Molecular Biology Data
Nr1a2 conditional knock out model
ICS references K252

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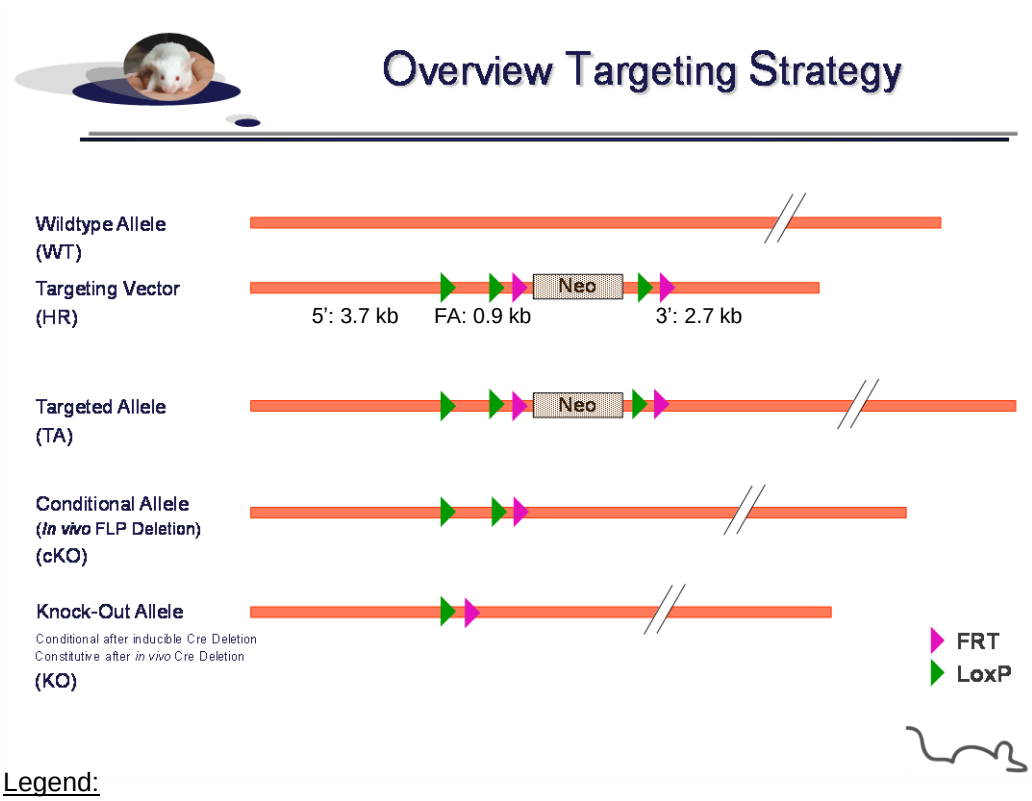
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

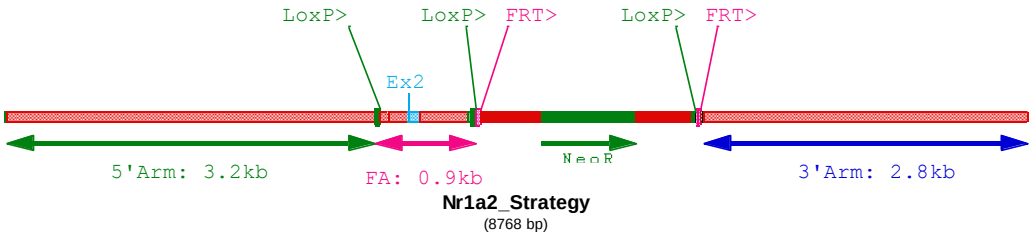


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exon 2

Nr1a2 gene (also named Thrb) 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=markerDetail&key=14126

Strategy used to generate the conditional knock out model

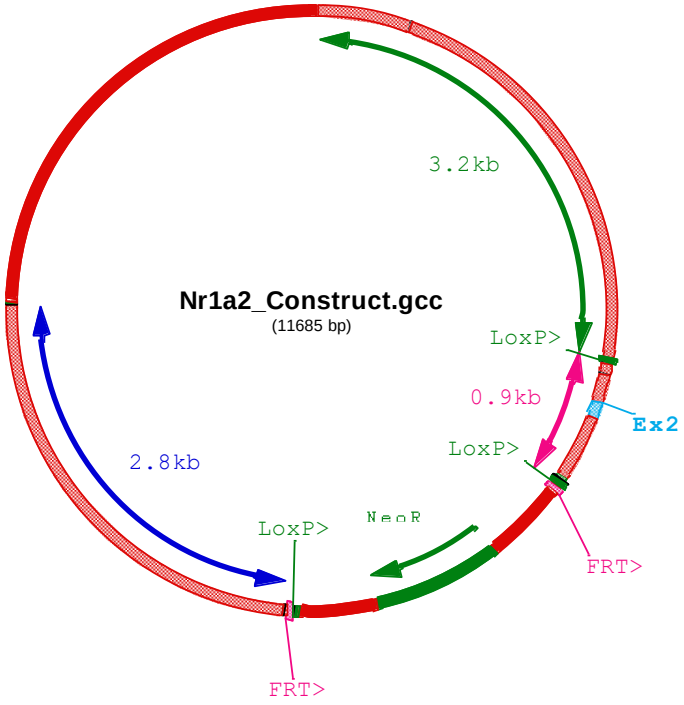


2. Construct used for homologous recombination in ES cells: Nr1a2 project

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. Regions sequenced are indicated in bolds.

2.2. Map of targeting vector plasmid



2.3. 5' homology arm (3.7 kb)

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2.4. Floxed fragment (0.9 kb)

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2.5. PGK-Neo region

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tgcagg

2.6. 3’ homology arm (2.7 kb)

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TA

2.7. Vector backbone sequence

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ggaaacagctatgaccatgattacgccaagcgcgcattaaccctcactaaagggaacaaaagctggagctcgcgg
ccgcggcgcgcc

3. ES cell lines targeted and validation data:

3.1. ES cell lines targeted

The targeting vector was electroporated in P1 ES cells [MCI-129Sv/Pas background]

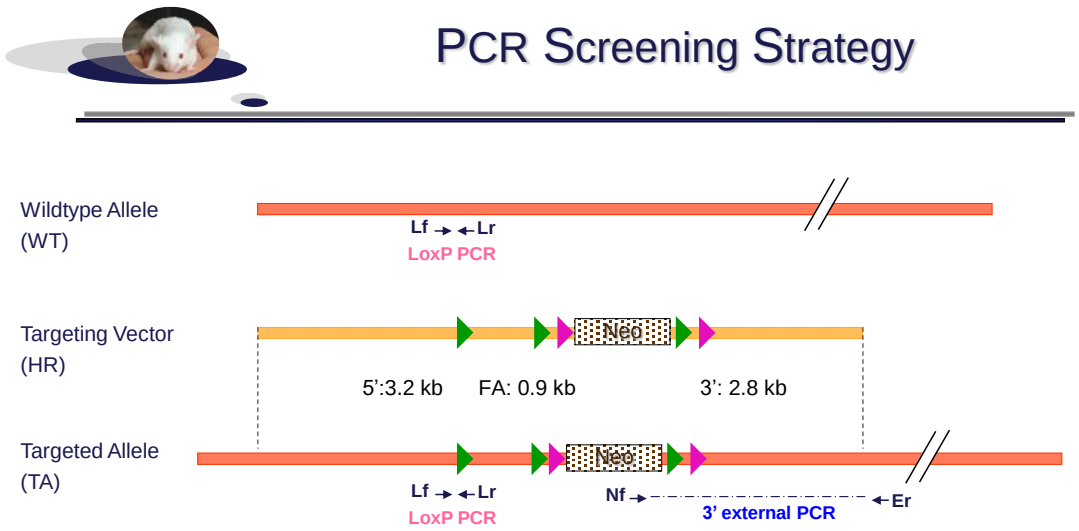
Number of clones screened: 744
Number of positives: 3

Reference of clone used to generate the mouse line:

- clone **K251P1-248**

3.2. PCR data on positive clone

3.2.1.PCR screening strategy

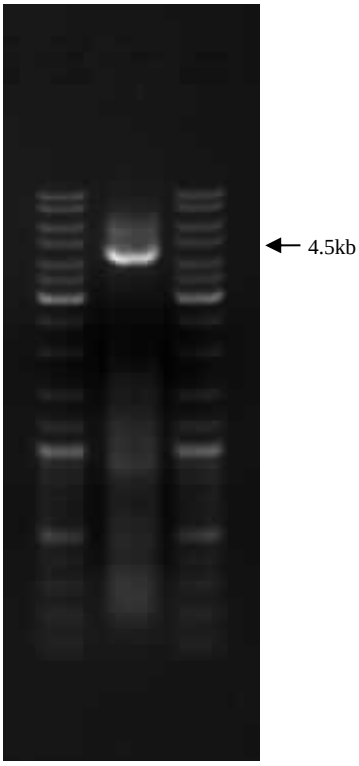


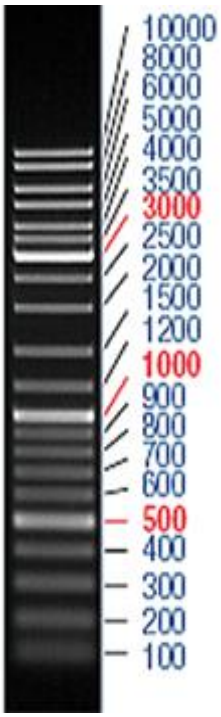
Primers used for PCR validation of ES clone

PCR	Primer Name	Primer sequences	PCR product size
3' external	Nf	AGGGGCTCGCGCCAGCCGAACTGTT	TA: 4.5kb
	Er	GCCCTCTTTGTCTATGTTGTGACTG	
LoxP	Lf	GCATTCAGCAATGTTGGCTGTGTG	WT: 0.19kb
	Lr	CATCGGCTCTAAGGAGTTGAACTTC	TA: 0.24kb

3.2.2.Picture of PCR on positive clone

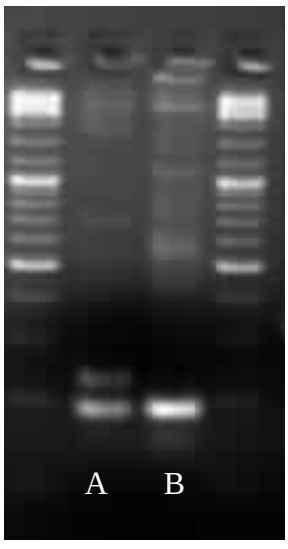
3' external PCR ladder

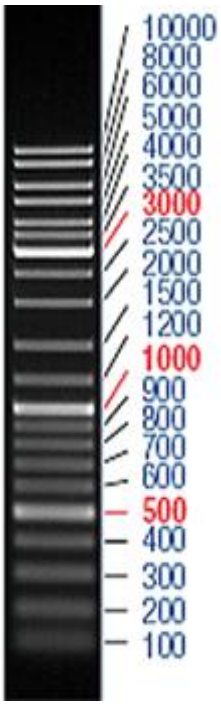




LoxP PCR ladder

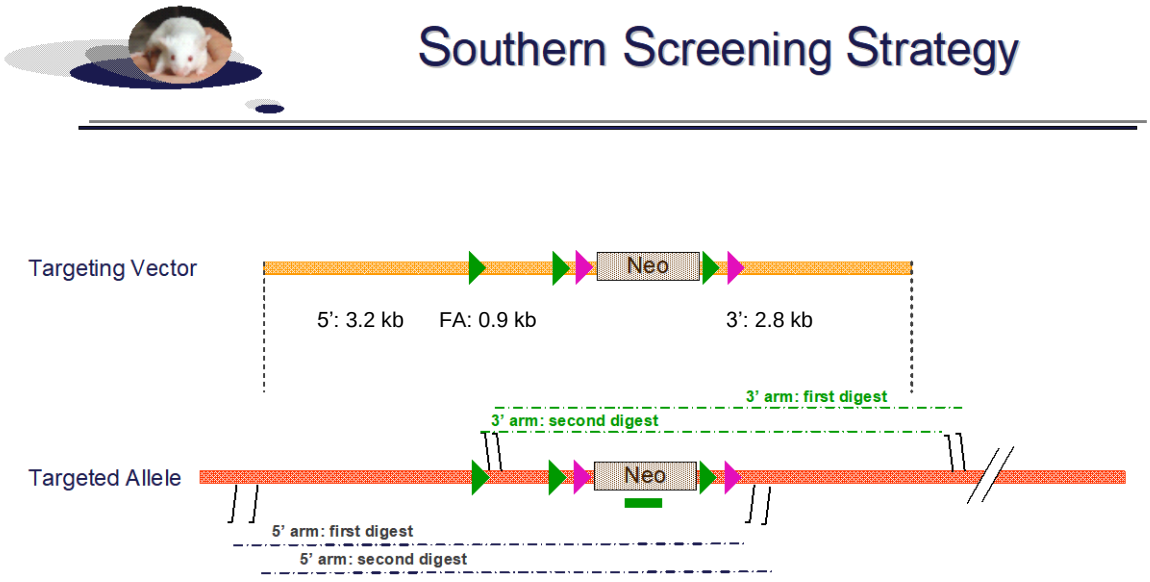
A: positive clone
B: WT clone





3.3. Southern data on positive clone

3.3.1.Neo Southern strategy



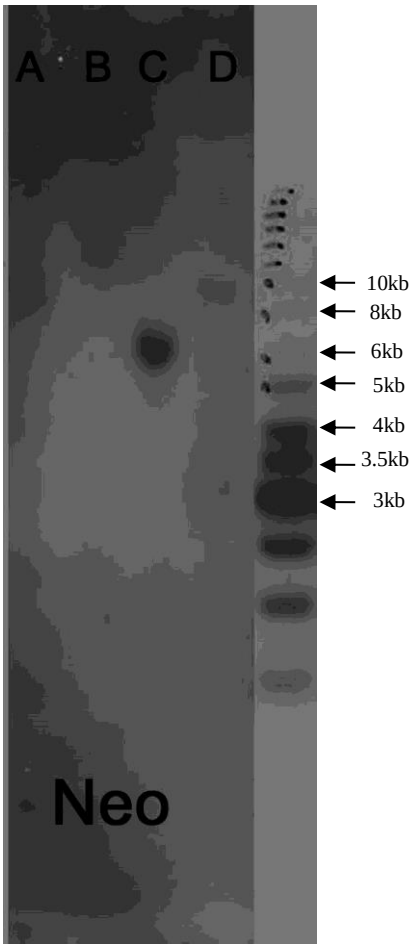
Digestions used to validate the 5' and 3' insertion

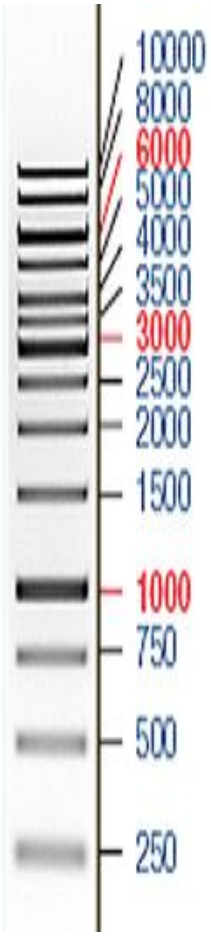
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	NdeI	/	10.5
	5' second digest	HindIII	/	12.9
	3' arm first digest	KpnI	/	6.6
	3' arm second digest	SexAI	/	9.7

Four different digests are used to validate correct HR event. Two digests validate the 5' insertion, 2 other digests validate the 3' insertion

3.3.2.Picture of Neo Southern

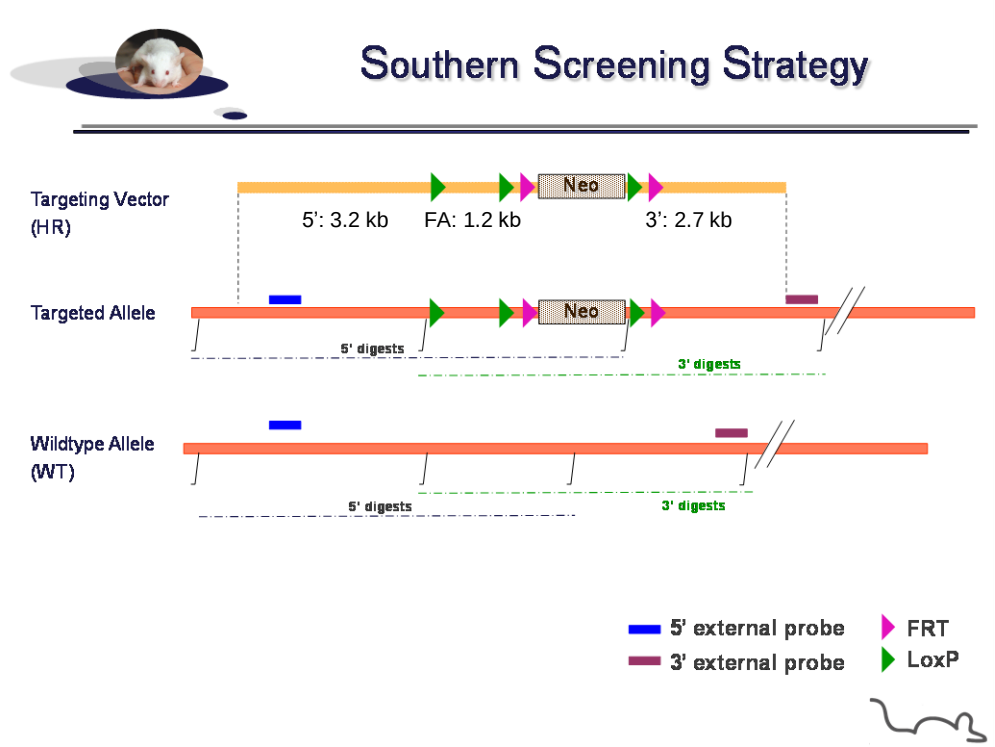
Neo southern blot: 5’ and 3’ arm validation ladder





A: NdeI 10.5kb
B: HindIII 12.9kb
C: KpnI 6.6kb
D: SexAI 9.7kb

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	HindIII	10.8	12.9
3' external	first digest	DraIII	8.1	6.5
	second digest	PshAI	10.1	12.1

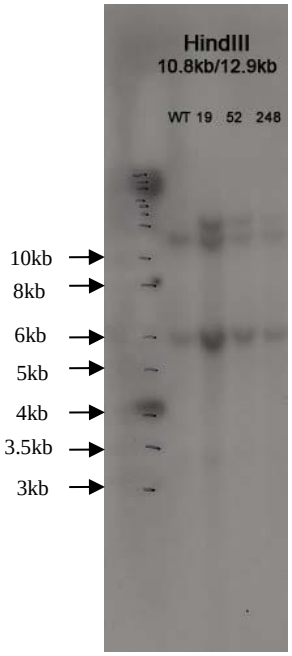
Primers for probe synthesis:

5' probe
TGGCTGGTATCTGAGTGTGGTCTCC
GTGTTCGTGGGGTTAGTATGTACAC

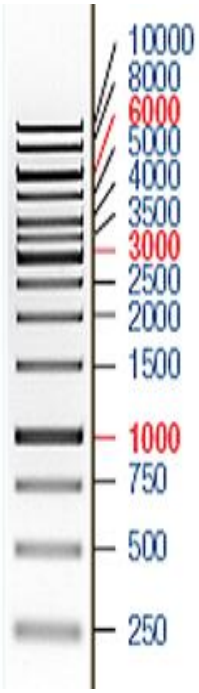
3' probe
CTACTCTGGACCCTATTATTGCAAG
ATGGCCTTTACCAACTCTCTTAACC

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

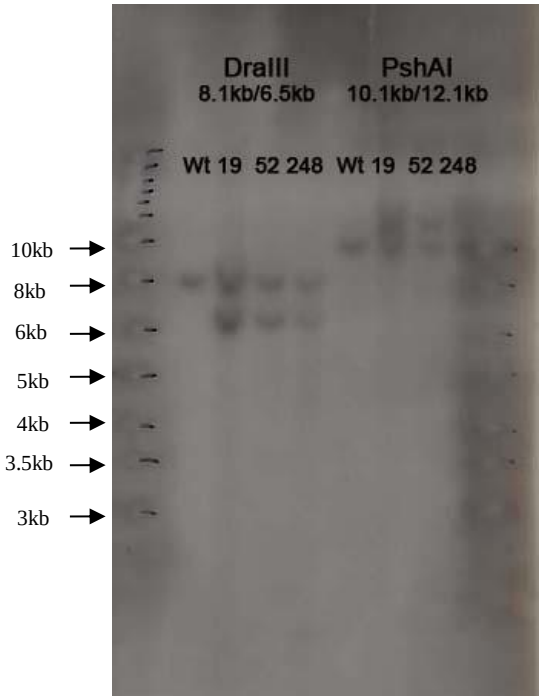
5’ external probe validation



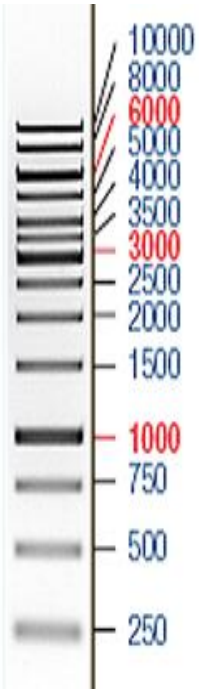
Ladder



3’ external probe validation



Ladder

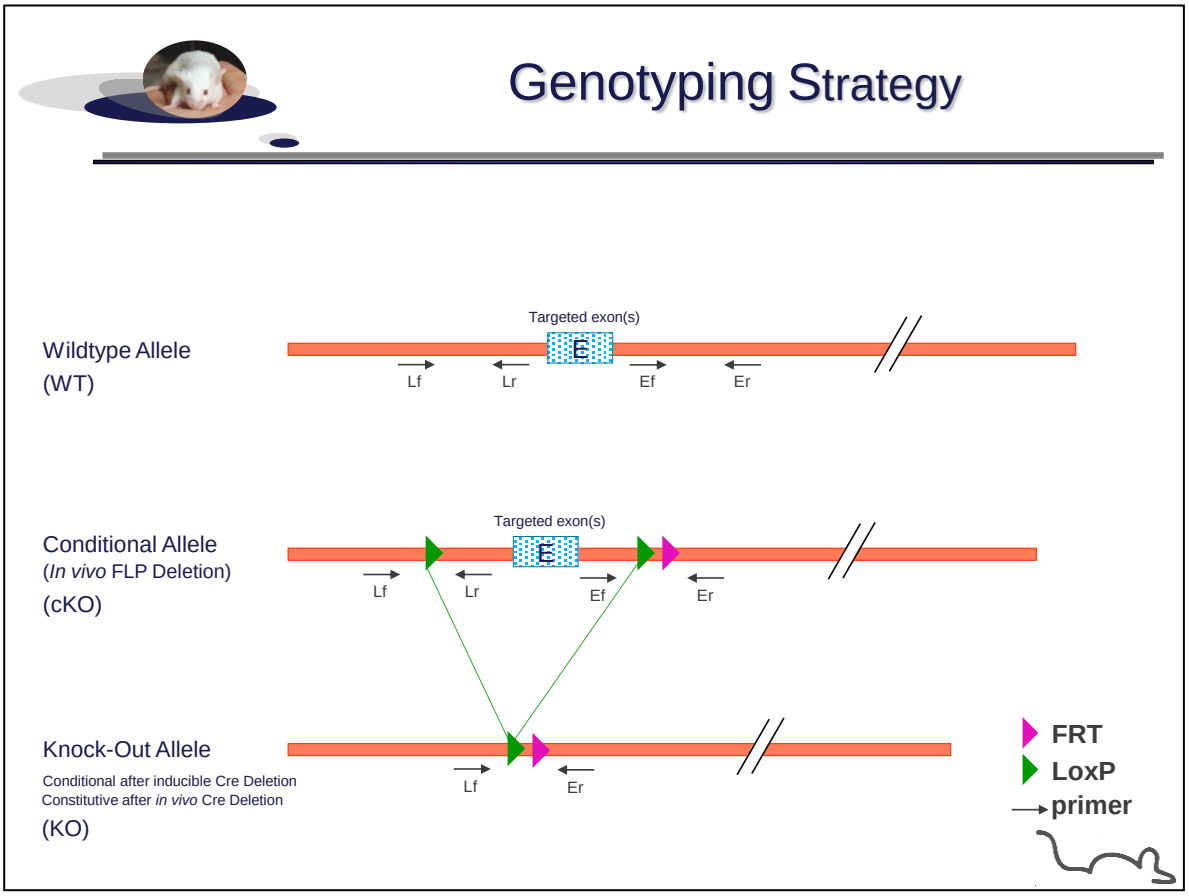


4. Genotyping protocol and data on conditional and knock-out animals

Both conditional and knock-out mouse models were backcrossed in C57BL/6J background.

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	Primers	Sequence
Ef	3472	AATGGAAGTTTGCAATAGTGTGGTG
Er	3474	CAGGCTCTGCTGAAAGCCAC
Lf	3470	TGCATTCAGCAATGTTGGCTGTGTG
Lr	3471	CATCGGCTCTAAGGAGTTGAACTTC

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 5'loxP	3470-3471	Lf / Lr	240	---	190
Excision of the selection marker	3472-3474	Ef / Er	421	---	317
Total Excision (excision of the floxed exon(s), i.e. knock out)	3470-3474	Lf / Er	1028*	204	882*

* 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:
-10x Buffer (Roche)	2.5µl
-dNTPs 10mM (Amersham Biosciences)	0.5µl
-Taq DNA Polymerase (Roche)	0.2µl
-DNA (50ng/µl)	3µl
-5' primer (100 µM)	0.125µl
-3' primer (100 µM)	0.125µl
-Sterile H2O	up to 25 µl

Cycling conditions:

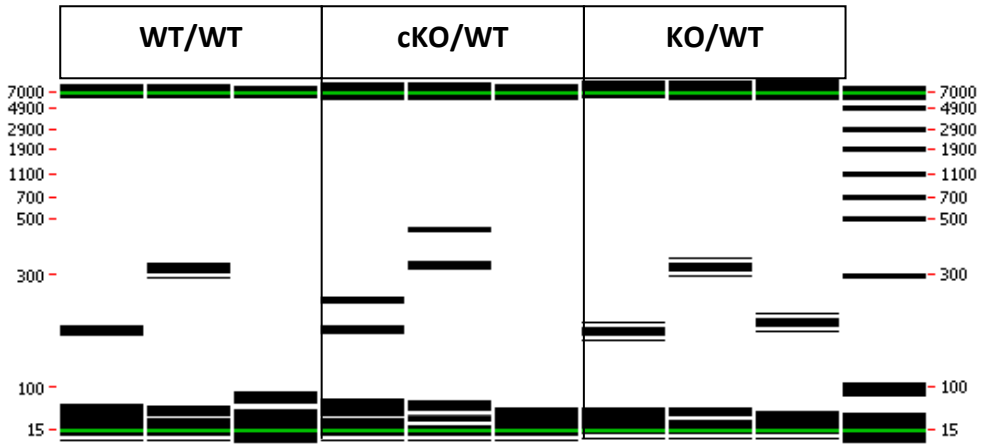
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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

4.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was not done by gel electrophoresis but 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.