Molecular Biology Data
Nr3b1 conditional knock out model
ICS reference K167/DG24

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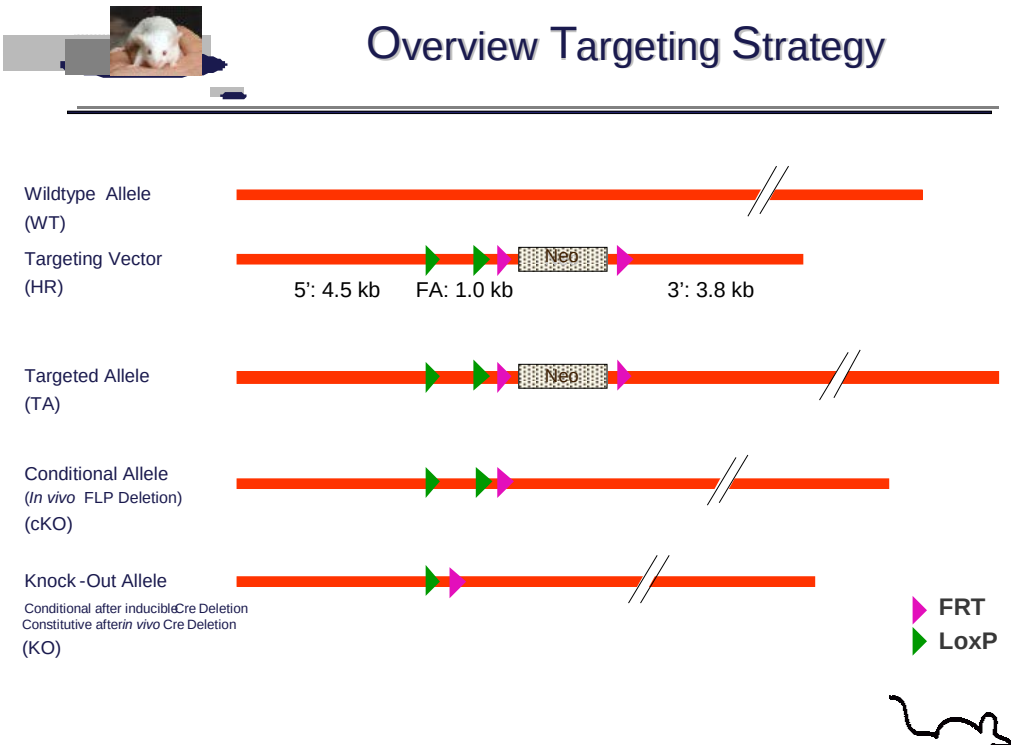
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

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:

Nr3b1 gene (also named ERR1) 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=43302

1.2.1.Relevant informations

This section provides additional information that can be useful for the comprehension of the strategy.

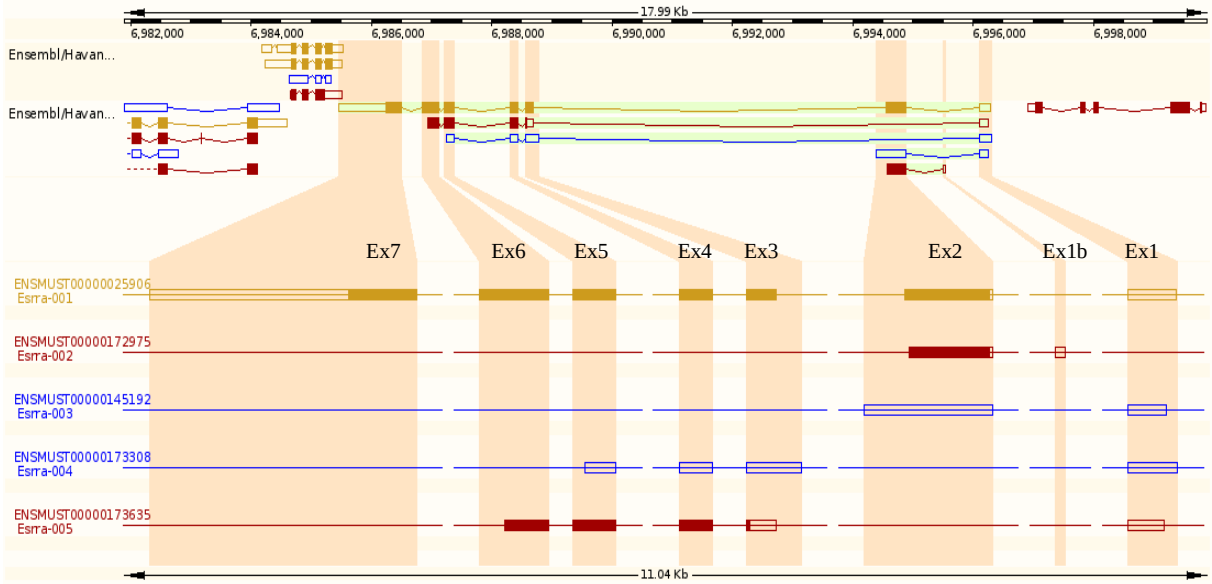
1.2.1.1. Know transcripts

Ensembl data (in Feb 2012) indicate that 5 splice variants are known for this gene (see below).

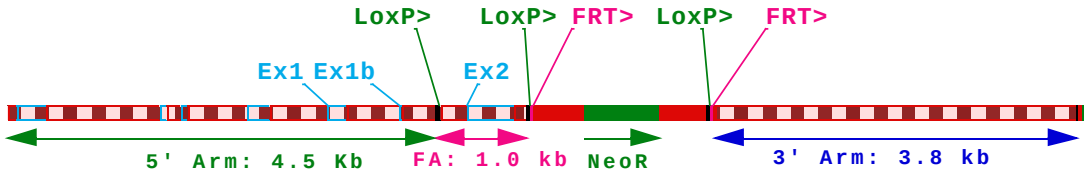
Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CCDS
Esrra-001	ENSMUST00000025906	2242	ENSMUSP00000025906	422	Protein coding[1]	CCDS29509
Esrra-002	ENSMUST00000172975	358	ENSMUSP00000133916	103	Protein coding[1]	-
Esrra-005	ENSMUST00000173635	727	ENSMUSP00000134587	161	Protein coding[1]	-
Esrra-004	ENSMUST00000173308	654	No protein product	-	Processed transcript[2]	-
Esrra-003	ENSMUST00000145192	641	No protein product	-	Retained intron[3]	-

[1] A protein coding transcript is a spiced mRNA that leads to a protein product.
[2] Noncoding transcript that does not contain an open reading frame (ORF).
[3] Noncoding transcript containing intronic sequence.

1.2.1.2. Splice variants and exons nomenclature



1.2.2.Strategy used to generate the conditional knock out model



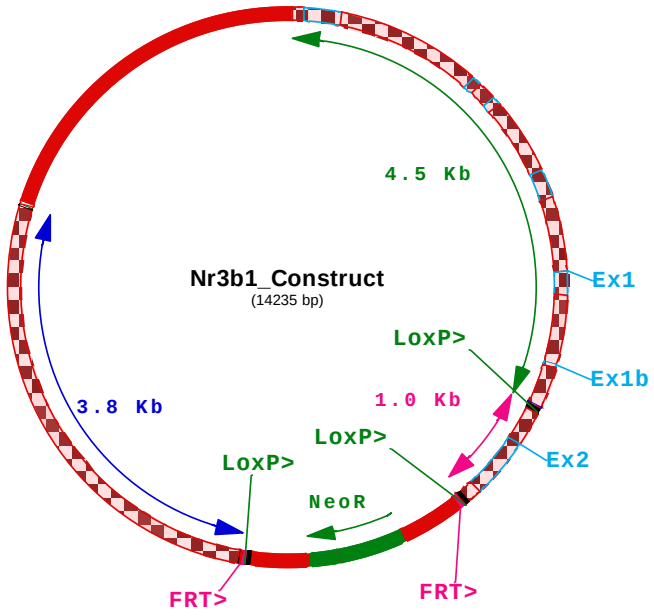
Nr3b1_Strategy
(11361 bp)

2. Construct used for homologous recombinaison in ES: Nr3b1 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 (4.5kb)

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GGCAAATCTGTAAGAAACAGGATTCGCAAGTACAGCCAGACCATTTCTATTAGGGACAGCCTCAACCTCGAGCCA
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2.5. PGK-Neo region

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2.6. 3’ homology arm (3.8kb)

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2.7. Vector backbone sequence

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cgccacctctgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcctatggaaaaacgccagcaac
gcggcctttttacggttcctggccttttgctggccttttgctcacatgttctttcctgcgttatcccctgattct
gtggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcagcgagtca
gtgagcgaggaagcggaagagcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagc
tggcacgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattag
gcaccccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacaca
ggaaacagctatgaccatgattacgccaagcgcgcaattaaccctcactaaagggaacaaaagctggagctcgcg
gccgcggcgcgcc

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]

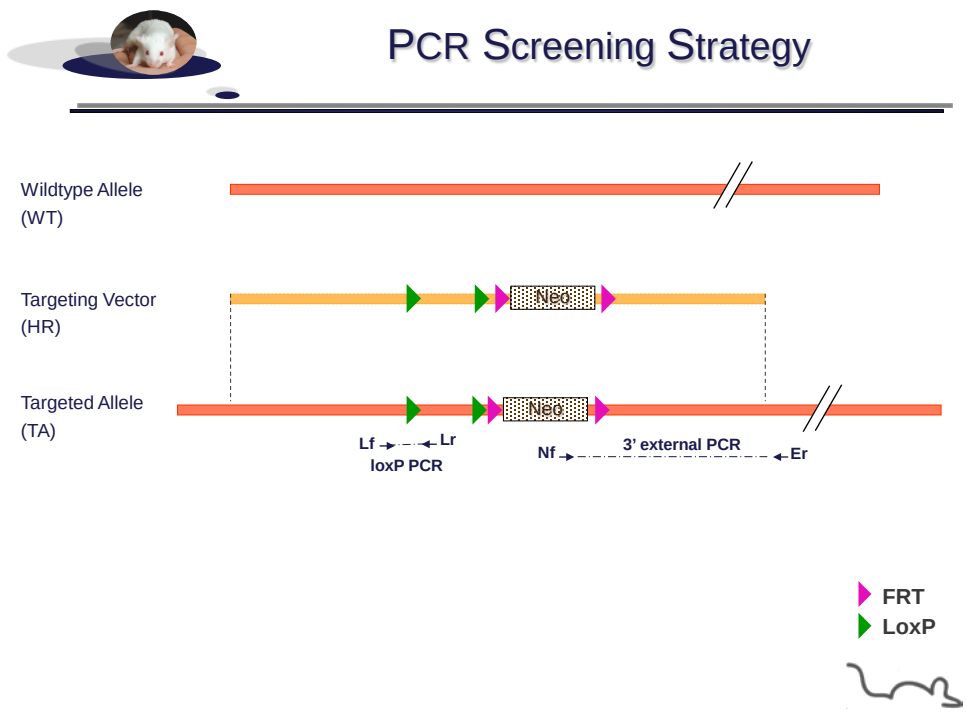
Number of clones screened: 372
Number of positives: 2

Reference of clone used to generate the mouse line:

- clone **K167-227**

3.2. PCR on positive clone:

3.2.1.PCR screening strategy

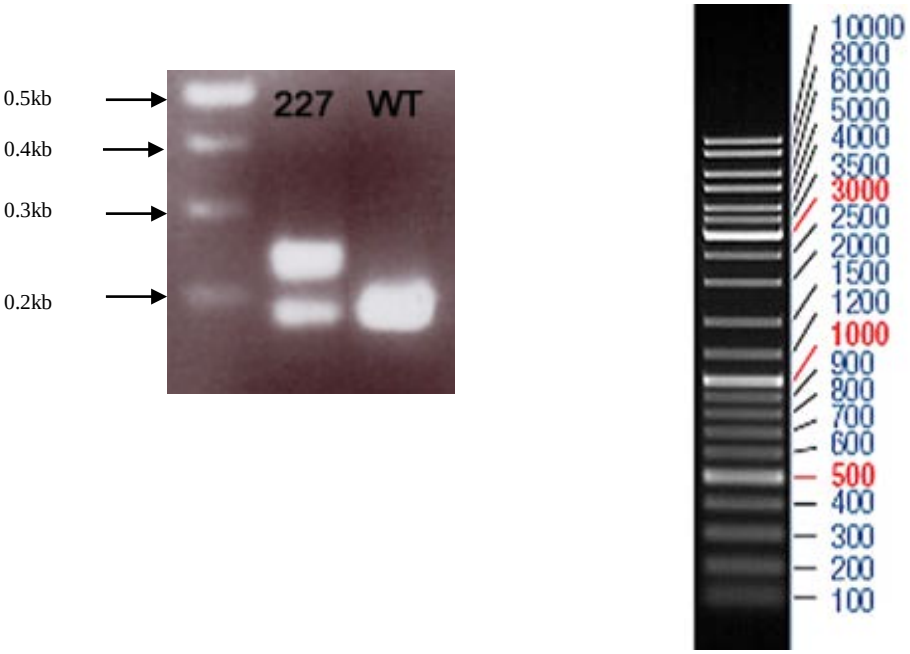


Primers used for PCR validation of ES clone

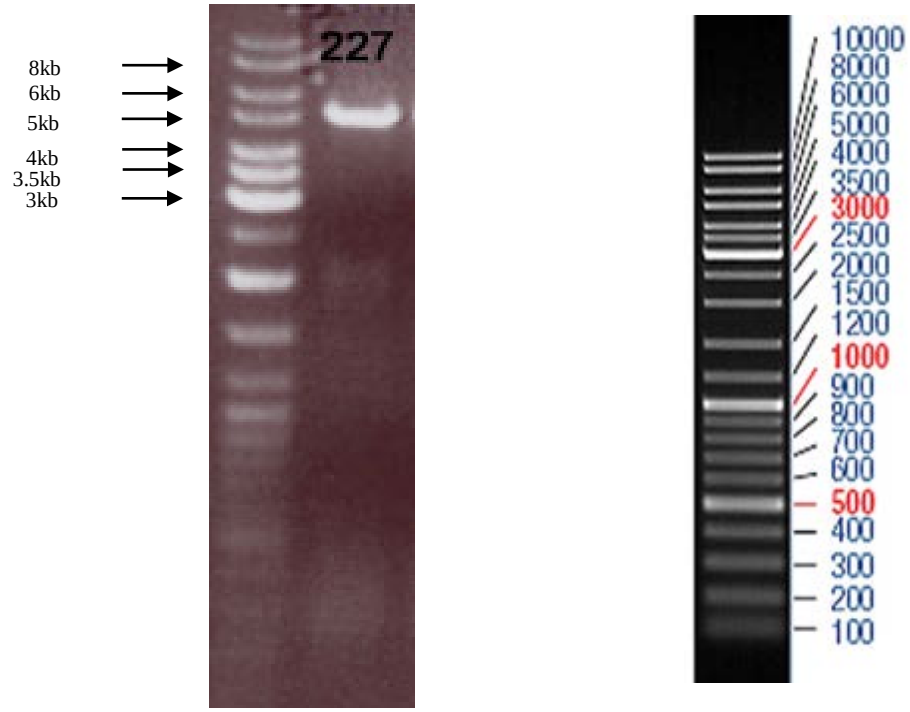
PCR	Primer Name	Primer sequences	PCR product size (kb)
loxP	Lf	AGGATCATGCTTATGCTCGGGGTTG	WT: 0.18
	Lr	CCCATGGCGACAGCTGGGGGCCCTC	TA: 0.25
3' external	Nf	AGGGGCTCGCGCCAGCCGAACTGTT	TA: 5.1
	Er	GTTATGTATCGGATAGTGGTAAGGC	

3.2.2.Picture of PCR on positive clone

LoxP PCR (WT: 0.18kb / TA: 0.25kb) Ladder

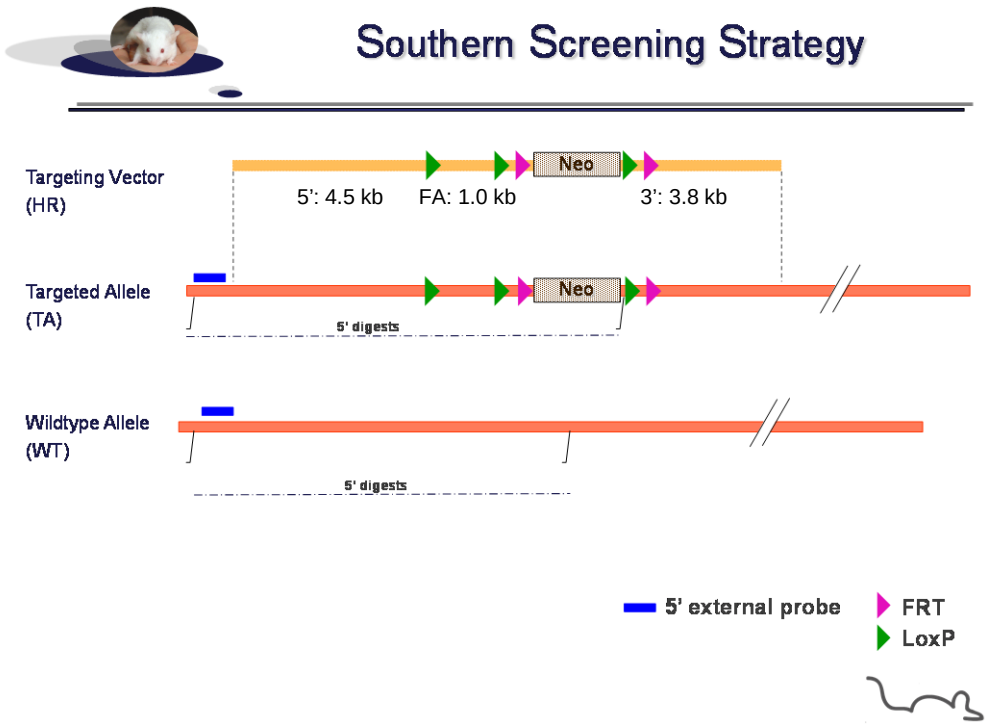


3' external PCR (5.1kb) Ladder



3.3. Southern data on positive clone:

3.3.1.External probes Southern



Digestion used to validate with 5'

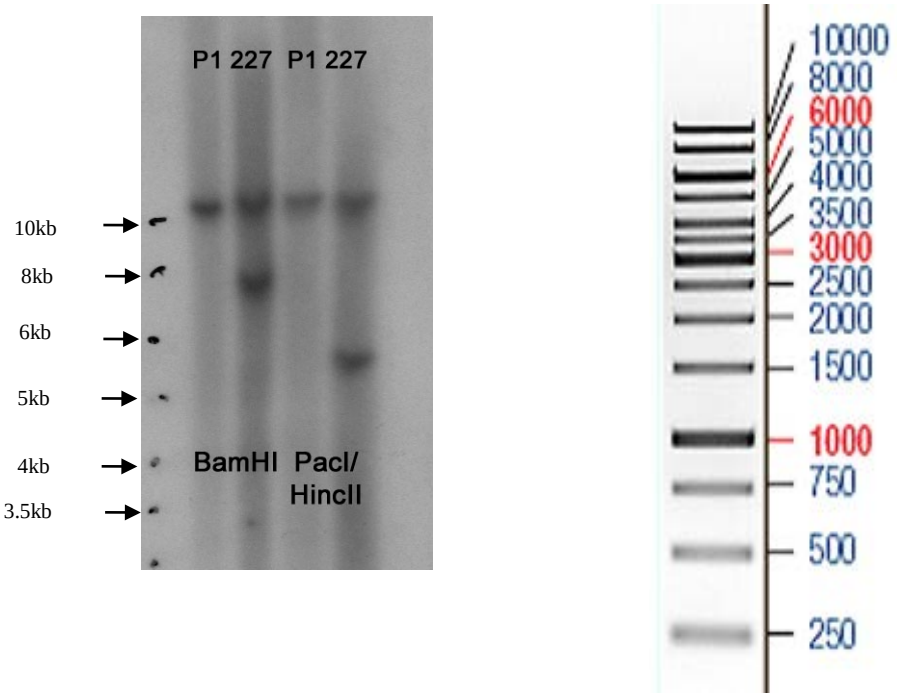
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
5' external	5'first digest	BamHI	10.7	7.7
	5' second digest	PacI/HincII	11.3	5.8

Primers for probe synthesis:

5' probe
CACGCCAGGGCTTTCGCTCTTGCTG
GCAGAGACAAGAGTCTGAATACCTG

3.3.2.Picture of Southern with external 5' probe

5' external probe ladder

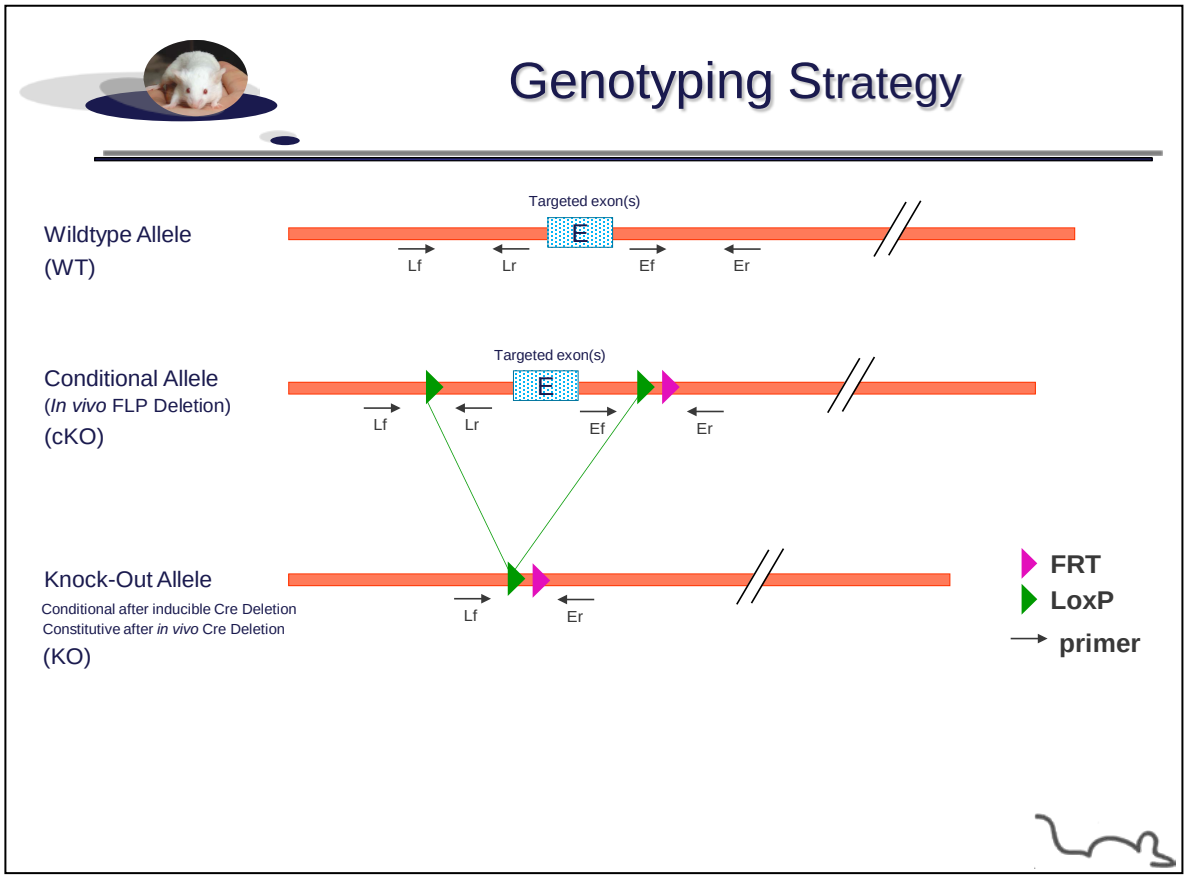


4. Genotyping protocol and data on conditional and knock-out animals

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	2097	CCCTGCTTCTGTGCCCTTTGC
Er	2098	CCACCACTGCCCAGCTTCAC
Lf	2095	GCCCCCCTTGGCCCCCCTTAGCCCCCTCCC
Lr	2101	GCTCTGCCTTGATGTAGAGAGGC

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	2095-2101	Lf / Lr	539	---	473
Excision of the selection marker	2097-2098	Ef / Er	506	---	396
Excision of the floxed exon(s), i.e. knock out	2095-2098	Lf / Er	1341*	386	1165*

* 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 µM)	0.06µl
- 3' primer (100 µM)	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

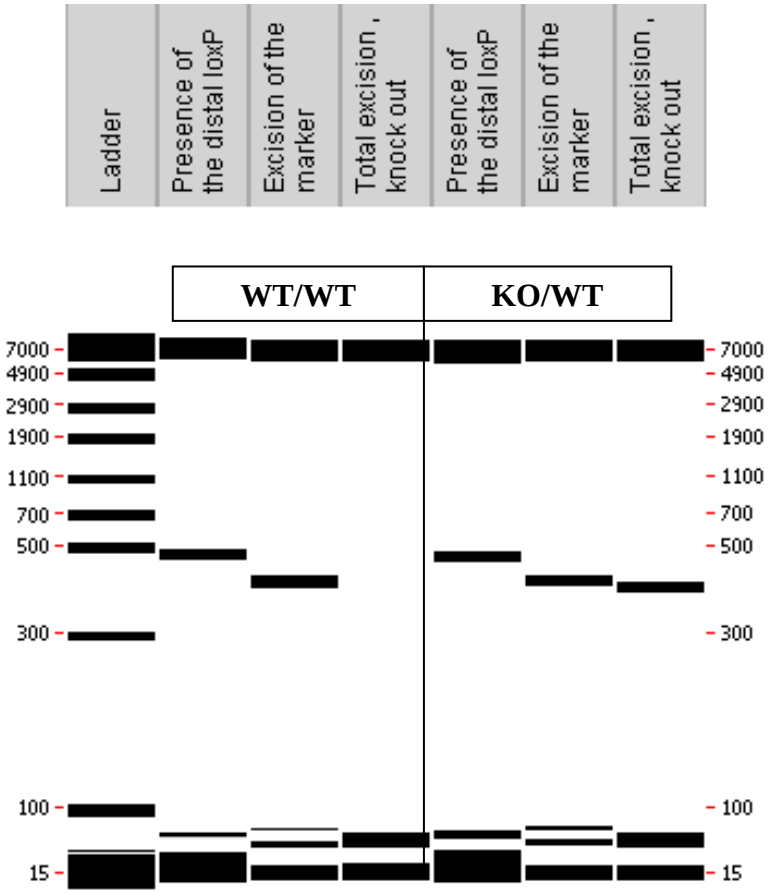
- Picture of genotyping with conditional knock-out (cKO) allele

Data not shown

- Picture of genotyping with knock-out (KO) allele

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.