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EXCELLENCE IN MOUSE PHENOGENOMICS

MODEL GENERATION TECHNICAL REPORT

Generation of mouse model : Mir137 conditional Knock-Out

Project code: G17 / IR3731

Report updated: 07/09/2023

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

1 PROJECT PROCESS & QUALITY CONTROLS

2 GENETIC STRATEGY

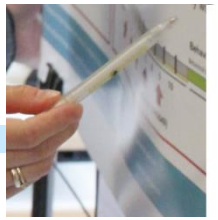
3 HOMOLOGOUS RECOMBINATION VECTOR CONSTRUCTION

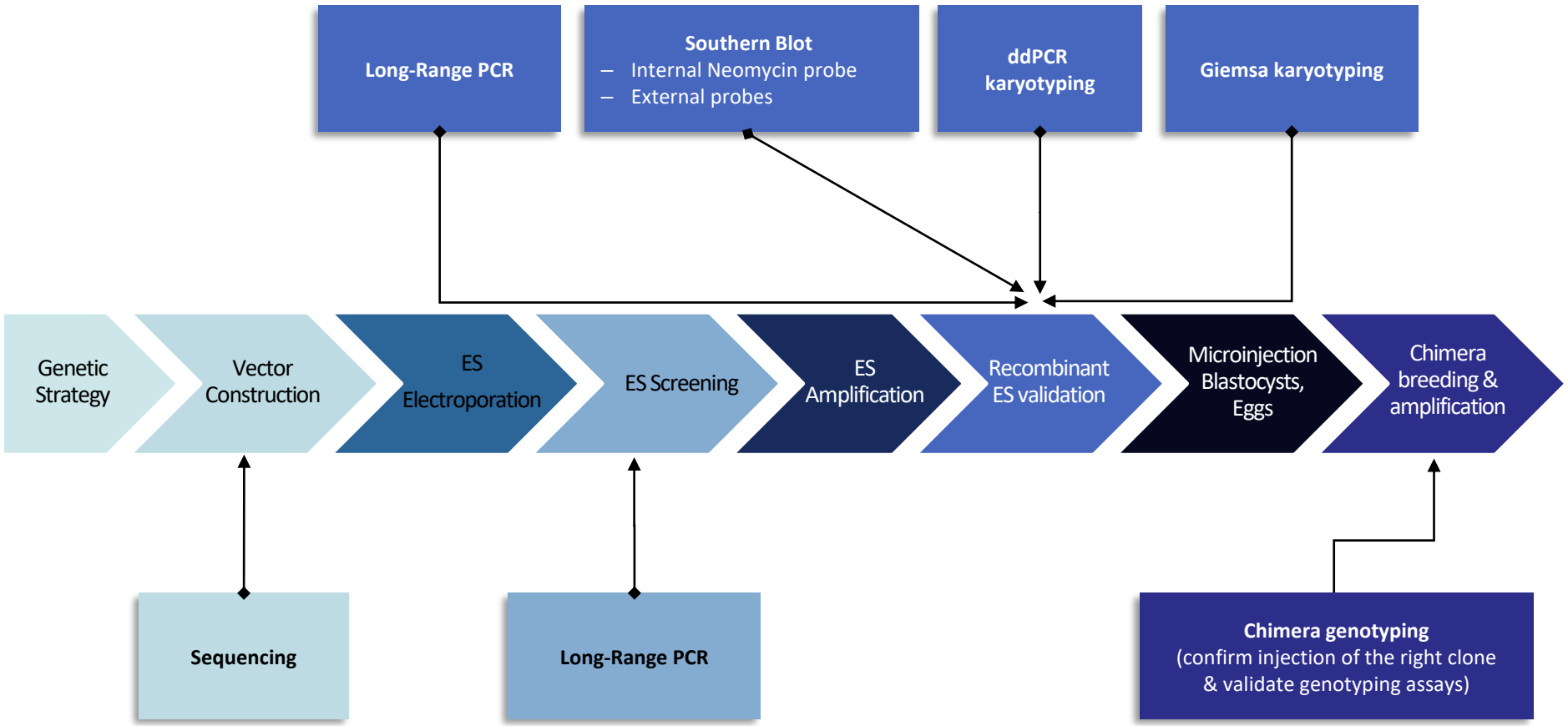
4 ES ELECTROPORATION & SCREENING OF RECOMBINANT CLONES

5 MICROINJECTION & BREEDING

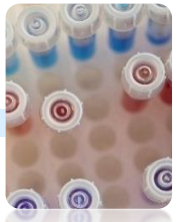
6 SEQUENCE OF THE DELIVERED ALLELE

PROJECT PROCESS & QUALITY CONTROLS



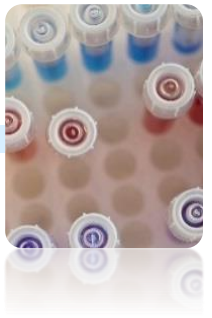


2 GENETIC STRATEGY

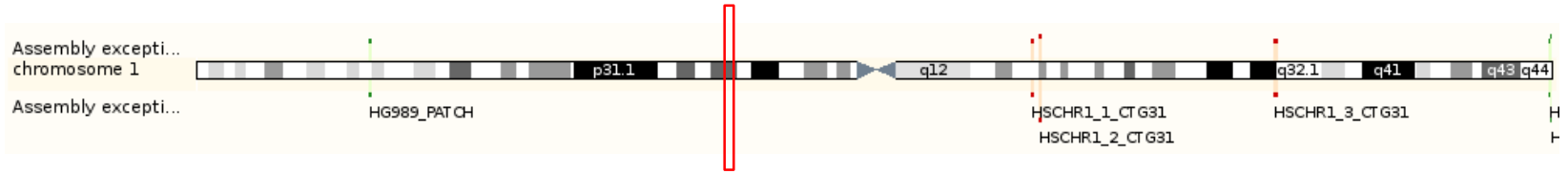


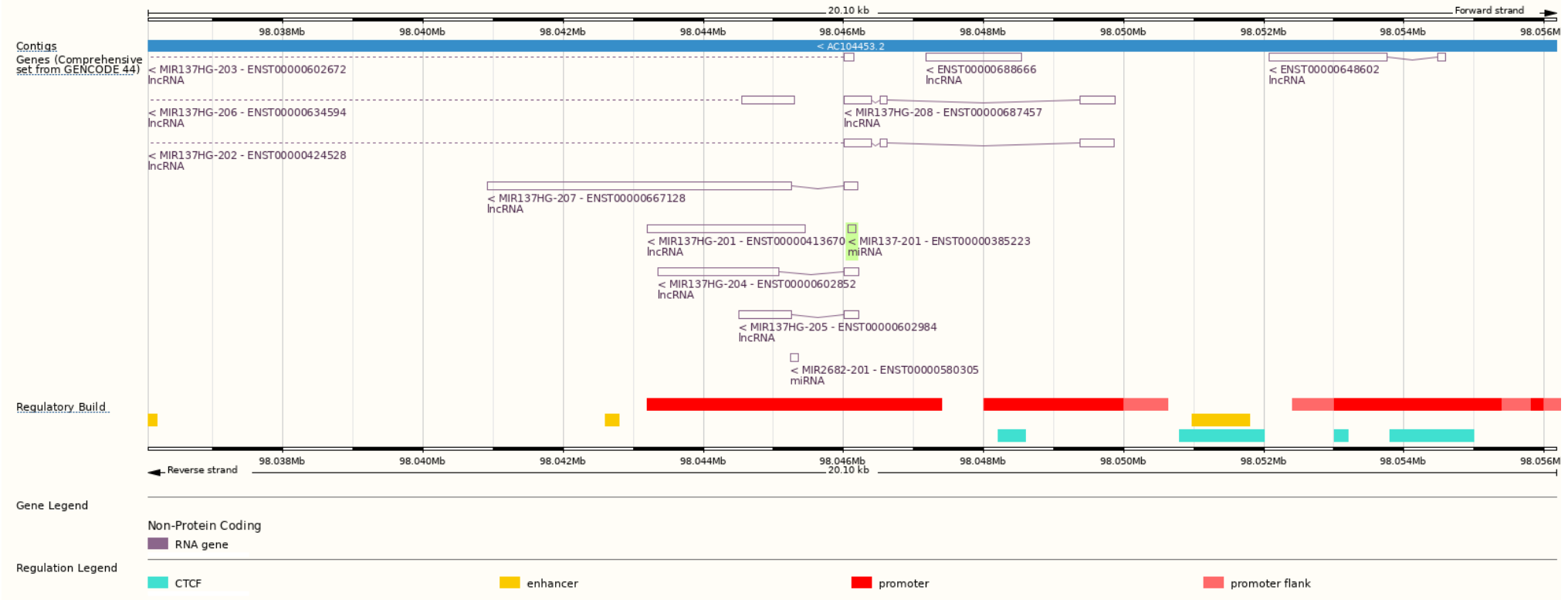
- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

Human MIR-137 genomic locus – structure

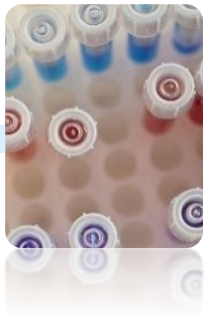


Chromosome 1: 98,042,735-98,049,518

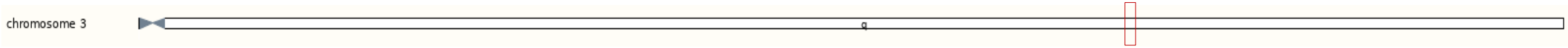




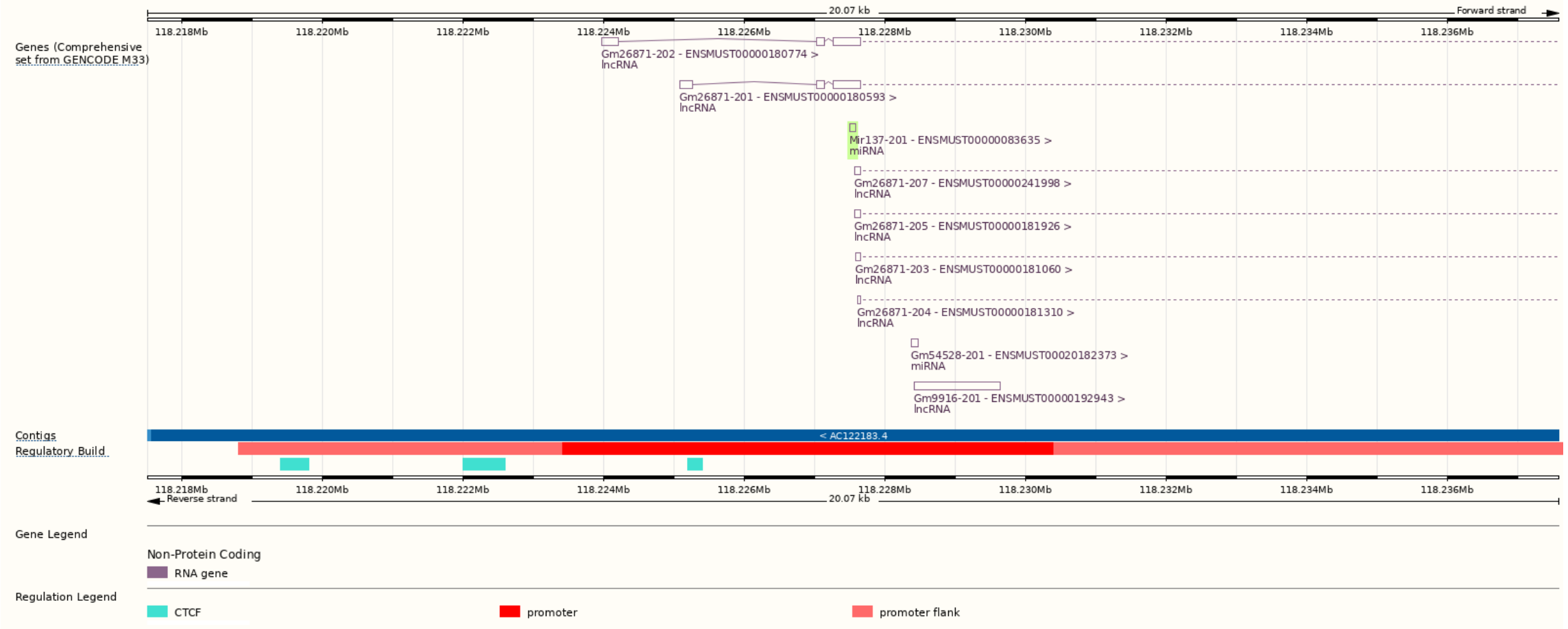
Mir-137 mouse genomic locus – structure



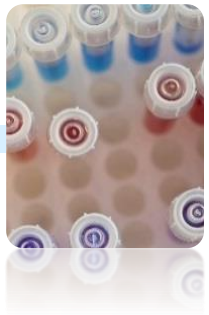
Chromosome 3: 118,227,506-118,227,578



Gene: Mir137 ENSMUSG00000065569

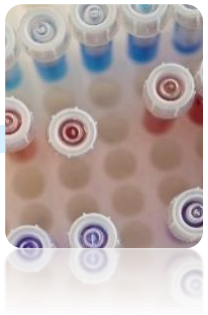


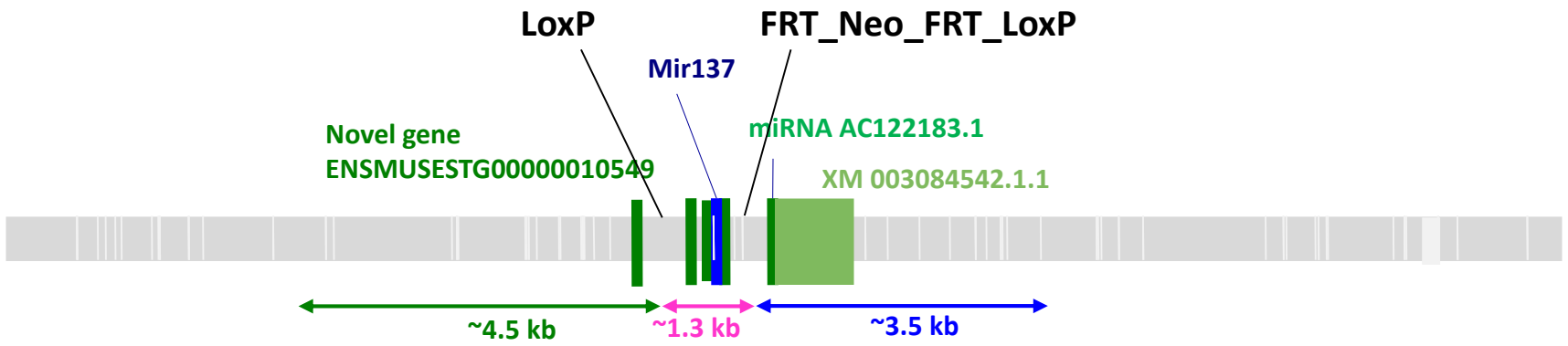
Mir137 mRNA and protein



Transcript ID	Name	bp	Protein	Biotype	CCDS
ENSMUST00000083635.4	Mir137-201	73	No protein	miRNA	

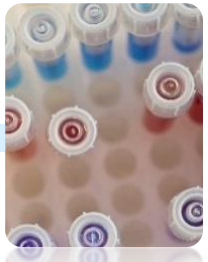
Strategy





Repeated regions

PROs& CONs evaluation of the strategy



Pros

- miR137 will be deleted after Cre mediated excision

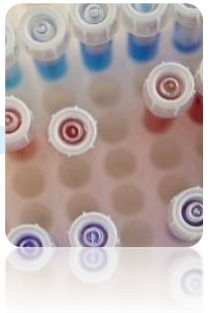
Cons

- *Other putative genes (EST and microRNA) might be disturbed before and after Cre mediated excision. These new genes have recently be described in mouse*

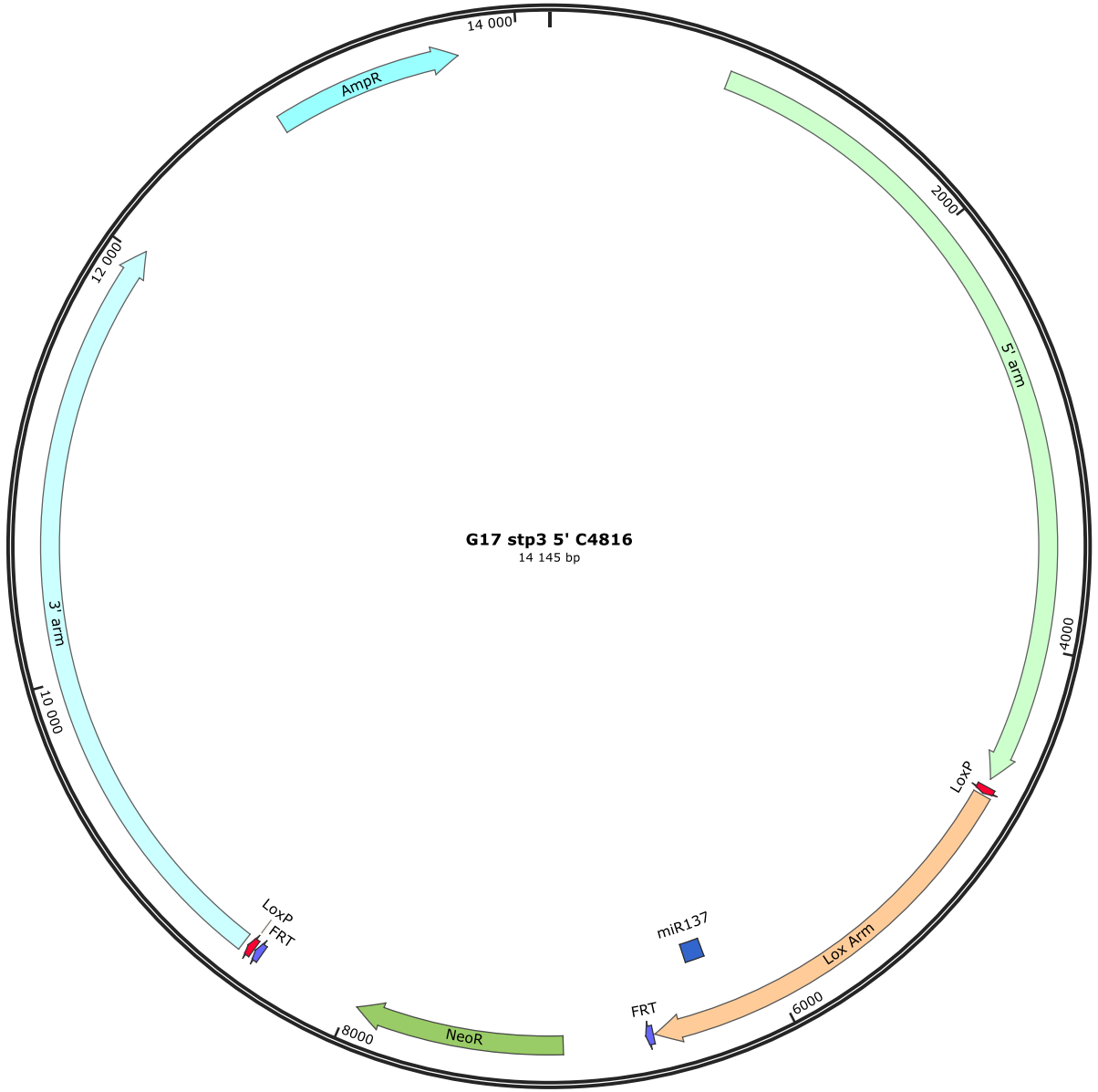
The selection cassette (FRT-Neo-FRT) will be removed by breeding male chimera with a flp deleter line which shows maternal contribution (*Birling et al.*, 2012)

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



Created by SnapGene



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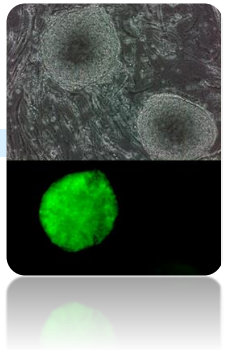
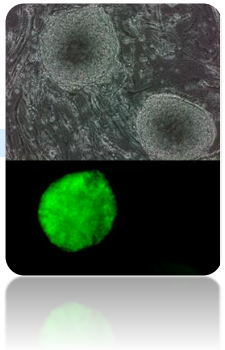
4 ES cell electroporation & Screening of recombinant clones



- 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



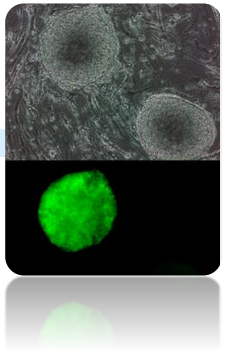
The targeting vector was electroporated in the proprietary C57BL/6N BD10 cell line.

Transfected ES clones were submitted to neomycin selection (G418) and 104 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.

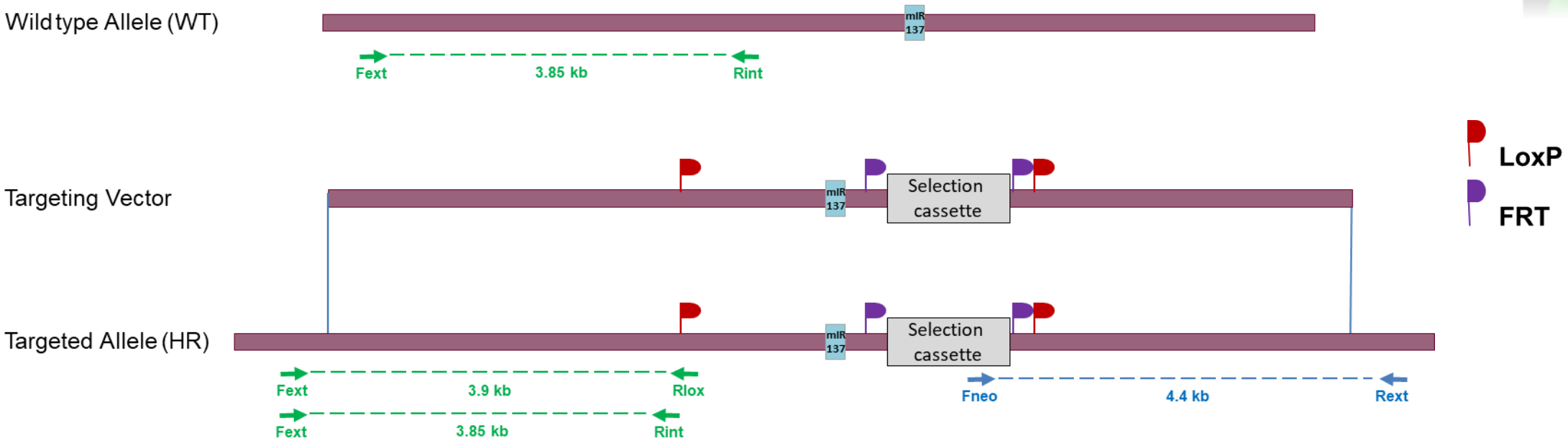
Screening process steps:

1. Identification of candidate recombinant clones by initial 5’ Long-Range PCR
2. Seven 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 probe
4. The karyotype of at least 2 validated clones is verified using ddPCR aneuploidy screening and Giemsa staining

Long range PCR screening – strategy

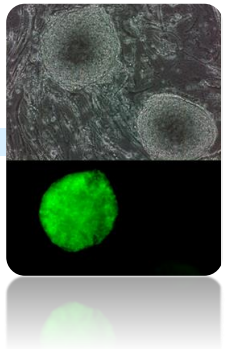


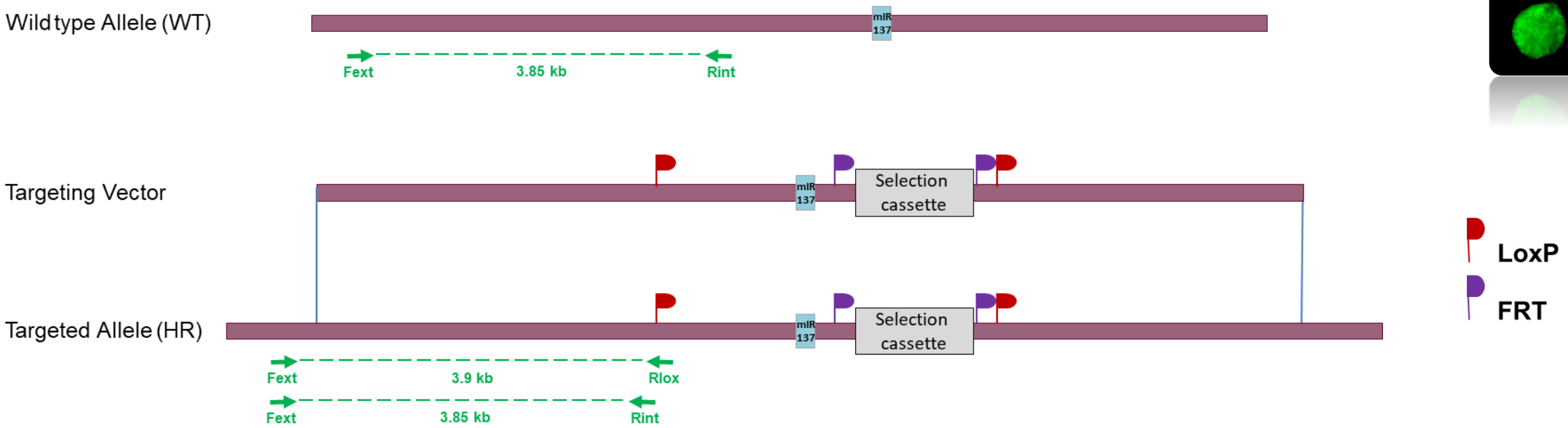
Schematic 5’ and 3’ PCR screening strategy



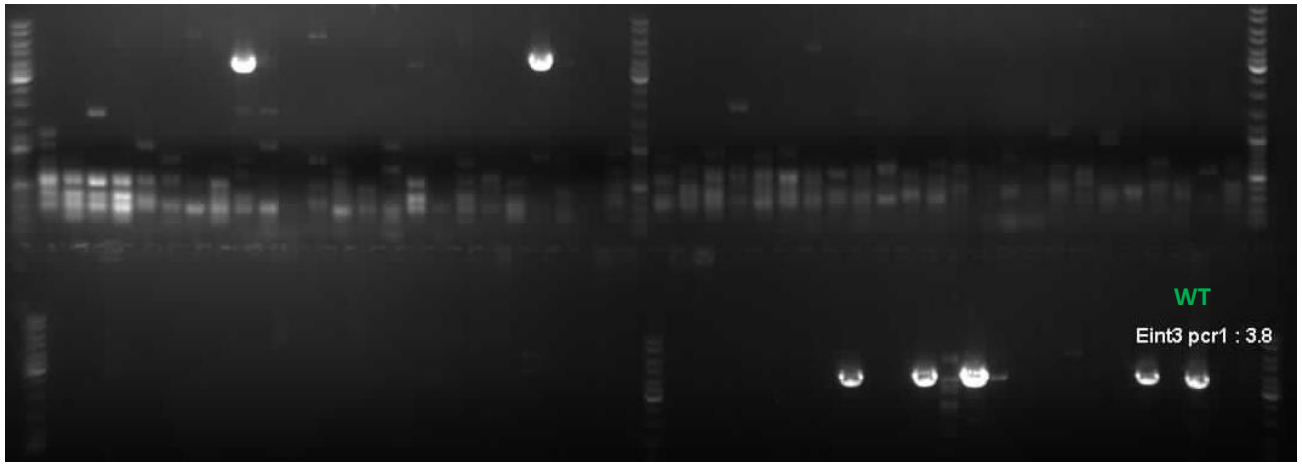
PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	AGGACAGACAGTGTTGAGTGCACC	3.9 kb
	Rlox	GTTATCTGCAGGTCGACCTTAAGCT	
5’ PCR	Fext	AGGACAGACAGTGTTGAGTGCACC	3.85 kb
	Rint	CCTATCTTTGCAGTTATCTTTTTAG	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	4.4 kb
	Rext	CAAAGATGGCTACAGCAGTTTAGTG	

Long-Range 5’ PCR screening – results

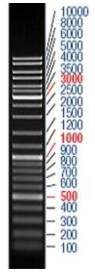




PCR Fext – RLox : 3.9 kb



WT : Controls DNA



Ladder pattern

PCR Fext – Rint : 3.85 kb

Seven candidate clones out of the 7 positive clones were selected for 3’ Long-Range PCR and Southern blot validation.

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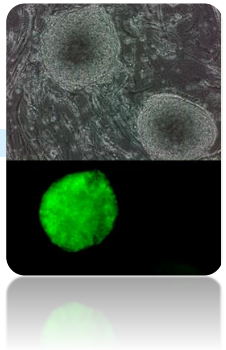
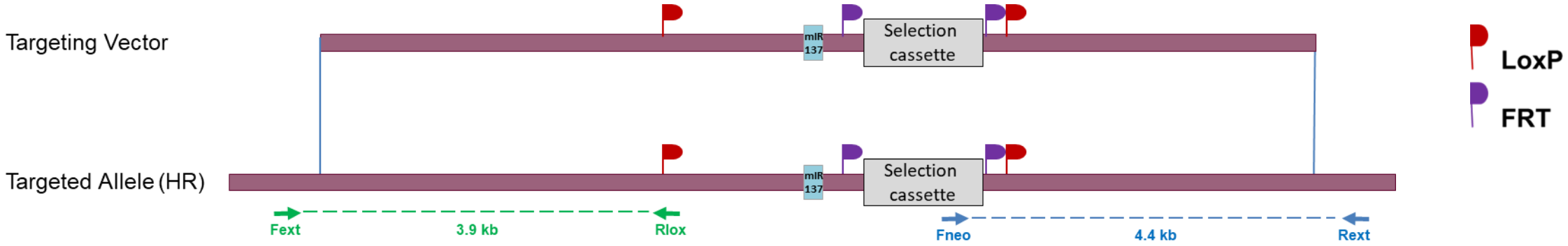
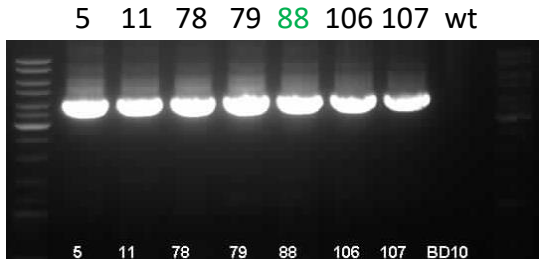
Recombinant ES validation by Long Range PCR

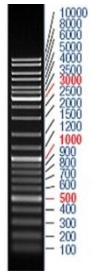


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

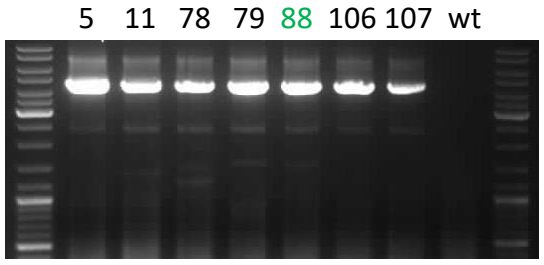




Pcr Fext – Rlox : 3.9 kb



Ladder pattern



Pcr Fneo – Rext : 4.4 kb

Seven candidate clones identified by 5’ PCR screening were further analysed by 3’ PCR screening.
Seven clones (clones #5, #11, #78, #79, #88, #106 and #107) were confirmed.

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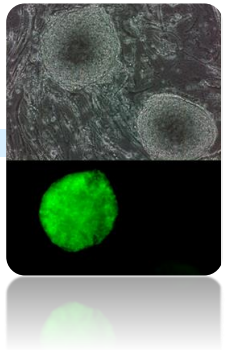
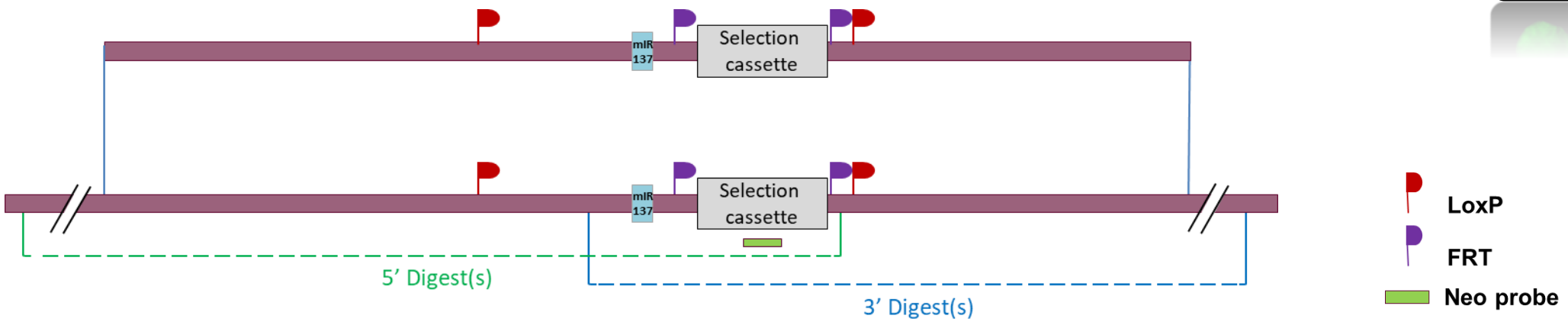
Recombinant ES clones validation by Southern Blot – Internal probe



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.

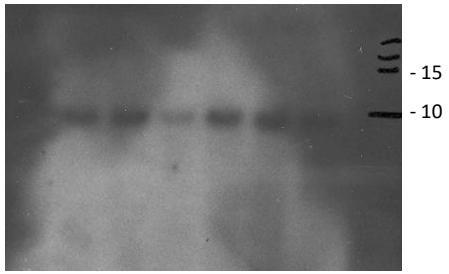


Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest		
	3' digest	AseI	7.8

Southern blot - Neo 5'

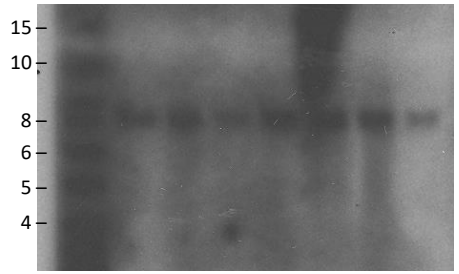
5 11 78 79 88 106 107



DraIII

Southern blot - Neo 3'

5 11 78 79 88 106 107



AseI

Neo probe sequence

CTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTG
CTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGAT
CTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGG
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GCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGG
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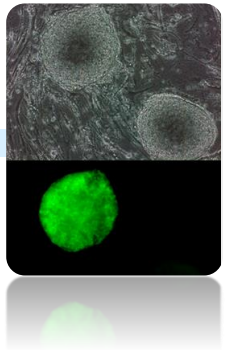
ATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGAT
ATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCT
CCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTC

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	Giemsa
#5	Not done
#11	**Pass**
#78	Not done
#79	Failed
#88	**Pass**
#106	Not done
#107	Not done

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 #11 and #88 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
#11	1	2	2	5
#88	1	0	4	5

Breeding to F1 generation

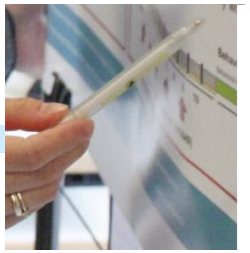
- Four highly chimeric males generated in the previous phase by blastocyst injection of the ES clones were mated with wild-type C57BL/6NCrl females (health status SPF – Specific Pathogen Free) to investigate whether the recombined ES cells have contributed to the germ layer.

- Germ line transmission was obtained with clone #88 the 5/12/2012.

- The line issued from clone #88 was cryopreserved

- Allele nomenclature (following MGI guidelines) :**Mir37$^{tm1.1Ics}$**

6 SEQUENCE OF THE FROZEN ALLELE



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GCTCGTGACTTCGCAAATCTCTGGCCTGGGGCGGAGCTGGAGCCCCACCCGCGACCCGCCGAGCTGCTCAGCAAGCAGACATGGAAACCTTGCTAAGGAAGGGGCGGGGAACAGGGGGCTGGGTCCCGGT
GGAACTTTCTGATTACAAGTGTTGTGGGTCTCTGAGGAACCCCTATTTTCTTTTTGGATTCTCCGCGAAGATGTGGAGA

LoxP FRT mIR137

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EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION

Report finalized on 2023/09/07

Prepared by Romain LORENTZ, IE

Verified and finalized by Marie-Christine BIRLING, PhD

CONTACT US

By email at mutagenesis@igbmc.fr

By phone at +33 (0)3 88 65 56 57

www.phenomin.fr