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# MODEL GENERATION TECHNICAL REPORT

## Conditional overexpression of TEV protease by targeted transgenesis into the Rosa Locus 26

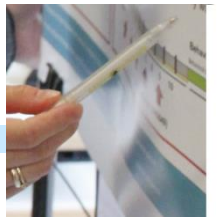
Project code: K5430 / IR5430

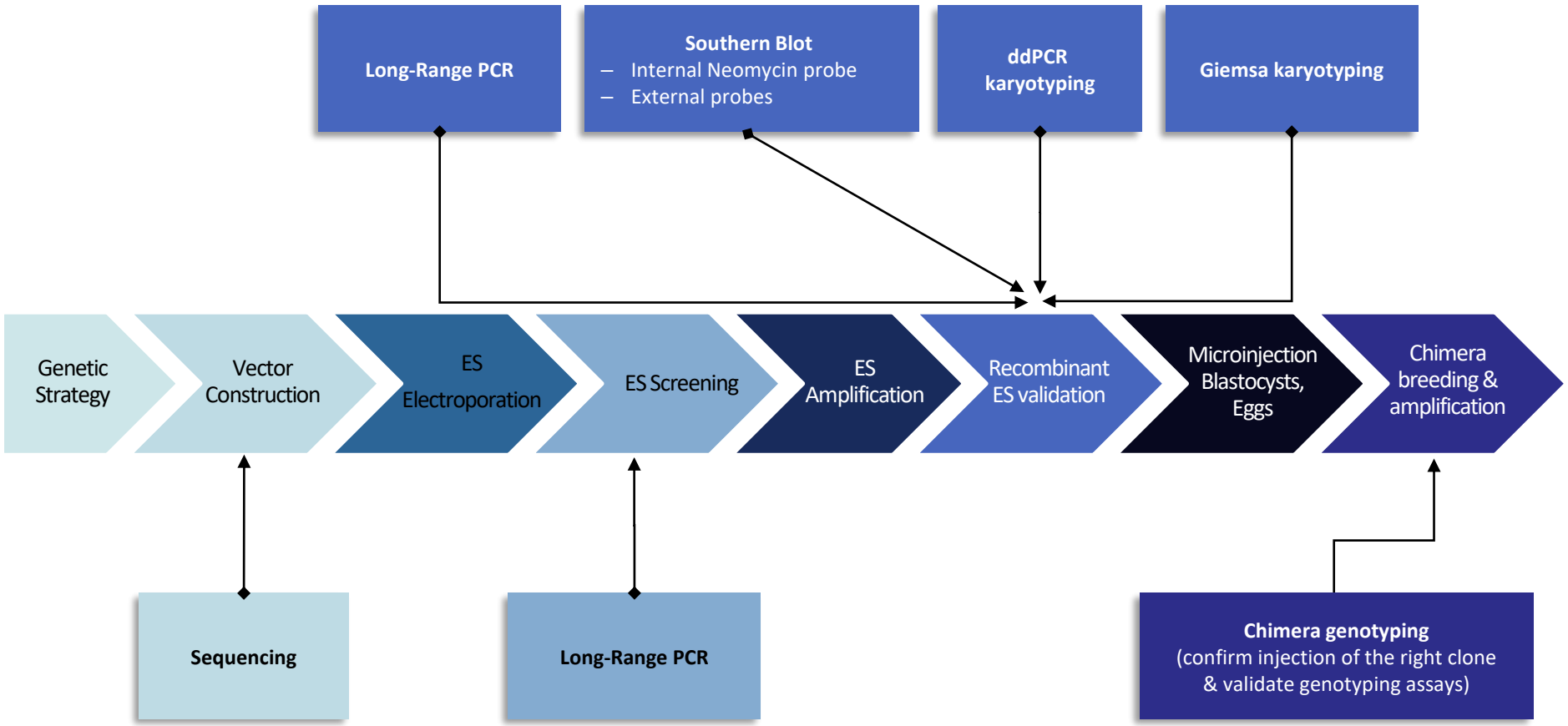
Report finalized: 2024/12/05

cnrs
Université de Strasbourg
Inserm
CERBM centre européen de recherche en biologie et en médecine gie
Aix*Marseille université

# MODEL GENERATION TECHNICAL REPORT

# Project process & quality controls



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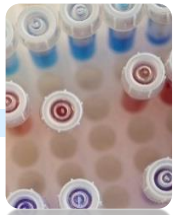
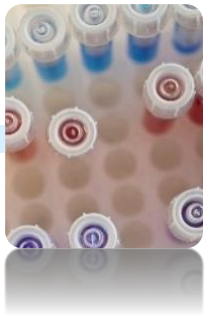
# GENETIC STRATEGY

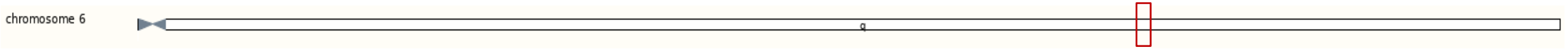


- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

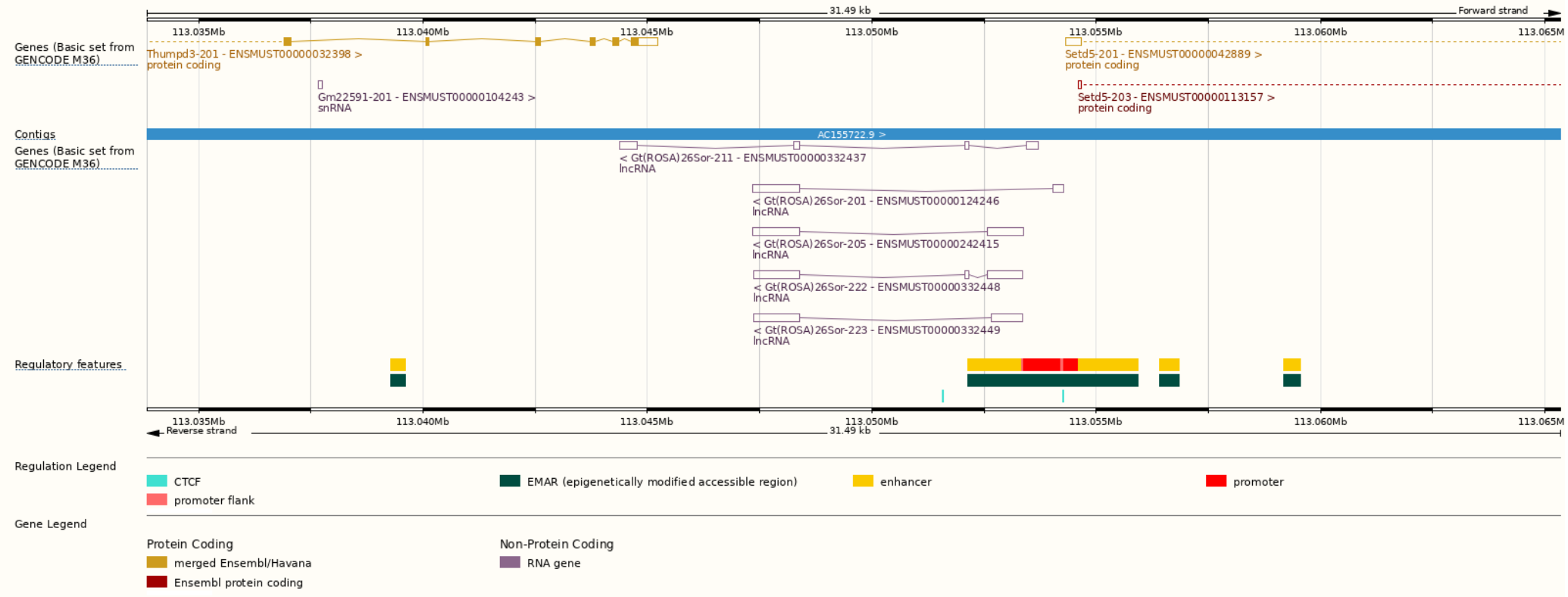
# Rosa26 mouse genomic locus – structure (GRCm39)



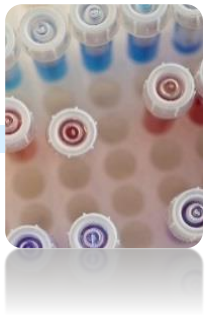
## Location : Chromosome 6: 113,043,843-113,055,336



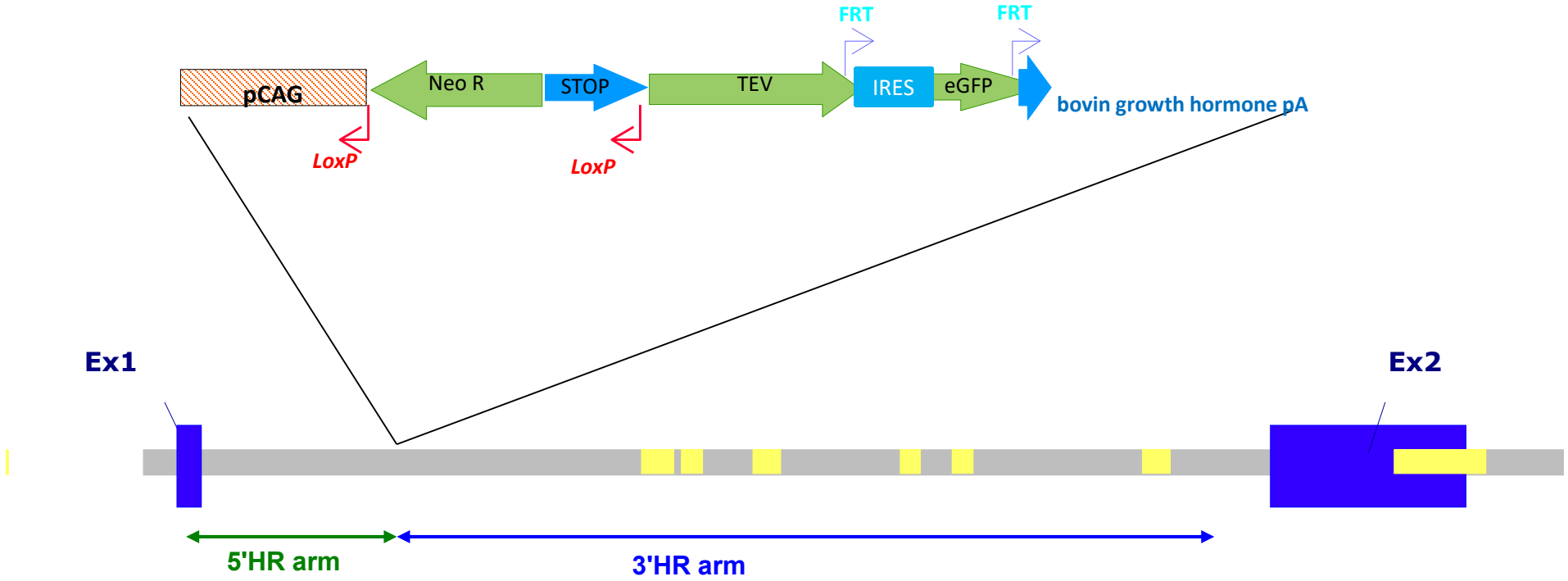

## Gene: Gt(ROSA)26Sor ENSMUSG00000086429



# Strategy



## Targeted locus



# Strategy

## TEV cDNA

AscI

GAATTCagatctatacattatacgaagttatcggcgcgccgccgccac atg ggc gag agc ctt ttc aag ggc ccg agg gac tac aac ccg atc tcc

M G E S L F K G P R D Y N P I S

agc acc atc tgt cac ctg acc aac gag agc gac ggt cac acc act agt ctg tac ggc atc ggc ttc ggc ccc ttc atc atc acc

S T I C H L T N E S D G H T T S L Y G I G F G P F I I T

aac aag cat ctg ttc agg agg aat aac ggc aca ctg ctg gtg caa agc ctg cac ggc gtg ttc aaa gtg aag aac aca acc acc

N K H L F R R N N G T L L V Q S L H G V F K V K N T T T

ctg caa cag cac ctg atc gac ggc agg gac atg att atc atc agg atg ccc aag gac ttc ccc ccc ttt ccc cag aaa ctg aag

L Q Q H L I D G R D M I I I R M P K D F P P F P Q K L K

ttc agg gag cca caa agg gag gag cga atc tgc ctg gtg acc acc aac ttc cag acc aag tcc atg agc agc atg gtc tct gat

F R E P Q R E E R I C L V T T N F Q T K S M S S M V S D

acc agc tgc acc ttc ccc agc agc gac ggc atc ttc tgg aag cac tgg att cag acg aag gat ggc caa tgc ggc agc cca ttg

T S C T F P S S D G I F W K H W I Q T K D G Q C G S P L

gtg agc act agg gac ggc ttc atc gtg ggc atc cac agc gcc agc aat ttt acc aat acc aac aac tac ttc acg agc gtg ccg

V S T R D G F I V G I H S A S N F T N T N N Y F T S V P

aaa aac ttc atg gag ctg ttg acc aat caa gag gcg cag cag tgg gtg agc ggc tgg agg ctg aac gcc gac agc gtt ctt tgg

K N F M E L L T N Q E A Q Q W V S G W R L N A D S V L W

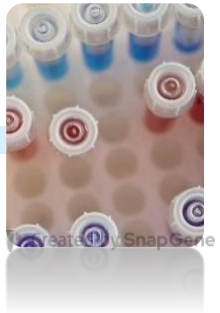
AscI

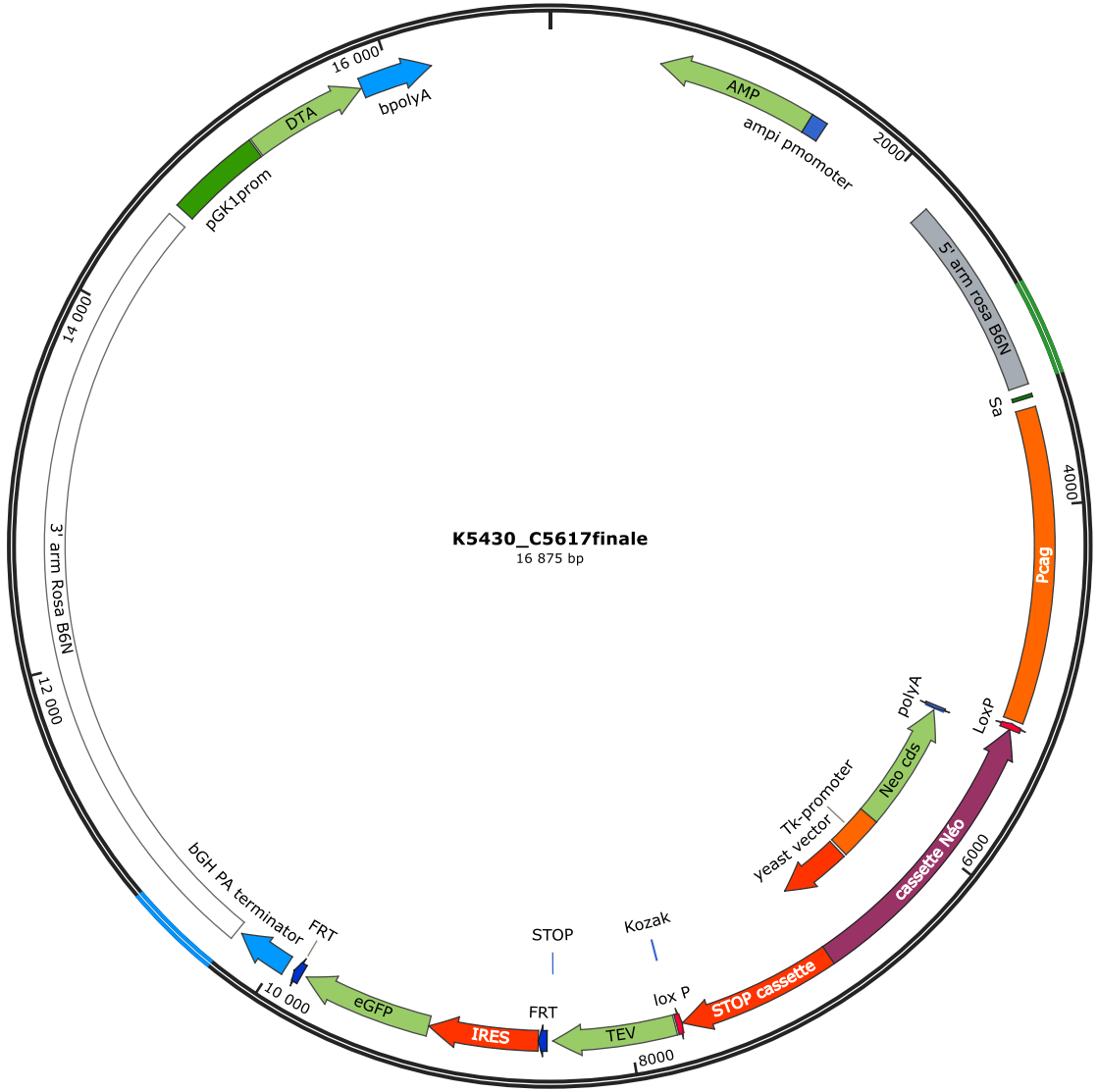
ggc gga cat aag gtg ttc atg tgaggcgcgccgcggccgcgatcaattcggtaccgaagatctGGATCC

G G H K V F M

Cloned using AscI site into CTV vector (https://www.addgene.org/15912/)

# 3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION



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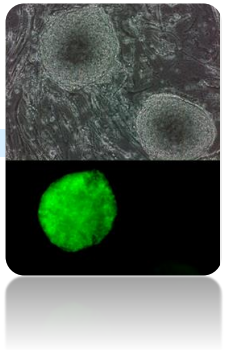
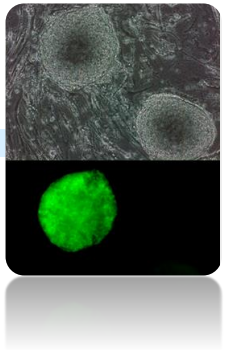
# ES cell electroporation & Screening of recombinant clones



- Electroporation and screening process
- Long range PCR screening – strategy
- Long-Range 5’ PCR screening – results
- Recombinant ES validation by Long Range PCR
- Recombinant ES clones validation by Southern Blot – internal probe
- Recombinant ES clones validation by Southern Blot – External probe
- Aneuploidy screening in ES recombinant clones

# Electroporation and screening process



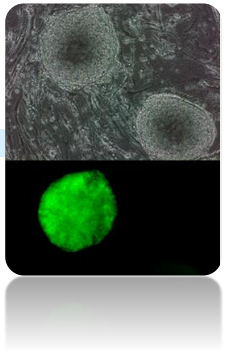
The targeting vector was electroporated in the proprietary C57BL/6NCrl S3 embryonic stem cell line.

Transfected ES clones were submitted to neomycin selection (G418) and 24 resistant ES clones were isolated. The clones were then submitted to the screening process allowing secured identification of those harbouring the expected recombination events at both ends of targeting vector.

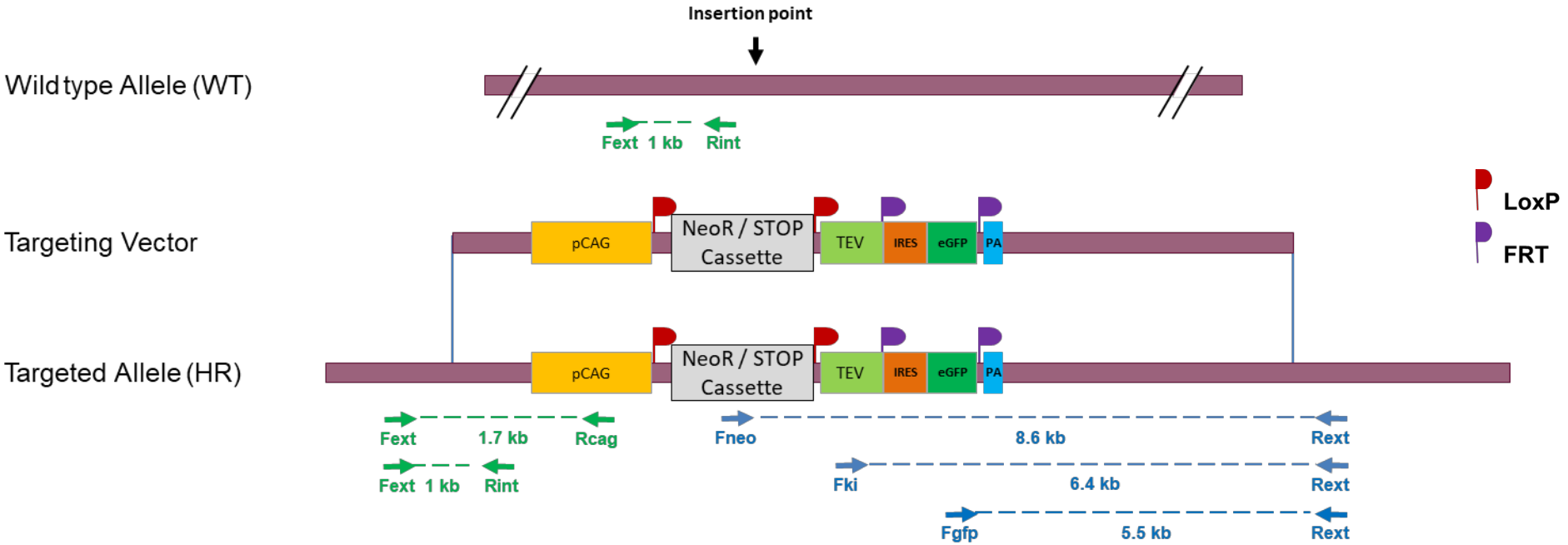
Screening process steps:

1. Identification of candidate recombinant clones by initial 5’ Long-Range PCR
2. Nine of 5’ PCR positive clones are confirmed for 3’ recombination event by Long-Range PCR
3. Positive clones in step 2 are further validated by Southern blot analysis using internal and external probes
4. The karyotype of at least 2 validated clones is verified using ddPCR aneuploidy screening and Giemsa staining

# Long range PCR screening – strategy

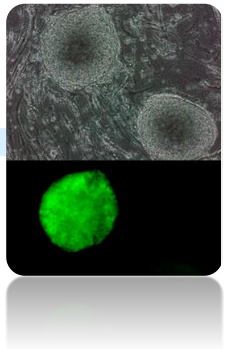


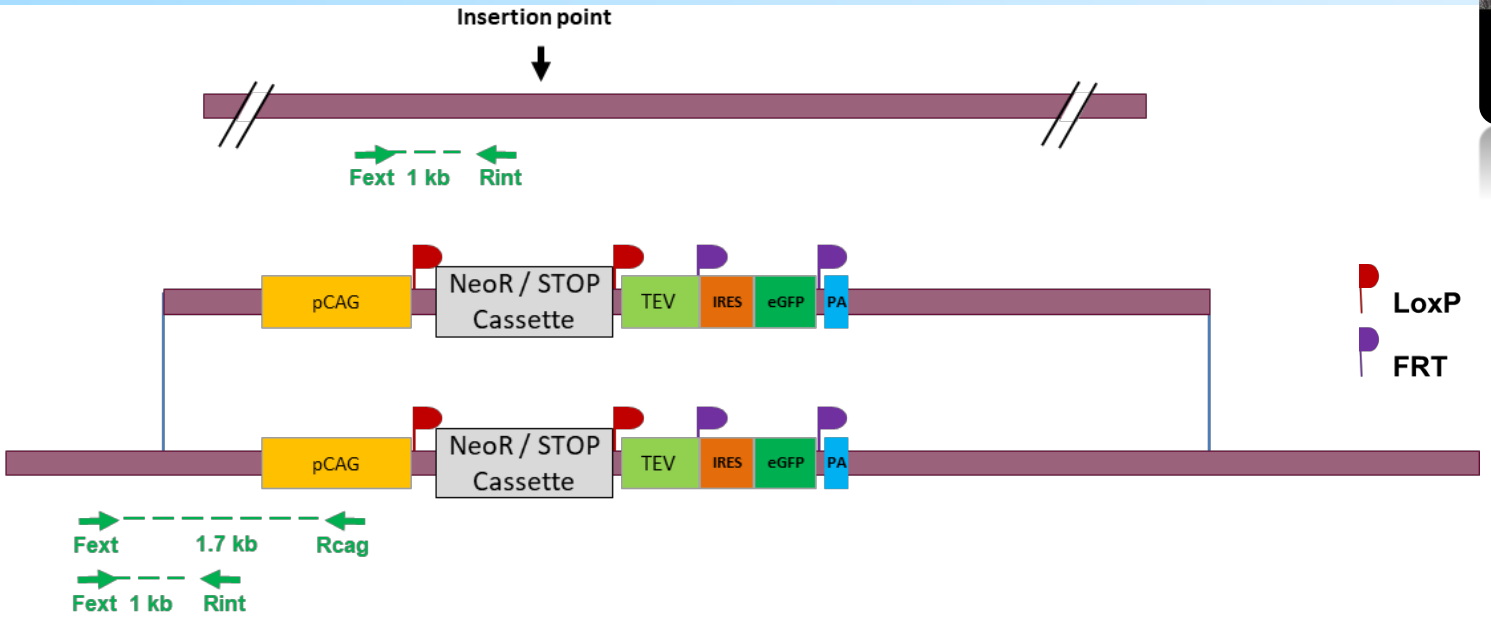
## Schematic 5’ and 3’ PCR screening strategy



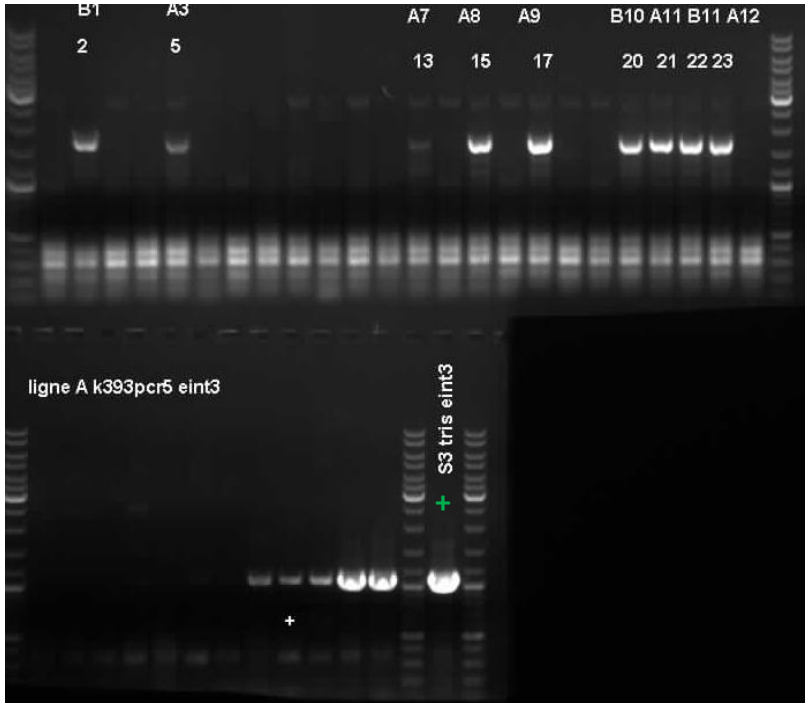

| PCR | Primer Name | Primer sequences | PCR product size |
|---|---|---|---|
| 5' PCR | Fext | GGTAGGGGATCGGGACTCTGGCGGG | 1 kb |
| | Rext | CTCCCAGAAAGGTATTGCAACACTC | |
| 5’ PCR | Fext | GGTAGGGGATCGGGACTCTGGCGGG | 1.7kb |
| | Rcag | GGAGAGTGAAGCAGAACGTGGGGCT | |
| 3’ PCR | Fneo | GCGGCCGGAGAACCTGCGTGCAATC | 8.6 kb |
| | Rext | CTCAGTGGCTCAACAACACTTGGTC | |
| 3’ PCR | Fki | GGGTGAGCGGCTGGAGGCTGAACGC | 6.4 kb |
| | Rext | CTCAGTGGCTCAACAACACTTGGTC | |
| 3’ PCR | Fgfp | GGCGACGTAAACGGCCACAAGTTCA | 5.5 kb |
| | Rext | CTCAGTGGCTCAACAACACTTGGTC | |

# Long-Range 5’ PCR screening – results



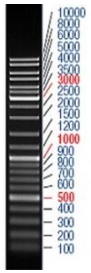



**PCR Fext – Rcag : 1.7 kb**




\+ : Controls DNAs

**PCR Fext – Rint : 1 kb**




Ladder pattern

Nine candidate clones out of the positive clones were selected for 3’ Long-Range PCR and Southern blot validation.

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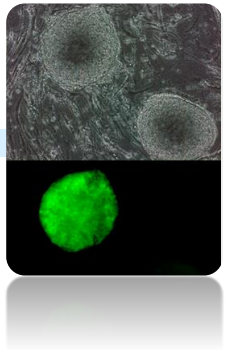
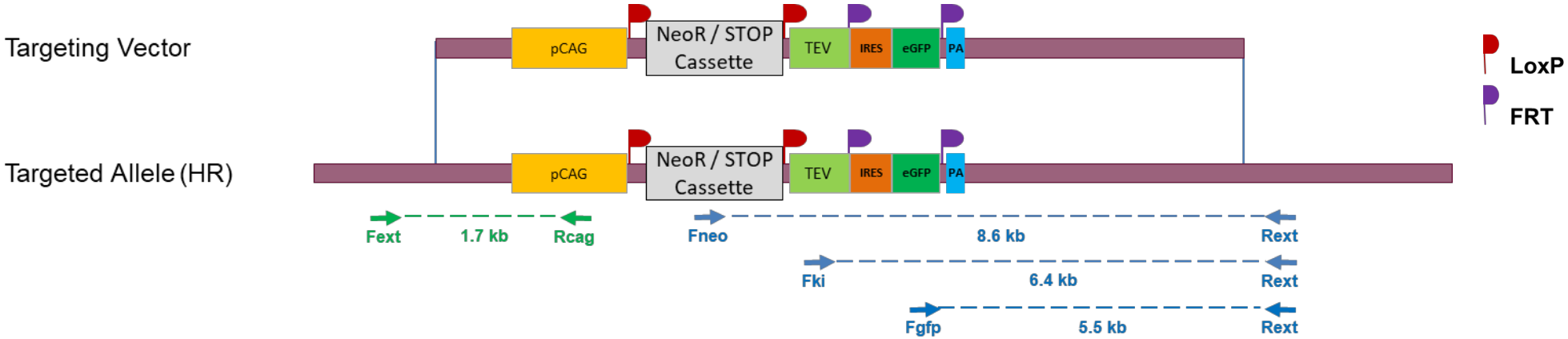
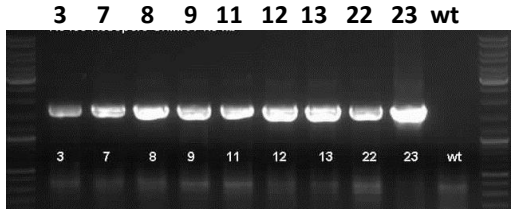
# Recombinant ES validation by Long Range PCR

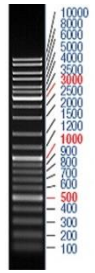


## Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

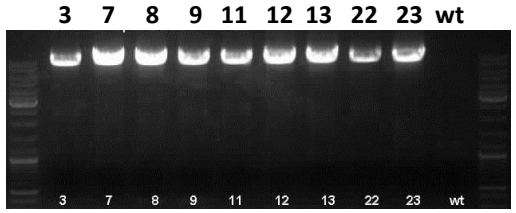


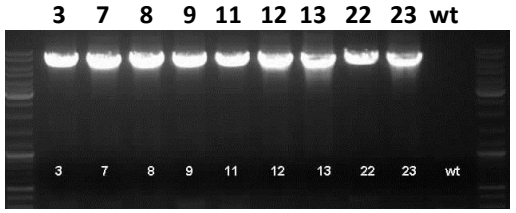

PCR Fext – Rki : 1.7 kb



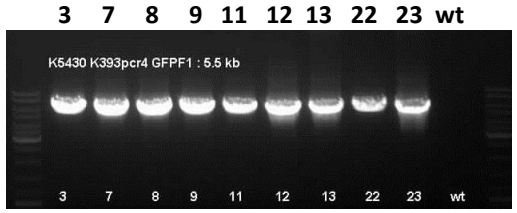
Ladder pattern




PCR Fneo – Rext : 8.6 kb




PCR Fki – Rext : 6.4 kb




PCR Fgfp – Rki : 5.5 kb

Nine candidate clones identified by 5’ PCR screening were analysed by 3’ Long-Range PCR screening.
Eight clones (clones #3, #7, #8, #9, #11, #12, #22, and #23) were further analysed.

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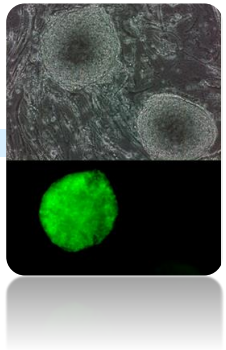
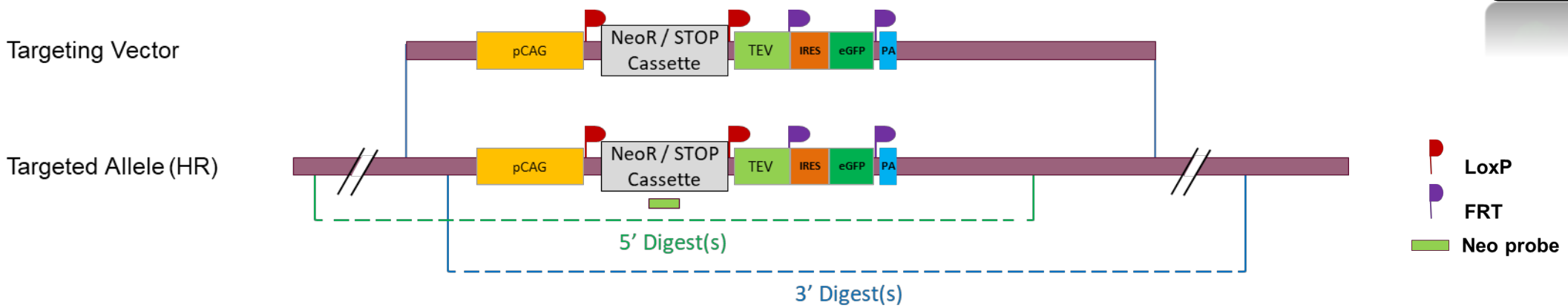
# Recombinant ES clones validation by Southern Blot – Internal probe



## Schematic Southern Blot validation strategy

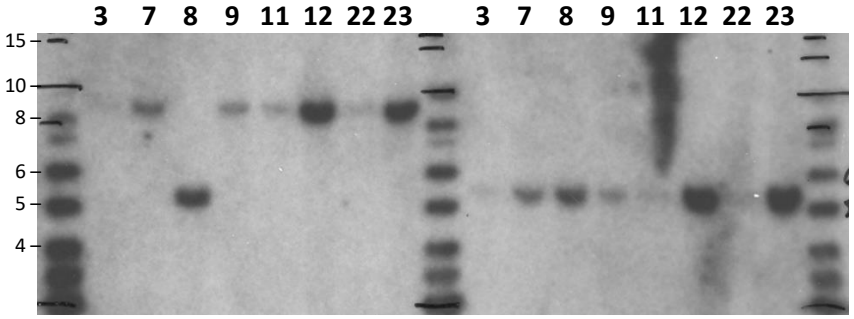
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



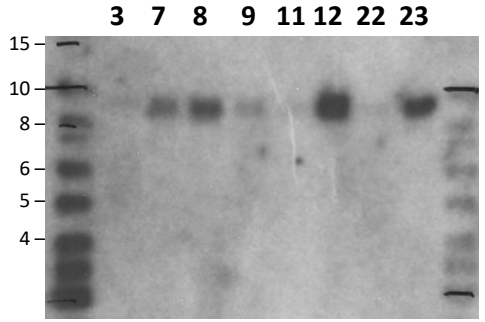

## Digestions used to validate the 5' and 3' insertion

| Probe | | Genomic DNA digest | Targeted Allele (kb) |
|---|---|---|---|
| Neo | 5' digest | XcmI | 9.4 |
| | | EcoNI | 5.6 |
| | 3' digest | PacI | 9.6 |

### Southern blot - Neo 5'



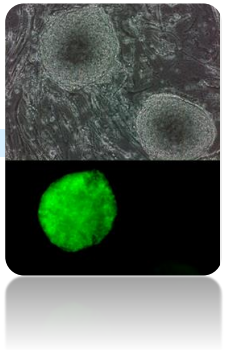

### Southern blot - Neo 3'




### Neo probe sequence

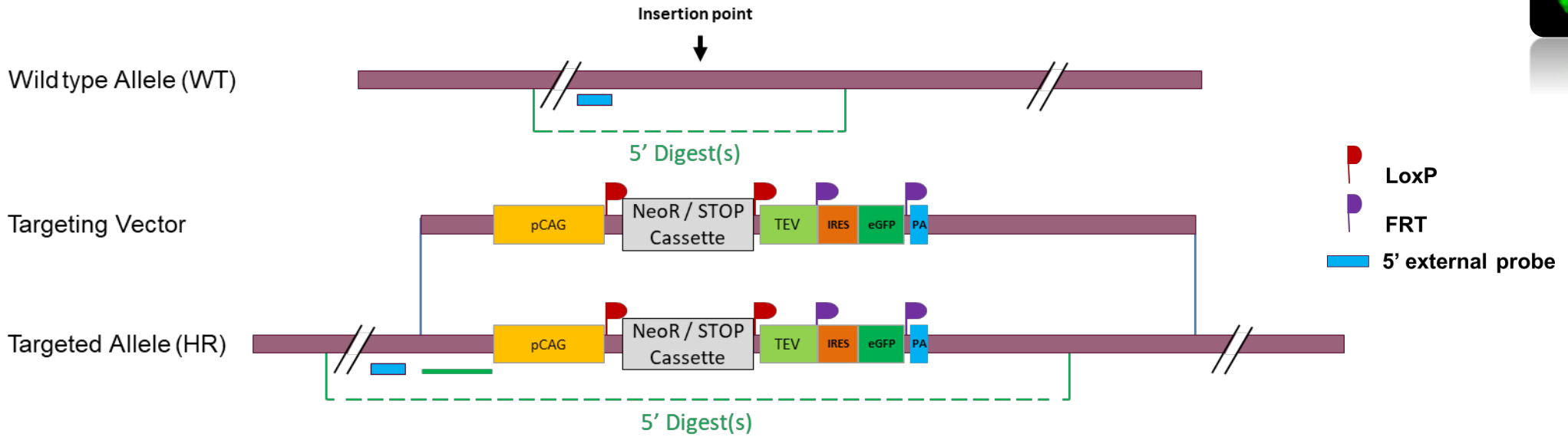
AGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGT
AAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCA
ACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGC
GGCCATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTCACGACGAGATCCTCGC
CGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCTT
CGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGAT
GTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTGCAT
CAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCA
CTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAG
GAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTCGTCCTGCAG

# Recombinant ES clones validation by Southern Blot – External probe

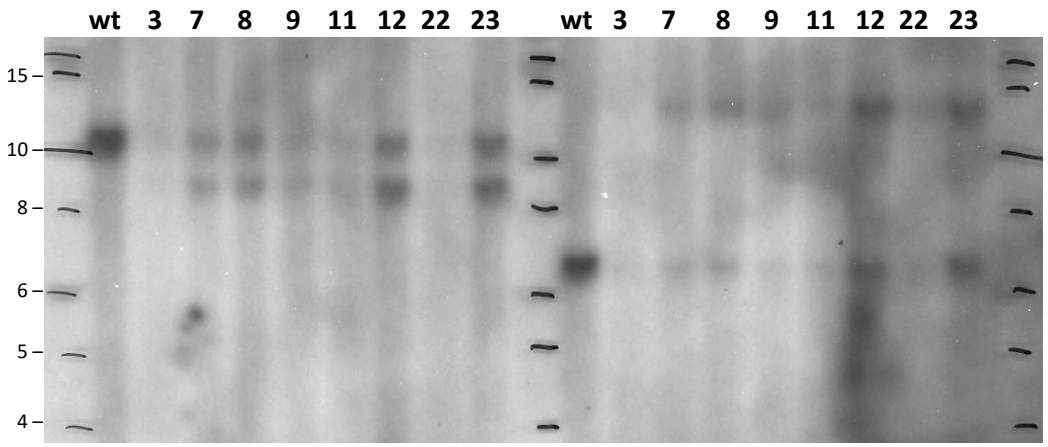


## Schematic Southern Blot validation strategy

Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.




### Southern blot – 5' probe




### 5' probe sequence

TATGTGTATTTTGAGAGCAGGGTTGGGAG
GCCTCTCCTGAAAAGGGTATAAACGTGGA
GTAGGCAATACCCAGGCAAAAAGGGGAGA
CCAGAGTAGGGGGAGGGGAAGAGTCCTGA
CCCAGGGAAGACATTAAAAAGGTAGTGGG
GTCGACTAGATGAAGGAGAGCCTTTCTCT
CTGGGCAAGAGCGGTGCAATGGTGTGTAA
AGGTAGCTGAGAAGACGAAAAGGGCAAGC
ATCTTCCTGCTACCAGGCTGGGGAGGCCC
AGGCCCACGACCCCGAGGAGAGGGAACGC
AGGGAGACTGAGGTGACCCTTCTTTCCCC
CGGGGCCCGGTCGTGTGGTTCGGTGTCTC
TTTTCTGTTGGACCCTTACCTTGACCCAG
GC

### Digestions used to validate the 5' and 3' insertion

| Probe | Name | Genomic DNA digest | WT allele (kb) | Targeted Allele (kb) |
|---|---|---|---|---|
| 5' external probe | 5' first digest | EcoRV | 11.5 | 9.2 |
| | 5' second digest | SexAI | 6.7 | 13.7 |

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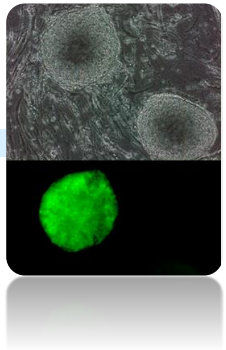
# Aneuploidy screening in ES recombinant clones

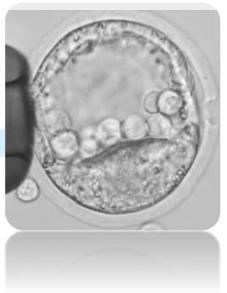


Selected recombinant ES cells clones were karyotyped by ddPCR as described in Codner *et al.* [1] and by Giemsa metaphase staining. Results of aneuploidy analysis are presented in the table below.

| Clone ID | ddPCR | Giemsa |
|---|---|---|
| **#7** | **Pass** | **Pass** |
| #9 | Pass | Not done |
| #11 | Pass | Not done |
| **#12** | **Pass** | **Pass** |
| #22 | Pass | Not done |
| #23 | Failed | Not done |

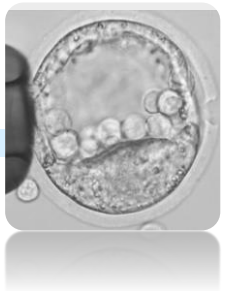
[1] Codner, G.F., Lindner, L., Caulder, A., Wattenhofer-Donzé, M., Radage, A., Mertz, A., Eisenmann, B., Mianné, J., Evans, E.P., Beechey, C.V., Fray, M.D., Birling, M.-C., Hérault, Y., Pavlovic, G., Teboul, L
Aneuploidy screening of embryonic stem cell clones by metaphase karyotyping and droplet digital polymerase chain reaction.
BMC Cell Biology 2016 **doi:10.1186/s12860-016-0108-6**

# MICROINJECTION & BREEDING



- Microinjection
- Breeding to F1 generation

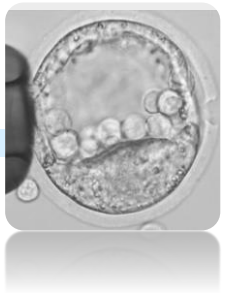
# Microinjection



- The ES cells used in the injection experiment were originally derived from a C57BL/6NCrl mouse strain (which have black coat colour). These cells were injected into blastocysts derived from an BALB/cN strain, which have a white coat colour. The resulting offspring are thus chimeras of two different cell types (ES cell-derived cells and host blastocyst-derived cells) and the degree of chimerism was monitored by the percentage of light and dark patches on these animals.
- Recipient blastocysts were isolated from mated BALB/cN females (Health status SPF Specific Pathogens Free).
- Recombinant ES clones #7 and #12 validated in previous project phase were injected into blastocysts to generate chimeric males. The results are presented in the table below.

| Clone ID | Number of chimeric males identified according to chimerism rate (Number of chimeric males bred to F1 generation) | | | |
|---|---|---|---|---|
| | 5 - 40% | 45% - 55% | 60-100% | Total |
| #7 | 0 | 0 | 0 | 0 |
| #12 | 5 | 0 | 2 | 7 |

# Breeding to F1 generation



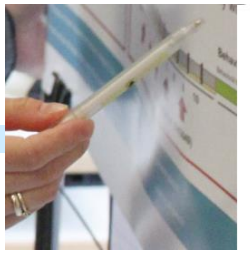
- Two highly chimeric males generated in the previous phase by blastocyst injection of the ES clone #12 were mated with wild-type C57BL/6NCrl females (health status SPF – Specific Pathogen Free) to investigate whether the recombined ES cells have contributed to the germ layer.

- Germ line transmission was obtained the : 26/08/2015

- MGI allele ID: MGI:7788441

- Allele nomenclature (following MGI guidelines) : **Gt(ROSA)26Sor$^{tm18(CAG-TEVgp1*,-EGFP)Ics}$**

# SEQUENCE OF THE DELIVERED ALLELE





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LoxP pCAG NeoR – STOP casette TEV cDNA FRT IRES eGFP bGH pA

phenomin

EXCELLENCE IN MOUSE PHENOGENOMICS

**REPORT REDACTION & VALIDATION**

Protocol finalized on 2024/12/05

Prepared by Romain LORENTZ, IE

Verified by Marie-Christine BIRLING, PhD

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# Genotyping protocol

## Conditional overexpression of TEV under the pCAG promoter / KI in Rosa26

This report has been prepared by: **David Moulaert**
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This report has been validated by: **Sylvie Jacquot, PhD, Head of Genotyping Service**
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The first version of this report was generated the: 23 Feb 2016

For any question, please contact:

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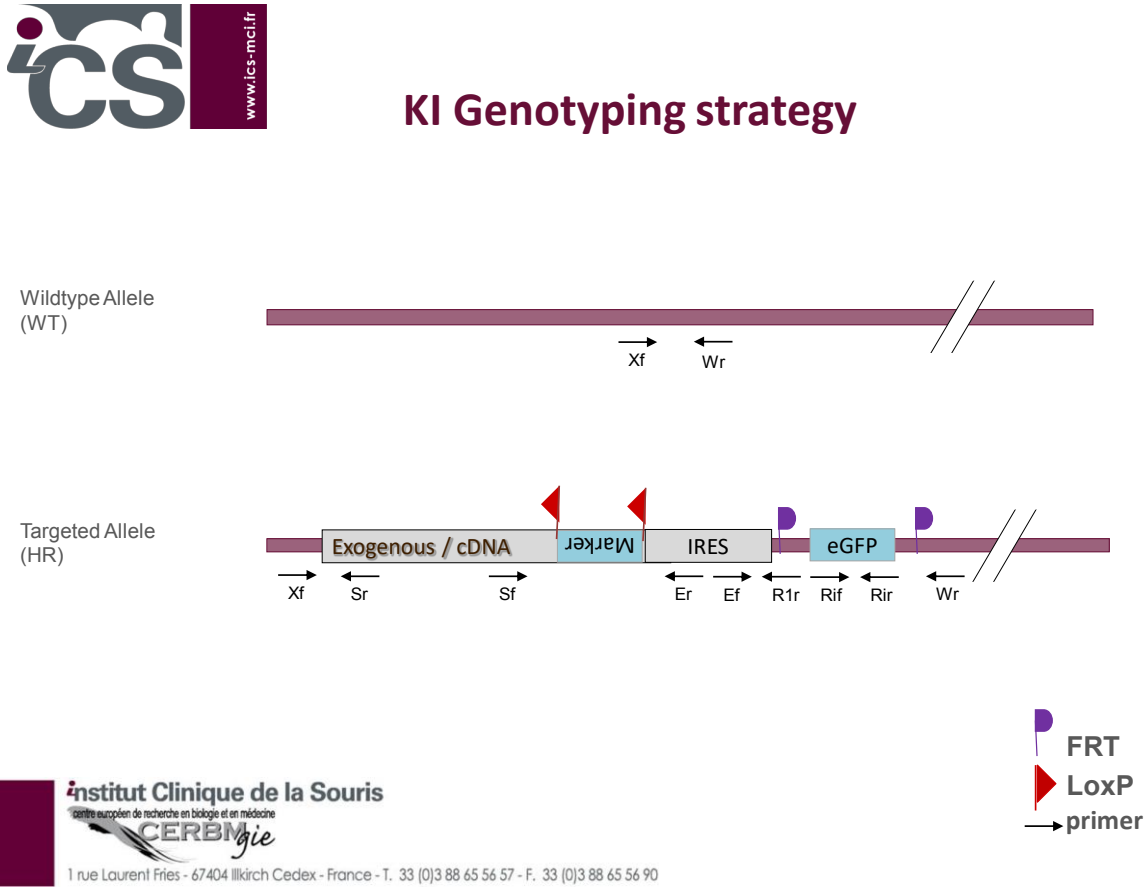
## 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **TEV KI in Rosa26** Knockin (KI) project.

### 1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.



Sequence of primers used for genotyping:

| Position | Primers | Sequence |
|---|---|---|
| Xf | 4036 | AAAGTCGCTCTGAGTTGTTAT |
| Sf | 1857 | GCGGAGCCGAAATCTGGGAG |
| Ef | 8820 | TCATGGAGCTGTTGACCAA |
| Wr | 4035 | CCTTTAAGCCTGCCCAGAAG |
| Er | 8821 | TGGTGTGACCGTCGCTCTCGTT |
| R1r | 5735 | CAAGCGGCTTCGGCCAGTAACGTTAG |
| Rif | 164 | CAGCCGCTACCCCGACCACA |
| Rir | 165 | CACCTTGATGCCGTTCTTCT |
| Sr | 5723 | TGGGCTATGAACTAATGACCCCGTA |

PCR fragments expected size (bp):

| Region analyzed | Primers used | Position on the primer *(see the map above)* | Targeted allele (HR) | Targeted allele (cKO) | KI allele | WildType allele |
|---|---|---|---|---|---|---|
| WildType allele specific PCR (5' part of the targeted locus) | 4036-4035 | Xf / Wr | 7168 | 4663 | 3270 | 239 |
| Excision of the selection marker | 1857-8821 | Sf / Er | 2986 | 481 | 481 | --- |
| 5' reporter | 8820-5735 | Ef / R1r | 240 | 240 | --- | --- |
| Internal reporter | 164-165 | Rif / Rir | 277 | 277 | --- | --- |
| Exogenous/cDNA specific PCR | 4036-5723 | Xf / Sr | 389 | 389 | 389 | --- |

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)
**: this PCR is only verified if mice are generated
---: no Amplicon should be obtained

## 1.2. PCR protocol

This section describes the composition of the mix and cycling conditions used for genotyping.

| Reagents: | Volume: |
|---|---|
| - FastStart PCR Master (Roche) | 7.5µl |
| - DNA (50ng/µl) | 1.5µl |
| - 5' primer (100 µM) | 0.06µl |
| - 3' primer (100 µM) | 0.06µl |
| - Sterile $H_2O$ | up to 15 µl |

Cycling conditions:

| Temp | Time | #Cycles |
|---|---|---|
| 95°C | 4min | 1 |
| 94°C | 30s | |
| 62°C | 30s | 34 |
| 72°C | 1min | |
| 72°C | 7min | 1 |
| 20°C | 5min | 1 |

**NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.**

## 2. Cre and Flp genotyping method

You will find the genotyping protocol in the publication:

Highly-efficient, fluorescent, locus directed cre and FlpO deleter mice on a pure C57BL/6N genetic background.
Birling MC, Dierich A, Jacquot S, Hérault Y, Pavlovic G.
Genesis. 2012 Jun;50(6):482-9. doi: 10.1002/dvg.20826. Epub 2012 Mar 20.