

Three basic vector backbones

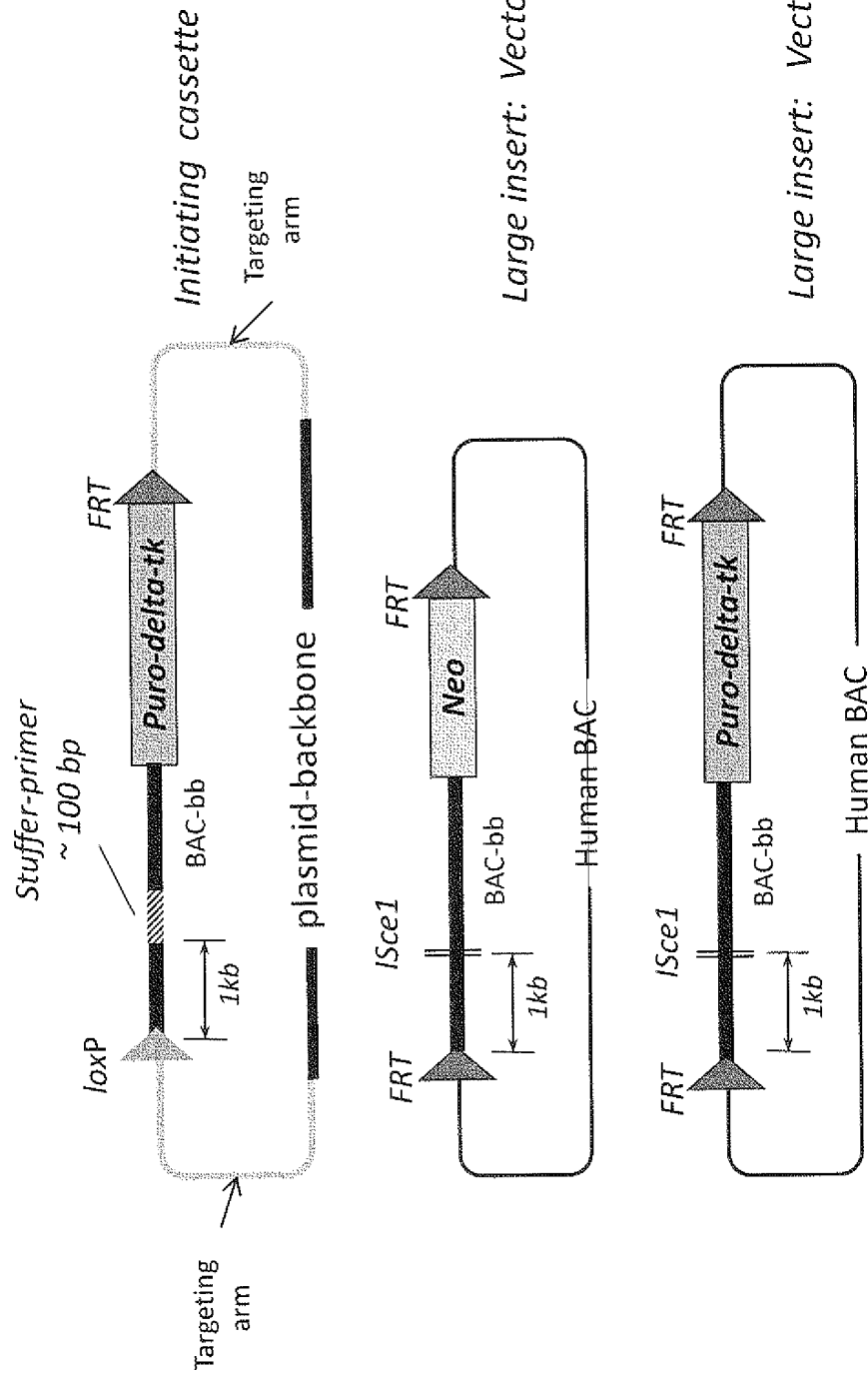
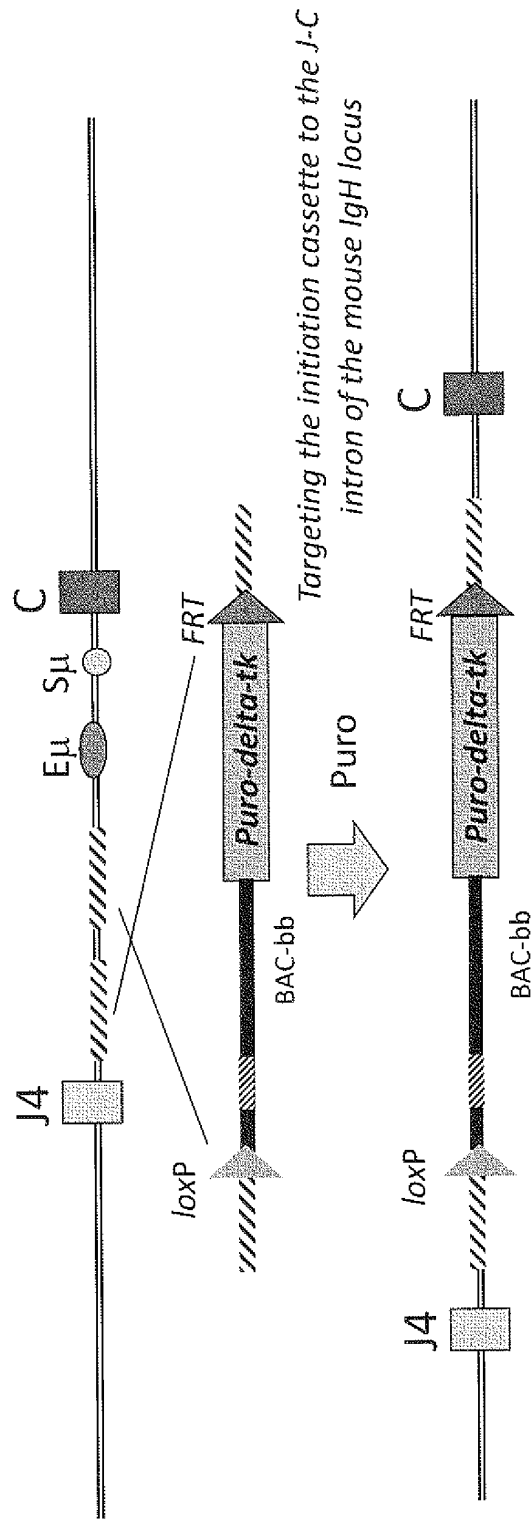


Fig. 1

Entry / Initiation vector: Step 1

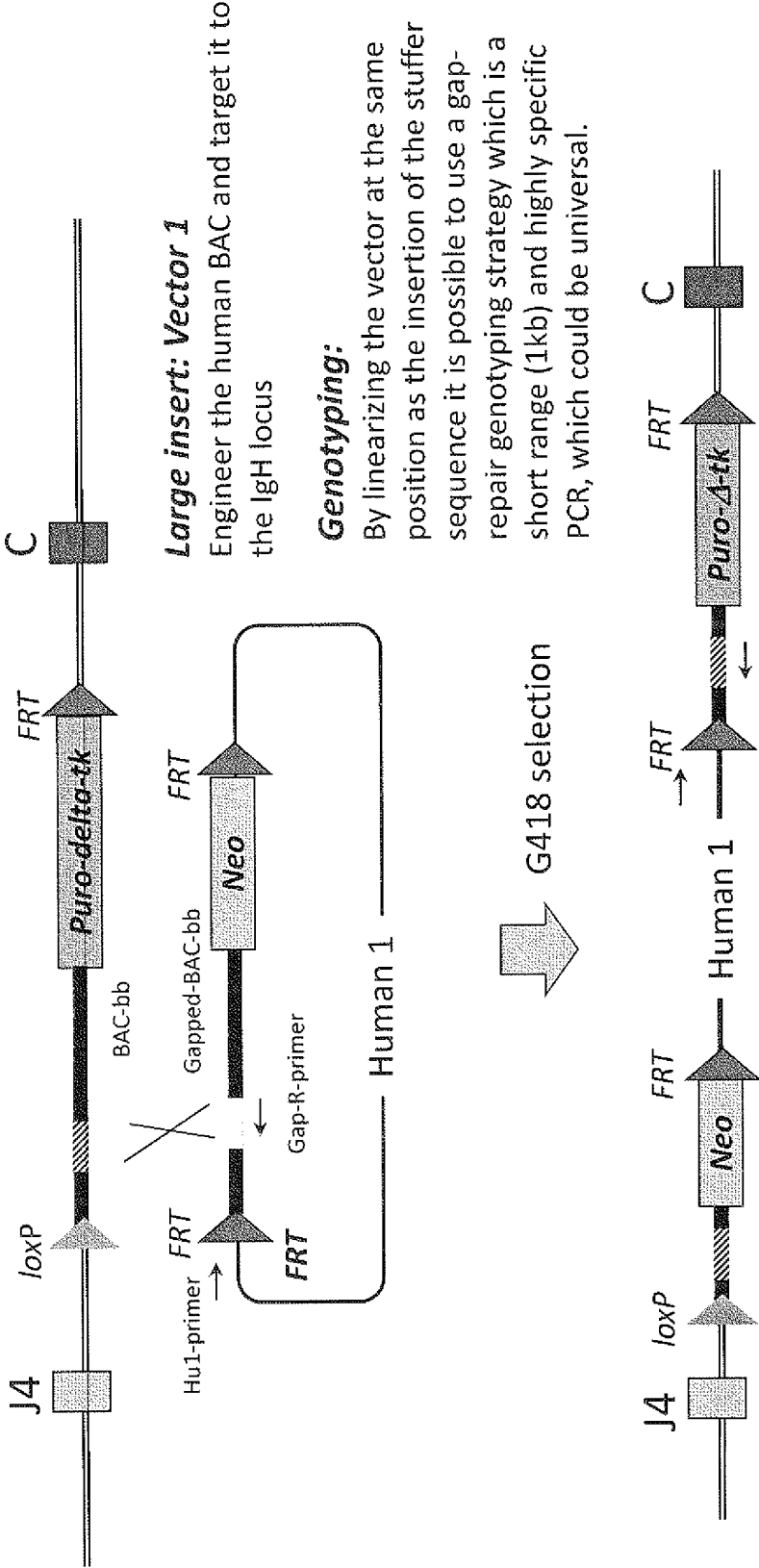


Validation:

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

Fig. 2

Addition vector: Step 2



Large insert: Vector 1

Engineer the human BAC and target it to the IgH locus

Genotyping:

By linearizing the vector at the same position as the insertion of the stuffer sequence it is possible to use a gap-repair genotyping strategy which is a short range (1kb) and highly specific PCR, which could be universal.

Validation:

- 1. CGH tiling array across the BAC sequence.
- 2. PCR verification of all critical V, D & J regions

Fig. 3

Addition vector: Step 2

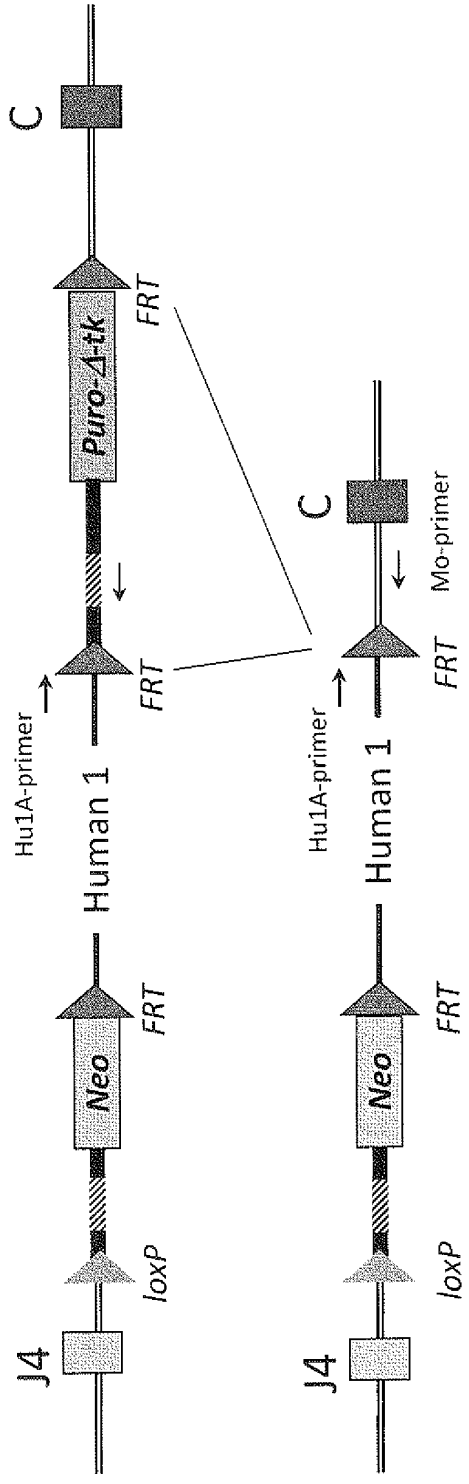
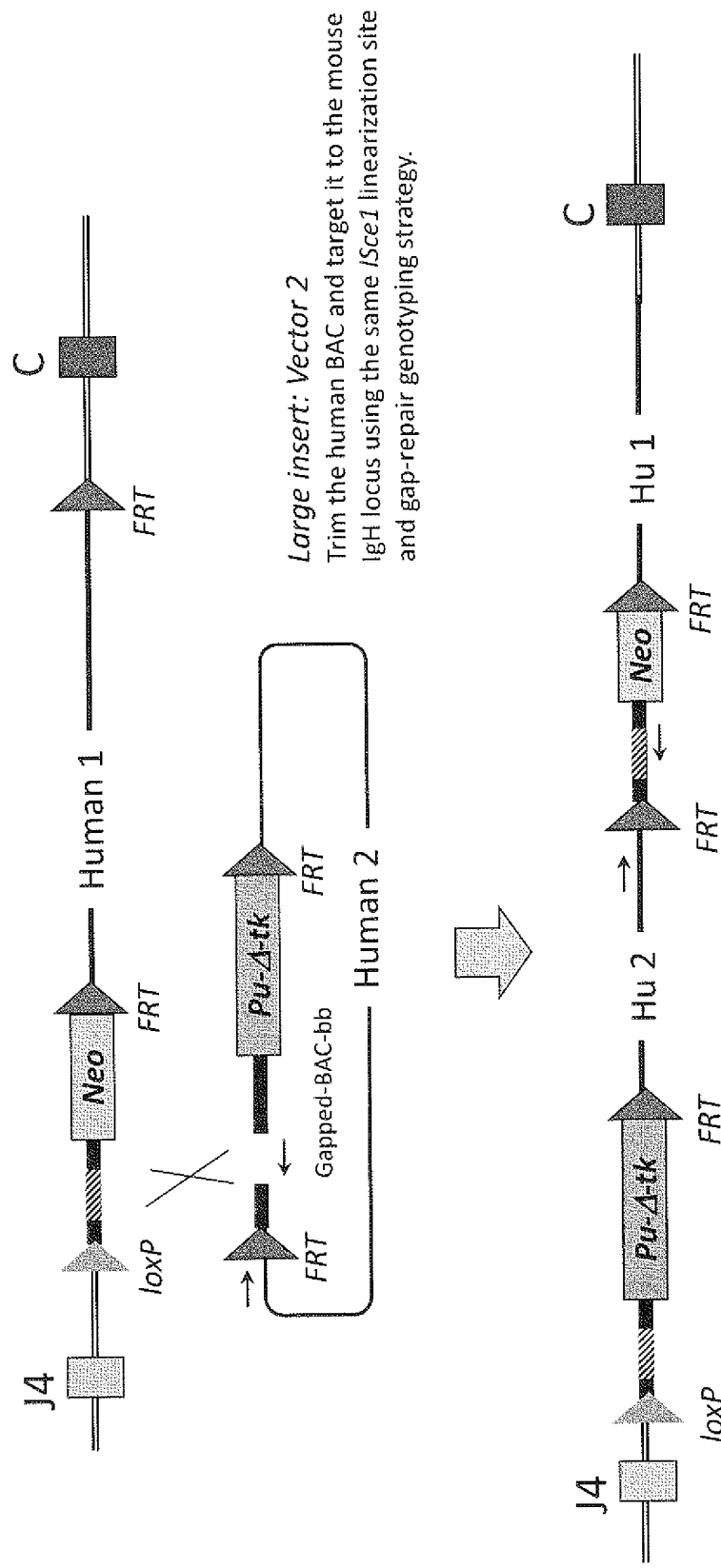


Fig. 4

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

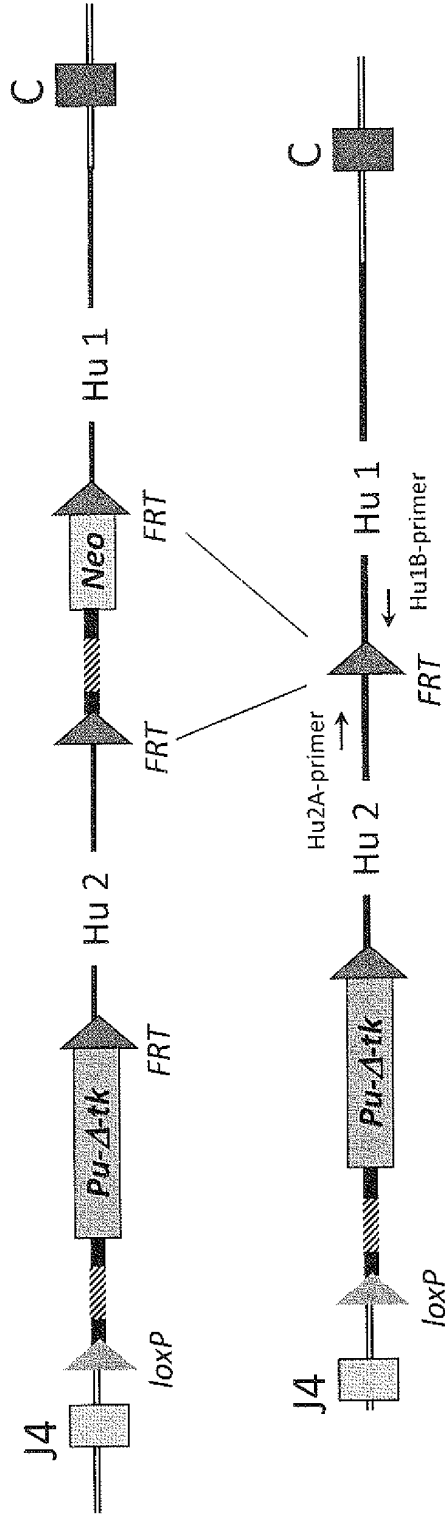
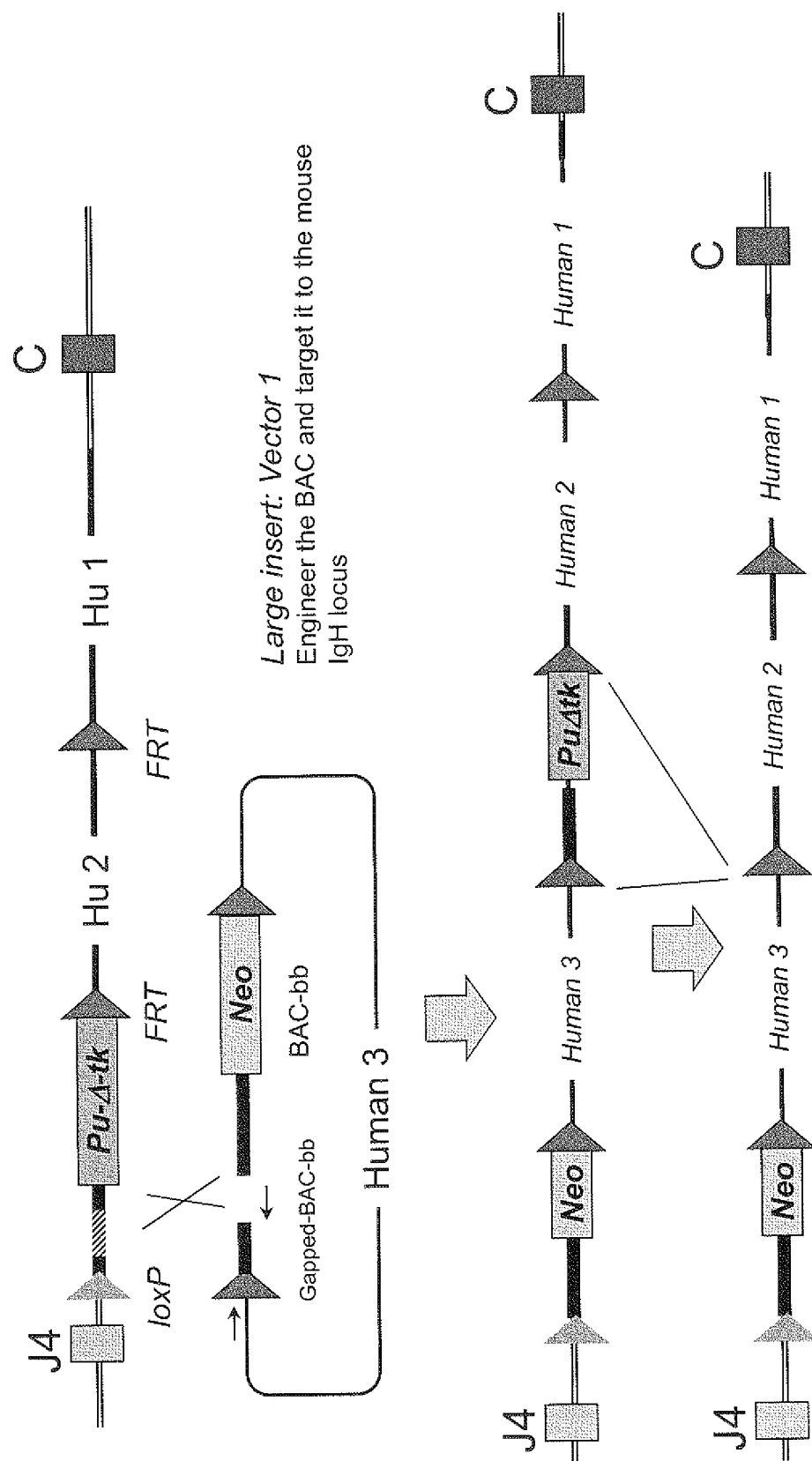


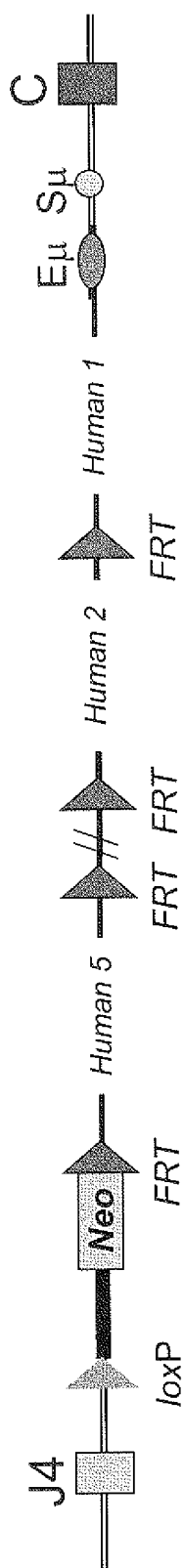
Fig. 6

Addition vector: Step 4



7
6
5

A humanized mouse IgH locus



00
00
a
L

BAC landing pad inserted at the IgH locus

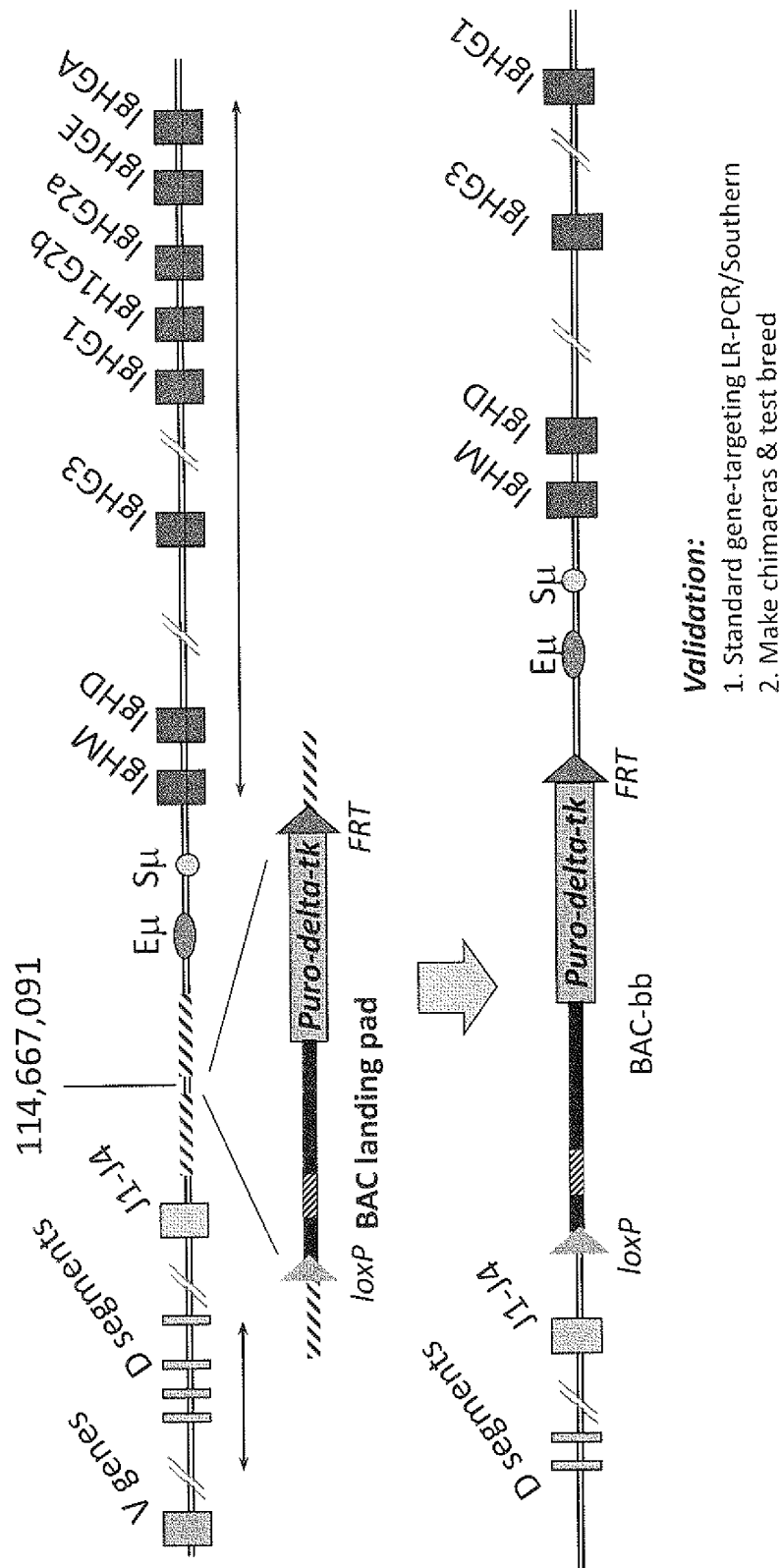


Fig. 9

1st BAC insertion – Step 1

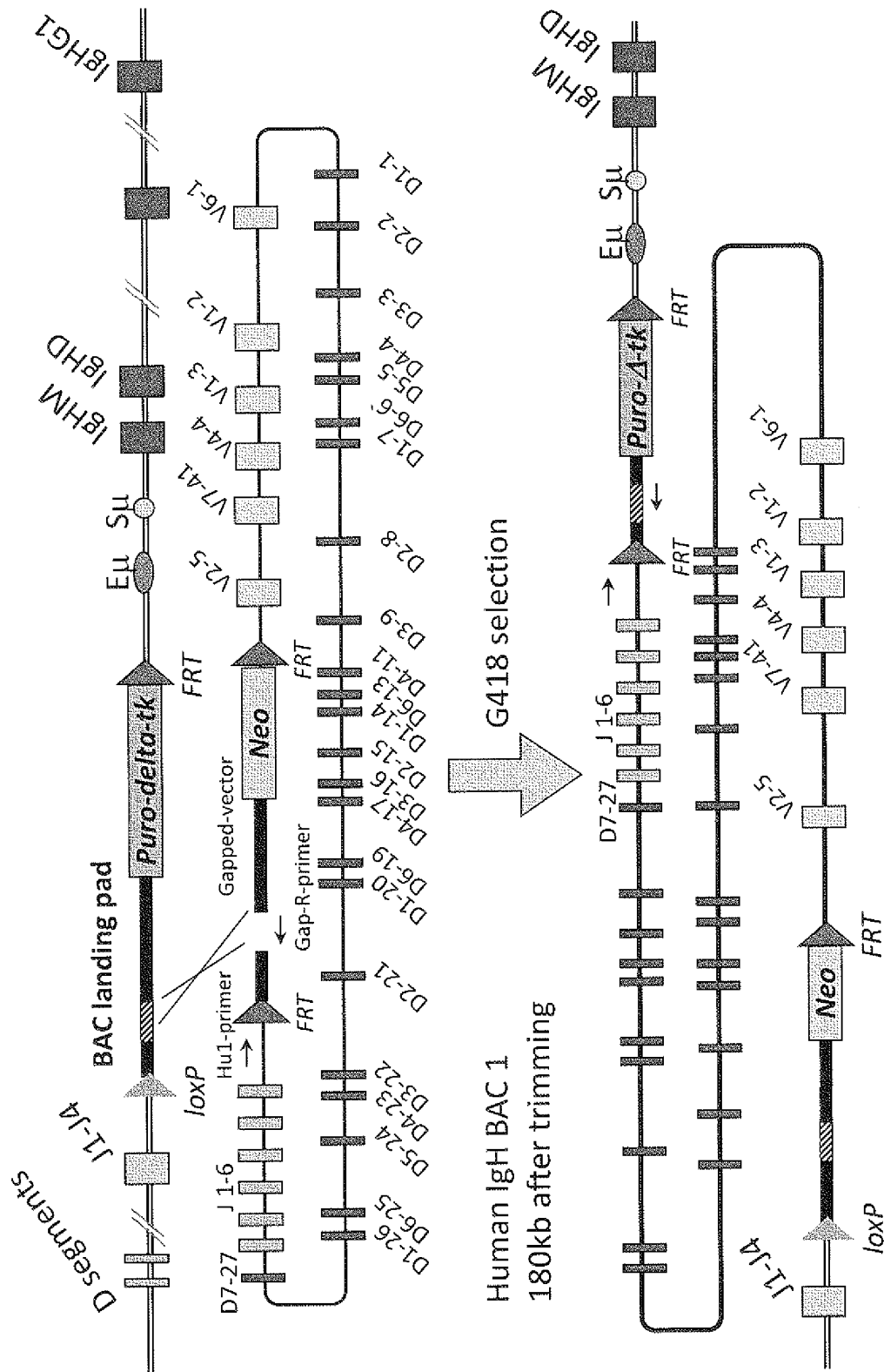


Fig. 10

1st BAC insertion – Step 2

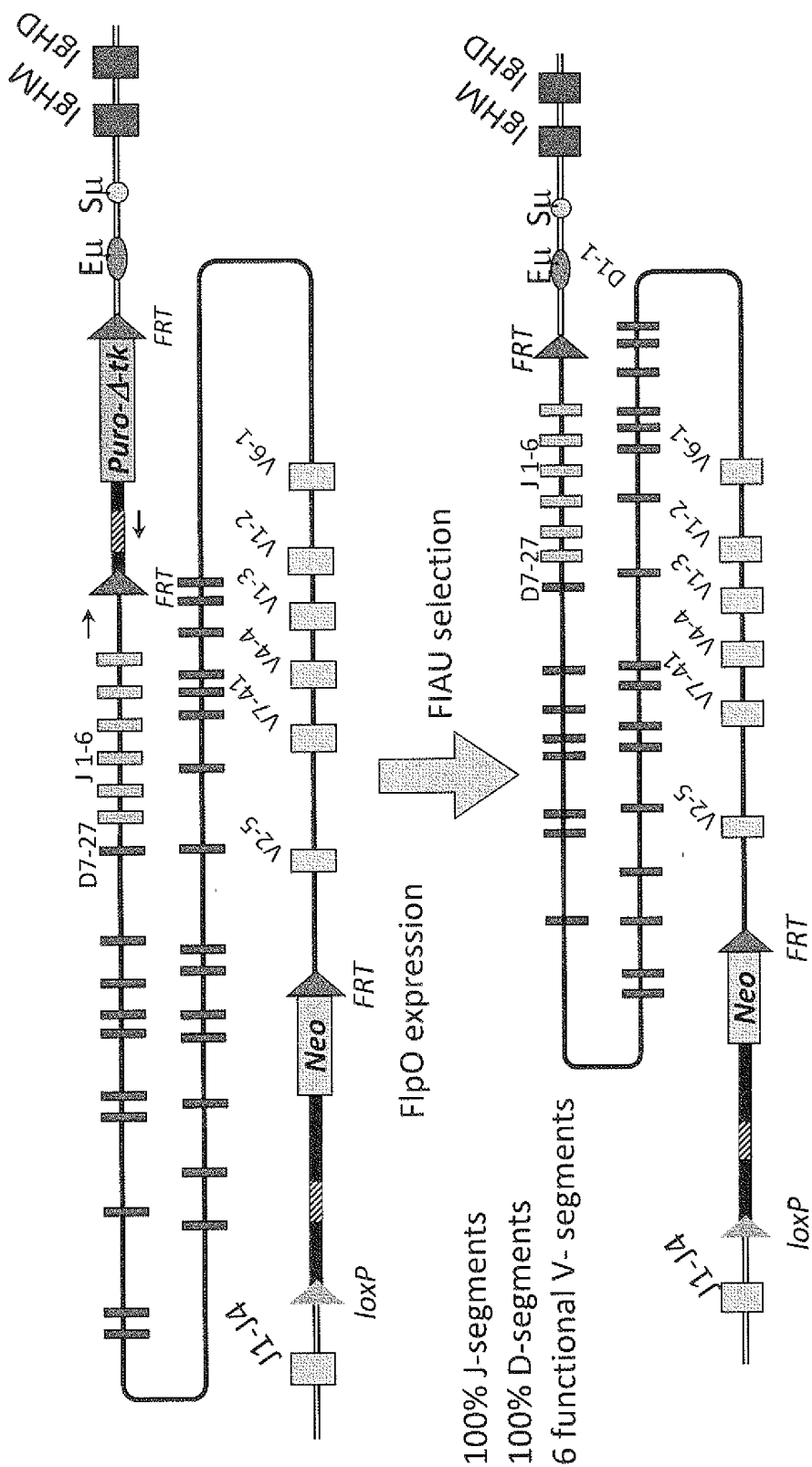
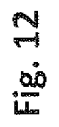


Fig. 11



2nd BAC insertion – Step 4

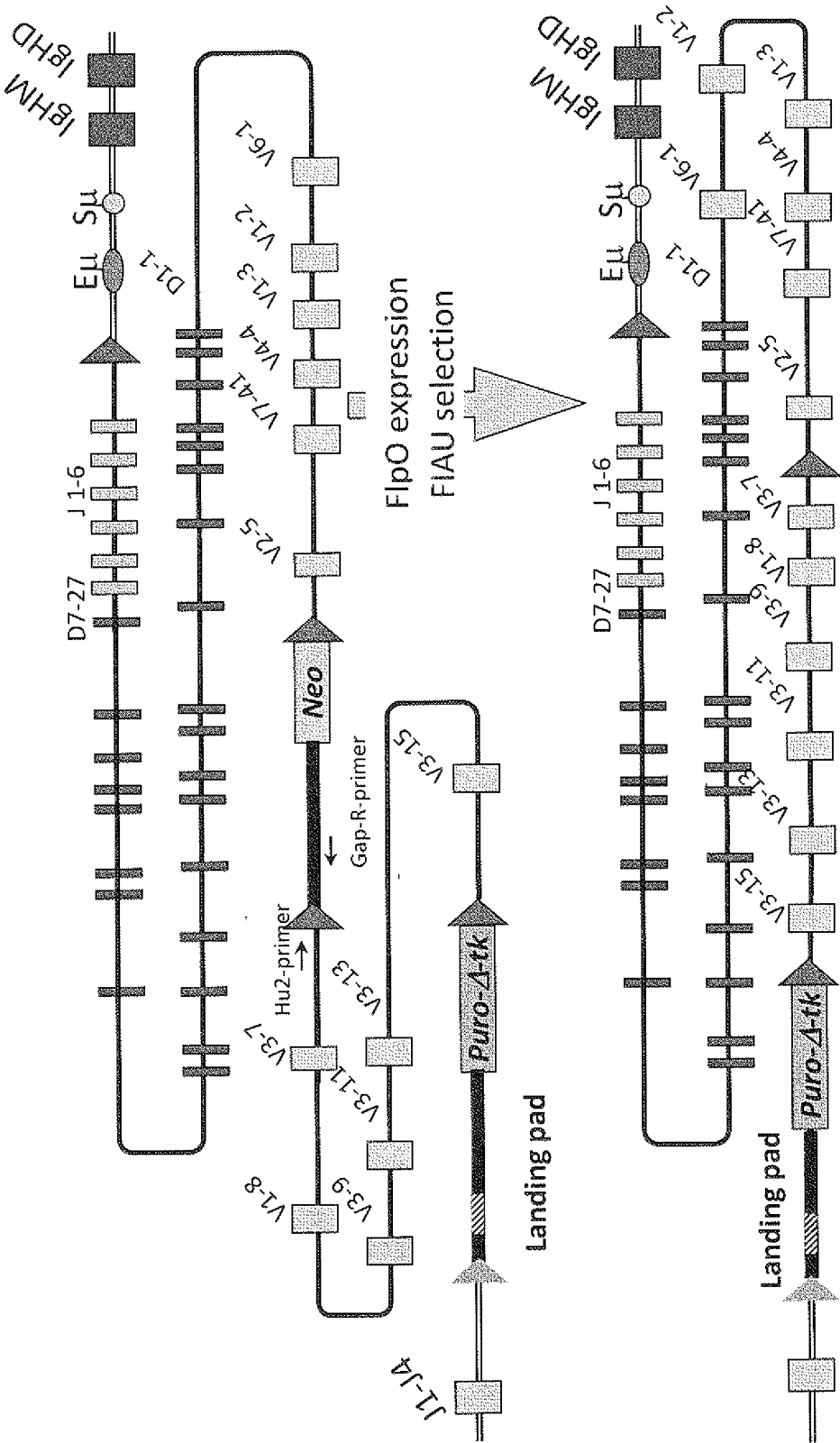


Fig. 13

BAC Landing Pad at IgK Locus

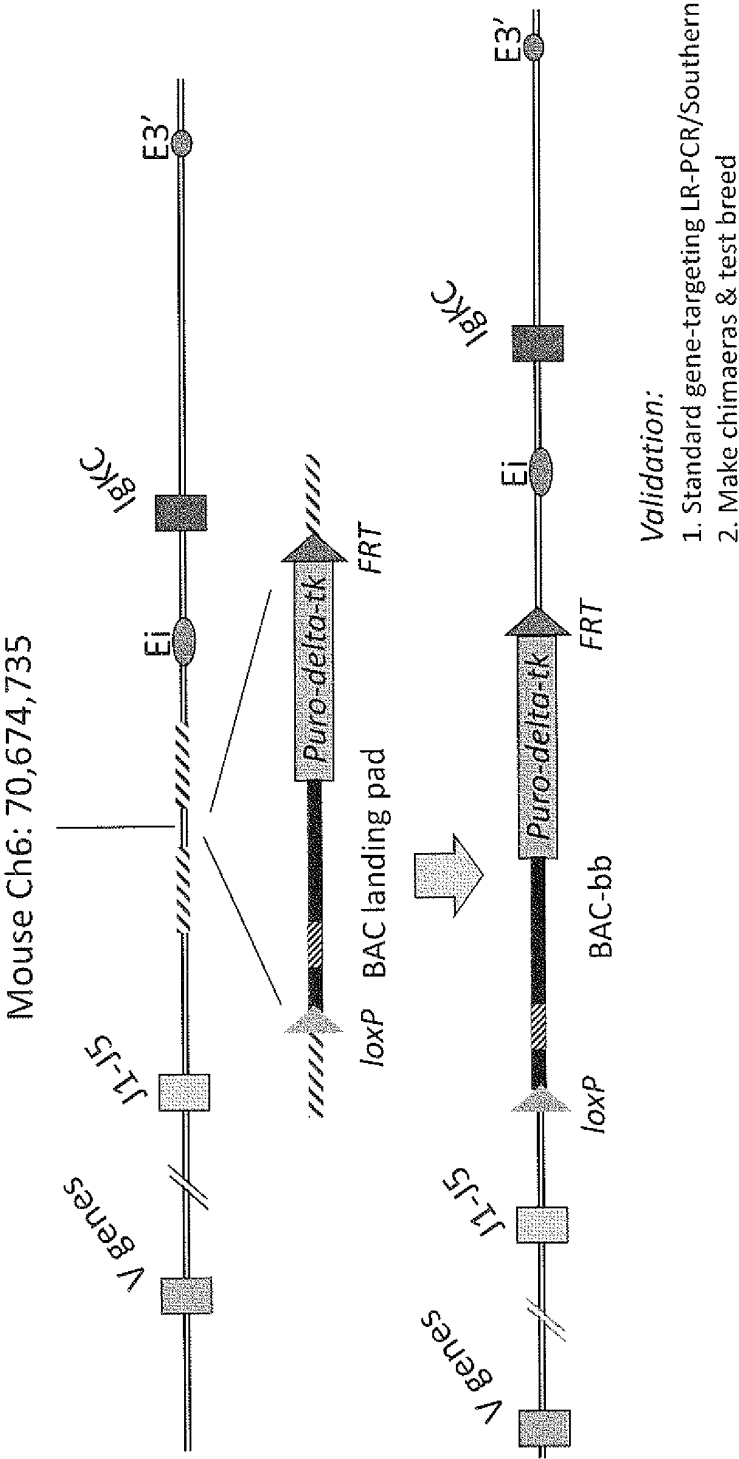


Fig. 14

1st BAC Insertion – Step 1

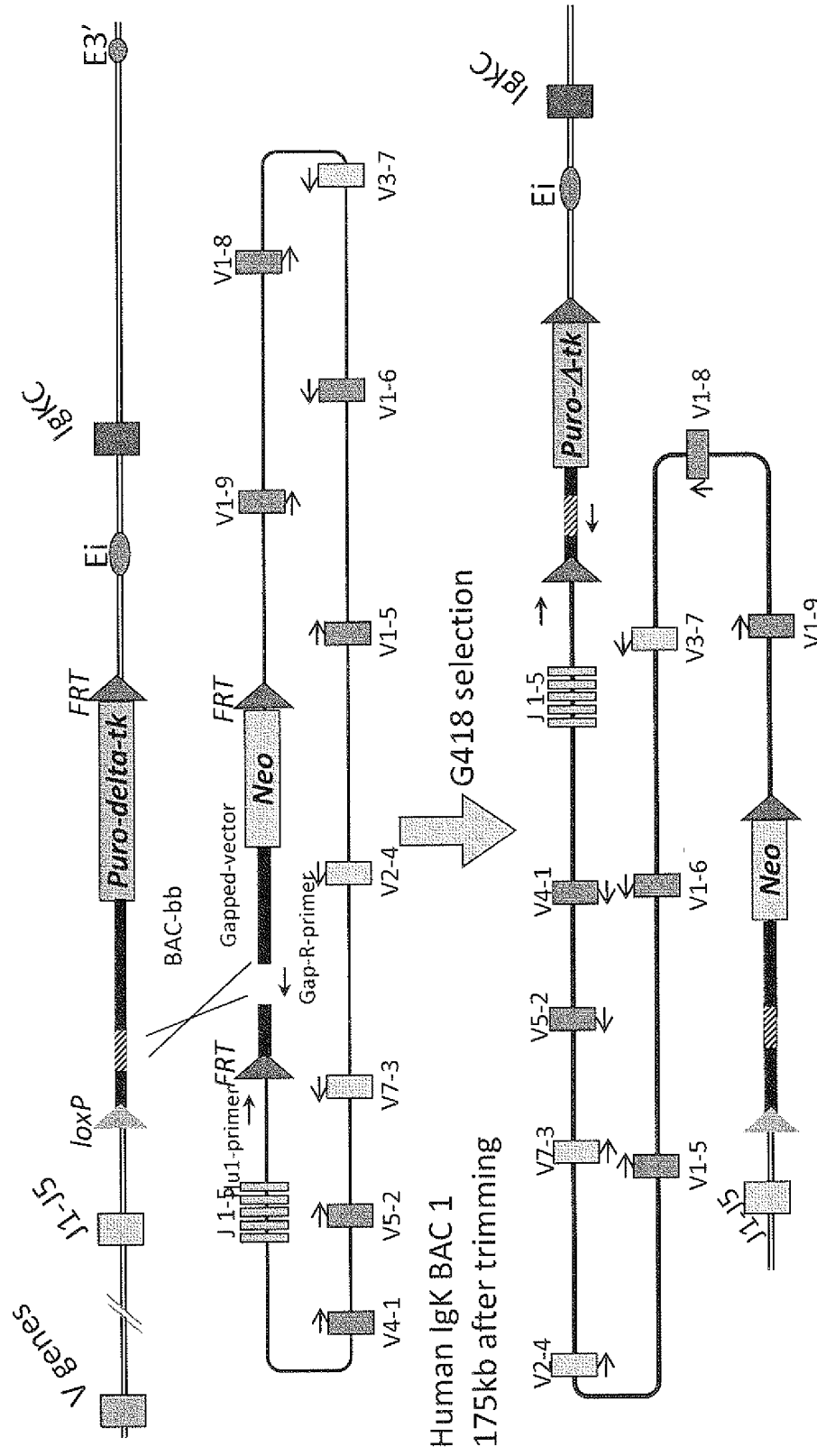


Fig. 15

1st BAC Insertion – Step 2

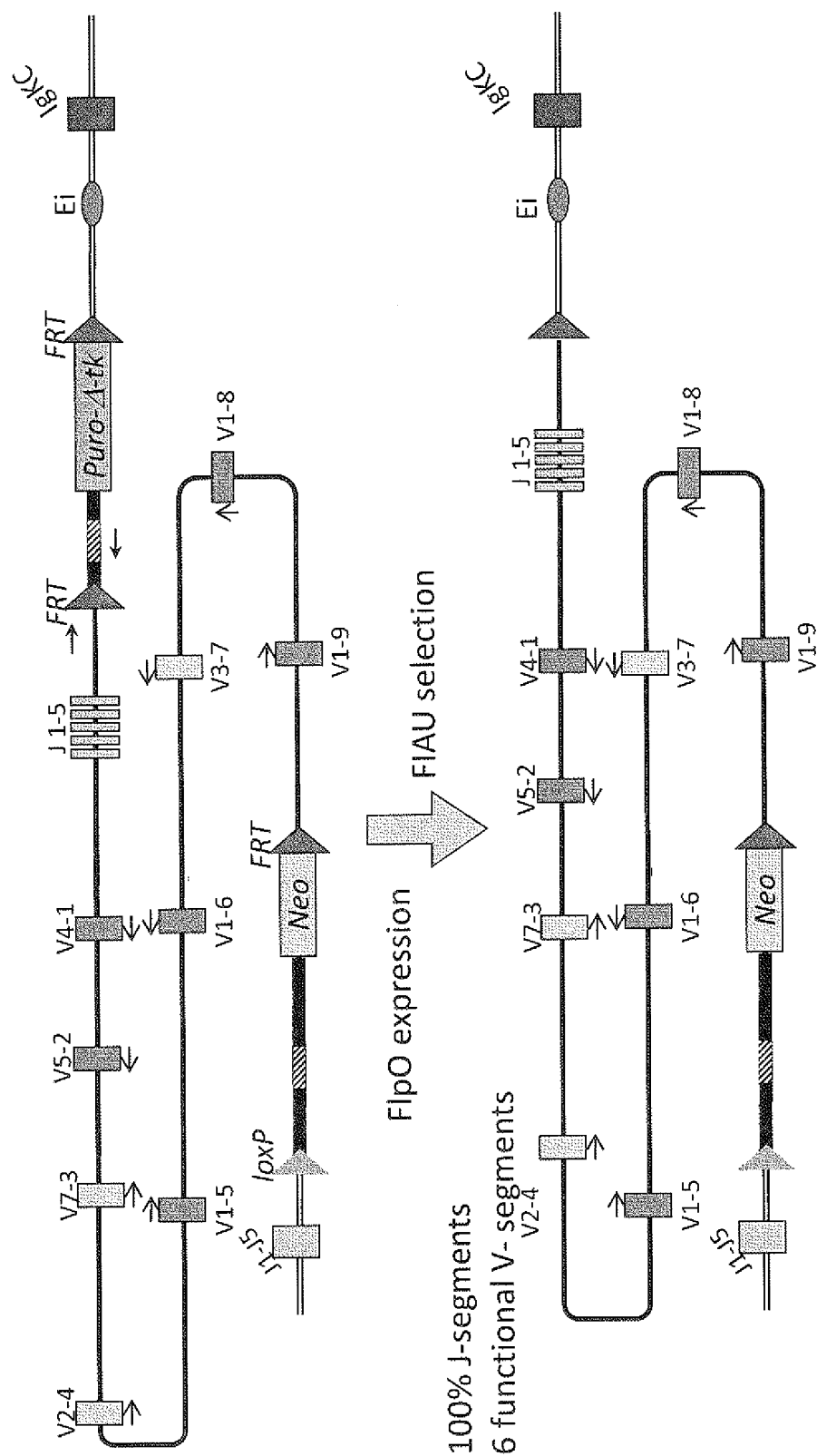


Fig. 16

2nd BAC Insertion – Step 3

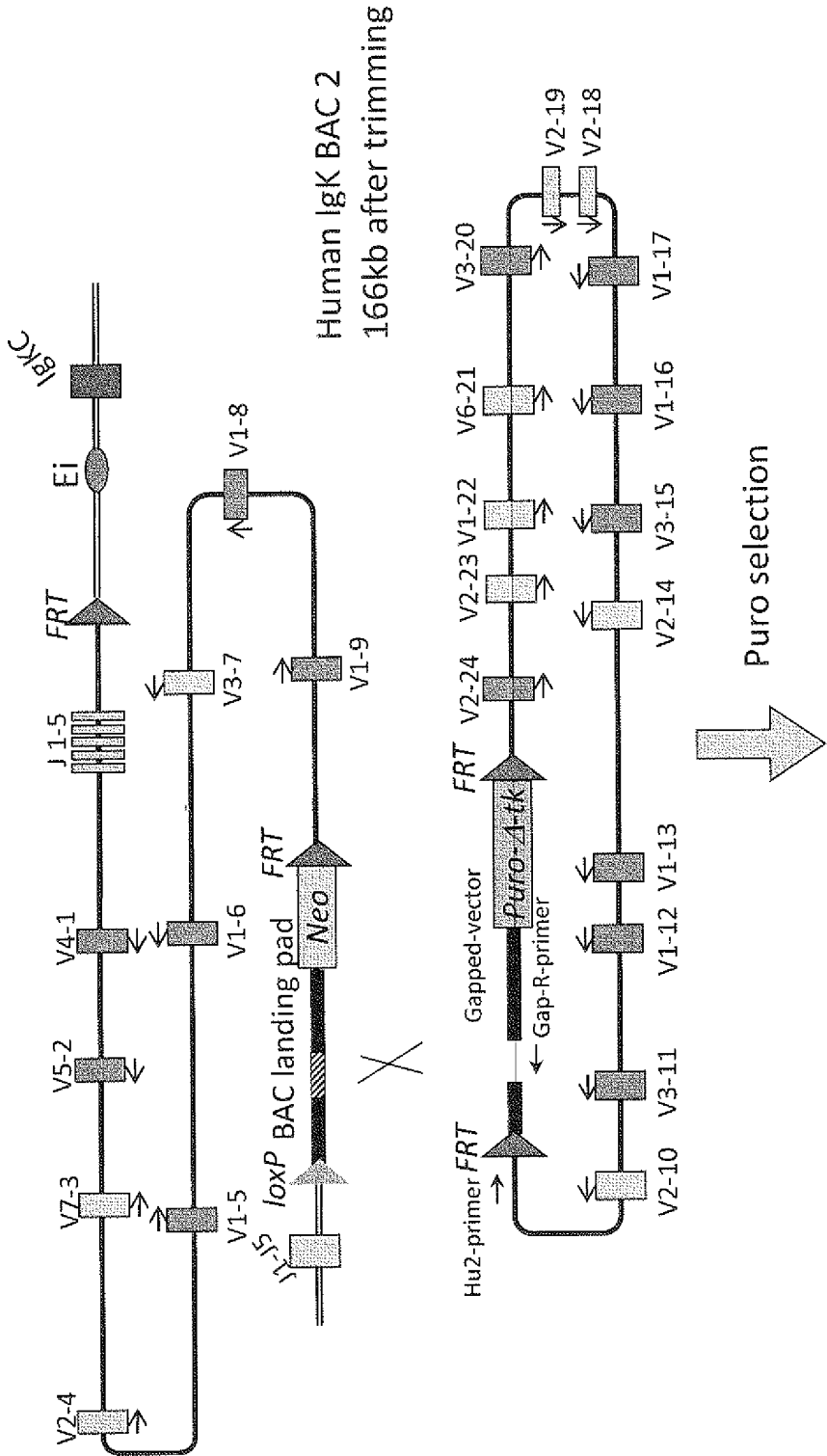


Fig. 17

2nd BAC insertion – Step 4

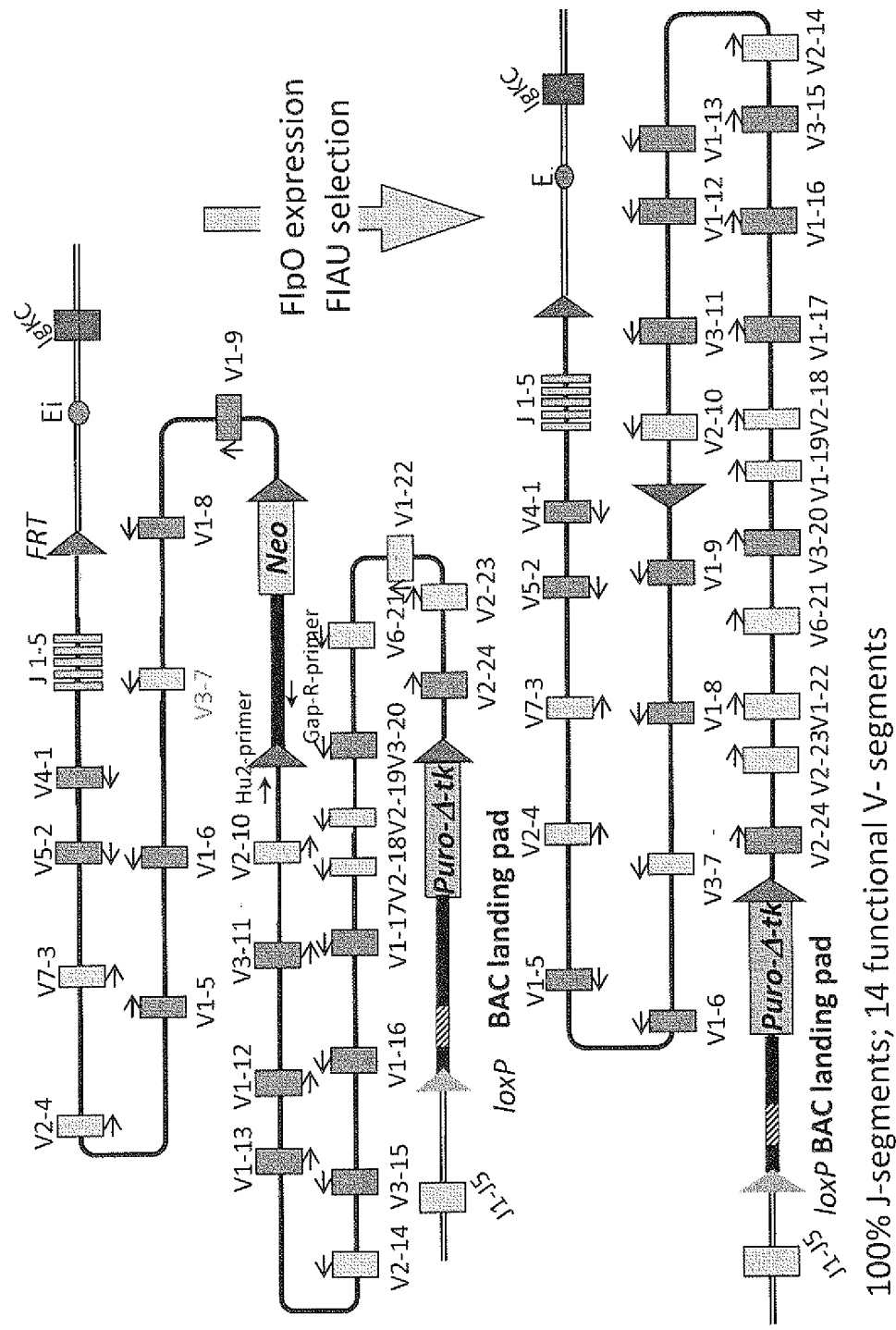


Fig. 18

Rapid Testing of ES Cells with Humanized IgH and IgK Loci

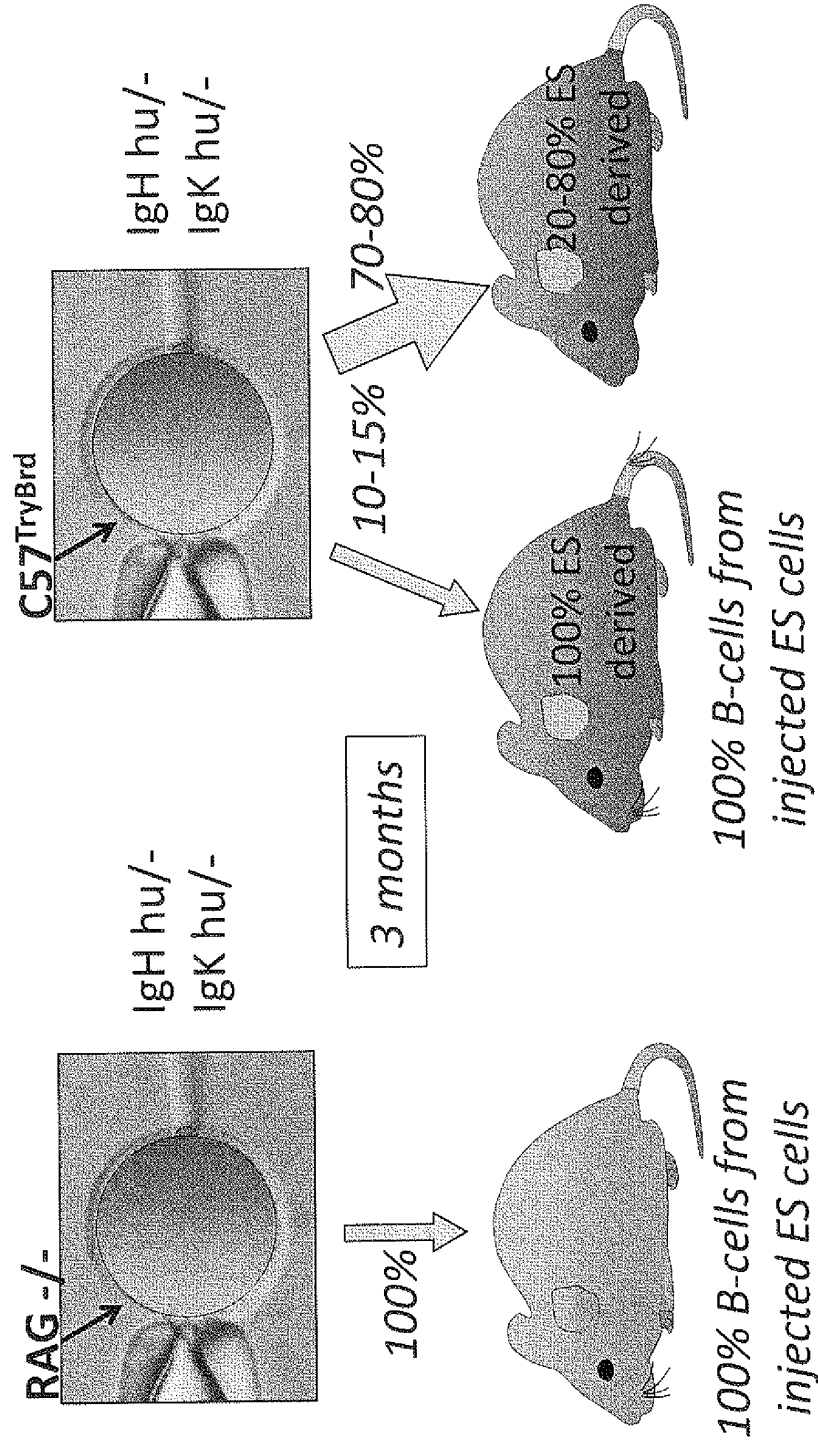


Fig. 19

Antibody Generation in Chimaeric Mice with Humanized IgH and IgK Loci

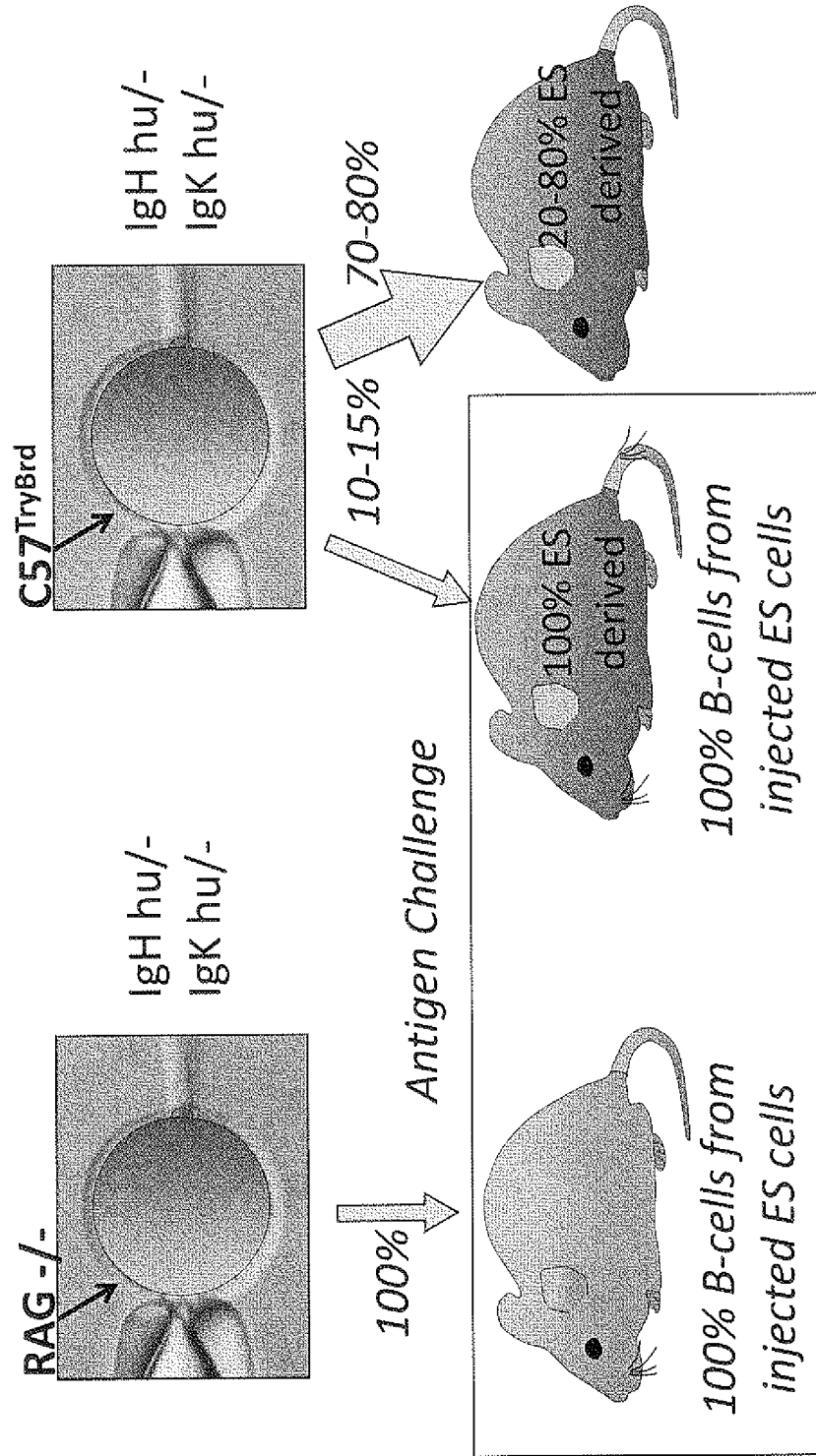


Fig. 20

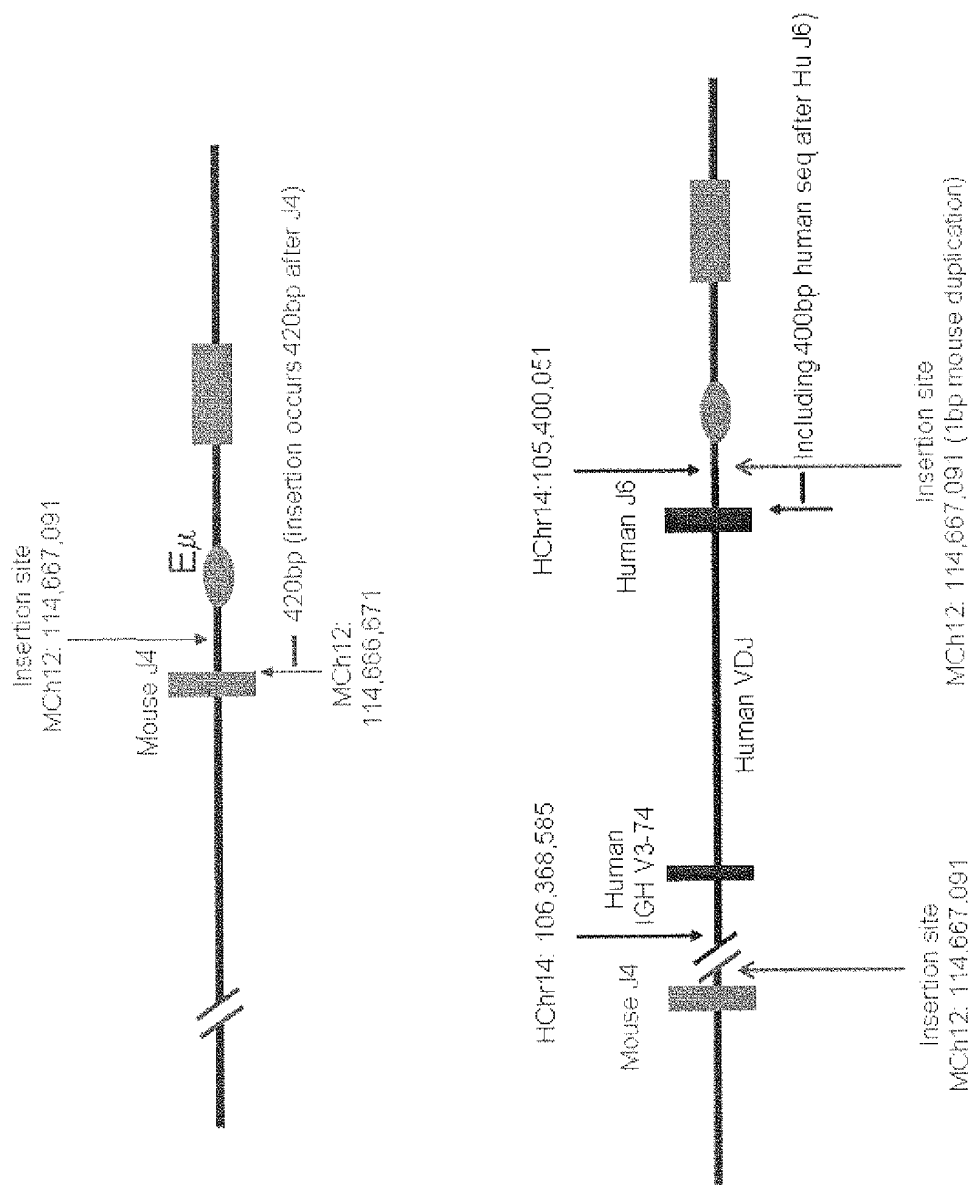


Fig. 21

Targeting of the Landing Pad

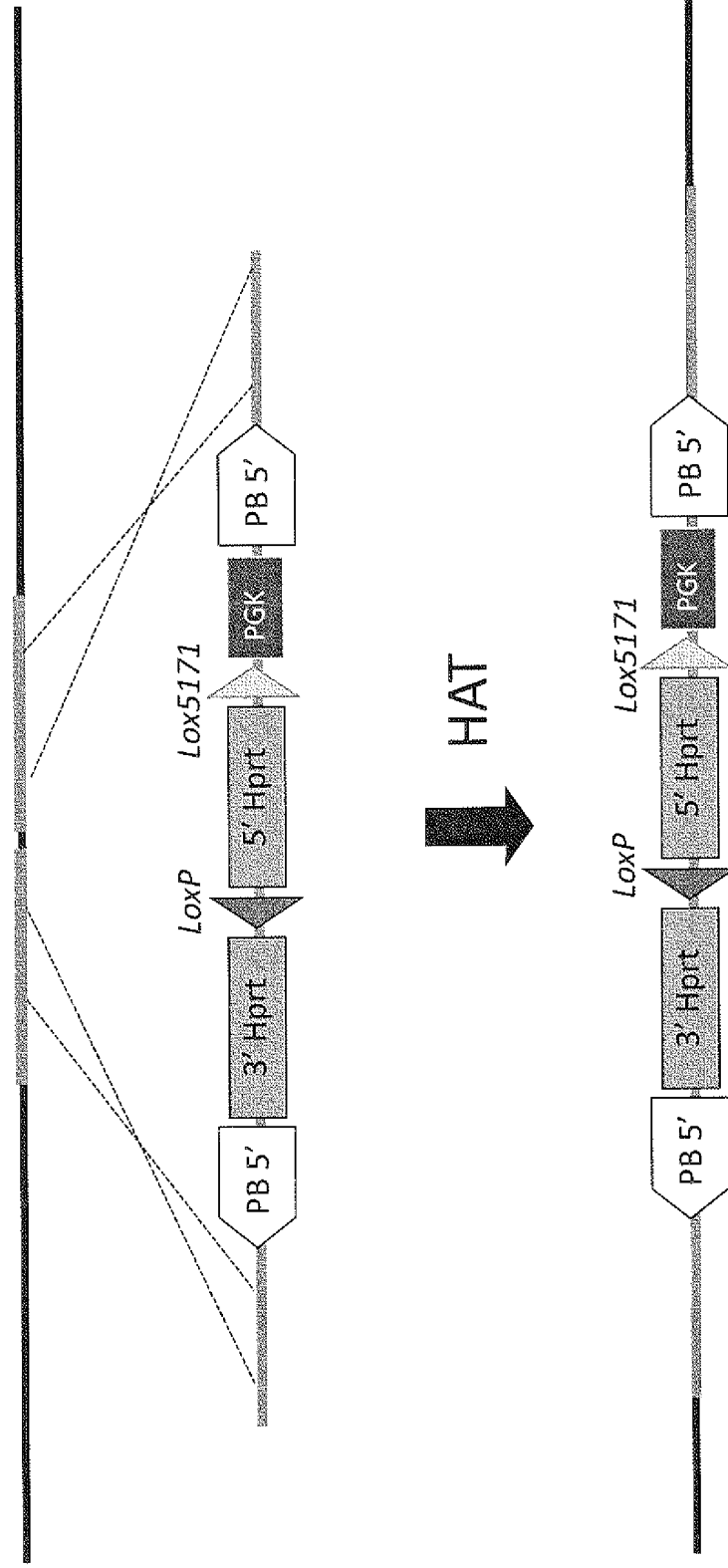


Fig. 22

Insertion of the 1st BAC

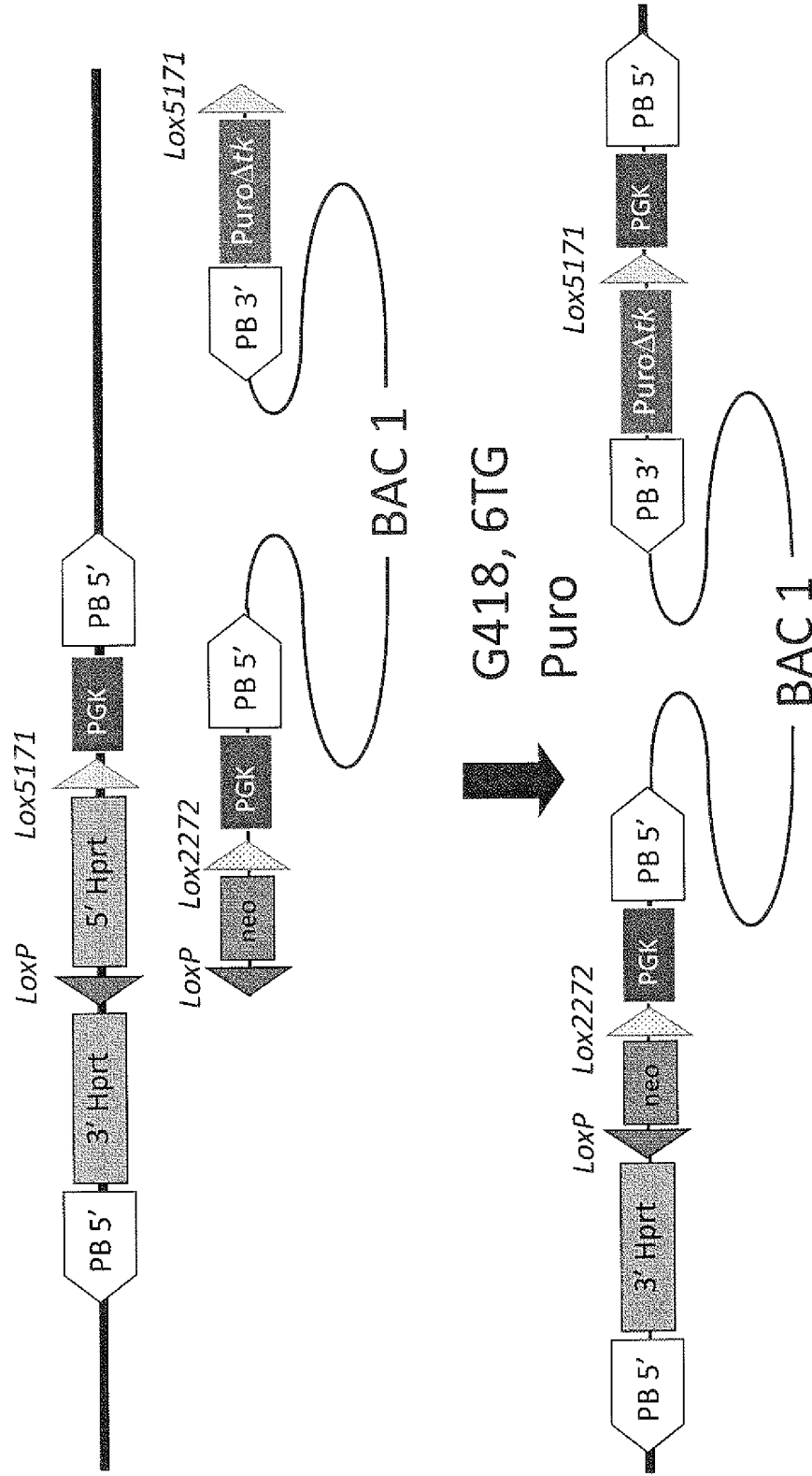


Fig. 23

Curing of the 3' Modification

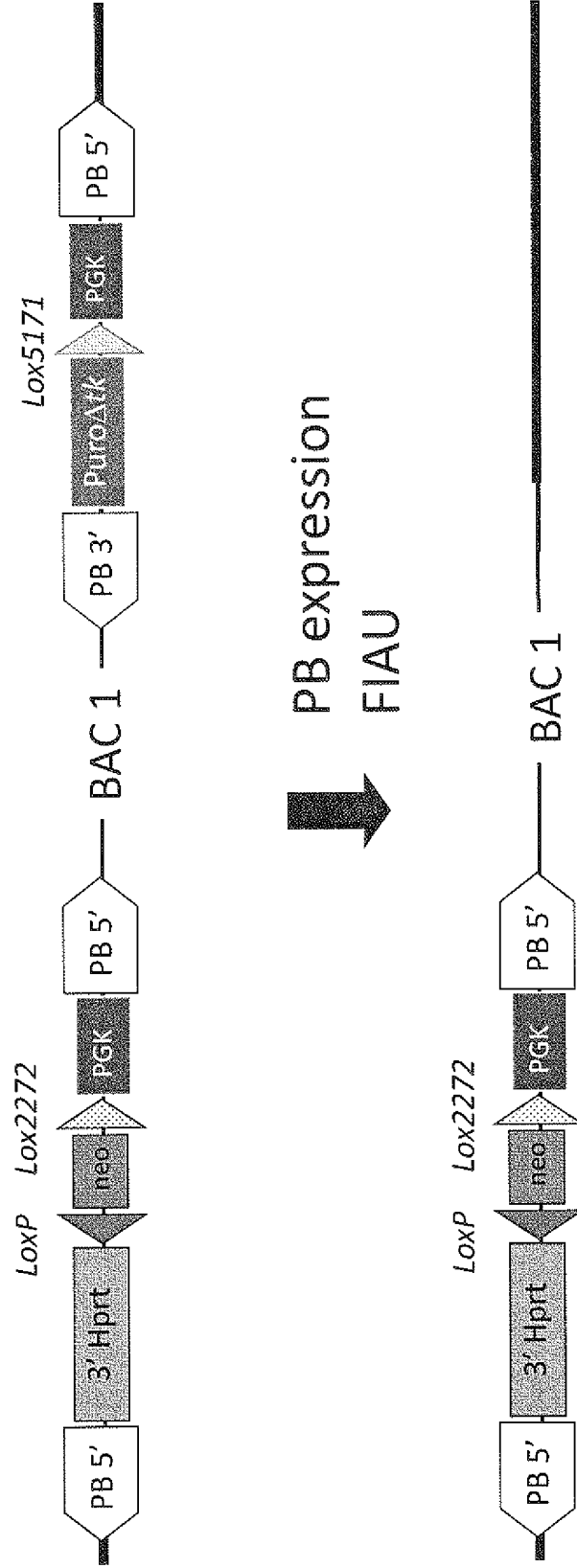


Fig. 24

Insertion of the 2nd BAC

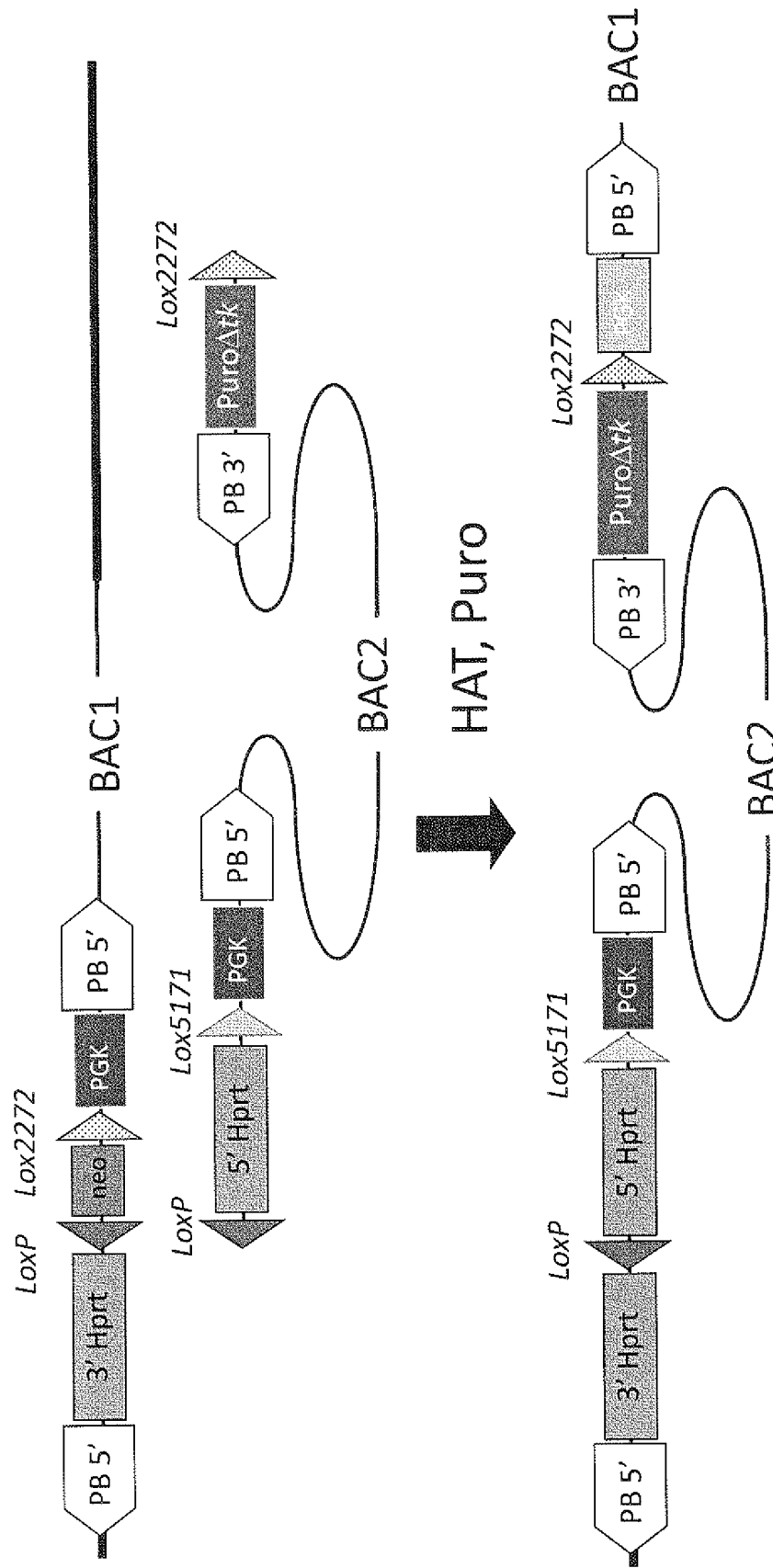


Fig. 25

Removing the 3' Modification

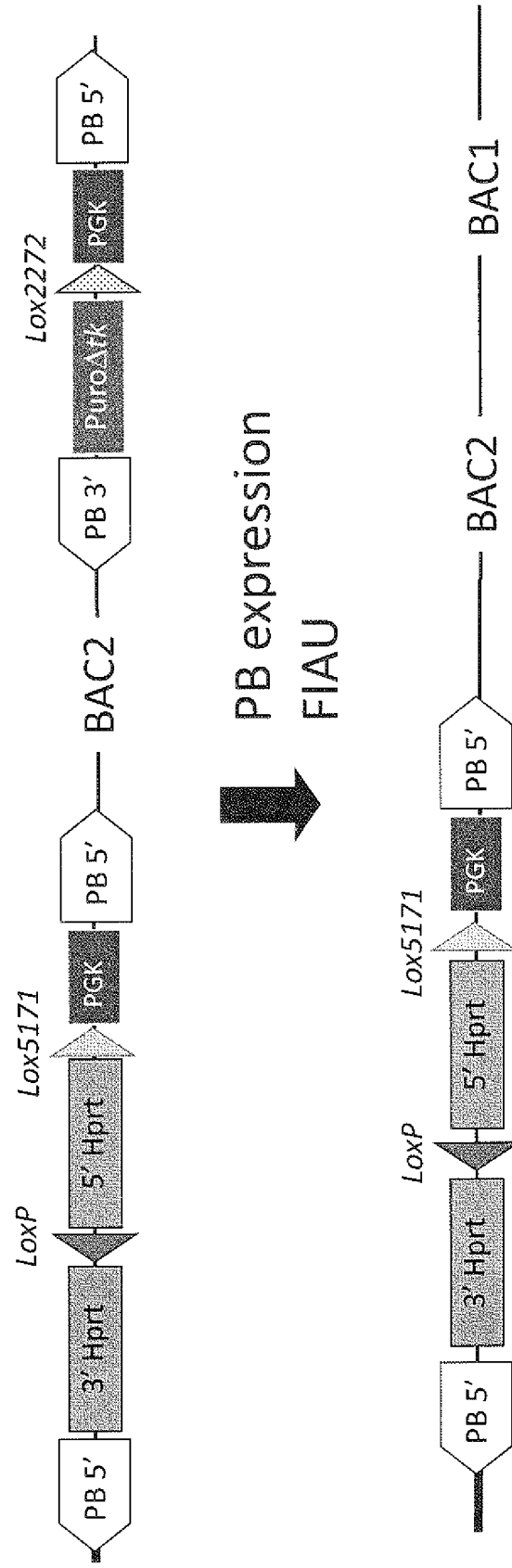


Fig. 26

Targeting the “Flip-Over” Vector

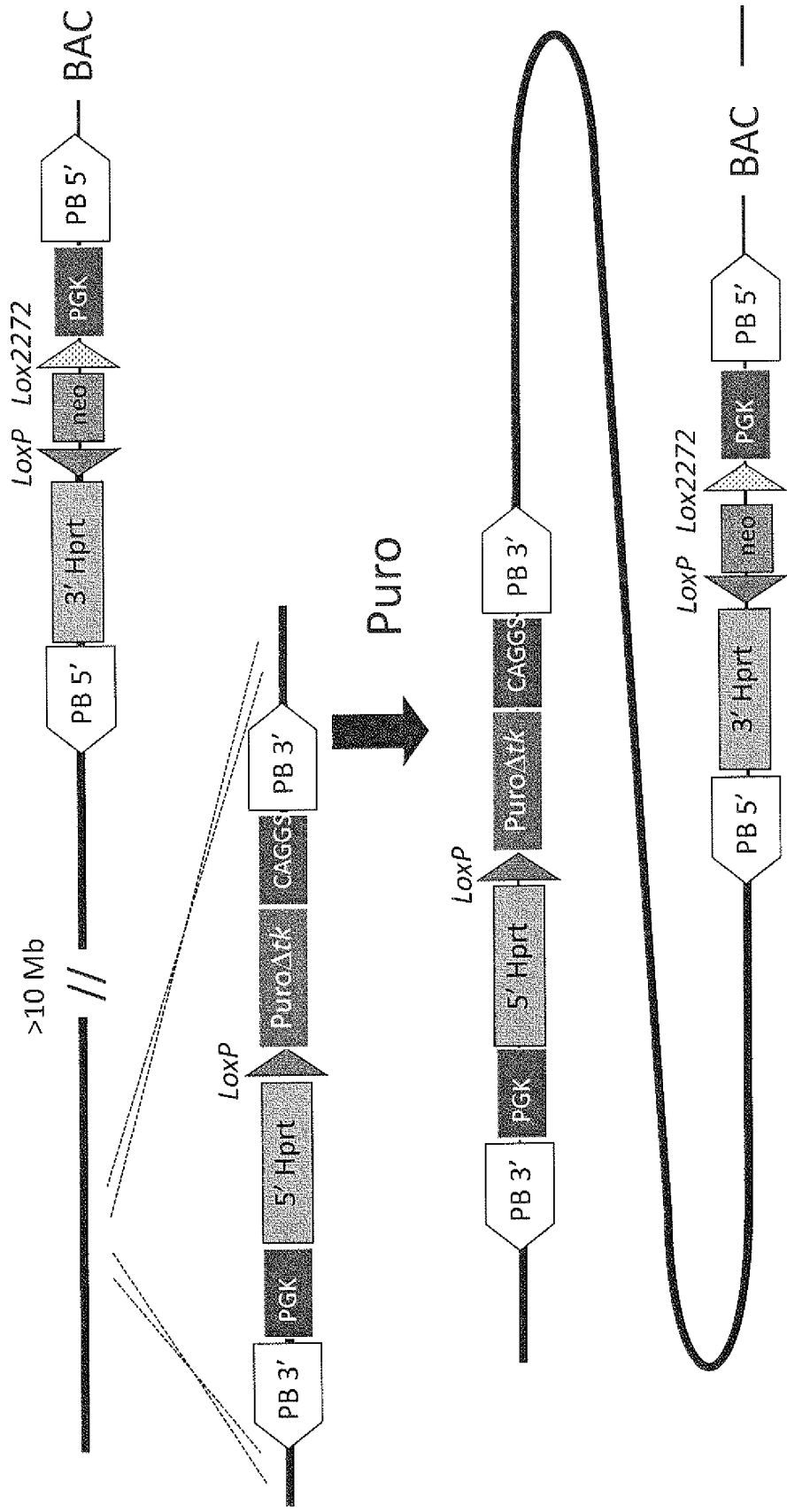


Fig. 27

Inversion

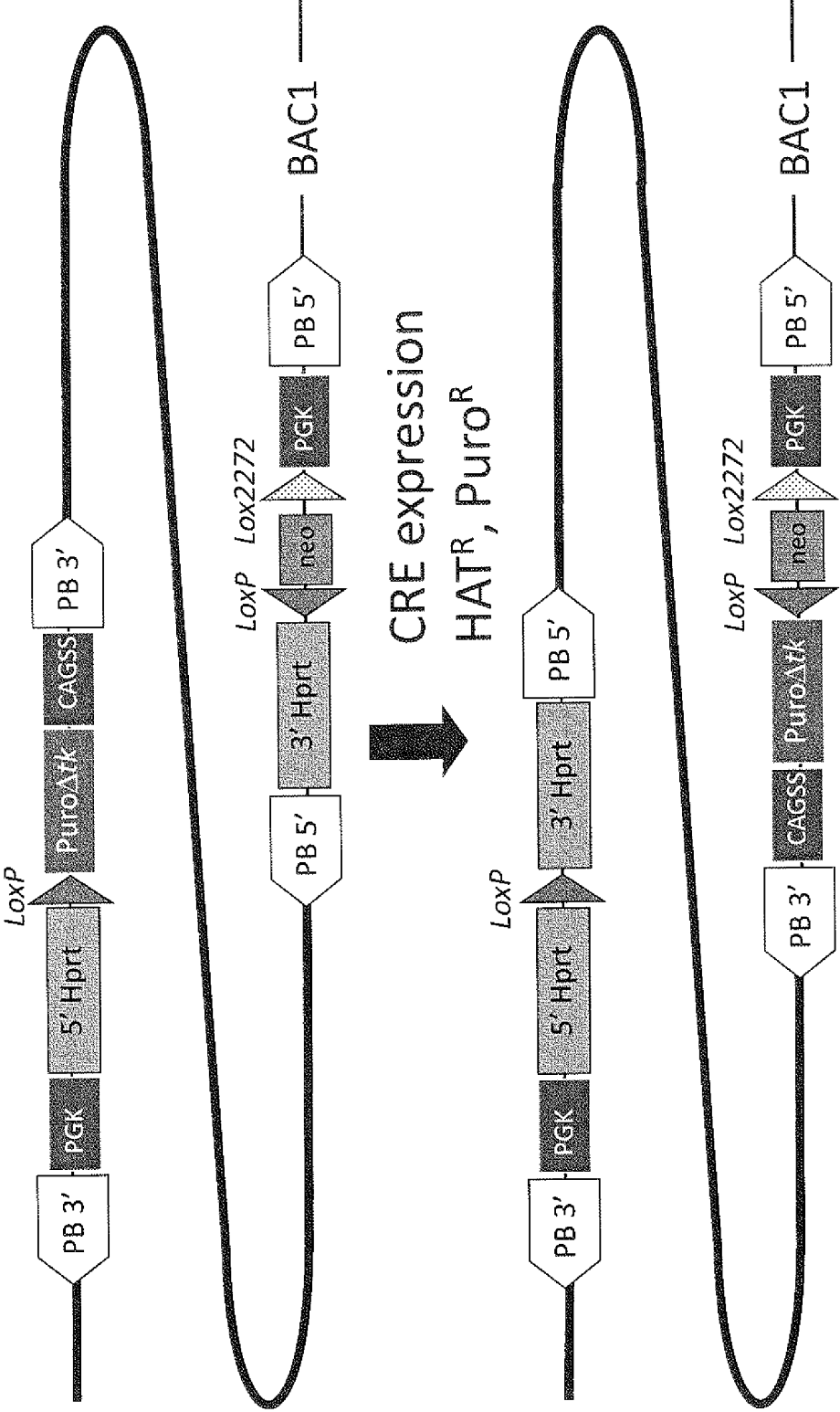


Fig. 28

Removing All Modifications

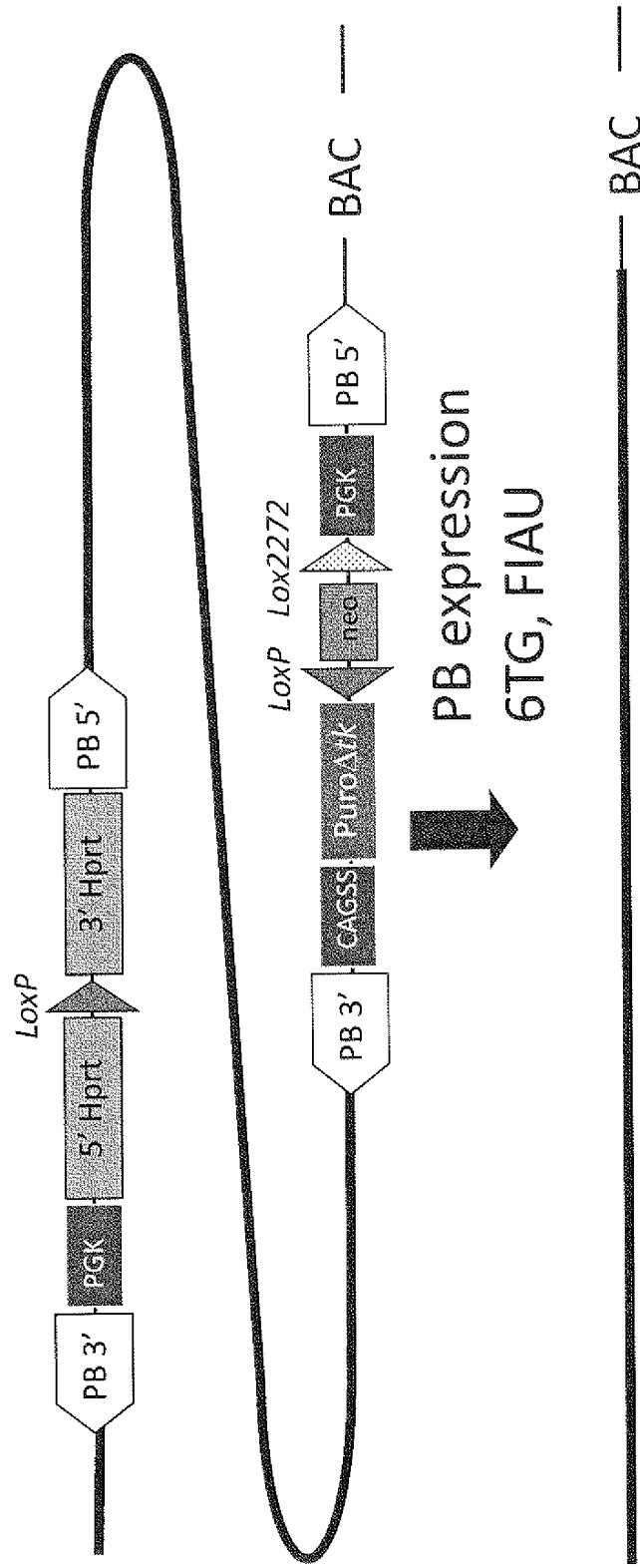


Fig. 29

Sequential RMCE – Integration into Landing Pad

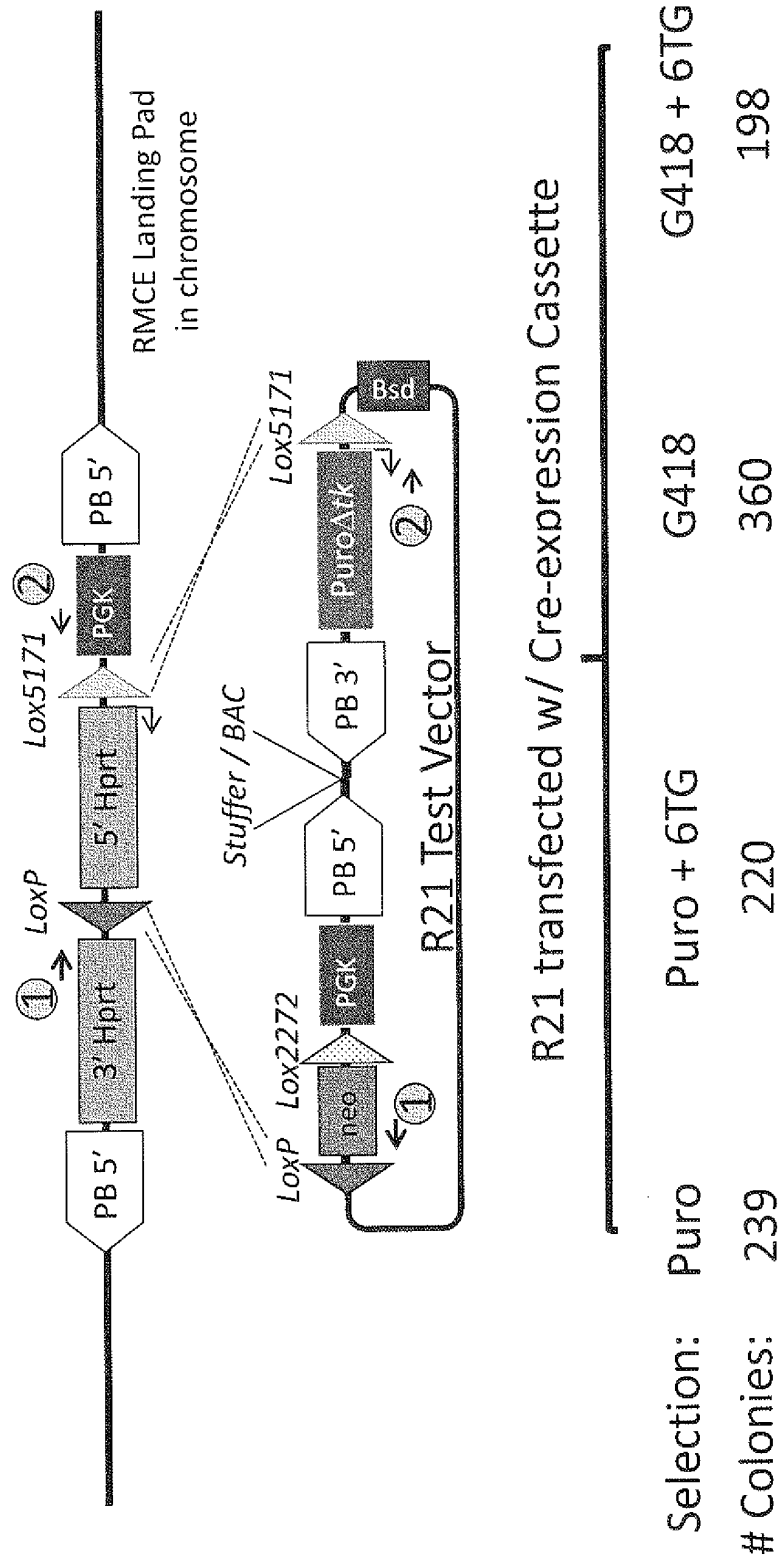
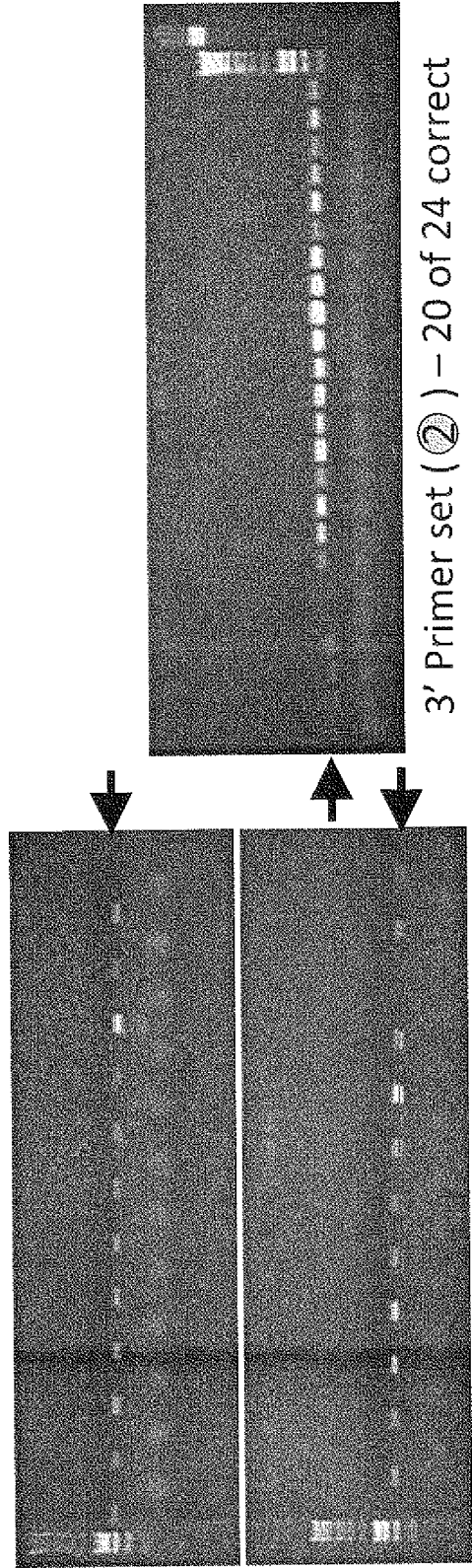


Fig. 30

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

PCR Confirmation of 3' End Curing

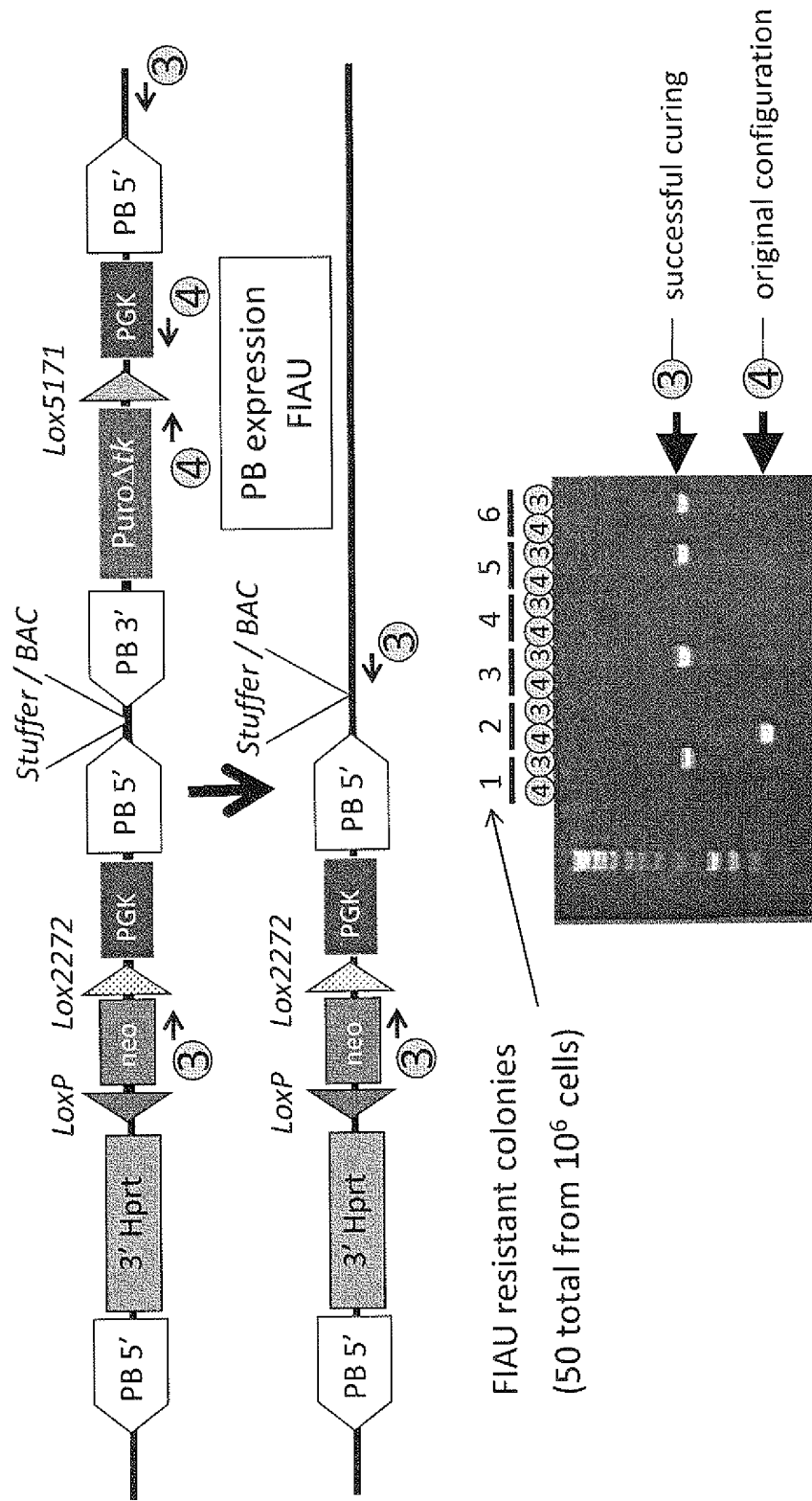


Fig. 32

Insertion of BAC#1 – PCR Diagnostics

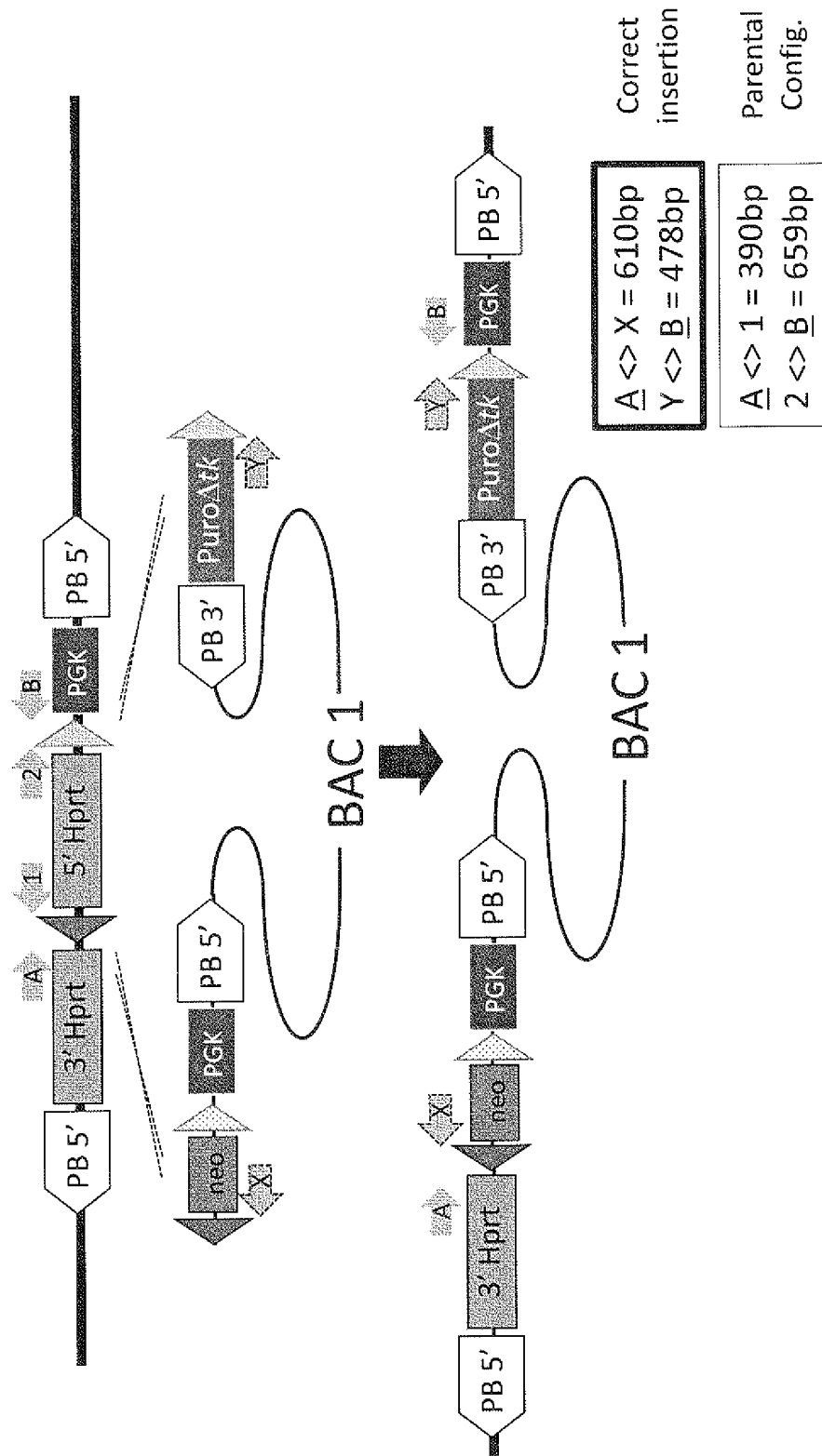
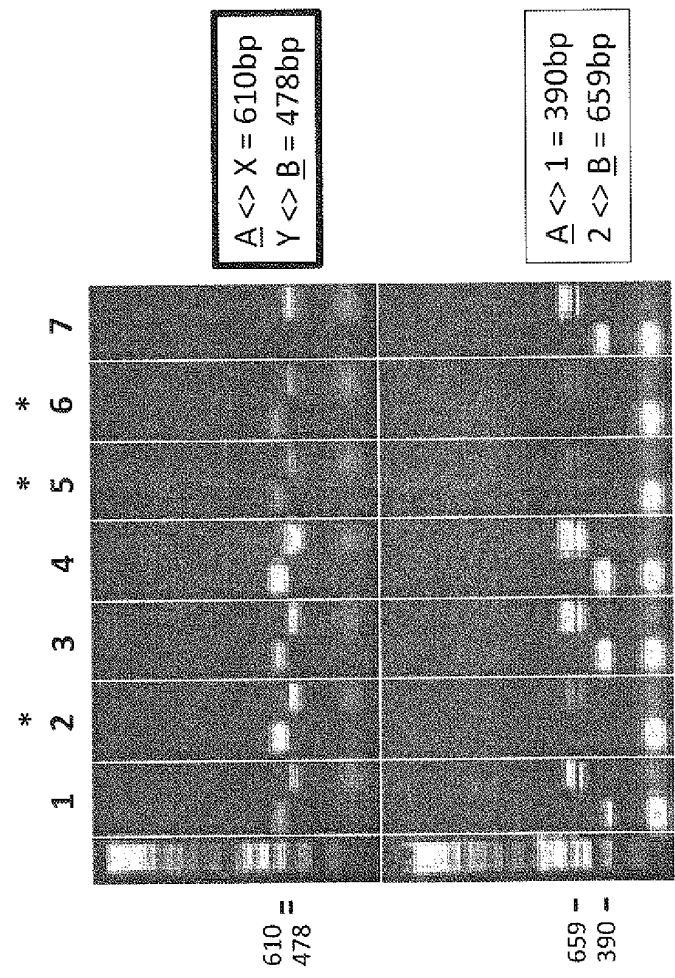


Fig. 33

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