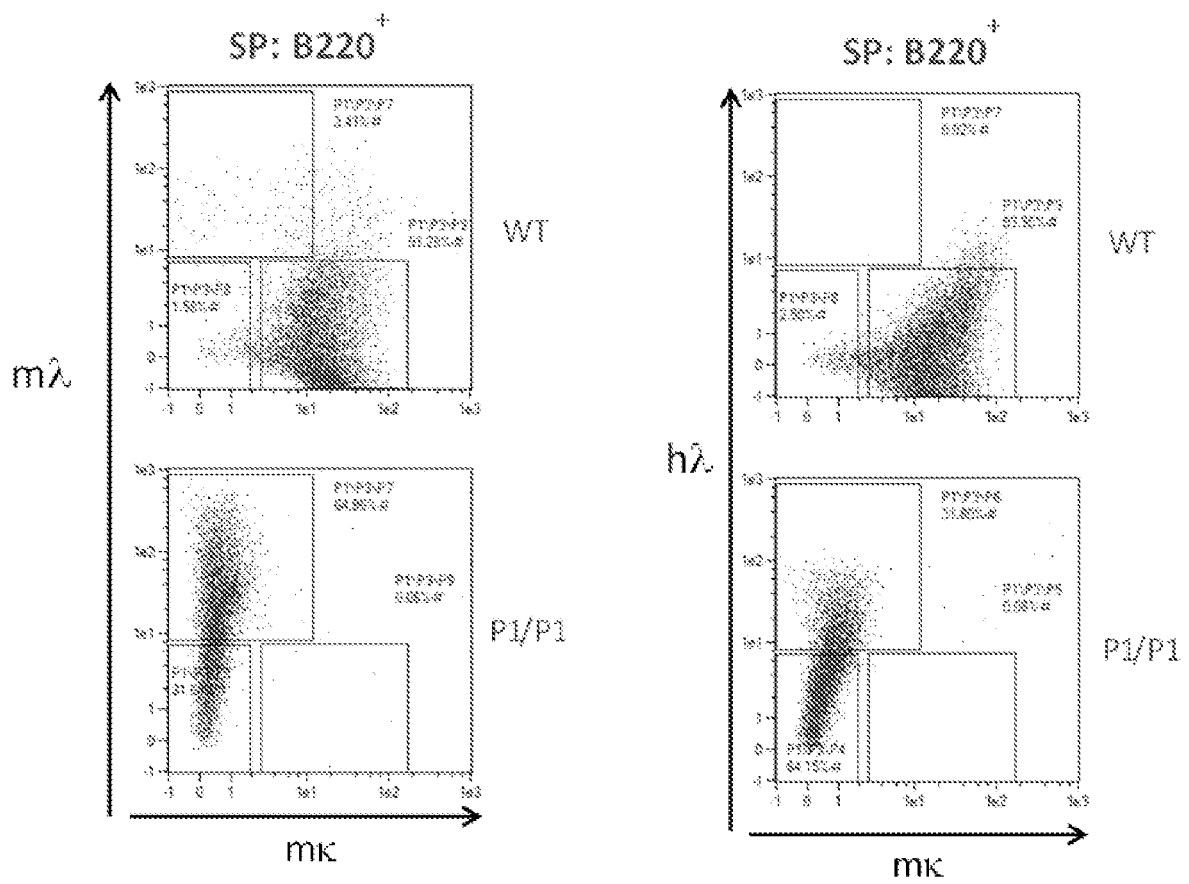


Fig. 57

60/75

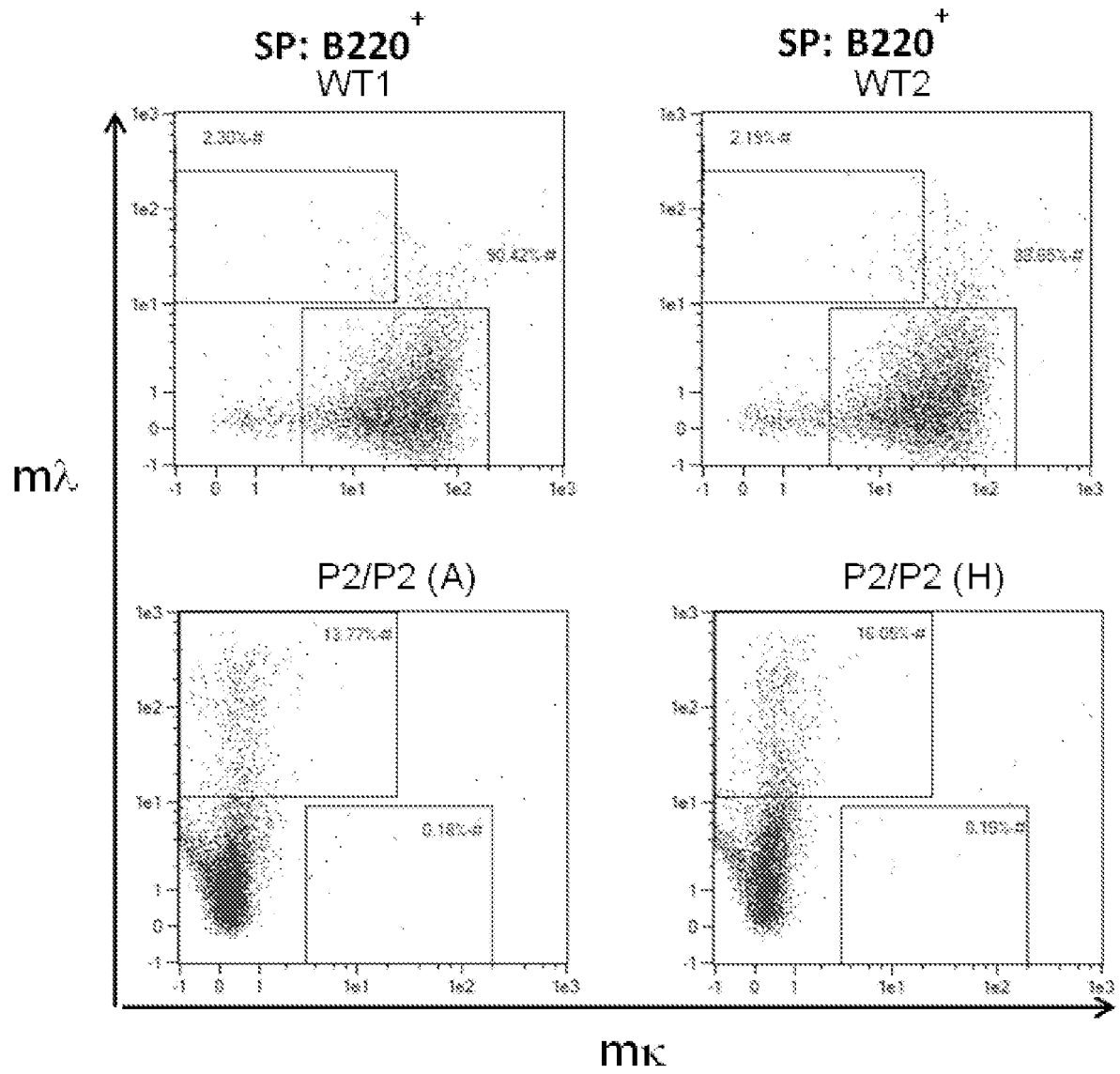


Fig. 58A

61/75

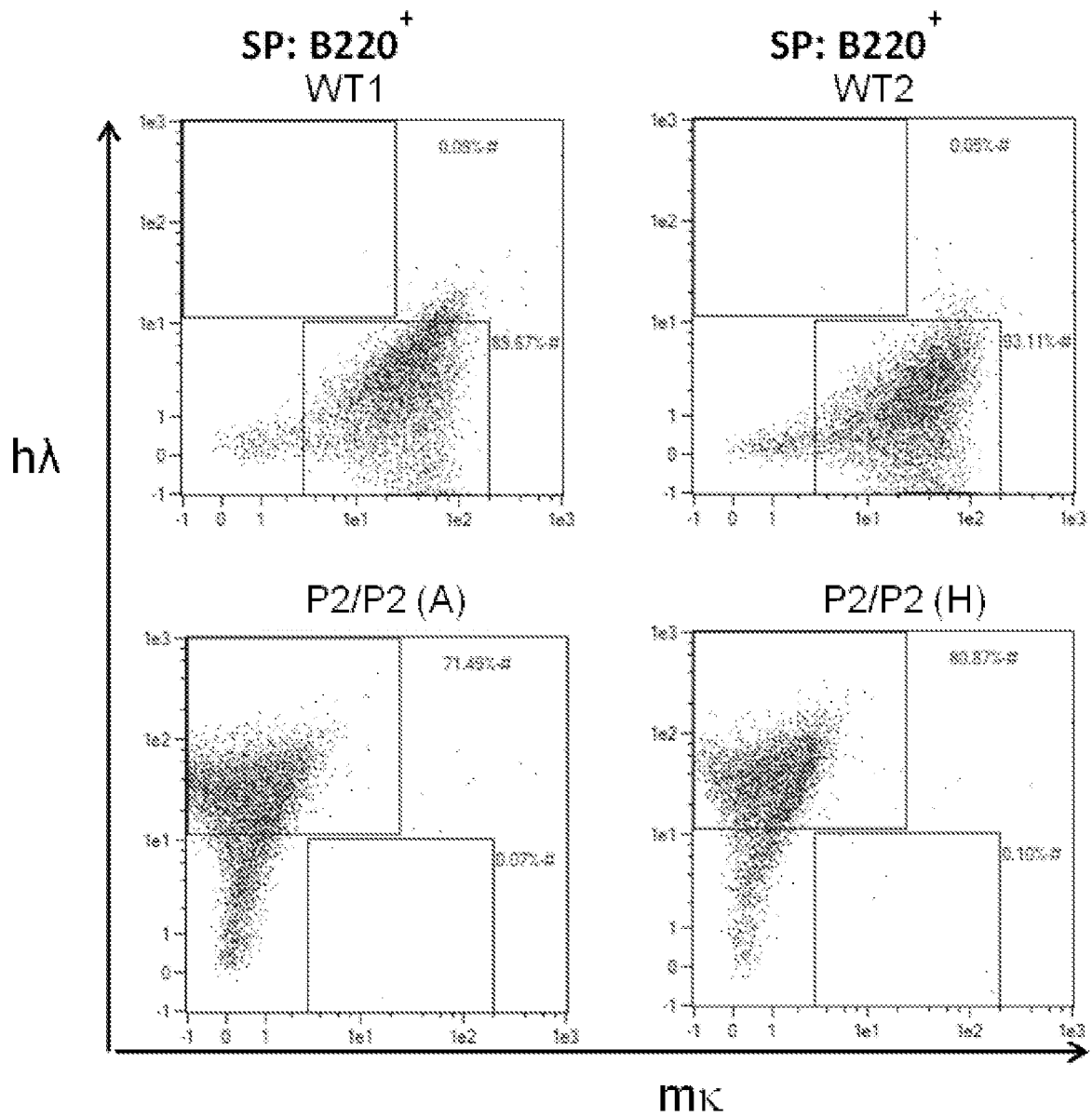


Fig. 58B

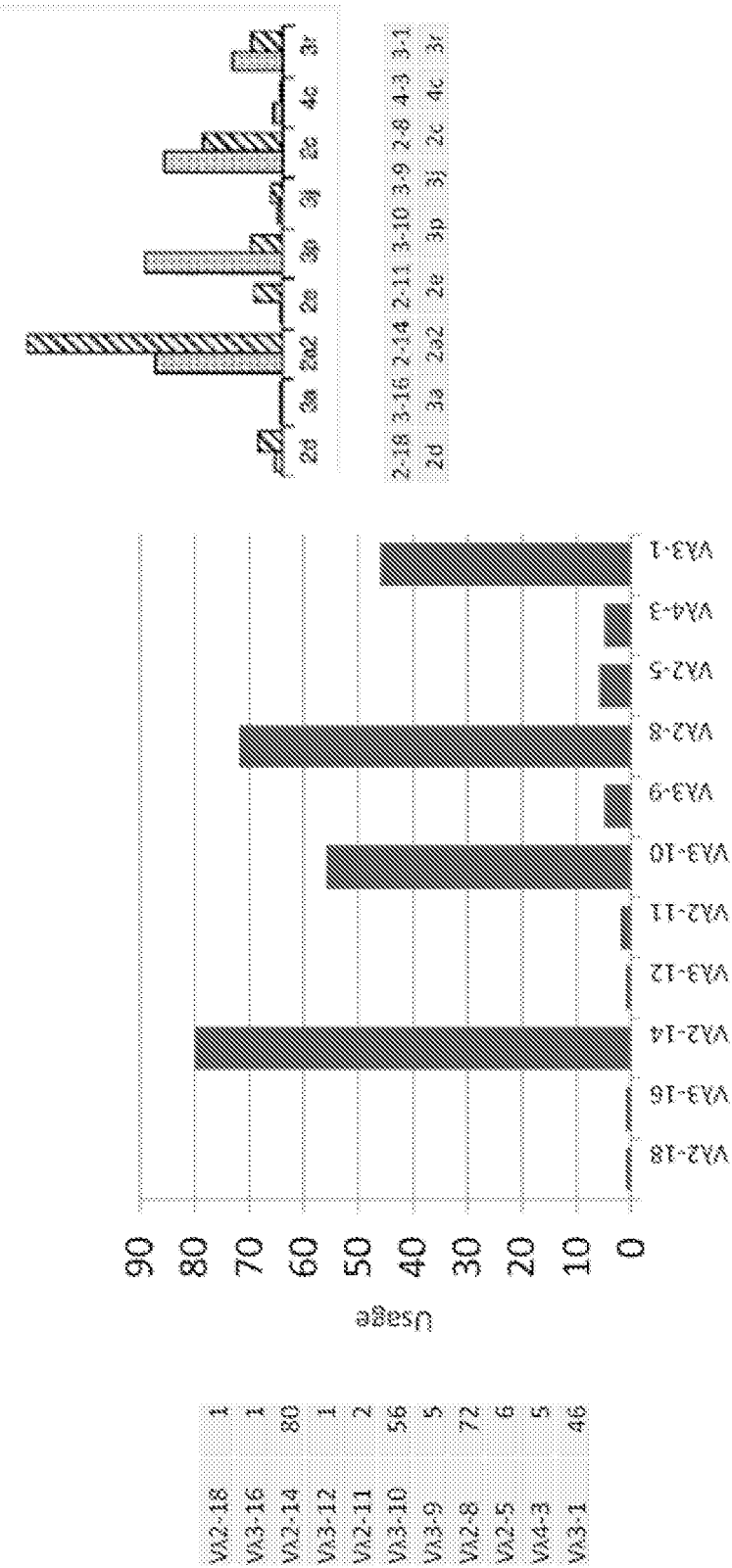


Fig. 59

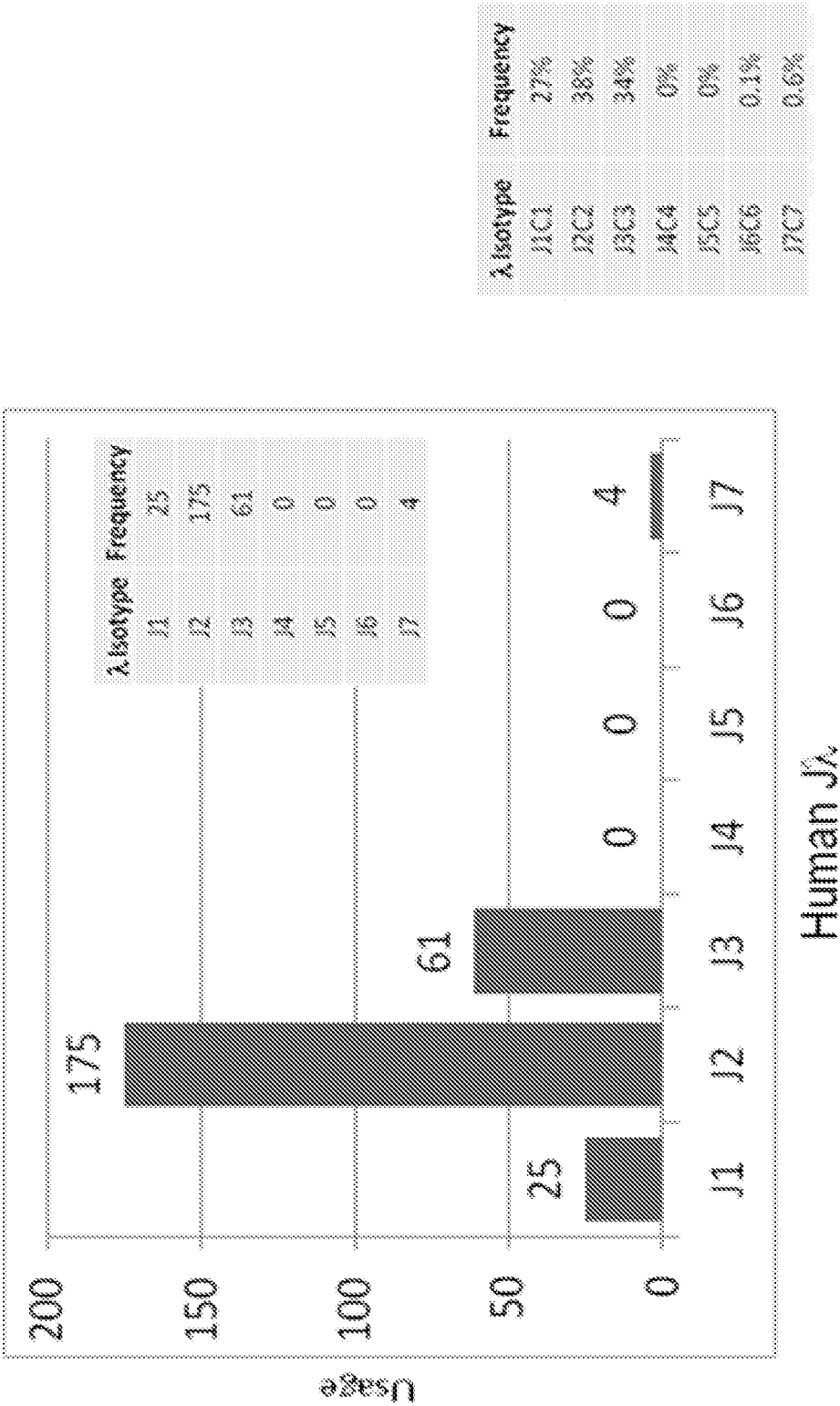


Fig. 60

64/75

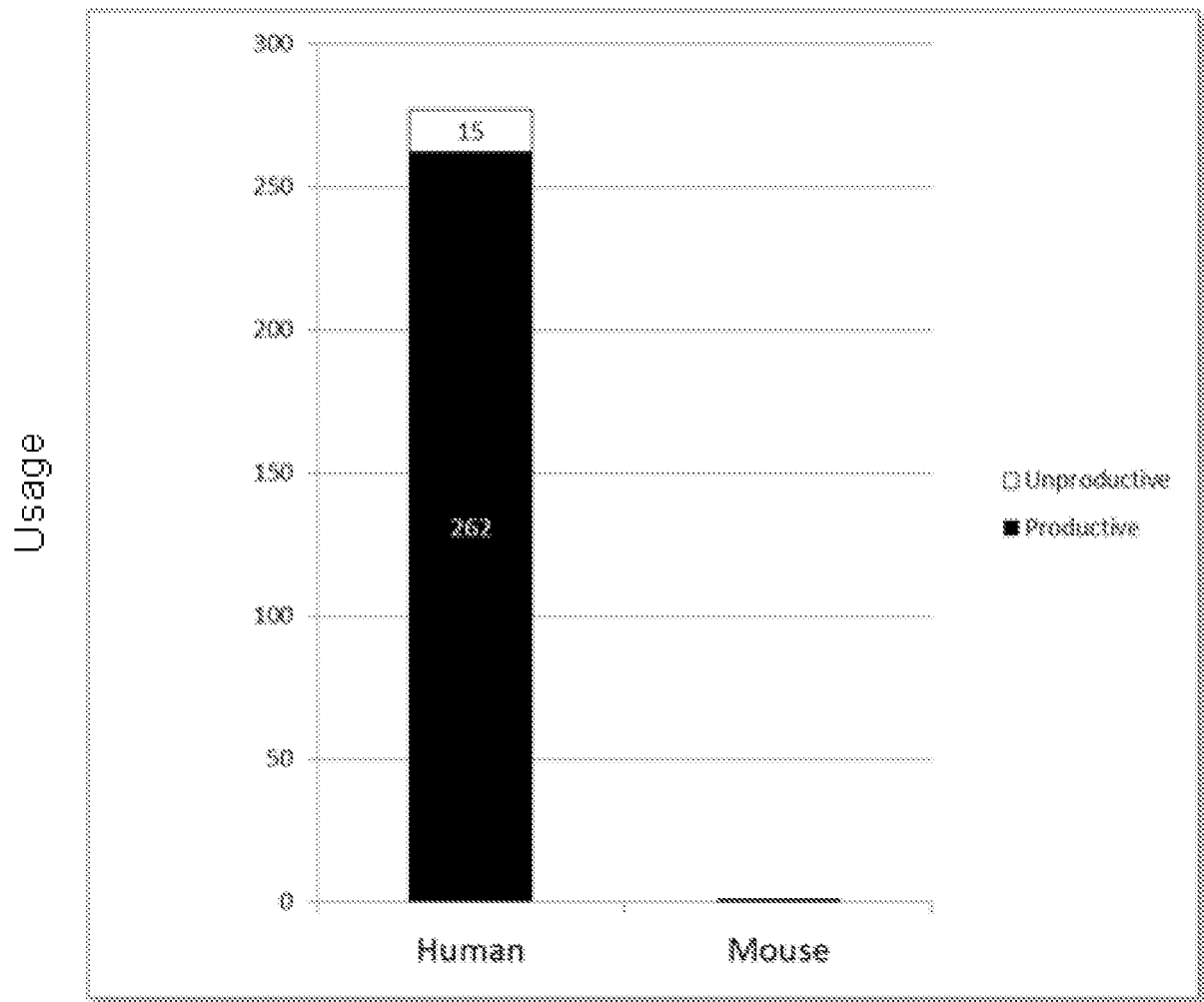


Fig. 61

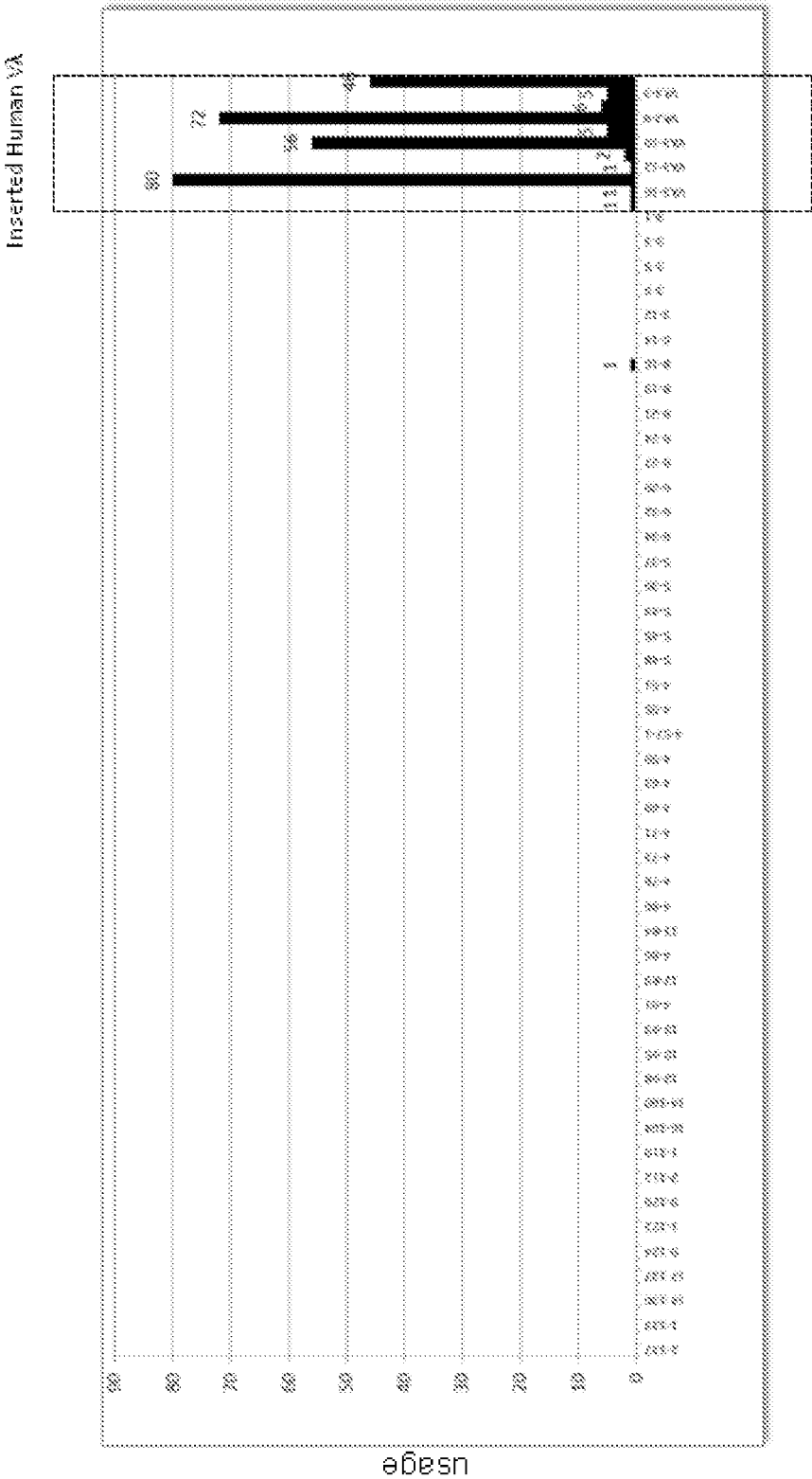


Fig. 62

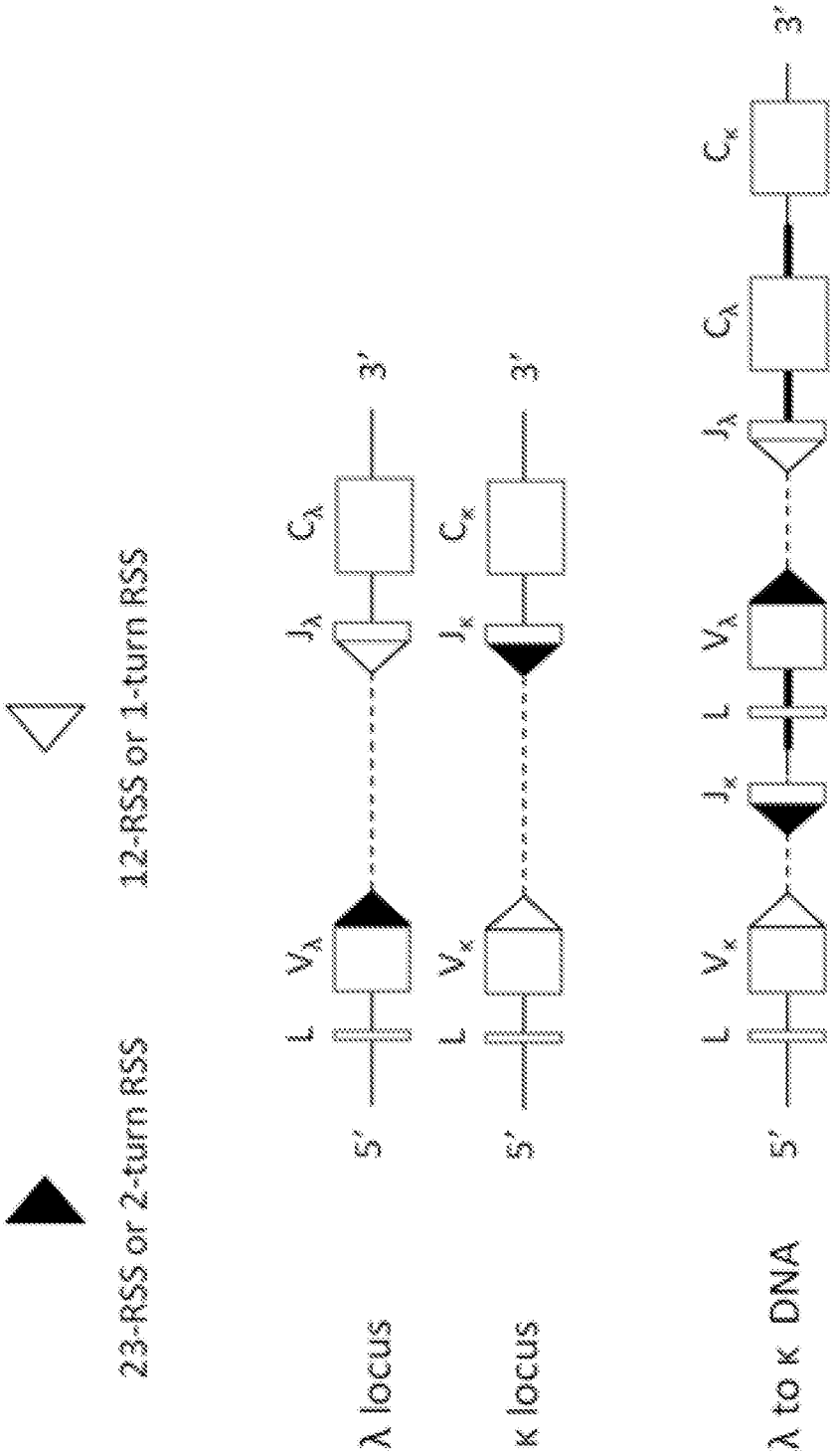


Fig. 63

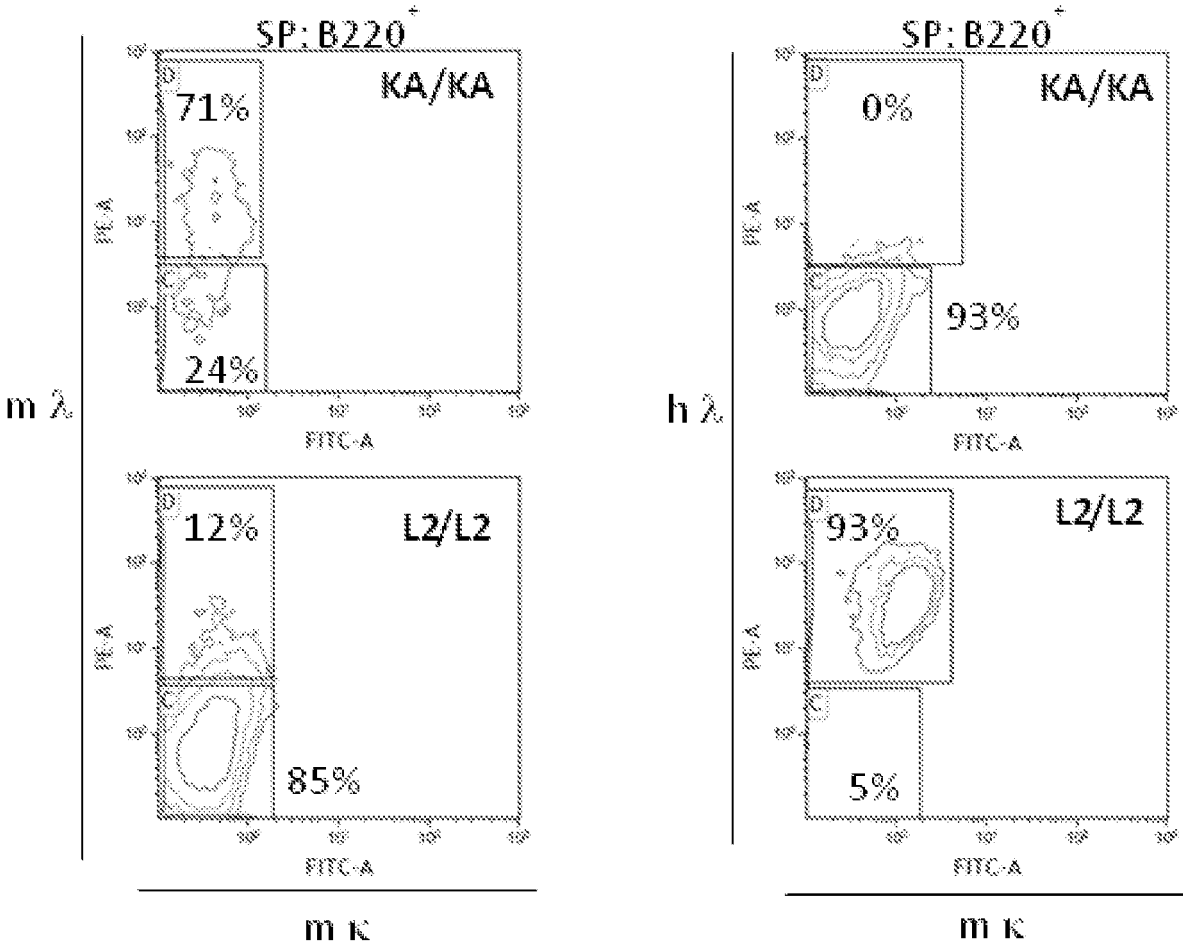


Fig. 64A

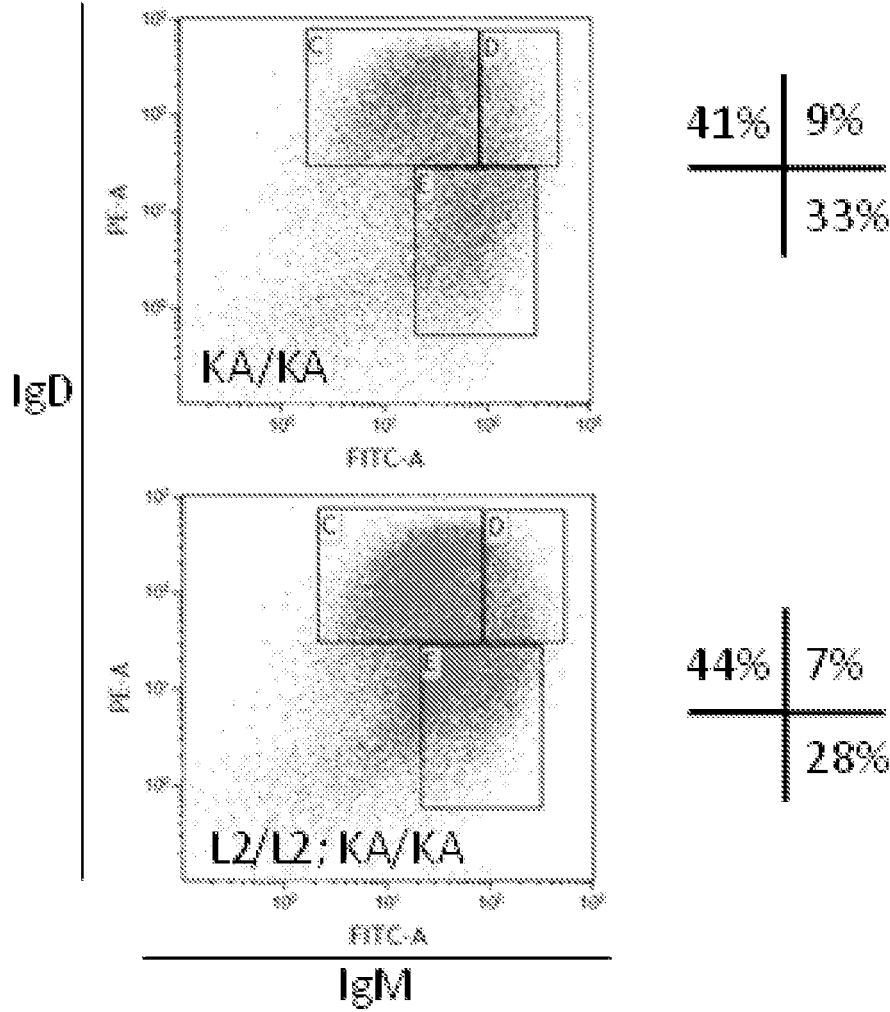


Fig. 64B

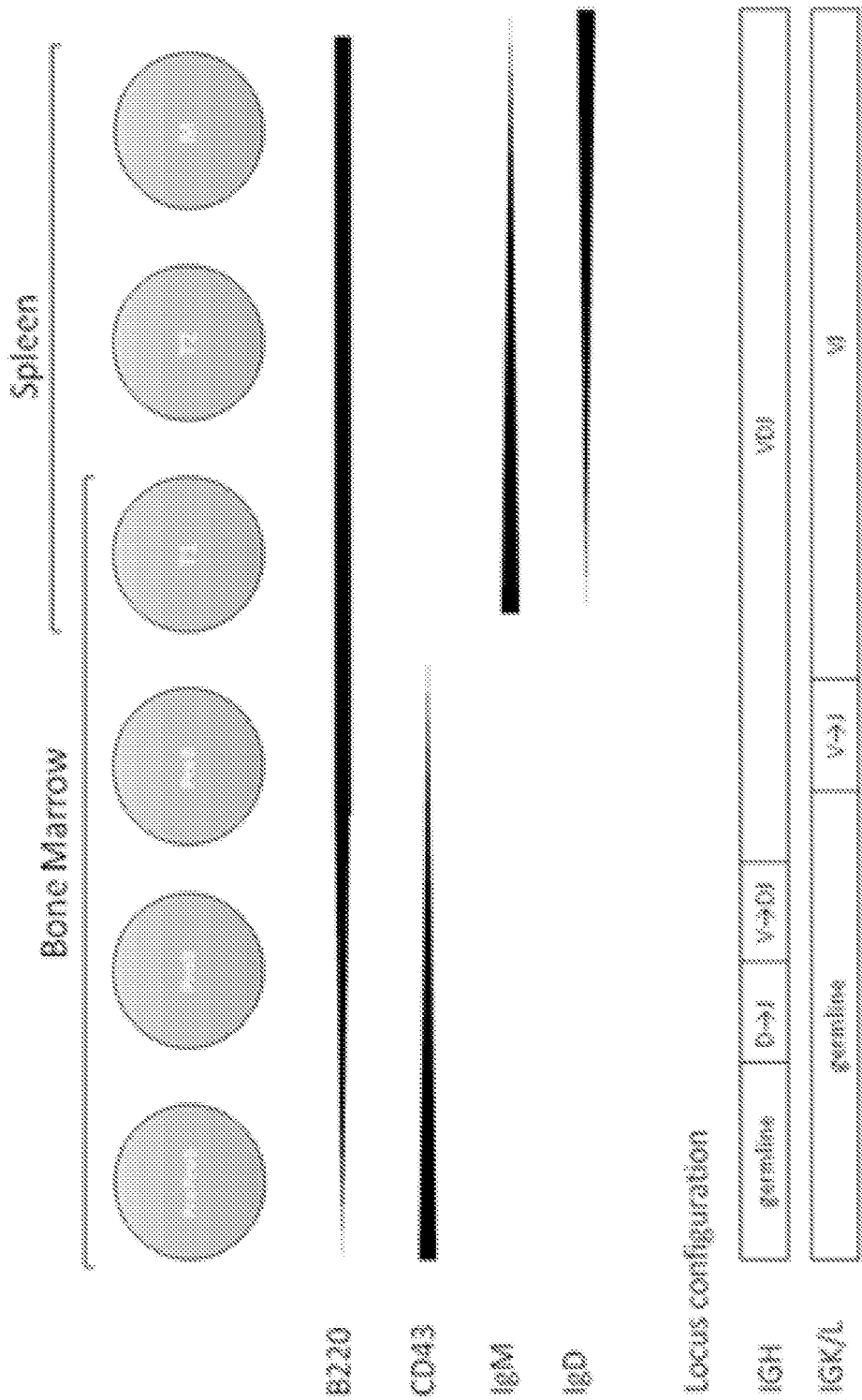


Fig 65

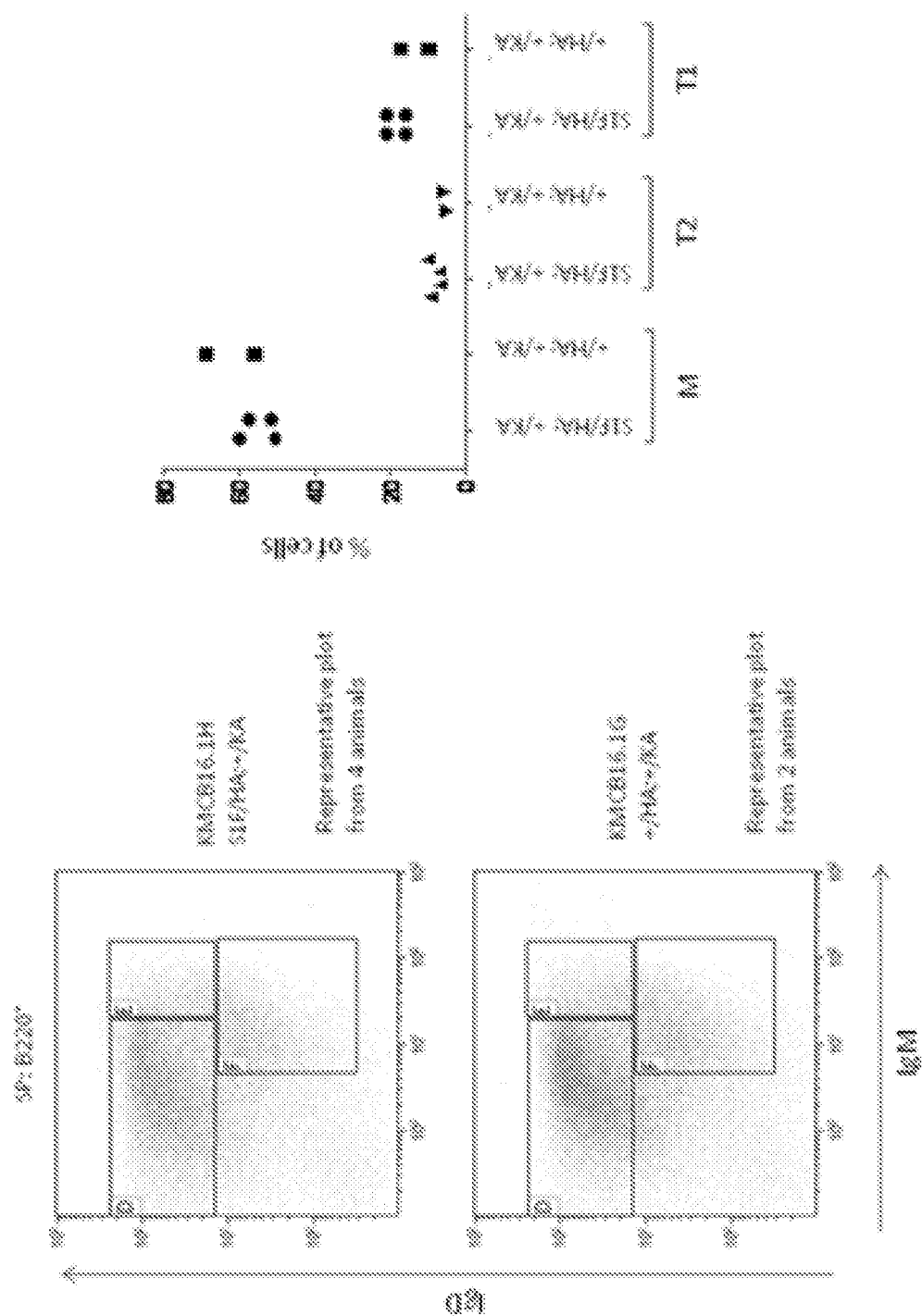


Fig. 66A

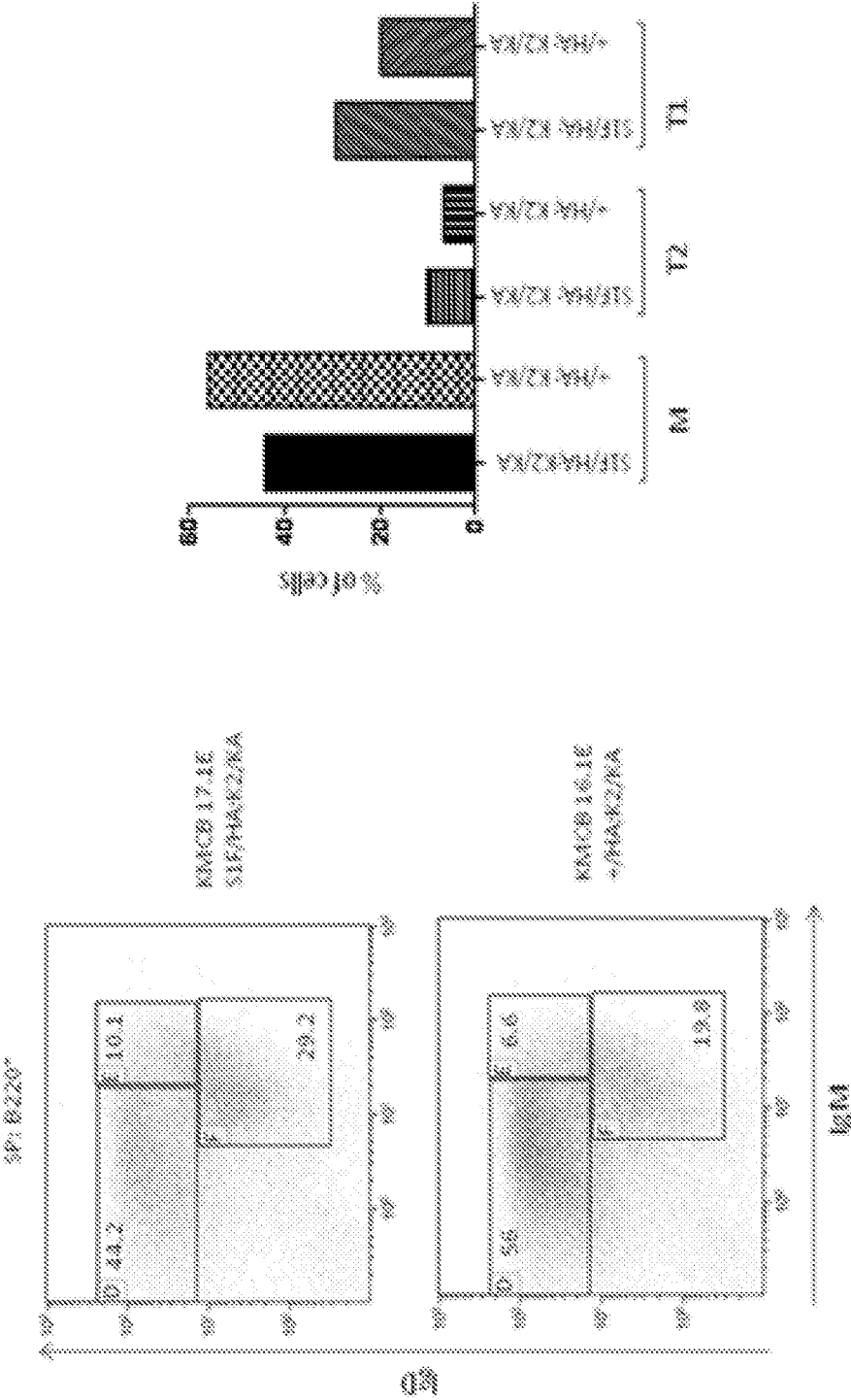


Fig. 66B

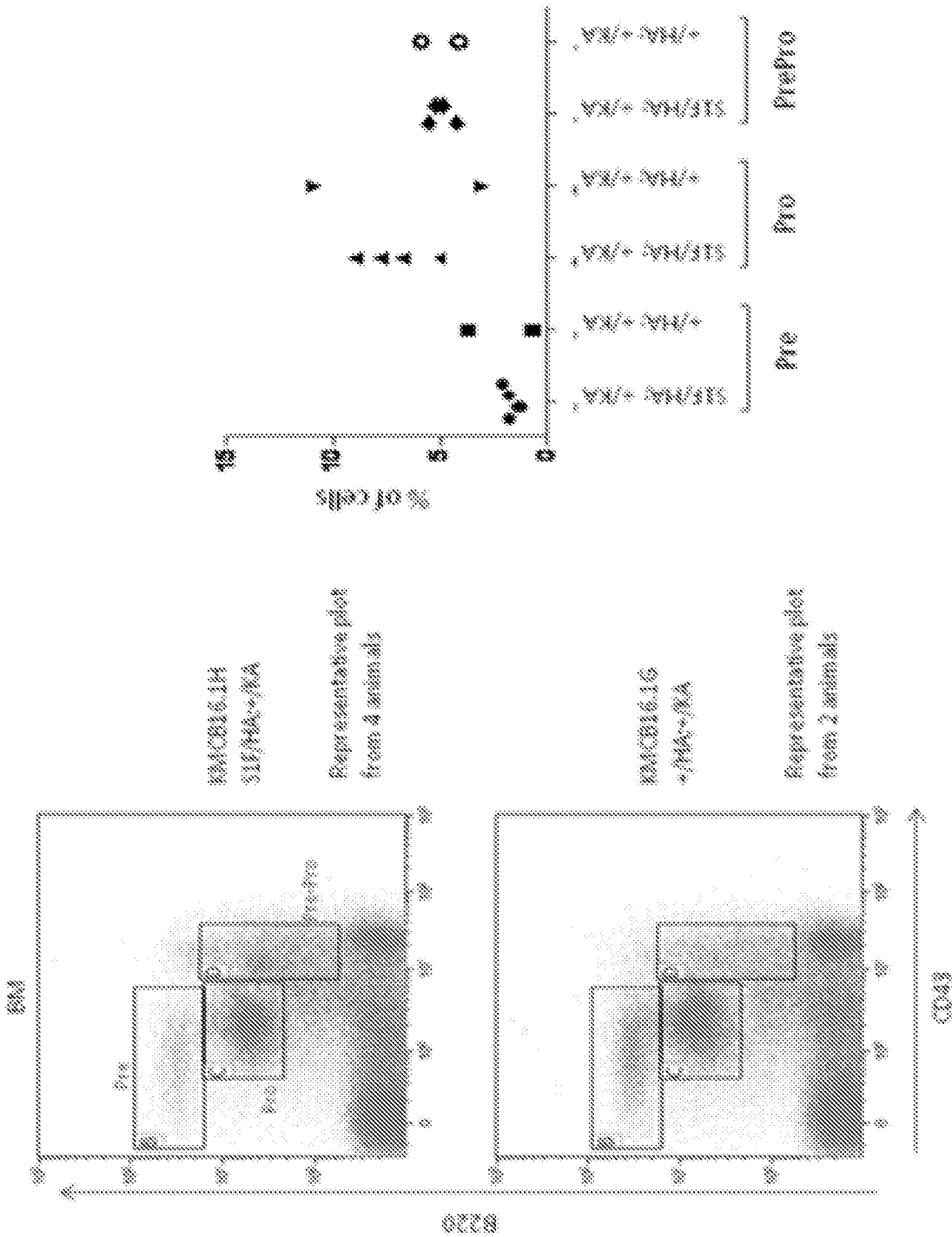


Fig. 67A

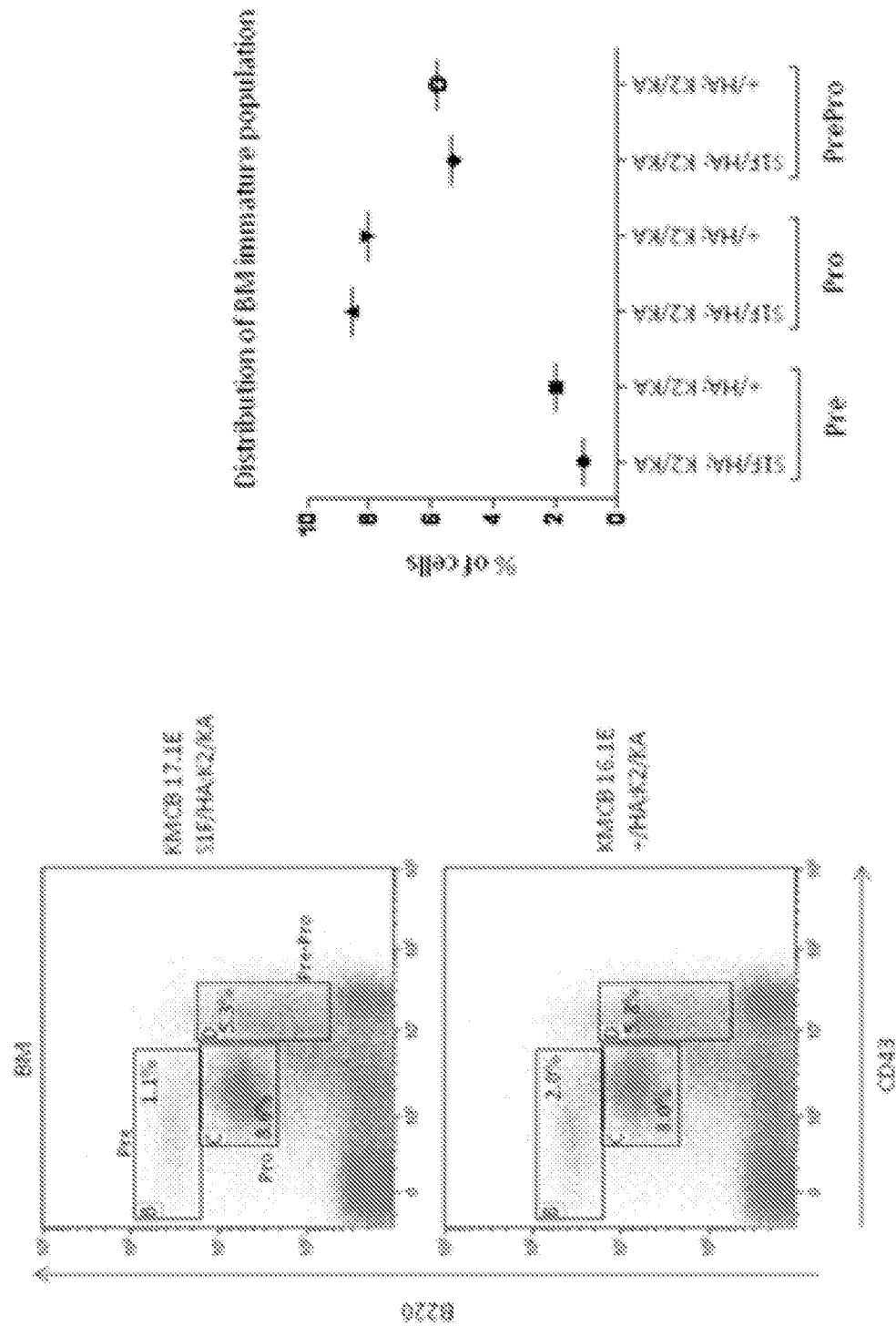


Fig. 67B

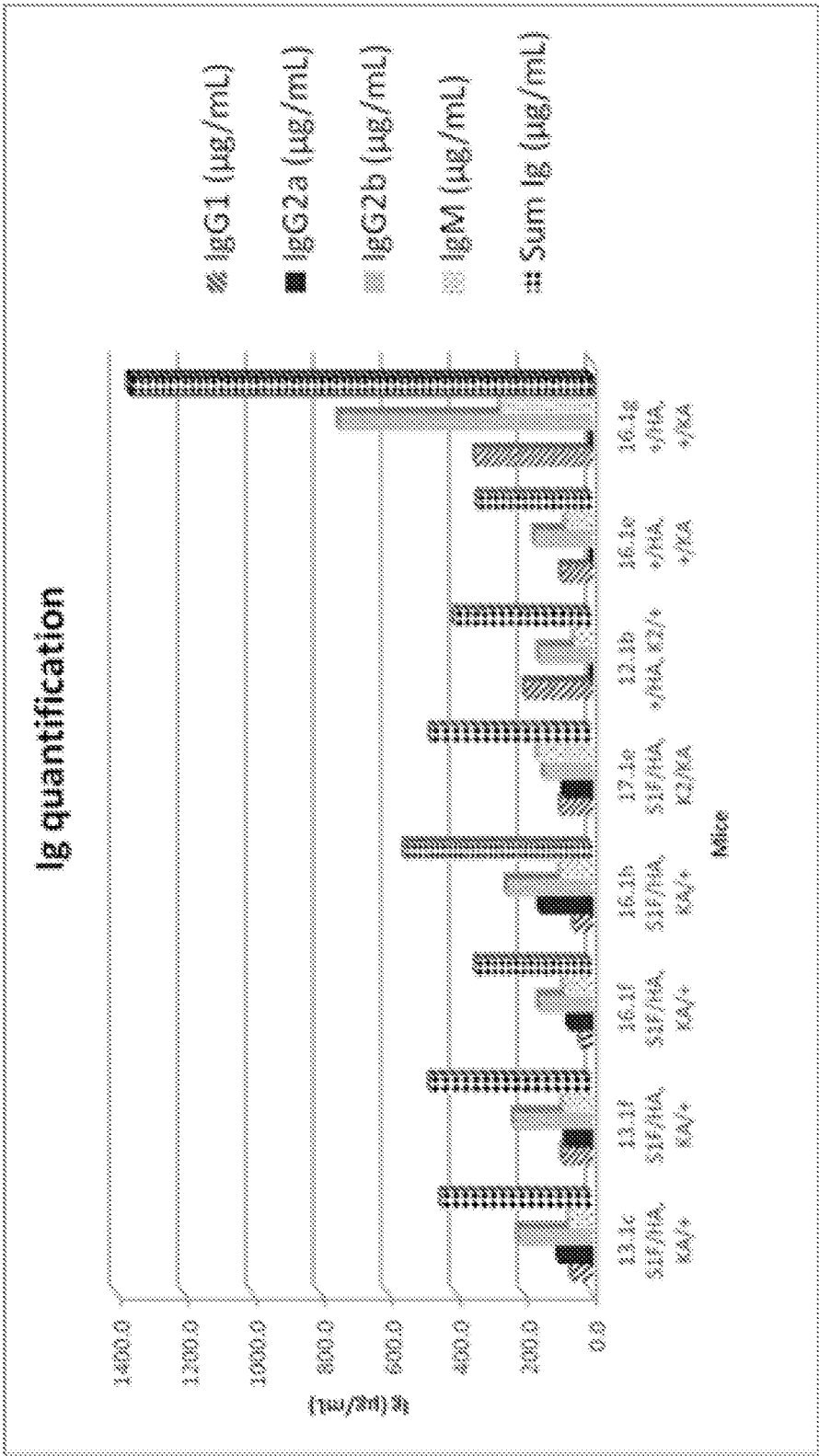


Fig. 68

