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EXCELLENCE IN MOUSE PHENOGENOMICS

MODEL GENERATION TECHNICAL REPORT

Generation of mouse model :

Phf8 Conditional Knot-out

Project code: G25 / IR00004556

Report finalized: 19/09/2023

cnrs
Université de Strasbourg
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CERBM centre européen de recherche en biologie et en médecine gie
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MODEL GENERATION TECHNICAL REPORT

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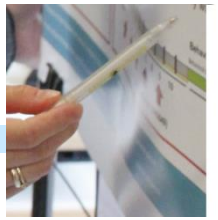
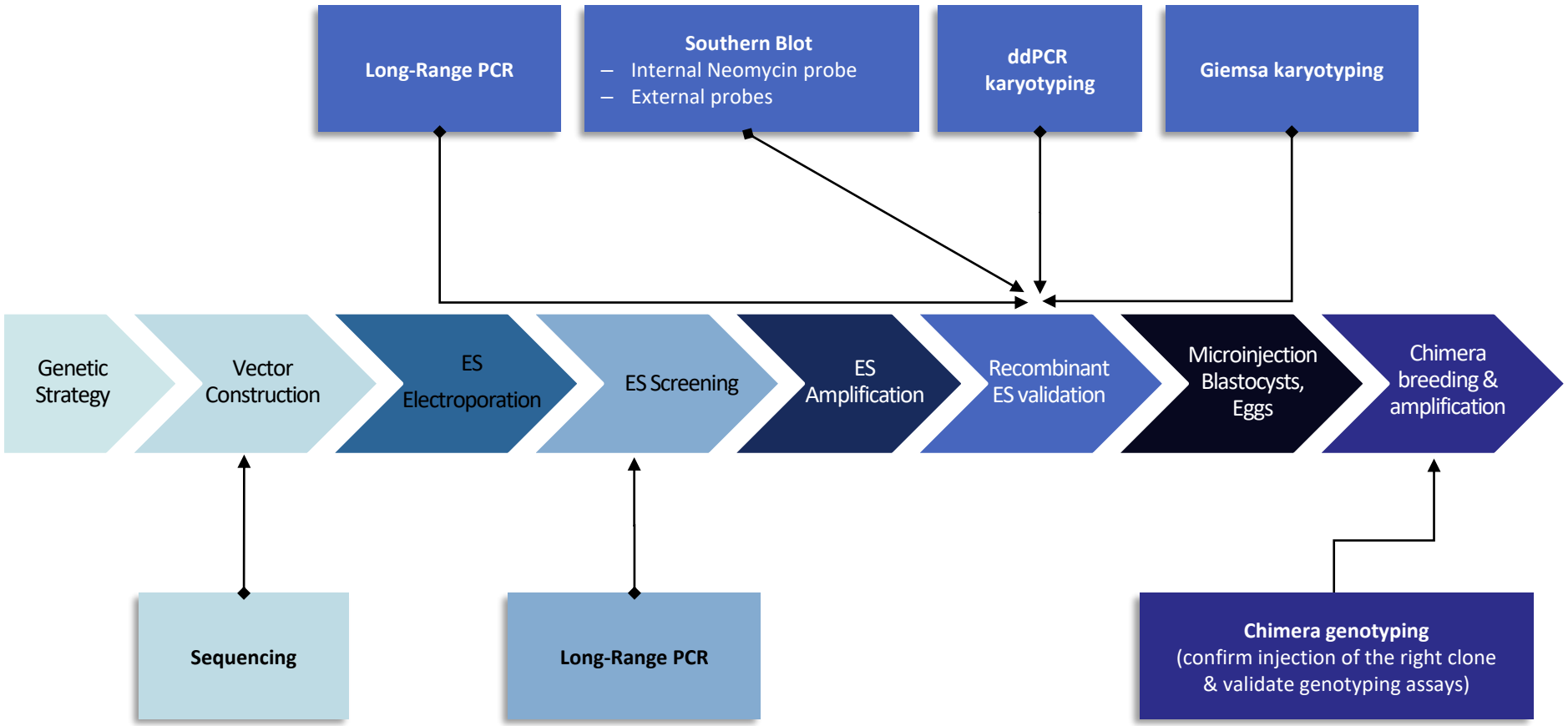
4 ES ELECTROPORATION & SCREENING OF RECOMBINANT CLONES

5 MICROINJECTION & BREEDING

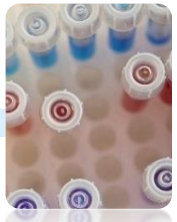
6 SEQUENCE OF THE DELIVERED ALLELE

PROJECT PROCESS & QUALITY CONTROLS



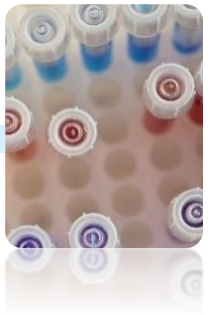


2 GENETIC STRATEGY

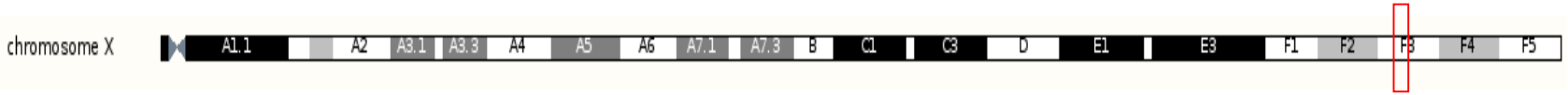


- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

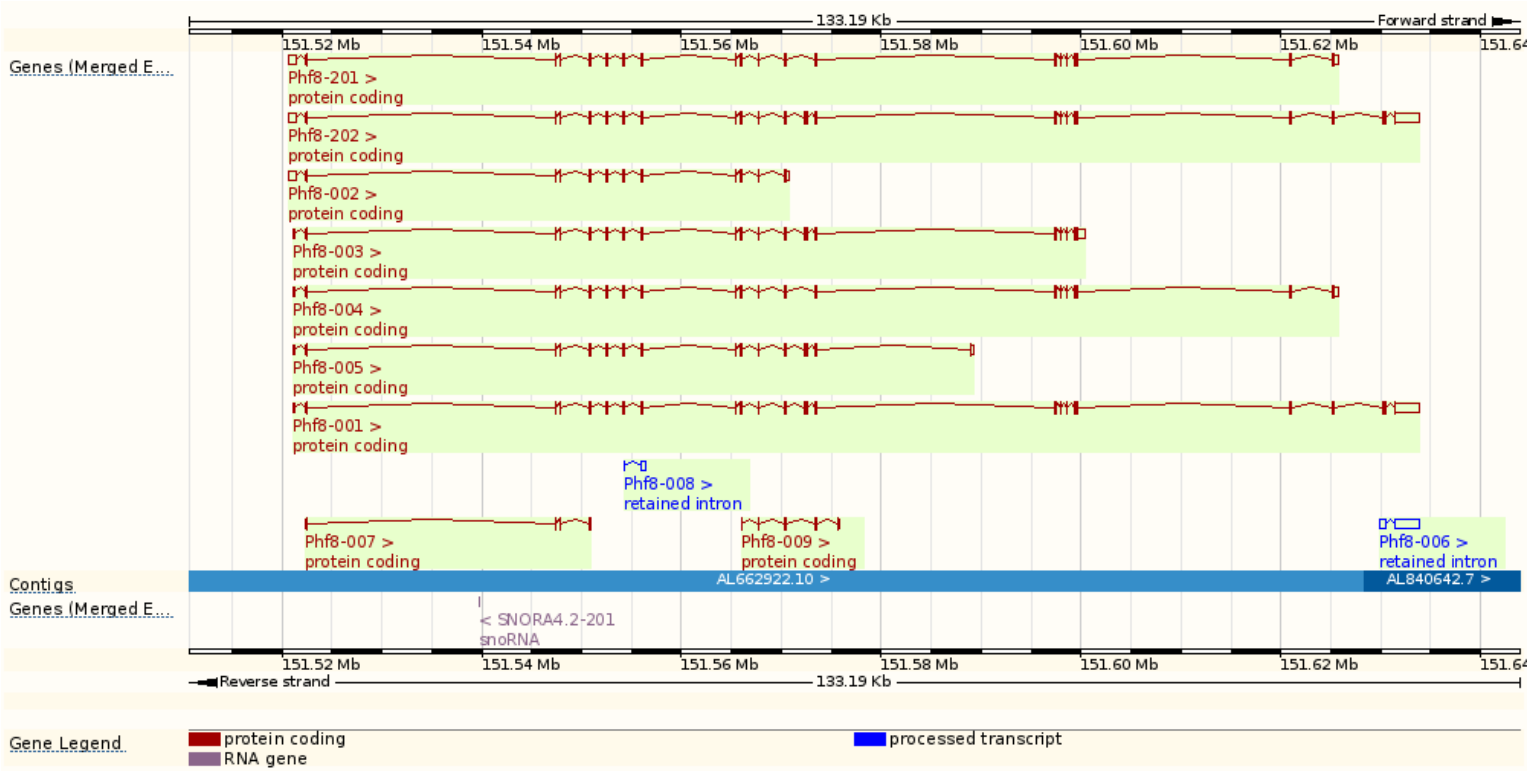
Phf8 mouse genomic locus – structure



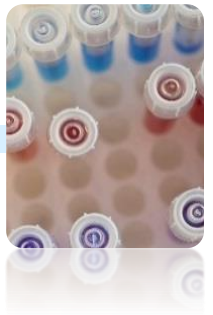
Location:



Ensembl Gene ID: ENSMUSG00000041229

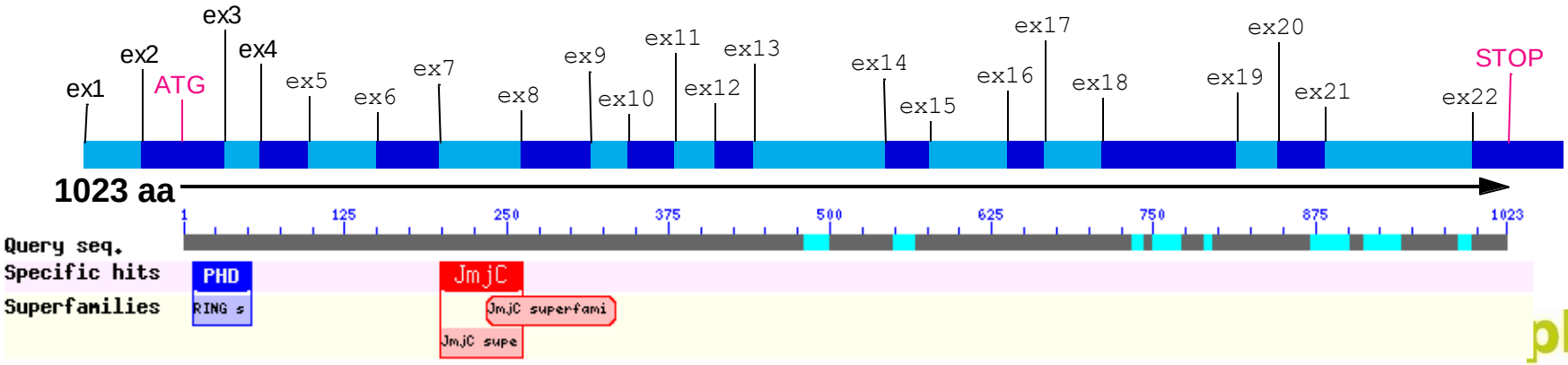


Phf8 mRNAs and proteins

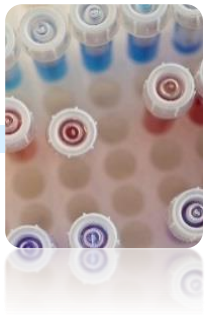


Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CDS incomplete	CCDS
Phf8-001	ENSMUST00000046950	5728	ENSMUSP00000040765	1023	Protein coding	-	CCDS53223
Phf8-002	ENSMUST00000112666	2556	ENSMUSP00000108285	464	Protein coding	-	-
Phf8-003	ENSMUST00000112670	3484	ENSMUSP00000108289	820	Protein coding	-	-
Phf8-004	ENSMUST00000046962	3105	ENSMUSP00000041312	795	Protein coding	-	CCDS30470
Phf8-005	ENSMUST00000112668	2291	ENSMUSP00000108287	602	Protein coding	-	-
Phf8-006	ENSMUST00000138318	3191	No protein product	-	Retained intron	-	-
Phf8-007	ENSMUST00000148622	573	ENSMUSP00000122974	151	Protein coding	3'	-
Phf8-008	ENSMUST00000141715	572	No protein product	-	Retained intron	-	-
Phf8-009	ENSMUST00000151941	516	ENSMUSP00000116792	124	Protein coding	5'	-
Phf8-201	ENSMUST00000112662	3732	ENSMUSP00000108281	795	Protein coding	-	CCDS30470
Phf8-202	ENSMUST00000168501	6359	ENSMUSP00000127653	1023	Protein coding	-	CCDS53223

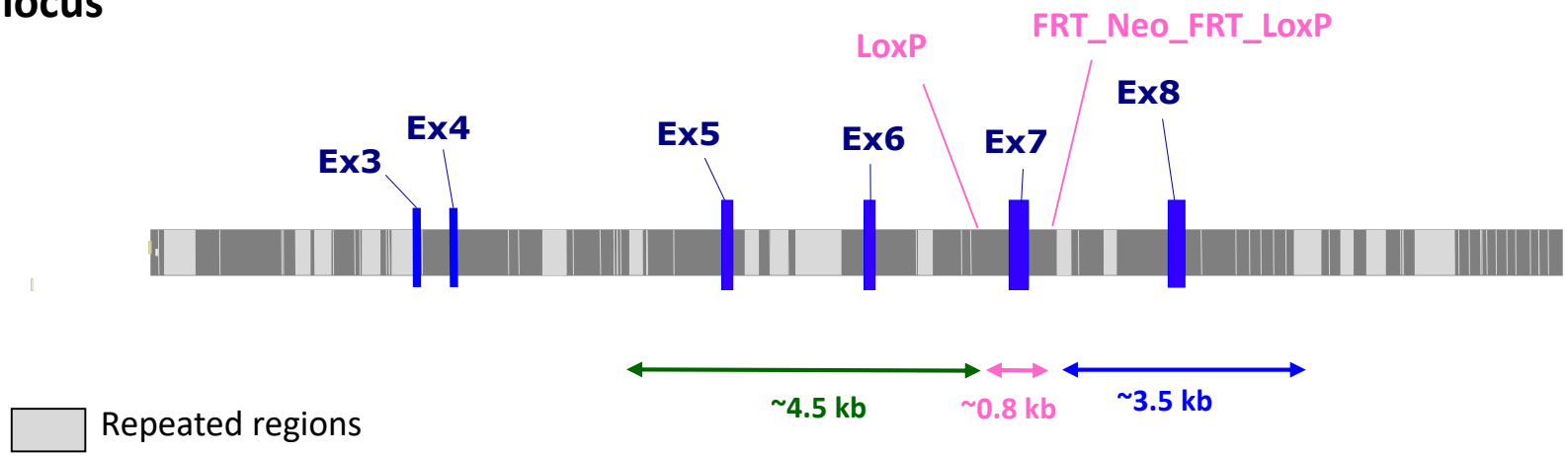
Phf8-001 ENSMUST00000046950



Approach selected: flox Ex7 (7/11/2012)

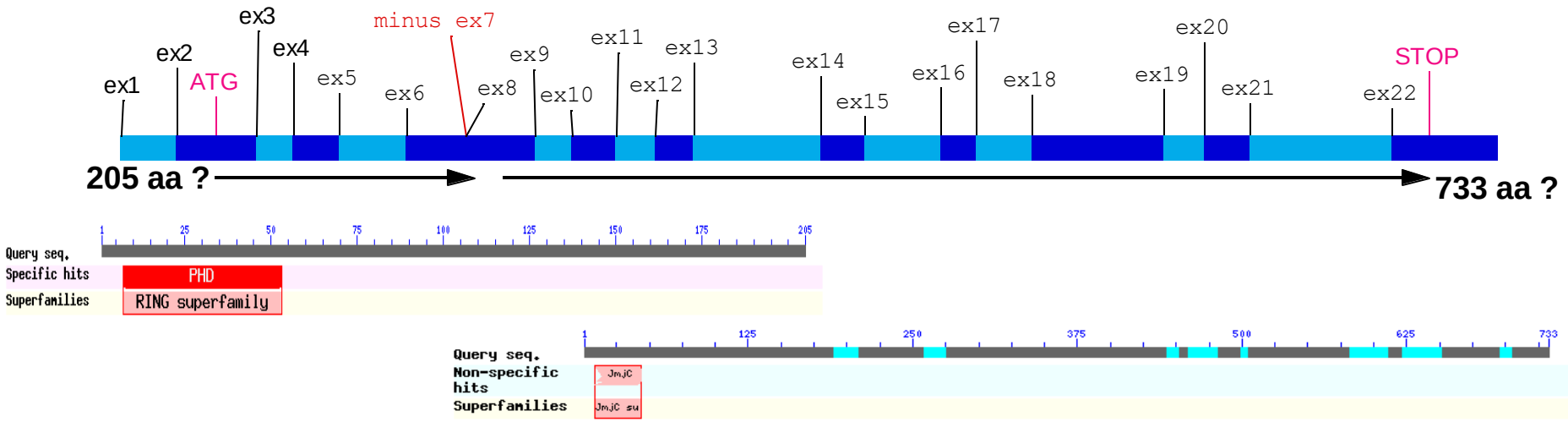


Targeted locus



Exon 7 Ensembl ID:
ENSMUSE00000246773

mRNA and protein expected after Cre mediated recombination



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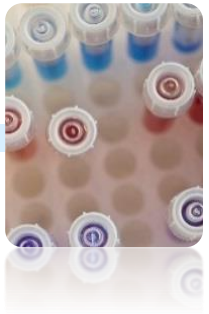
PROs& CONs evaluation of the strategy



Pros

- The JmjC domain will be disrupted after Cre mediated excision
- Reasonnable size of the floxed fragment

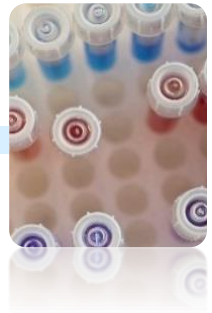
Cons

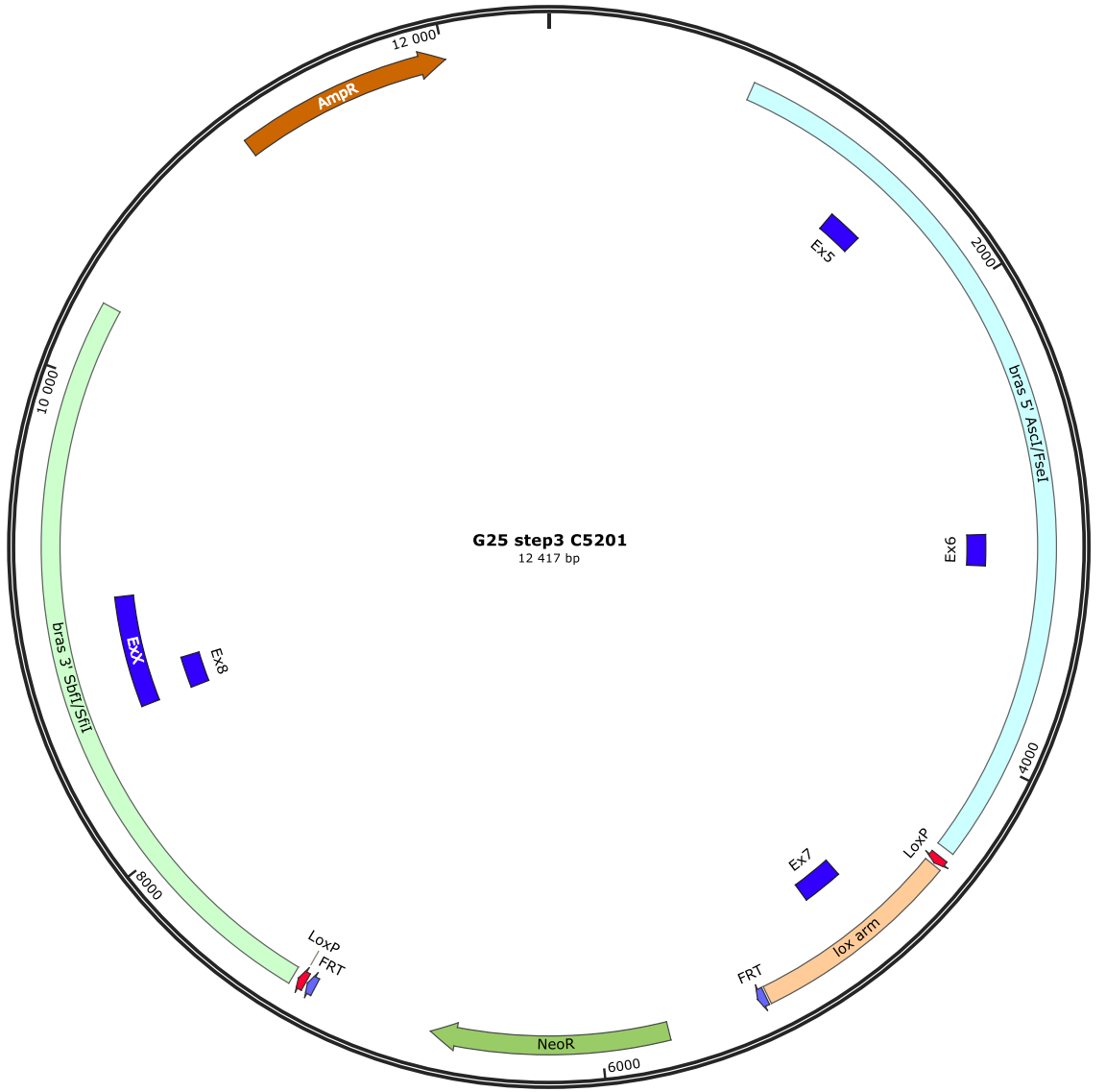
- A protein of 205 aa might be expressed after Cre mediated excision if RNA decay does not occur
- A protein of, at most, 733 aa might be expressed if reinitiation occur at one of the in frame ATG present in exon 8 or further exons (if RNA decay does not occur)
- Presence of repeated sequences (in yellow) might render PCR amplification or PCR screening 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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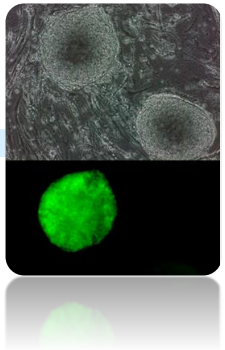
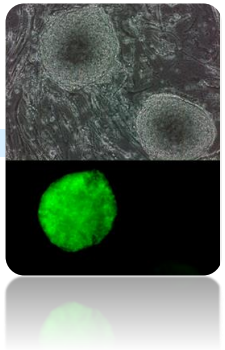
4 ES cell electroporation & Screening of recombinant clones



- 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
- Aneuploidy screening in ES recombinant clones

Electroporation and screening process



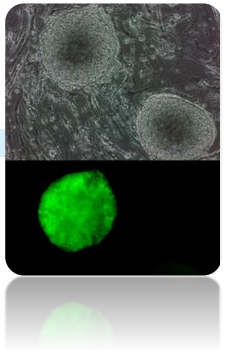
The targeting vector was electroporated in the proprietary C57BL/6NTac TB1 ES cell line.

Transfected ES clones were submitted to neomycin selection (G418) and 81 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.

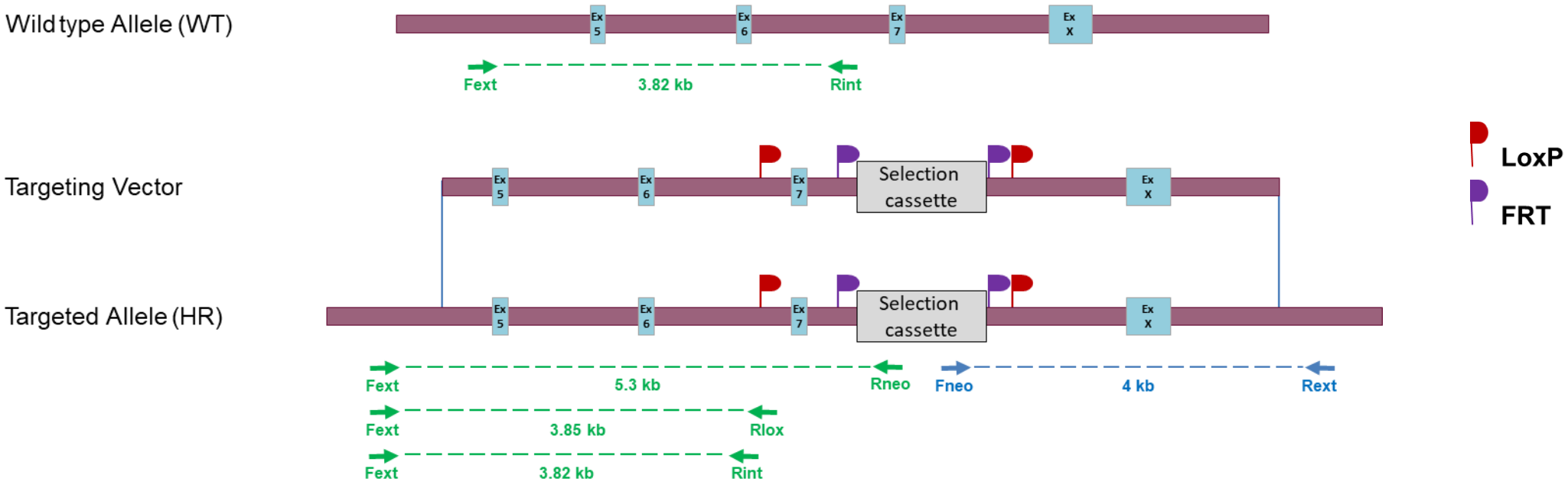
Screening process steps:

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 step 2 are further validated by Southern blot analysis using internal probe
4. The karyotype of at least 2 validated clones is verified using Giemsa staining

Long range PCR screening – strategy

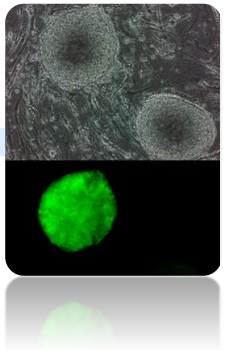


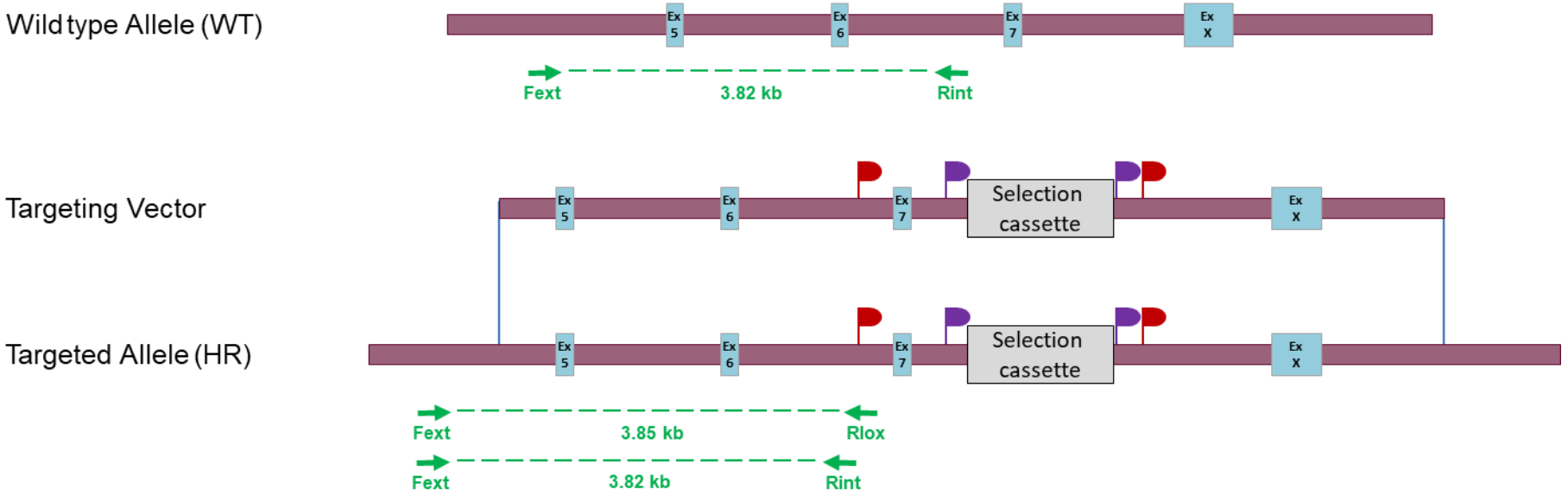
Schematic 5’ and 3’ PCR screening strategy



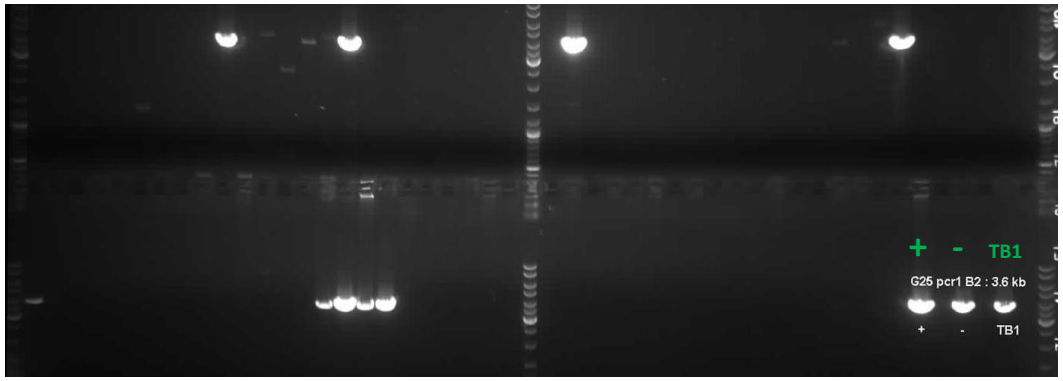
PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	ATAACAGCTTTCCTTGCAGACAATT	3.85 kb
	Rlox	GTTATCTGCAGGTCGACCTTAAGCT	
5’ PCR	Fext	ATAACAGCTTTCCTTGCAGACAATT	3.82 kb
	Rint	AATATGGCCGGCCCTGTCCTTTCCCTACTTTAATATCT	
5’ PCR	Fext	ATAACAGCTTTCCTTGCAGACAATT	5.3 kb
	Rneo	GCGGCCGGAGAACCTGCGTGCAATC	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	4 kb
	Rext	GCCTAGACTATGTGAGAGTCTATCC	

Long-Range 5’ PCR screening – results



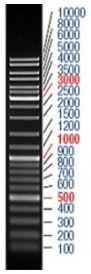


PCR Fext – Rlox : 3.85 kb



+ / - / TB1 : Controls DNAs

PCR Fext – Rint : 3.82 kb



Ladder pattern

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

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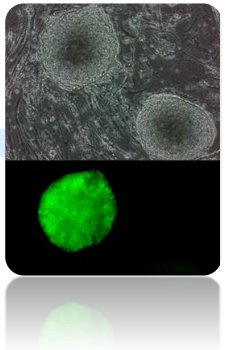
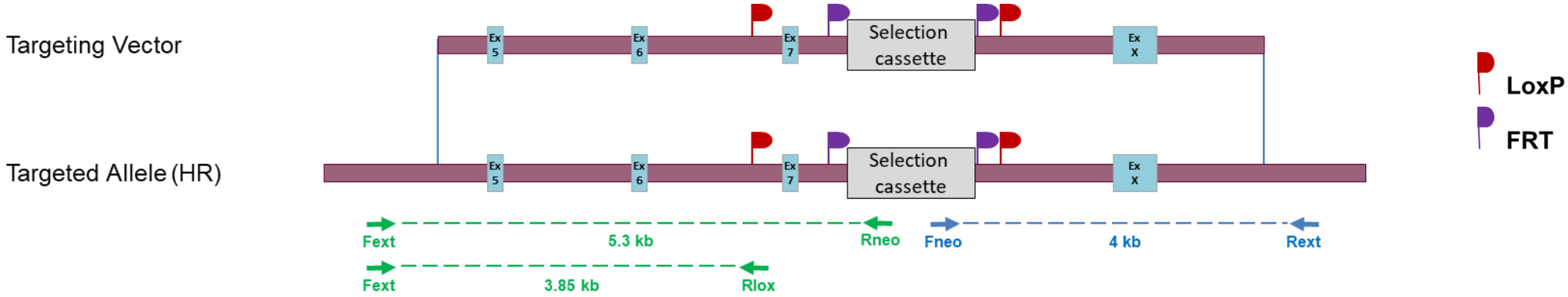
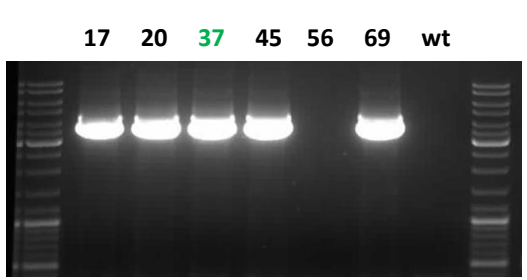
Recombinant ES validation by Long Range PCR

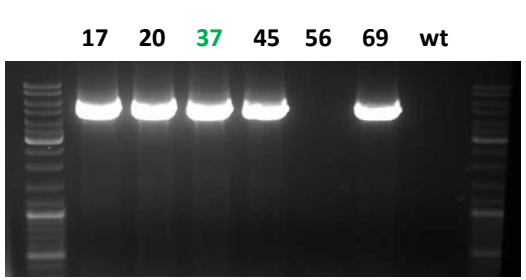


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

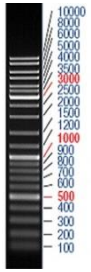




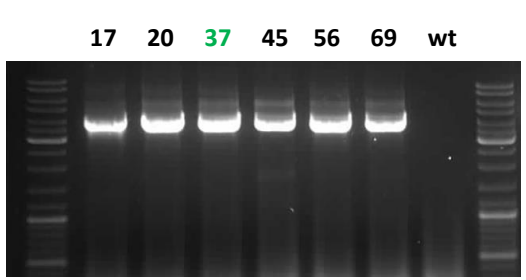
PCR Fext – Rlox : 3.85 kb



PCR Fext – Rneo : 5.3 kb



Ladder pattern



PCR Fneo – Rext : 4 kb

Six candidate clones identified by 5’ PCR screening were further analysed by 3’ PCR screening.
Five clones (clones #17, #20, #37, #45, #56 and #69) were confirmed.

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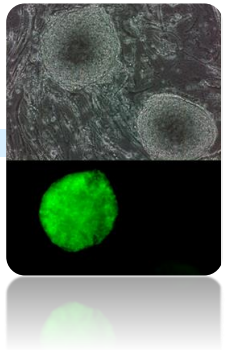
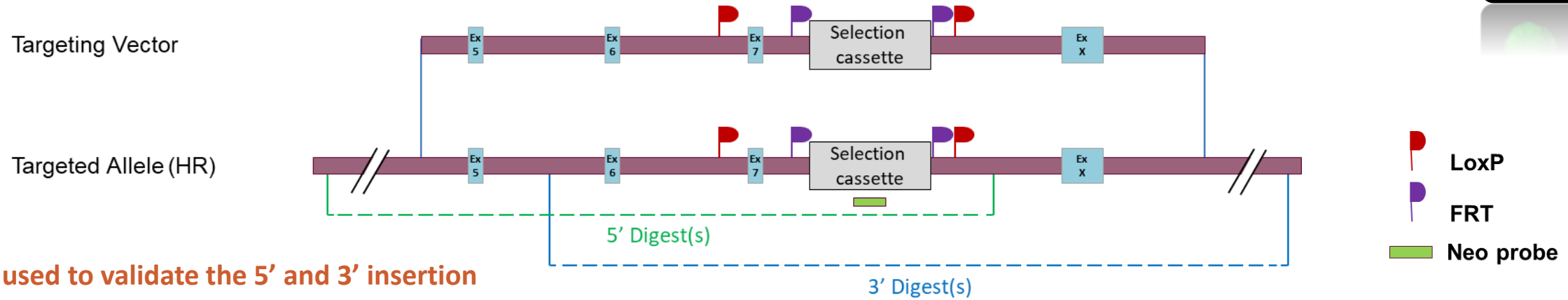
Recombinant ES clones validation by Southern Blot – Internal probe



Schematic Southern Blot validation strategy

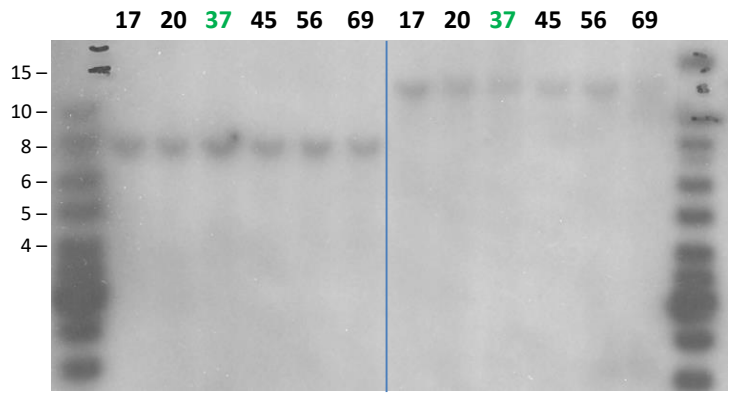
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



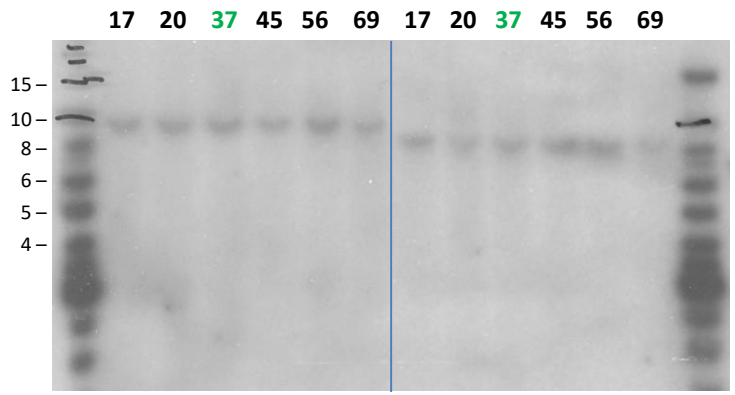
Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	EcoRV	8.3
		BamHI	13.4
	3' digest	BglI	10.3
		NdeI	8.8

Southern blot - Neo 5'



Southern blot - Neo 3'



Neo probe sequence

CTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCT
CGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCC
TGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCAT
ACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTAC
TCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAG
CCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGC
GATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCG
GCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTG
GCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATC
GCCTTCTATCGCCTTCTTGACGAGTTCTTC

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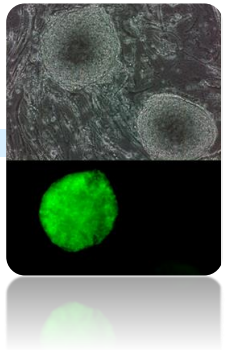
Aneuploidy screening in ES recombinant clones



Selected recombinant ES cells clones were karyotyped by Giemsa metaphase staining.

Results of aneuploidy analysis are presented in the table below.

Clone ID	Giemsa
#17	Not done
#20	Not done
#37	**Pass**
#45	Failed
#69	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/6 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 #37 validated in previous project phase was 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
#37	4	3	2	9

Breeding to F1 generation

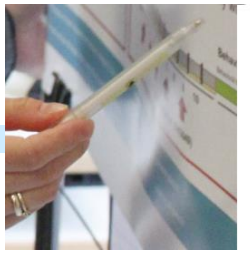
- Eleven chimeric males generated in the previous phase by blastocyst injection of the ES clones were mated with C57BL/6NCrl Flp deleted females showing maternal contribution* (health status SPF – Specific Pathogen Free) to investigate whether the recombined ES cells have contributed to the germ layer.

- Germ line transmission was obtained the : 11/06/2014

- Allele nomenclature (following MGI guidelines) : **Phf8$^{tm1.1Ics}$**

***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 DELIVERED ALLELE



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CTGCAGGTAAGAGGTCAGCCTGAGCTTCATGGTAAGTTTCAGATCAGCTTCAGTTTAGCTTGAGCTAAAGAATGAGAGACTGTCTCAAAAAAACCCAAATTAAAAAAAAAAAAAAAA
CCACGGAGAGGAAGGCATGCAGCAGGCCCTAGGTTGCACCCCTAGTACTAAAACTACTTTTTTGAGGTCTAGGGACATAGCTTACTGGTGTAGTGCTTTCTTAACGTGTGCAAGGTC
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GTCTATATTAATTGTAGAAATTTCTTGGTAACATGAGTTTTCTCTCCCTCAAACTTTAATATATGTACTGTAGAATTCATTTACTTTCTCCAAAGTAACCAAAGTAAAGTGGCTGTA
TTTGTTTTTTTTTTTTCTGAGACATTTGGGAGTATTTCC

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REPORT REDACTION & VALIDATION

Protocol finalized on 2023/09/19

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