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For any question, please contact:

Mouse Clinical Institute – Institut Clinique de la Souris (ICS)

1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex France

Email: ics@igbmc.fr

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

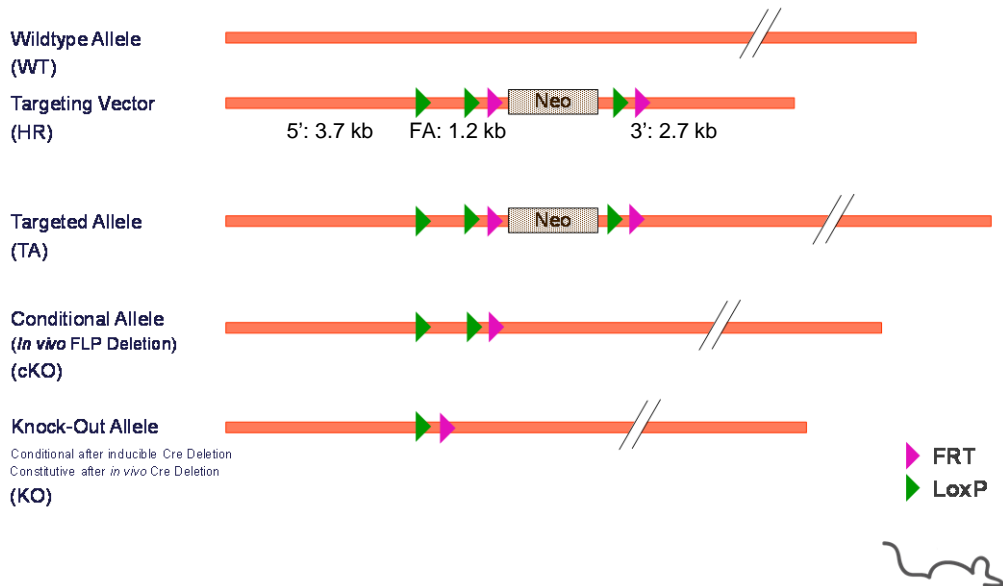
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview



Overview Targeting Strategy



Legend:

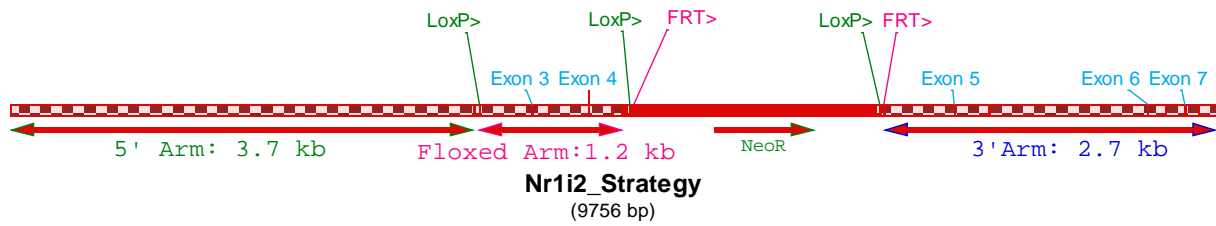
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
 This schematic representation is not on scale

1.2. Strategy chosen: flox of exons 3-4

Nr1i2 gene (also named PXR) 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=40490>

Strategy used to generate the conditional knock out model



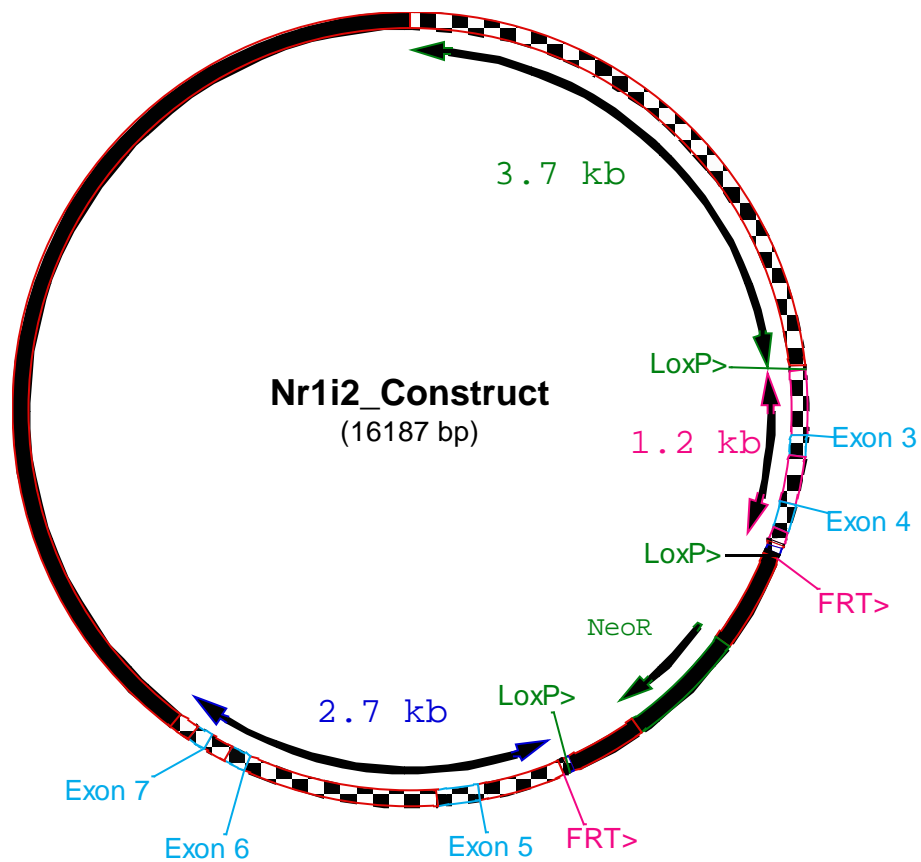
2. Construct used for homologous recombination in ES cells: Nr1i2 project

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 (on knock-out animals) are indicated in bolds.

2.2. Map of targeting vector plasmid





2.3. 5' homology arm (3.7 kb)

GAACACCAATGGCTTATGCTCTAAGATCAAGAATTGGCAAATGGGACCTCATAAAAATTGCAAAGCTTCTGTAAGG
CAAAGACTGTCAATAGGACAAAATGGTAGCCAACAAATTGAGAAAAGATCTTTACCAATCTACACCCGATA
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2.5. PGK-Neo region

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2.6. 3' homology arm (2.7 kb)

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```

2.7. Vector backbone sequence

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Molecular Biology Data
Nr1i2 conditional knock out model
-ICS reference K177/DG55/EUMO13

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ccgcccagcctagccgggtcctcaacgacaggagcagctcatgcccaccgctggccaggaccacaacgctgcccga
gatgcccgcgctgcccgtgctggagatggcggacgcgatggatattgttctgccaagggcgtttaaaccttaataag
tcgacggccggccctcagggcc

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: ~ 400

Number of positives: 1

Reference of clone used to generate the mouse line:

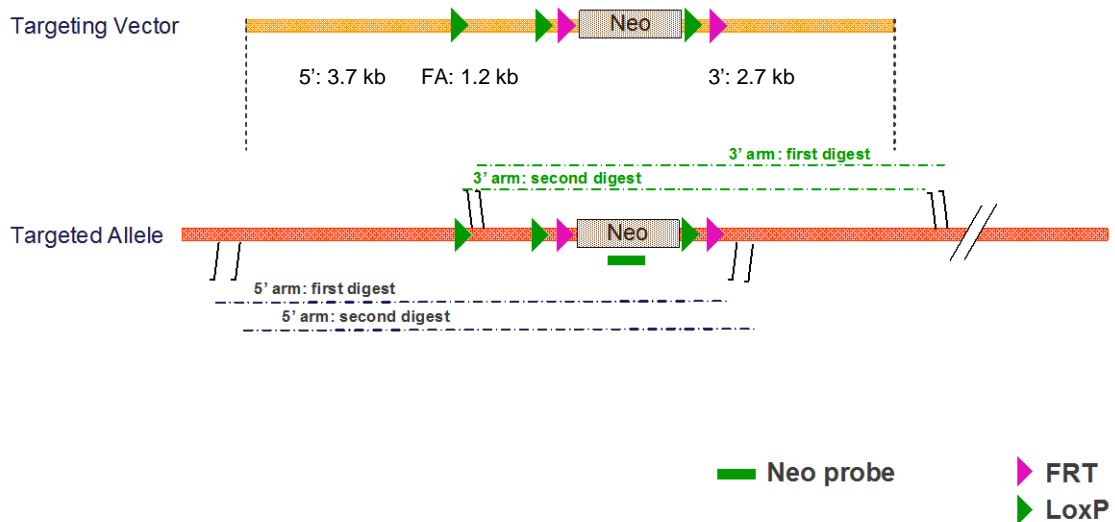
- clone **K177P1-27**

3.2. Southern data on positive clone

3.2.1. Neo Southern strategy



Southern Screening Strategy



Digestions used to validate the 5' and 3' insertion

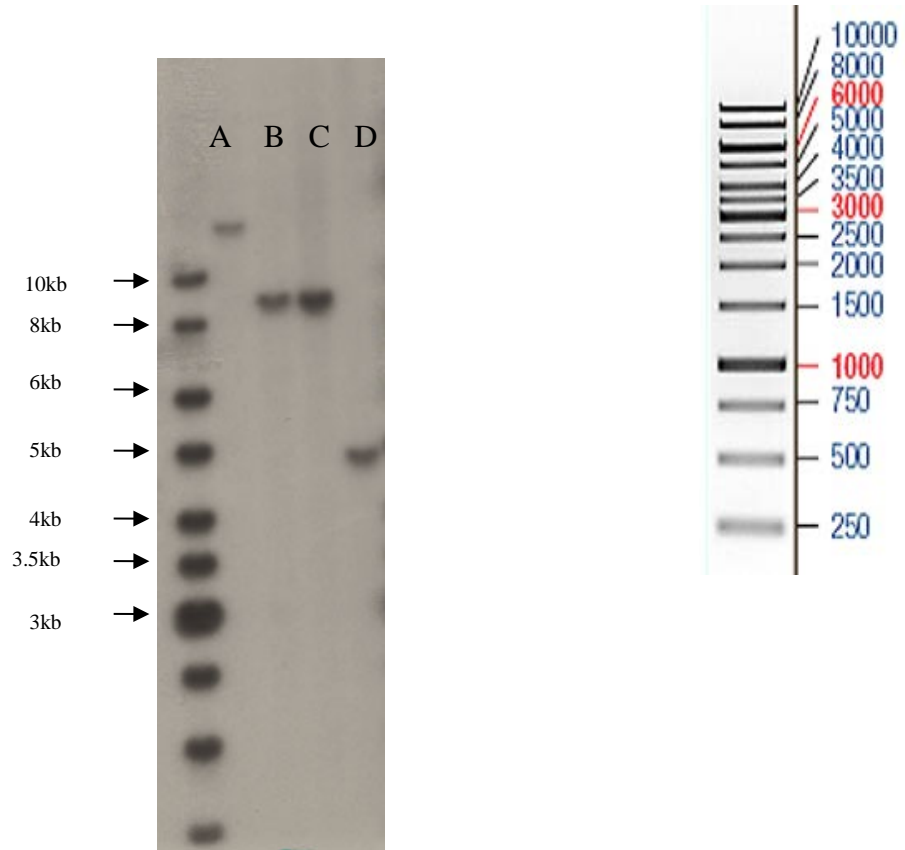
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	PshAI	/	13.3
	5' arm second digest	Scal	/	8.9
	3' arm first digest	SpeI	/	8.8
	3' arm second digest	HindIII	/	4.8

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

3.2.2. Picture of Neo Southern

Neo southern blot: 5' and 3' arm validation

ladder



A: PshAI 13.3kb
B: ScaI 8.9kb
C: SpeI 8.8kb
D: HindIII 4.8kb

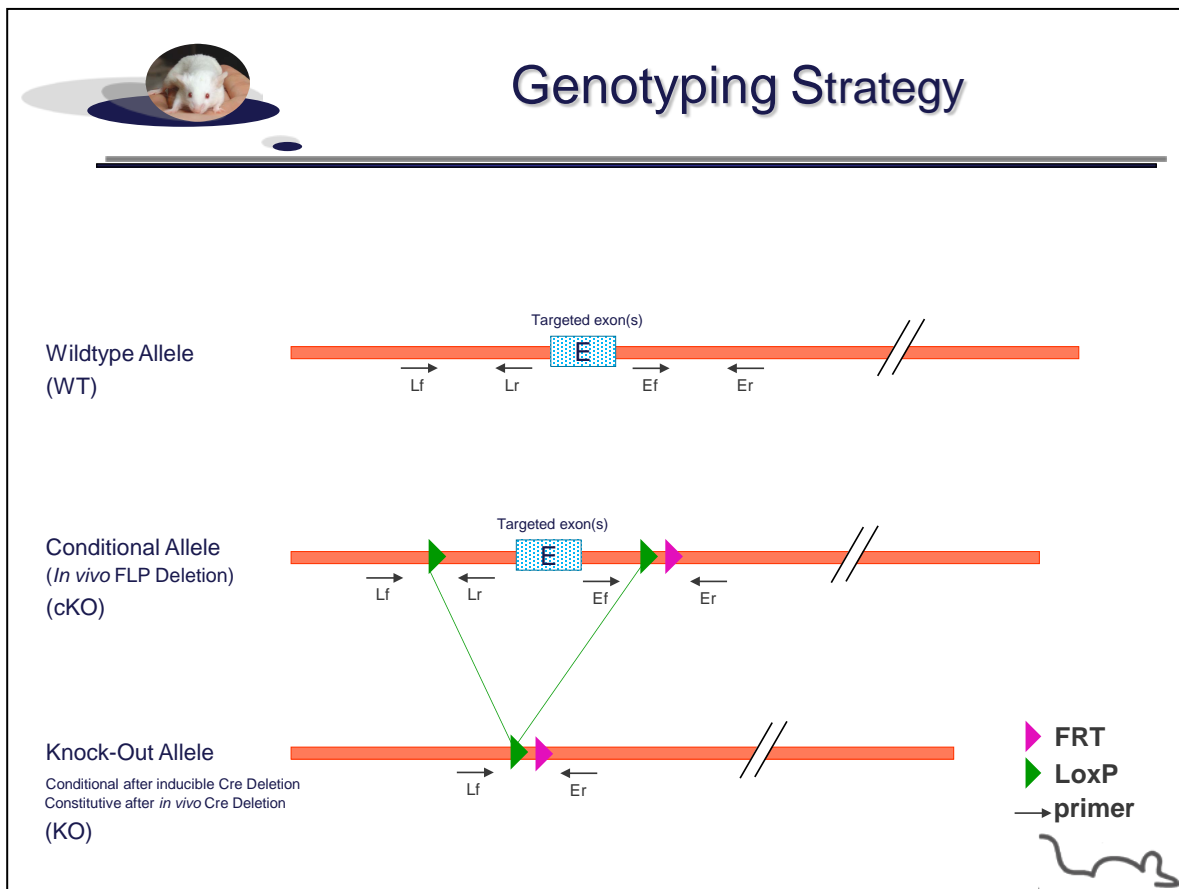
4. Data on conditional and knock-out animals

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

4.1. Genotyping protocol and data

4.1.1. Genotyping strategy

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



Sequence of primers used for genotyping

Primers	Sequence
Lf	CCCTTCAGAACCCTTCAGGCTG
Lr	AATACCTCCCATCCTTCCTCGGAG
Ef	CTTAGAGCTGTGACCACATCCATGG
Er	TATCCA CTGAGGACAGTCAGCTGG



Molecular Biology Data
Nr1i2 conditional knock out model
 -ICS reference K177/DG55/EUMO13

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	661-664	Lf / Lr	352	---	306
Excision of the selection marker	665-666	Ef / Er	268	---	187
Total Excision (excision of the floxed exon(s), i.e. knock out)	661-666	Lf / Er	1555*	338	1392*

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

4.1.2.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	2
62°C	1min	
72°C	1min	
94°C	30s	30
62°C	30s	
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.

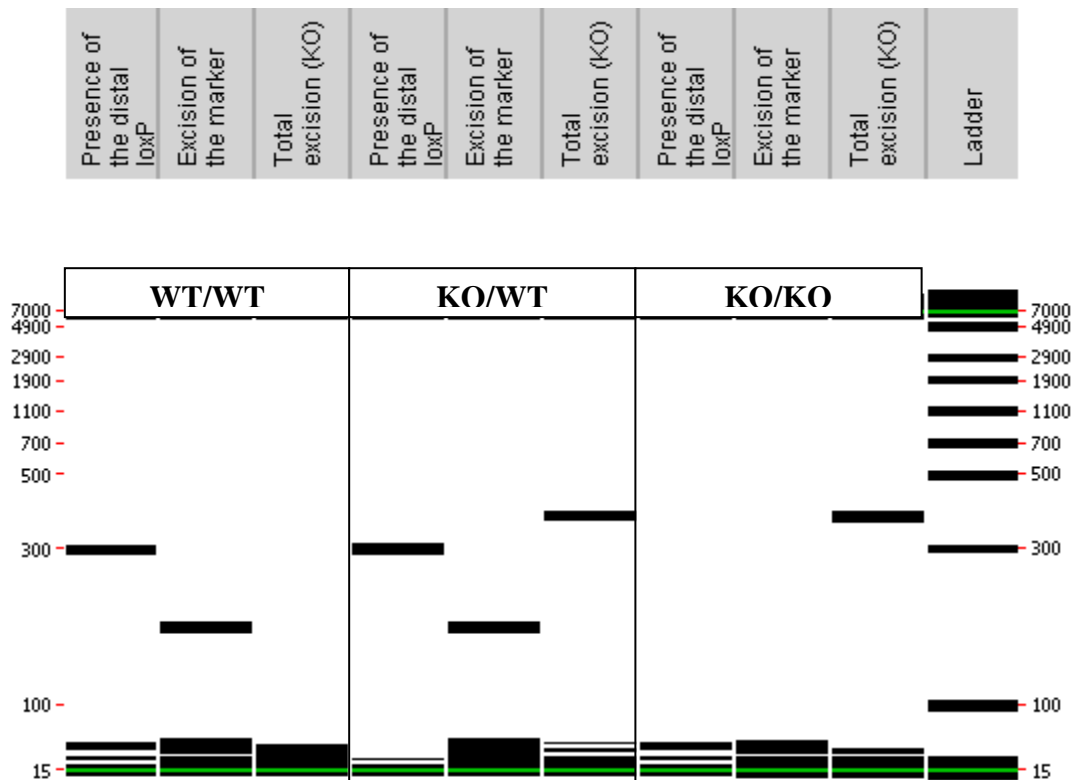
4.1.3. Picture of genotyping with various alleles

- 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.

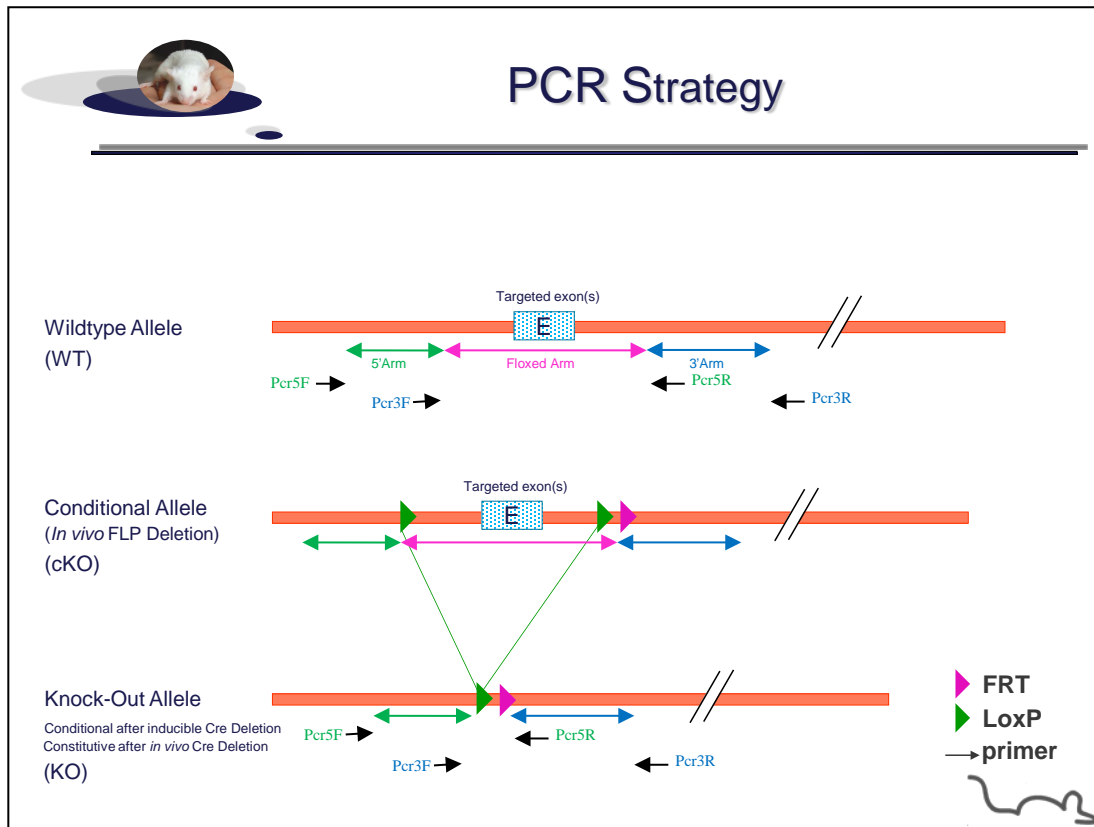
Representative genotyping picture



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

4.2. PCR data on mouse DNA

4.2.1. PCR strategy for validation at the mouse level



Primers used for PCR validation of ES clone

PCR	Primer Name	Primer sequences	PCR product size
5' external	Pcr5F	ttcattctccgctccattg	Wt: 6.8kb KO: 5.8kb
	Pcr5R	ccattctaggggagtgagg	
3' external	Pcr3F	tgaacaccagcccccttag	Wt: 4.4kb KO: 3.4kb
	Pcr3R	gagcaaaggaaagttcagtccag	

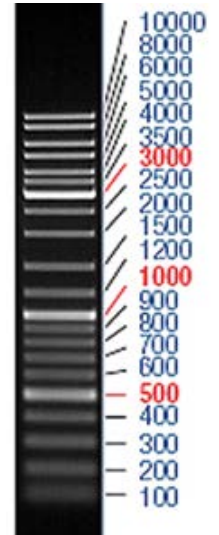
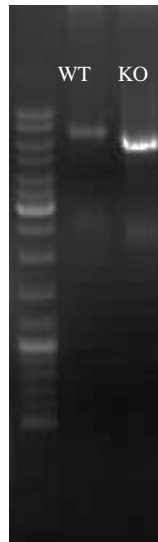
4.2.2. Picture of PCR

5' and 3' external PCR

ladder

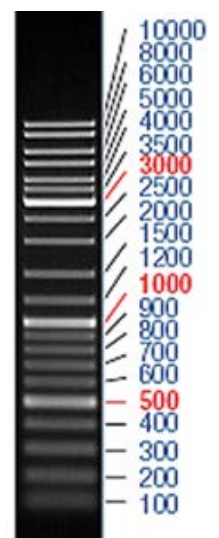
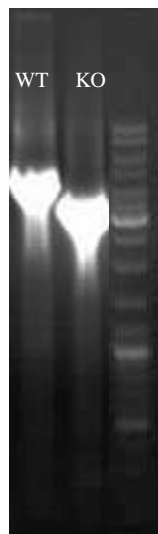
5' external PCR

Wt: 6.8kb
 KO: 5.8kb



3' external PCR

Wt: 4.4kb
 KO: 3.4kb



4.3. Evaluation of lethality of homozygote KO (KO/KO)

Males knock-out heterozygotes (KO/WT) were crossed with females knock-out heterozygotes (KO/WT). Offspring was genotyped to evaluate the ratio of the different genotypes. Results are provided in the table below.

Genotype	WT/WT	KO/WT	KO/KO	Total
Number of pups obtained	20	44	27	91
Experimental Ratio	22,0%	48,4%	29,7%	100%
Theoretical Ratio	25%	50%	25%	100%
Theoretical Ratio if L-/L- are not viable	33%	66%	0%	100%
Homozygote Viability	Viable			

The Nr1i2 knock-out homozygotes are viable.

Legend:

- >13% Homozygous = Viable
- >0% and ≤13% = Subviable
- 0% = Lethal