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MODEL GENERATION TECHNICAL REPORT

Tnfsf10 (Trail) Conditional overexpression under the Rosa26 promoter (KI in Rosa26)

ICS project code: Kos393 / IR1437

Report finalized: 2024/11/18

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 CONTROL

2 GENETIC STRATEGY

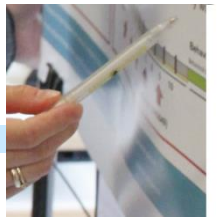
3 HOMOLOGOUS RECOMBINATION VECTOR CONSTRUCTION

4 ES ELECTROPORATION & SCREENING OF RECOMBINANT CLONES

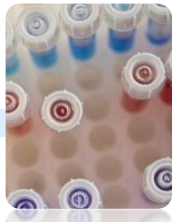
5 MICROINJECTION & BREEDING

6 SEQUENCE OF THE DELIVERED ALLELE

Project process & quality controls

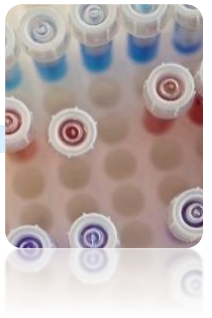


GENETIC STRATEGY

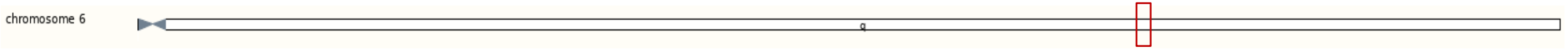


- Target locus structure
- mRNA(s) and protein(s)

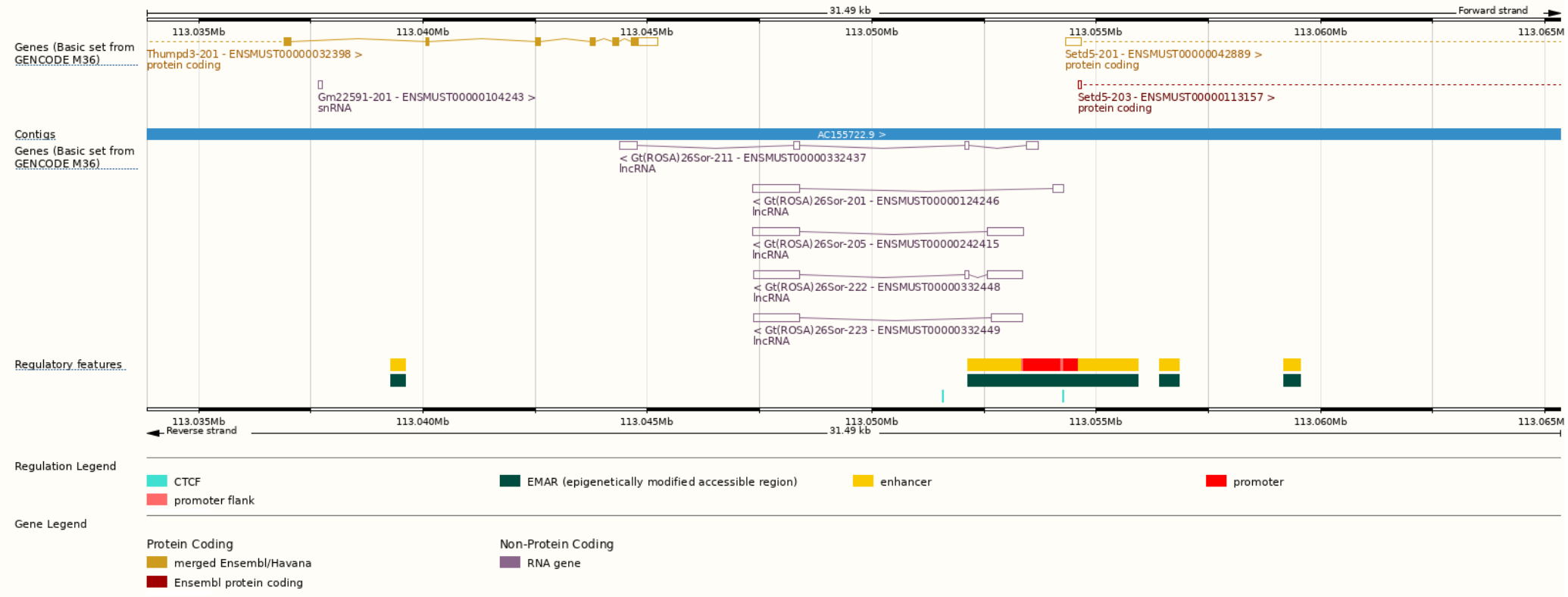
Rosa mouse genomic locus – structure



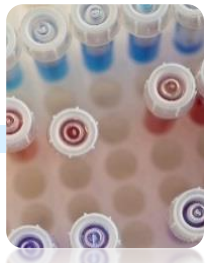
Location : Chromosome 6: 113,043,843-113,055,336



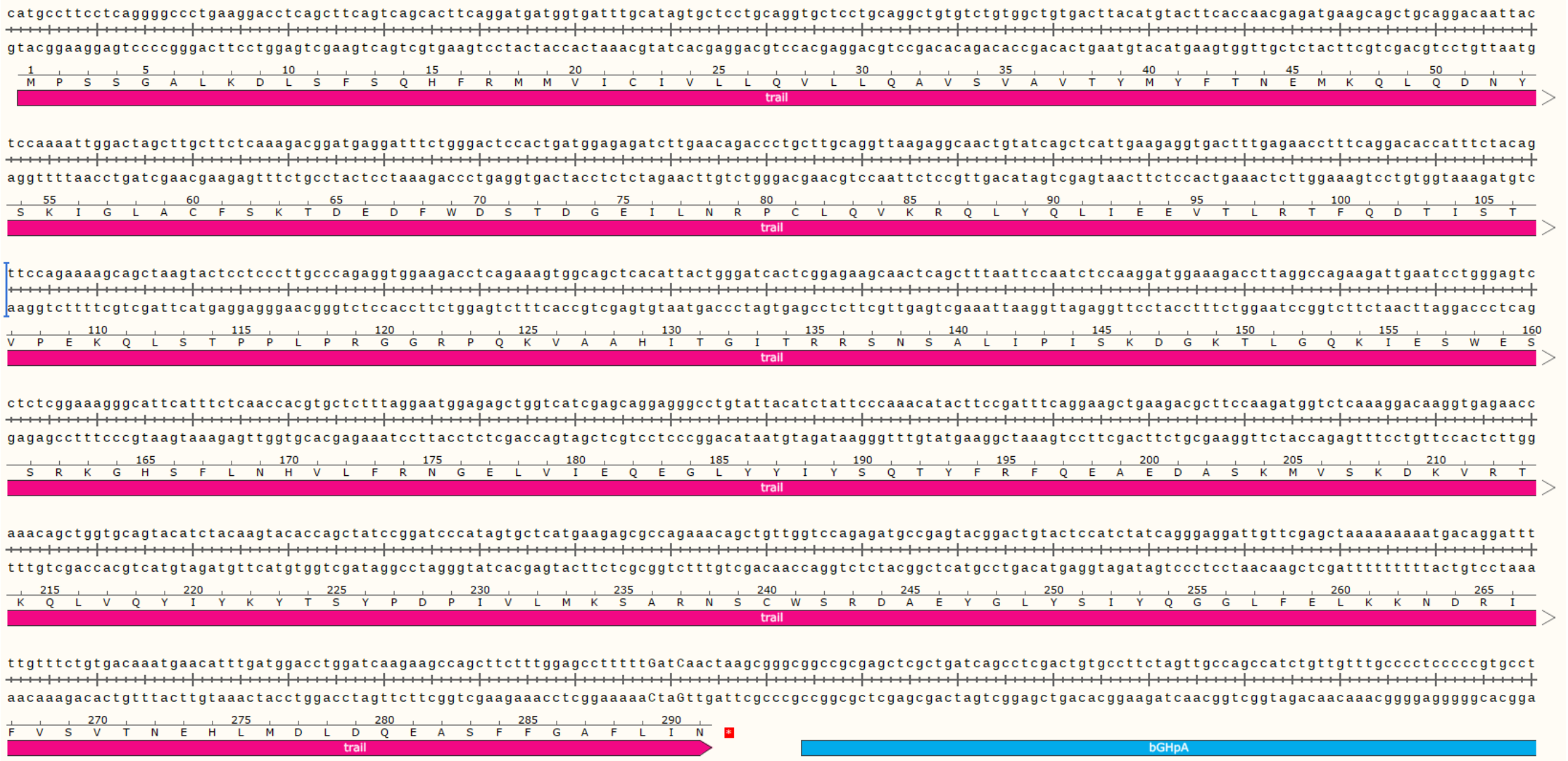
Gene: Gt(ROSA)26Sor ENSMUSG00000086429



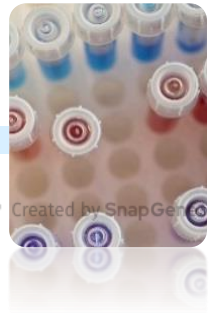
cDNA that will be overexpressed upon Cre mediated excision



Trail cDNA :



3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION



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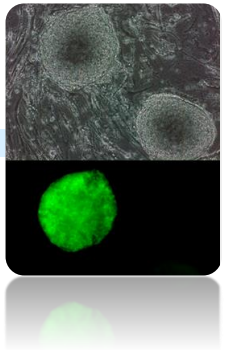
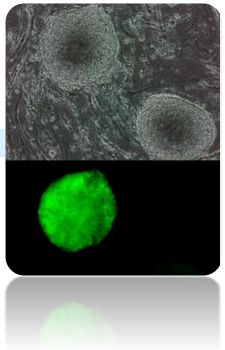
ES cell electroporation & Screening of recombinant clones



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



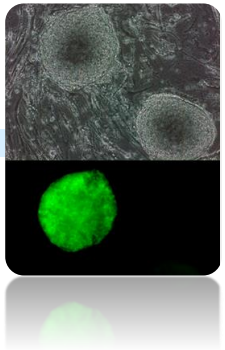
The targeting vector was electroporated in the proprietary 129SV/Pas P1 cell line.

Transfected ES clones were submitted to neomycin selection (G418) and 372 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.

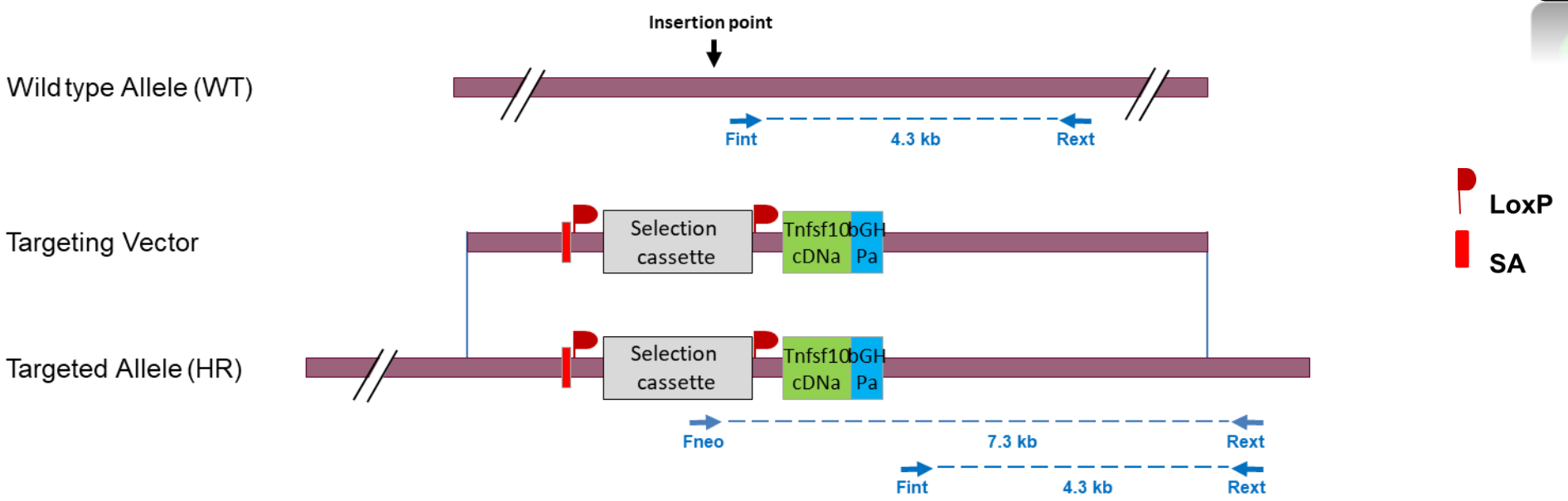
Screening process steps:

1. Identification of candidate recombinant clones by initial 3’ Long-Range PCR
2. Positive clones in step 1 are further validated by Southern blot analysis using internal and external probes
3. The karyotype of at least 2 validated clones is verified Giemsa staining

Long range PCR screening – strategy

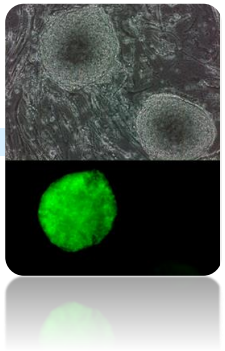


Schematic 5’ and 3’ PCR screening strategy

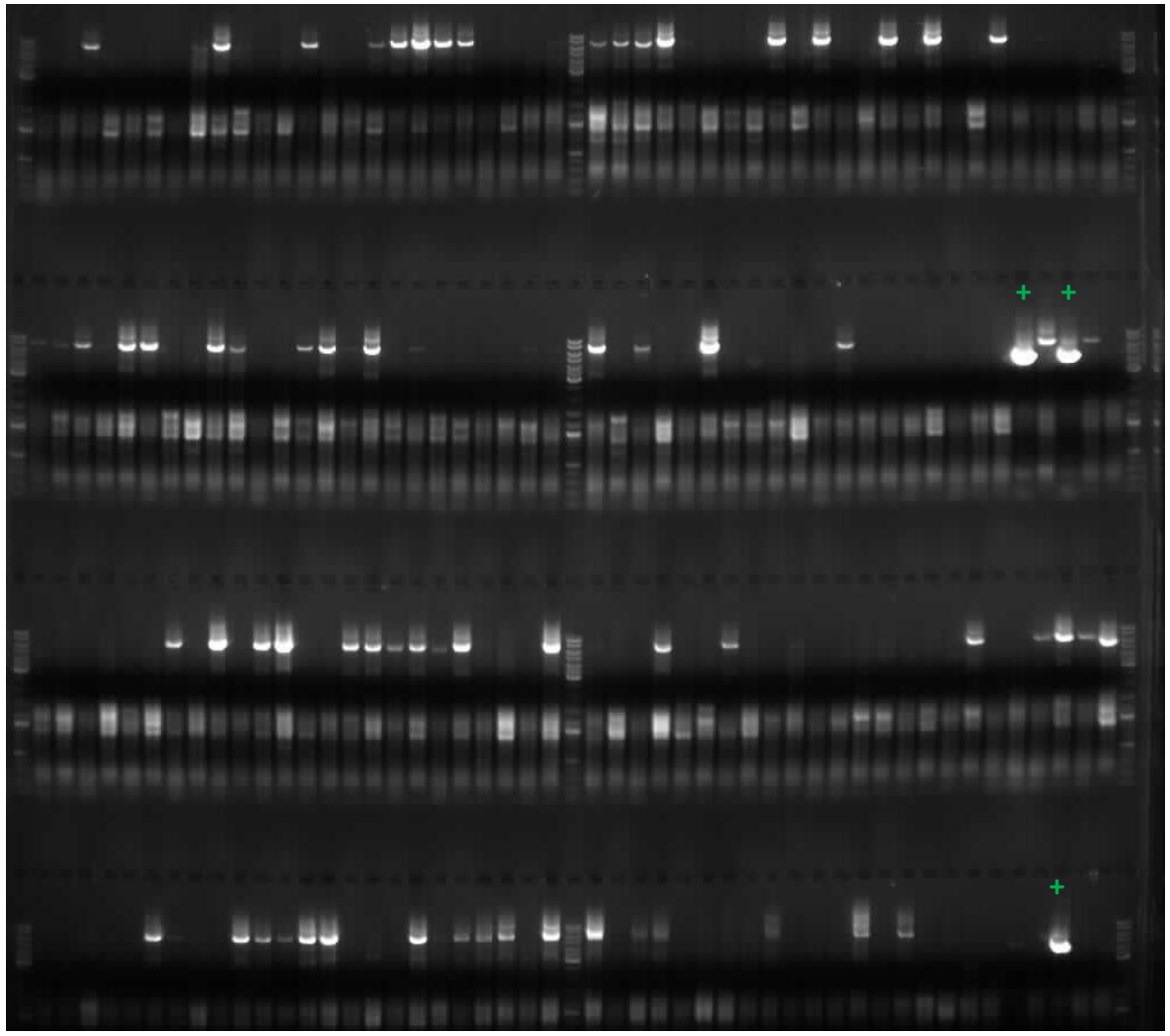


PCR	Primer Name	Primer sequences	PCR product size
3’ PCR	Fint	CTGGTGTGTGGGCGTTGTCCTGCAG	4.3 kb
	Rext	CTCAGTGGCTCAACAACACTTGGTC	
3’ PCR	Fneo	GGTCTTGTCGATCAGGATGATCTGG	7.3 kb
	Rext	CTCAGTGGCTCAACAACACTTGGTC	

Long-Range 3’ PCR screening – results

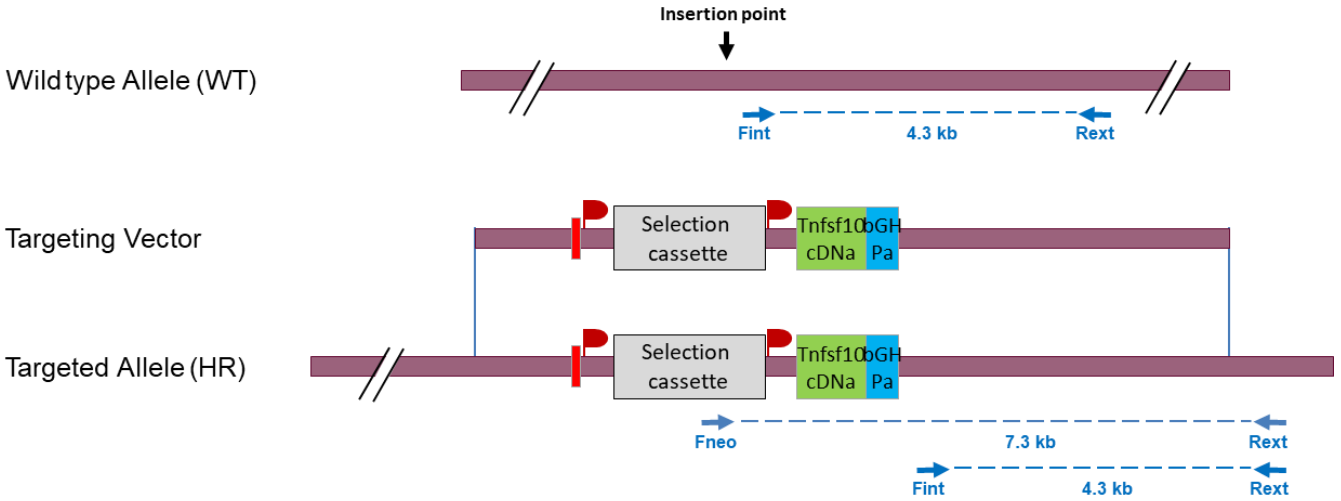


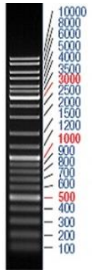
PCR Fneo – Rext : 7.3 kb



+ : Controls DNAs

PCR Fint – Rext : 4.3 kb





Ladder pattern

Six candidate clones (#21, #25, #26, #29, #38 and #47) were selected for Southern blot validation.

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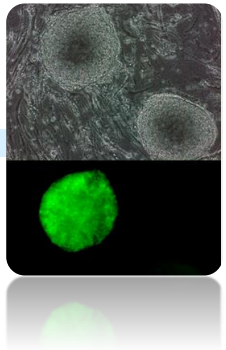
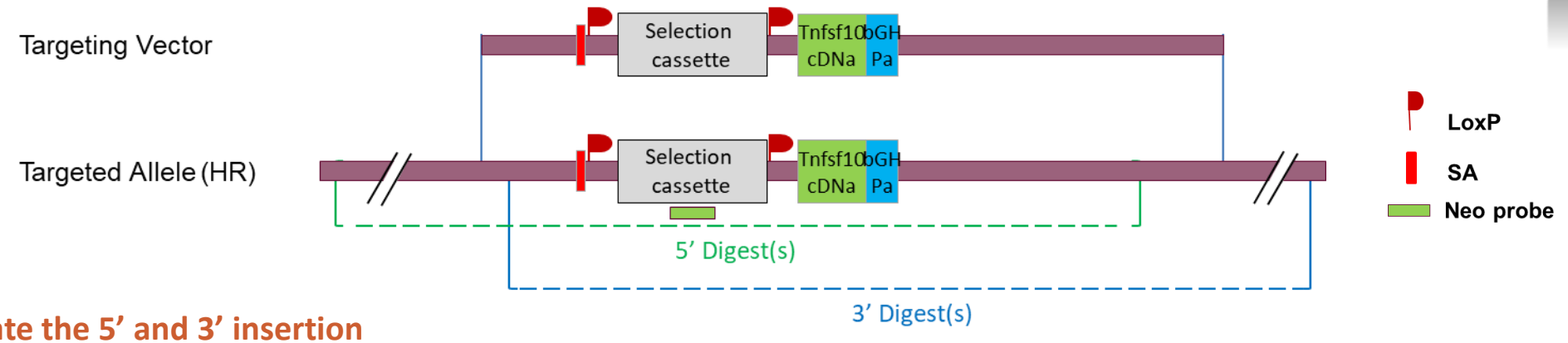
Recombinant ES clones validation by Southern Blot – Internal probe



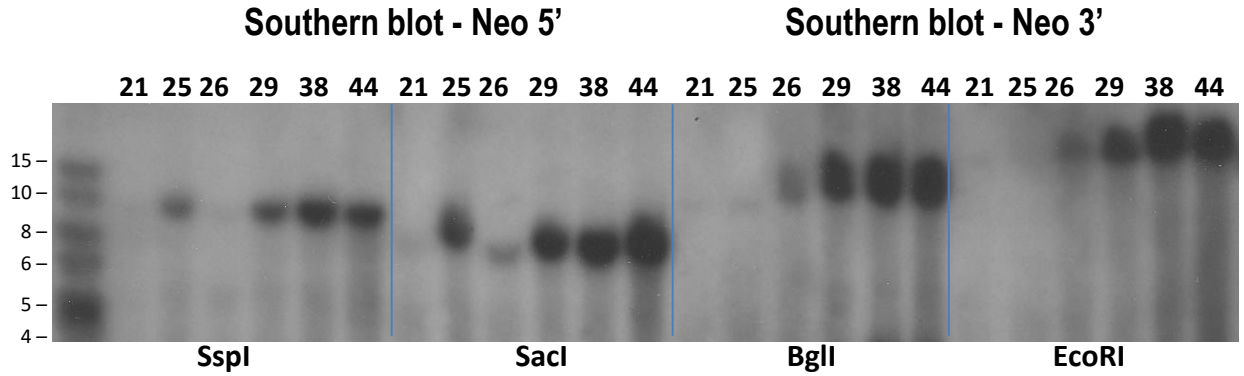
Schematic Southern Blot validation strategy

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	SspI	9.6
		SacI	6.5
	3' digest	BglI	9.5
		EcoRI	15.4



Neo probe sequence

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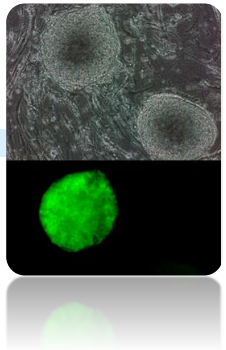
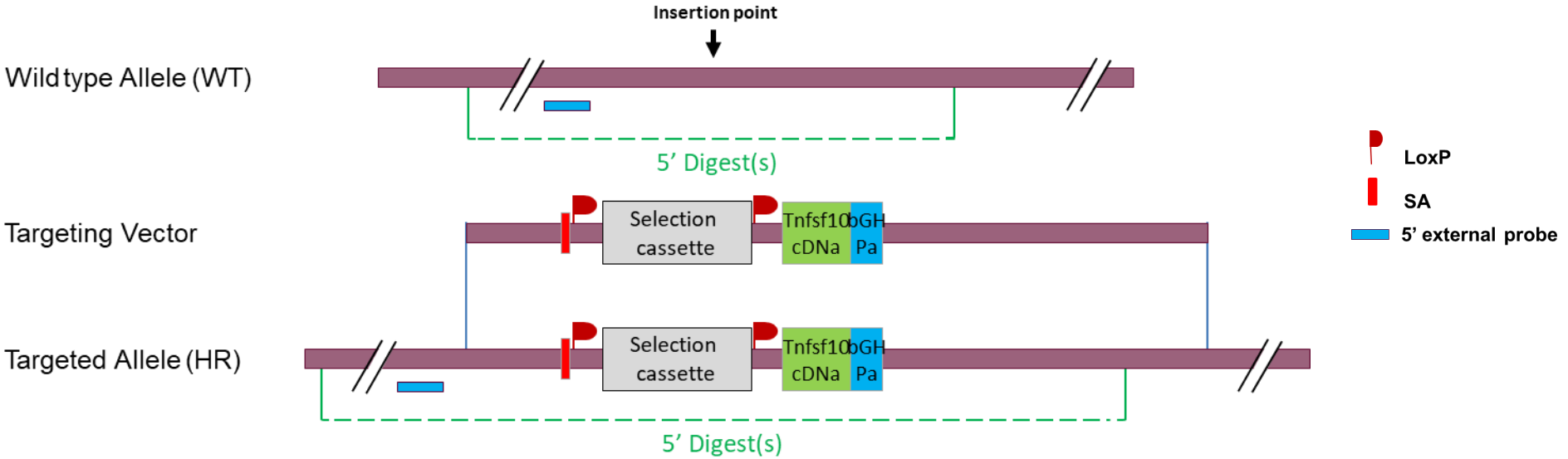
TTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTGCATCAGCCATGATGGATA
CTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCC
CGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTCG
TCCTGCAG

Recombinant ES clones validation by Southern Blot – External probe

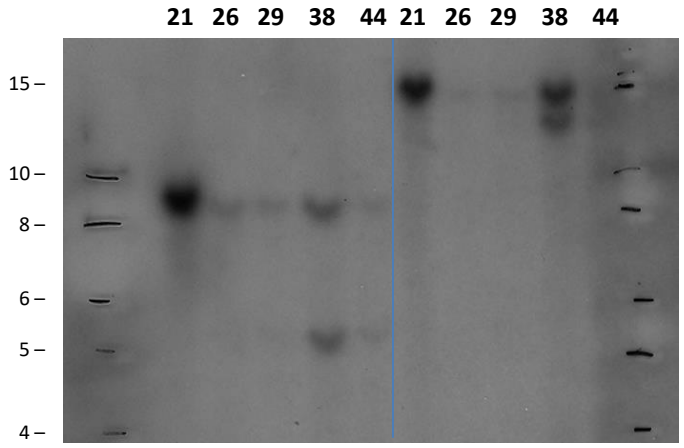


Schematic Southern Blot validation strategy

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



SpeI : 8.9 / 5.4 SpHI : 16.2 / 13.5

5' probe sequence

TATGTGTATTTTGAGAGCAGGGTTGGGAGGC
CTCTCCTGAAAAGGGTATAAACGTGGAGTAG
GCAATACCCAGGCAAAAAGGGGAGACCAGAG
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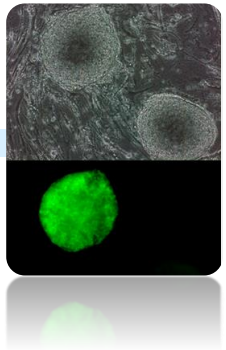
GGTGCAATGGTGTGTAAAGGTAGCTGAGAAG
ACGAAAAGGGCAAGCATCTTCCTGCTACCAG
GCTGGGGAGGCCCAGGCCCACGACCCCGAGG
AGAGGGAACGCAGGGAGACTGAGGTGACCCT
TCTTTCCCCCGGGGCCCGGTCGTGTGGTTCG
GTGTCTCTTTTCTGTTGGACCCTTACCTTGA
CCCAGGC

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	SpeI	8.9	5.4
	5' second digest	SphI	16.2	13.5

Aneuploidy screening in ES recombinant clones



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

Results of aneuploidy analysis are presented in the table below.

Clone ID	Giemsa
#22	Failed
#38	**Pass**
#44	Not Done

MICROINJECTION & BREEDING

- Microinjection
- Breeding to F1 generation

Microinjection

- The ES cells used in the injection experiment were originally derived from a 129SV/Pas mouse strain (which have agouti coat colour). These cells were injected into blastocysts derived from an C57BL/6J strain, which have a black 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 C57BL/6J females (Health status SPF Specific Pathogens Free).
- Recombinant ES clone #38 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
#38	3	1	2	6

Breeding to F1 generation

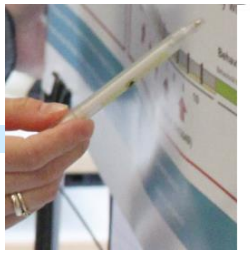
- Two highly chimeric males generated in the previous phase by blastocyst injection of ES clone #38 were mated with wild-type C57BL/6J females (health status SPF – Specific Pathogen Free) to investigate whether the recombined ES cells have contributed to the germ layer.

- Germ line transmission was obtained the : 19/12/2007

- Allele nomenclature (following MGI guidelines) : **Gt(ROSA)26Sor$^{tm7(Trail)Ics}$**

- Allele ID in MGI: MGI:7788424

SEQUENCE OF THE DELIVERED ALLELE



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ACAGAGCATTGGCATTTTCCCTTTCCTGATCTTAGAAGTCT

LoxP Splice Acceptor Neo/STOP cassette Trail cDNA bGHpA

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EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION
Protocol finalized on 2024/11/18
Prepared by Romain LORENTZ, IE
Verified by Marie-Christine BIRLING, PhD

CONTACT US
By email at mutagenesis@igbmc.fr
By phone at +33 (0)3 88 65 57 43

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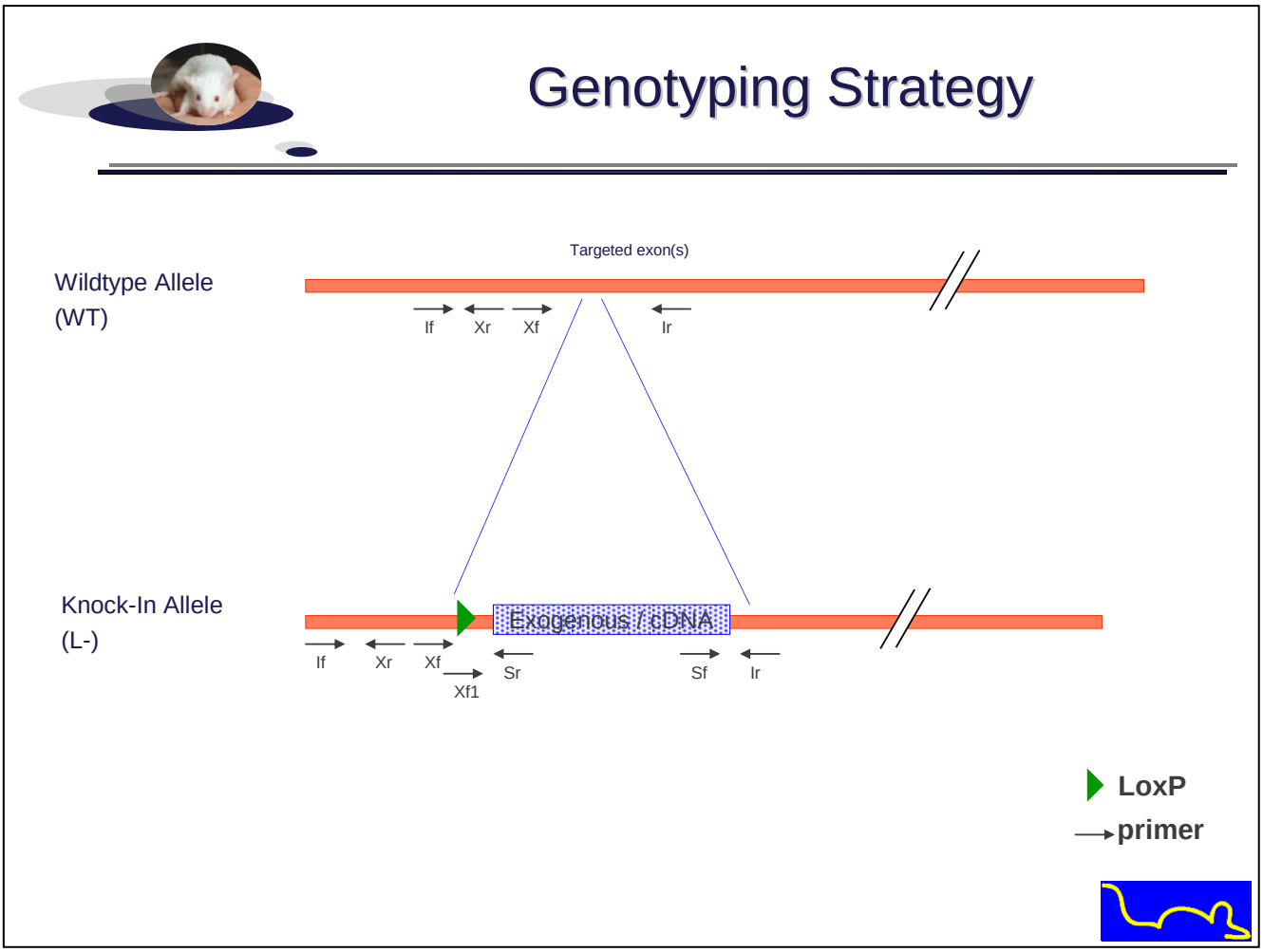
ROSA26 locus (IR1437 /ICS internal reference K393) mouse line genotyping protocol

This protocol describes the strategy and the PCR conditions used for ROSA26 locus knock in (KI) line genotyping.

PCR genotyping strategy



Genotyping protocol
ROSA26 (1437/ICS internal reference K393)

Sequence of primers used for genotyping

Position	Primer	Sequence
If	2999	GCCCACCGCCCCACACTTATTGGCC
Xr	3000	CTACCTAGCCGAGGCTCTCTG
Xf	3002	GAGTTCTCTGCTGCCTCCTGG
Ir	3003	CCCACACACCAGGTTAGCCTTTAAG
Sf	3005	GATCAAGAAGCCAGCTTCTTTGGAGC
Xf1	3010	GATCGACGGTATCGTAGAGTCGAG
Sr	3008	GAGCACCTGCAGGAGCACTATG

PCR fragments expected size (bp):

Primer pair used	**2999-3000**	**3002-3003**	**3005-3003**	**3010-3008**
Position	If/Xr	Xf/Ir	Sf/Ir	Xf1/Sr
Region analyzed	Presence of WT allele	Presence of WT allele	Presence of Ki seq	Cre mediated excision of Neomycin cassette
WT allele (WT)	117	148	/	/
Knock-Out Allele (L-)	117	1589	408	209

* This PCR product will not be observed using our PCR genotyping conditions (see description below)

Genotyping protocol
ROSA26 (1437/ICS internal reference K393)

PCR protocol:

Reagents:	Volume:
-10x Thermopol Reaction Buffer (Biolabs)	2.5µl
-dNTPs 10mM (Amersham Biosciences)	0.5µl
-Taq DNA Polymerase (Sigma can be used)	0.2µl
-DNA (50ng/µl)	2µl
-5' primer (100 µM)	0.25µl
-3' primer (100 µM)	0.25µl
-Sterile H2O	up to 25 µl

Cycling conditions:

Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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