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# MODEL GENERATION TECHNICAL REPORT

## Generation of mouse model : Med23 cKO

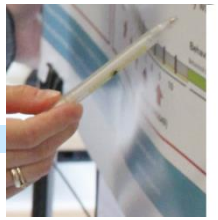
Project code: G4622 / IR4622

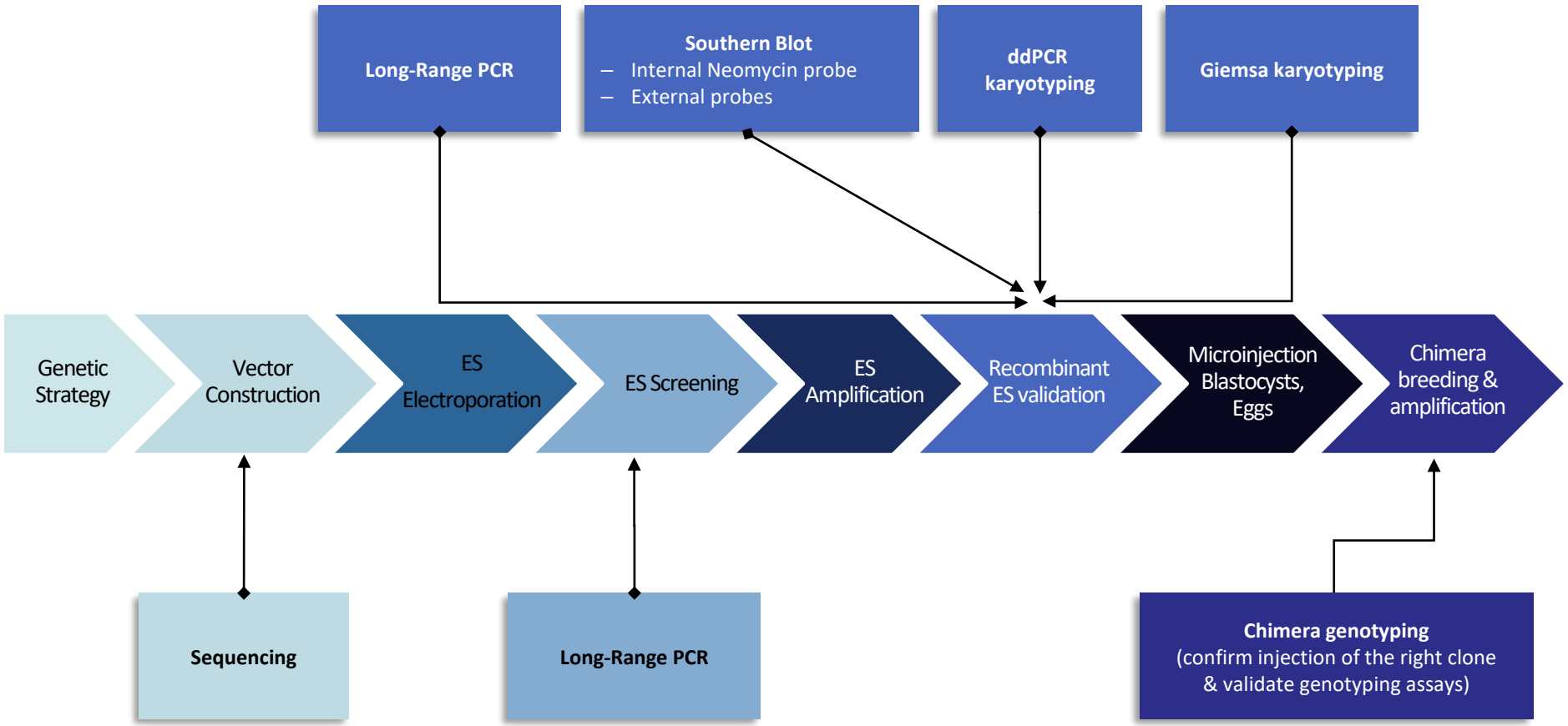
Report finalized: 06/10/2023

cnrs
Université de Strasbourg
Instituts thématiques Inserm institut national de la santé et de la recherche médicale
CERBM centre européen de recherche en biologie et en médecine gie
Aix*Marseille université

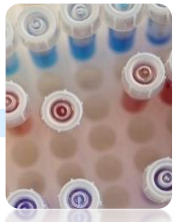
# MODEL GENERATION TECHNICAL REPORT

# PROJECT PROCESS & QUALITY CONTROLS



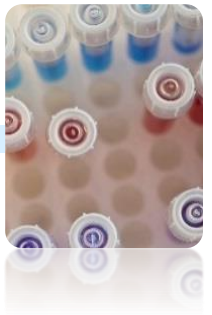


# 2 GENETIC STRATEGY

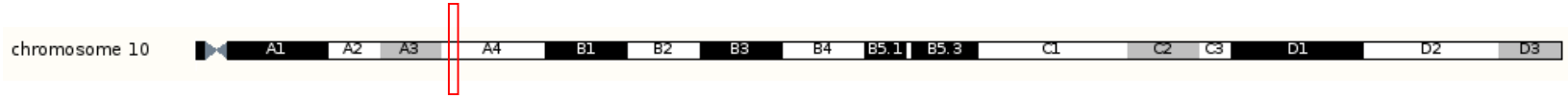


- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

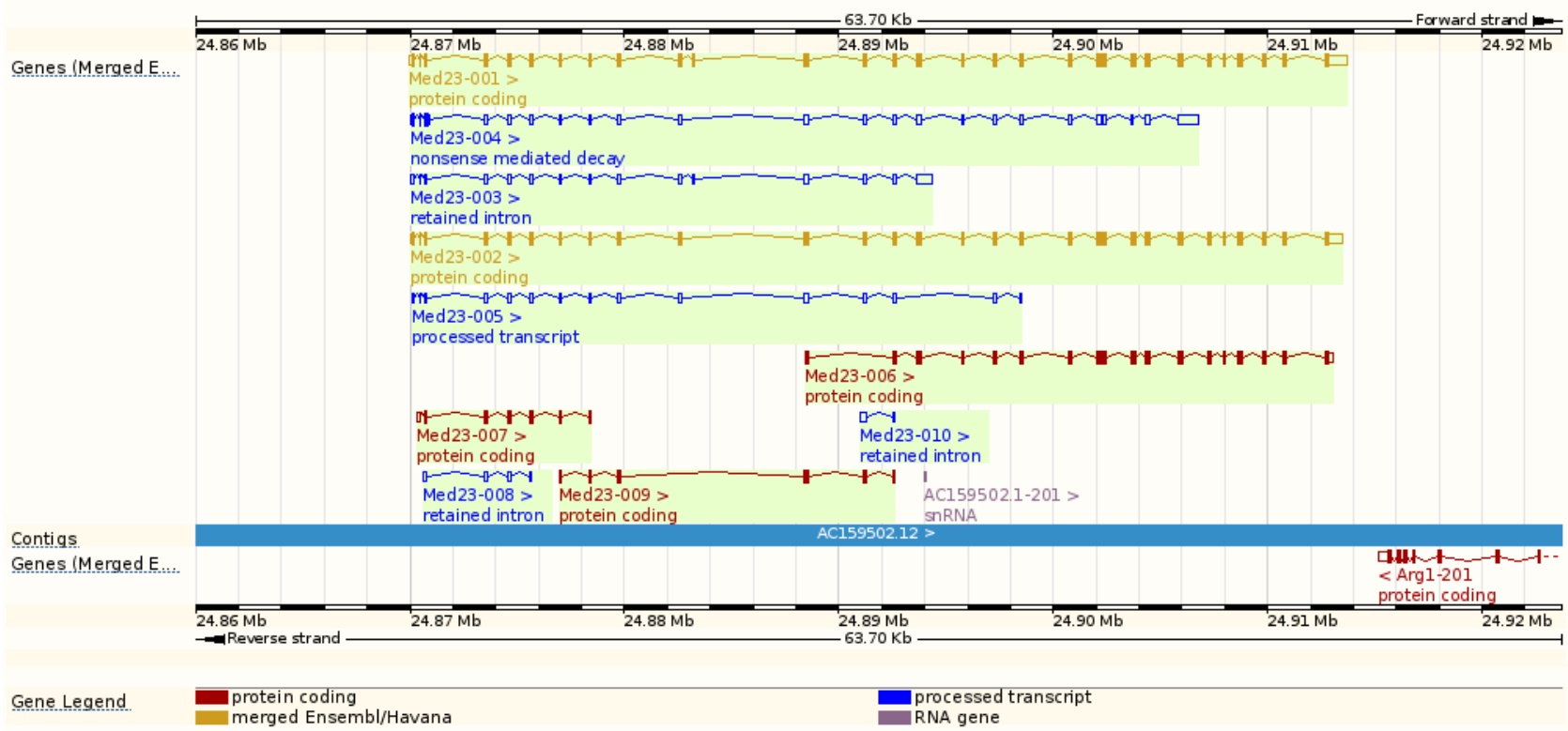
# Med23 mouse genomic locus – structure



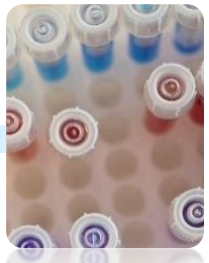
## Location




## Ensembl Gene ID: **Med23 ENSMUSG00000019984**

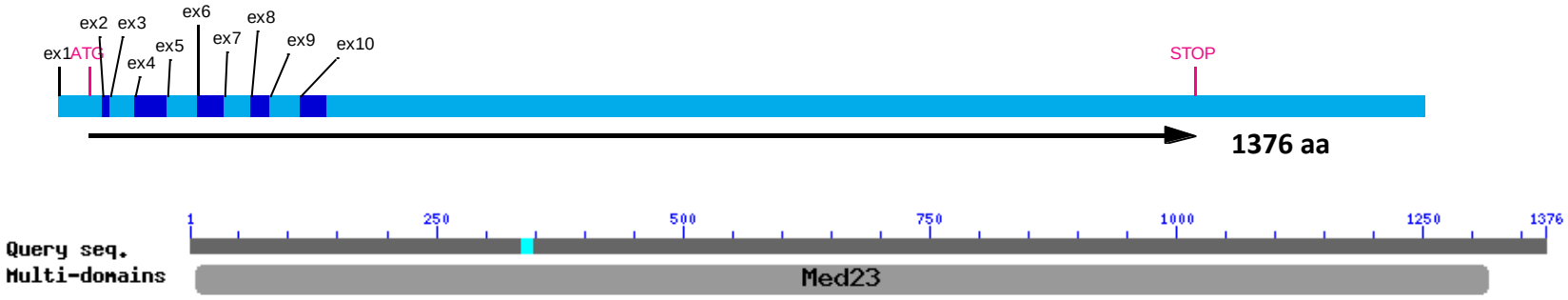


# Med23 mRNA(s) and protein(s)

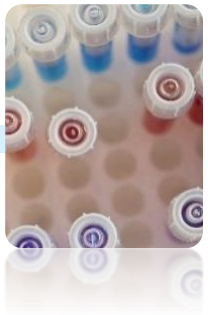


| Name | Transcript ID | Length (bp) | Protein ID | Length (aa) | Biotype | CDS incomplete | CCDS |
|---|---|---|---|---|---|---|---|
| Med23-001 | ENSMUST00000092646 | 5098 | ENSMUSP00000090316 | 1376 | Protein coding | - | CCDS48522 |
| Med23-002 | ENSMUST00000020159 | 4798 | ENSMUSP00000020159 | 1370 | Protein coding | - | CCDS35871 |
| Med23-006 | ENSMUST00000176285 | 3226 | ENSMUSP00000135232 | 1010 | Protein coding | 5' | - |
| Med23-007 | ENSMUST00000176313 | 770 | ENSMUSP00000135751 | 197 | Protein coding | 3' | - |
| Med23-009 | ENSMUST00000176502 | 704 | ENSMUSP00000134836 | 234 | Protein coding | 5' and 3' | - |
| Med23-004 | ENSMUST00000177232 | 3953 | ENSMUSP00000134866 | 58 | Nonsense mediated decay | - | - |
| Med23-005 | ENSMUST00000176827 | 1635 | No protein product | - | Processed transcript | - | - |
| Med23-003 | ENSMUST00000177522 | 2192 | No protein product | - | Retained intron | - | - |
| Med23-008 | ENSMUST00000177175 | 419 | No protein product | - | Retained intron | - | - |
| Med23-010 | ENSMUST00000175786 | 368 | No protein product | - | Retained intron | - | - |

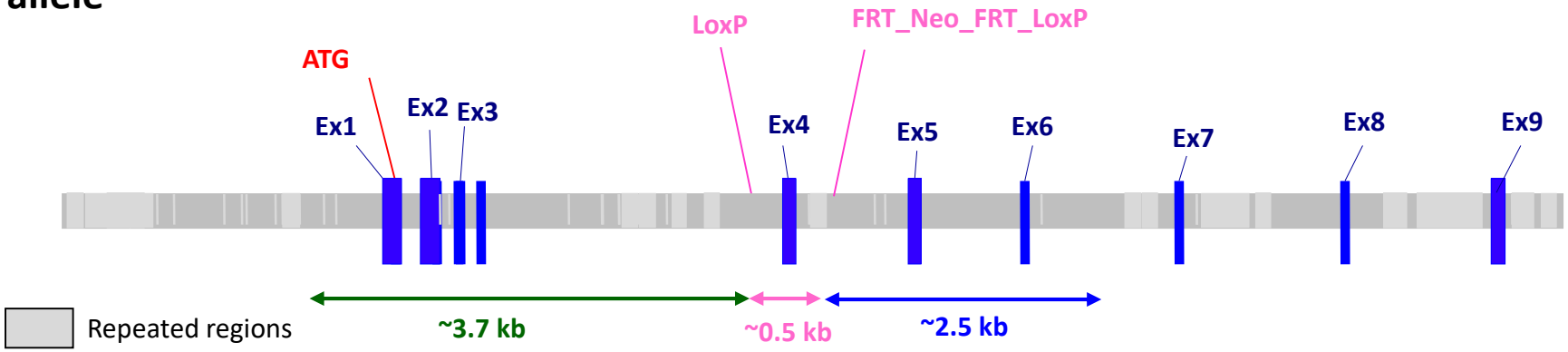
## Med23-001 ENSMUST00000092646



# Approach selected: flox exon 4



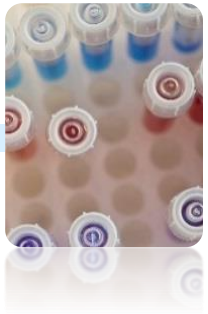
## Targeted allele




Ex4: ENSMUSE00001034764

## mRNA and protein obtained after Cre mediated excision (-001)

# PROs& CONs evaluation of the strategy



## Pros

- Appropriate size of the floxed fragment

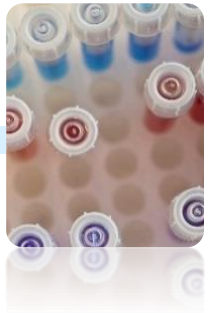
## Cons

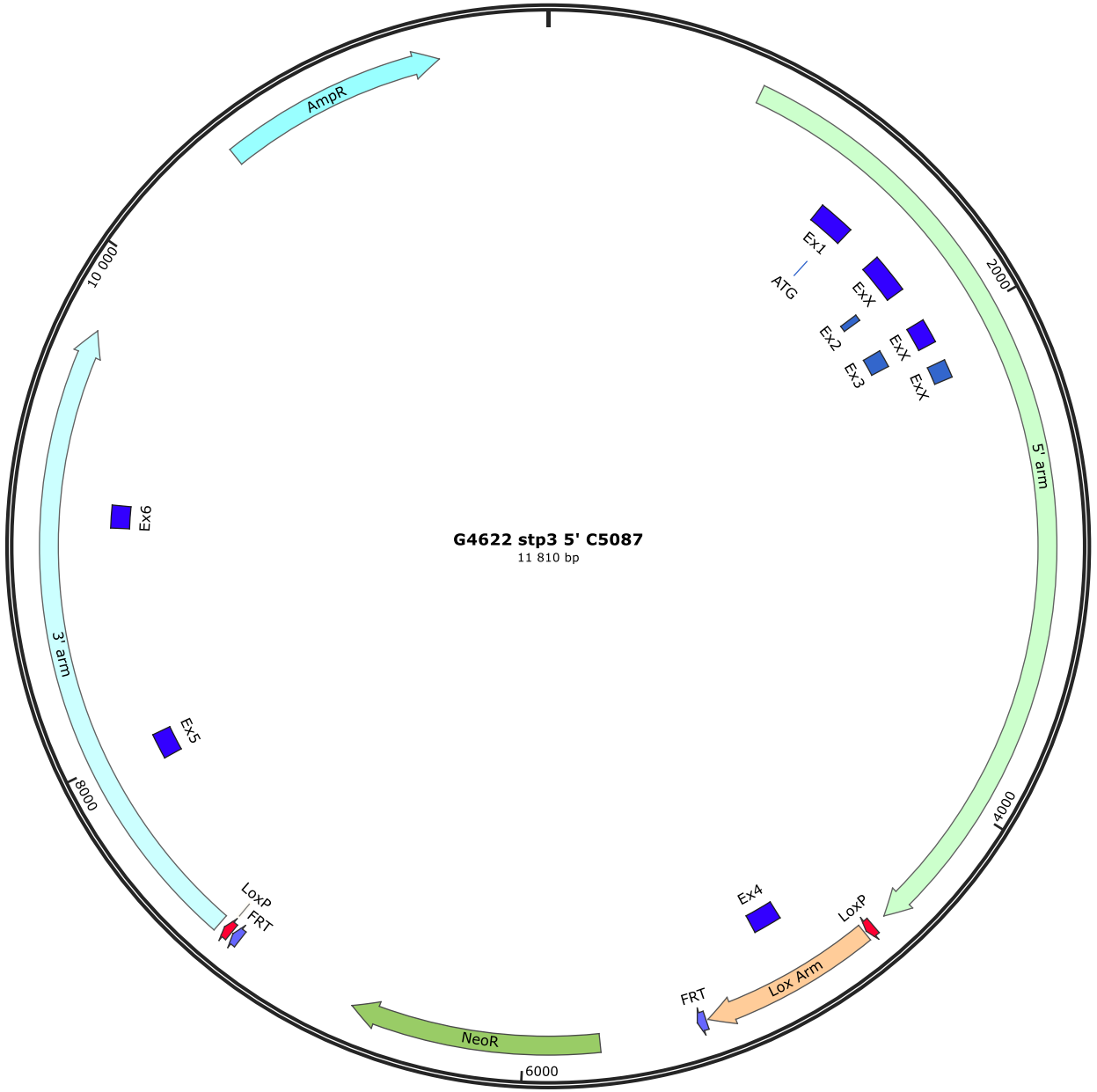
- A protein of 50 aa might be expressed after Cre mediated excision if RNA decay does not occur
- Presence of repeated regions (in light grey) in both homology arms (green and blue arrows) might render PCR amplification and/or homologous recombination at the locus difficult

The selection cassette (FRT-Neo-FRT) will be removed by breeding male chimera with a flp deleter line which shows maternal contribution (*Birling et al.*, 2012)

**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.

# 3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION



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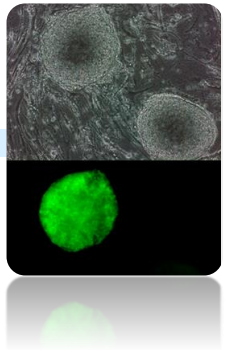
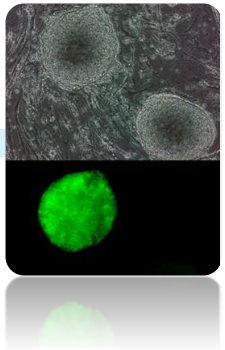
# 4 ES cell electroporation & Screening of recombinant clones



- 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



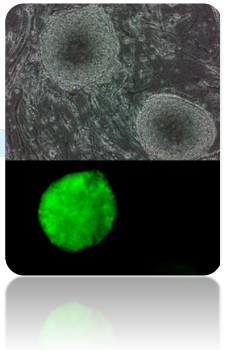
The targeting vector was electroporated in the proprietary C57BL/6N TB1 cell line.

Transfected ES clones were submitted to neomycin selection (G418) and 93 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.

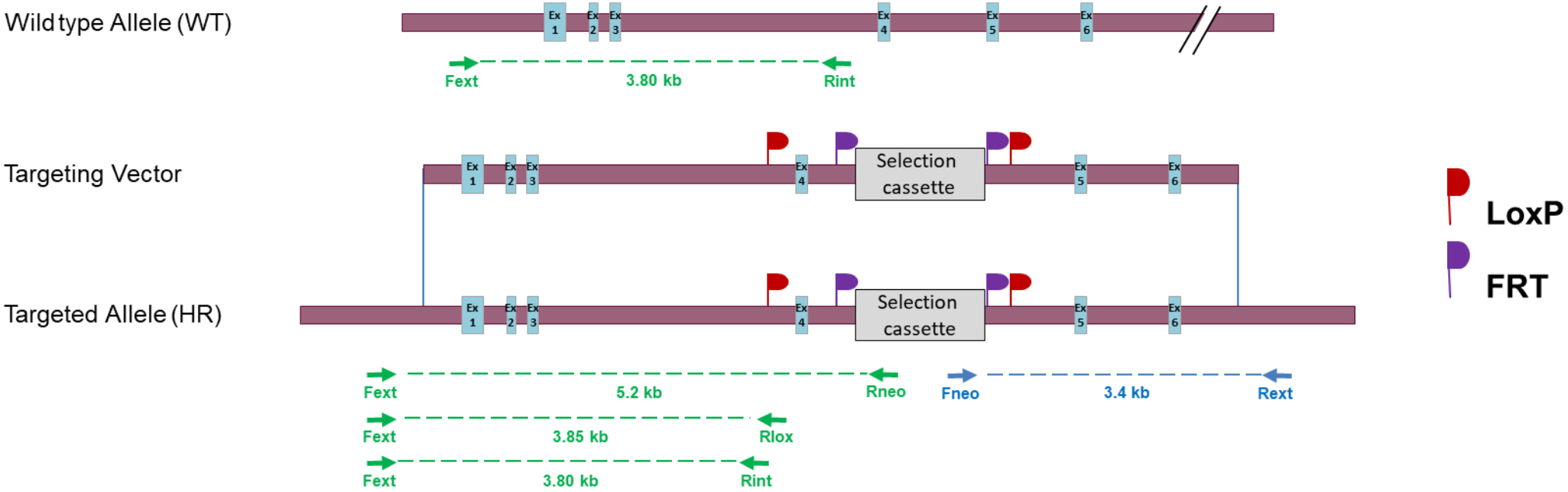
Screening process steps:

1. Identification of candidate recombinant clones by initial 5’ Long-Range PCR
2. Six of 5’ PCR positive clones are confirmed for 3’ recombination event by Long-Range PCR
3. Positive clones in step2 are further validated by Southern blot analysis using internal and external probes
4. The karyotype of at least 2 validated clones is verified 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 | AAGTTTCTGGAAGAAAACATTGACG | 5.2 kb |
| | Rneo | GCGGCCGGAGAACCTGCGTGCAATC | |
| 5’ PCR | Fext | AAGTTTCTGGAAGAAAACATTGACG | 3.85 kb |
| | Rlox | GTTATCTGCAGGTCGACCTTAAGCT | |
| 5’ PCR | Fext | AAGTTTCTGGAAGAAAACATTGACG | 3.80 kb |
| | Rint | CTTCCAGAGCATCCTGGGAGGATCT | |
| 3’ PCR | Fneo | AGGGGCTCGCGCCAGCCGAACTGTT | 3.4 kb |
| | Rext | CCACAAGGTATATGCACACATATGT | |

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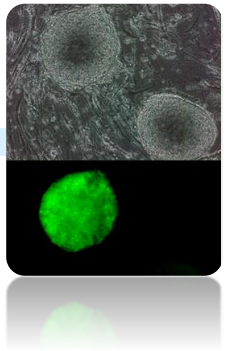
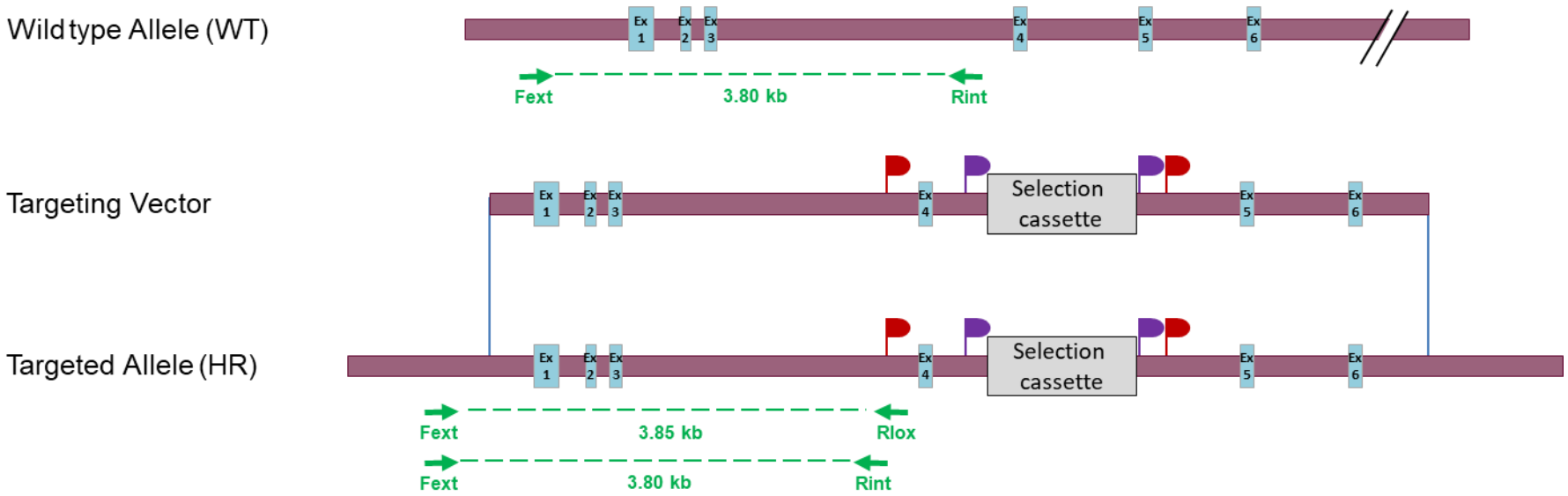
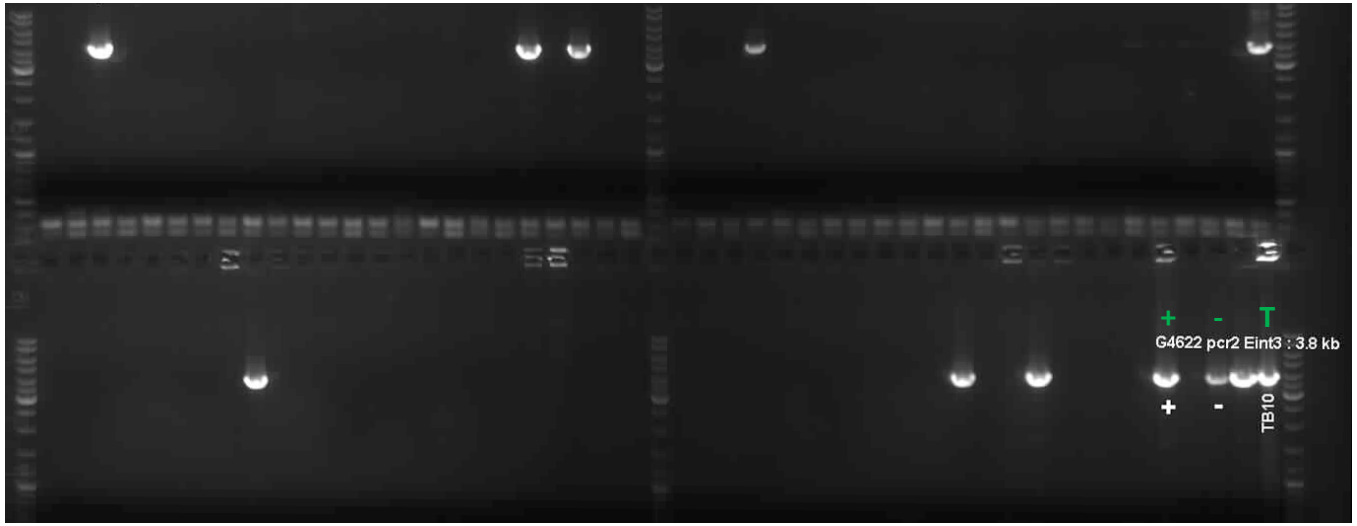
# Long-Range 5’ PCR screening – results



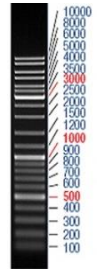



**PCR Fext – Rlox : 3.85 kb**




+ / - / T : Controls DNAs

**PCR Fext – Rint : 3.80 kb**




Ladder pattern

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

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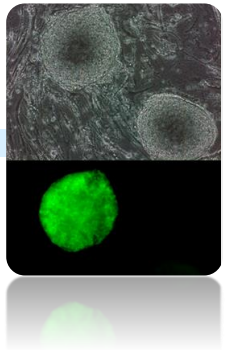
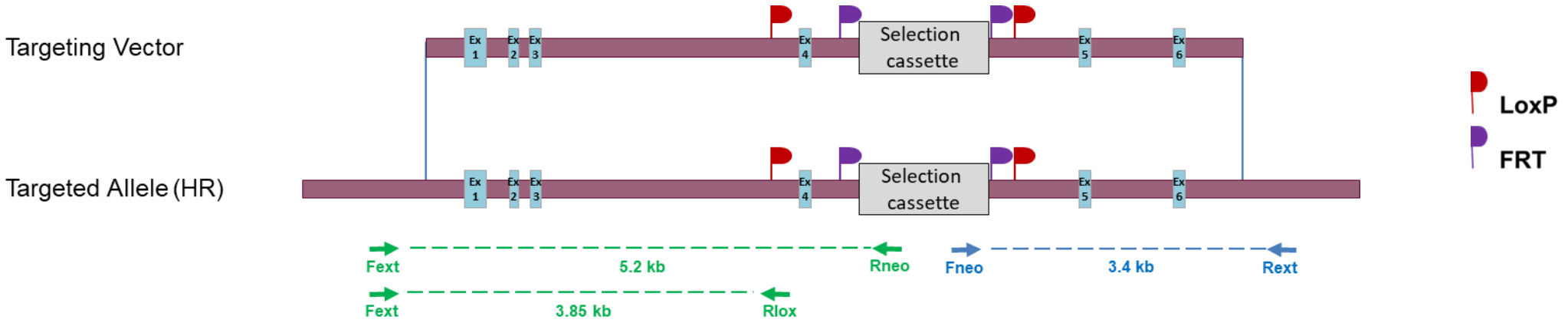
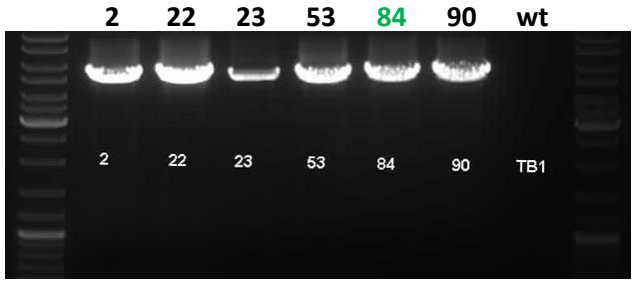
# Recombinant ES validation by Long Range PCR

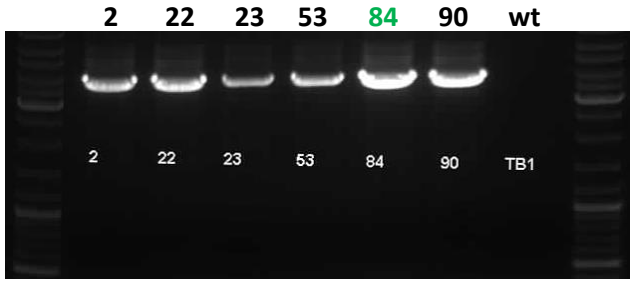


## Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

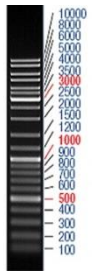


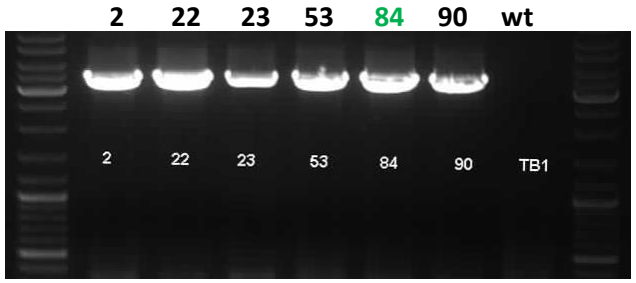

PCR Fext – Rneo : 5.2kb




PCR Fext – Rlox : 3.85 kb



Ladder pattern




PCR Fneo – Rext : 3.4 kb

Six candidate clones identified by 5’ PCR screening were further analysed by 3’ PCR screening.
Six clones (clones #2, #22, #23, #53, #84 and #90) were confirmed.

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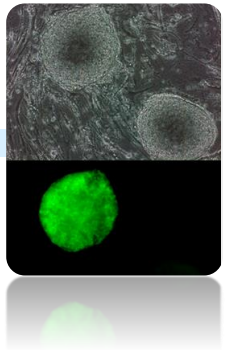
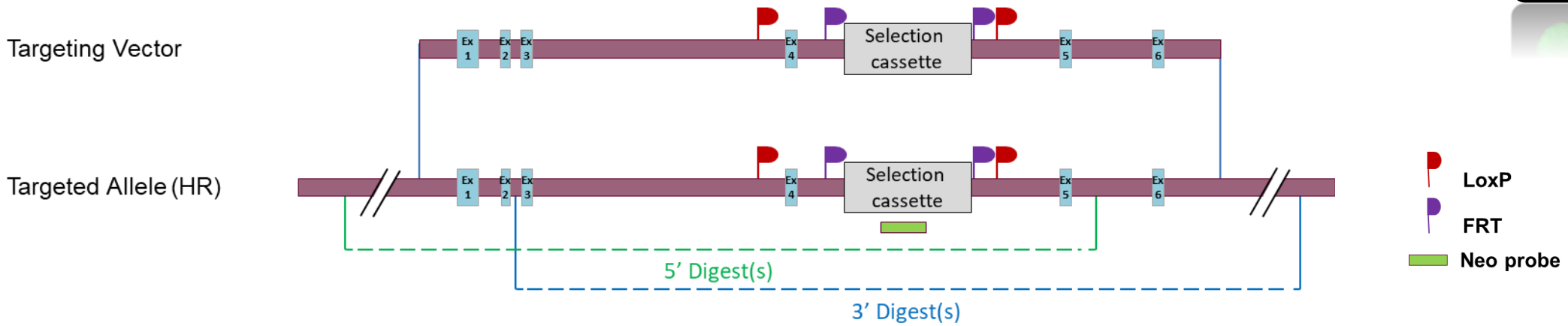
# Recombinant ES clones validation by Southern Blot – Internal probe



## Schematic Southern Blot validation strategy

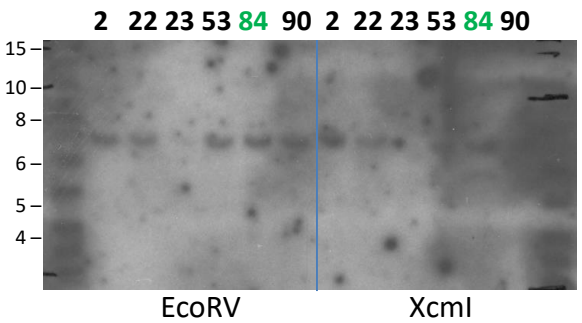
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



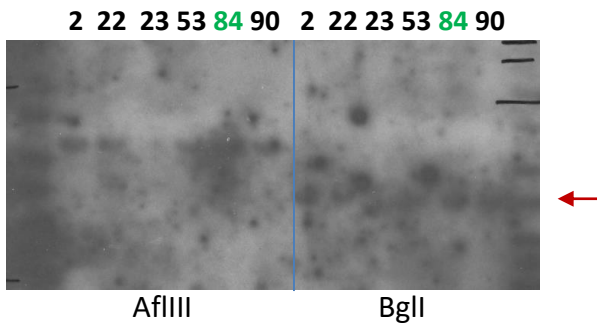

## Digestions used to validate the 5' and 3' insertion

| Probe | | Genomic DNA digest | Targeted Allele (kb) |
|---|---|---|---|
| Neo | 5' digest | EcoRV | 7.1 |
| | | XcmI | 7.5 |
| | 3' digest | AflIII | 6.9 |
| | | BglI | 5 |

### Southern blot - Neo 5'




### Southern blot - Neo 3'




### Neo probe sequence

CTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTC
ACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCT
GCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGAC
CACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGAC
GAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTC
GTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGT
GGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGC
GAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTT
CTTGACGAGTTCTTCTGAGGGGATCCGCTGTAAGTCT

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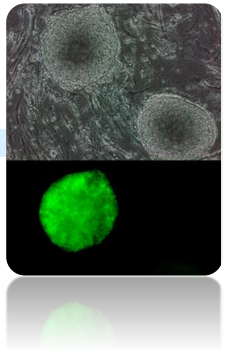
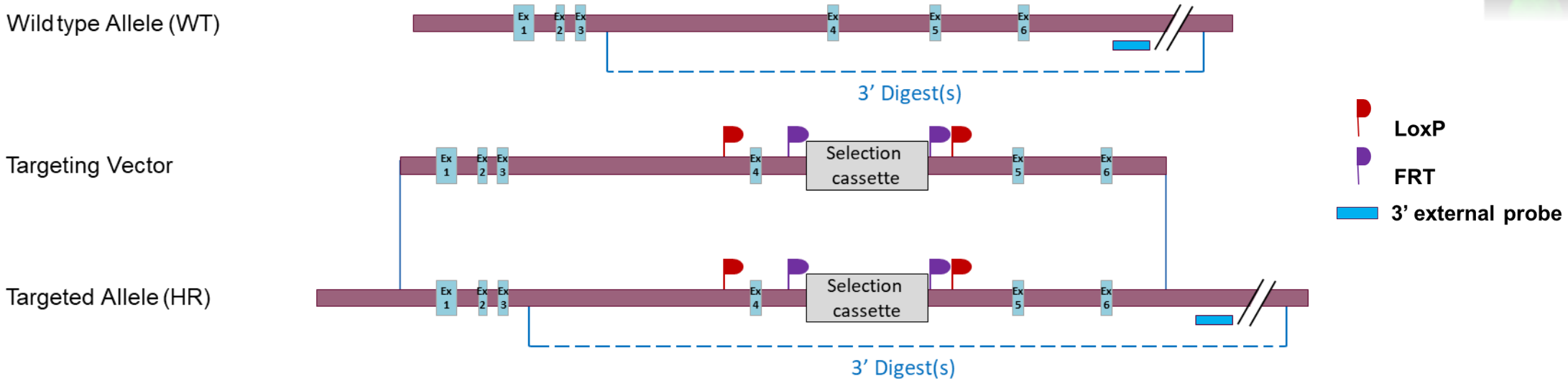
# Recombinant ES clones validation by Southern Blot – External probe

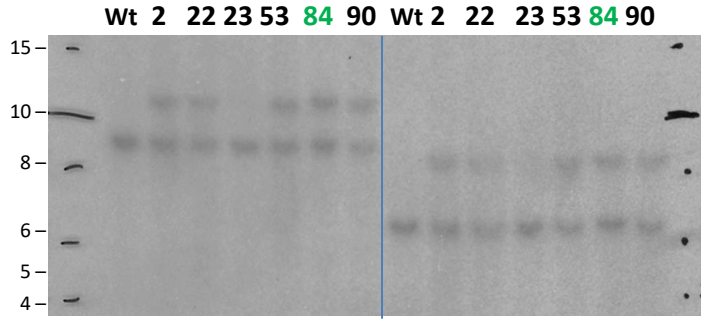


## Schematic Southern Blot validation strategy

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 – 3' probe




### 3' probe sequence

GCTCCCTCCCACACAGATACAAGCACACTTTGCCA
ACTAAGCTATTGTCCCAGCCCCATTTAGTCATTTG
TAAATATACTTTTTTGTTGTTGCCGTCGTTGTATT
TATAGGTTGTTTGTGTGAGCCTAGTCAAGAATAAT
GCTTTTTGCTTGTTTTAGGTTATAGCTTATATCTT
AGAACGAAATGCCTGCCTATTGCCGGCCTACTTTG
CAGTCACAGAGATCAGAAAACTATATCCTGAGGGA
AAACTTCCACACTGGG

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

| Probe | Name | Genomic DNA digest | WT allele (kb) | Targeted Allele (kb) |
|---|---|---|---|---|
| 3' external probe | 3' first digest | NheI | 9.1 | 11.2 |
| | 3' second digest | HincII | 6.6 | 8.6 |

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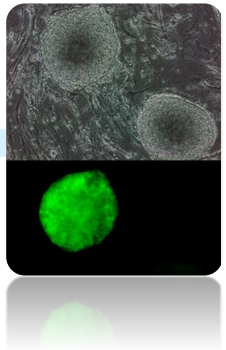
# Aneuploidy screening in ES recombinant clones



Selected recombinant ES cells clones were karyotyped by Giemsa metaphase staining. Results of aneuploidy analysis are presented in the table below.

| Clone ID | qPCR | Giemsa |
|---|---|---|
| **#2** | **Pass** | **Pass** |
| #22 | Pass | Not done |
| #53 | Pass | Failed |
| **#84** | **Pass** | **Pass** |
| #90 | Pass | 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**

# 5 MICROINJECTION & BREEDING

- Microinjection
- Breeding to F1 generation

# Microinjection

- The ES cells used in the injection experiment were originally derived from a C57BL/6N 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 #2 and #84 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 |
| #2 | 5 | 0 | 3 | 8 |
| **#84** | **2** | **0** | **3** | **5** |

# Breeding to F1 generation

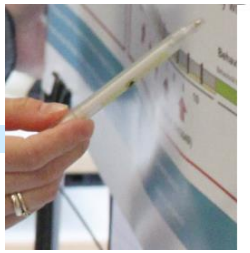
- Three highly chimeric males generated in the previous phase by blastocyst injection of the ES clones were mated with Flp deleted C57BL/6N females that show maternal contribution* (health status SPF – Specific Pathogen Free) to investigate whether the recombined ES cells have contributed to the germ layer.

- Germ line transmission was obtained the : 18/06/2014

- Allele nomenclature (following MGI guidelines) : **Med23$^{tm1.1Ics}$**

***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.

# 6 SEQUENCE OF THE ALLELE



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**REPORT REDACTION & VALIDATION**

Protocol finalized on 2023/10/06

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