

Three basic vector backbones

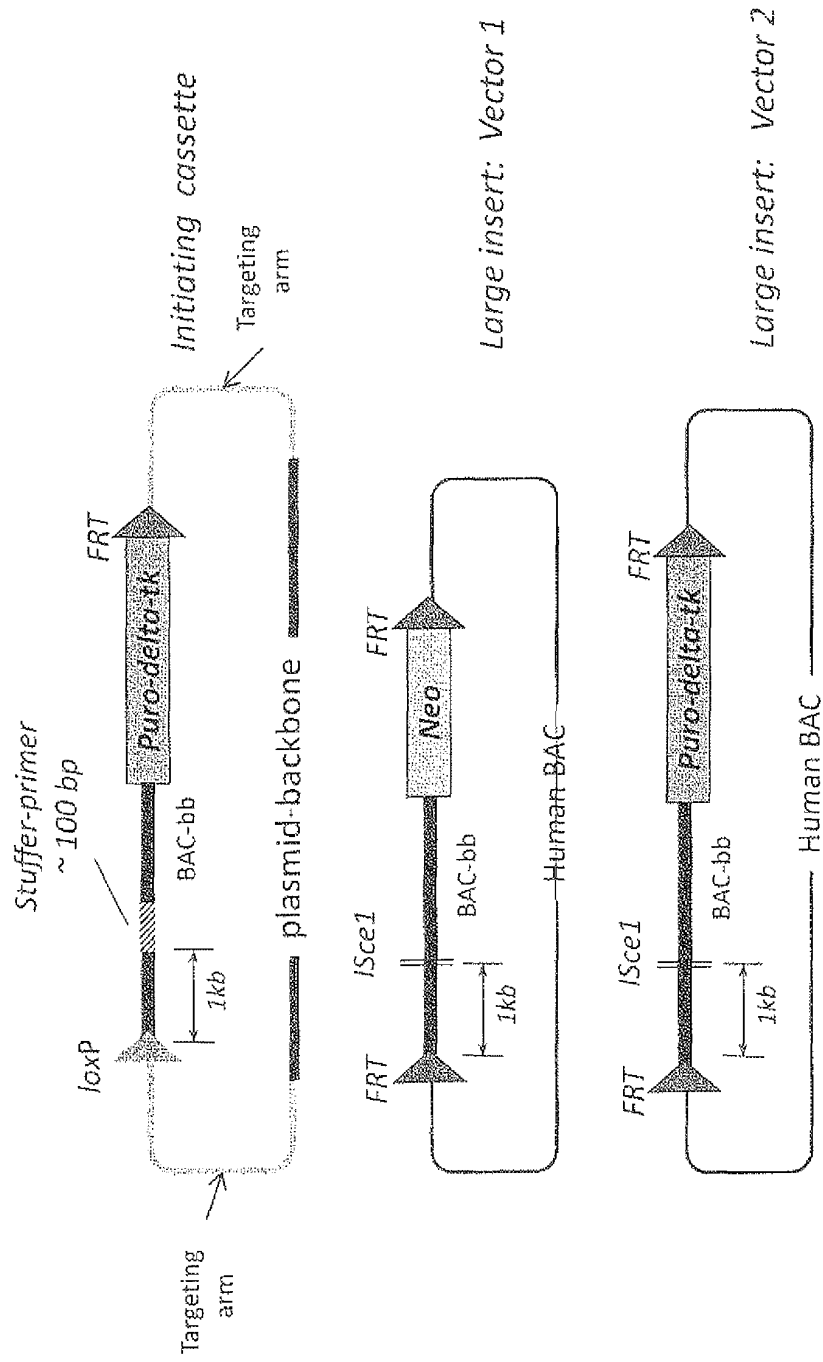
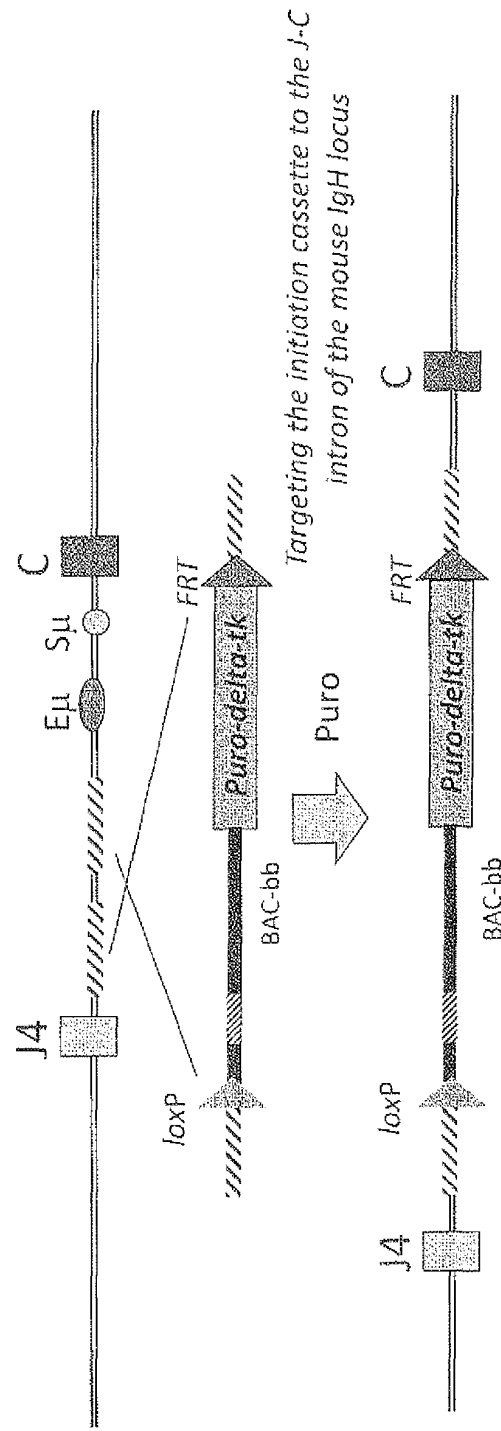


Fig. 1

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

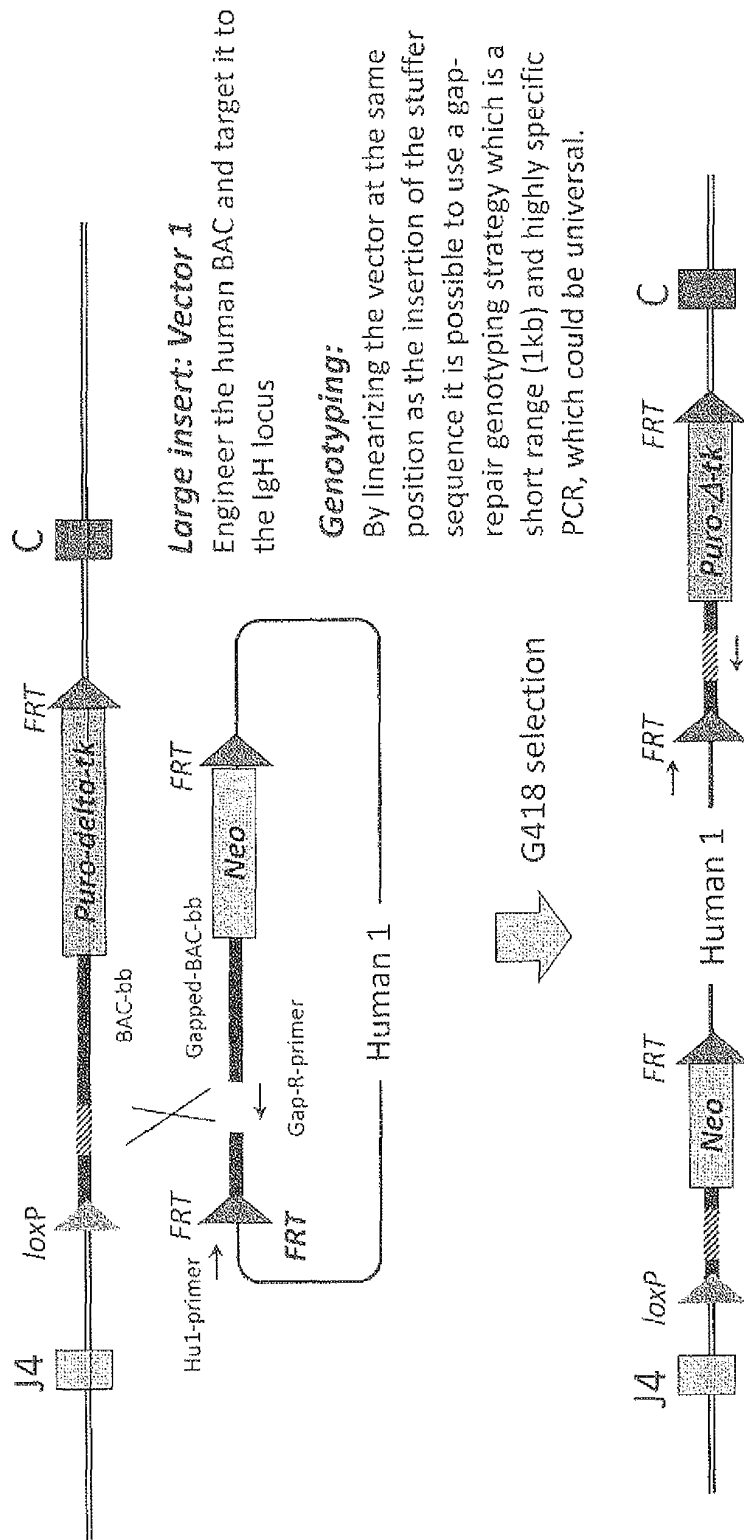


Fig. 3

Addition vector: Step 2

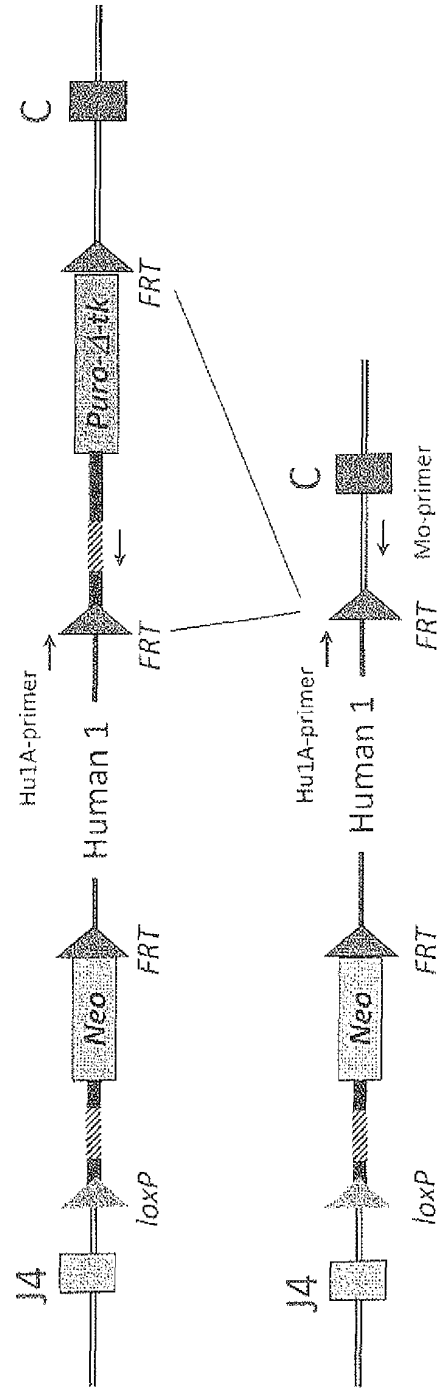
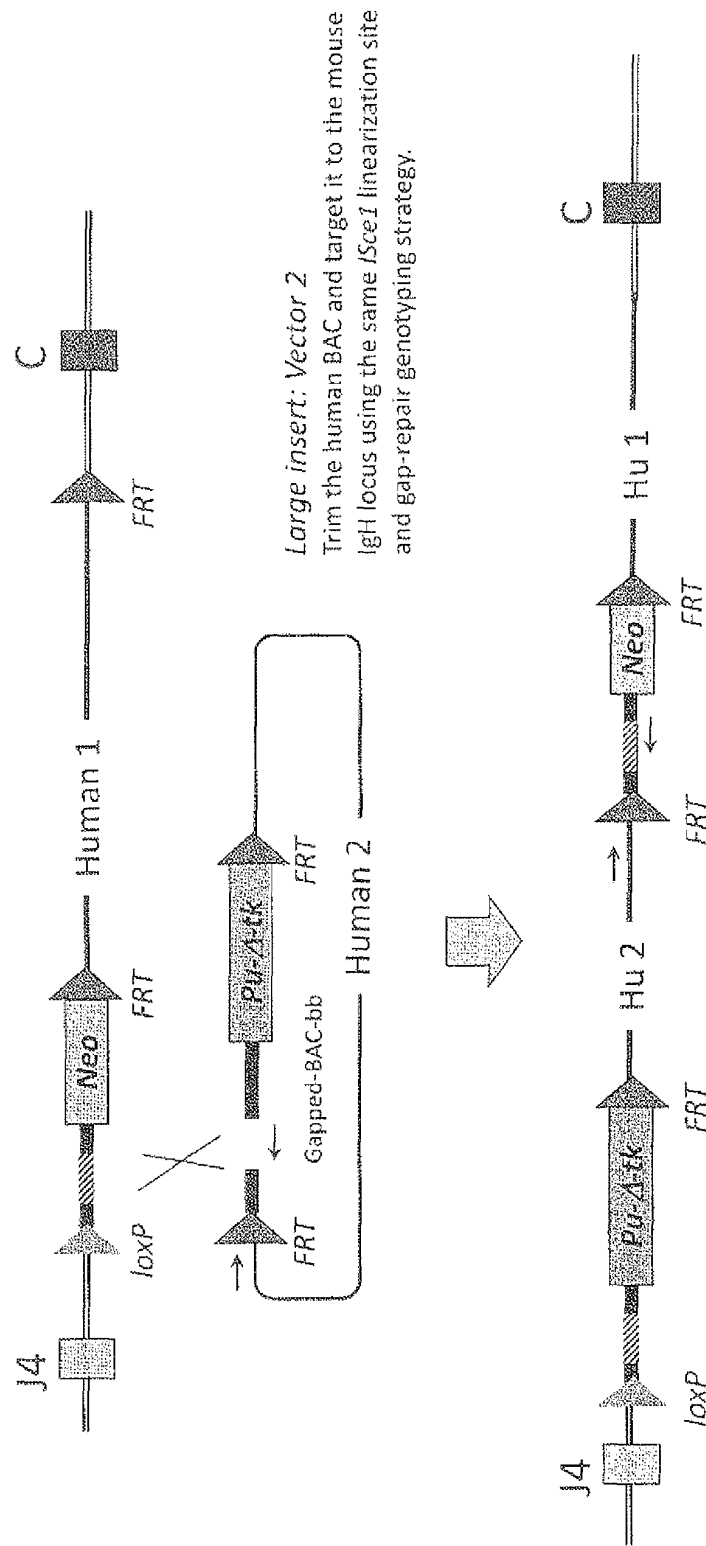


Fig. 4

Addition vector: Step 3A



- Validation:**
1. CGH tiling array across the BAC sequences for Hu1 and Hu2
 2. PCR verification of all critical V, D & J regions

Fig. 5

Addition vector: Step 3B

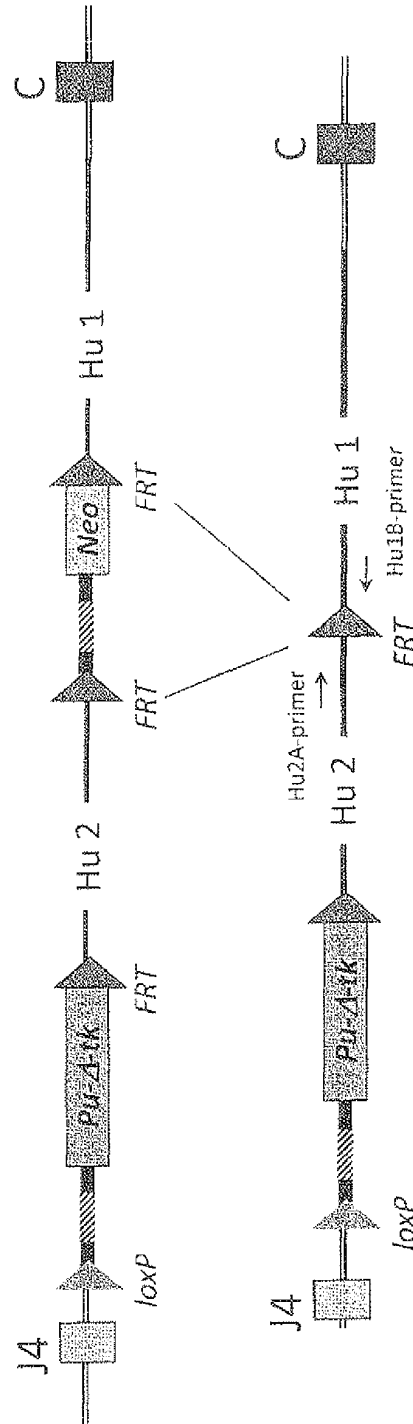


Fig. 6

Addition vector: Step 4

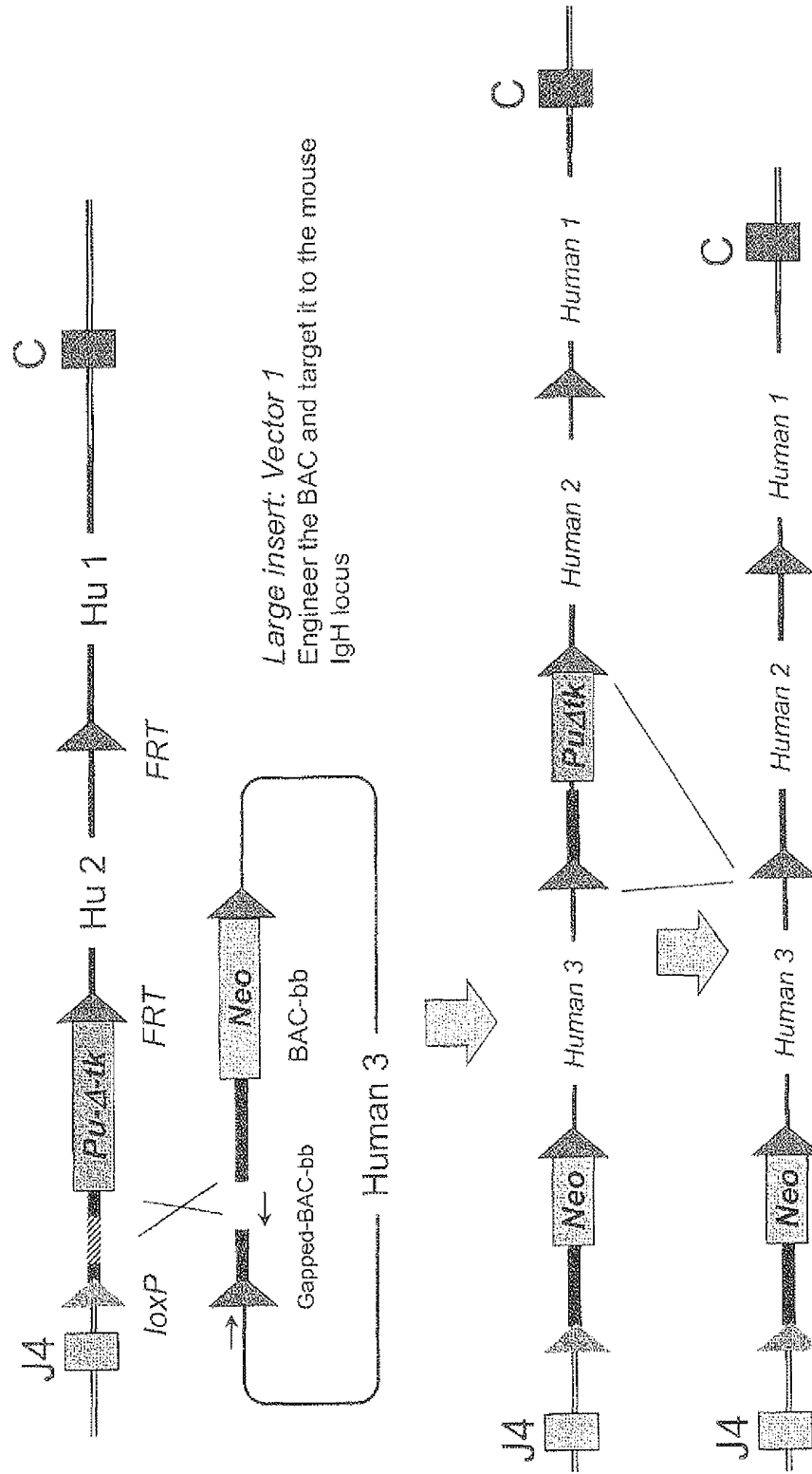


Fig. 7

A humanized mouse *IgH* locus

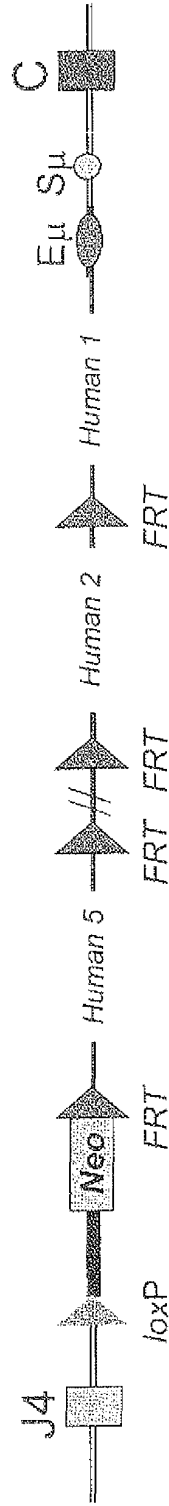


Fig. 8

BAC landing pad inserted at the IgH locus

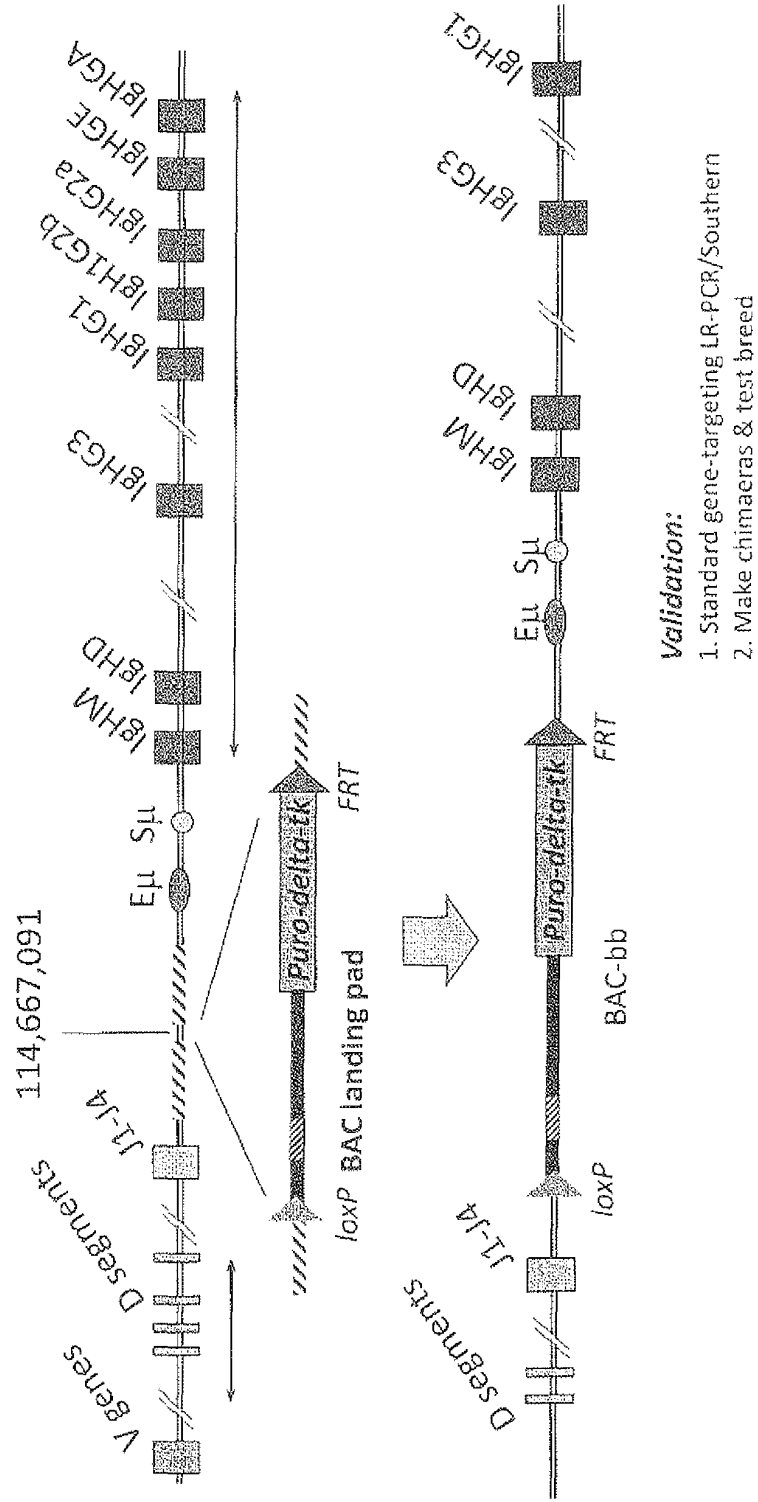
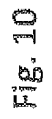


Fig. 9



1st BAC insertion – Step 2

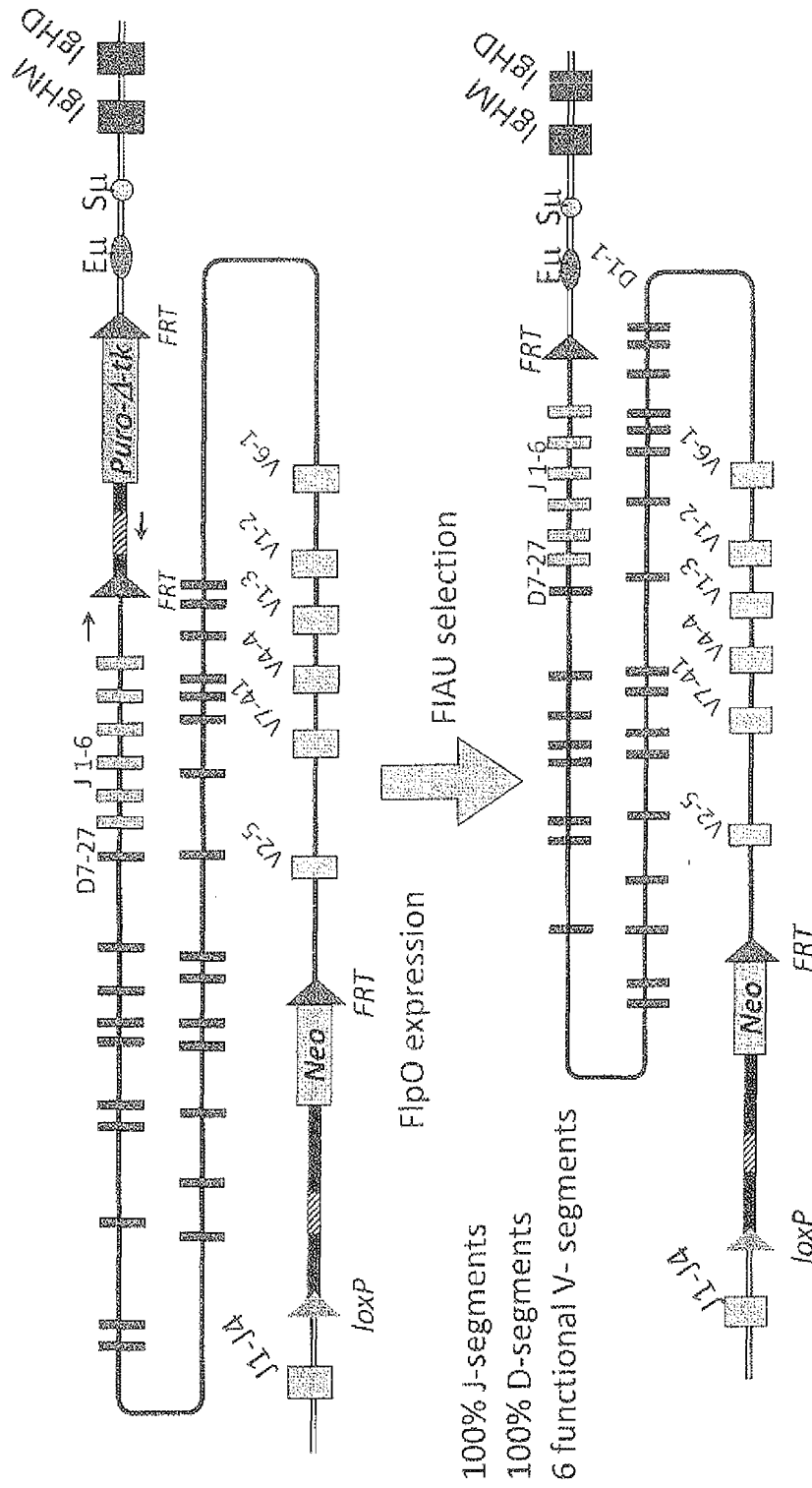


Fig. 11

2nd BAC insertion – Step 3

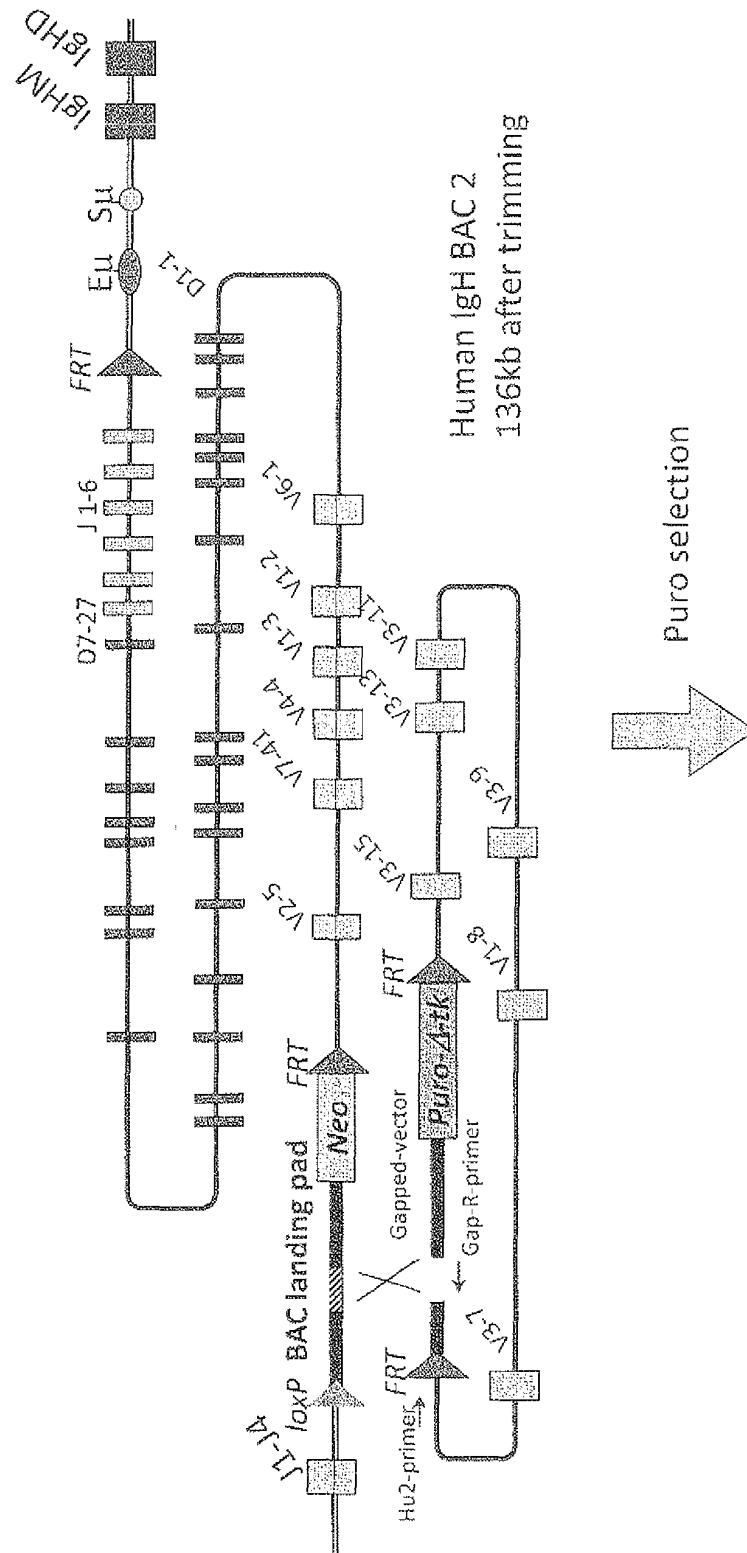
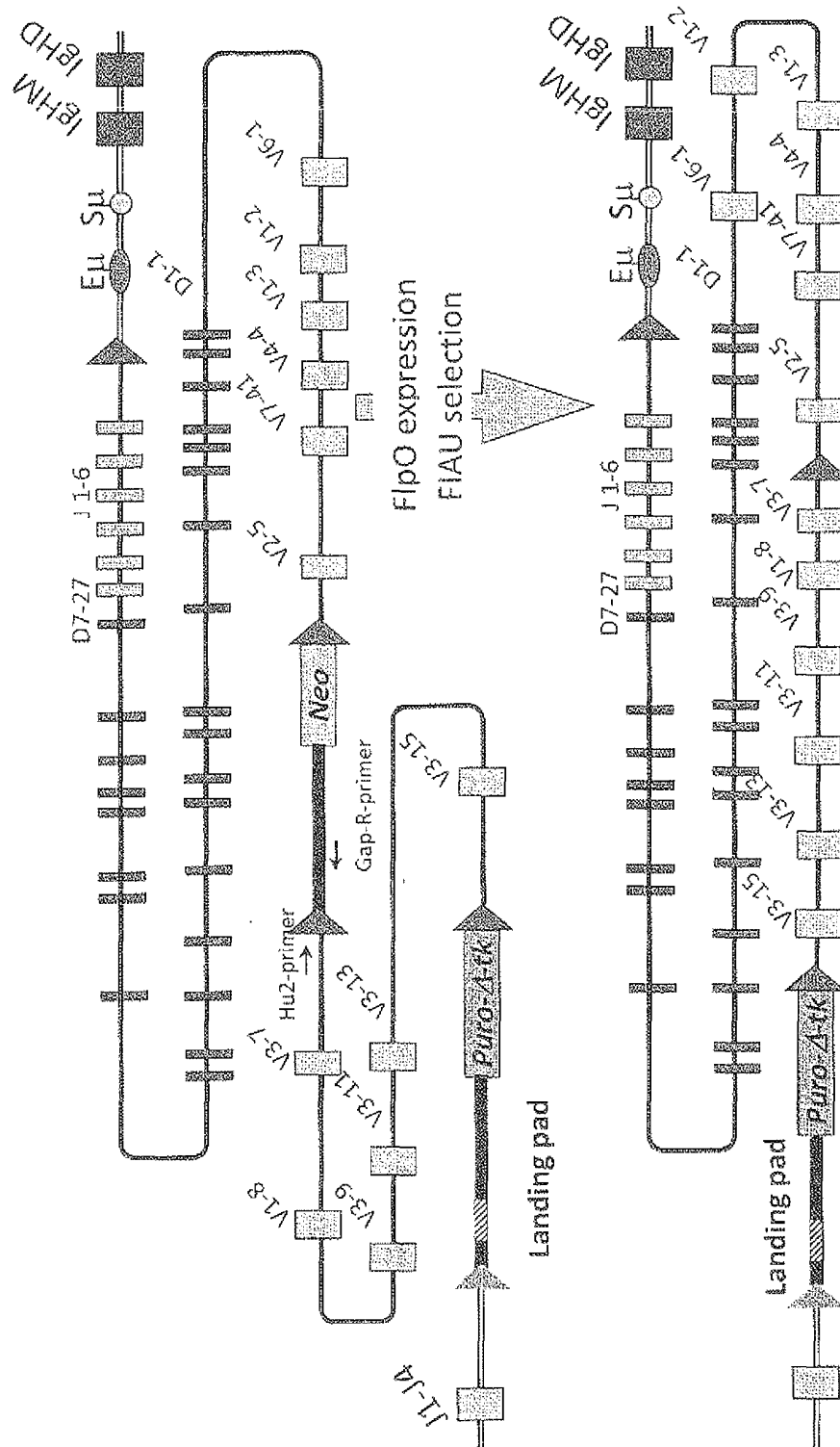


Fig. 12

2nd BAC insertion – Step 4



BAC Landing Pad at IgK Locus

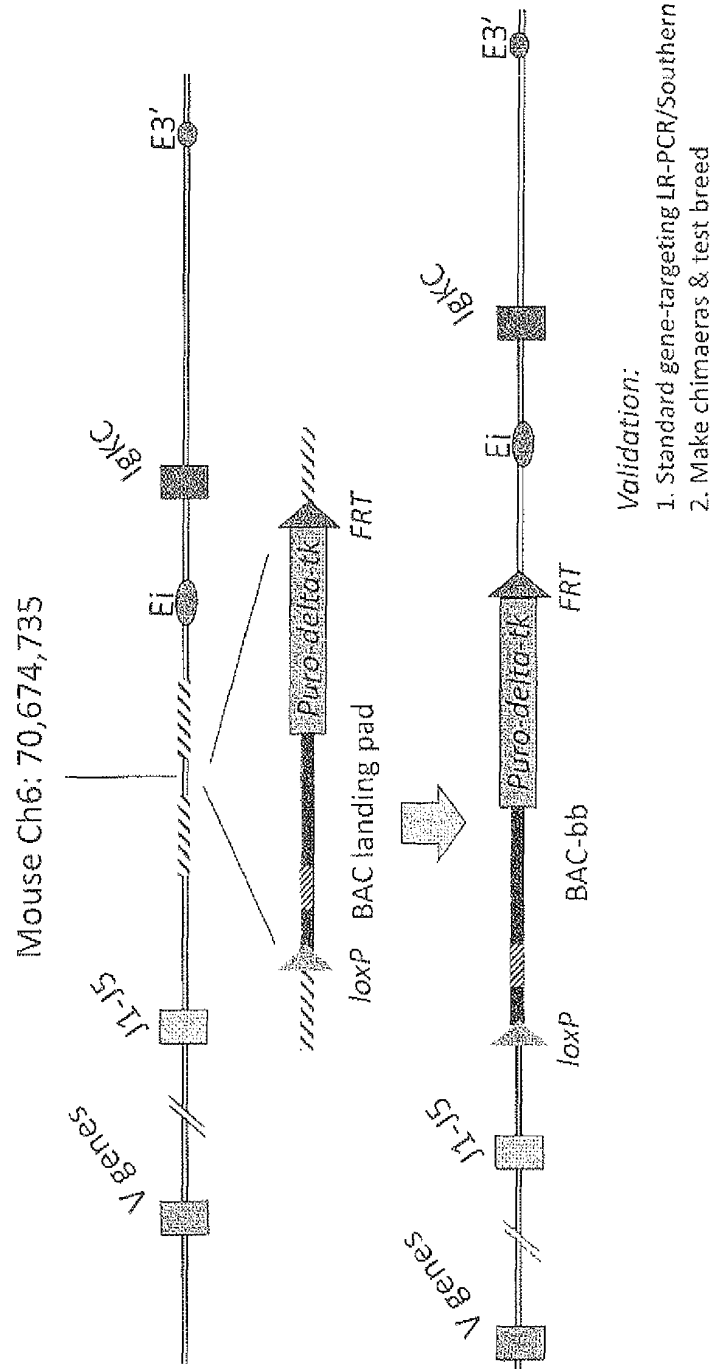
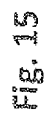


Fig. 14



1st BAC Insertion – Step 2

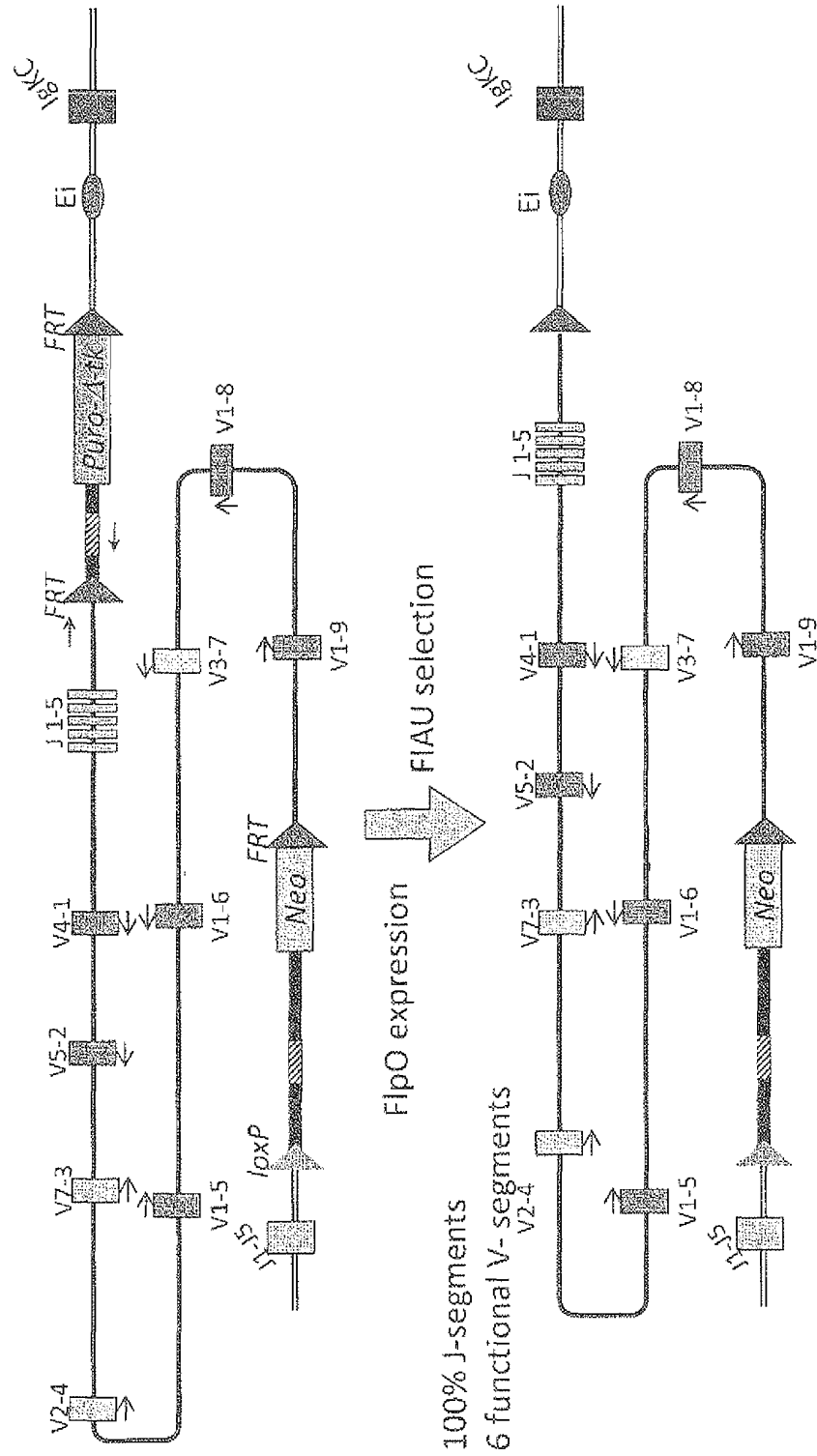


Fig. 16

2nd BAC Insertion – Step 3

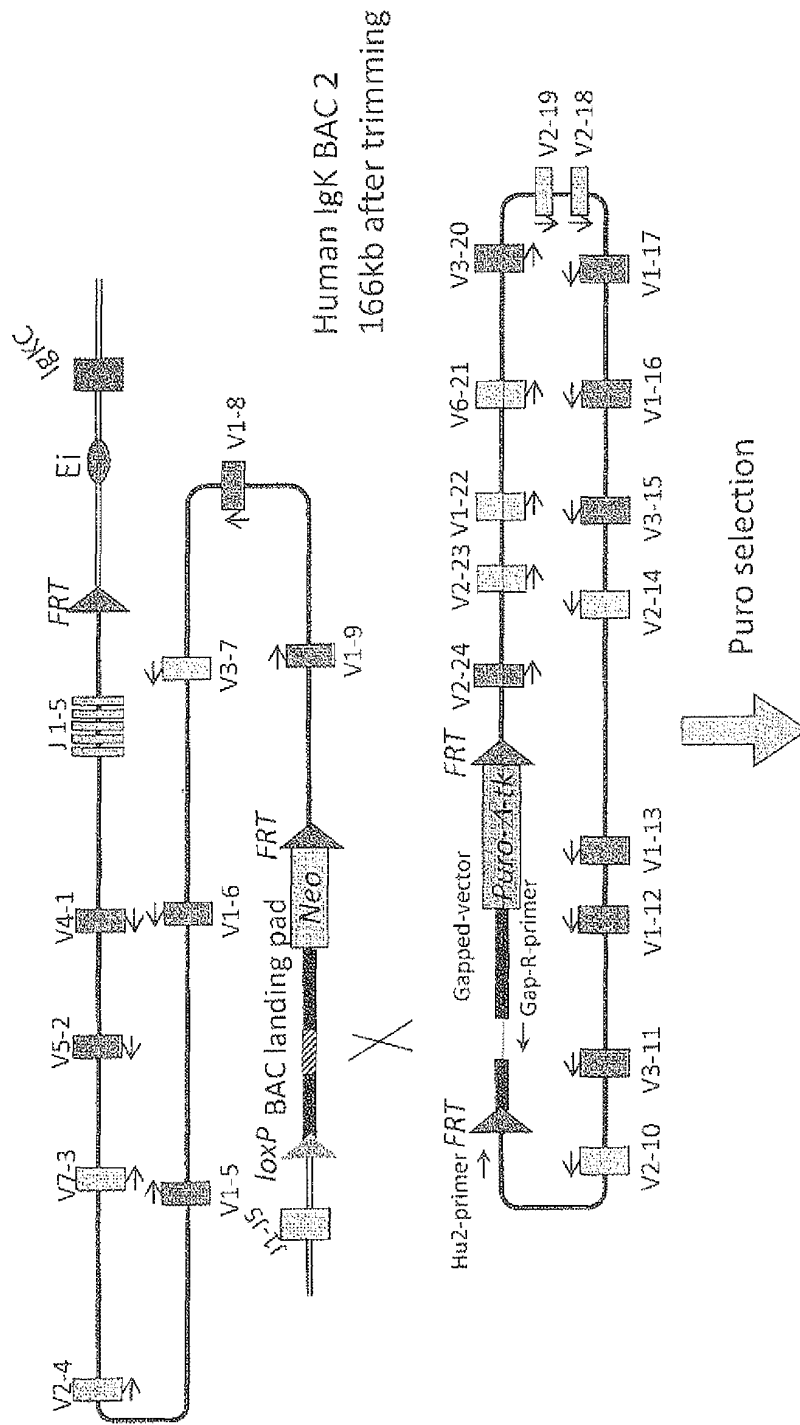
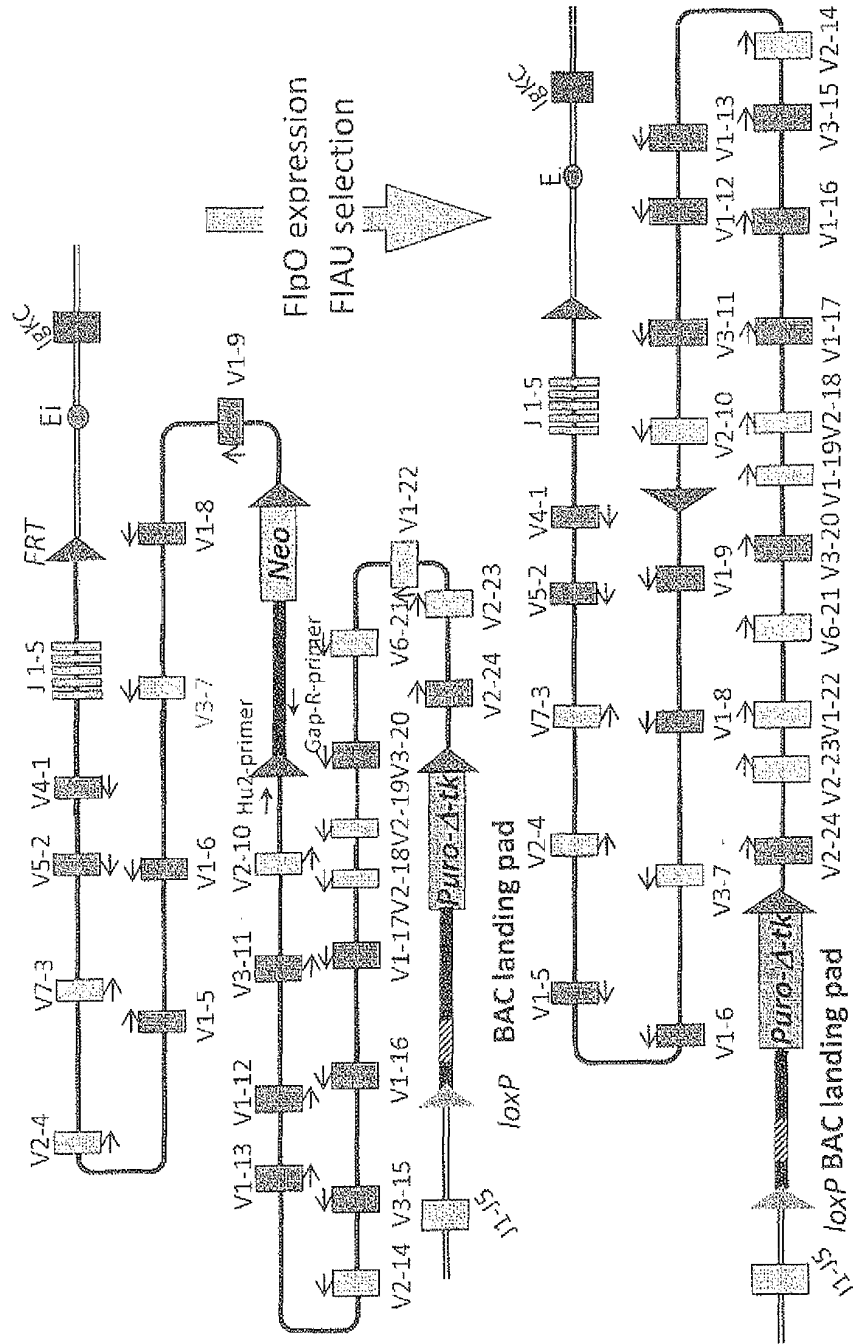


Fig. 17



CO
COPPER
BQ
BROOKLYN

Rapid Testing of ES Cells with Humanized IgH and IgK Loci

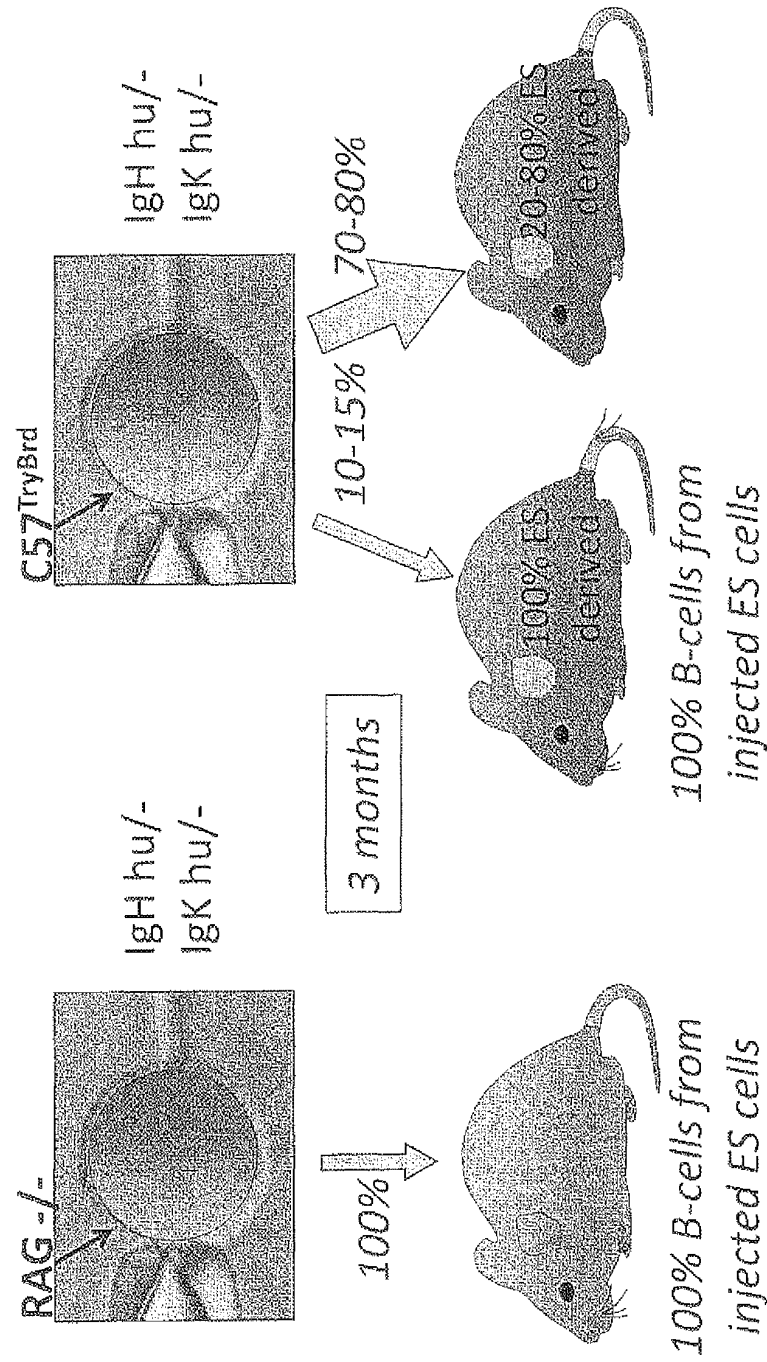


Fig. 19

Antibody Generation in Chimaeric Mice with Humanized IgH and IgK Loci

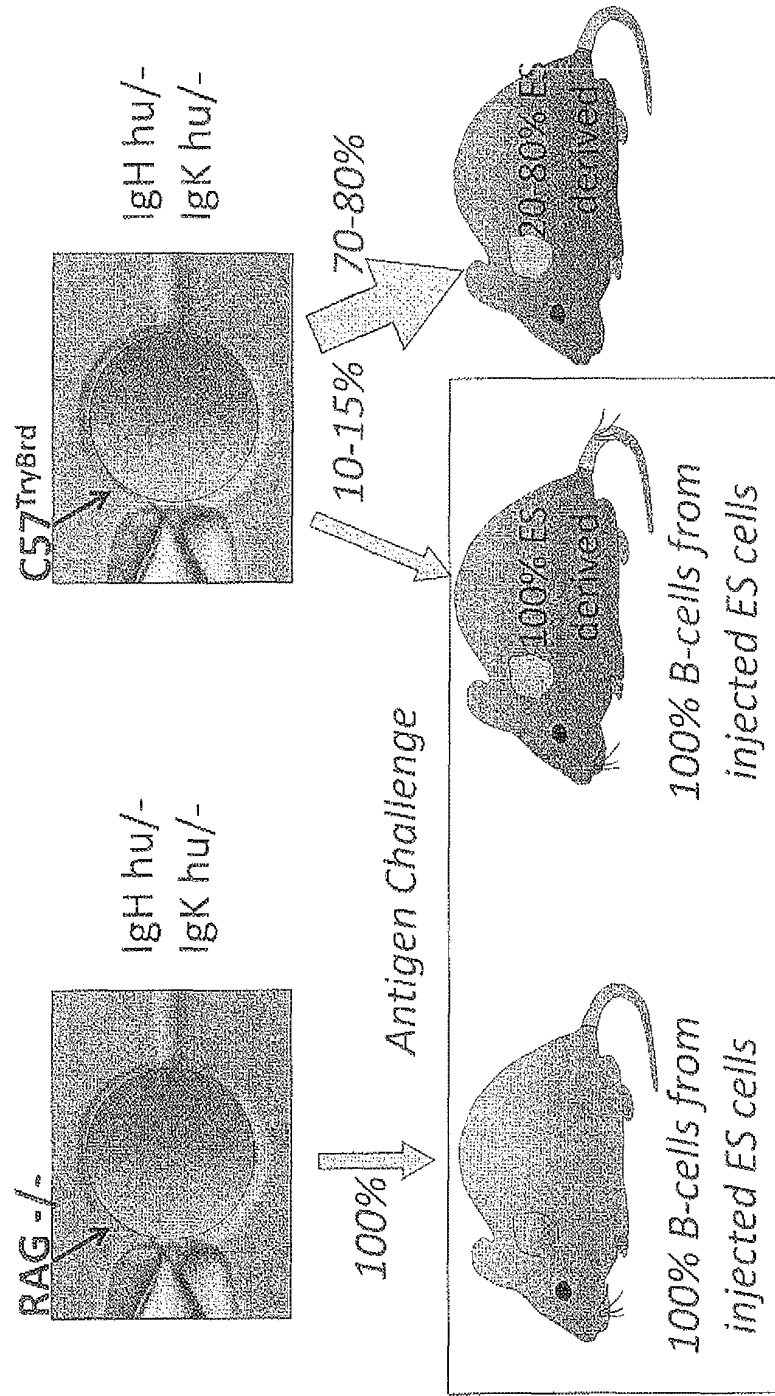


Fig. 20

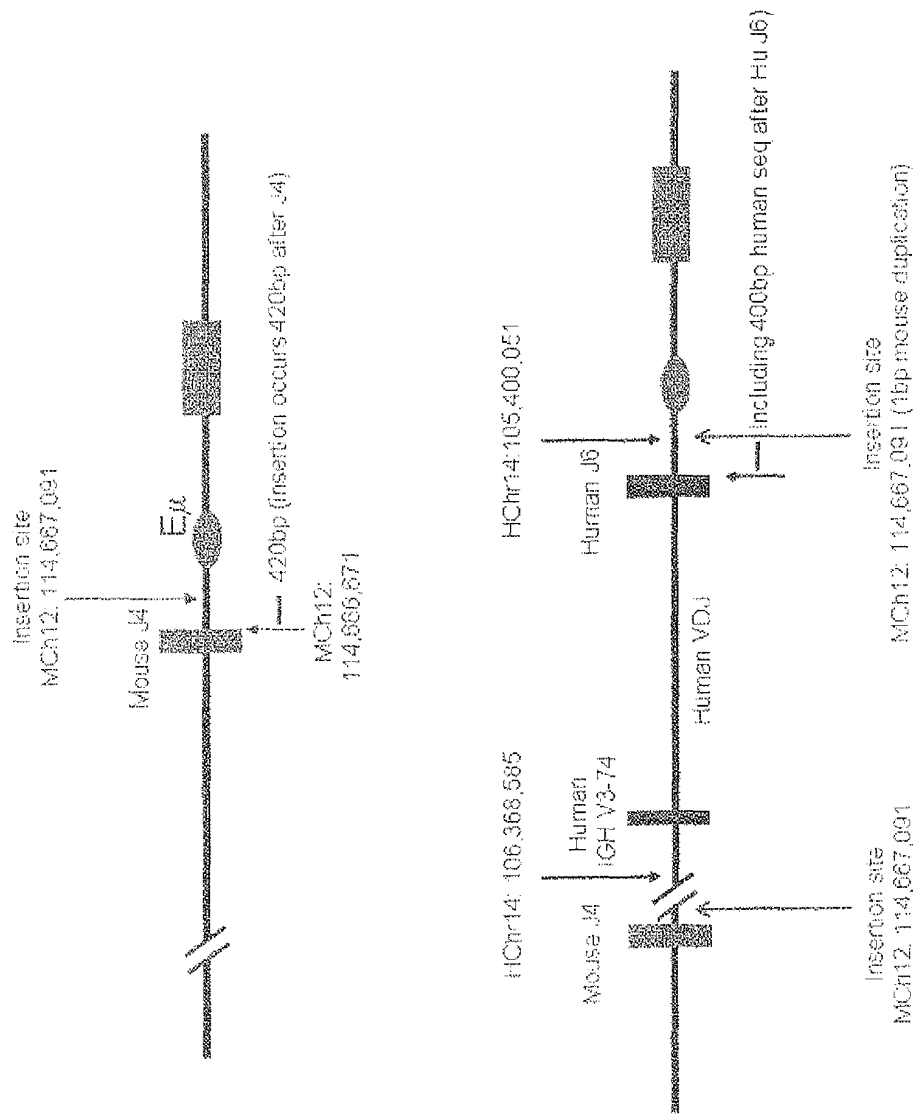


Fig. 21

Targeting of the Landing Pad

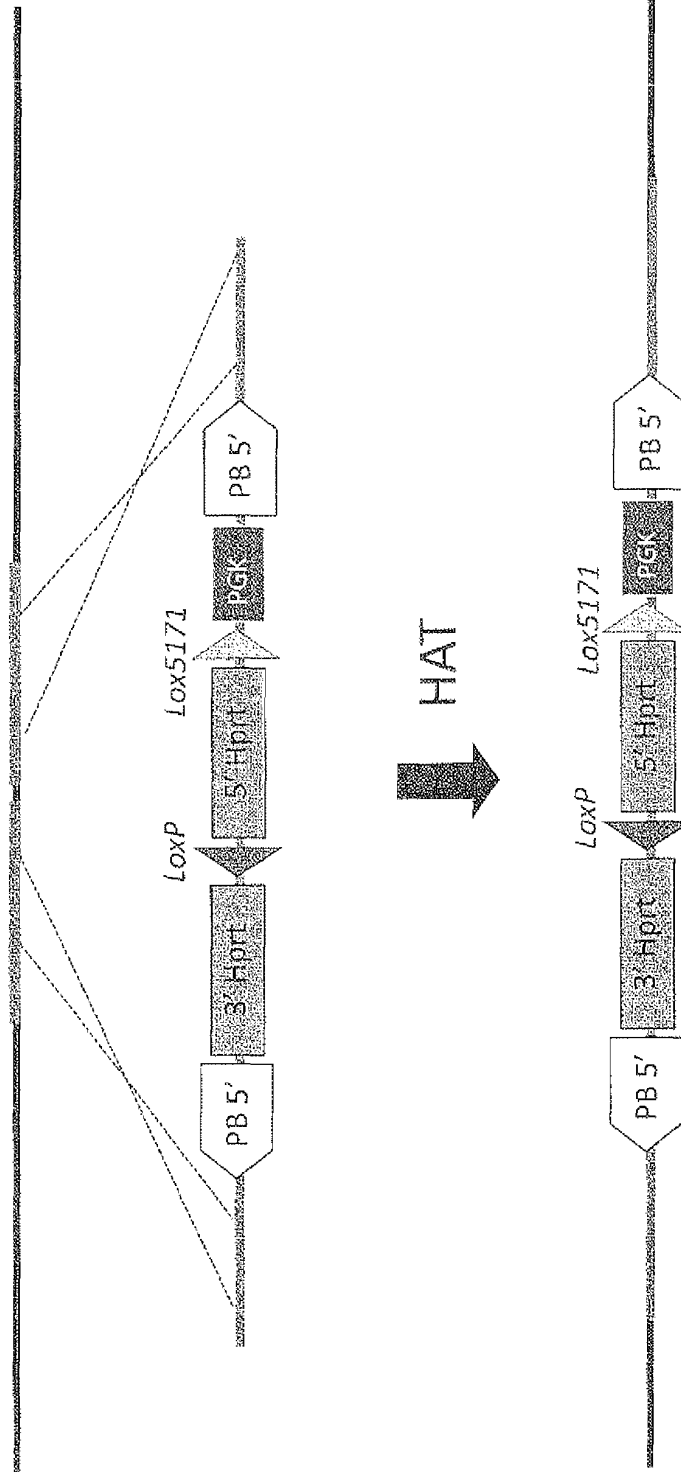


Fig. 22

Insertion of the 1st BAC

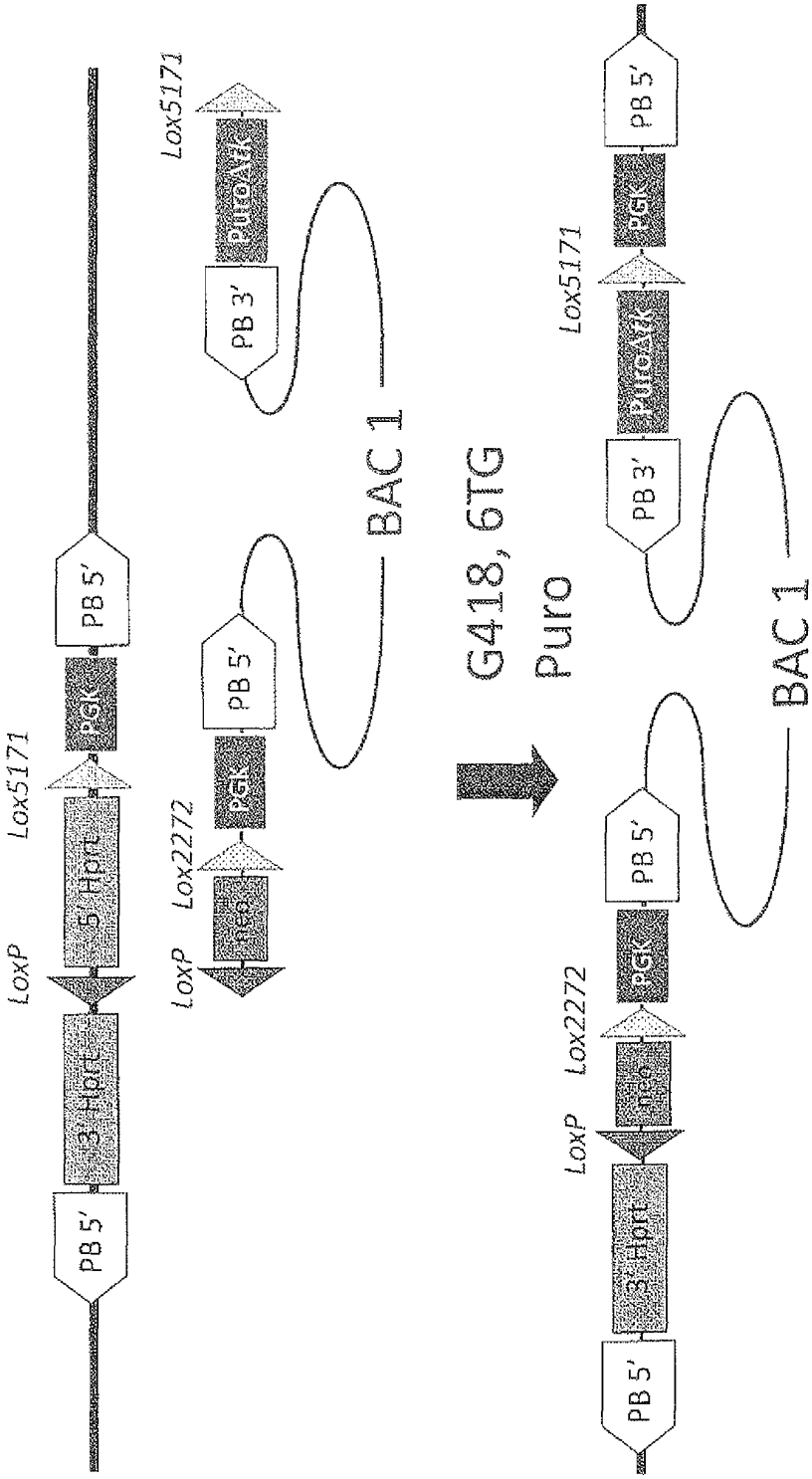


Fig. 23

Curing of the 3' Modification

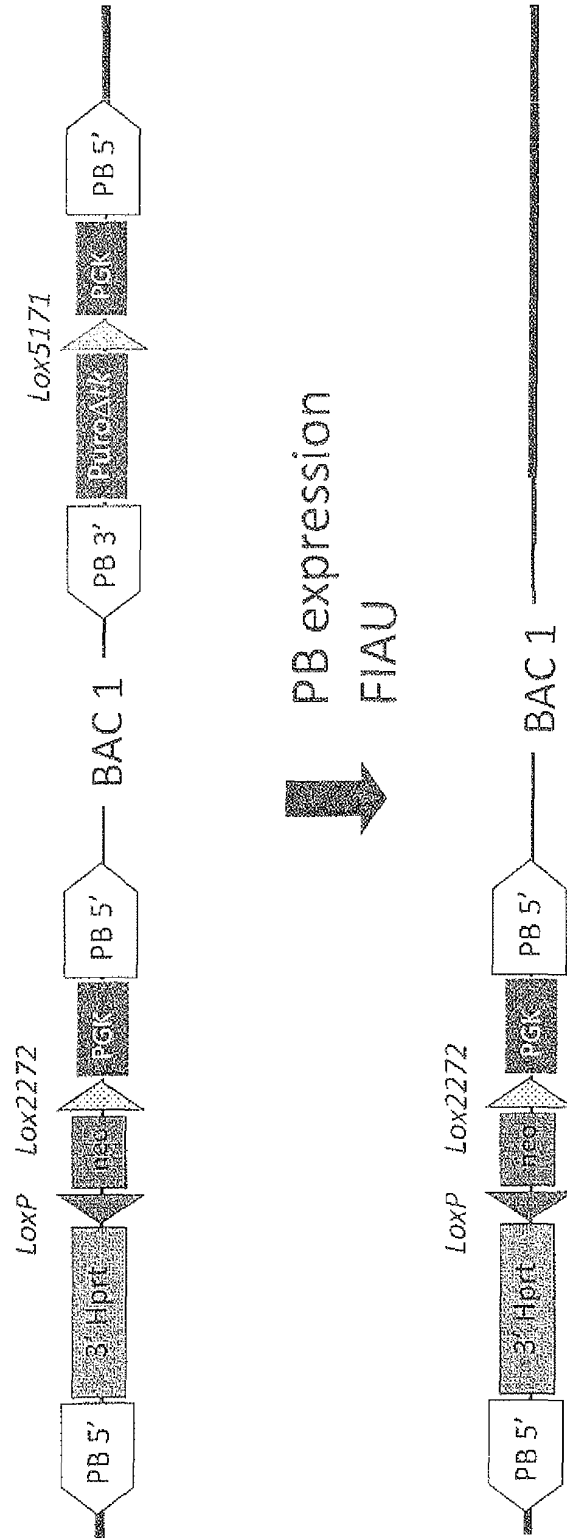


Fig. 24

Insertion of the 2nd BAC

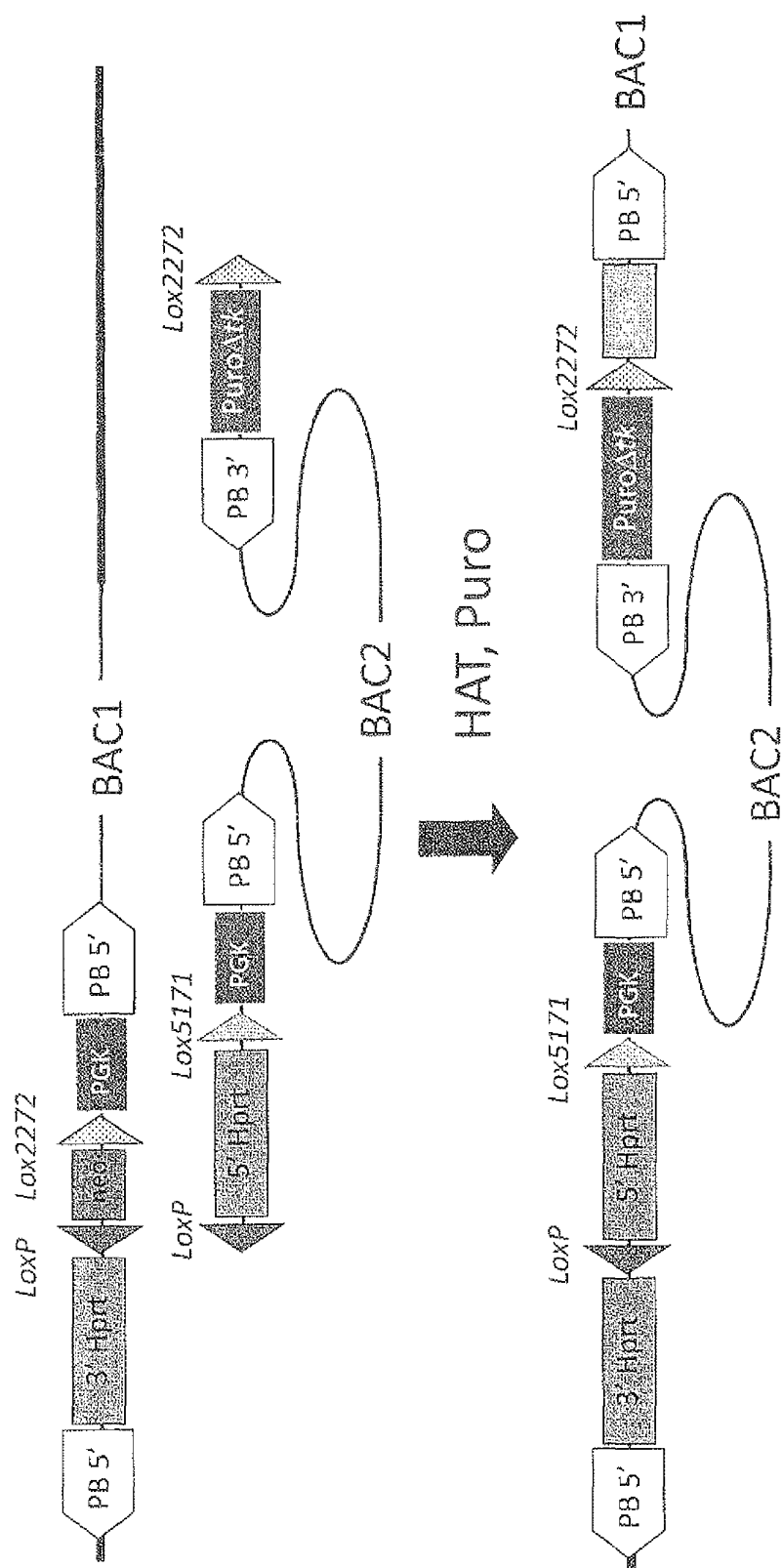


Fig. 25

Removing the 3' Modification

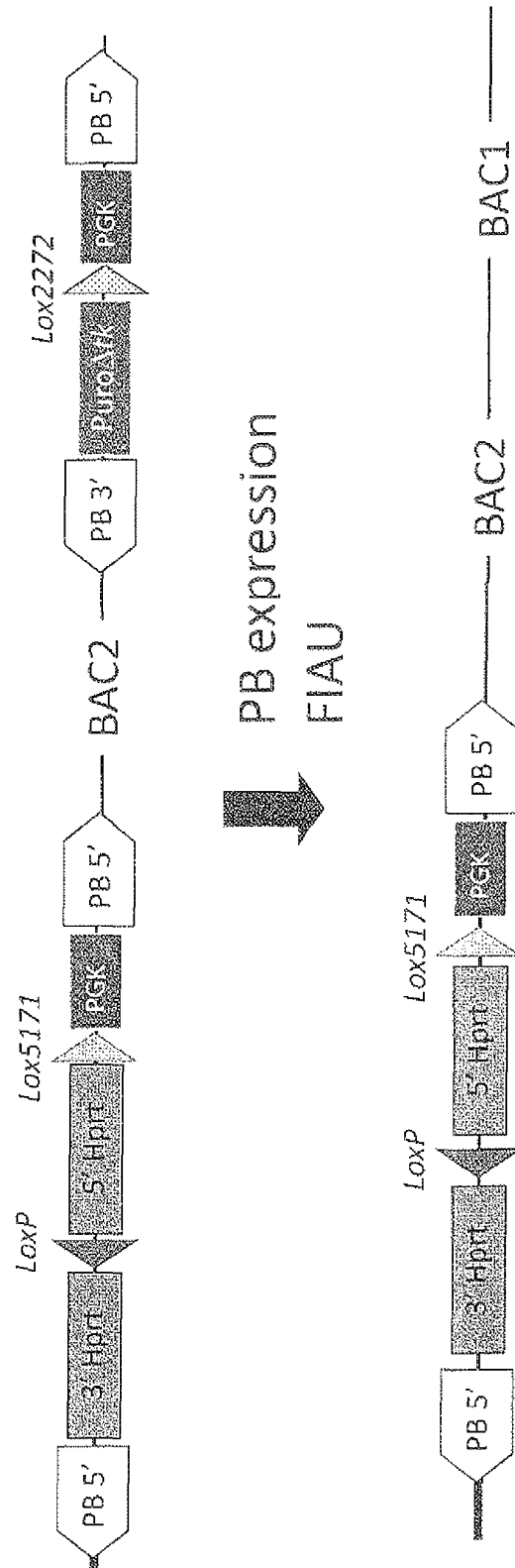


Fig. 26

Targeting the “Flip-Over” Vector

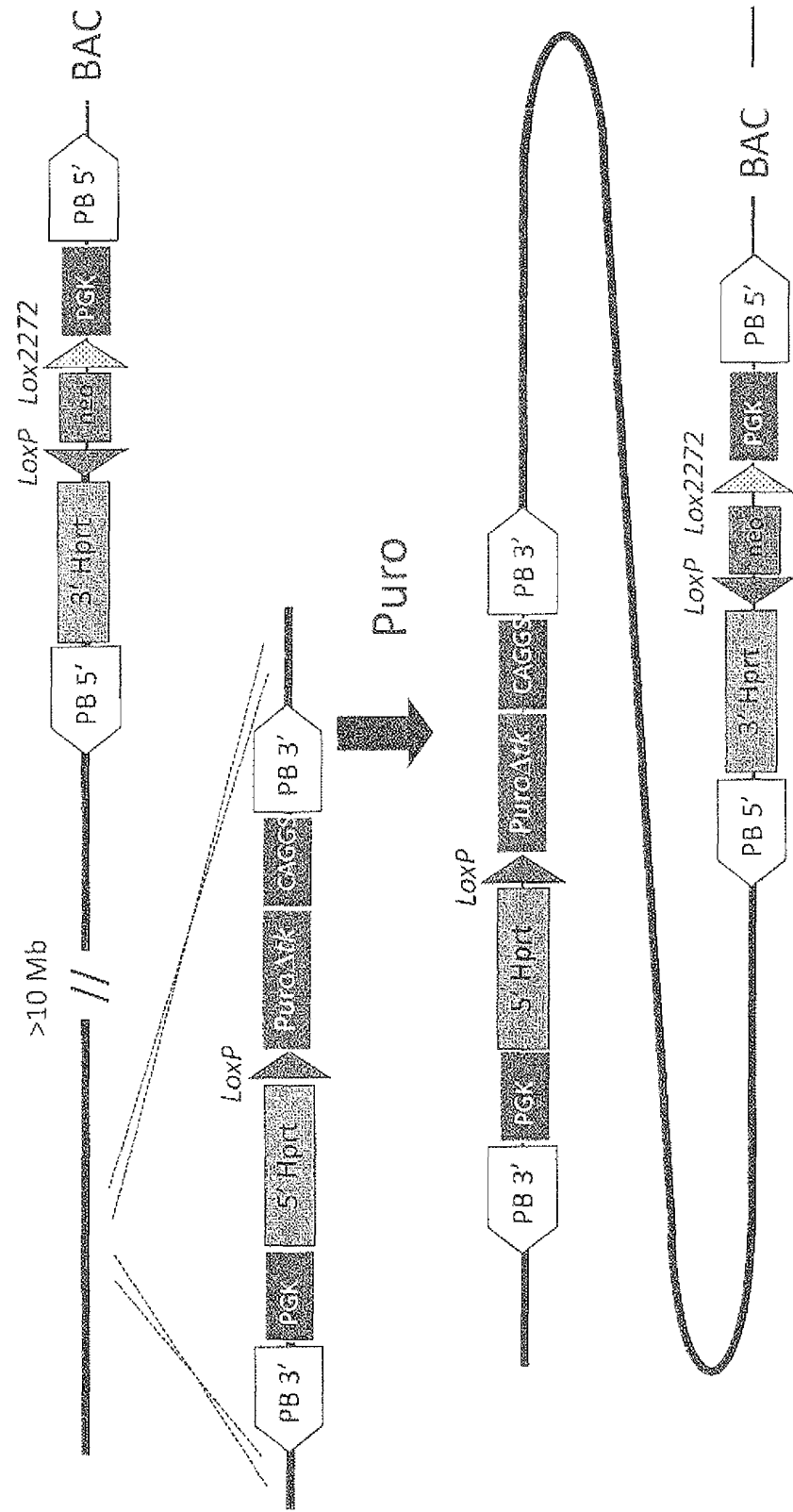


Fig. 27

Inversion

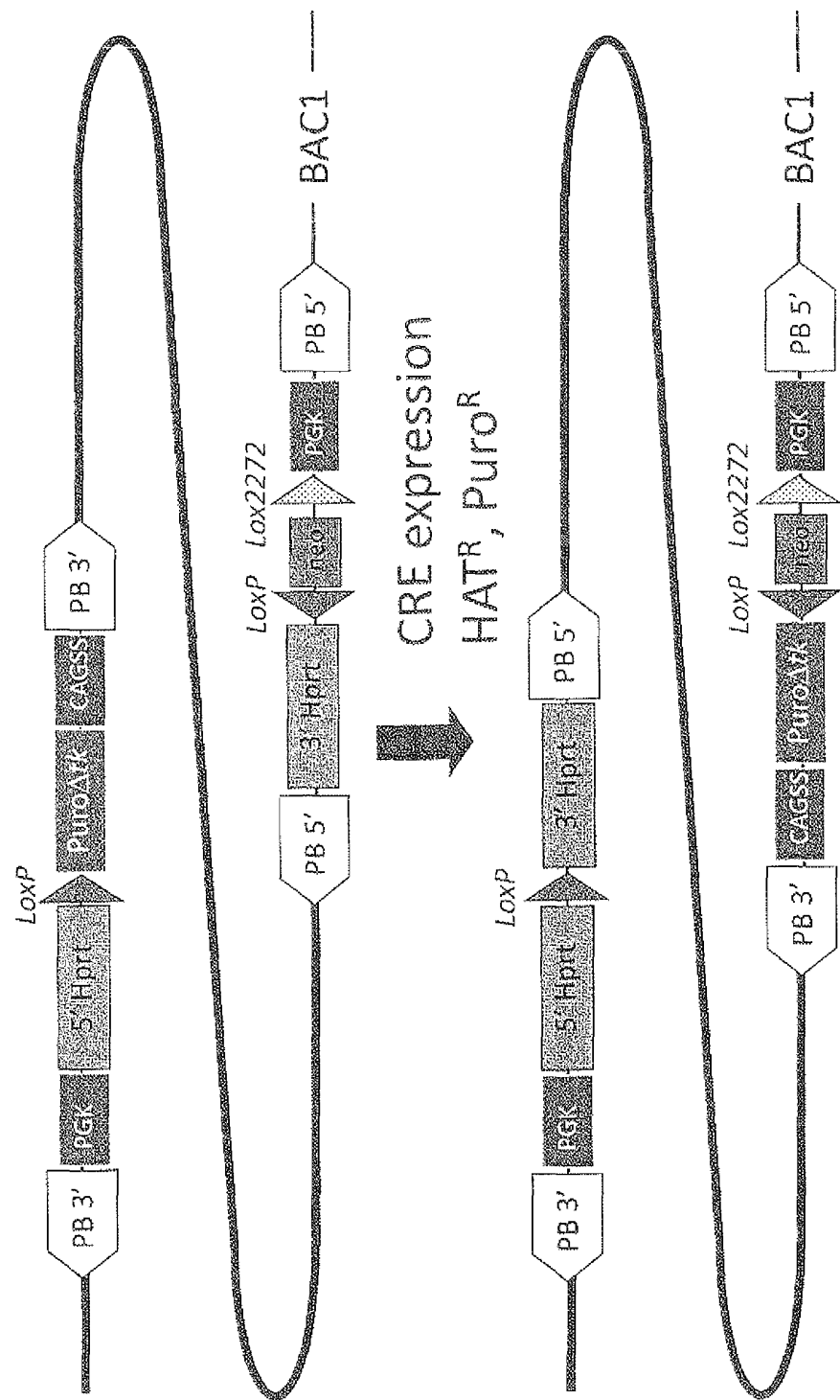
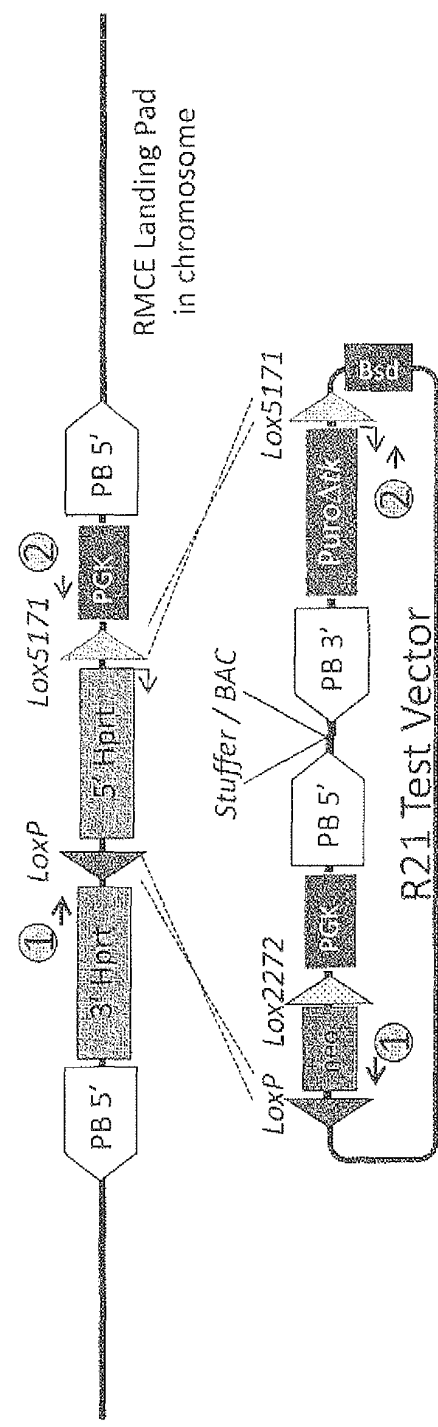


Fig. 28

Sequential RMCE – Integration into Landing Pad



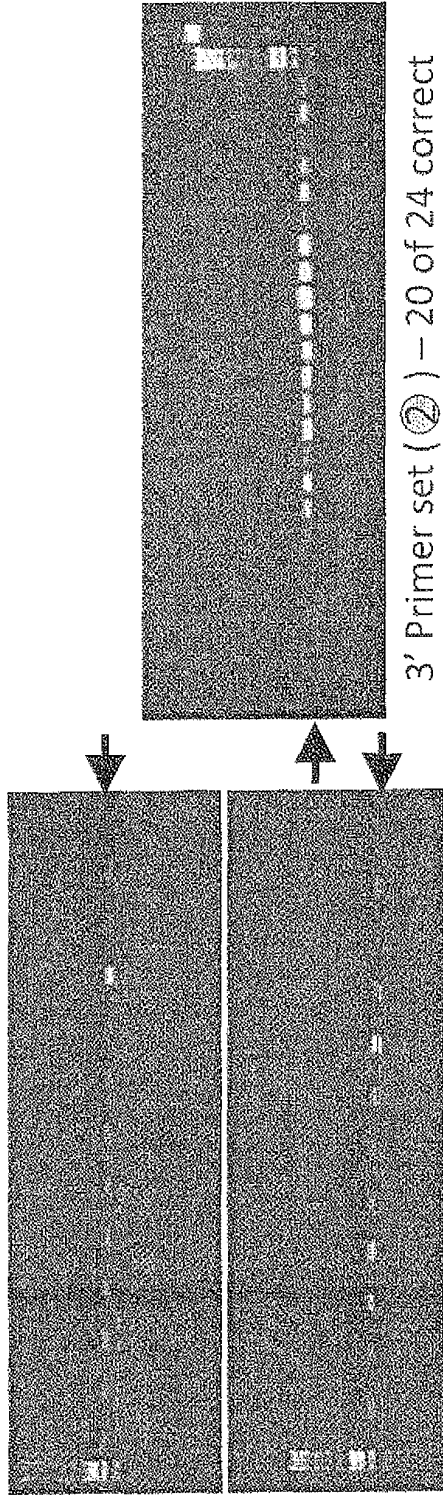
R21 transfected w/ Cre-expression Cassette

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:

	Puro + 6TG		Puro		G418 + 6TG		G418	
Colony #	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

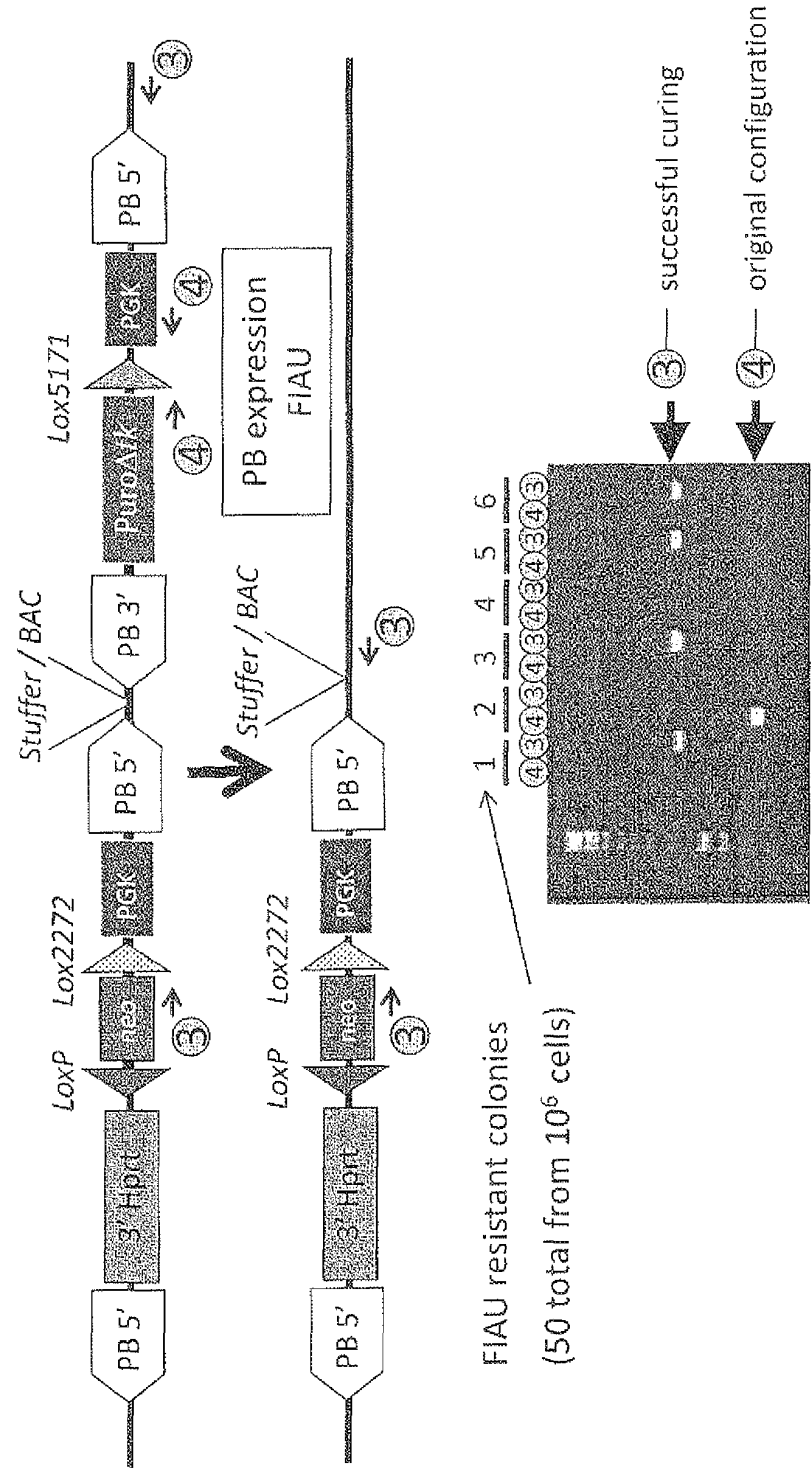


Fig. 32

Insertion of BAC#1 – PCR Diagnostics

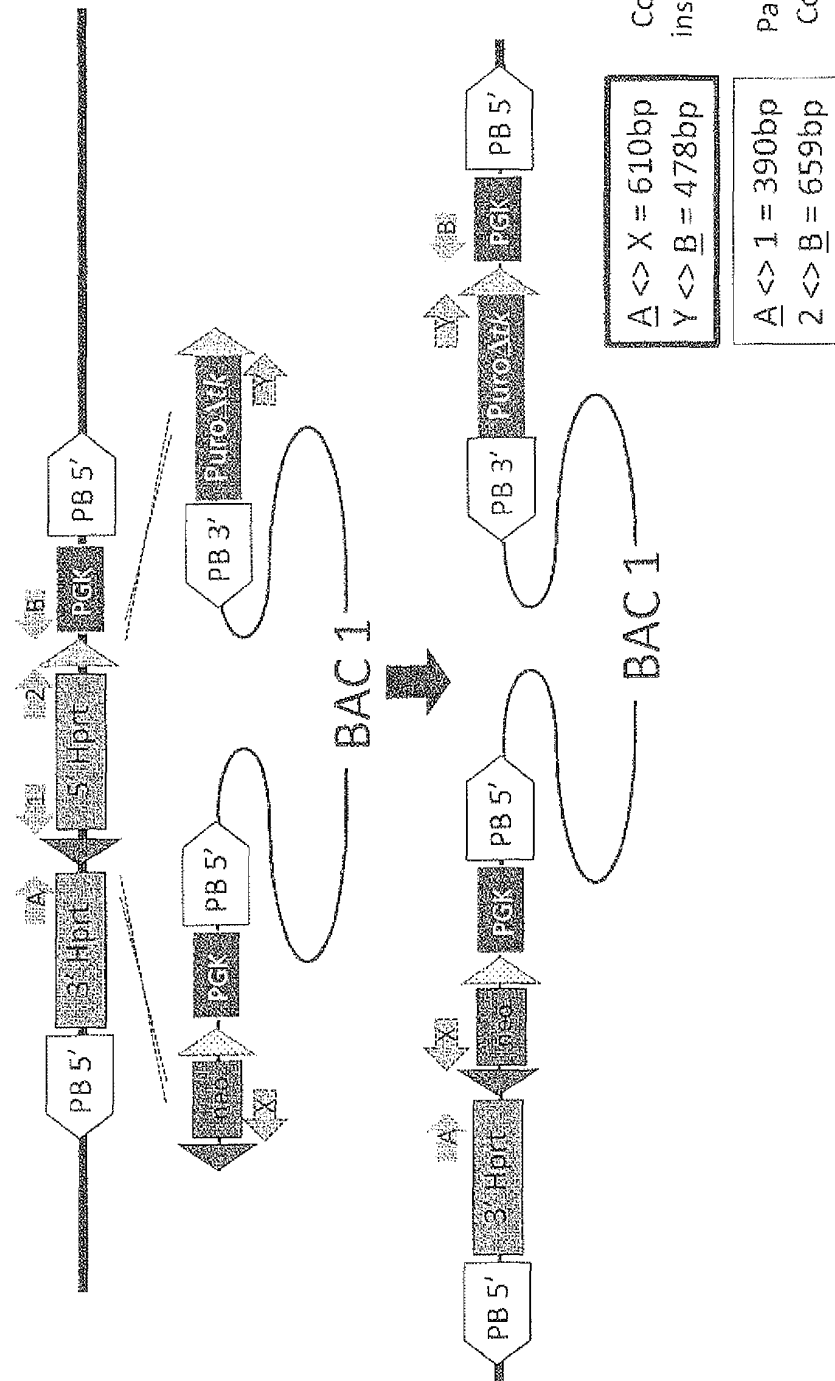
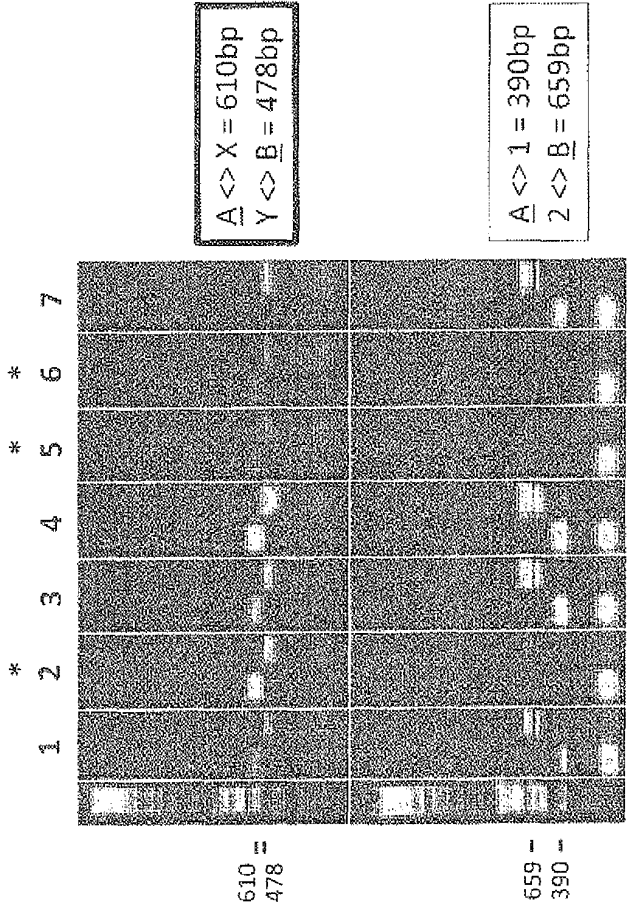


Fig. 33

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

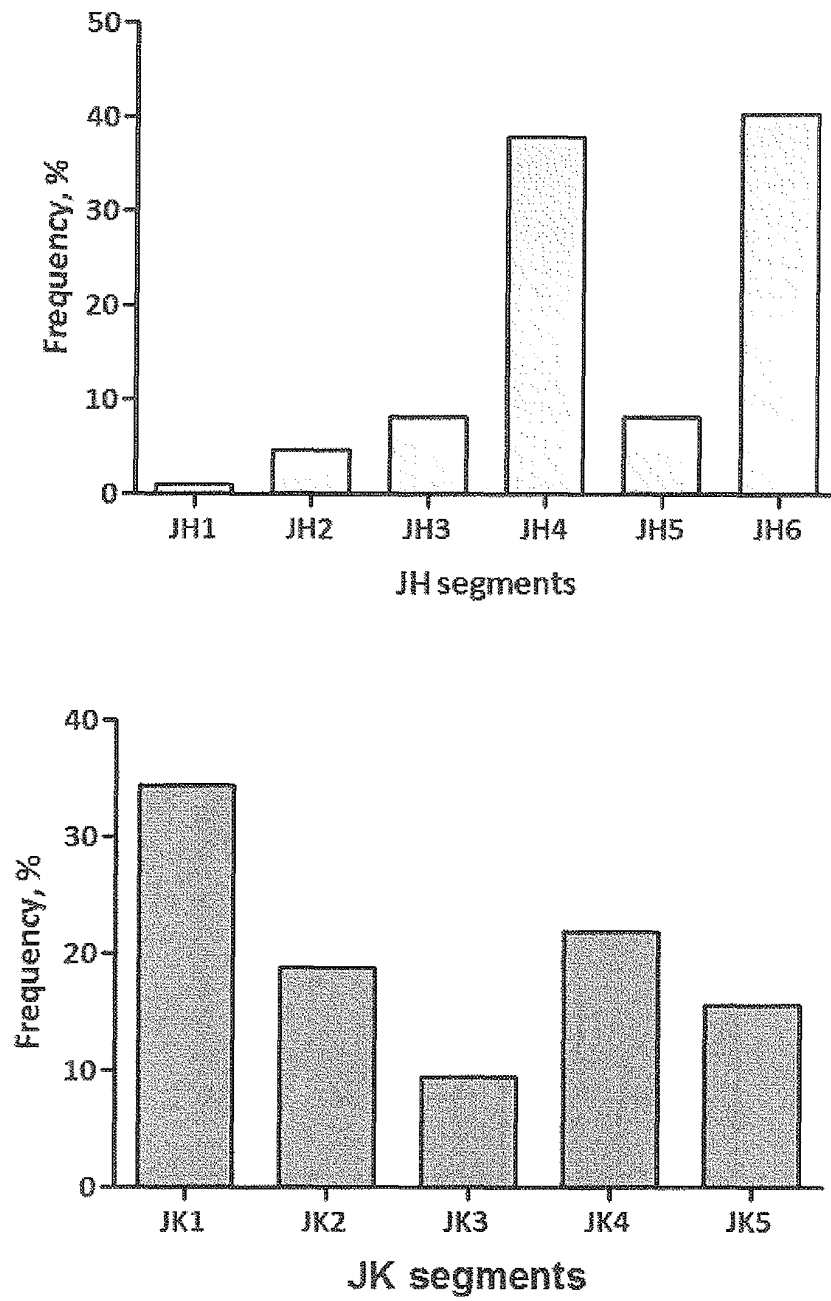


Fig.35.

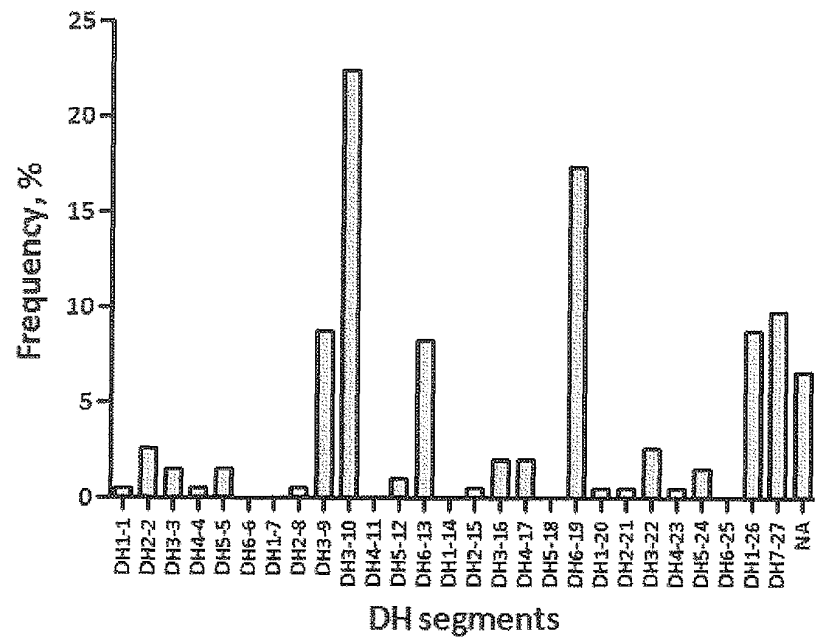


Fig. 36.

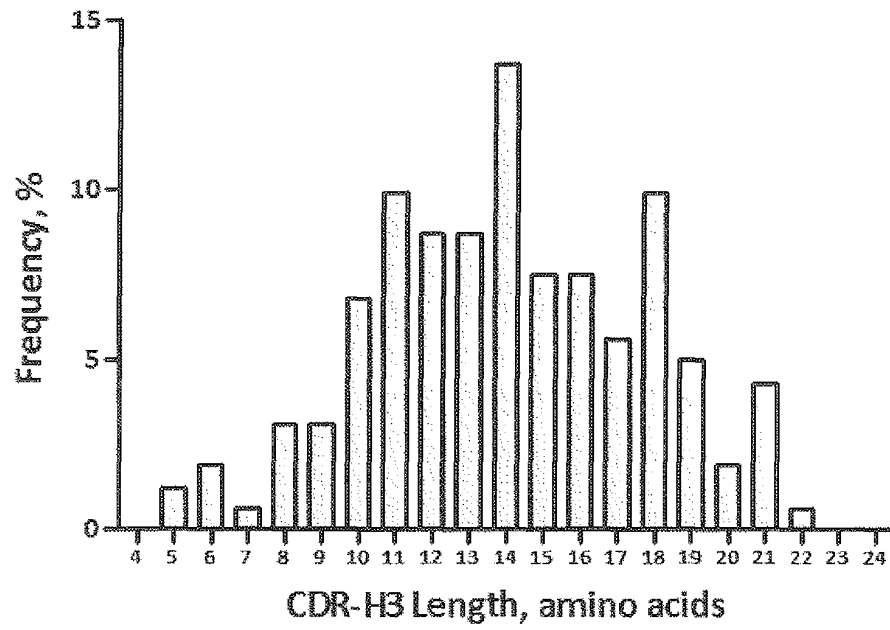


Fig. 37

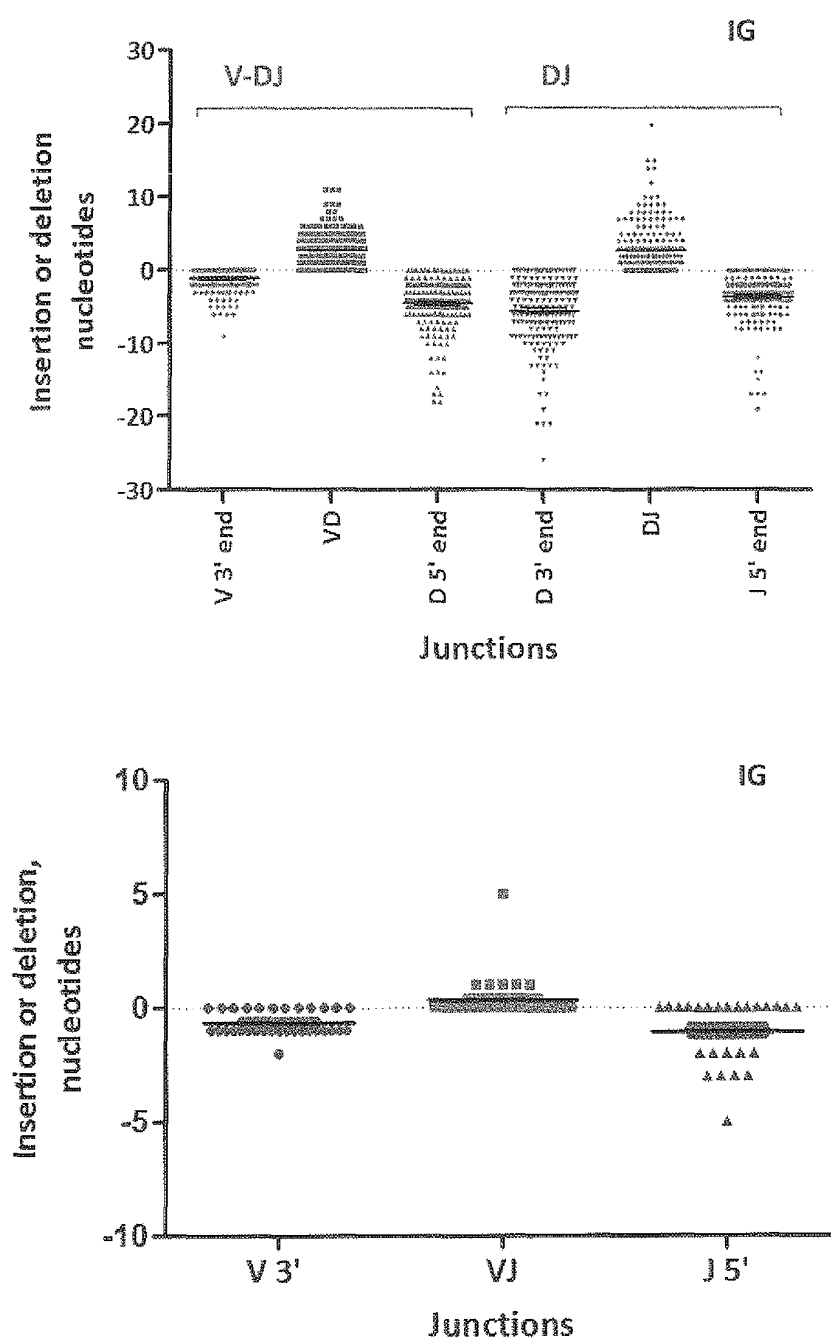


Fig. 38.

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.

Figure 39

Distribution of DH Usage Within Each VHS

[illegible]

The data includes 196 independent sequences.

Figure 40

Figure 42

Hypermutation in J Regions Generates IgK Variants

V1-6 J1 Ck

	150	160	170	180	190	
► Translate ► Consensus	I A C C C T I G T G G A C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G					
V1-6 J1 Ck (1x489)	→					
► T82_data_P2_a01.v2kM13F.scf(122>026)	←	T A C C C C - G T G G A C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a01.v2kM13F.scf(19>87)	→	I A C C C C - G T G G A C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a02.v2kM13F.scf(110>710)	→	I A C C C C - G T G G A C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a02.v2kM13F.scf(126>320)	←	I A C C C C - G T G G A C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a05.v2kM13F.scf(13>329)	←	I A C C C C - G T G G A C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a08.v2kM13F.scf(122>815)	→	I A C C C C - G T G G A C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a10.v2kM13F.scf(12>713)	→	I A C C C C - G T G G A C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a11.v2kM13F.scf(12>744)	→	I A C C C C - G T G G A C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a11.v2kM13F.scf(120>544)	→	I A C C C C - G T G G A C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a04.v2kM13F.scf(11>514)	→	I A C C C C T C - G G A C C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a05.v2kM13F.scf(121>705)	→	I A C C C C T C - G G A C C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a07.v2kM13F.scf(120>590)	→	I A C C C C T C - G G A C C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_a07.v2kM13F.scf(14>304)	→	T A C C C C T C - G G A C C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_b12.v2kM13F.scf(14>591)	→	T A C C C C T C - G G A C C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_b12.v2kM13F.scf(120>823)	→	T A C C C C T C - G G A C C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_c06.v2kM13F.scf(115>566)	→	T A C C C C T C - G G A C C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				
► T82_data_P2_c06.v2kM13F.scf(122>631)	→	T A C C C C T C - G G A C C G T T C G G C C A A G G G A C C A A G G T C G A A T C A A C C G C C T G				

A1, A2, A8, A10, A11, A12, B3, B5, B7, C8, D5, D6
KLLIYAASSLQSGVPSRFGSGSGTDFTLTISLQPEDFATYYCLQDYNYPHWTFGQGTKEIKRADAAPTVSIFPPSSEQLTSGGGASVVCFLNFPKDNVWKIDGSRQNGV

A4, A5, A7, B4, B9, B11, C3, C4, C7, C12, D7
KLLIYAASSLQSGVPSRFGSGSGTDFTLTISLQPEDFATYYCLQDYNYPHWTFGQGTKEIKRADAAPTVSIFPPSSEQLTSGGGASVVCFLNFPKDNVWKIDGSRQNGV

B12, C6, D3
KLLIYAASSLQSGVPSRFGSGSGTDFTLTISLQPEDFATYYCLQDYNYPHWTFGQGTKEIKRADAAPTVSIFPPSSEQLTSGGGASVVCFLNFPKDNVWKIDGSRQNGV

Figure 43

Joint Diversity Produces Functional CDS

V1-5_J1_Ck

VAGWPGISRNGGKPLSSStopSIRRLVStopKVGSHQGSAAVDLGQNSLSPSAAC
SLMetILQLITANSIIVILLTFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL
CFLNNFYPKDINVKWKIDGSSERQNGV

H12

SSWLAWYQQKPGKAPKLLIYKASSLESQVPSRFSGSGGTEFTLTISSLQPD
FATYYCQQYNSYSTFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL
NNFYPKDINVKWKIDGSSERQNGV

V1-5_J4_Ck

AGWPGISRNGGKPLSSStopSIRRLVStopKVGSHQGSAAVDLGQNSLSPSAAC
LMetILQLITANSIIVILWTFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL
CFLNNFYPKDINVKWKIDGSSERQNGV

E1

SWLAWYQQKPGKAPKLLIYKASSLESQVPSRFSGSGGTEFTLTISSLQPD
ATYYCQQYNSYSTFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL
NNFYPKDINVKWKIDGSSERQNGV

E2

SWLAWYQQKPGKAPKLLIYKASSLESQVPSRFSGSGGTEFTLTISSLQPD
ATYYCQQYNSYSTFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL
NFYPKDINVKWKIDGSSERQNGV

E8

SSWLAWYQQKPGKAPKLLIYKASSLESQVPSRFSGSGGTEFTLTISSLQPD
FATYYCQQYNSYSTFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL
LNNFYPKDINVKWKIDGSSERQNGV

kymab