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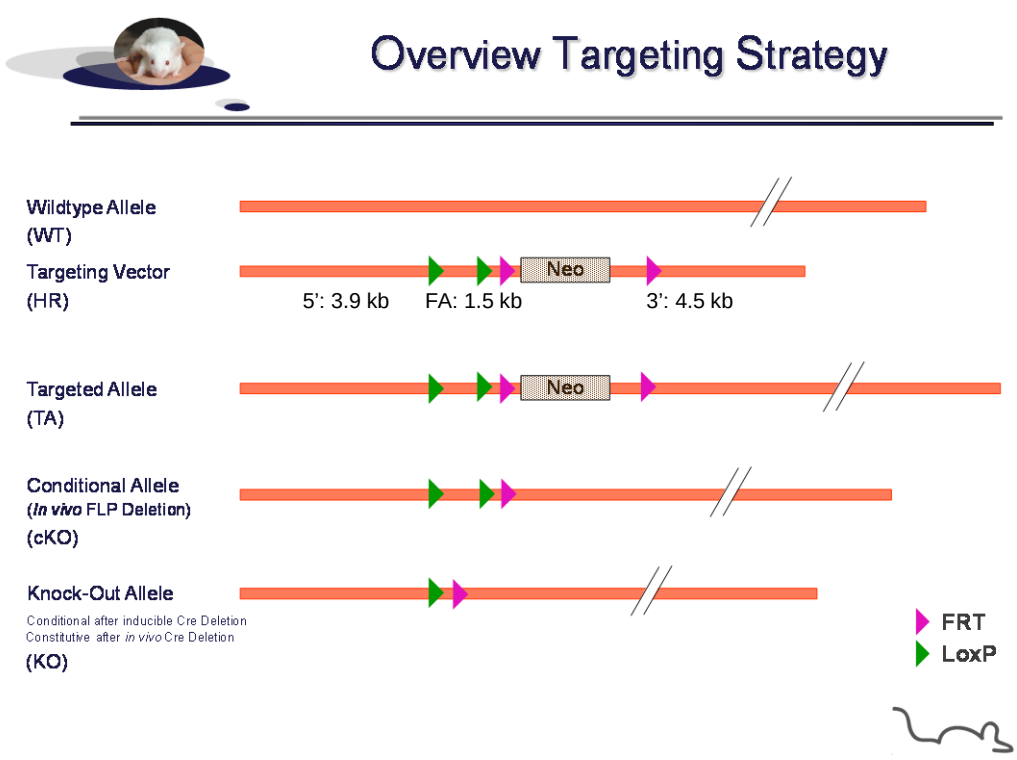
Web site: http://www.phenomin.fr/en-us//

This protocol has been prepared by Loic Lindner, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

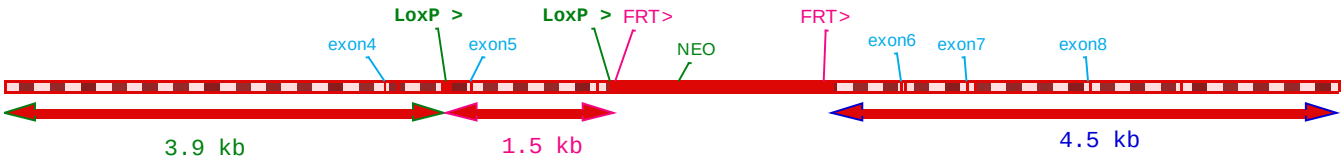


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exon 5

Ppargc1b gene (also named PGC-1β) is a member of the nuclear receptor family. Additional information on this gene can be accessed at:
http://www.informatics.jax.org/javawi2/servlet/WIFetch?page=marker Detail&key=85796

Strategy used to generate the conditional knock out model



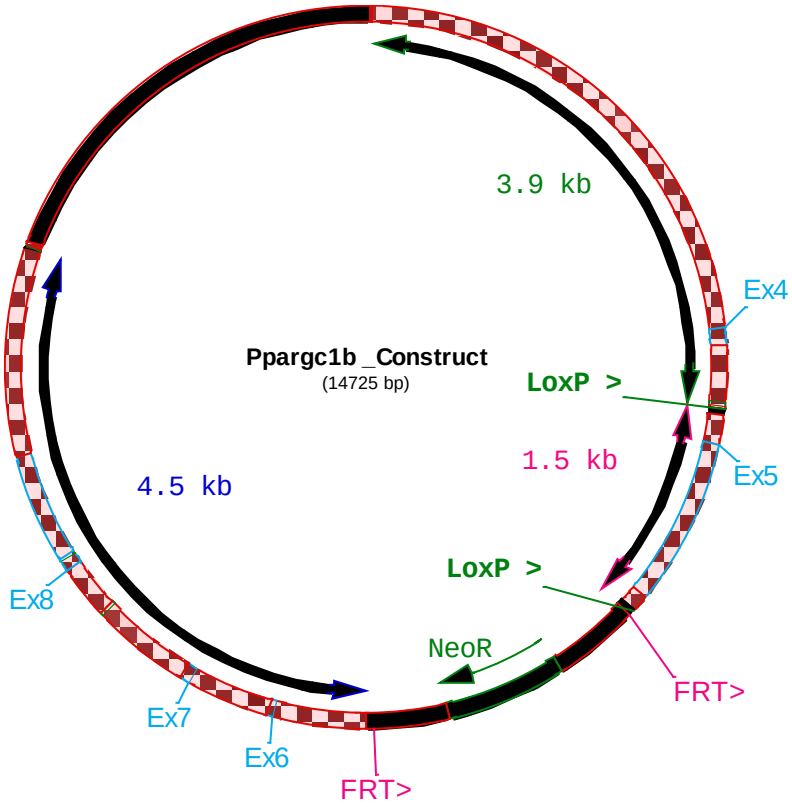
Ppargc1b Strategy
(11800 bp)

2. Construct used for homologous recombination in ES cells: Ppargc1b project

2.1. Legend

loxP sites are indicated in green ; FRT sites are indicated in purple; *Mus musculus* sequences are indicated in uppercase ; exogenous sequences are marked in lowercase.
The targeting vector was generated in 129Sv/Pas and was not fully sequenced. Sequenced regions are indicated in bold.

2.2. Map of targeting vector plasmid



2.3. 5' homology arm (3.9 kb)

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TCACCTTGCTTCCTCACTGAGCGCTGTGTGTGTGTGTGTGTGTGTATGTGTGTGTGTCCTGGAAACTCGTCAG
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2.4. Floxed fragment (1.5 kb)

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2.5. PGK-Neo region

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2.6. 3’ homology arm (4.5 kb)

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2.7. Vector backbone sequence

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3. ES cell lines targeted and validation data:

3.1. ES cell lines targeted

The targeting vector was electroporated in BD10 ES cells [MCI-C57BL/6NTac background]

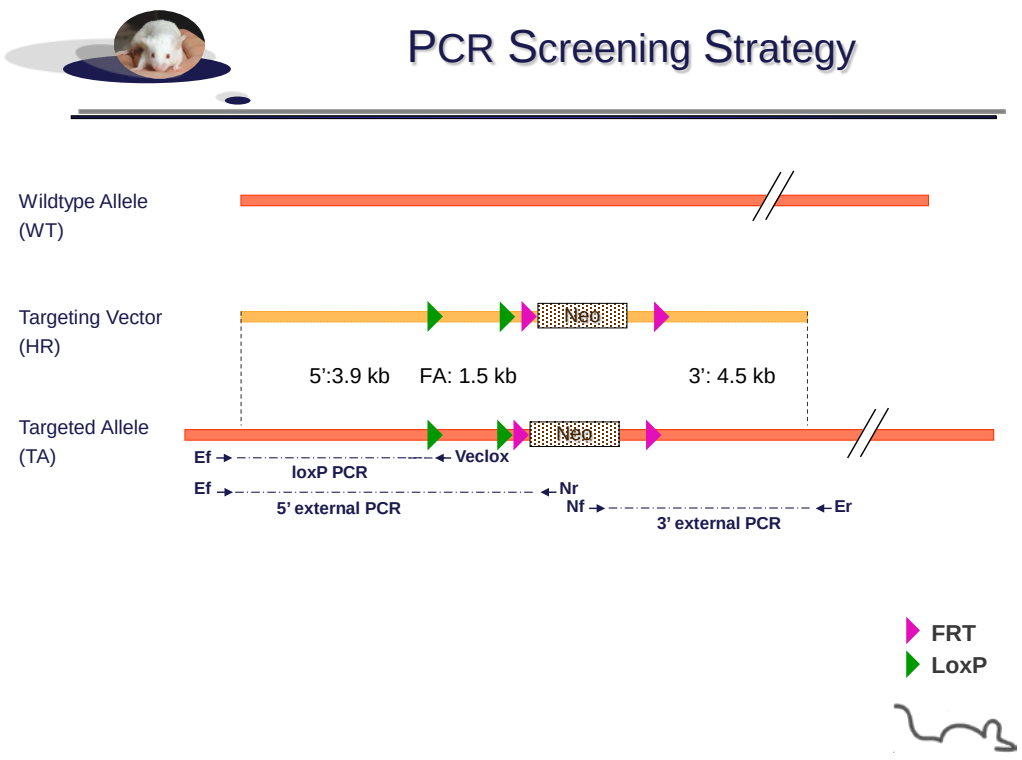
Number of clones screened: 372
Number of positives: 5

Reference of clone used to generate the mouse line:

- clone **K189BD10-271**

3.2. PCR data on positive clone

3.2.1.PCR screening strategy

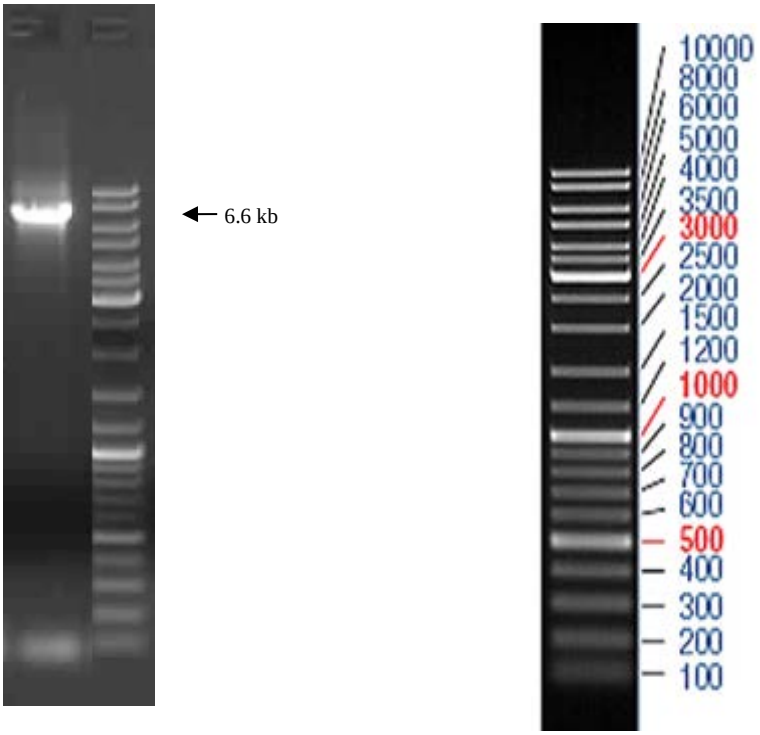


Primers used for PCR validation of ES clone

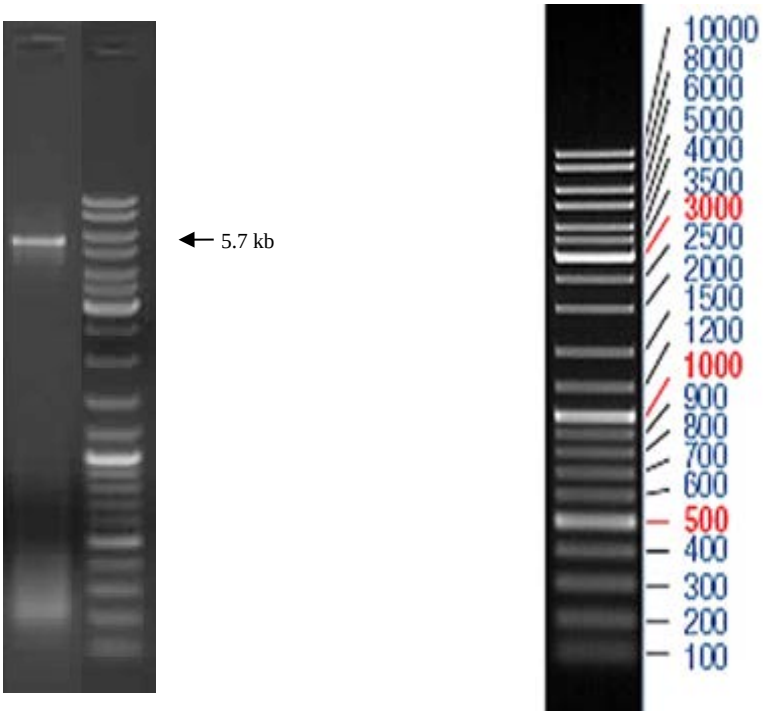
PCR	Primer Name	Primer sequences	PCR product size
5' external	Ef	ATCTCTAAATAGGCTACCTTCTGCC	6.6 kb
	Nr	GCGGCCGGAGAACCTGCGTGCAATC	
3' external	Nf	AGGGGCTCGCGCCAGCCGAACTGTT	5.7 kb
	Er	CTCGTGACTTGCTAACTTTCAACGC	
LoxP	Ef	ATCTCTAAATAGGCTACCTTCTGCC	4.5 kb
	Veclox	GGCCAGCCATATTCCATAACTTCGT	

3.2.2.Picture of PCR on positive clone

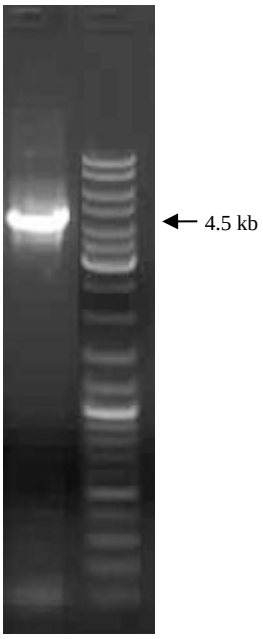
5' external PCR ladder



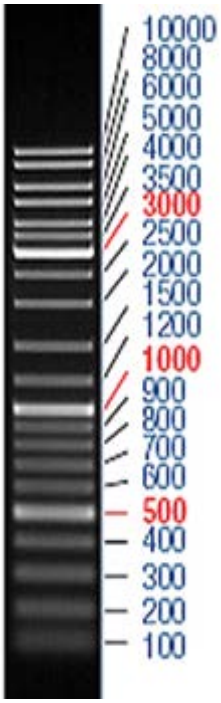
3' external PCR ladder



LoxP PCR

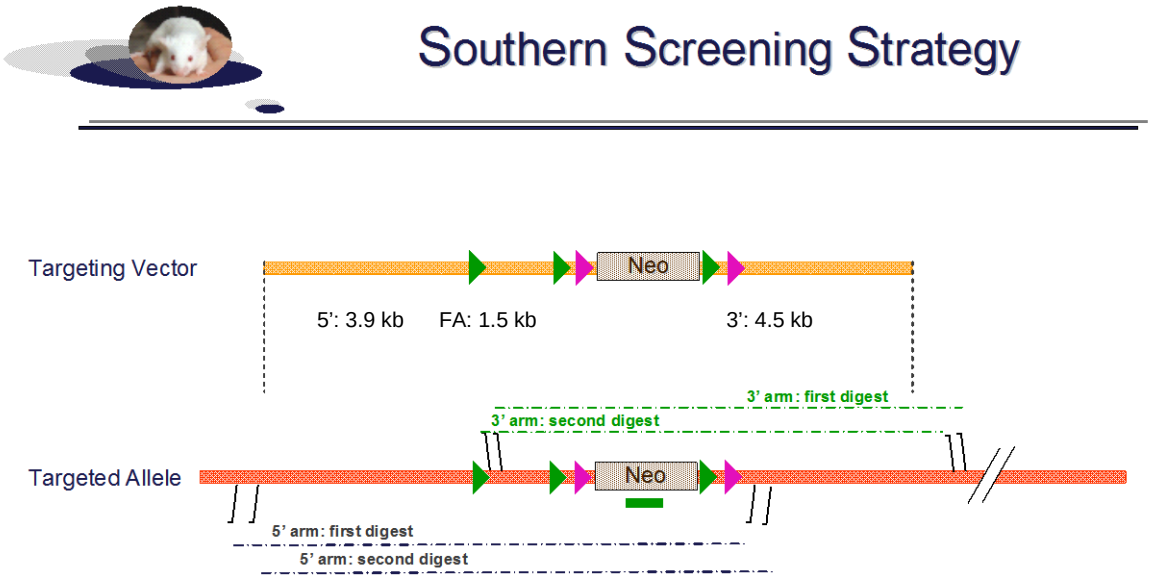


ladder



3.3. Southern data on positive clone

3.3.1.Neo Southern strategy



Digestions used to validate the 5' and 3' insertion

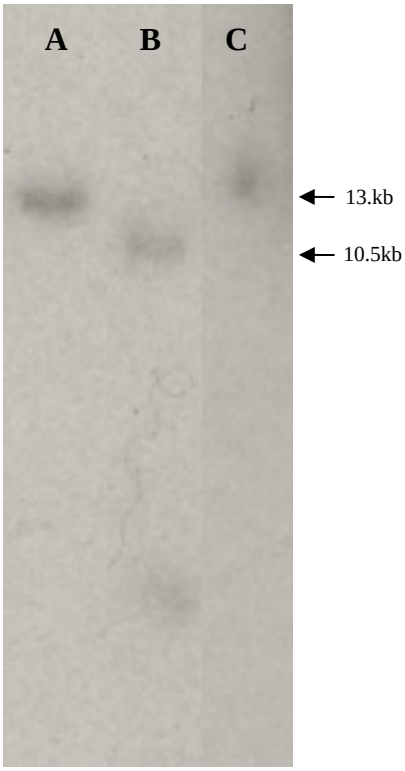
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	ScaI	/	13.4
	3' arm first digest	HpaI	/	10.5
	3' arm second digest	HindIII	/	13.3

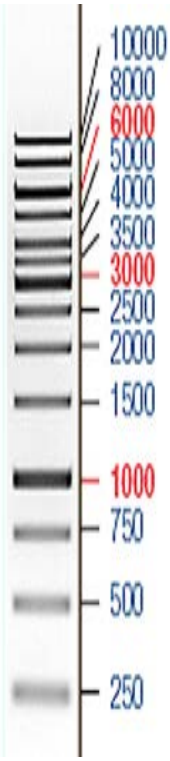
Three different digests are used to validate correct HR event. One digest validates the 5' insertion, 2 other digests validate the 3' insertion.

3.3.2.Picture of Neo Southern

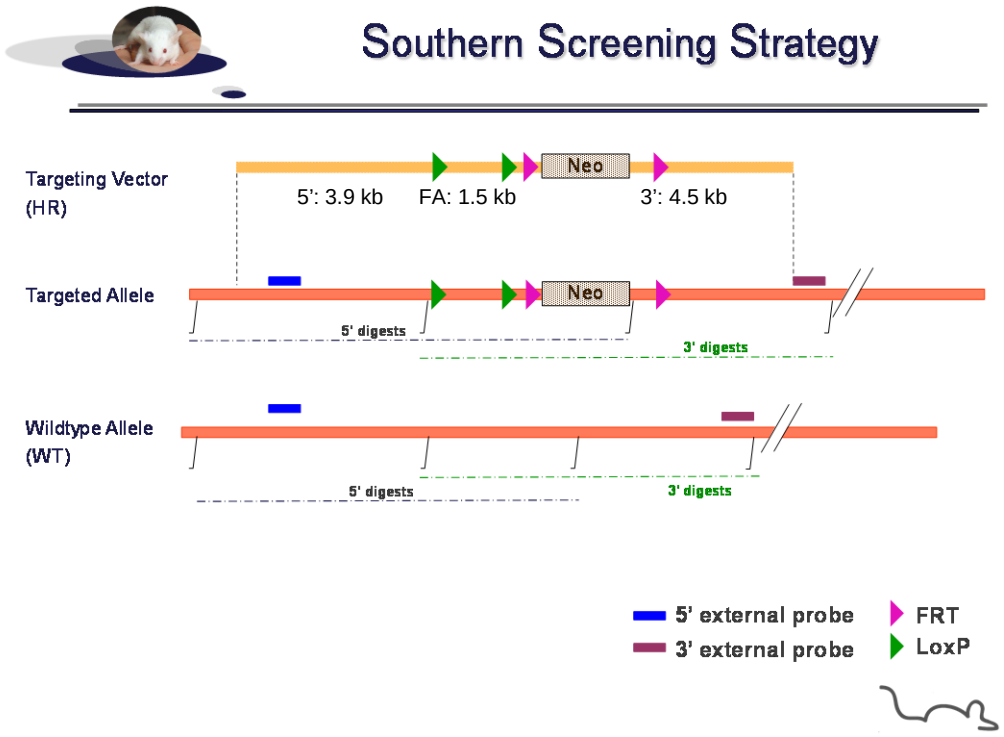
Neo southern blot: 5' and 3' arm validation ladder

A: ScaI : 13.4 kb
B: HpaI : 10.5 kb
C: HindIII : 13.3 kb





3.3.3.External probes Southern strategy



Digestions used to validate with 5' and 3' probes

Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
5' external	first digest	NheI/ScaI	15.9	10.0
3' external	first digest	HindIII	6.9	13.3
	second digest	NheI/SpeI	14.9	8.3

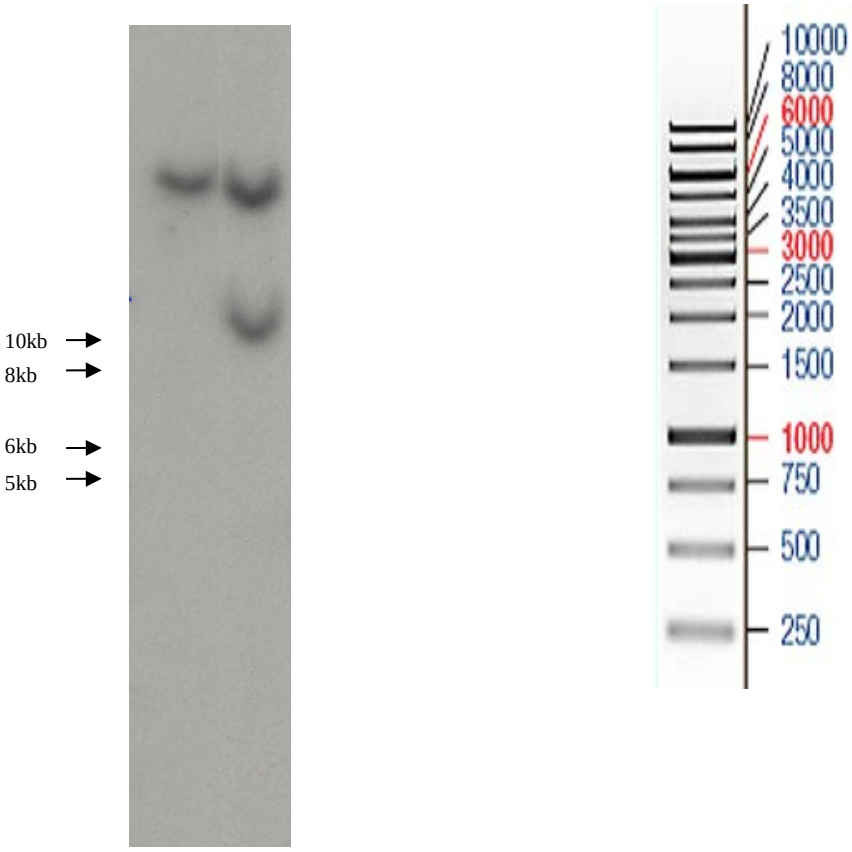
Primers for probe synthesis:

5' probe:
CACCCTTGGATGTGCCCCGGCTTGC
CTGAACCTACTACACAAGGTGAGGG

3' probe:
GGGTTTAAGTTCAACCCGTGGAGCT
TCTTCATGTTAGGGGTAGATAGCCC

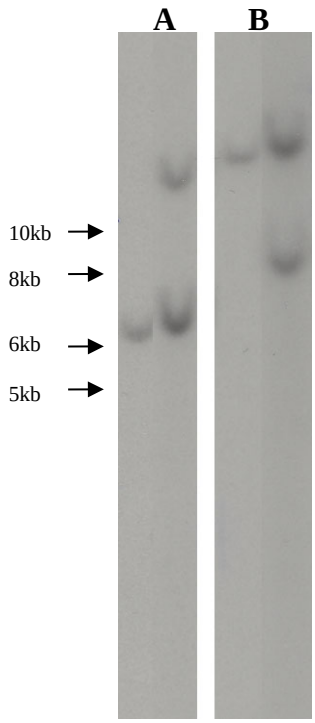
3.3.4.Picture of Southern with external 5’ and 3’ probes

5’ external probe validation Ladder

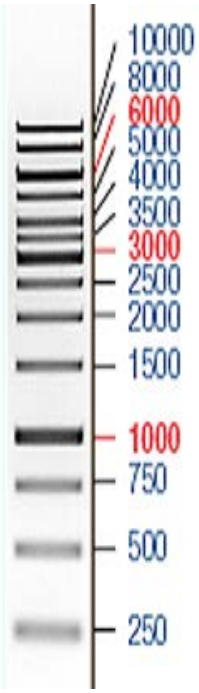


3' external probe validation

A: HindIII digest
B: NheI/SpeI digest



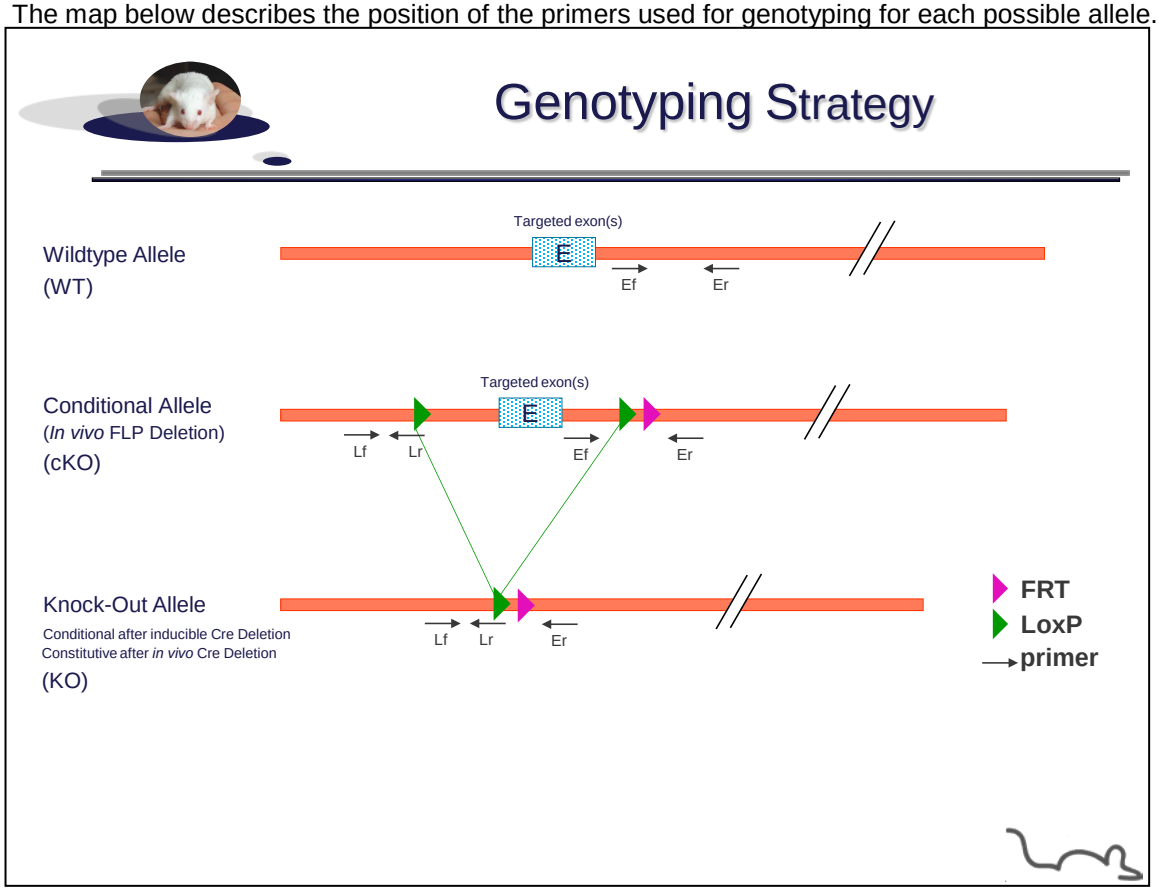
Ladder



4. Genotyping protocol and data on conditional and knock-out animals

4.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.



Sequence of primers used for genotyping

Position	Primer	Sequence
Ef	3039	GTGAGGTTGGATCTGCTTATCCACTG
Er	3041	CCTGTCTCTGCTTCCTAAGTATTGTGC
Lf	3038	CTCTGGGGCCTCATGAGCTAATG
Lr	3037	ACGAAGTTATGGAATATGGCTGGCC

PCR fragments expected size (bp):

Region analyzed	**Primers used**	Position on the primer (see the map above)	Conditional allele (cKO)	Knock-Out allele (KO)	WT allele (WT)
Presence of the distal loxP	3038-3037	Lf / Lr	164	164	---
Excision of the selection marker	3039-3041	Ef / Er	329	---	219
Excision of the floxed exon(s), i.e. knock out	3038-3041	Lf / Er	1898*	442	1781*

*: This PCR product will not be observed using our PCR genotyping conditions (see description below)
---: No Amplicon should be obtained

4.2. PCR protocol

This section describes the composition of the mix and cycling conditions used for genotyping.

Reagents:	Volume:
- FastStart PCR Master (Roche)	7.5µl
- DNA (50ng/µl)	1.5µl
- 5' primer (100 □M)	0.06µl
- 3' primer (100 □M)	0.06µl
- Sterile H_2O	up to 15 µl

Cycling conditions:

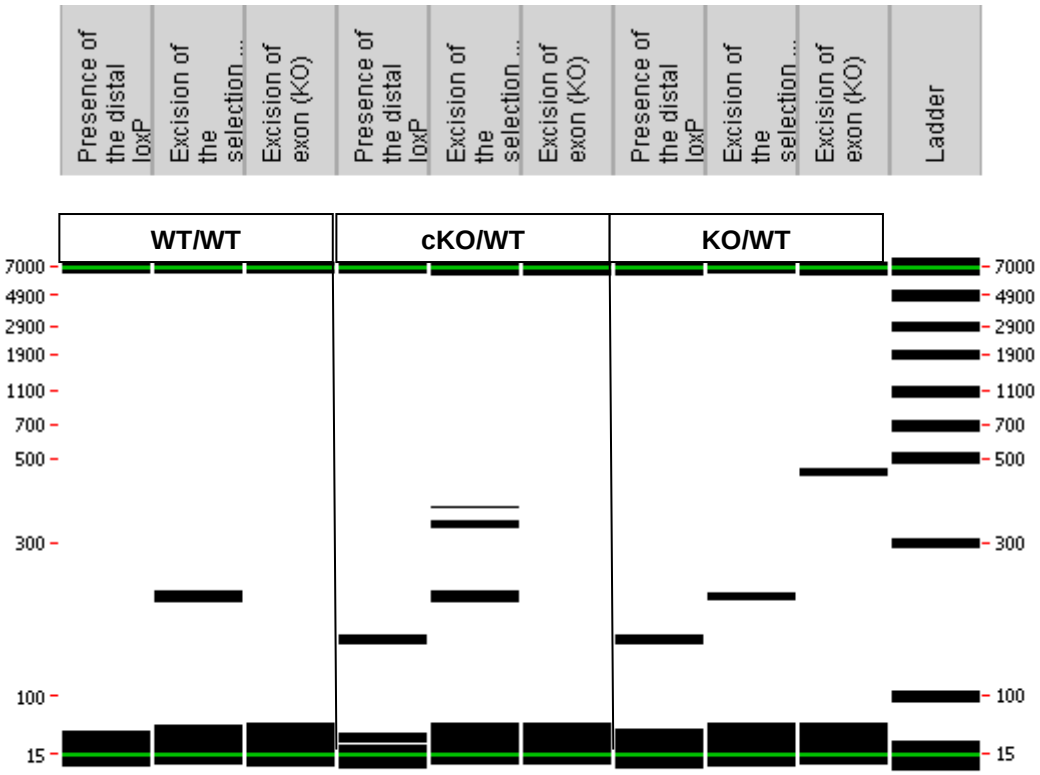
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

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

4.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was done by using LabChip® 90 microfluidic apparatus. PCR products were run on the HT DNA 5K LabChip® 90 Assay Kit.

Representative genotyping picture



Note that as this technology is more sensitive than gel analysis, non-specific signals and/or primer dimers may be visible on the picture.