phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

MODEL GENERATION TECHNICAL REPORT

Generation of mouse model : Med17 L369P point mutation (corresponding to hum L371P)

Project code: G2 / IR00003697

Report updated: 14/06/2023

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MODEL GENERATION TECHNICAL REPORT

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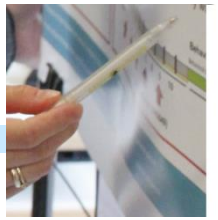
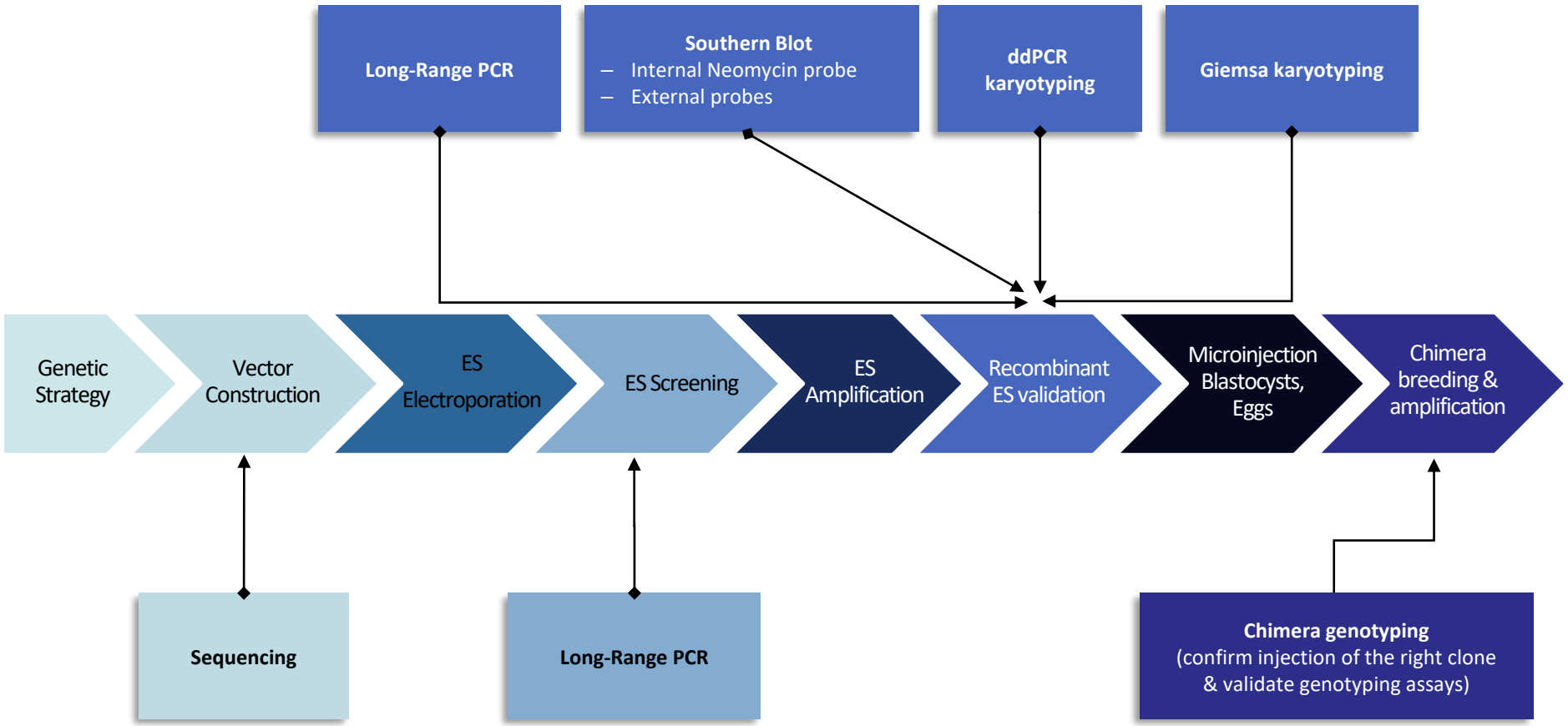
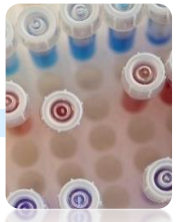
7 SEQUENCE OF THE DELIVERED ALLELE

PROJECT PROCESS & QUALITY CONTROL



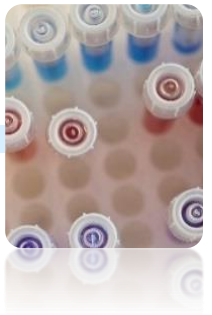


2 GENETIC STRATEGY

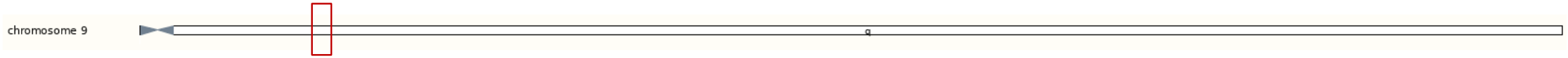


- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

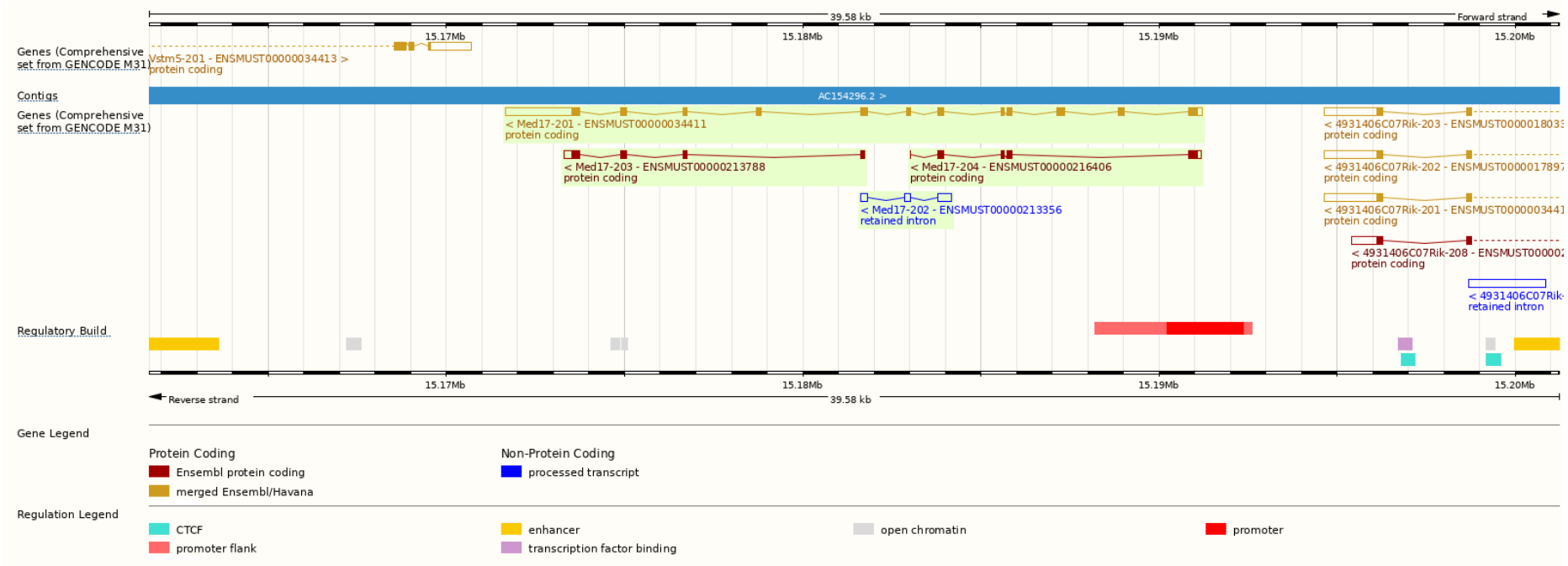
Med17 mouse genomic locus – structure



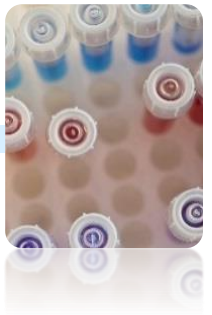
Location, Chromosome 9: 15,171,647-15,191,227



Gene: Med17 ENSMUSG00000031935

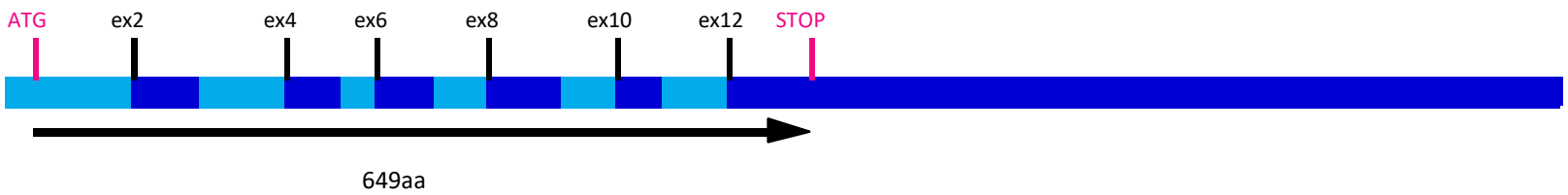


Med17 mRNA(s) and protein(s)

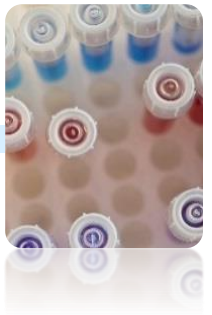


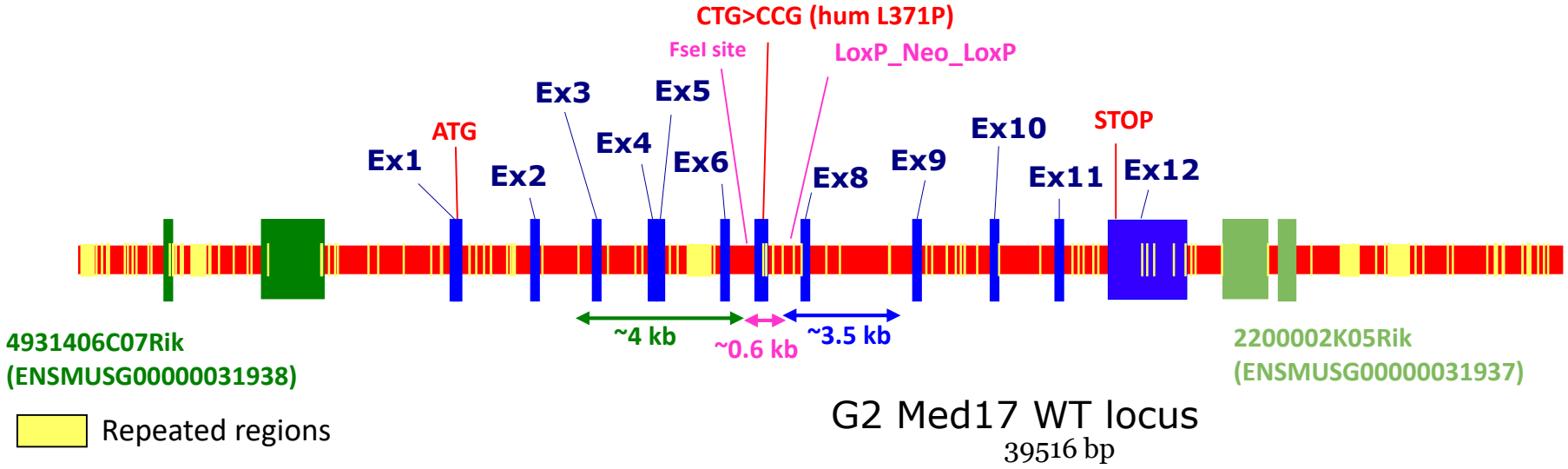
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match
ENSMUST00000034411.10	Med17-201	3964	649aa	Protein coding	CCDS22834	Q8VCD5
ENSMUST00000213788.2	Med17-203	820	199aa	Protein coding		A0A1L1SQB3
ENSMUST00000216406.2	Med17-204	743	211aa	Protein coding		A0A1L1SV12
ENSMUST00000213356.2	Med17-202	735	No protein	Retained intron		-

Transcript: ENSMUST00000034411.10 (Med17-201)



Strategy proposed : point mutation L369P



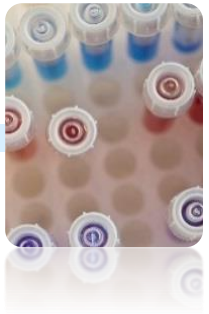


G2 Med17 WT locus
39516 bp

Sequence detail

```
GCA CAG CTC TCC CGG GAA GCC GTT CAG ATT AAG TCT CAG ATC CCT CAC ATT GTG GTG AAA AAC CAG ATC
 A   Q   L   S   R   E   A   V   Q   I   K   S   Q   I   P   H   I   V   V   K   N   Q   I
ATC TCT CAG CCC TTT CCA AGC TTG CAG TTG TCC ATT TCT CTG TGC CAC TCC TCA GAT GAT AAG AAG TCG
 I   S   Q   P   F   P   S   L   Q   L   S   I   S   L   C   H   S   S   D   D   K   K   S
                                                                 CTG>CCG (hum L371P)
CAG AAG TGT GCC GCA GAG AAG CCT GGG CAA GAG GAT CAC CTC TAC GTC CCG GAG CAC AAC CTG CAC CTG
 Q   K   C   A   A   E   K   P   G   Q   E   D   H   L   Y   V   P   E   H   N   L   H   L
             ex8
CTG ATC AGA GAG TTT CAT AAA CAG ACC TTG AGT TCC ATT GTG ATG CCA CAC CCA GCA AGT GCT CCC TTT
 L   I   R   E   F   H   K   Q   T   L   S   S   I   V   M   P   H   P   A   S   A   P   F
GGC CAC AAG AGG ATG AGA CTC TCA GGT CCT CAA GCT TTT GAT AAA AAT GAA ATT AAT TCA ATA CAG TCC
 G   H   K   R   M   R   L   S   G   P   Q   A   F   D   K   N   E   I   N   S   I   Q   S
ACA GAA GGA CTC CTA GAA AAA ATA ATT AAA CAG
 T   E   G   L   L   E   K   I   I   K   Q
```

PROs& CONs evaluation of the strategy



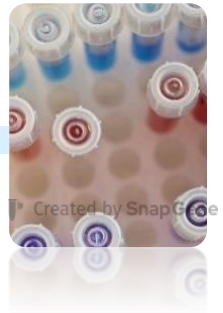
- Pros

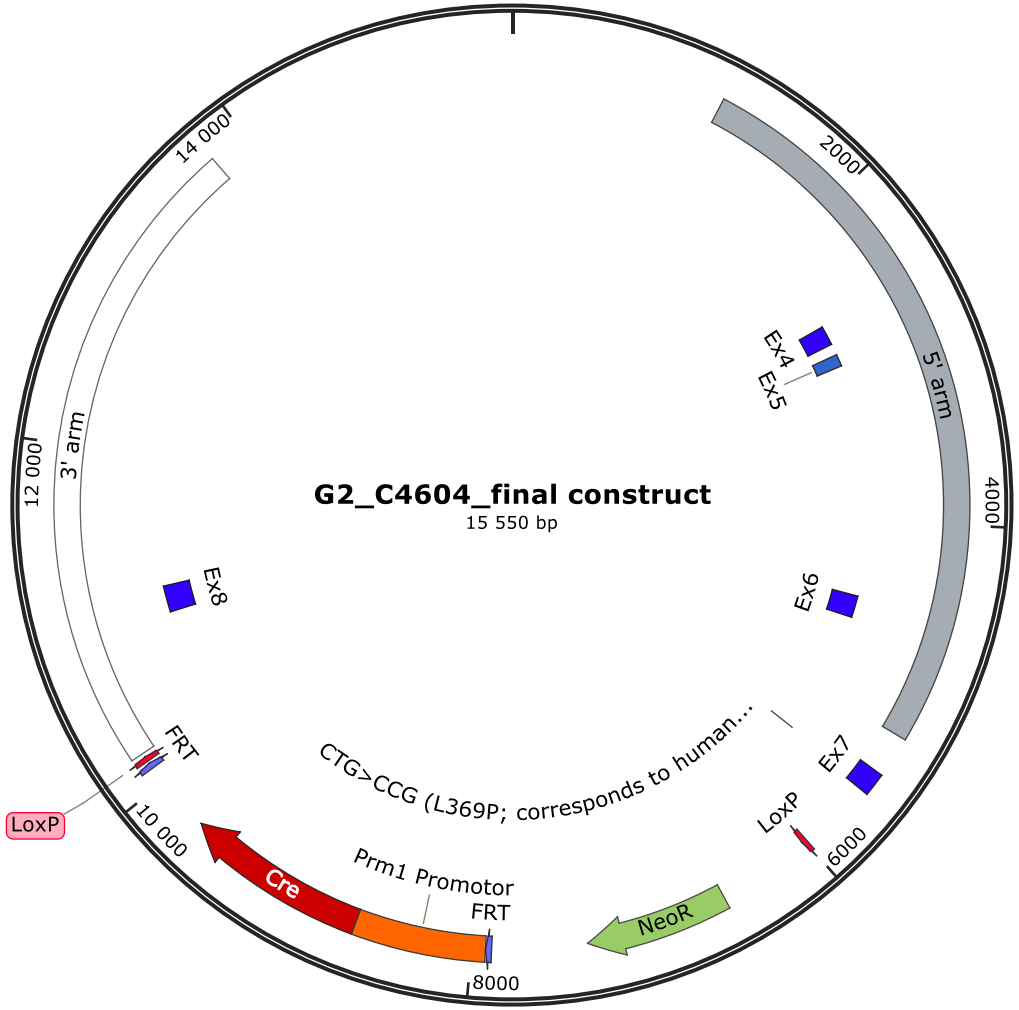
  Human L371P mutation introduced, in the mouse it correspond to the amino acid 369

- Cons

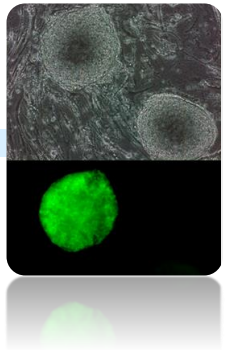
  An FseI restriction site will be insertion in intron 6 for cloning purpose

3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION



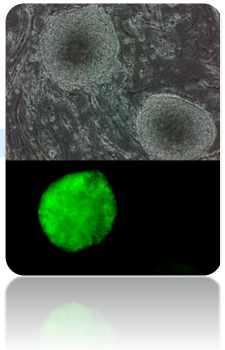


4 ES cell electroporation & Screening of recombinant clones



- Electroporation and screening process
- Long range PCR screening – strategy
- Long-Range 3’ 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



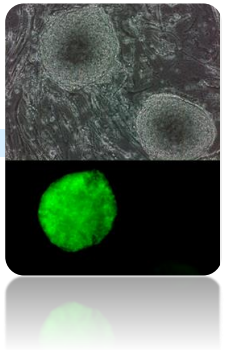
The targeting vector was electroporated in the proprietary C57BL/6N BD10 cell line.

Transfected ES cell clones were submitted to neomycin selection (G418) and 186 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.

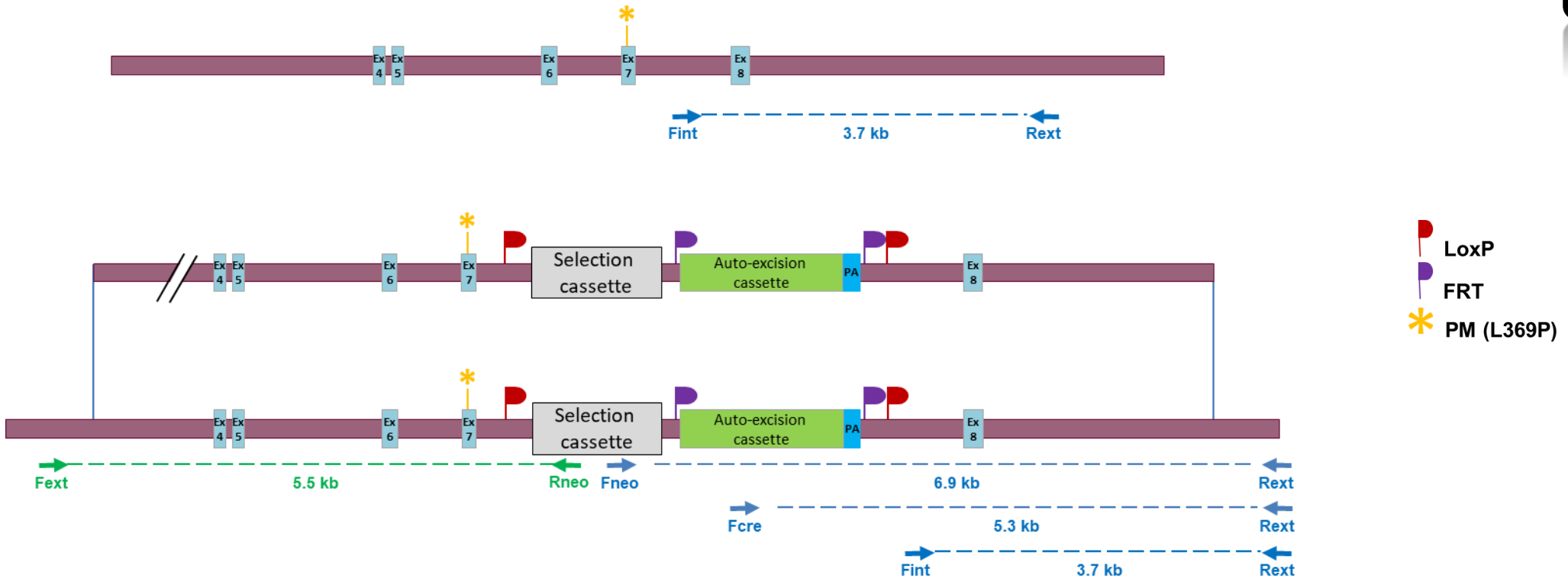
Screening process steps:

1. Identification of candidate recombinant clones by initial 3’ Long-Range PCR
2. Eight of 3’ PCR positive clones are confirmed for 5’ 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 using ddPCR aneuploidy screening and Giemsa staining

Long range PCR screening – strategy

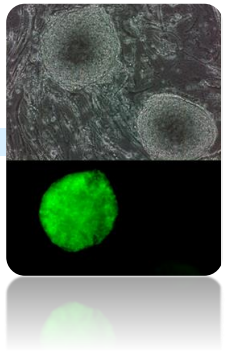


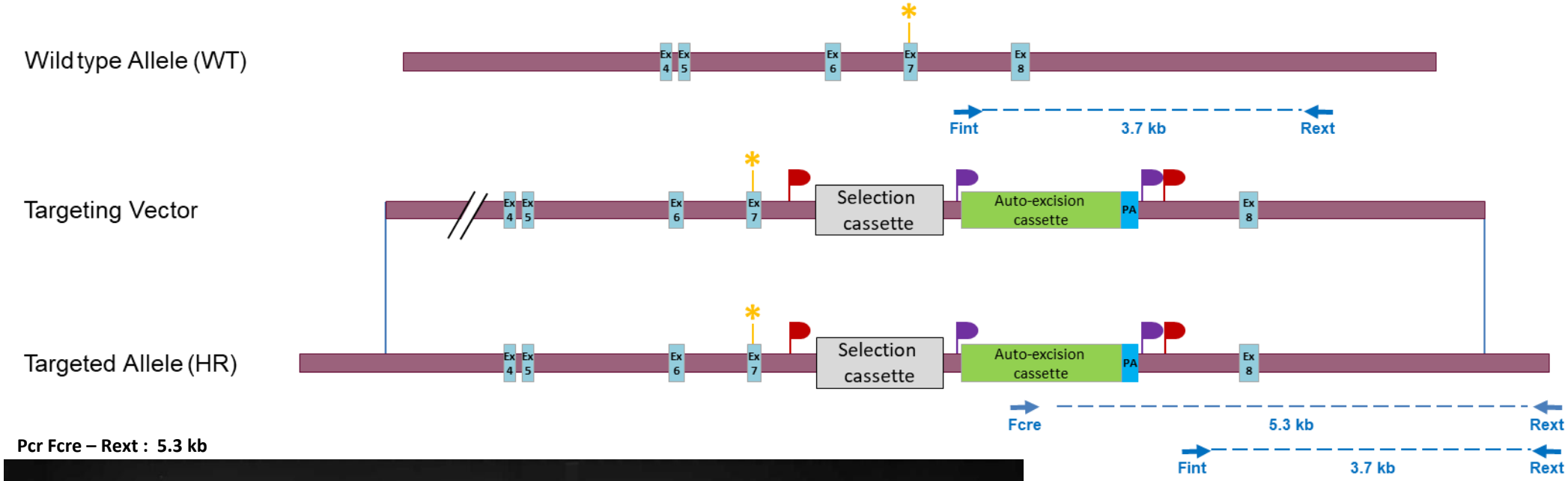
Schematic 5’ and 3’ PCR screening strategy



PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	AGAGTCCACAAACGCTGCAGCTGAT	5.5 kb
	Rneo	GCGGCCGGAGAACCTGCGTGCAATC	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	6.9 kb
	Rext	TGGATCTGGGGGTCCTCAATGCGAC	
3’ PCR	Fcre	GGCCAAGCCAGCACCATGTCCA	5.3 kb
	Rext	TGGATCTGGGGGTCCTCAATGCGAC	
3’ PCR	Fint	TGCCACTGTTCAGAGAGGGGCAGCT	3.7 kb
	Rext	TGGATCTGGGGGTCCTCAATGCGAC	

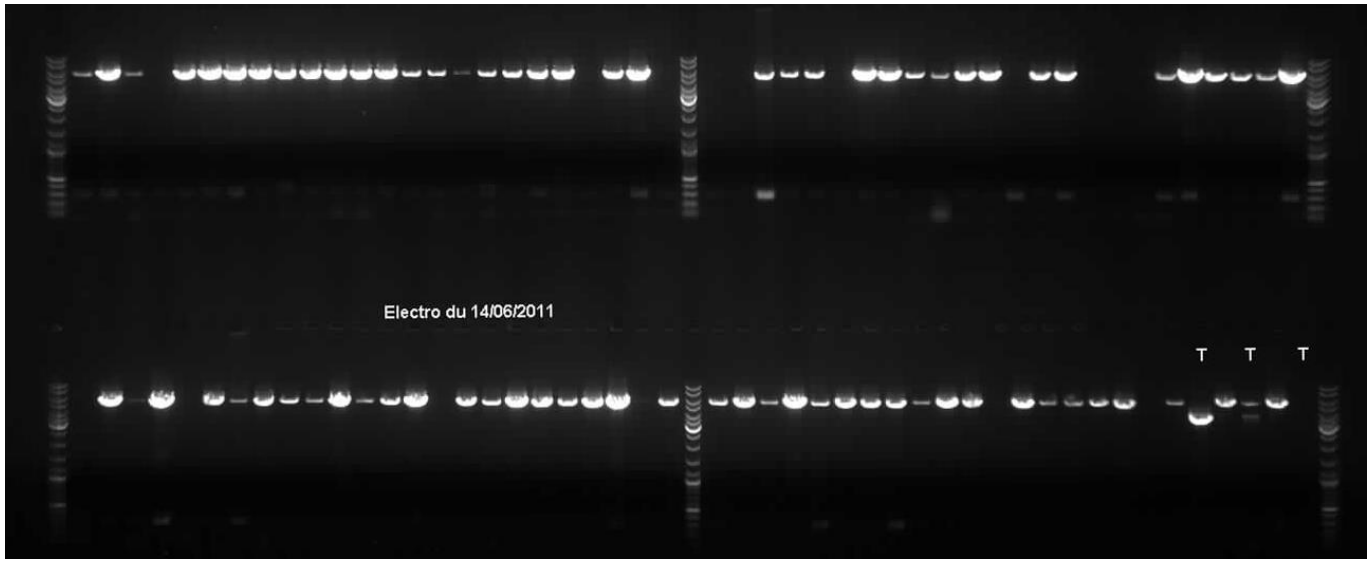
Long-Range 3' PCR screening – results



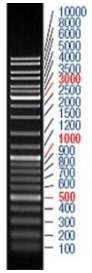


LoxP
FRT
PM (L369P)

Pcr Fcre – Rext : 5.3 kb



T = control DNA, PCR :Fint – Rext : 3.7 kb



Ladder pattern

Eight candidate clones out of the 33 positive clones were selected for 5' Long-Range PCR and Southern blot validation.

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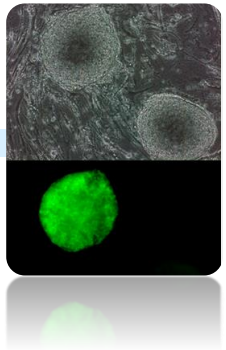
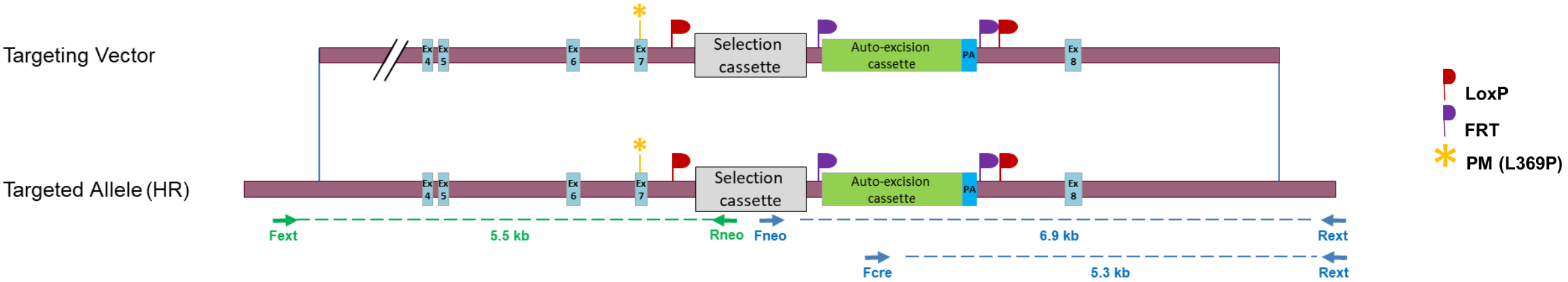
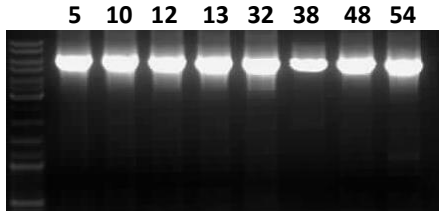
Recombinant ES validation by Long Range PCR

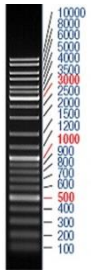


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

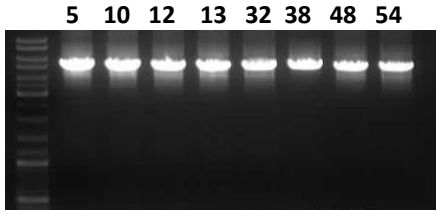




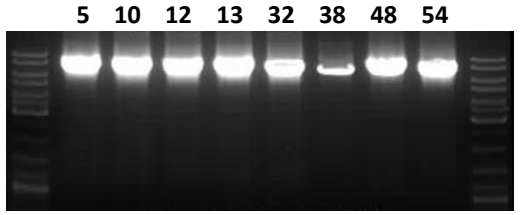
Pcr Fext – Rneo : 5.5 kb



Ladder pattern



Pcr Fcre – Rext : 5.3 kb



Pcr Fneo – Rext : kb

Eight candidate clones identified by 3’ PCR screening were further analysed by 5’ PCR screening.
Eight clones (clones #5, #10, #12, #13, #32, #38, #48, and #54) were confirmed.

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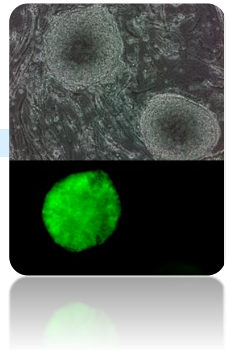
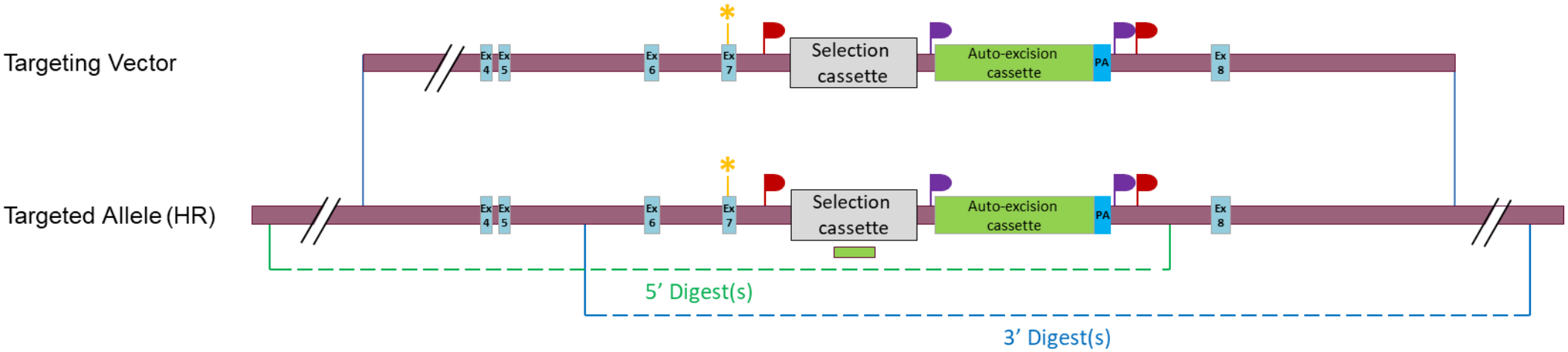
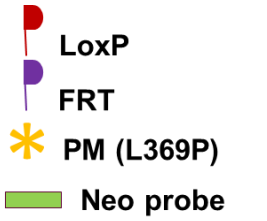
Recombinant ES clones validation by Southern Blot – Internal probe



Schematic Southern Blot validation strategy

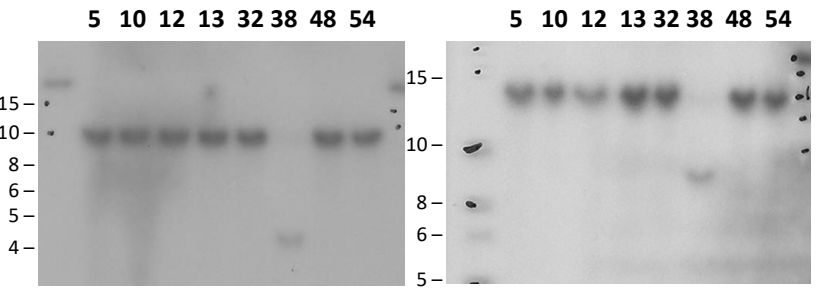
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



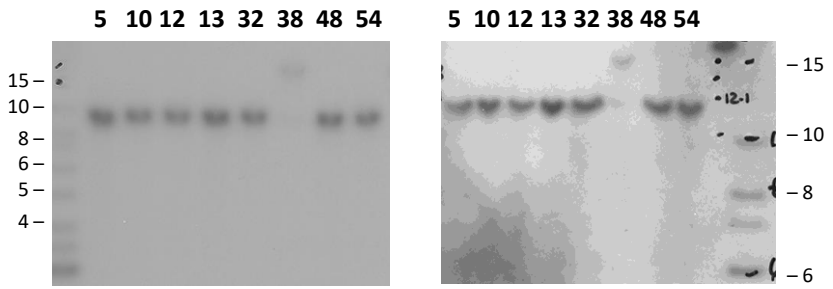


Digestions used to validate the 5' and 3' insertion

Southern blot - Neo 5'

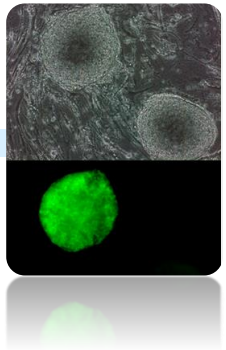


Southern blot - Neo 3'



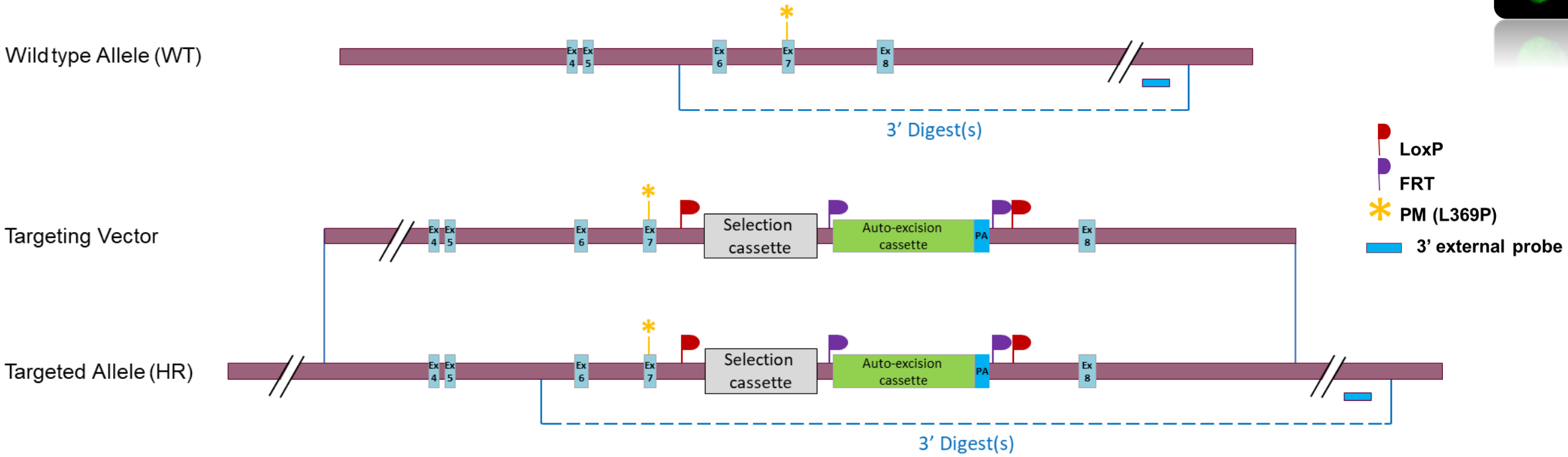
Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	BglII	9.8
		SexAI	13.3
	3' digest	KpnI	9.9
		EcoNI	11.9

Recombinant ES clones validation by Southern Blot – External probe

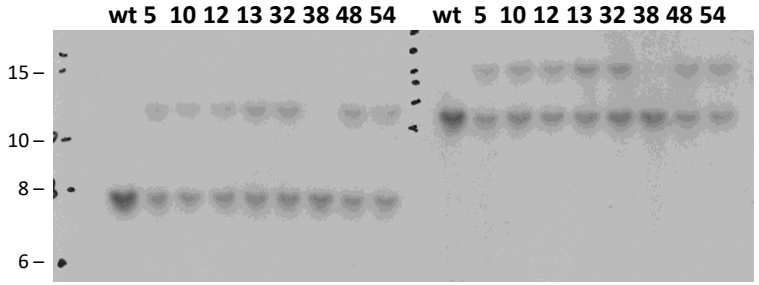


Schematic Southern Blot validation strategy

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



EcoNI 7.6 / 11.9 AflII 11.5 / 15.7

3' probe sequence

GGATCCCACATTGAGCAGAAAGCACCCTCACTTGCTA
AGATGTTCTCCAGGCTGGAGTCAGTTCCTGCCTAGAG
GCTGTGCAAGTCTGTTGTGTCAGGAGTGAGGACCATC
CAGGCCTGTACCCAGCCTAGTGTGCTGAGCATAGTCT
GAGTTCATAGTTCTCTCCTGACCTGGTGGTGTCCTTG
ATTCTGATCCATTAGTTGATGATTGTGCCTCTAAACC
AGGTTGACTATTGTGTGTCTGTGGAAAGCAGCATTGC
TCTTGCTCTTGTCCC

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	EcoNI	7.6	11.9
	3' second digest	AflII	11.5	15.7

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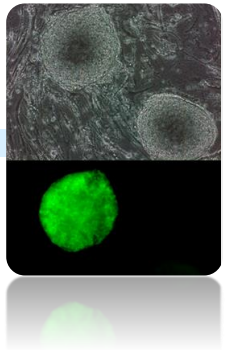
Aneuploidy screening in ES recombinant clones



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

Clone ID	Giemsa
#5	Not done
#10	Not done
#12	Not done
#13	**Pass**
#32	Failed
#48	**Pass**

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 #13 and #48 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
#13	0	0	2	2
#48	**1**	**4**	**9**	**14**

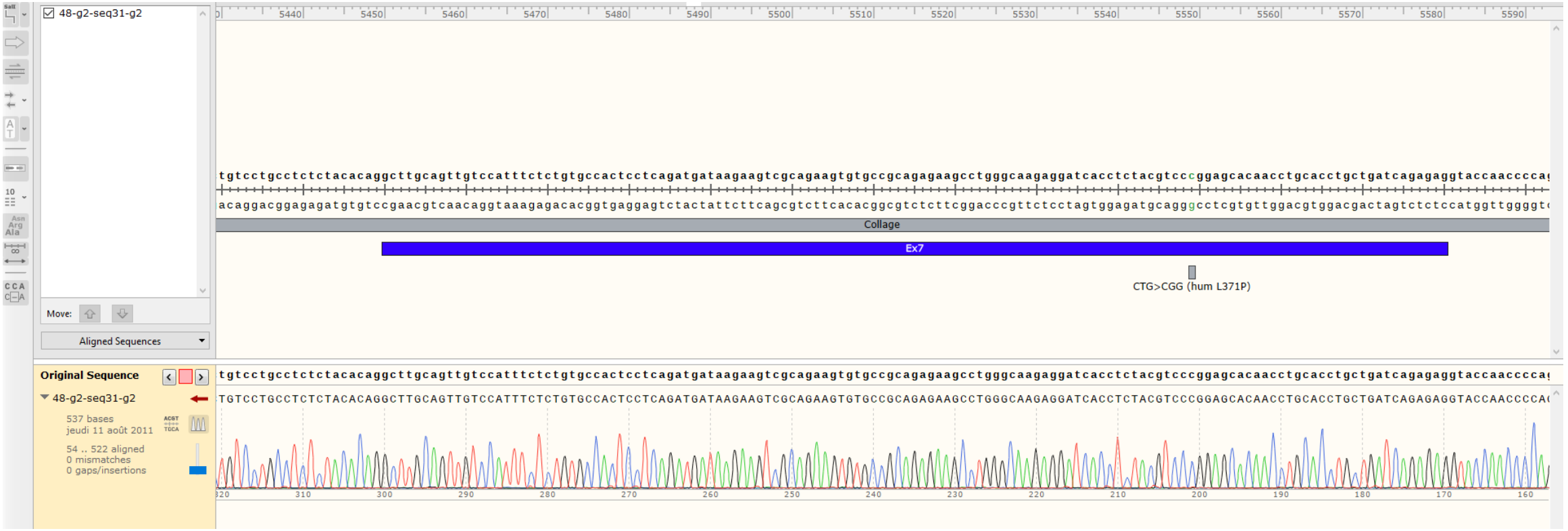
Breeding to F1 generation

- Six 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.

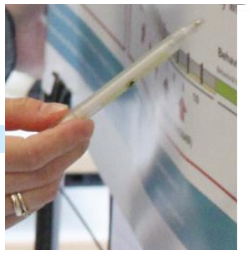
- Germ line transmission was obtained the : 29/02/2012

- Allele nomenclature (following MGI guidelines) : **Med17$^{tm1.1Ics}$**

6 SEQUENCE OF clone #48 : PM confirmation



7 SEQUENCE OF THE DELIVERED ALLELE



ATGTGCTGTAGGCATGTGTACATGTGCTACAGGCTGTGCTGGGCATCCGGGCGGCCAATGAAGCTAAAGCTGGCTCTGCTGCTCACTCCTCTGTGAGCTGAGAGTGGTGCCTTGGGCTTTCATGGCGCATGTTAAA
AAGTGTGCTGCTGTGGCTTCAGAAAGCAGCAAGCAGTGCTTTGGCAGTAGCGTTTCTTTCAAGGATACTTGCCTTTTCTAAATGAGGTACCAATTTAAAAAGAAAAATAGATGTTCCTAATTTGTAGAAATGAACTT
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GTGATCTGGGGACAGGATCTCACTAGCCCTGACTGACCTGCAACAC

LoxP Exon 7 PM: CTG > CGG

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EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION
Protocol finalized on 2023/06/14
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