

Three basic vector backbones

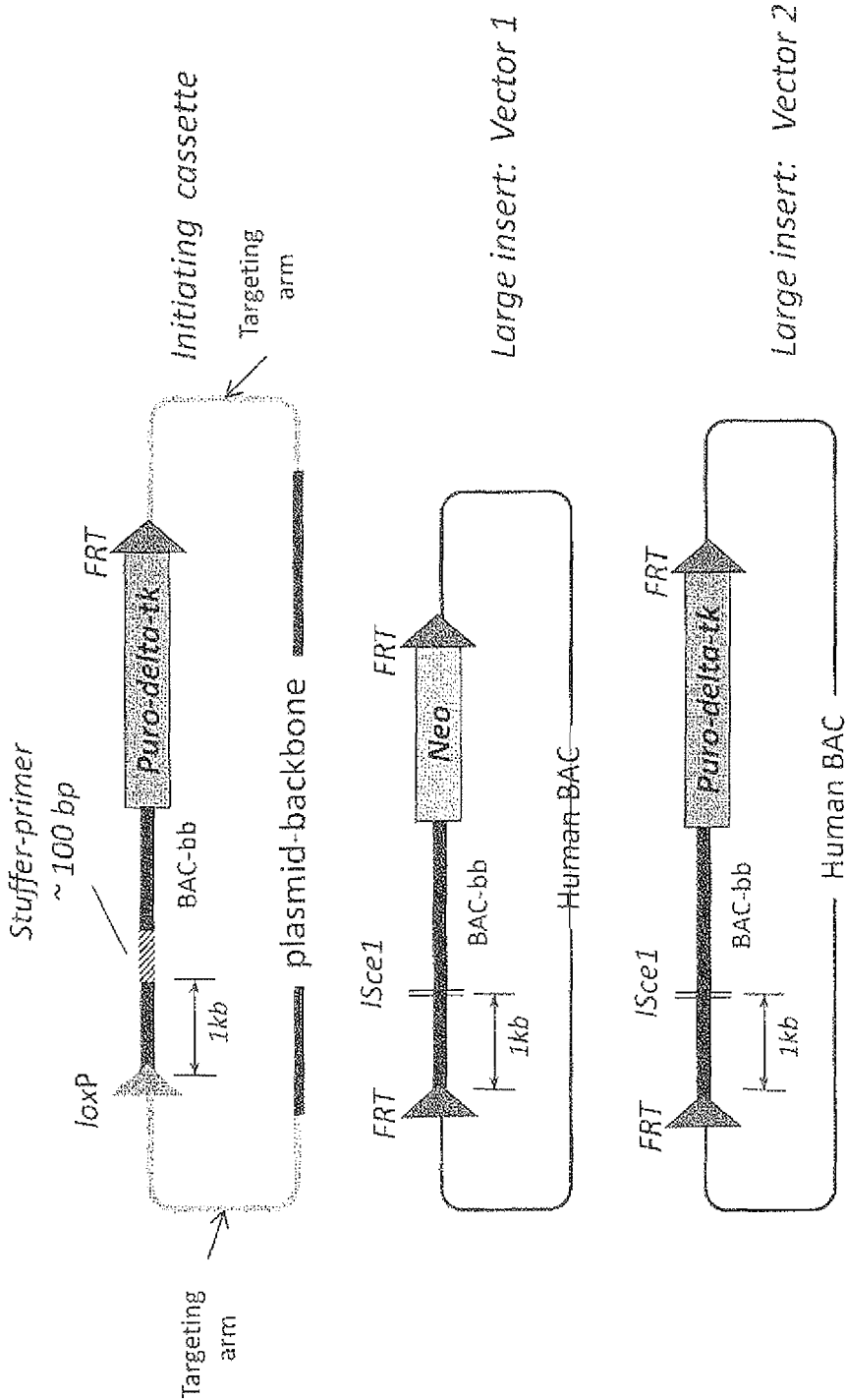
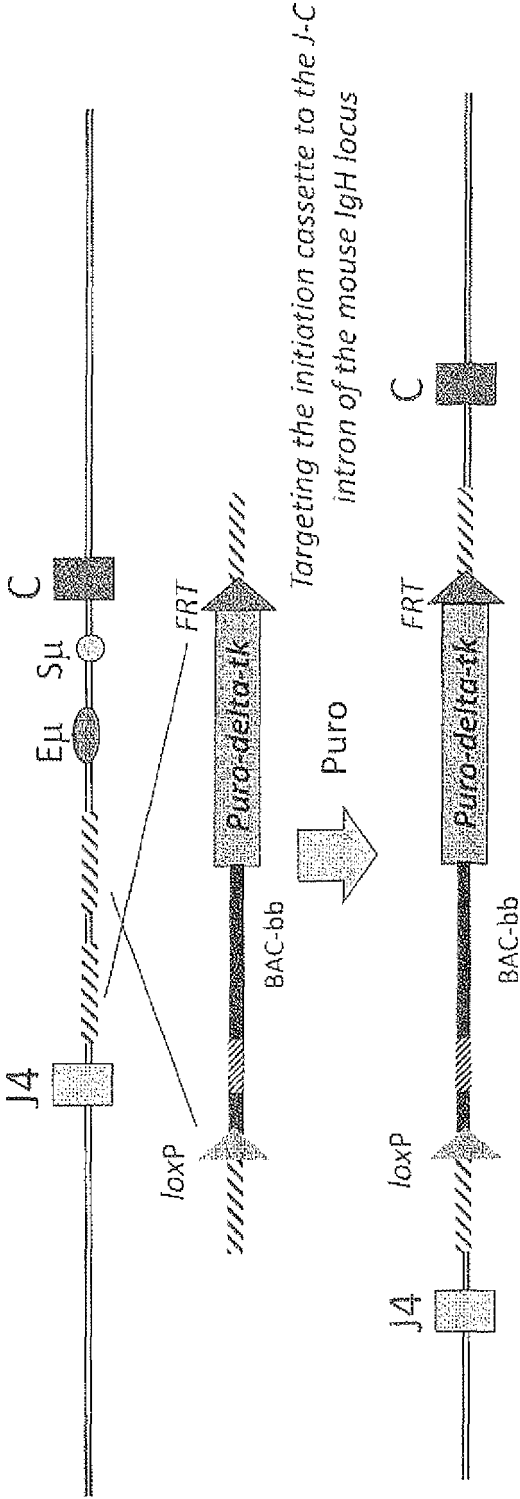


Fig. 1

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

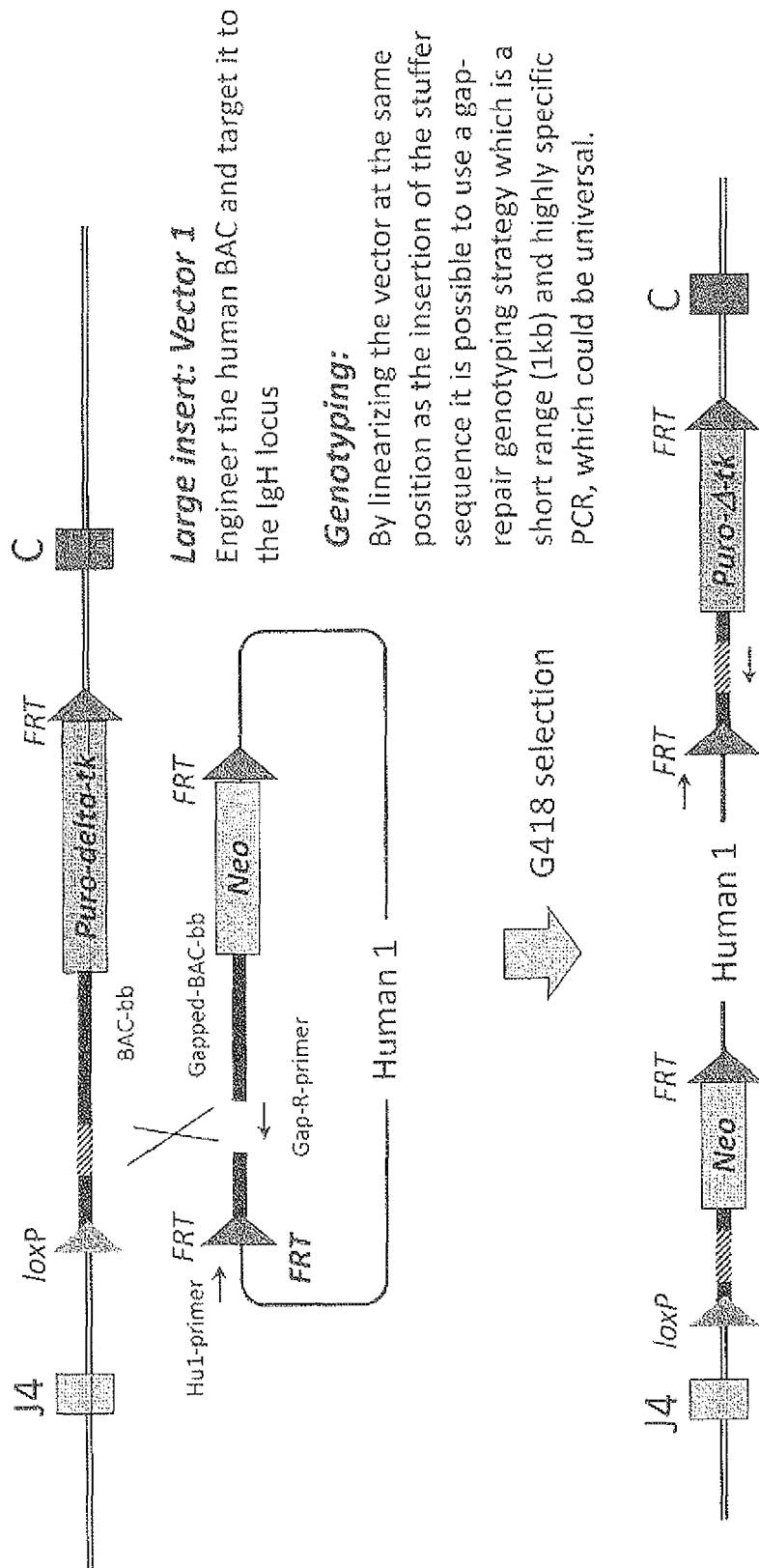


Fig. 3

Addition vector: Step 2

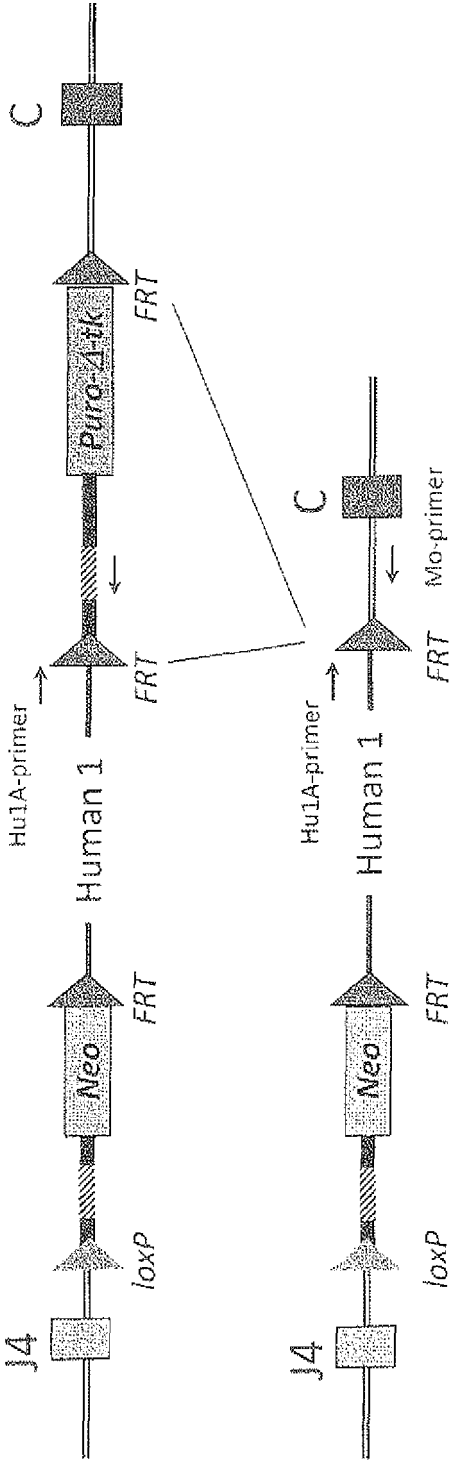


Fig. 4

Addition vector: Step 3A

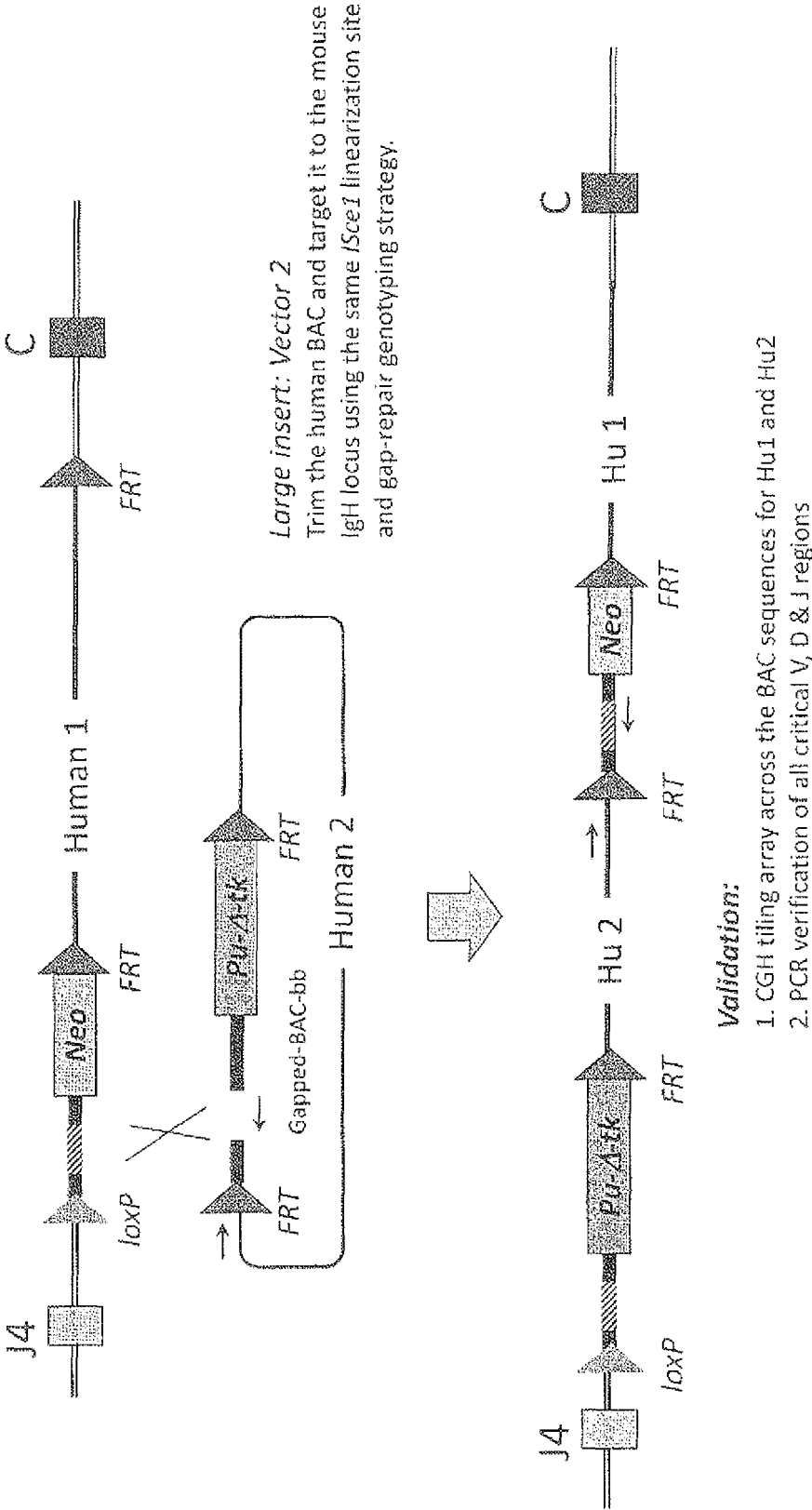


Fig. 5

Addition vector: Step 3B

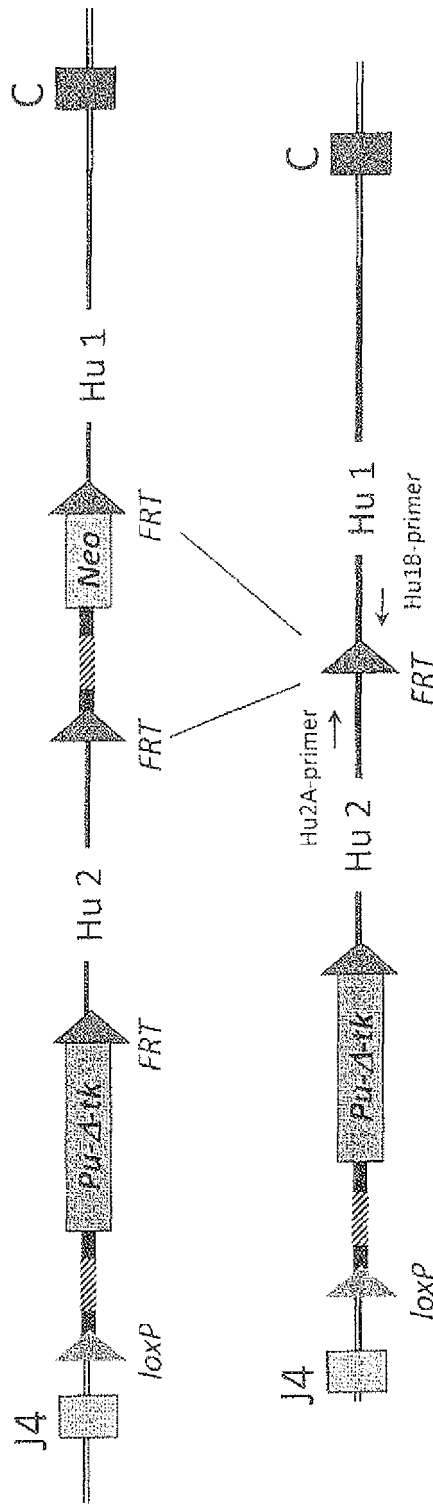


Fig. 6

Addition vector: Step 4

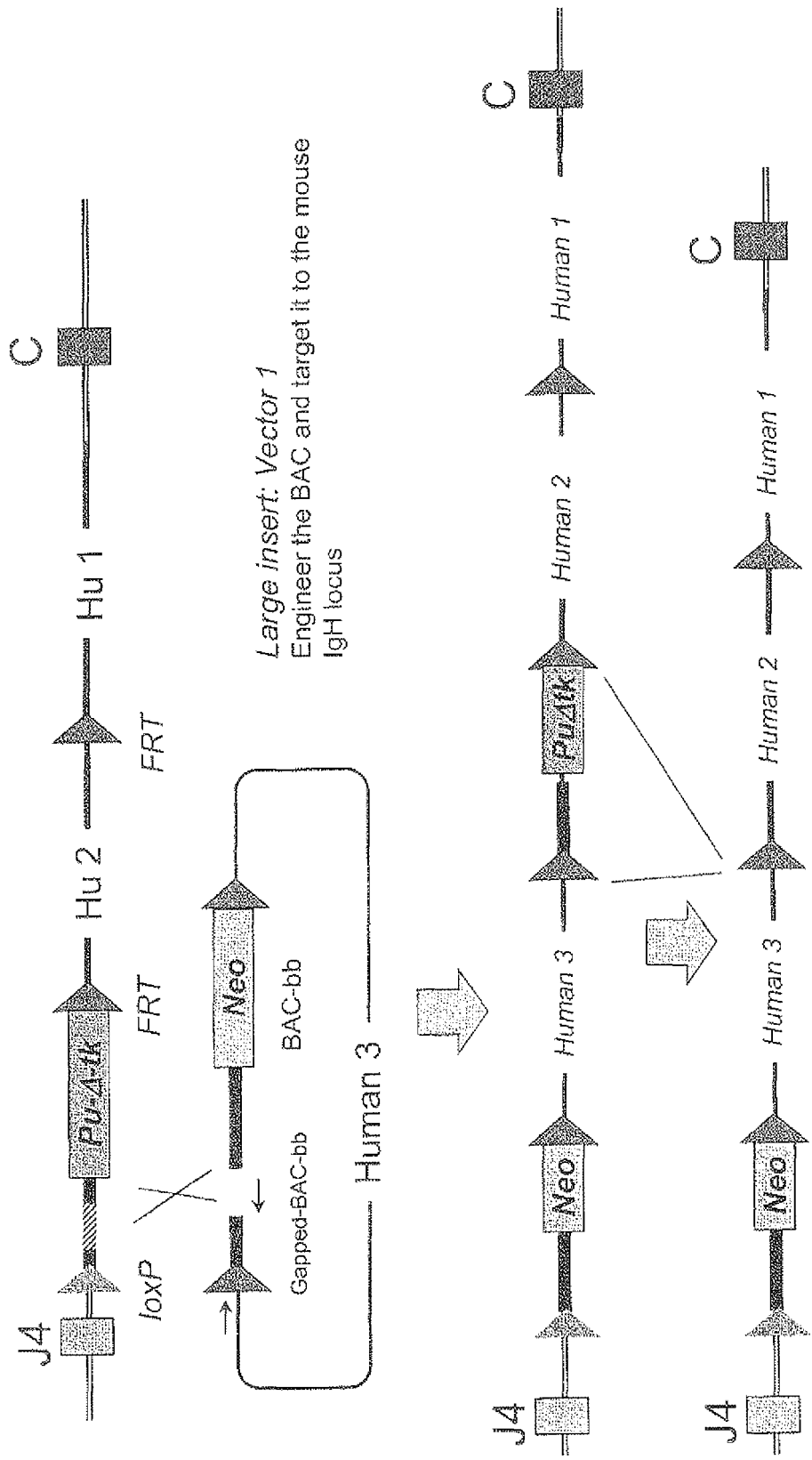


Fig. 7

A humanized mouse *IgH* locus

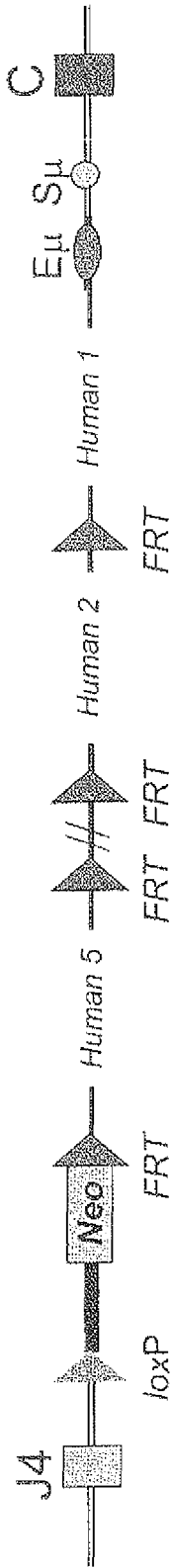


Fig. 8

BAC landing pad inserted at the IgH locus

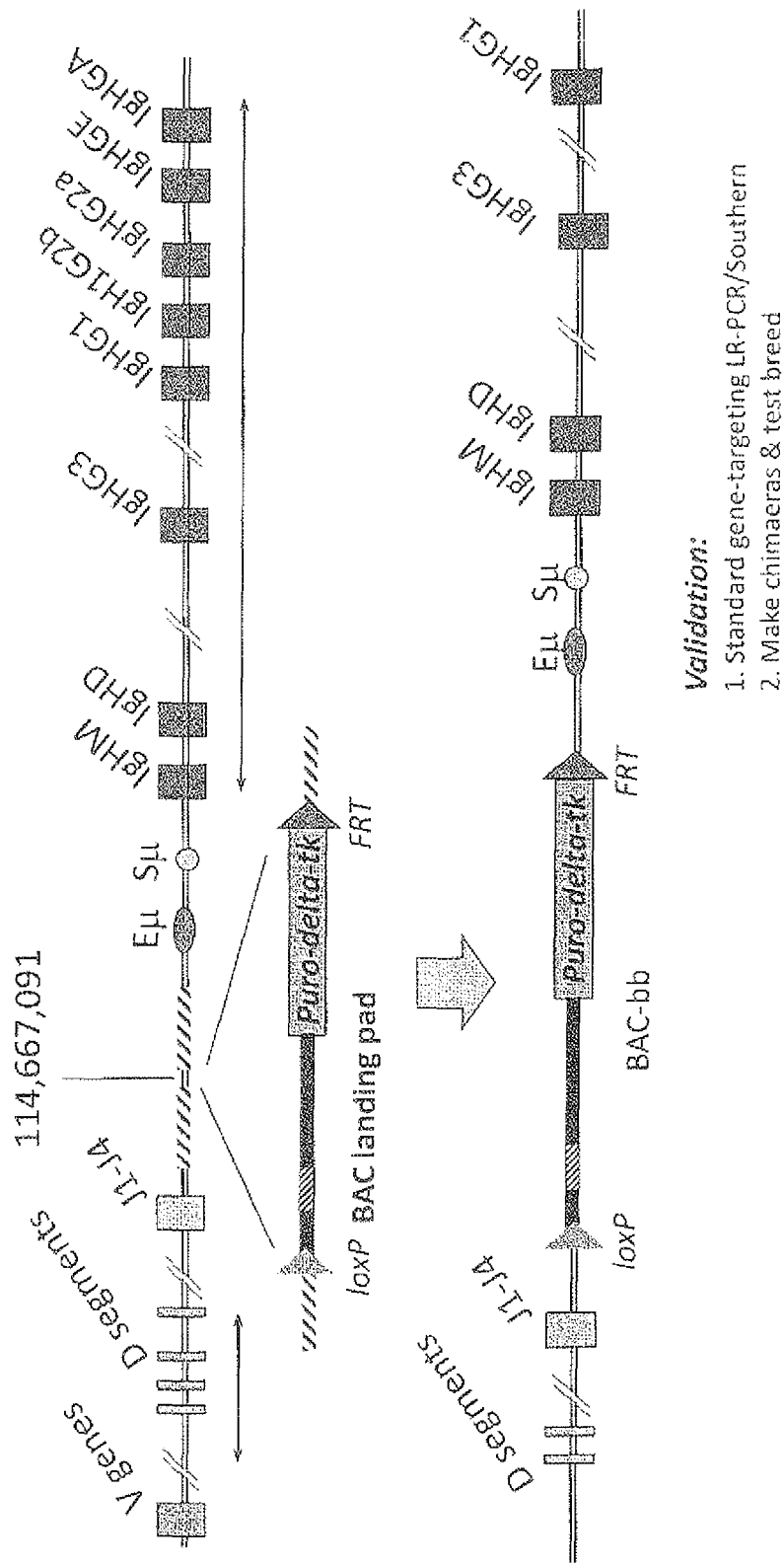


Fig. 9

1st BAC insertion – Step 2

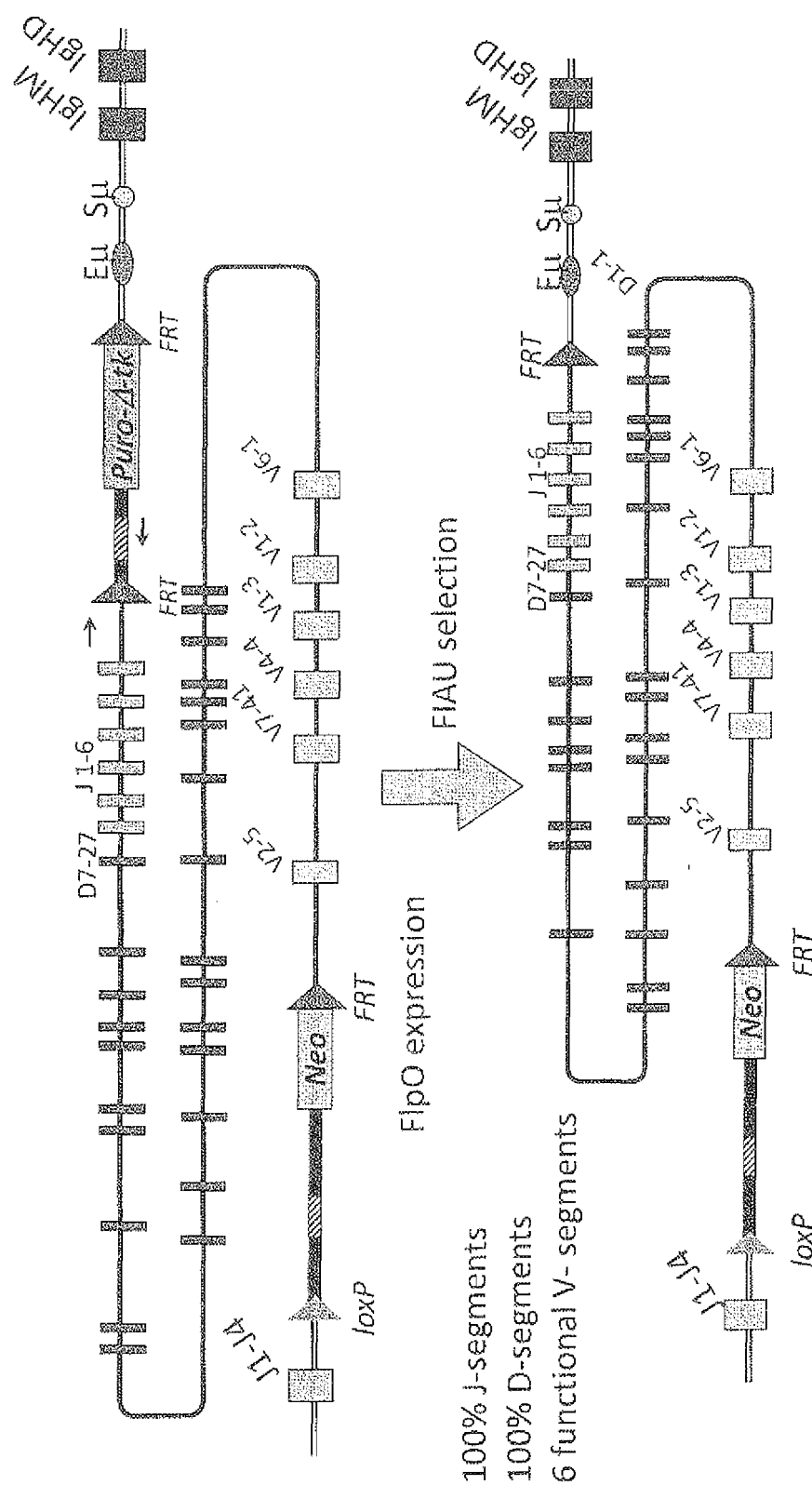


Fig. 11

2nd BAC insertion – Step 3

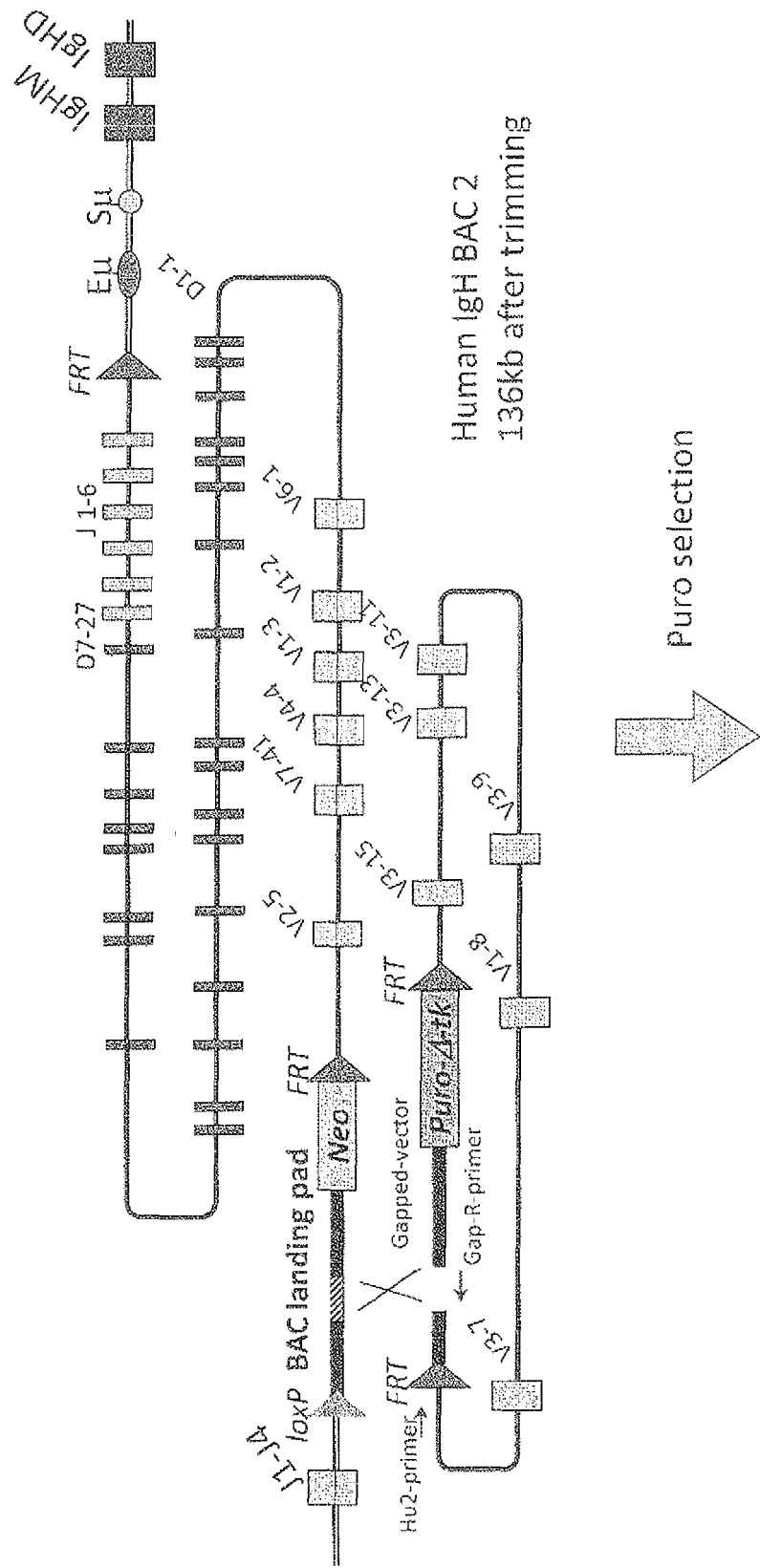


Fig. 12

2nd BAC insertion – Step 4

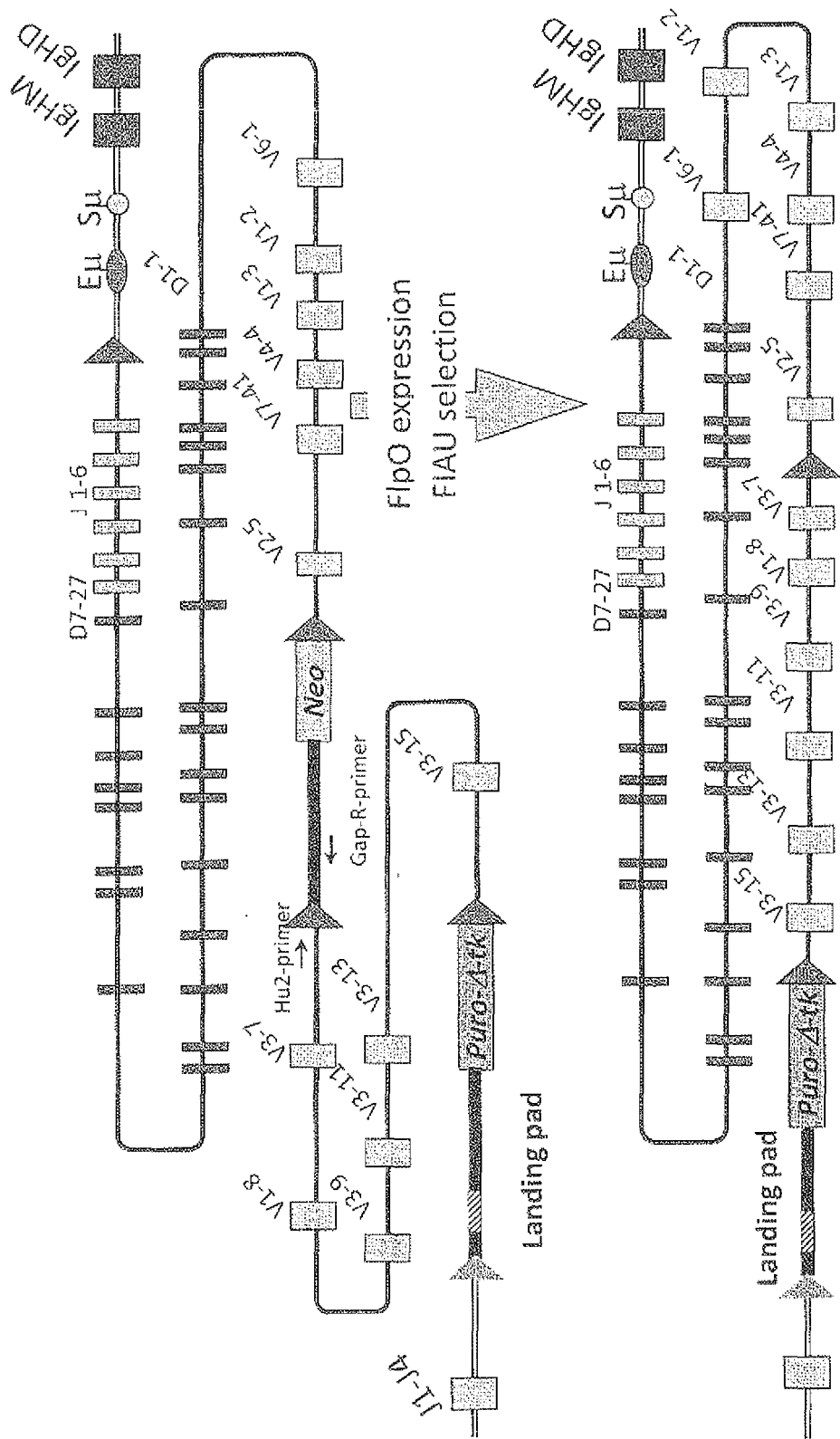


Fig. 13

BAC Landing Pad at Igk Locus

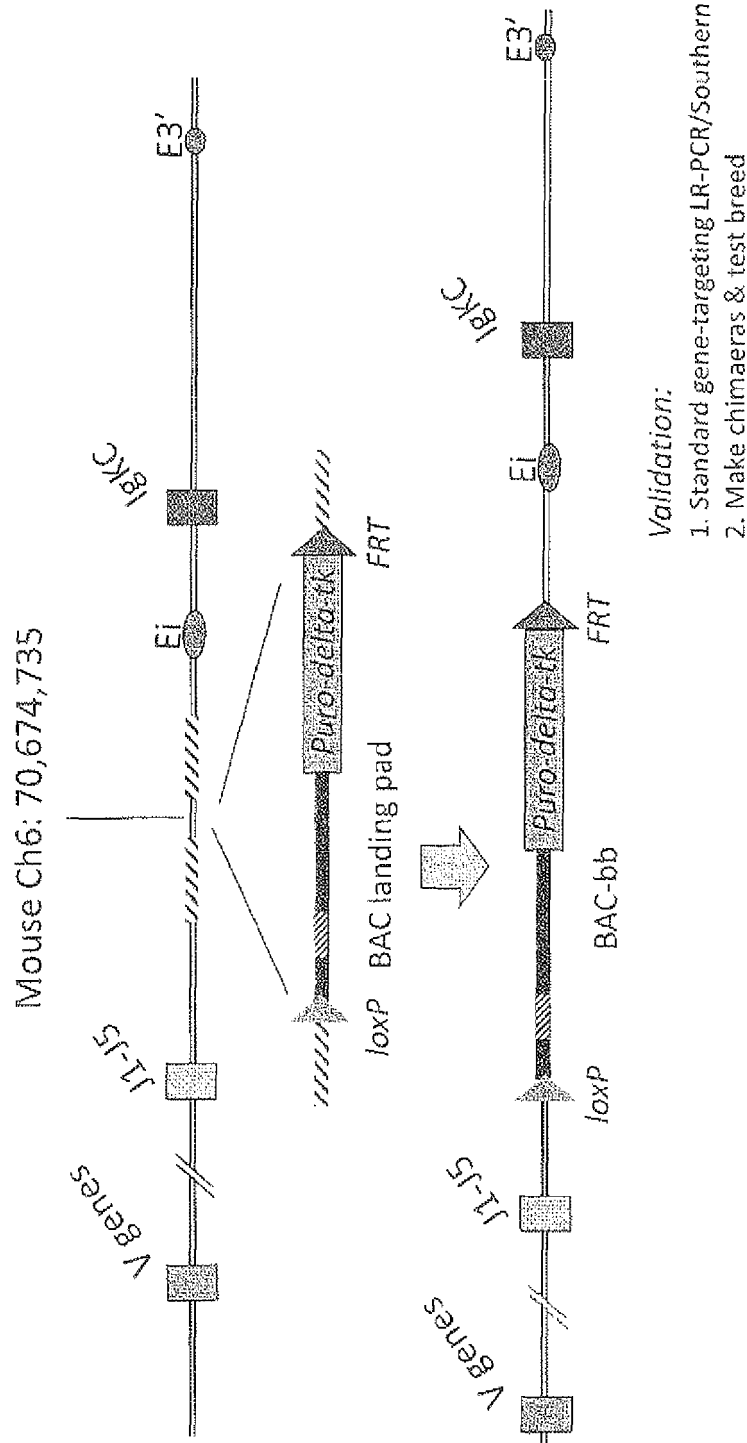


Fig. 14

1st BAC Insertion – Step 1

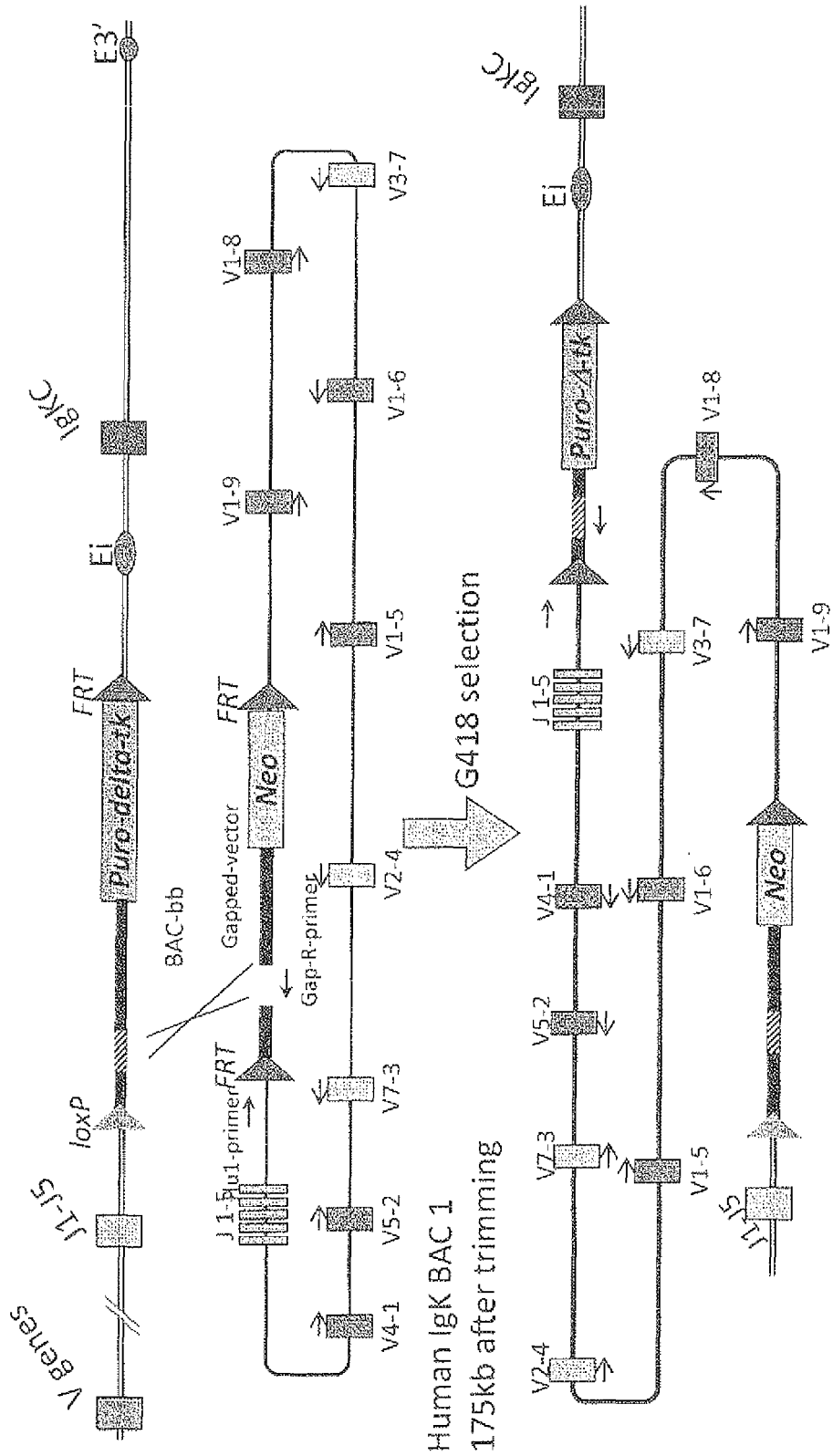


Fig. 15

1st BAC Insertion – Step 2

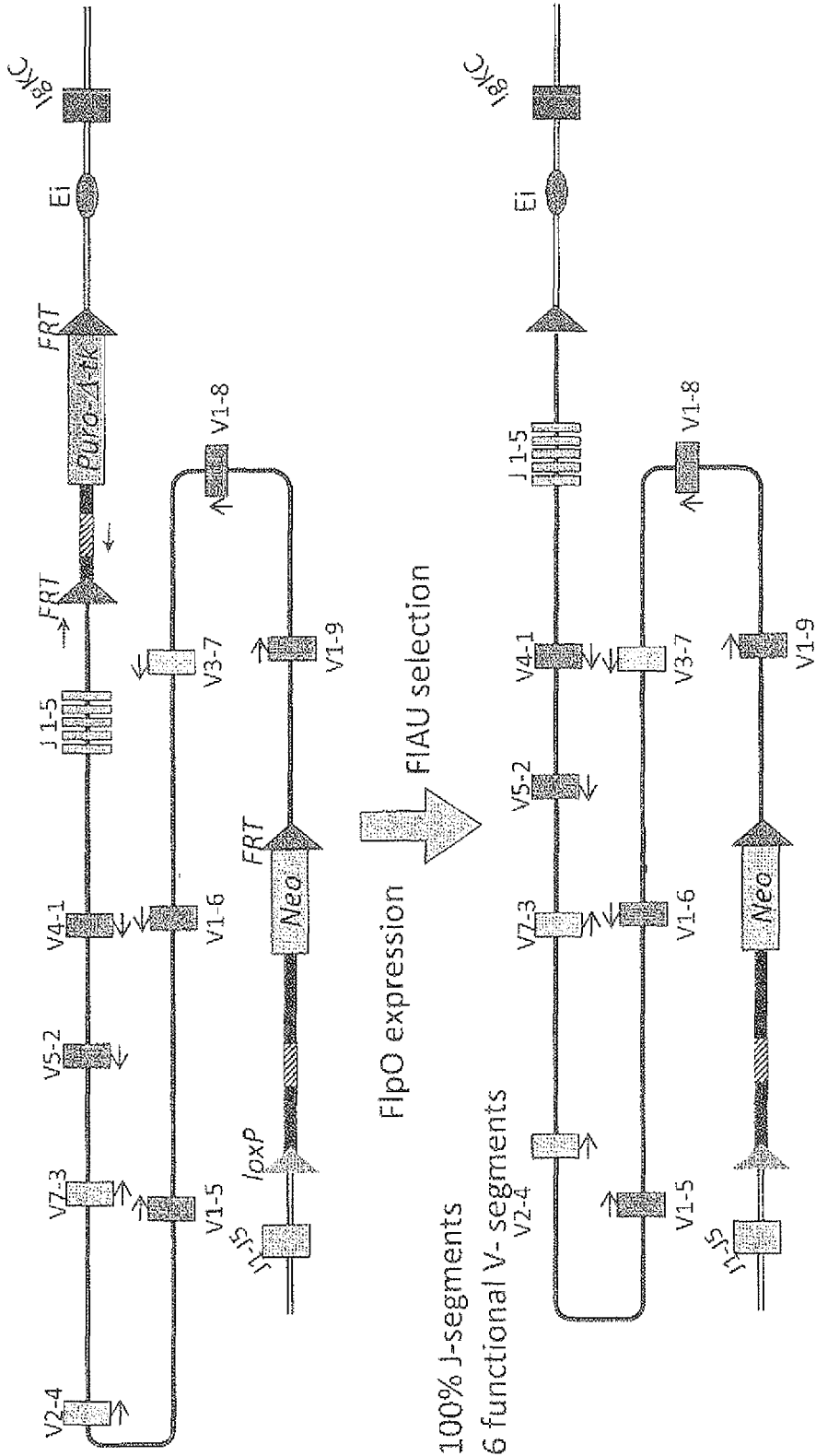


Fig. 16

2nd BAC Insertion – Step 3

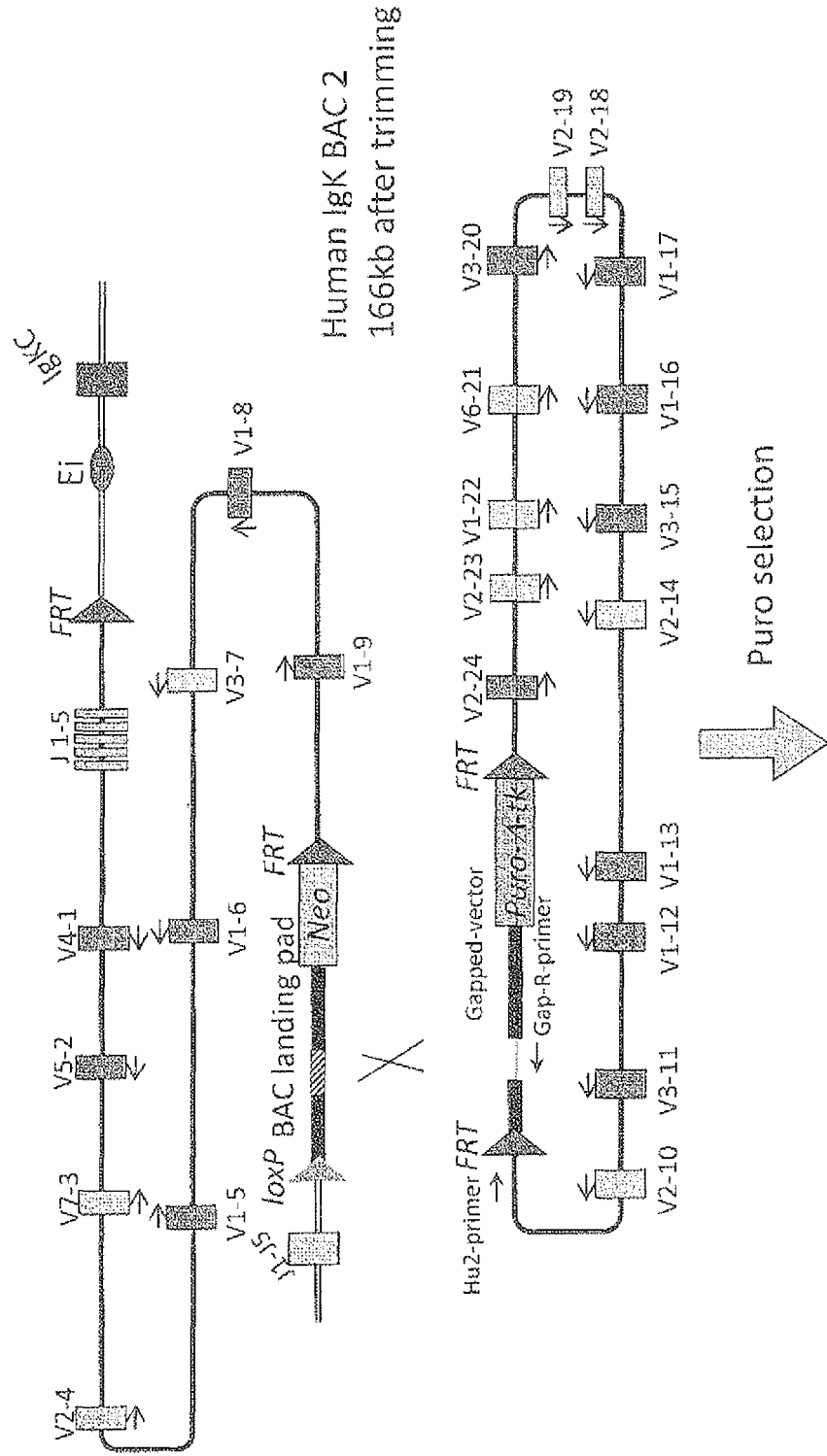
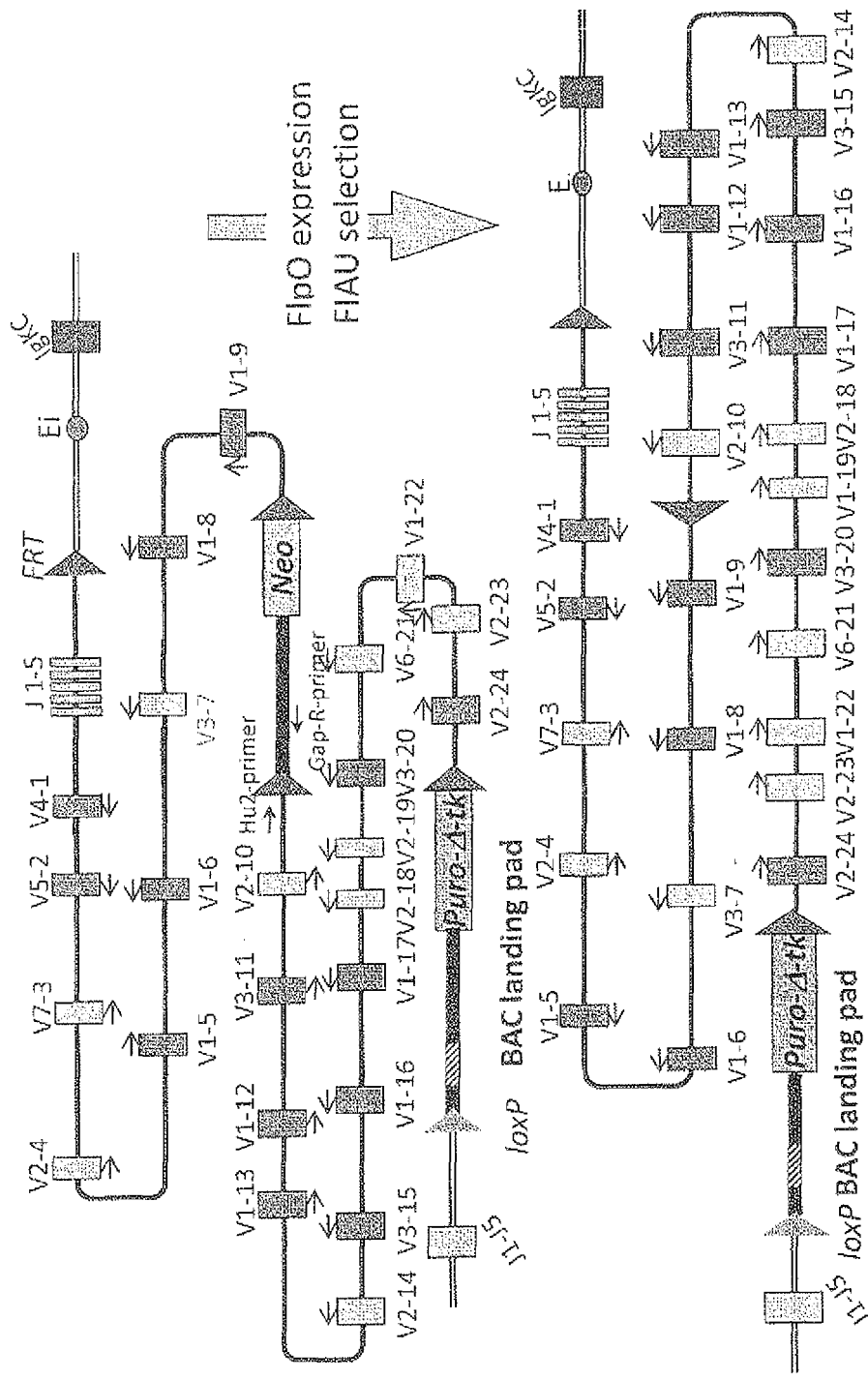


Fig. 17

2nd BAC insertion – Step 4



100% J-segments; 14 functional V-segments

Fig. 18

Rapid Testing of ES Cells with Humanized IgH and IgK Loci

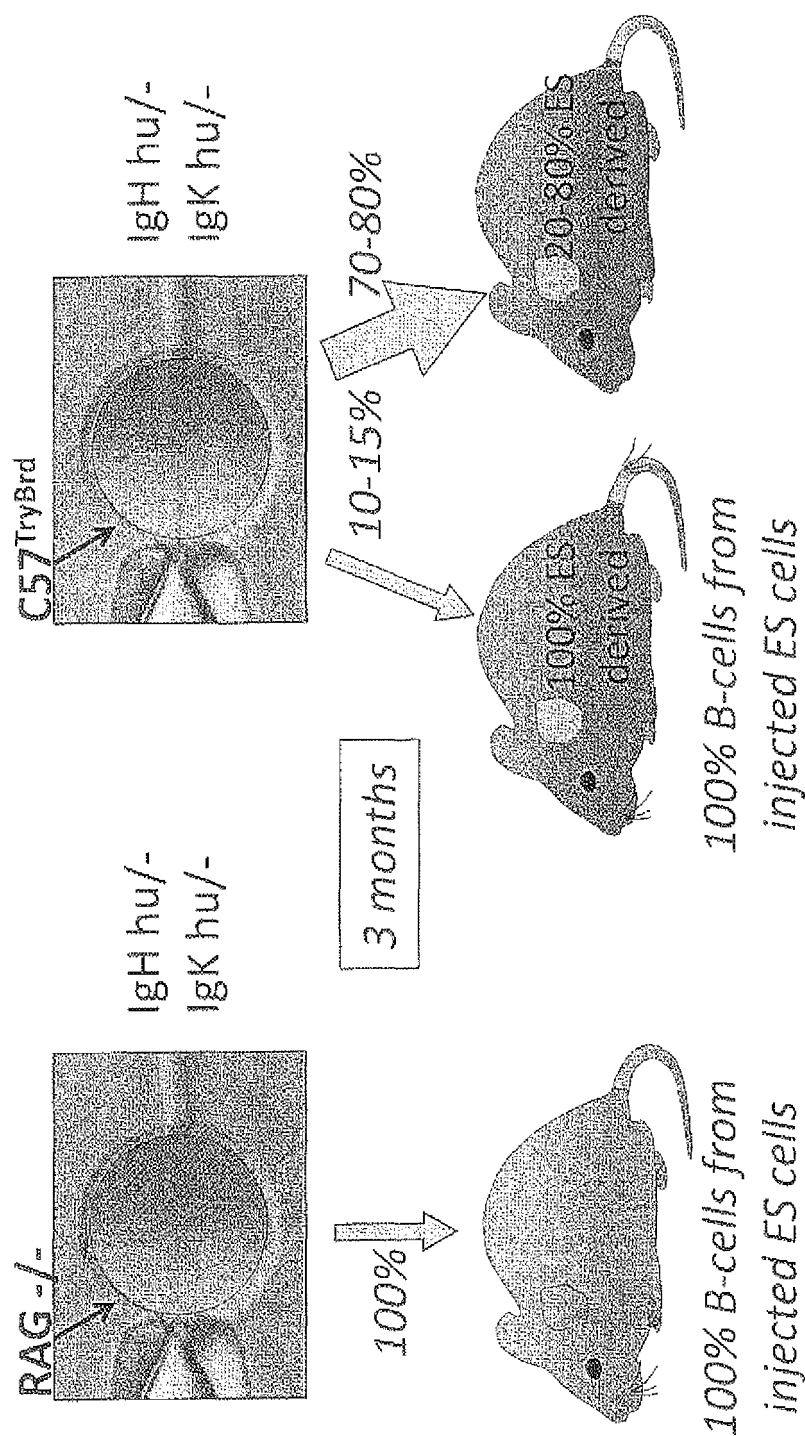


Fig. 19

Antibody Generation in Chimaeric Mice with Humanized IgH and IgK Loci

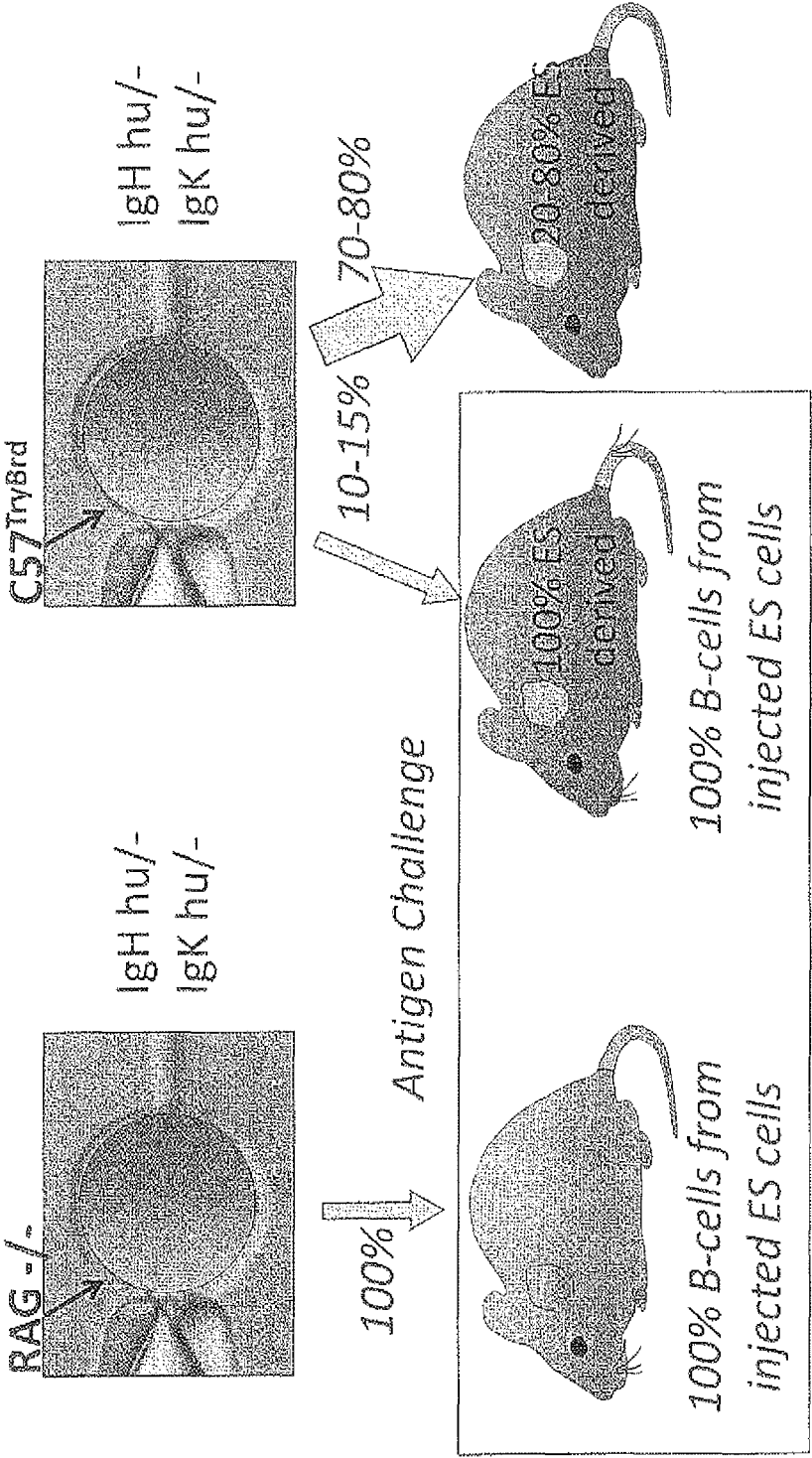


Fig. 20

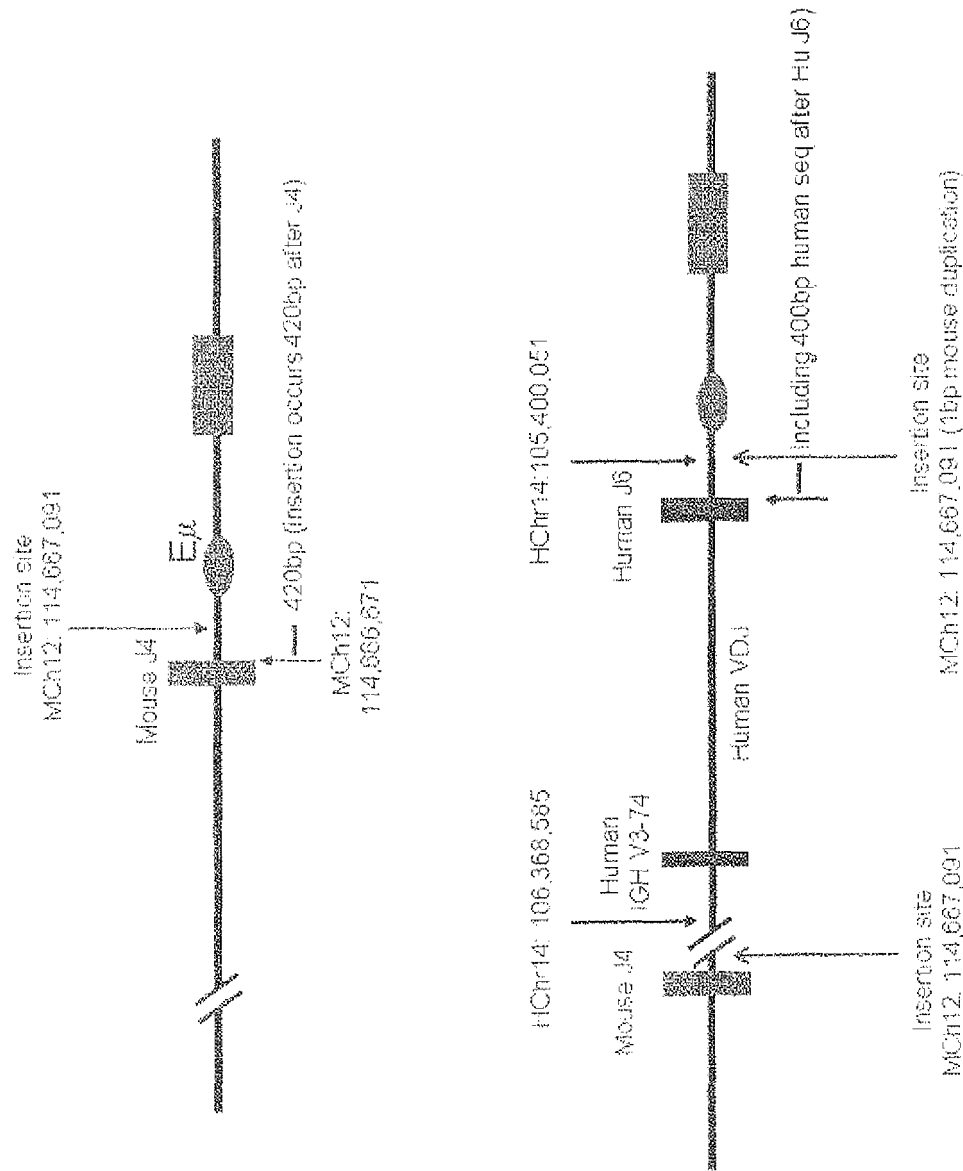


Fig. 21

Targeting of the Landing Pad

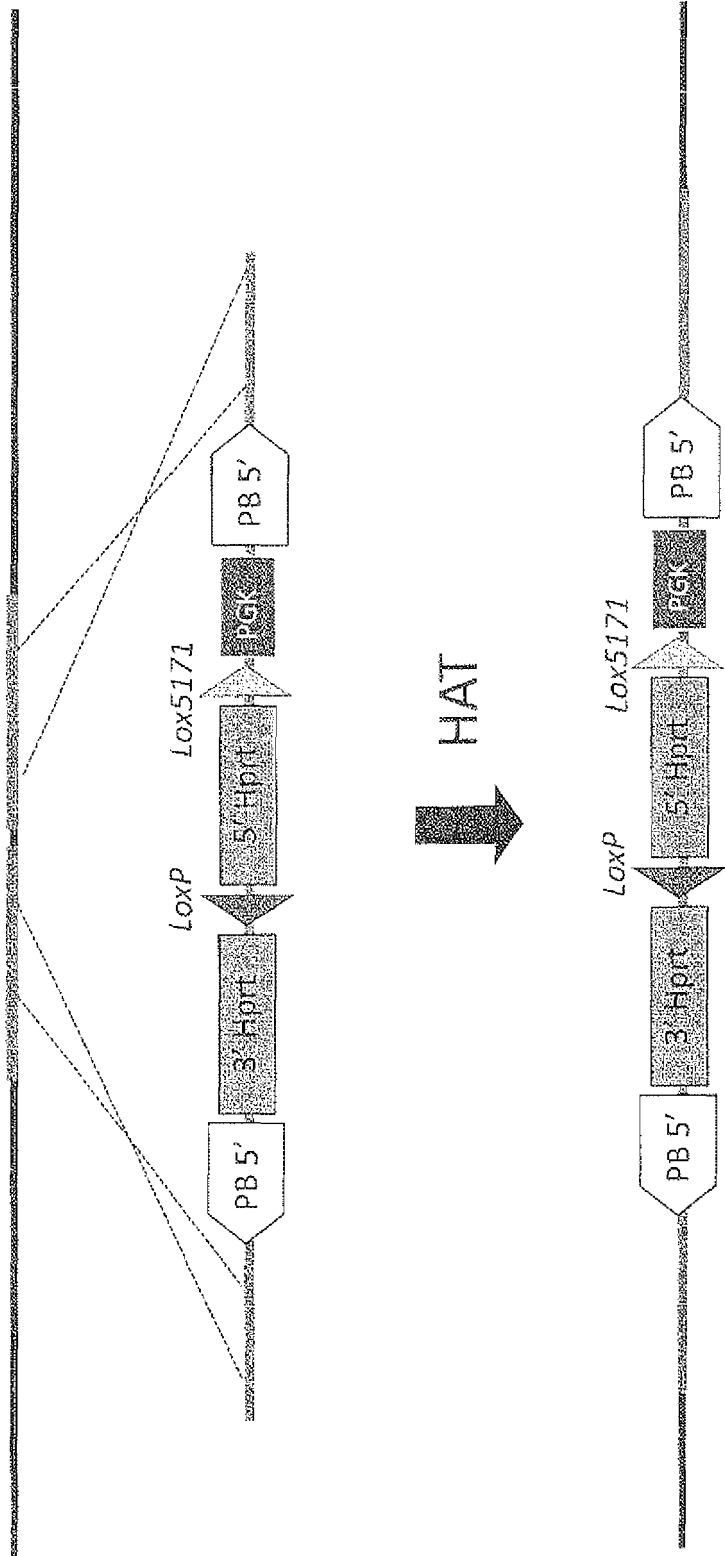


Fig. 22

Insertion of the 1st BAC

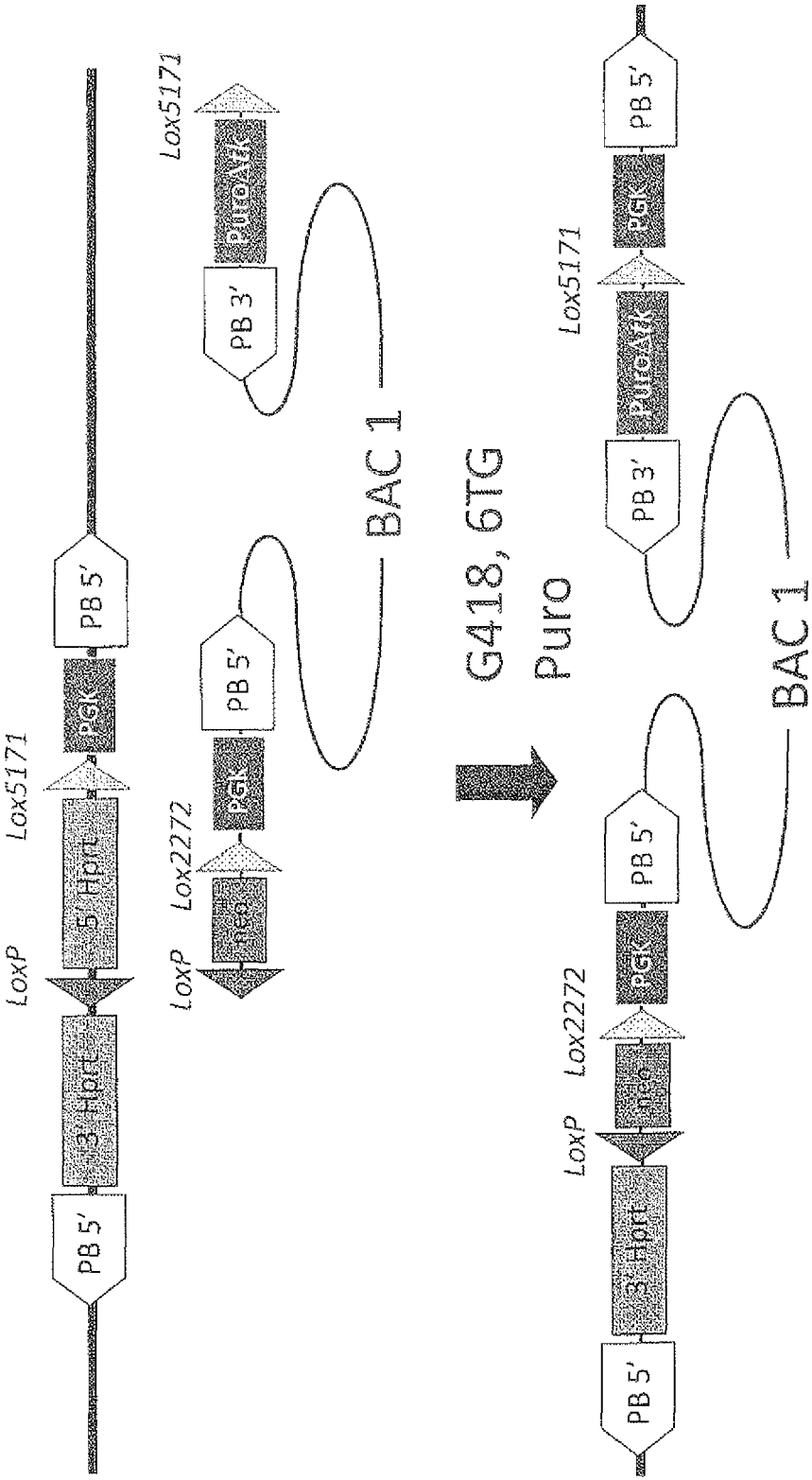


Fig. 23

Curing of the 3' Modification

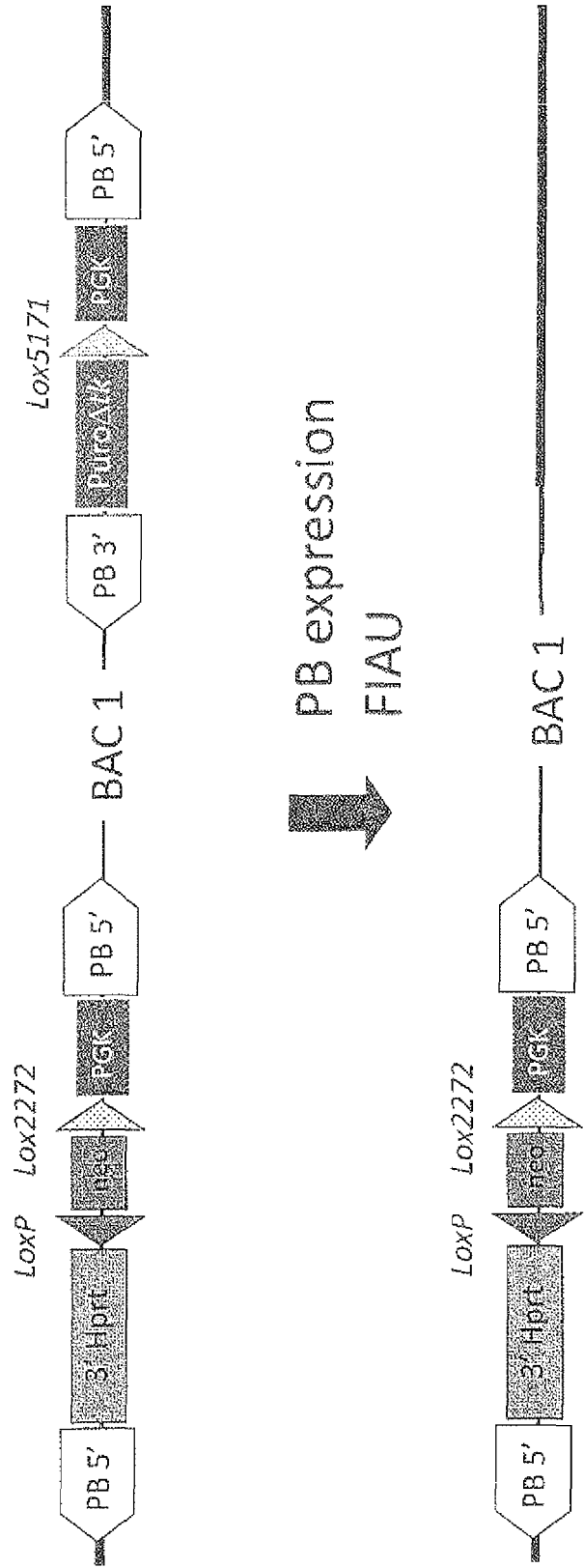


Fig. 24

Insertion of the 2nd BAC

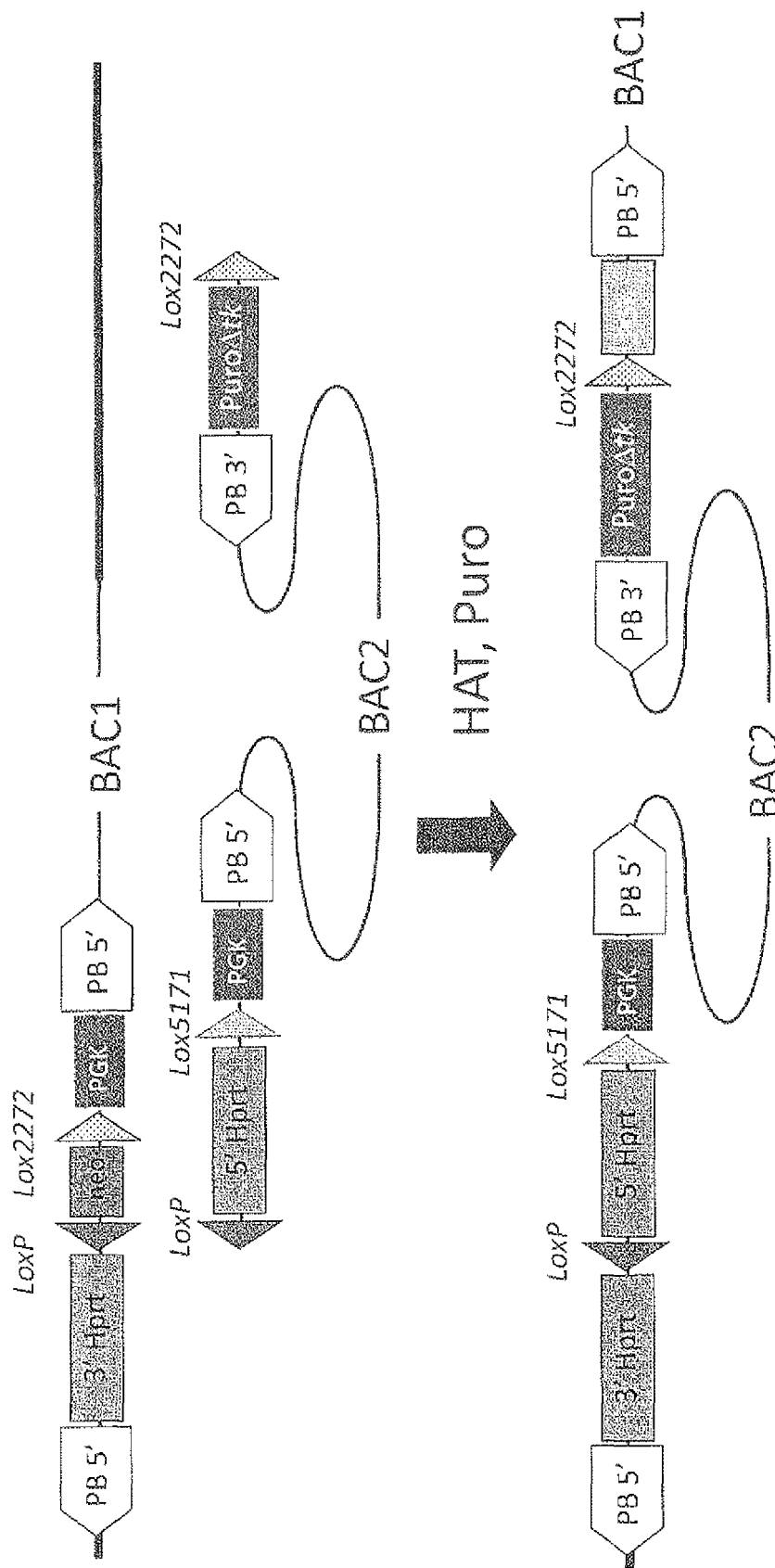


Fig. 25

Removing the 3' Modification

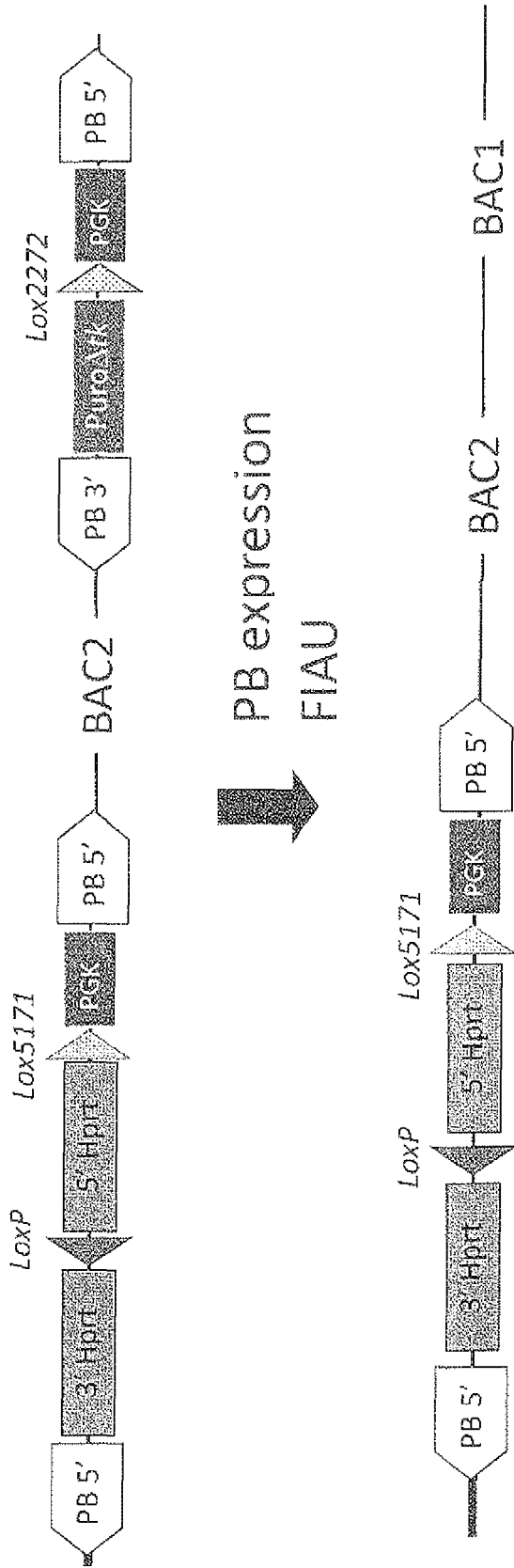


Fig. 26

Targeting the “Flip-Over” Vector

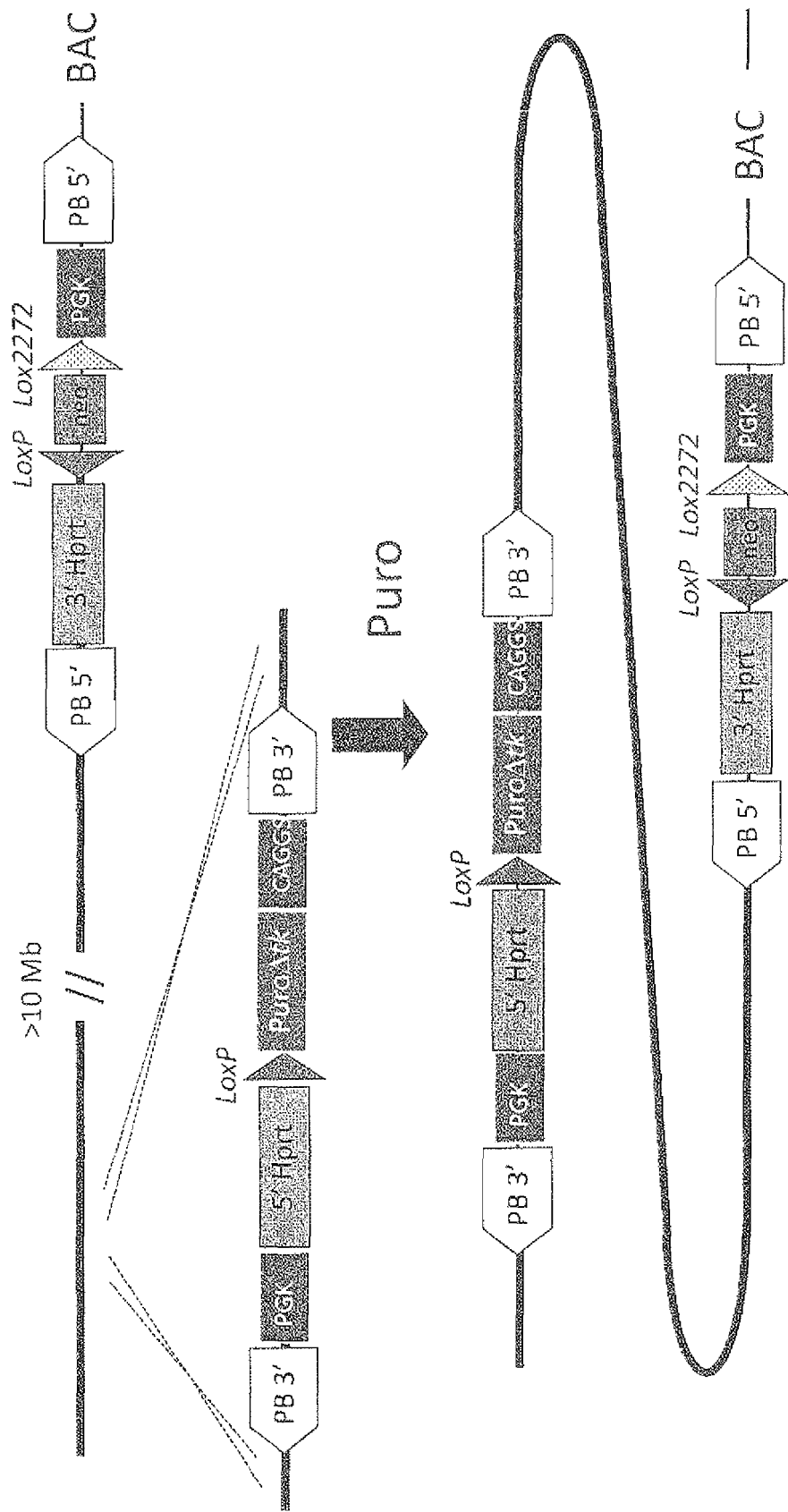


Fig. 27

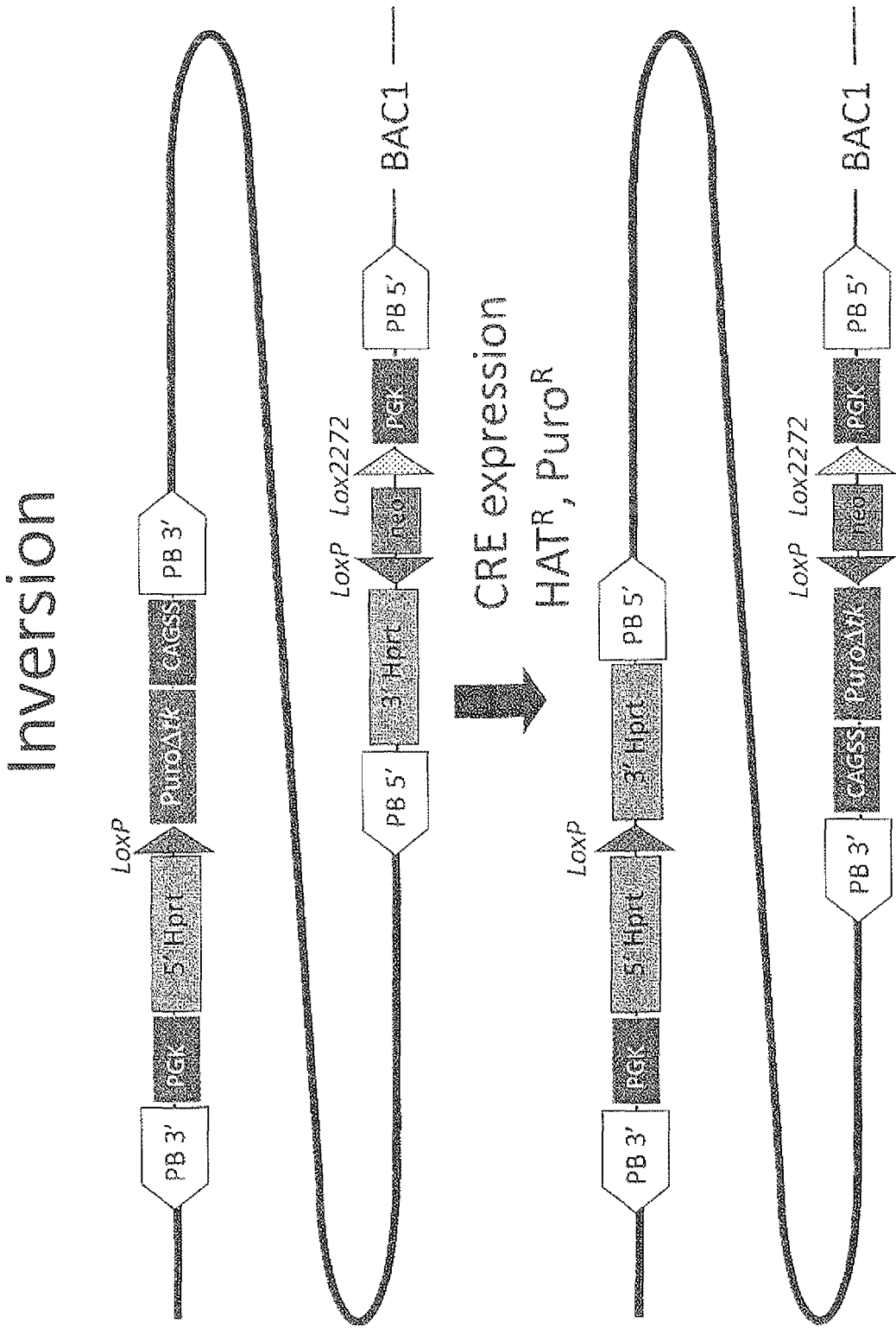


Fig. 28

Removing All Modifications

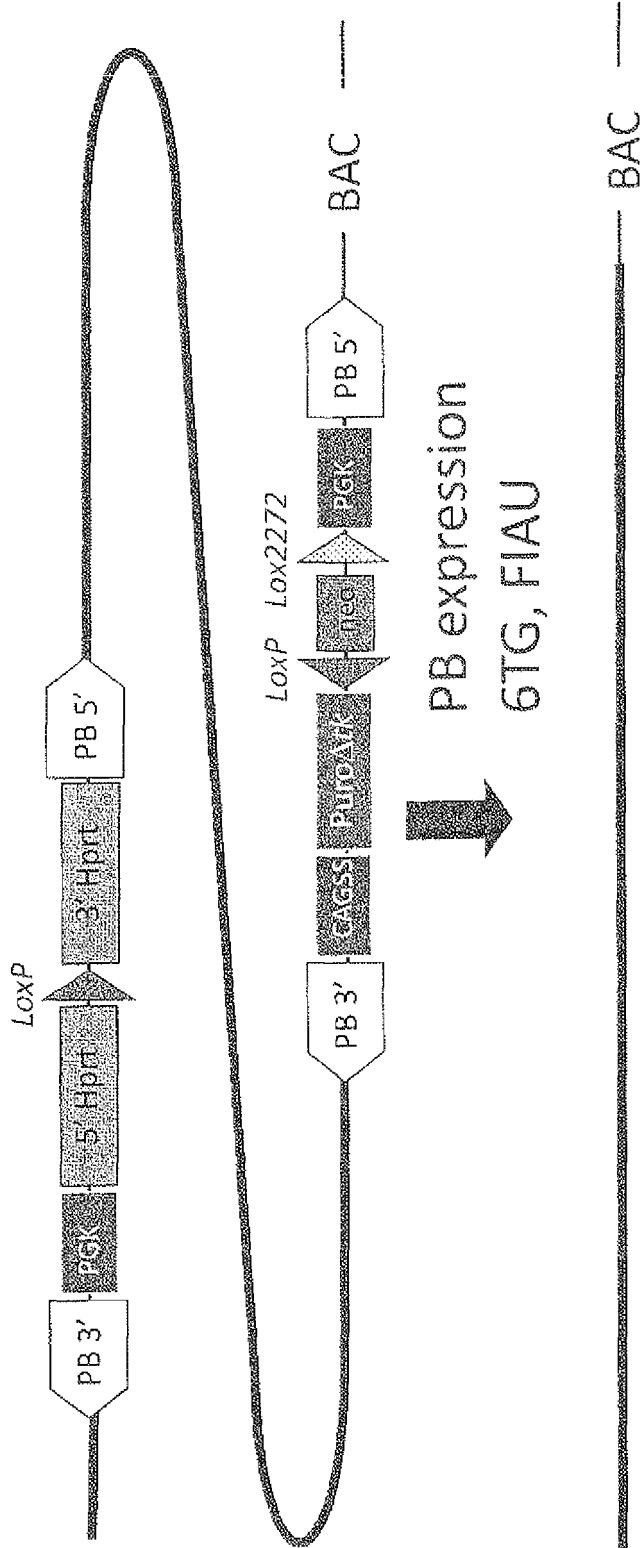
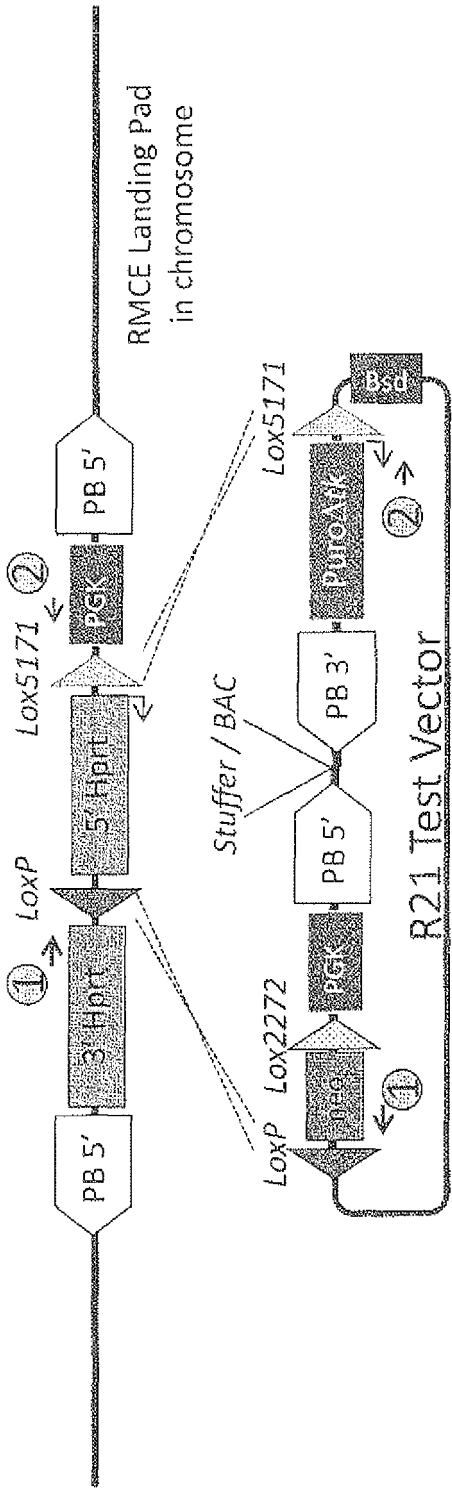


Fig. 29

Sequential RMCE – Integration into Landing Pad



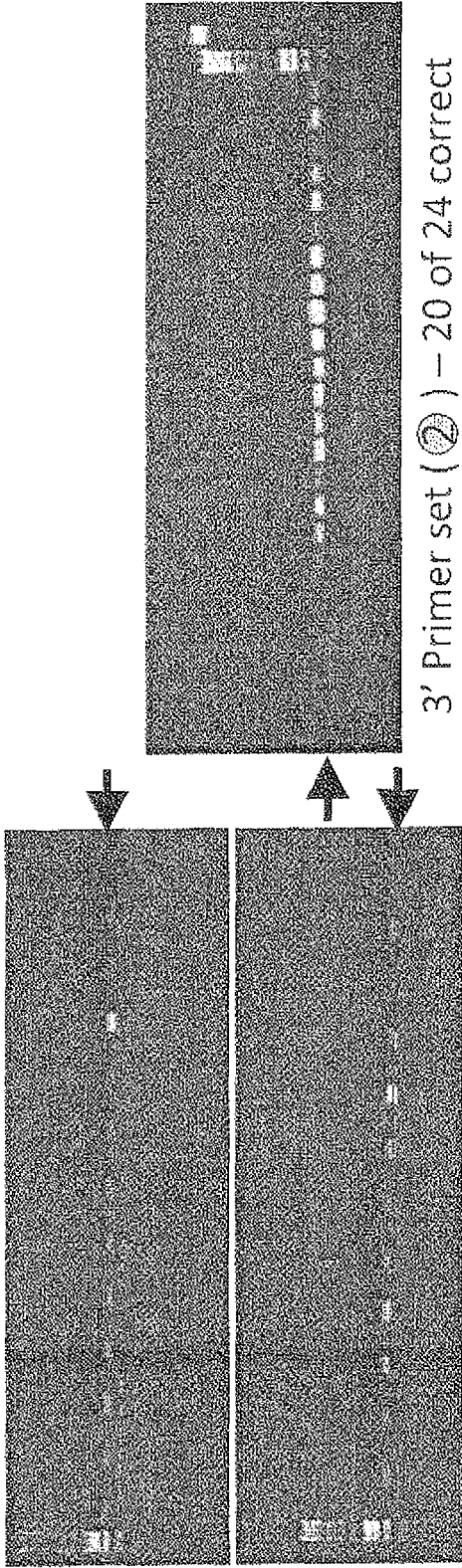
R21 transfected w/ Cre-expression Cassette

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

Fig. 30

Confirmation of Successful Insertion into Landing Pad

Colonies : 6-TG and Puro - Resistant



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

Insertion of BAC#1 – PCR Diagnostics

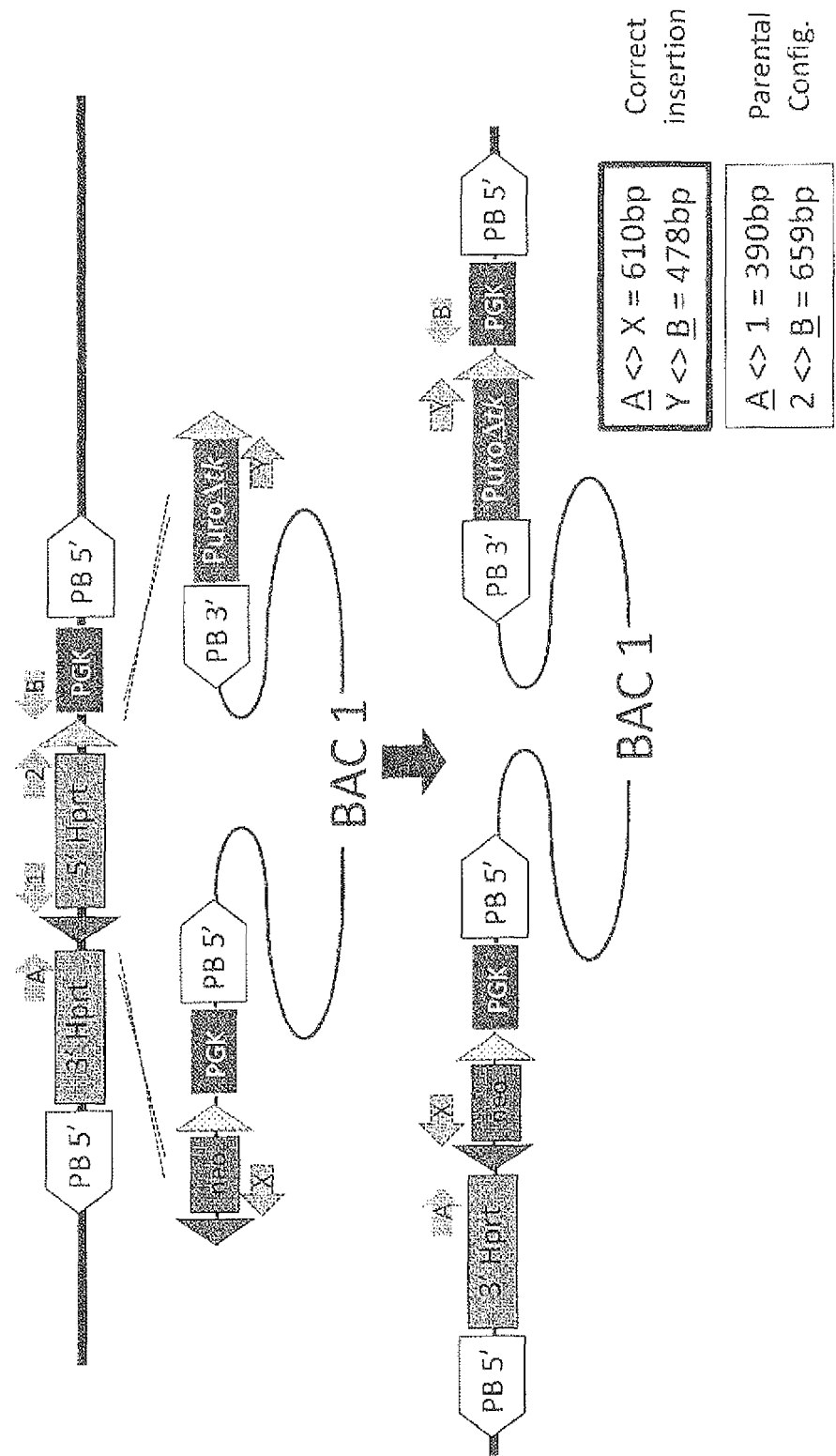
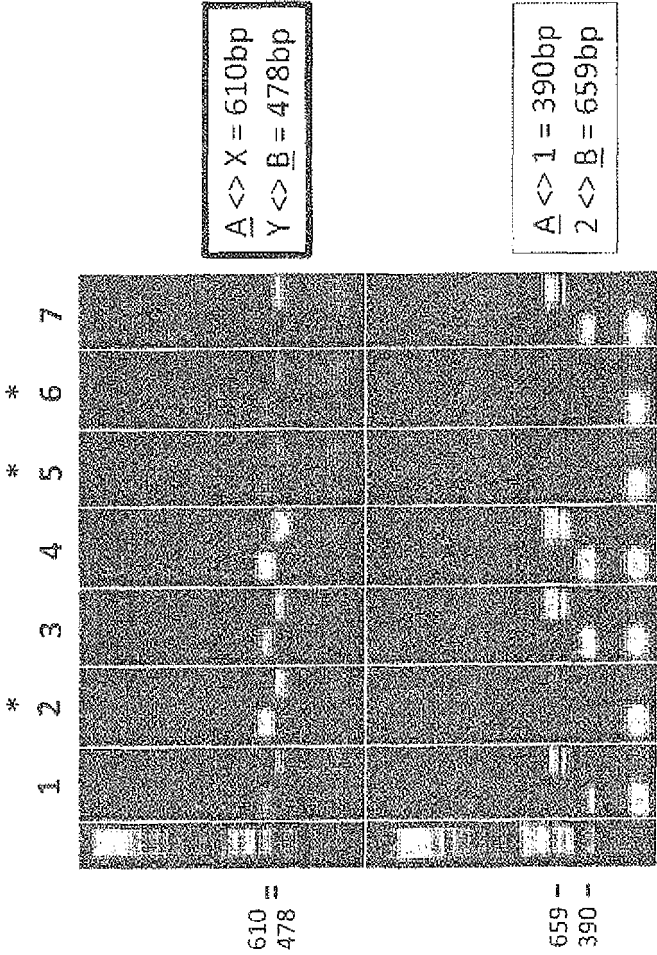


Fig. 33

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

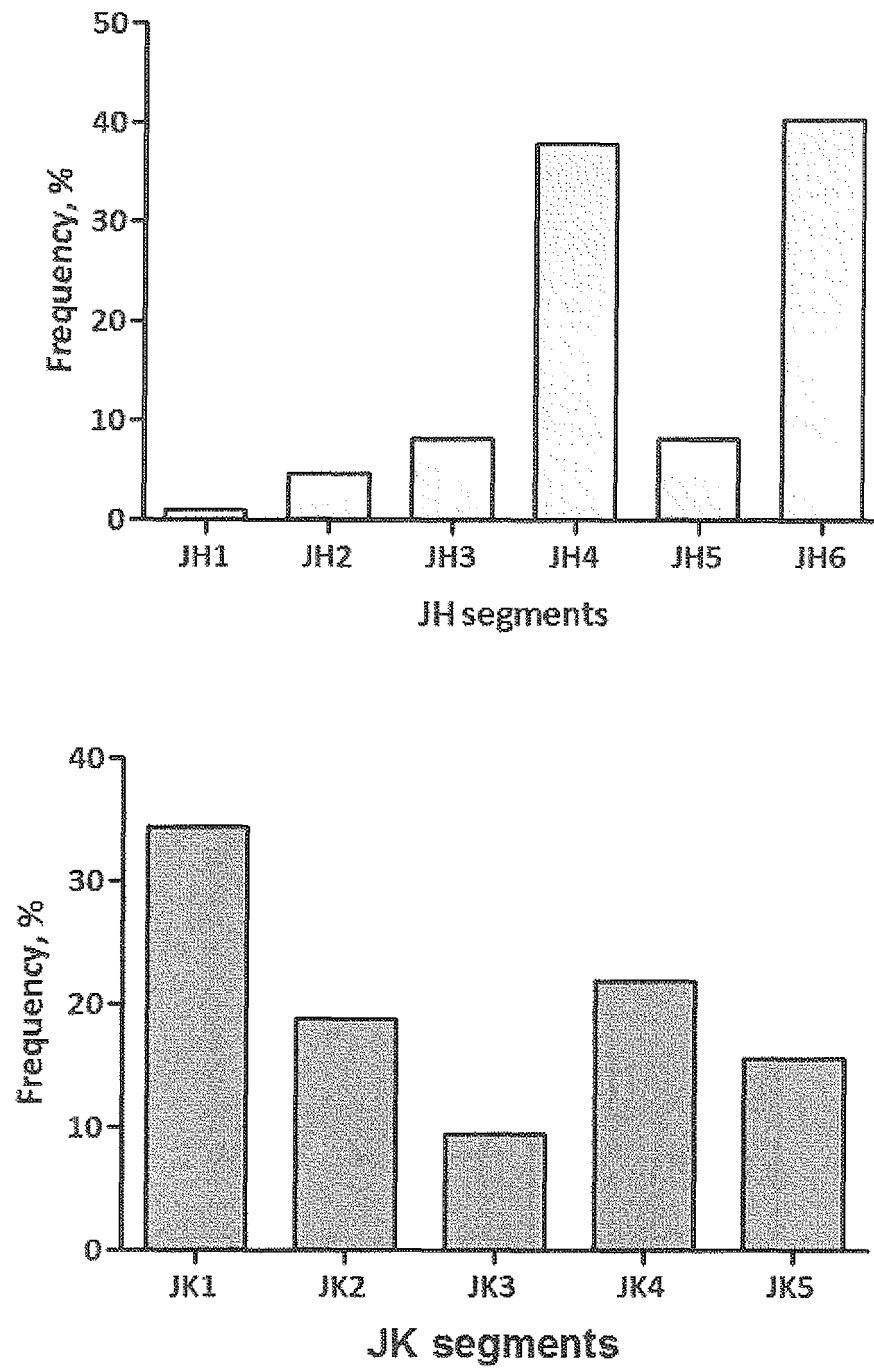


Fig.35.

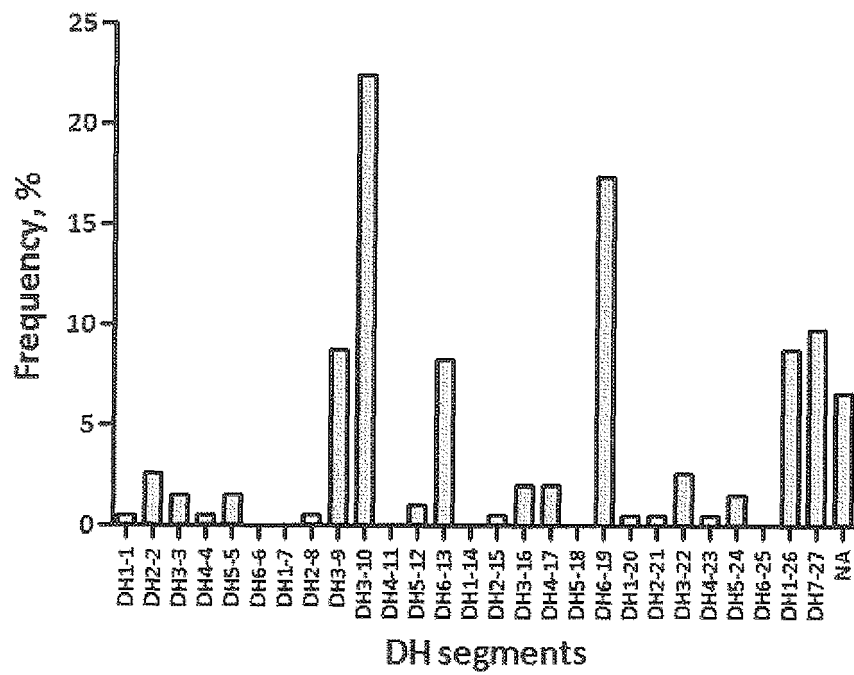


Fig. 36.

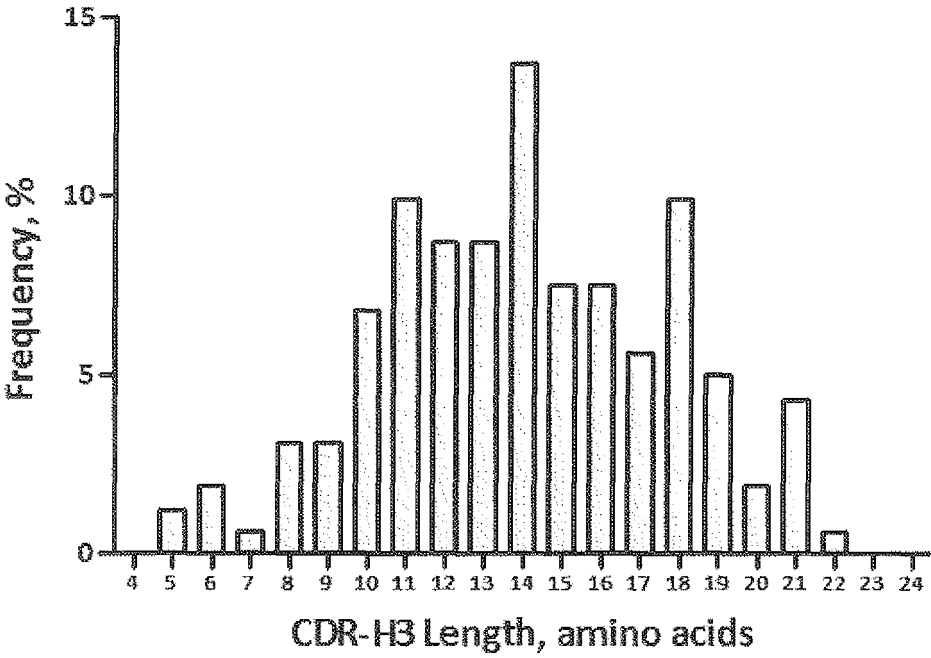


Fig. 37

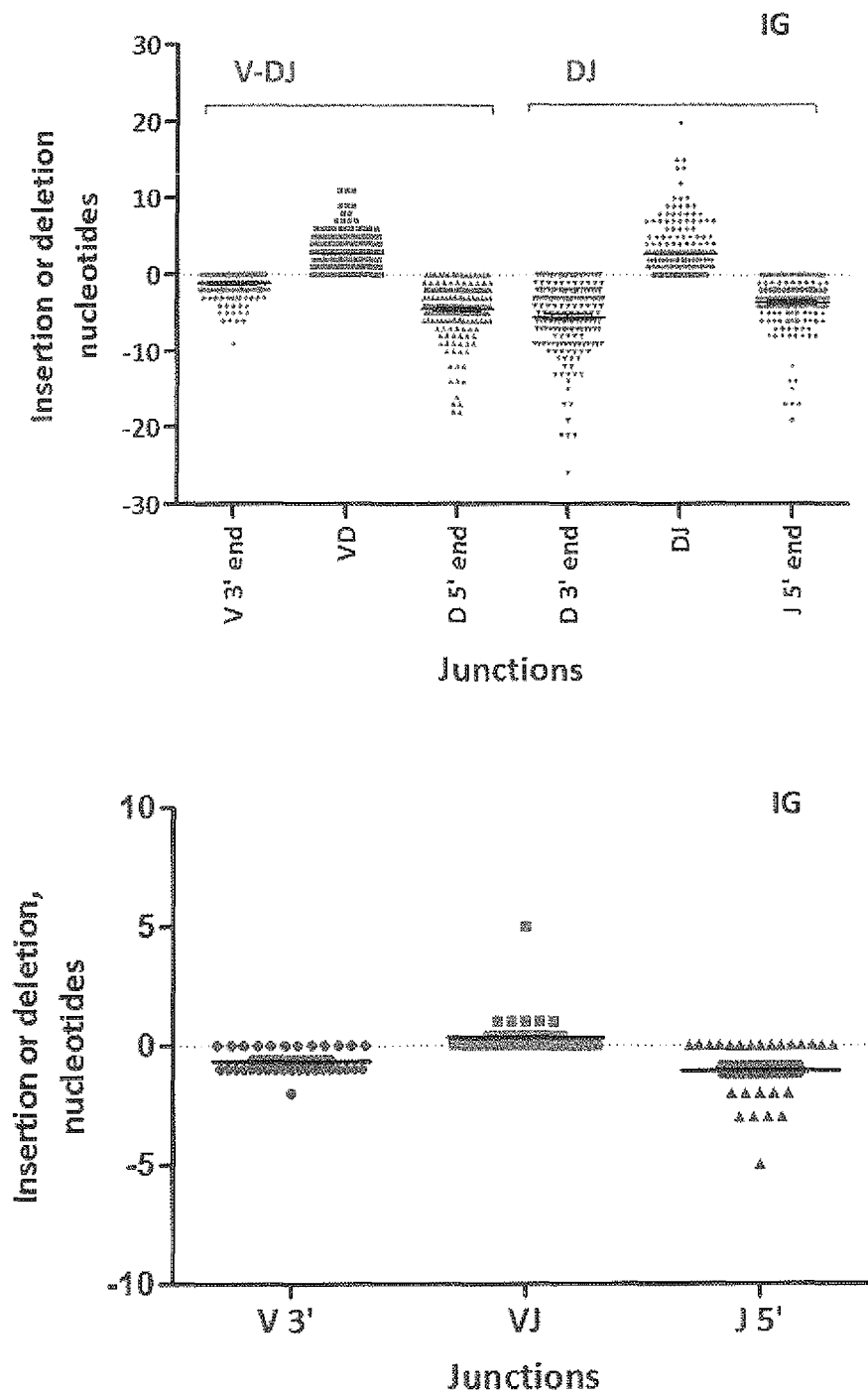


Fig. 38.

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

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		
V 2-5																														
V 4-4									1	3				3		2			1								1	3	14	
V 1-3										5				1		3											1	1	11	
V 1-2	1	5	1	1	2				1	13	25		1	12		2					1	3			1		7	8	13	115
V 6-1											1														1				3	
T			2		1						10		1	4		1	2					2			1		8	7		53
T	1	5	3	1	3	0	0	1	17	44	0	2	16	0	1	4	4	0	34	1	1	5	1	3	0	17	19	13	196	

The data includes 196 independent sequences.

Figure 40

Figure 71

Nucleotide Gain or Loss at VJ Joints Generates IGK Variants

V1-8 J1 CK

Translators	Consensus	250	270	280	290	300
V1-8 J1 CK(1499)						
T82_data_P1_e02.w2M13R.scf(17-624)	←	A C C A C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_e02.w2M13F.scf(20-744)	←	A C C C A C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_e12.w2M13F.scf(2B-749)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_e12.w2M13R.scf(17-685)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F03.w2M13F.scf(20-717)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F03.w2M13R.scf(18-572)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F08.w2M13R.scf(10-645)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F08.w2M13F.scf(15-846)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_e10.w2M13R.scf(15-700)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_e10.w2M13F.scf(14-574)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F05.w2M13R.scf(23-754)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F05.w2M13F.scf(14-540)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F06.w2M13R.scf(19-750)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F06.w2M13F.scf(16-421)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F06.w2M13R.scf(20-679)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F06.w2M13F.scf(10-466)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F01.w2M13R.scf(14-710)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			
T82_data_P1_F01.w2M13F.scf(42-653)	←	A C C C C	T G G A C C G T T C G G C C A A G G A C C A A G G T G G A A A T C A A A C G G C T G A			

E6, F5, G6

SSFSASIGDRVTITCRASQGISSYLAWYQKPKGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISCLQSEDFATYYCQQYYSY[PTFTGQ]
 GTKVEIKRADAA[PTVSIFPPSSEQLTSGGASVVCFLNFPKDNVKKWKIDGSRQNGV]

E2

ILILCIYRQSHHLSGESGYStopQLFSLVSAKTRSPStopAPOLCCIHFAKWGPIKVQRQWIWDRFHHQLPAVStopRFCNLLSLTVLStopL
 PTWTFGGGKVEIKRADAA[PTVSIFPPSSEQLTSGGASVVCFLNFPKDNVKKWKIDGSRQNGV]

E10, G8

SSFSASIGDRVTITCRASQGISSYLAWYQKPKGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISCLQSEDFATYYCQQYYSY[PTFTGQ]
 GTKVEIKRADAA[PTVSIFPPSSEQLTSGGASVVCFLNFPKDNVKKWKIDGSRQNGV]

F3, F12

SSFSASIGDRVTITCRASQGISSYLAWYQKPKGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISCLQSEDFATYYCQQYYSY[PTFTGQ]
 GTKVEIKRADAA[PTVSIFPPSSEQLTSGGASVVCFLNFPKDNVKKWKIDGSRQNGV]

H1

SSFSRSIGDRVTITCRASQGISSYLAWYQKPKGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISCLQSEDFATYYCQQYYSY[PTFTGQ]
 GTKVEIKRADAA[PTVSIFPPSSEQLTSGGASVVCFLNFPKDNVKKWKIDGSRQNGV]

CONFIDENTIAL

Figure 42

Hypermutation in J Regions Generates IGK Variants

		V1-6										J1										CK																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
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► Translate ► Consensus		I	A	C	C	C	T	G	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C	G	T	T	C

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

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

B12, C6, D3
KLLIYAASSLQSGVPSRFSGSGGTDFLTISLQPEDFATYYCLQDYNYPRTFGGGTKEIKRADAAPTVSIFPPSSEQLTS
GGASVVCFLNNFYPKDINVWKIDGSRQNGV

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Figure 43

Joint Diversity Produces Functional CDS

VI-5_J1_Ck
VAGWPGISRNGQKPLSSStopSIRRLVStopKVGSHQGSAAVDLGQNSLSPSAAC
SLMetILQLITANSIIVILTFGGGKVEIKRADAAPTVSIFPPSSEQLTSGGASVV
CFLNMFYPKDINVKKIDGSERQNGV

H12
SSWLAWYQQKPGKAPKLLIYKASSLESQVPSRFSGSGTEFTLTISSLQPD
FATYYCQQYNSYSTFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL
NNFYPKDINVKKIDGSERQNGV

VI-5_J4_Ck
AGWPGISRNGQKPLSSStopSIRRLVStopKVGSHQGSAAVDLGQNSLSPSAACS
LMetILQLITANSIIVILTFGGGKVEIKRADAAPTVSIFPPSSEQLTSGGASVV
CFLNMFYPKDINVKKIDGSERQNGV

E1
SWLAWYQQKPGKAPKLLIYKASSLESQVPSRFSGSGTEFTLTISSLQPD
FATYYCQQYNSYSTFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL
NNFYPKDINVKKIDGSERQNGV

E2
SWLAWYQQKPGKAPKLLIYKASSLESQVPSRFSGSGTEFTLTISSLQPD
FATYYCQQYNSYSTFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL
NNFYPKDINVKKIDGSERQNGV

E8
SSWLAWYQQKPGKAPKLLIYKASSLESQVPSRFSGSGTEFTLTISSLQPD
FATYYCQQYNSYSTFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVC
FLNMFYPKDINVKKIDGSERQNGV

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