

# Three basic vector backbones

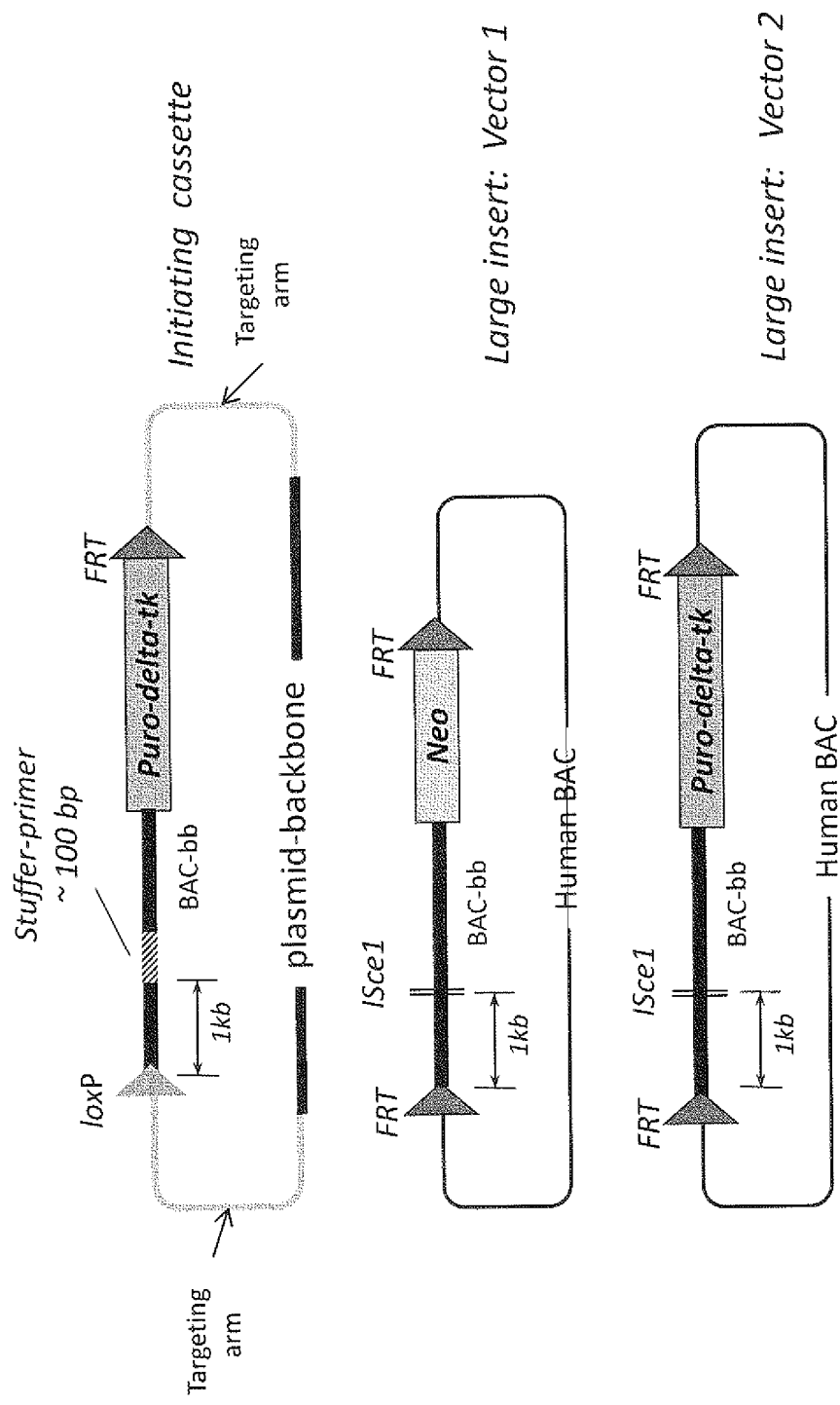
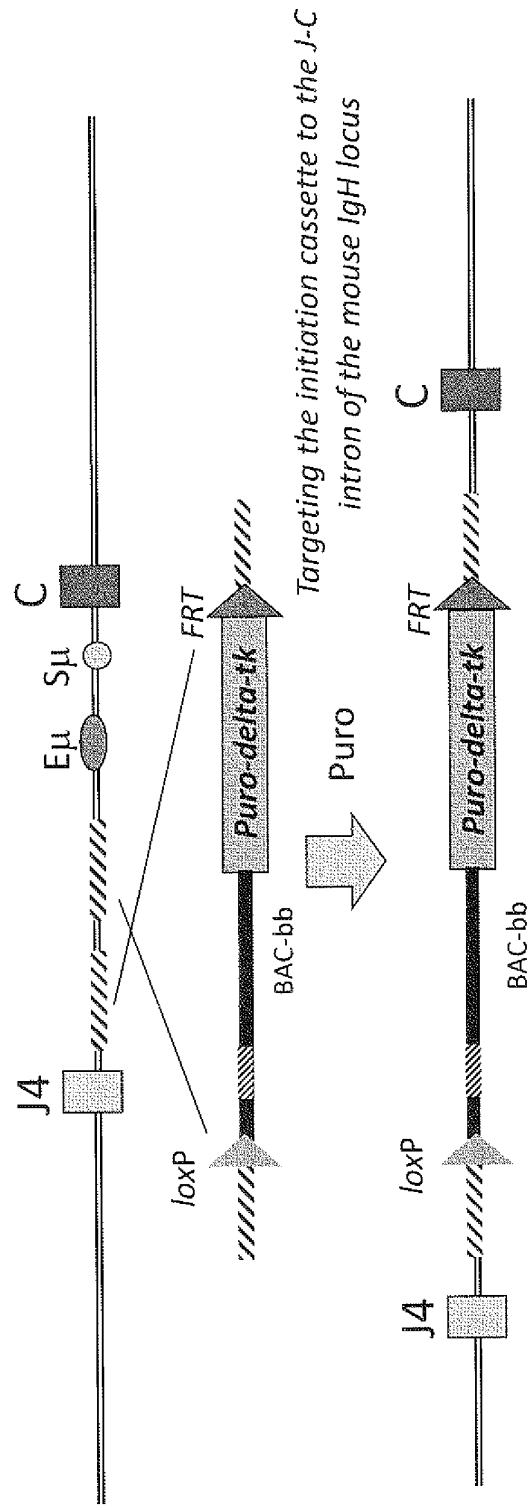


Fig. 1

# Entry / Initiation vector: Step 1

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## Validation:

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

Fig. 2

# Addition vector: Step 2

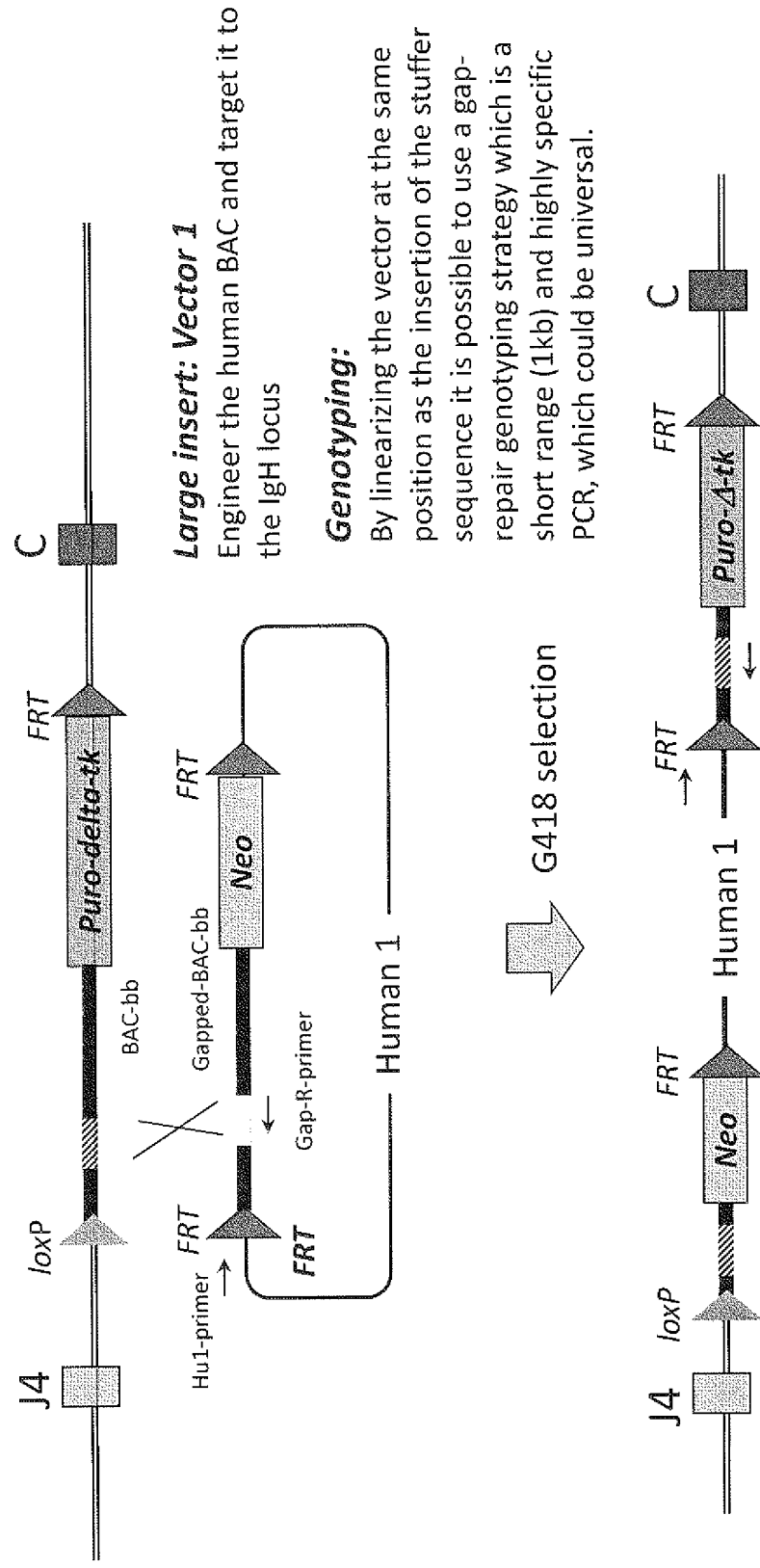


Fig. 3

# Addition vector: Step 2

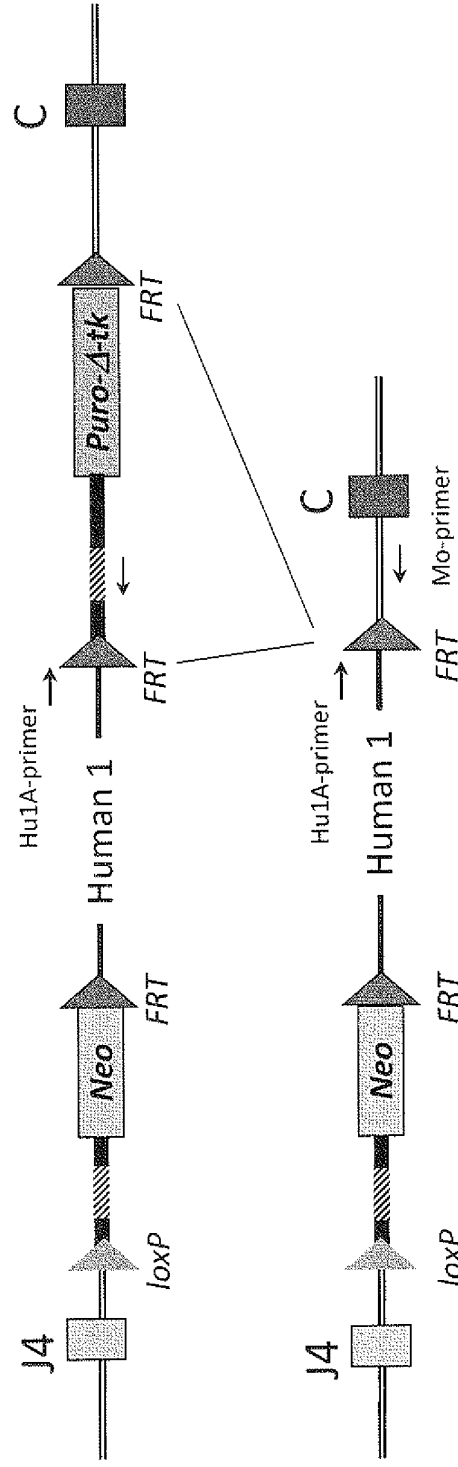


Fig. 4

# Addition vector: Step 3A

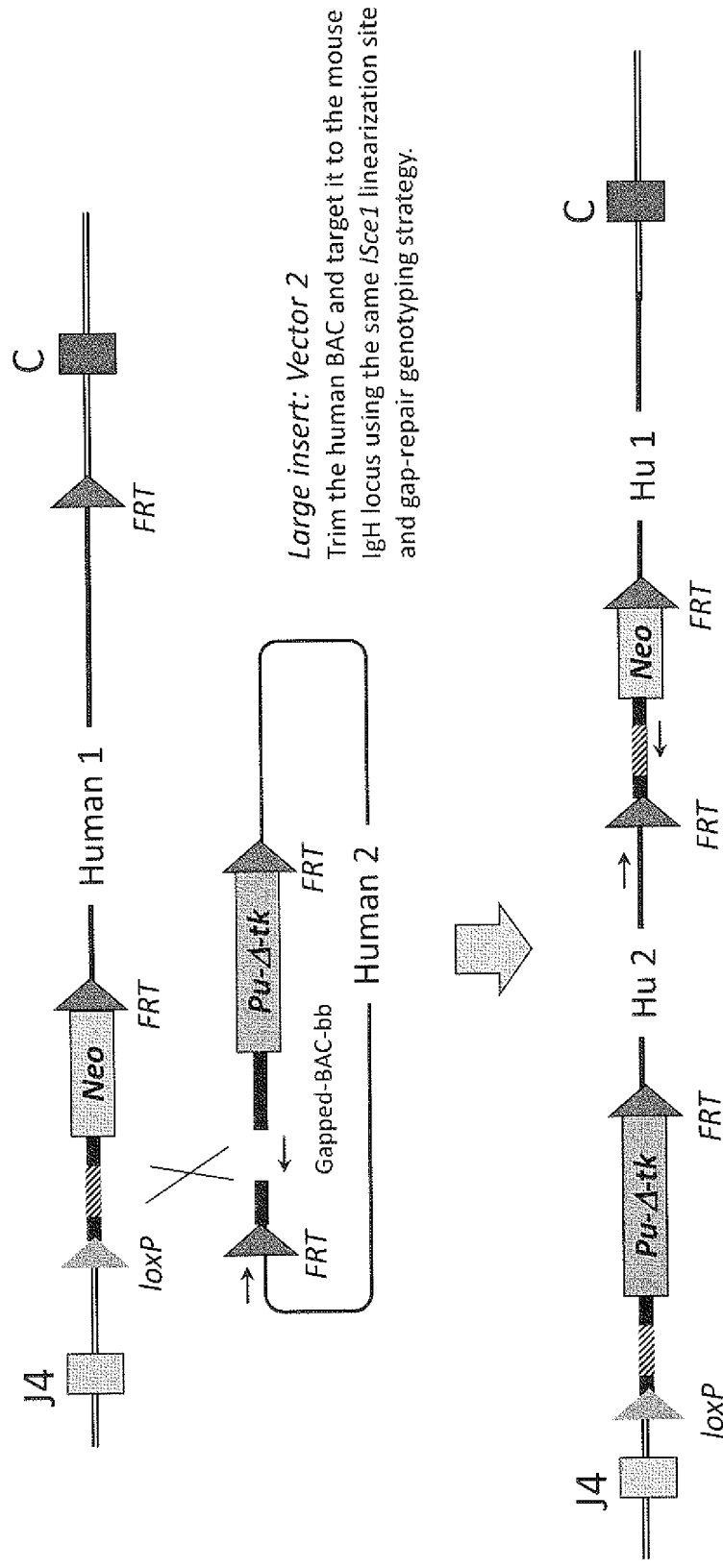


Fig. 5

# Addition vector: Step 3B

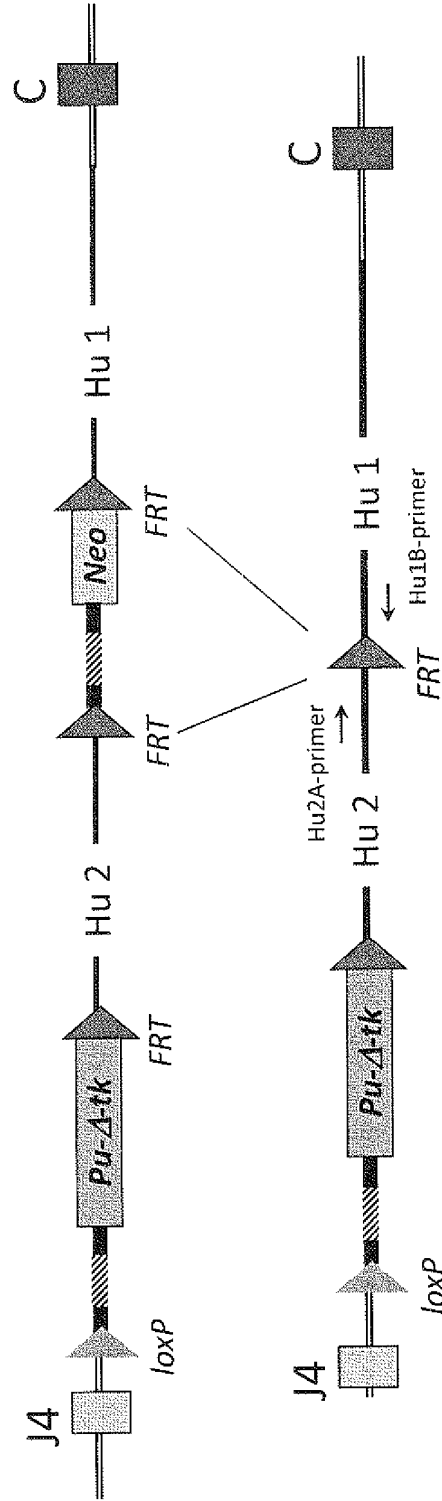


Fig. 6

# Addition vector: Step 4

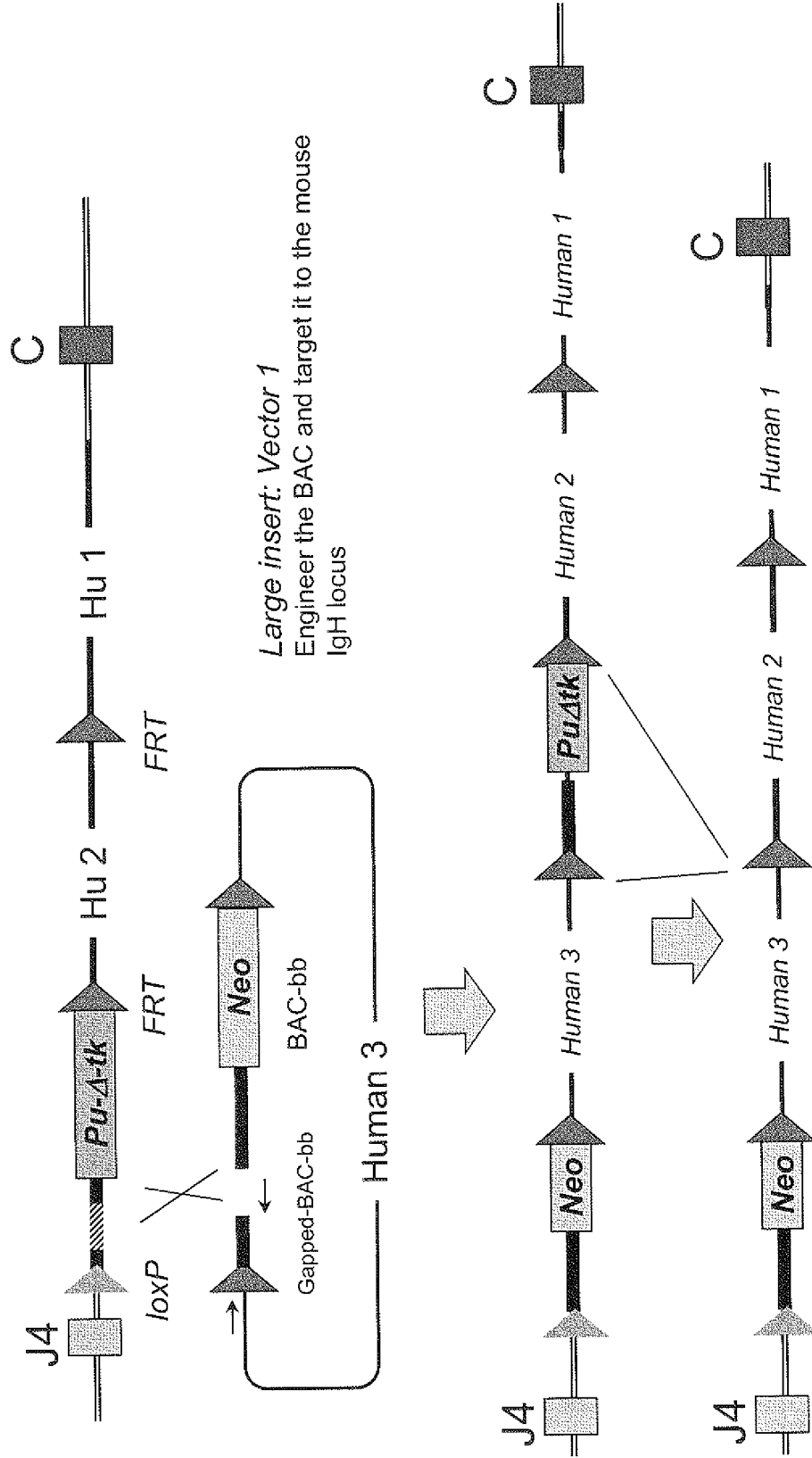


Fig. 7

# A humanized mouse *IgH* locus

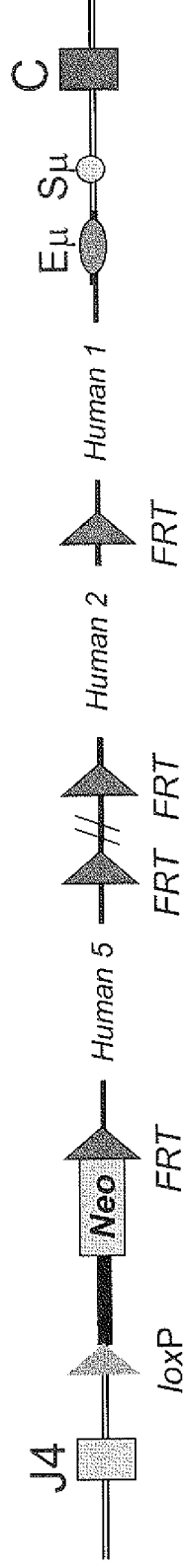


Fig. 8

# BAC landing pad inserted at the IgH locus

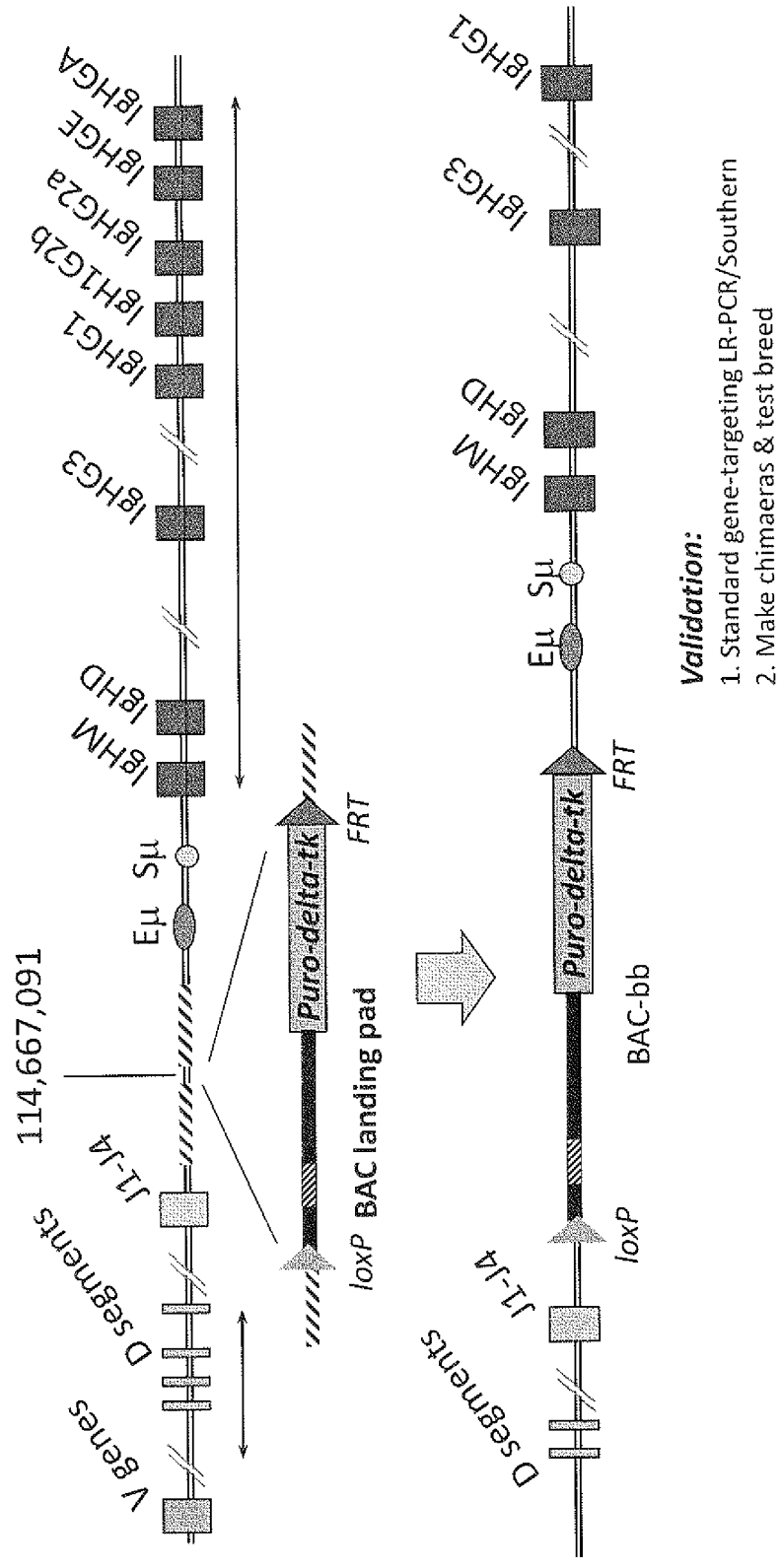


Fig. 9

# 1st BAC insertion – Step 1

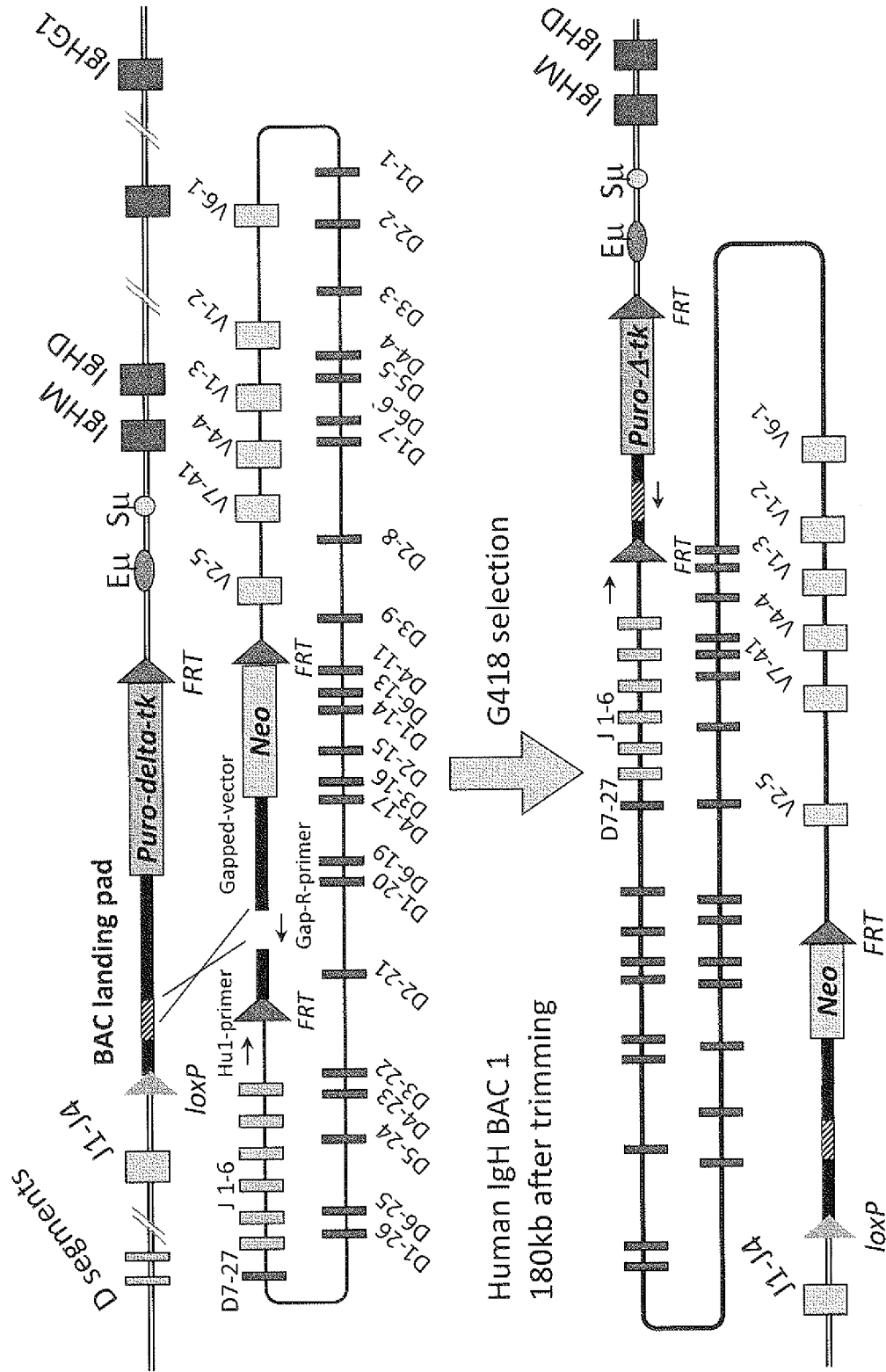


Fig. 10

# 1st BAC insertion – Step 2

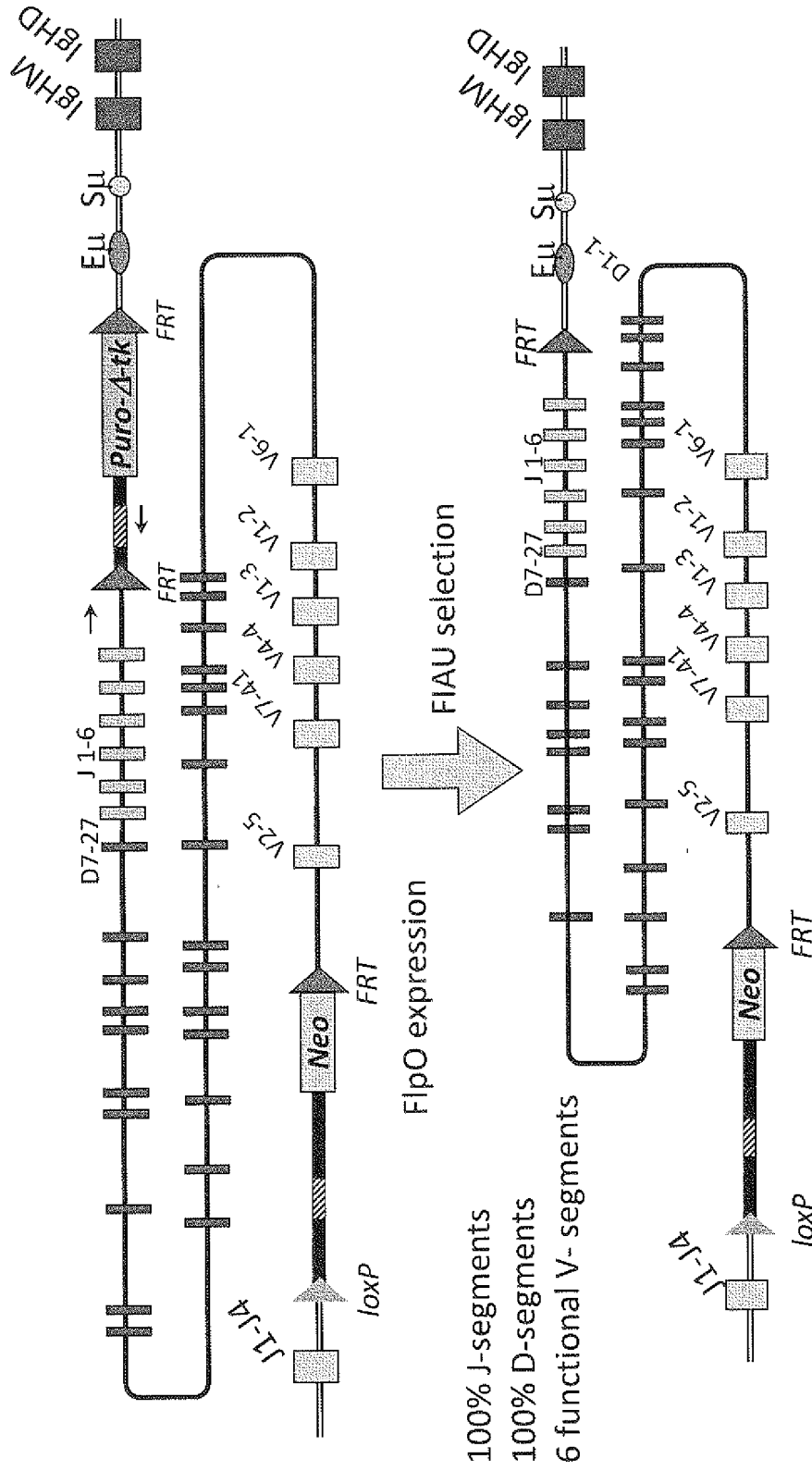


Fig. 11

## 2nd BAC insertion – Step 3

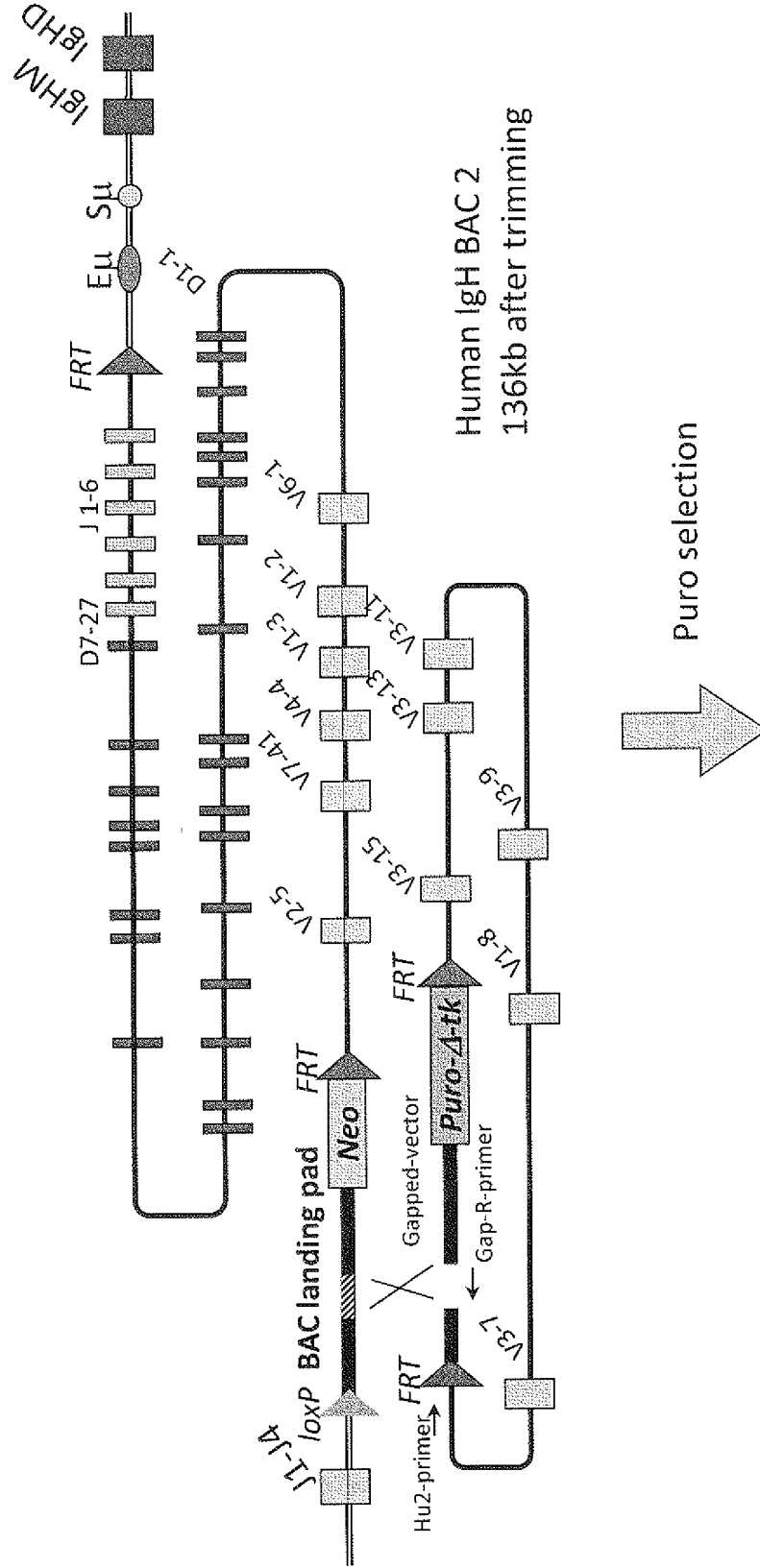
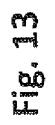


Fig. 12



# BAC Landing Pad at IgK Locus

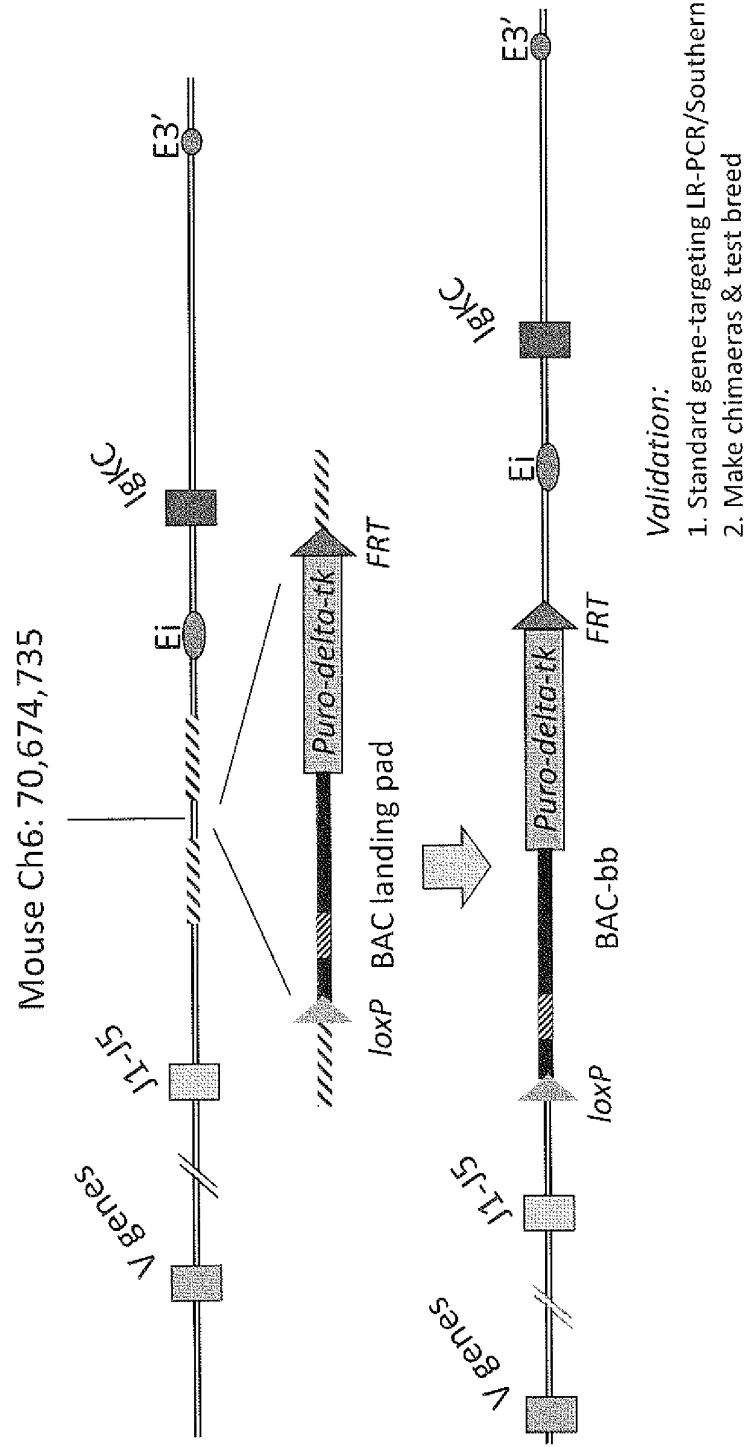


Fig. 14

# 1st BAC Insertion – Step 1

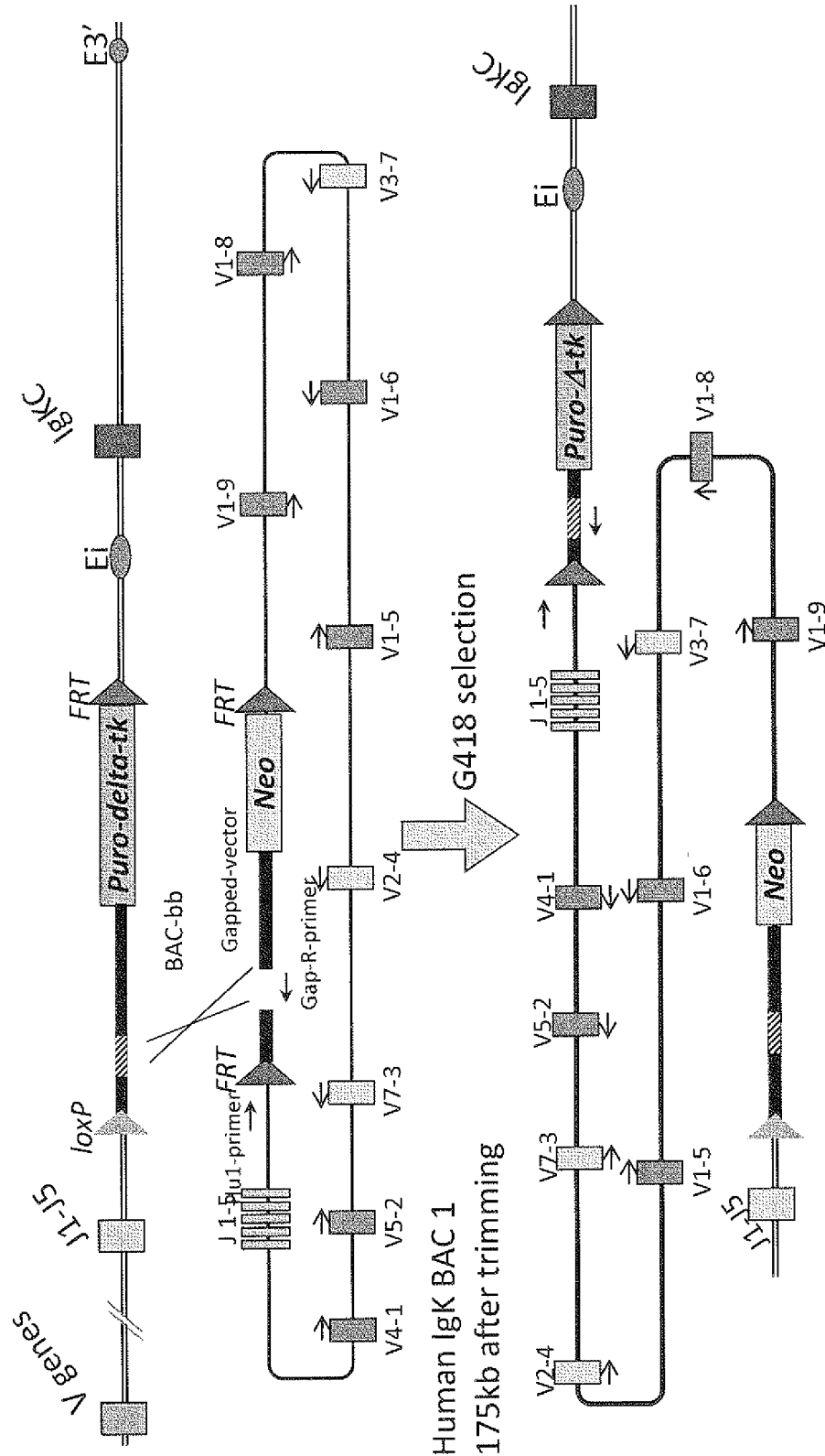


Fig. 15

# 1<sup>st</sup> BAC Insertion – Step 2

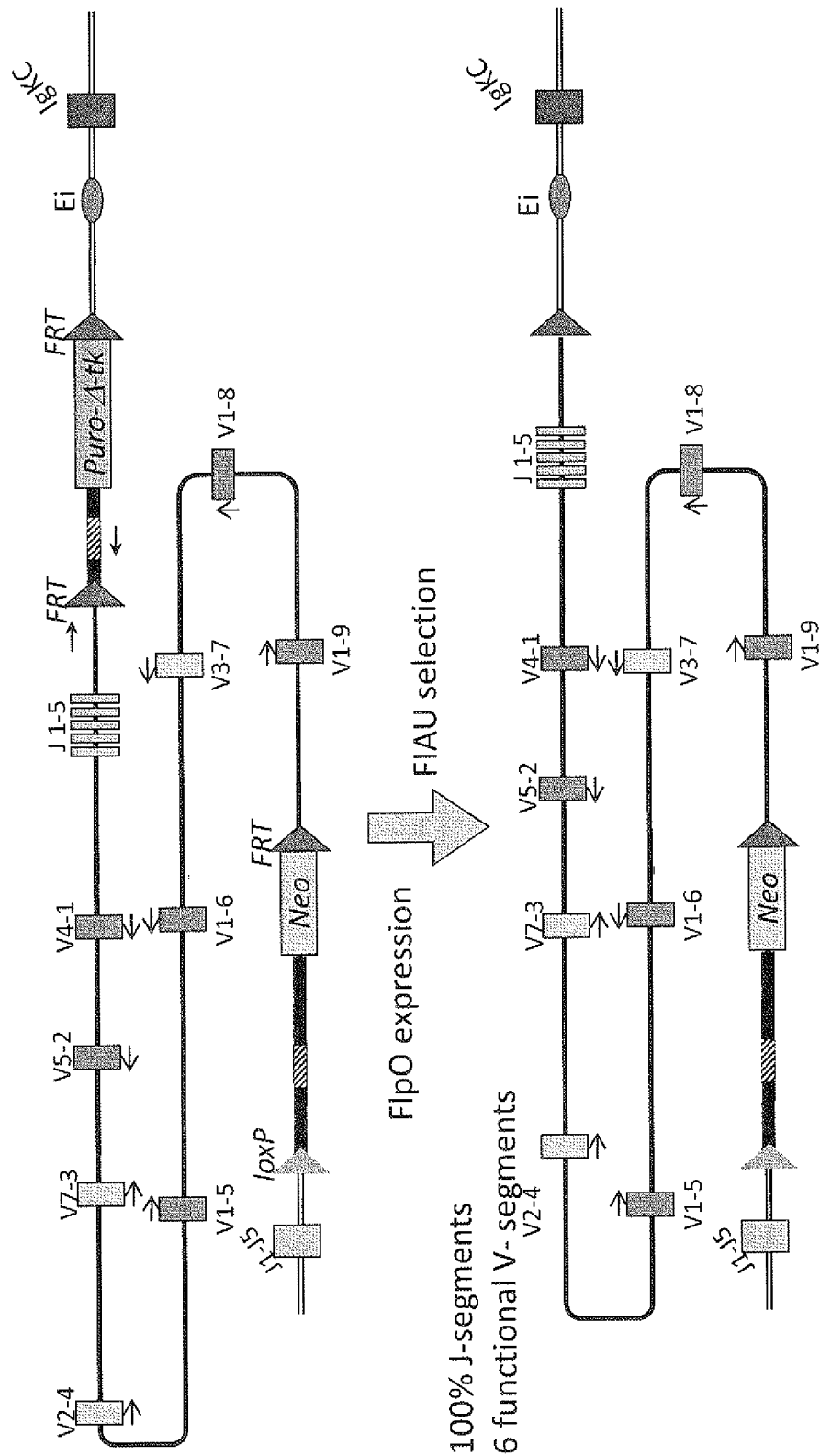


Fig. 16

## 2nd BAC Insertion – Step 3

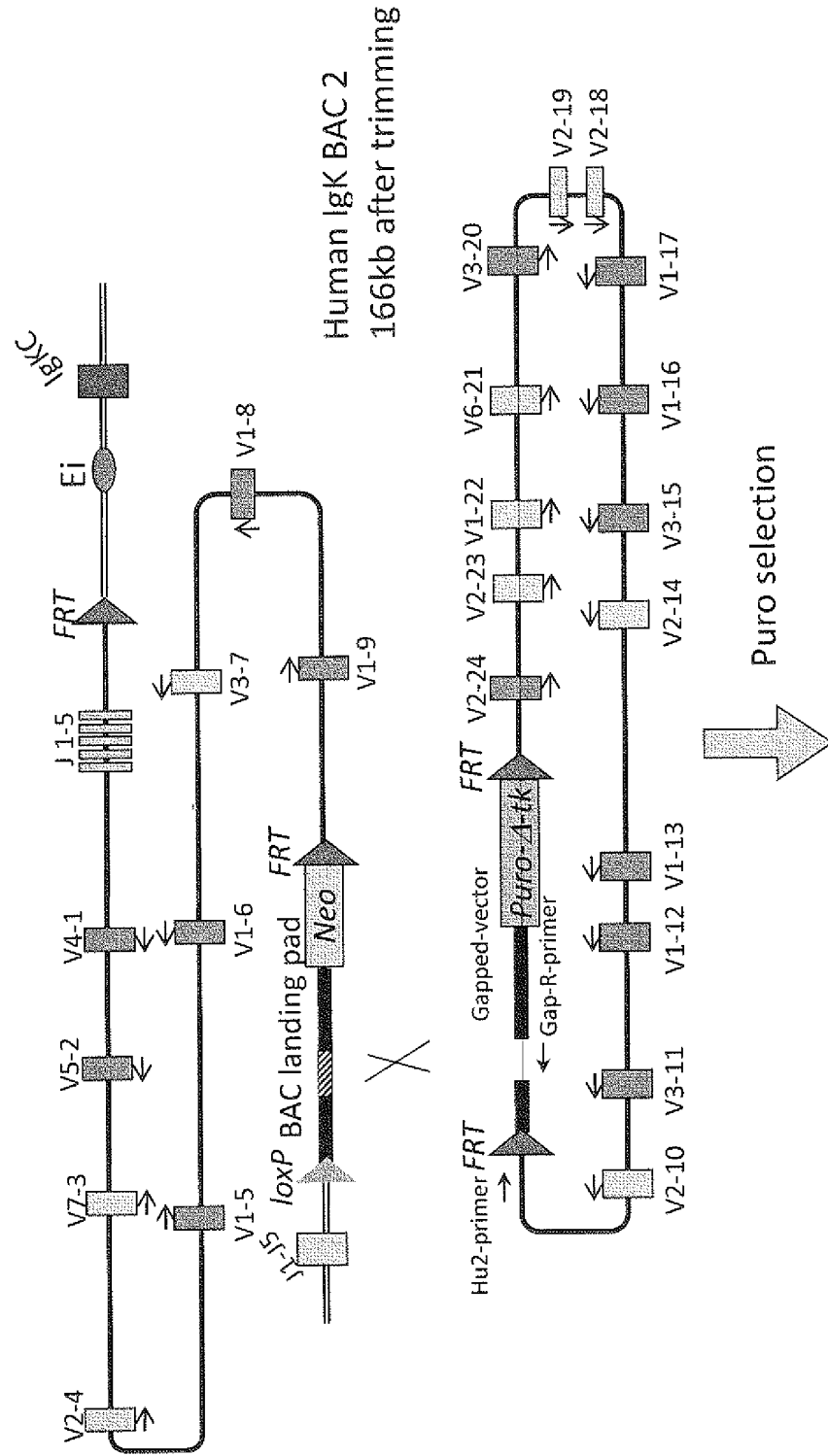


Fig. 17

## 2nd BAC insertion – Step 4

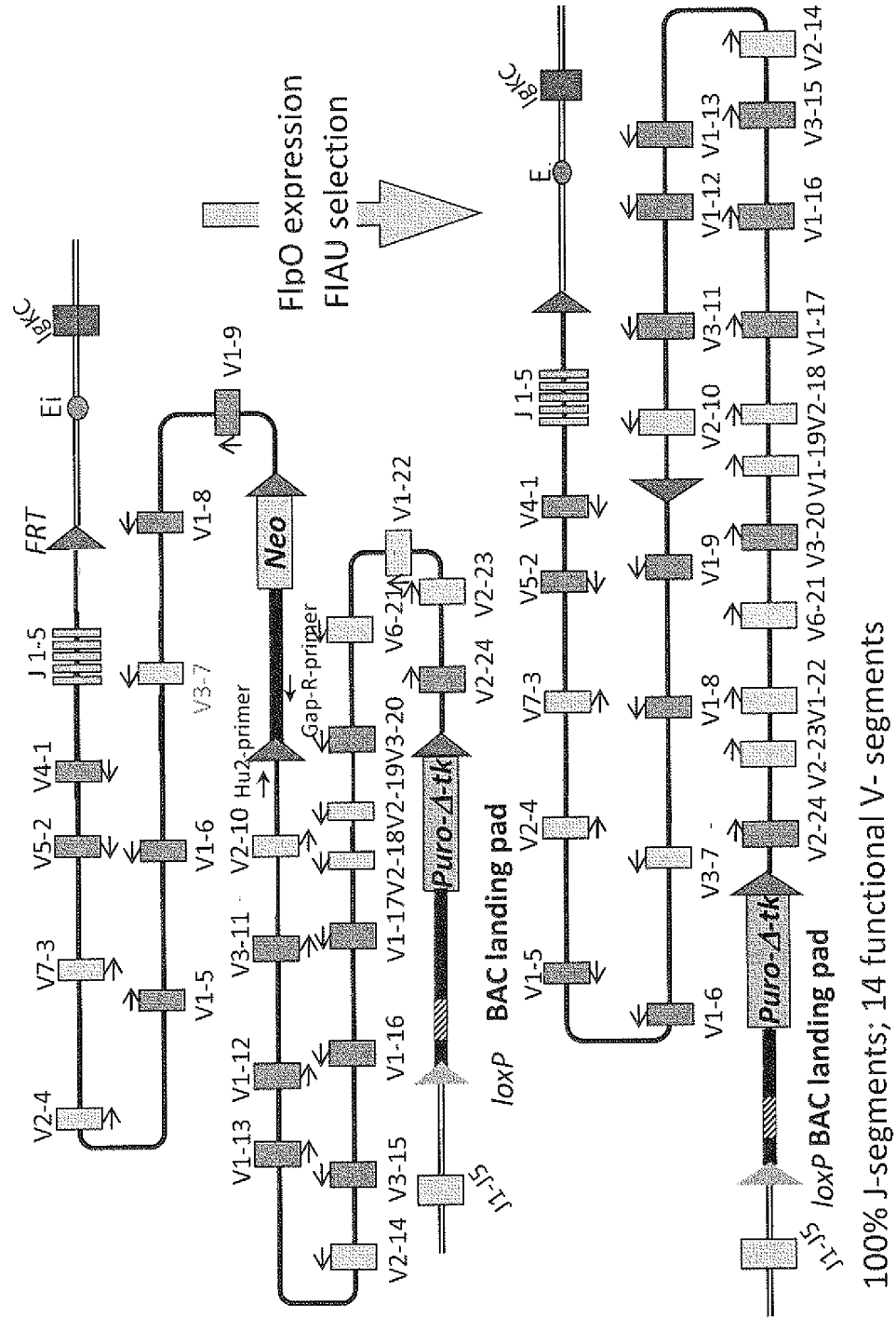


Fig. 18

# Rapid Testing of ES Cells with Humanized IgH and IgK Loci

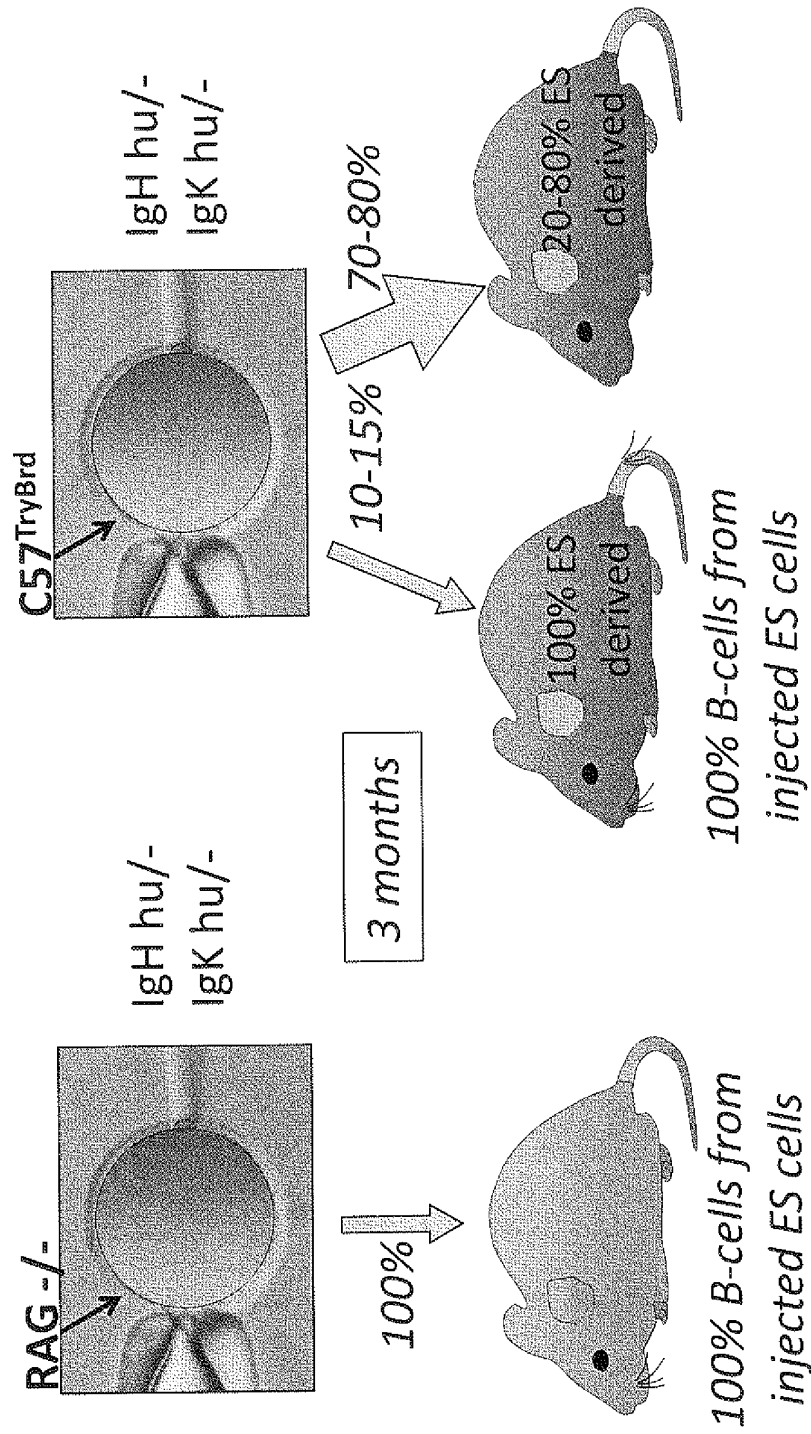


Fig. 19

# Antibody Generation in Chimaeric Mice with Humanized IgH and IgK Loci

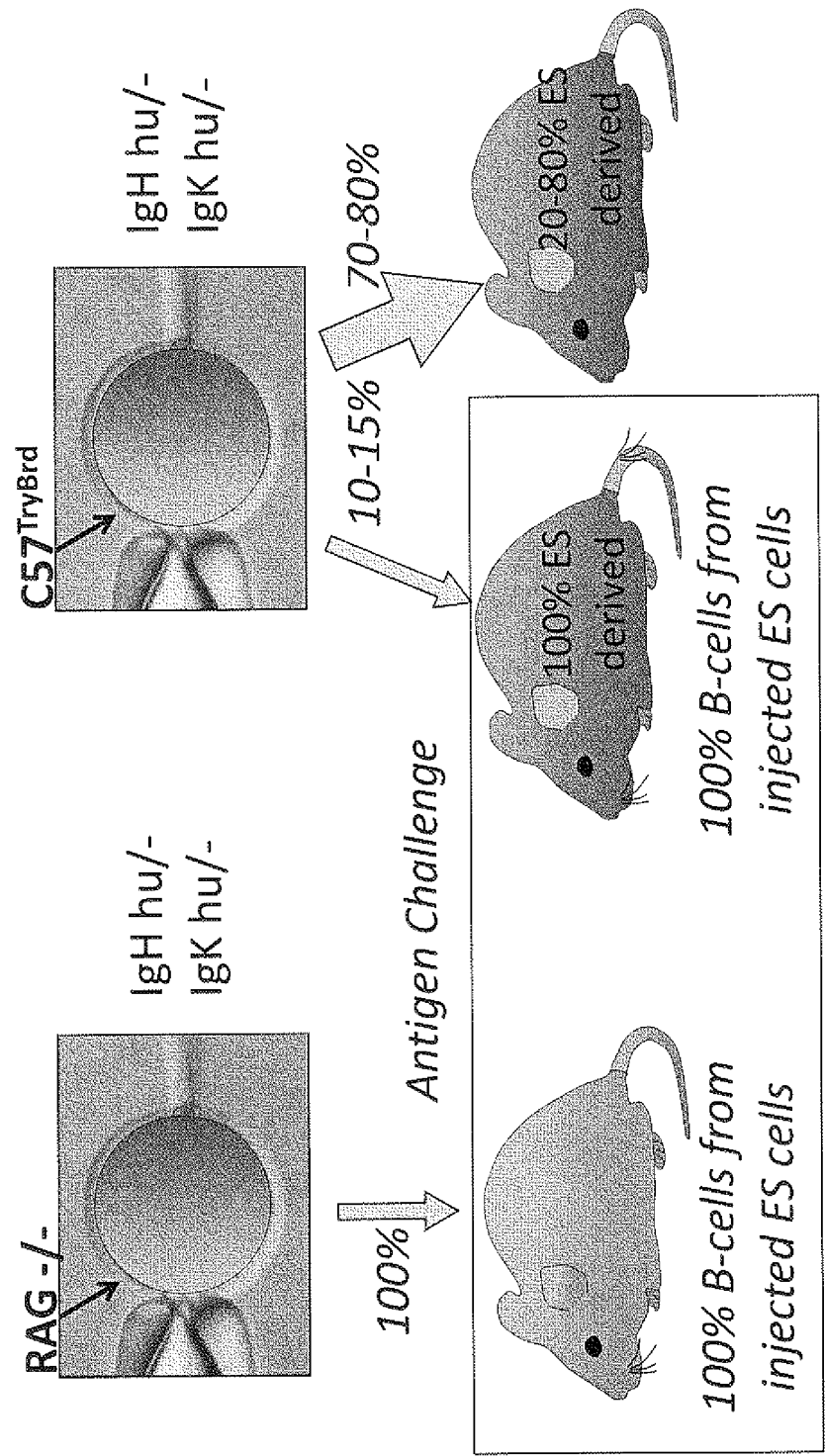


Fig. 20

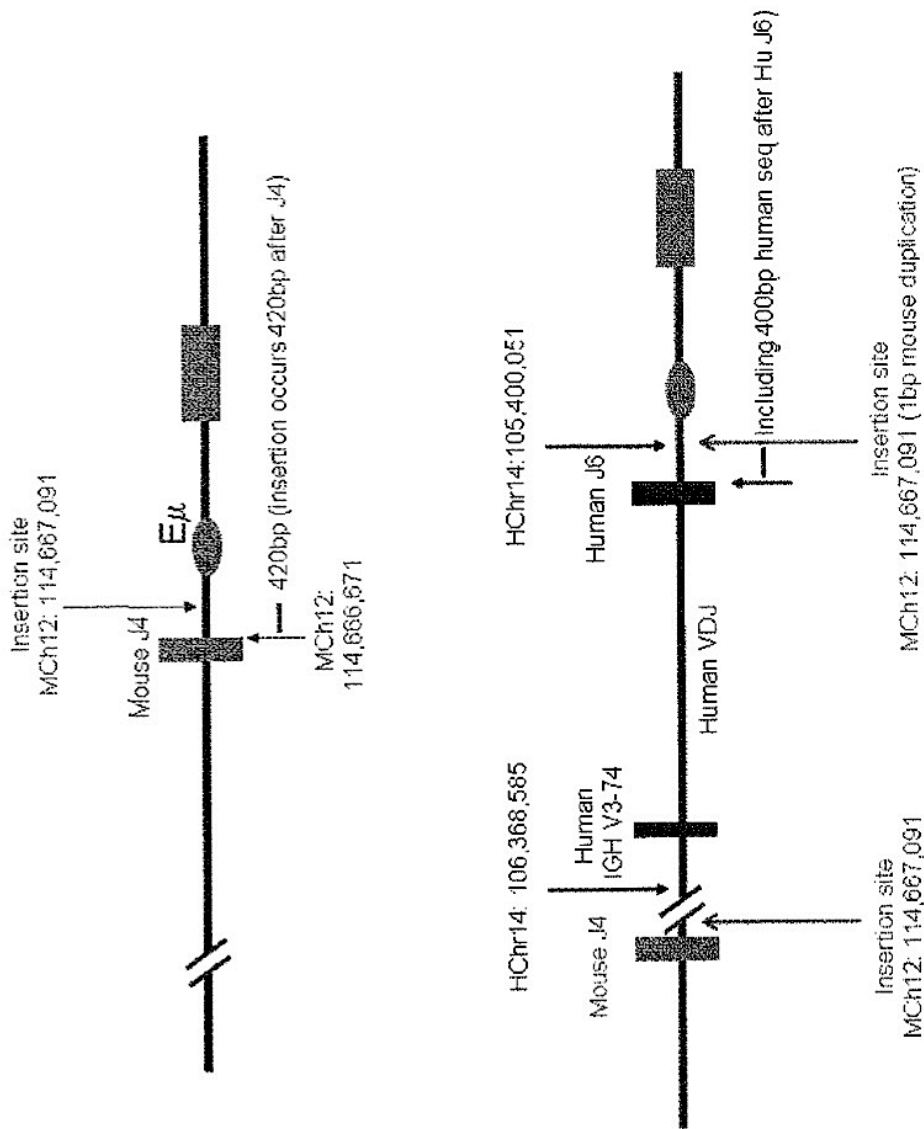


Fig. 21

# Targeting of the Landing Pad

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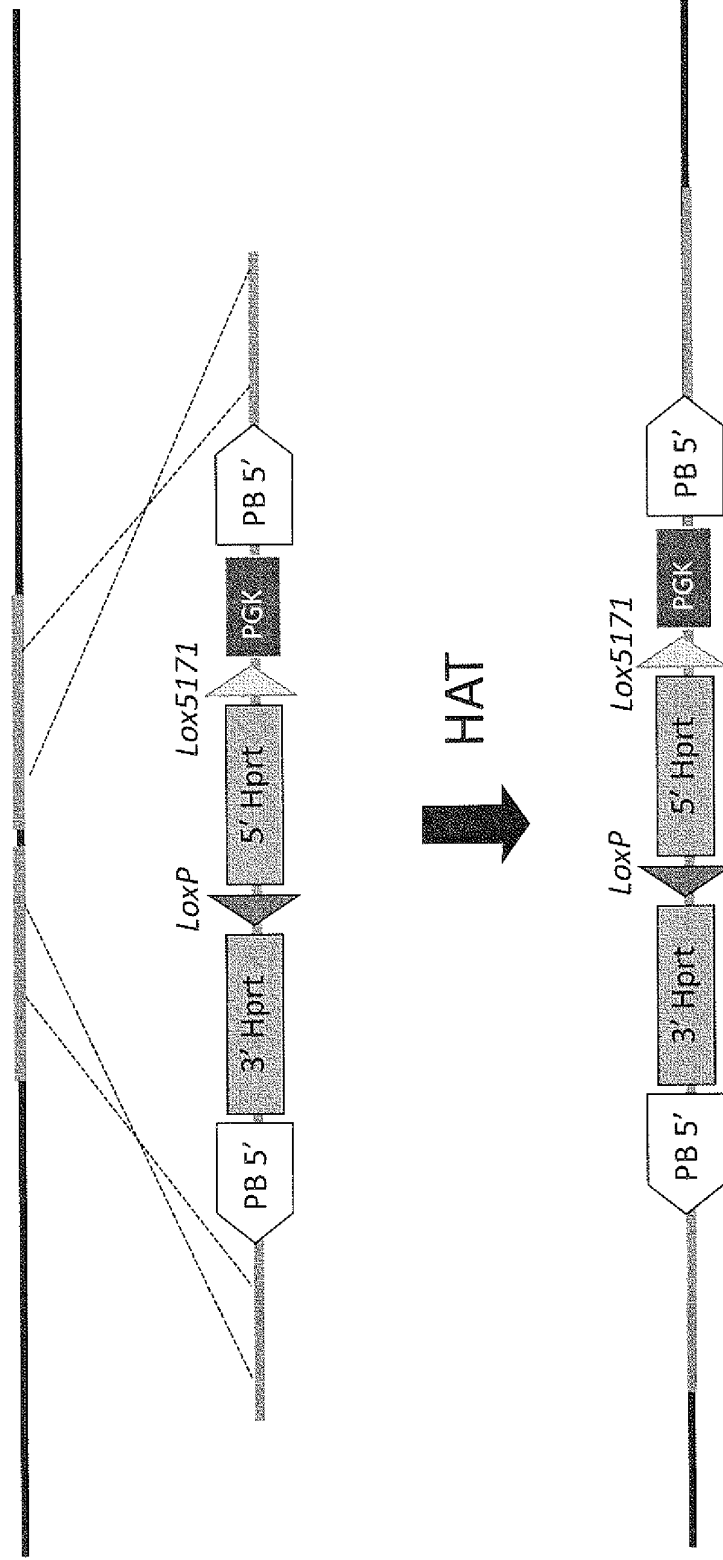


Fig. 22

# Insertion of the 1<sup>st</sup> BAC

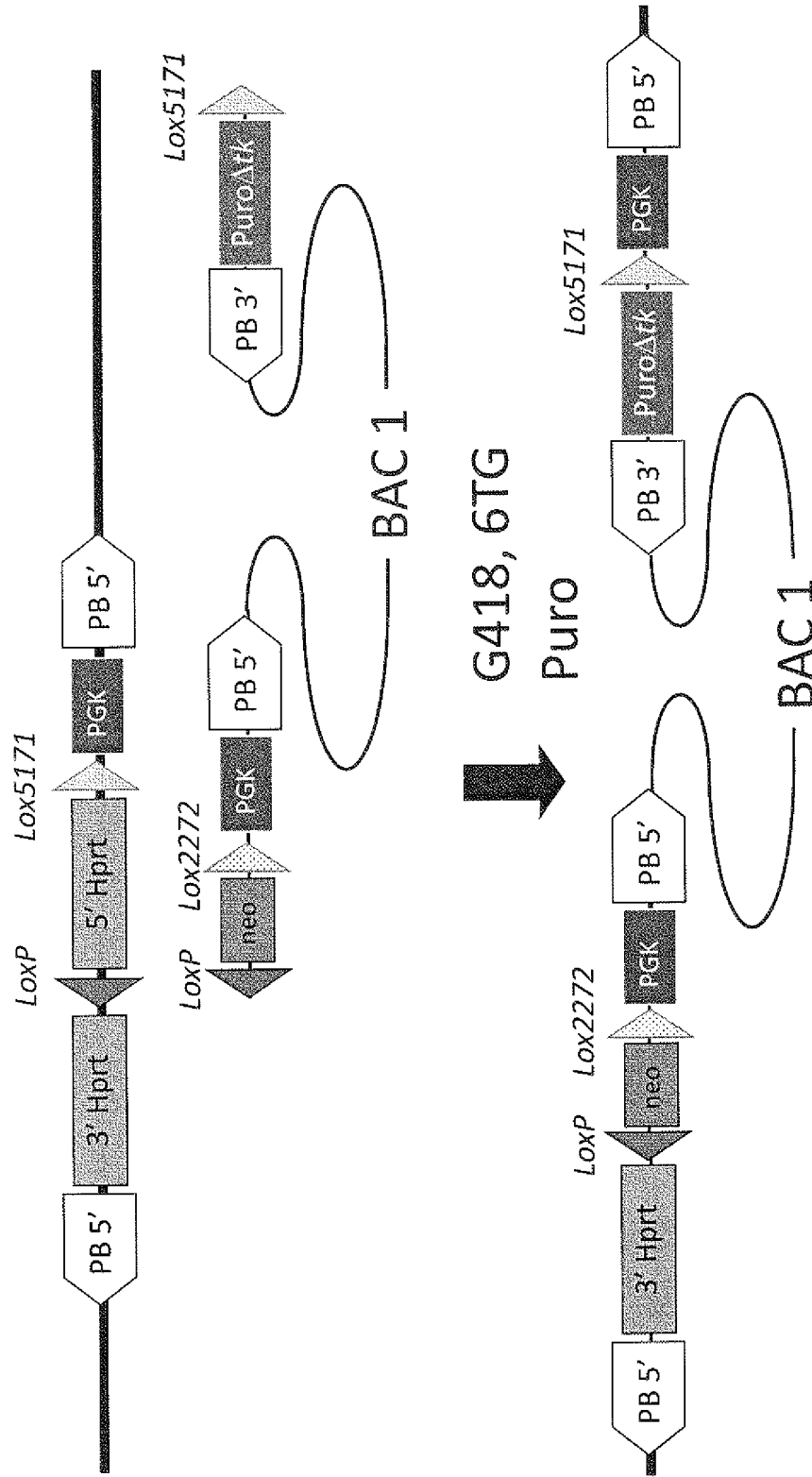


Fig. 23

# Curing of the 3' Modification

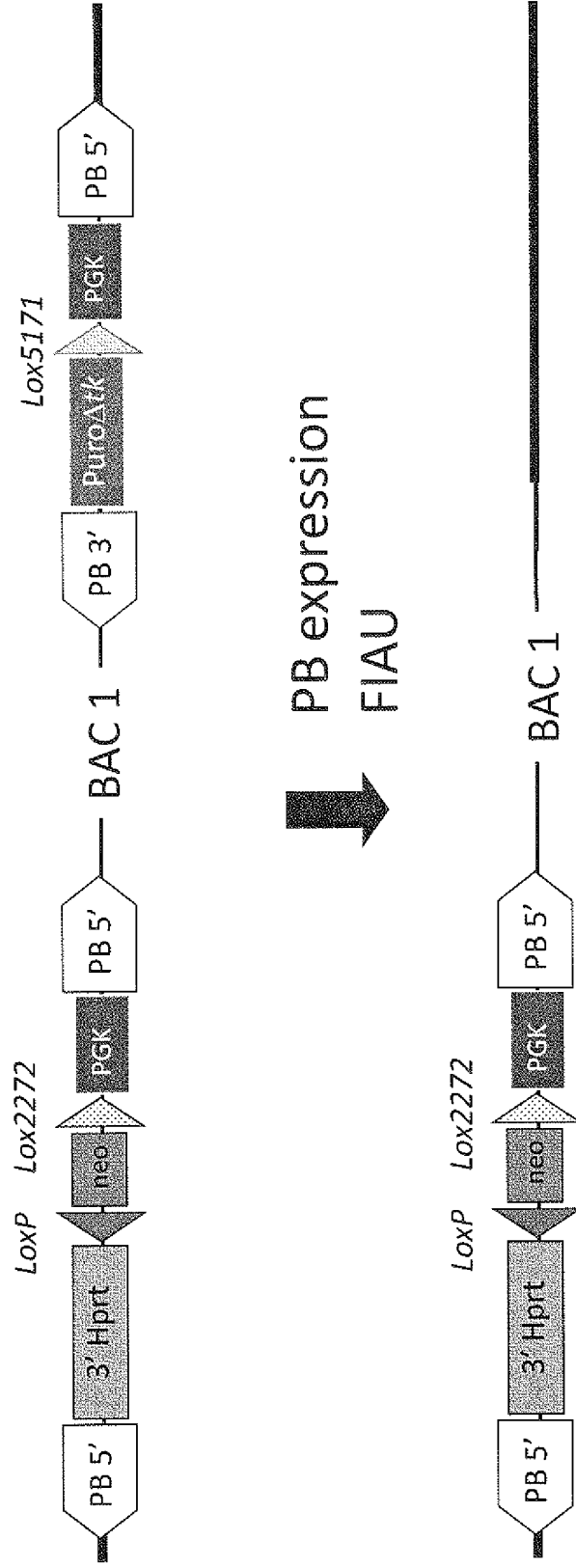


Fig. 24

# Insertion of the 2<sup>nd</sup> BAC

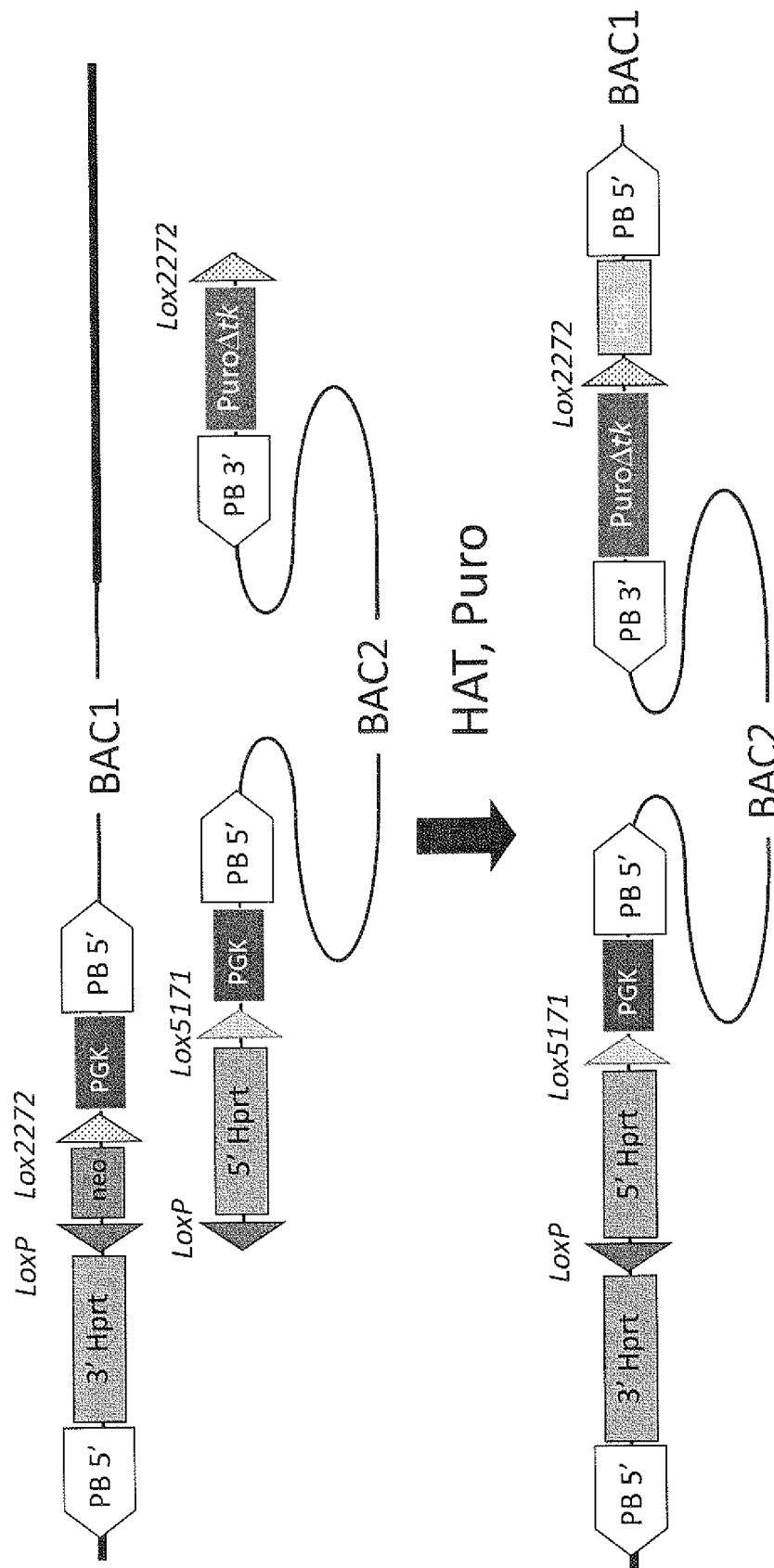


Fig. 25

# Removing the 3' Modification

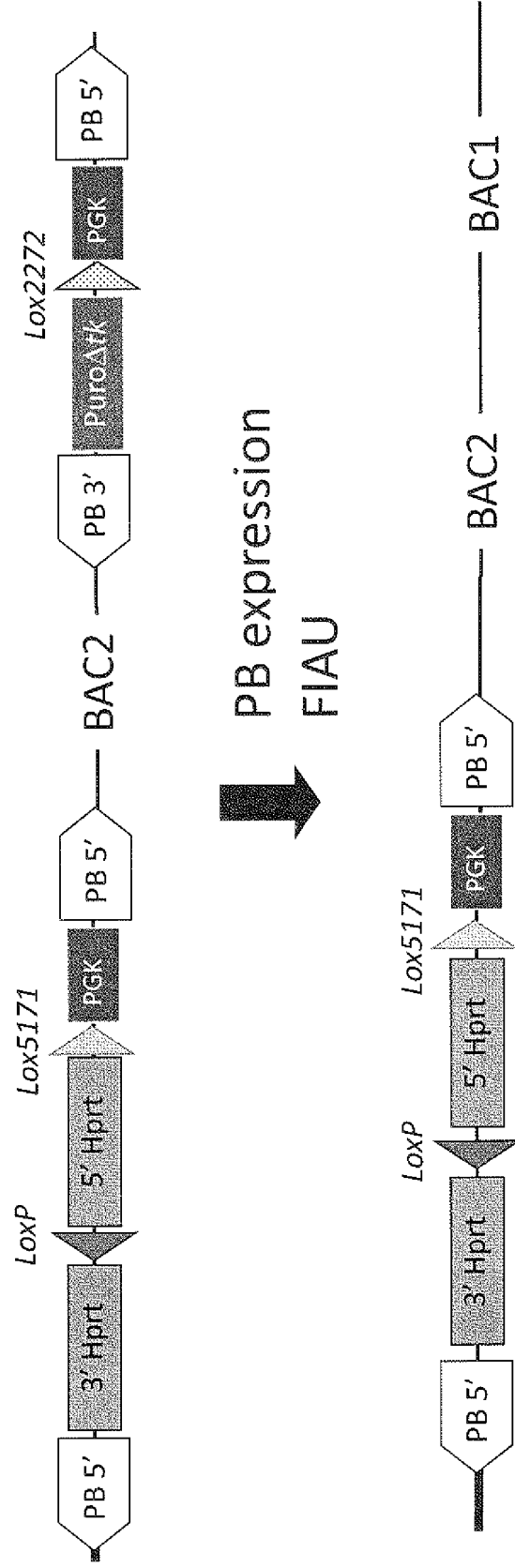


Fig. 26

# Targeting the “Flip-Over” Vector

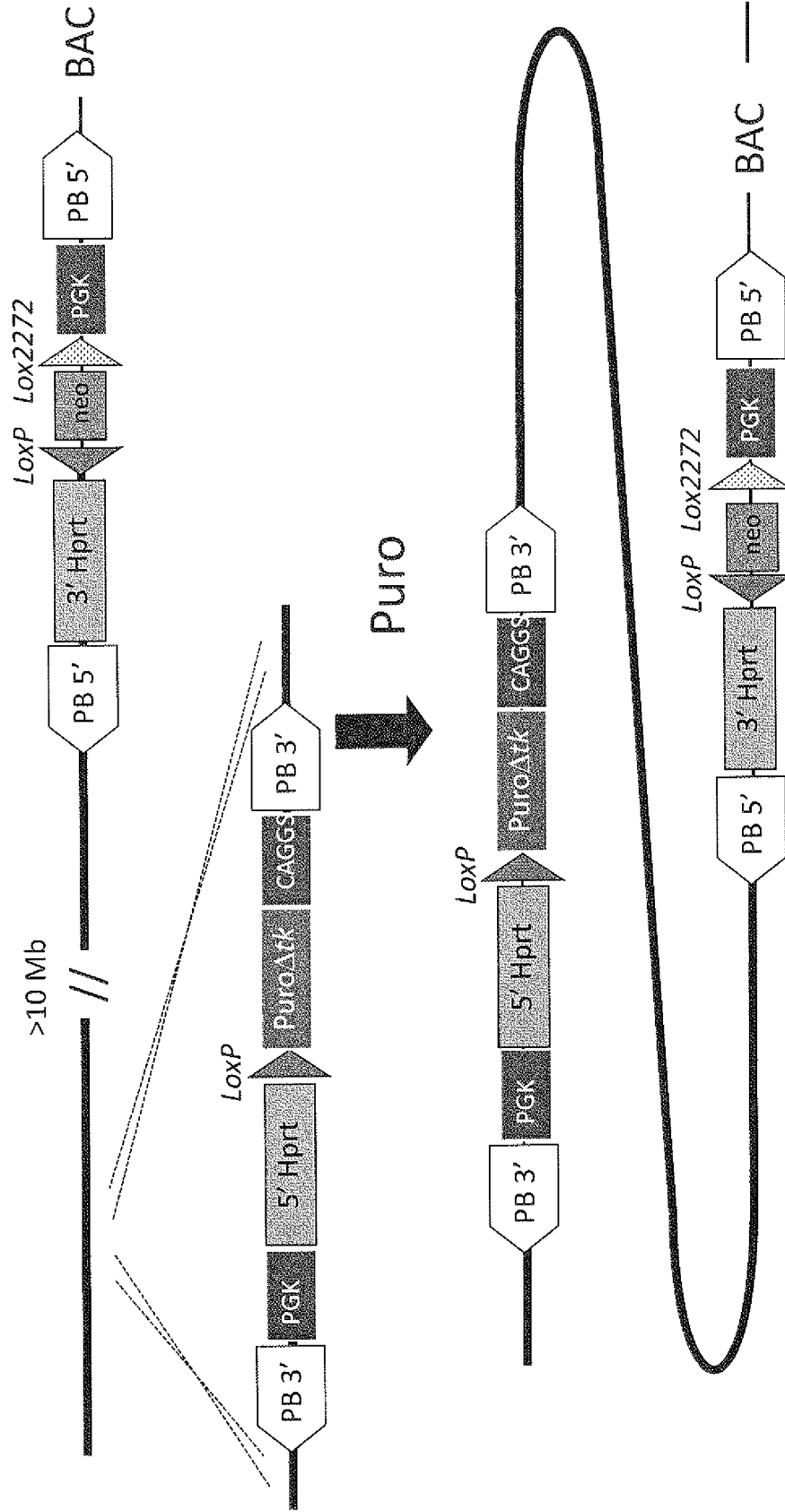


Fig. 27

# Inversion

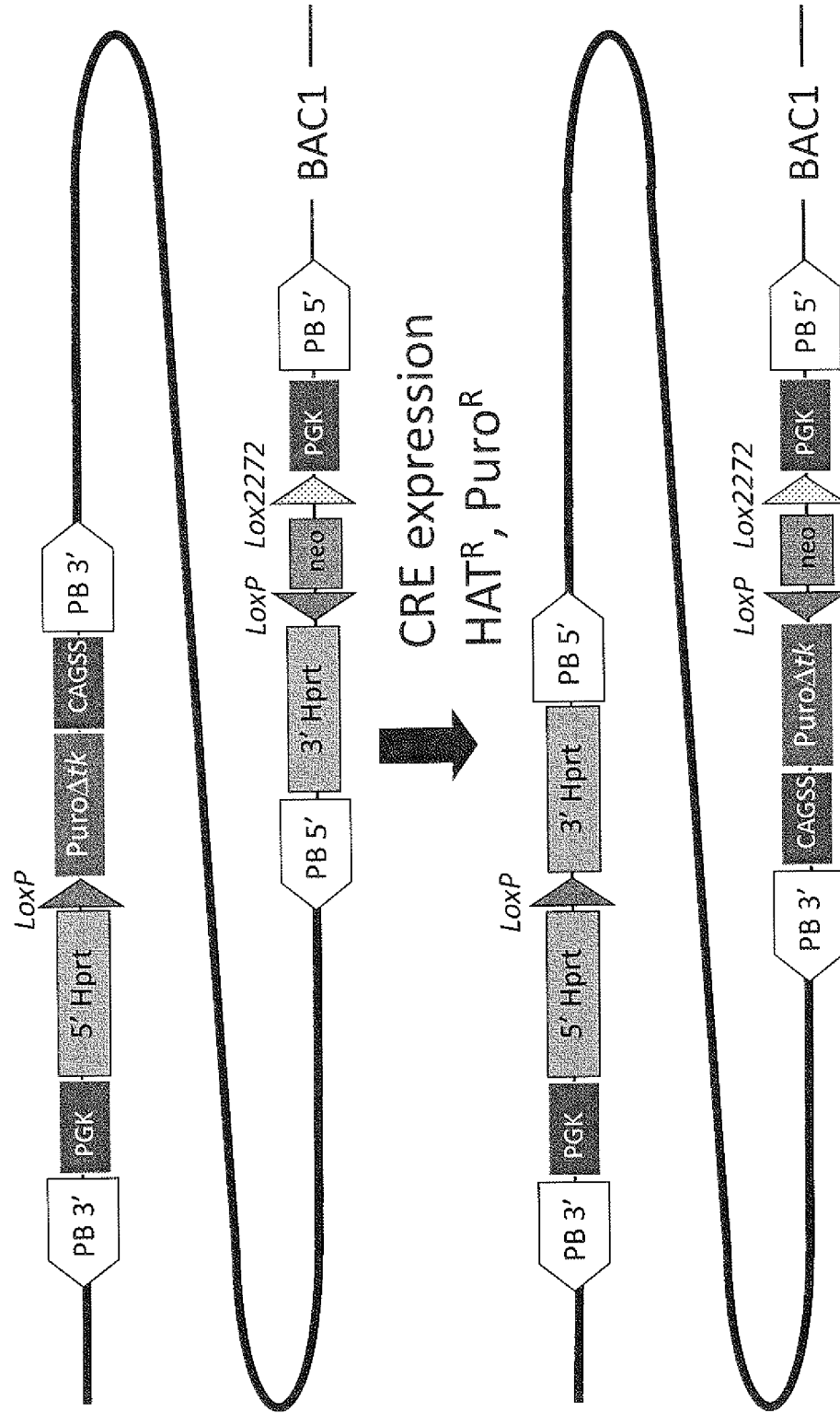


Fig. 28

# Removing All Modifications

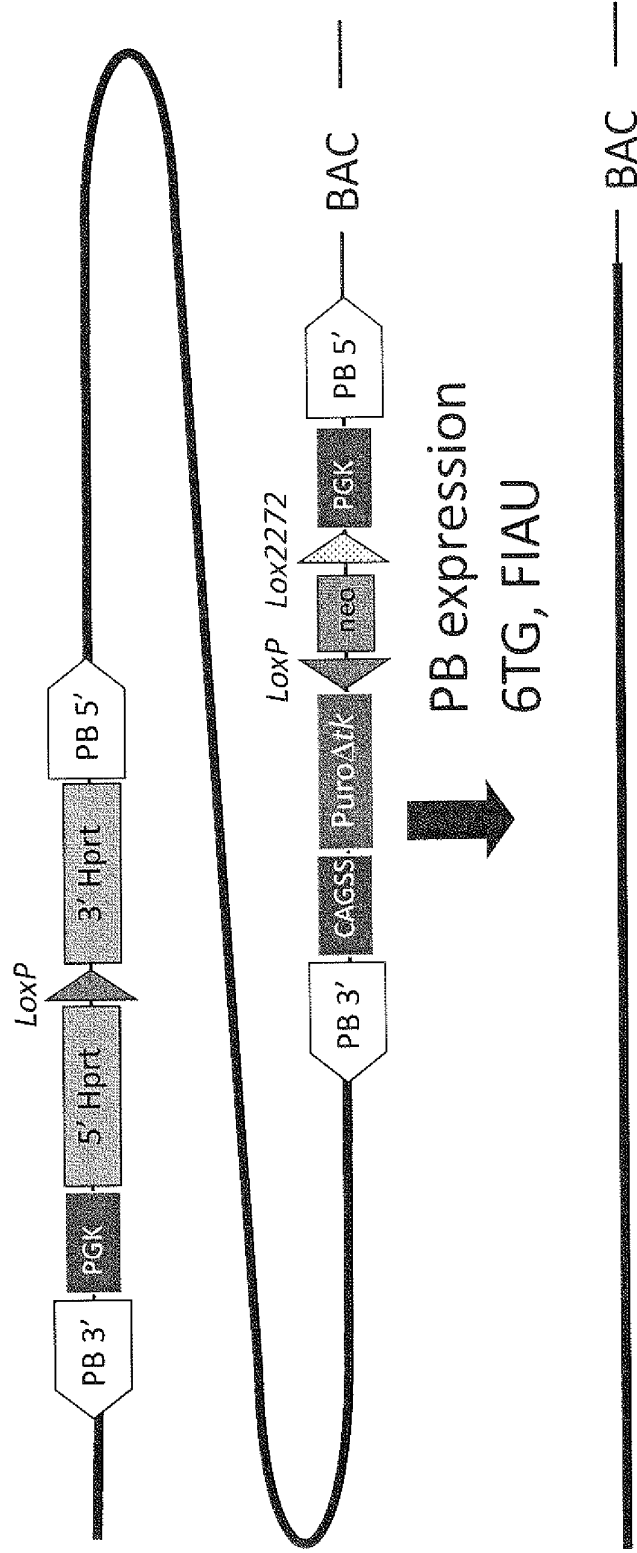
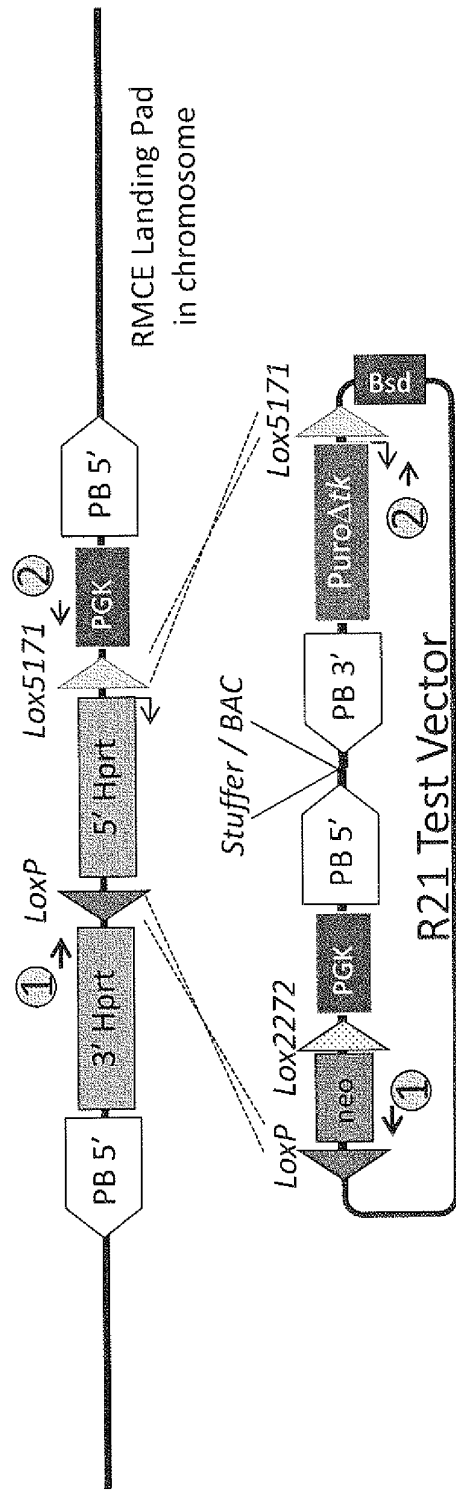


Fig. 29

# Sequential RMCE – Integration into Landing Pad



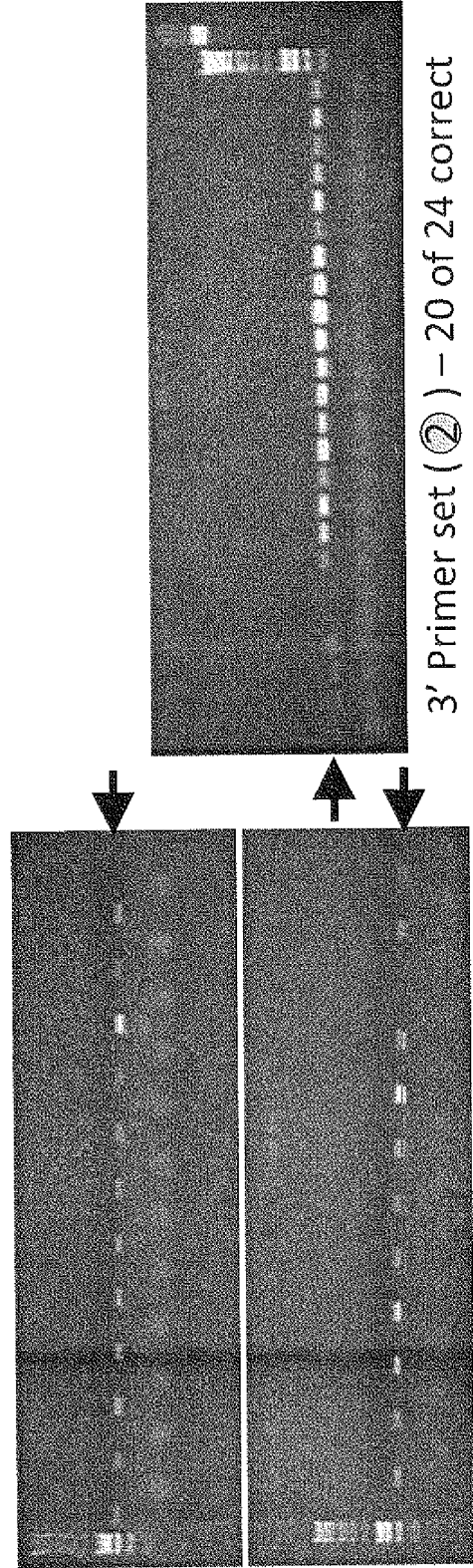
R21 transfected w/ Cre-expression Cassette

|             |      |            |      |            |
|-------------|------|------------|------|------------|
| 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

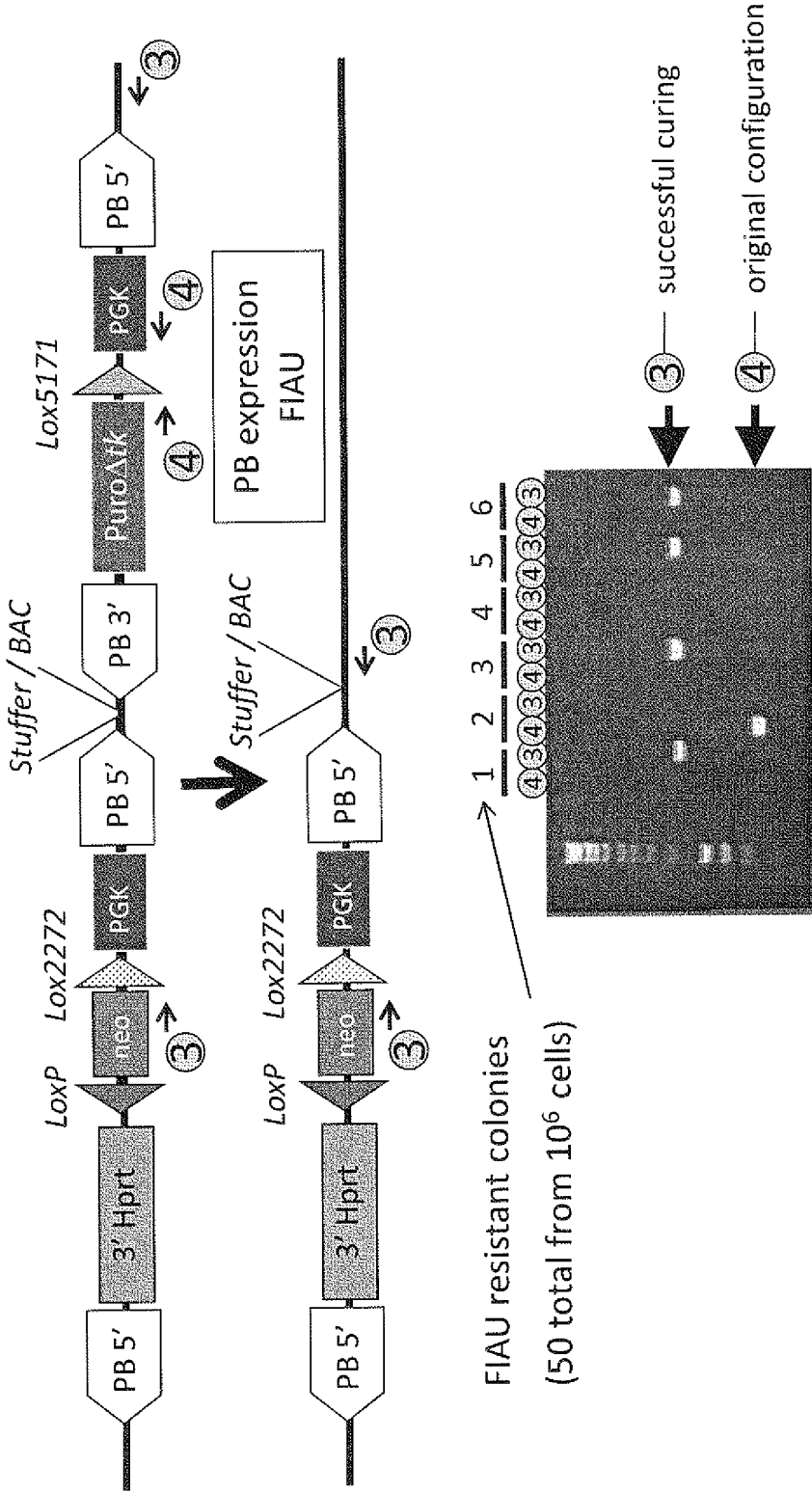


Fig. 32

# Insertion of BAC#1 – PCR Diagnostics

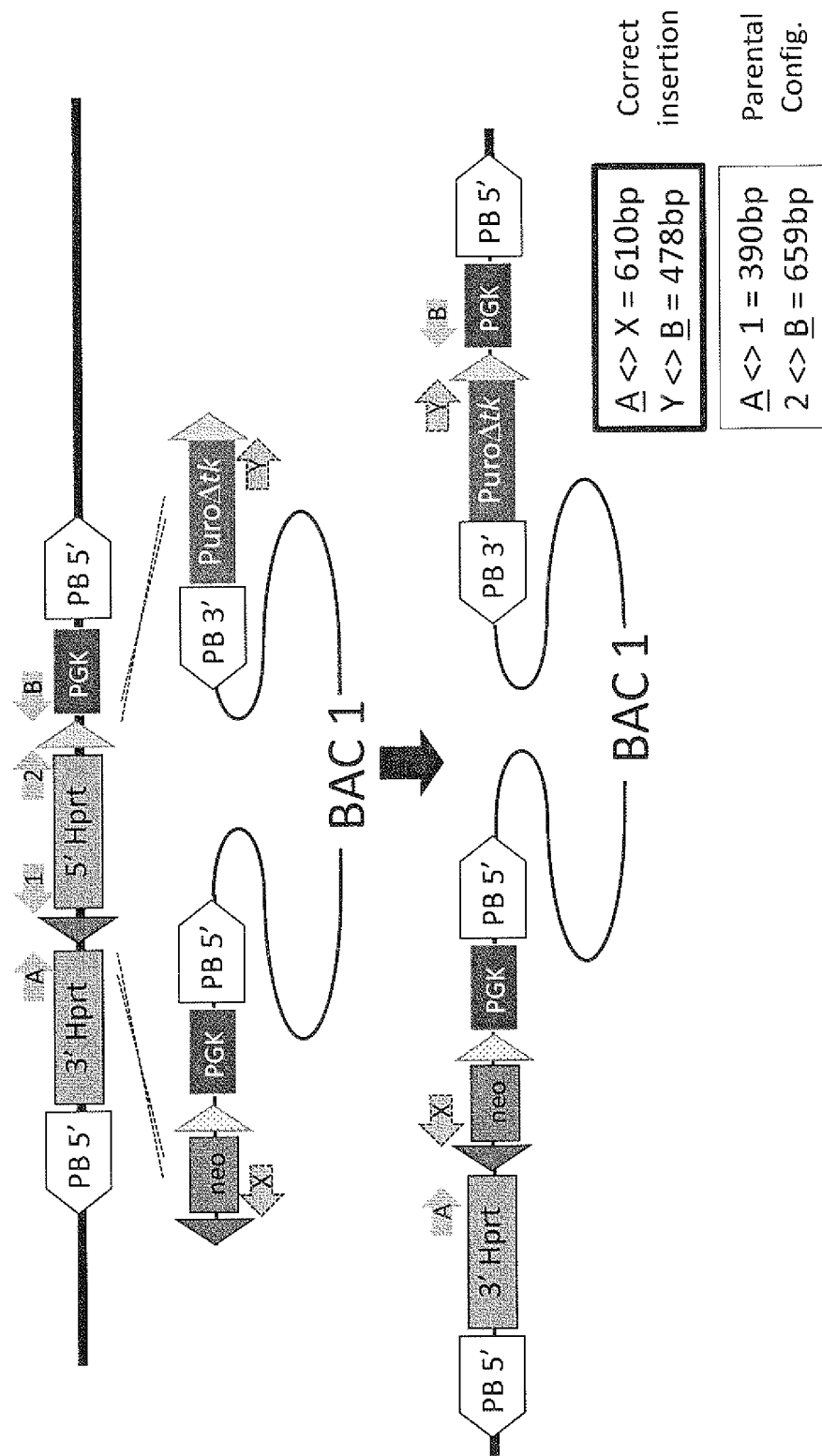
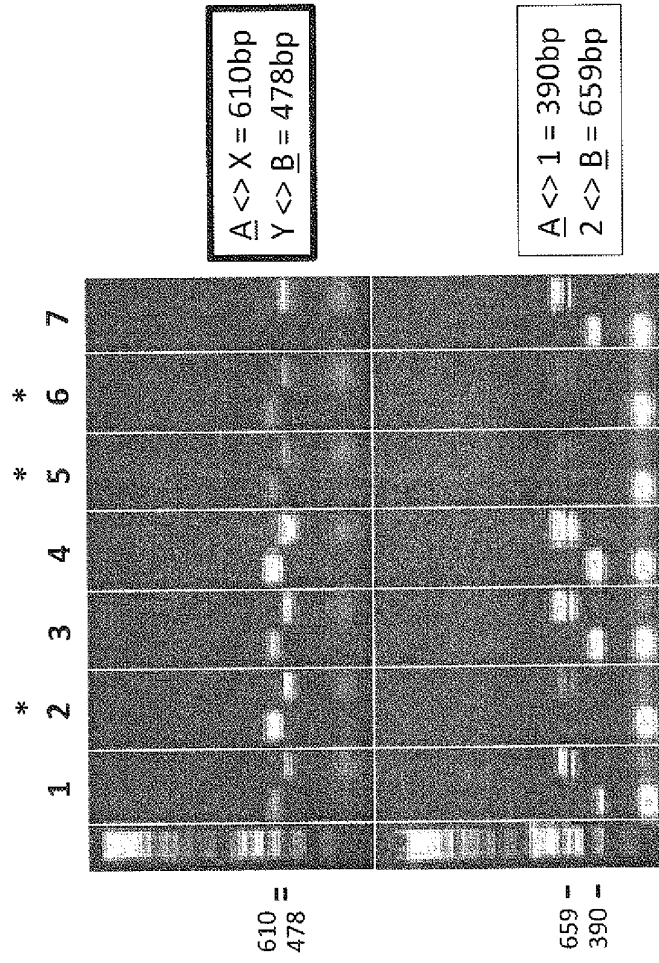


Fig. 33

# 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