Molecular Biology Data
Nr1i3 conditional knock out model
ICS reference DG1/K104

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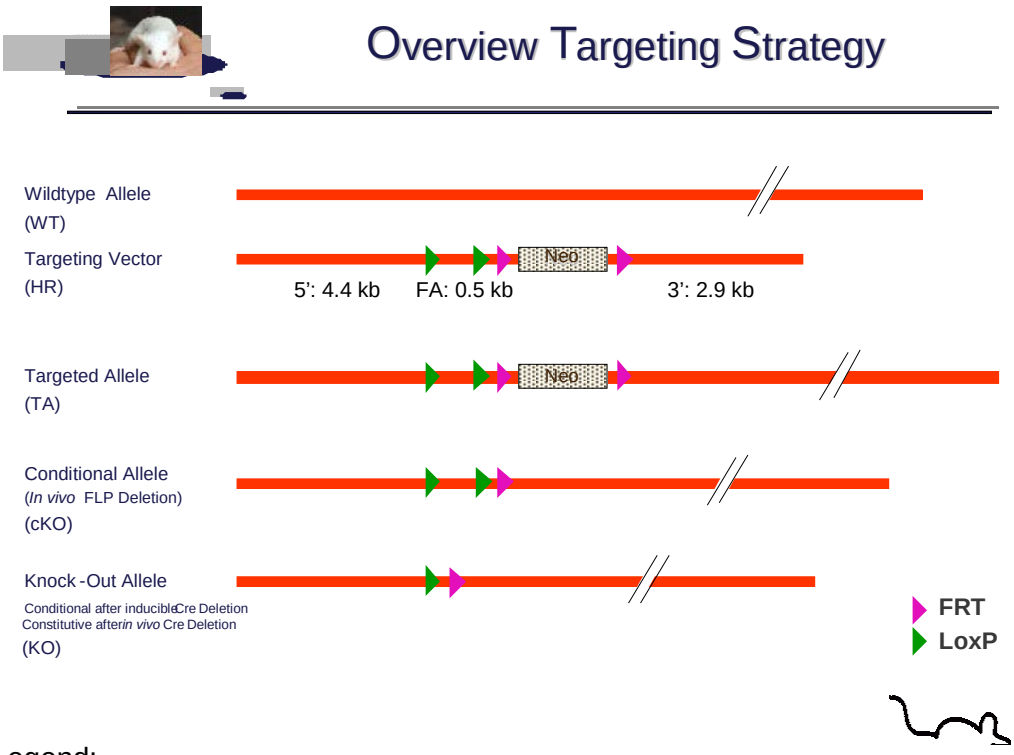
This protocol has been prepared by Alban Roudaut, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

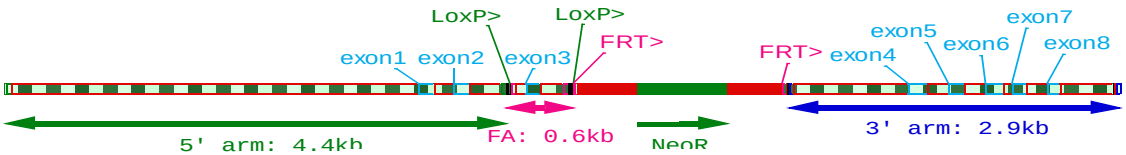


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exon 3

Nr1i3 gene (also named CARa) 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=42884

Strategy used to generate the conditional knock out model



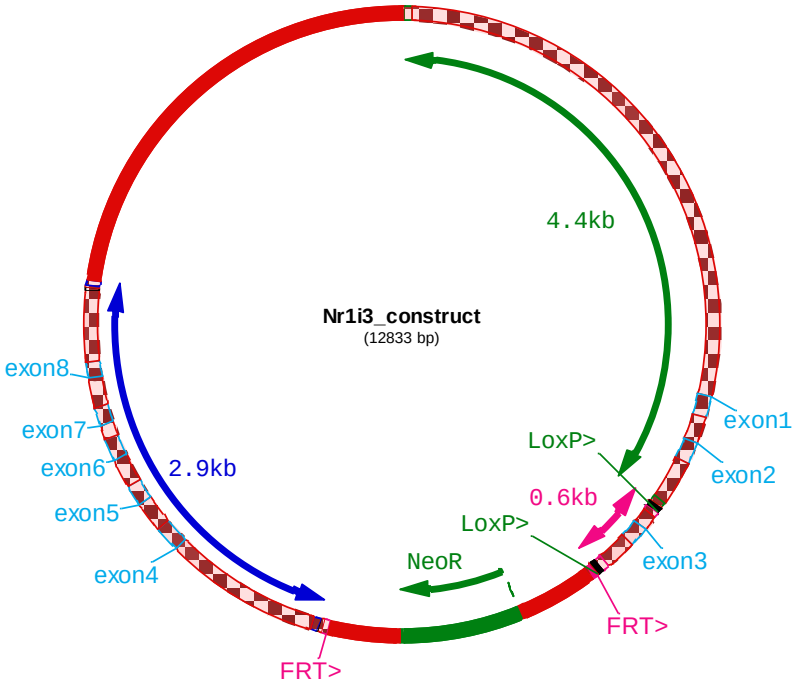
Nr1i3_Strategy
(9917 bp)

2. Construct used for homologous recombinaison in ES: Nr1i3 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 (4.4 kb)

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2.4. Floxed fragment (0.5 kb)

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TAAA**TCGATCCGTCTTTTAGGCCGCGTCCTTTCGGGGTGCTCTTAGACCCACCTGCATGTTCTCTGCCATCTAAA**
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2.5. PGK-Neo region

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2.6. 3’ homology arm (2.9 kb)

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CCGTTTGCTACGAGGGAGATG

2.7. Vector backbone sequence

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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]

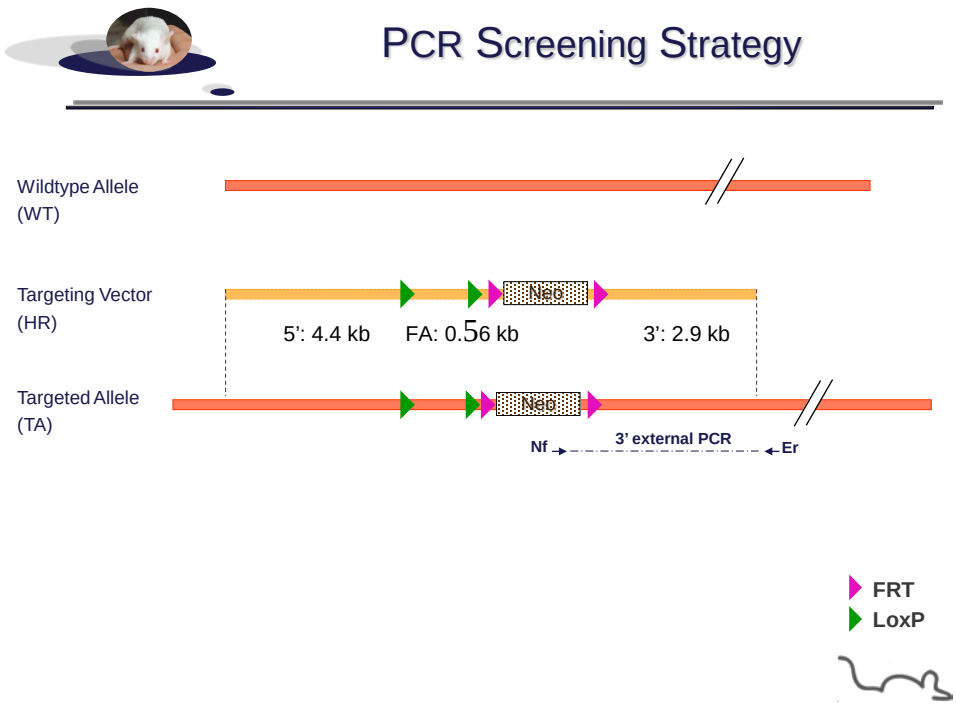
Number of clones screened: ~400
Number of positives: 7

Reference of clone used to generate the mouse line:

- clone **K104-168**

3.2. PCR data on positive clone

3.2.1.PCR screening strategy



Primers used for PCR validation of ES clone

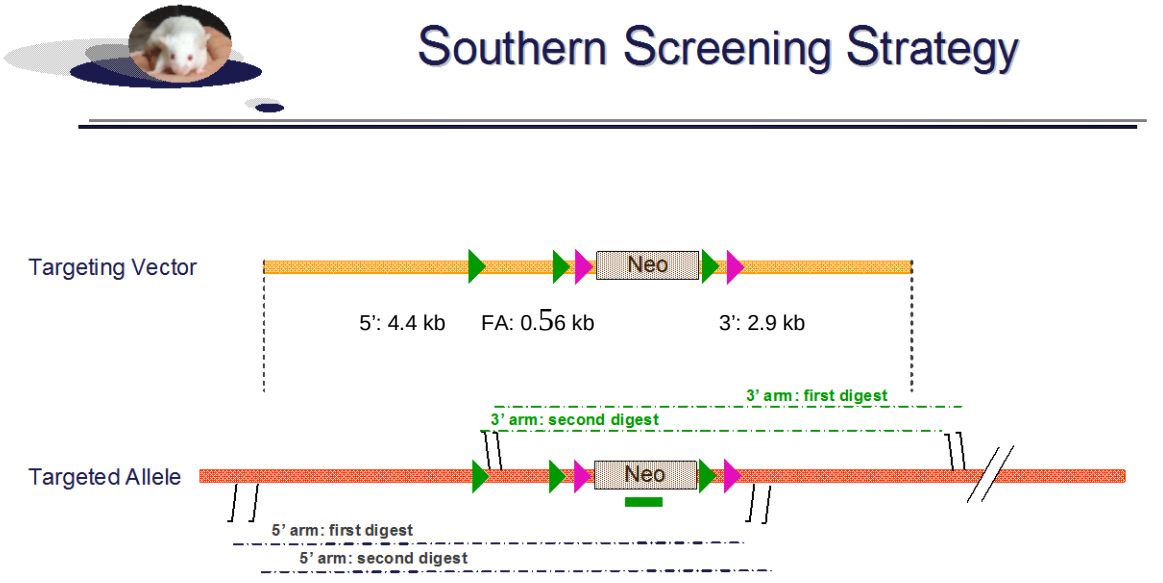
PCR	Primer Name	Primer sequences	PCR product size (kb)
3' external	Nf	AGGGGCTCGCGCCAGCCGAACTGTT	3.8
	Er	CACAACCGCCTTCTAGAACTGGATC	

3.2.2.Picture of PCR on positive clone

3' external PCR ladder

3.3. Southern data on positive clone

3.3.1.Neo Southern strategy



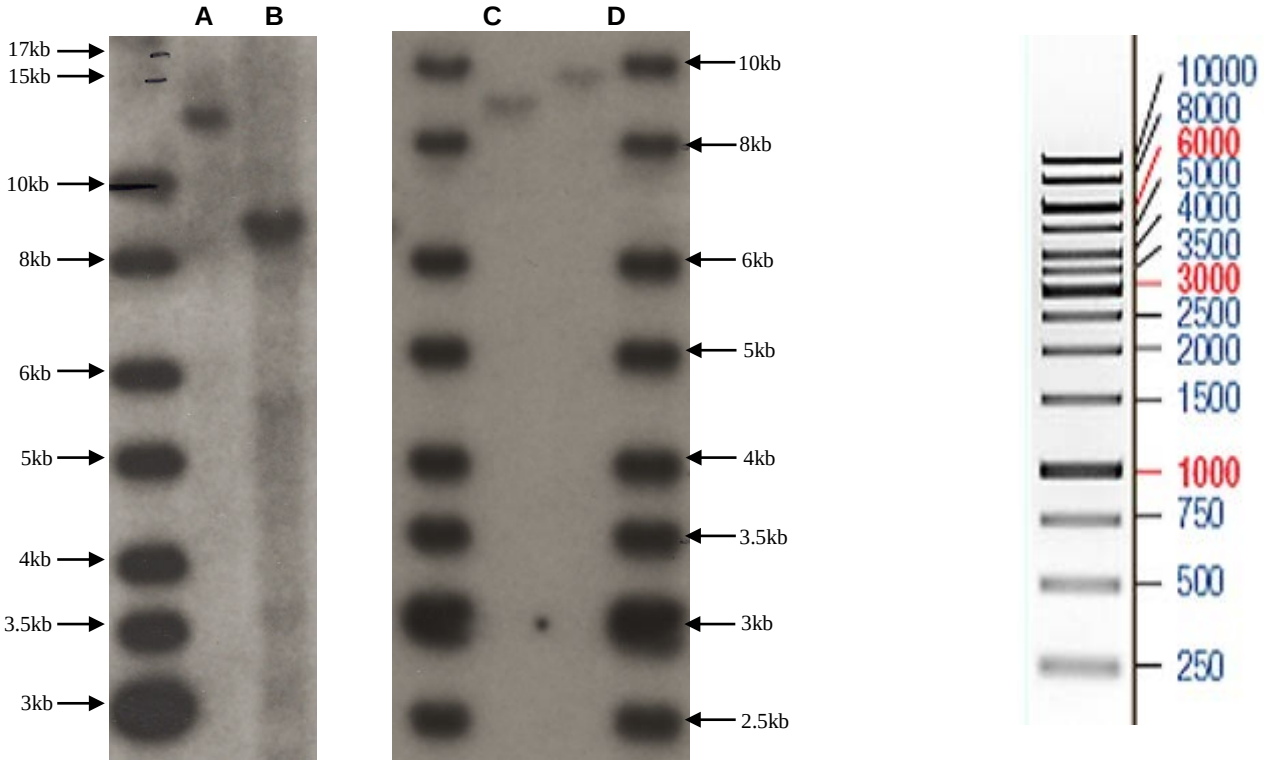
Digestions used to validate the 5' and 3' insertion

Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	AflII	/	12.8
	5' arm second digest	EcoRV	/	8.8
	3' arm first digest	SpeI	/	9.0
	3' arm second digest	EcoRI	/	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: AflII (12.8kb)
B: EcoRV (8.8kb)
C: SpeI (9.0kb)
D: EcoRI (9.7kb)

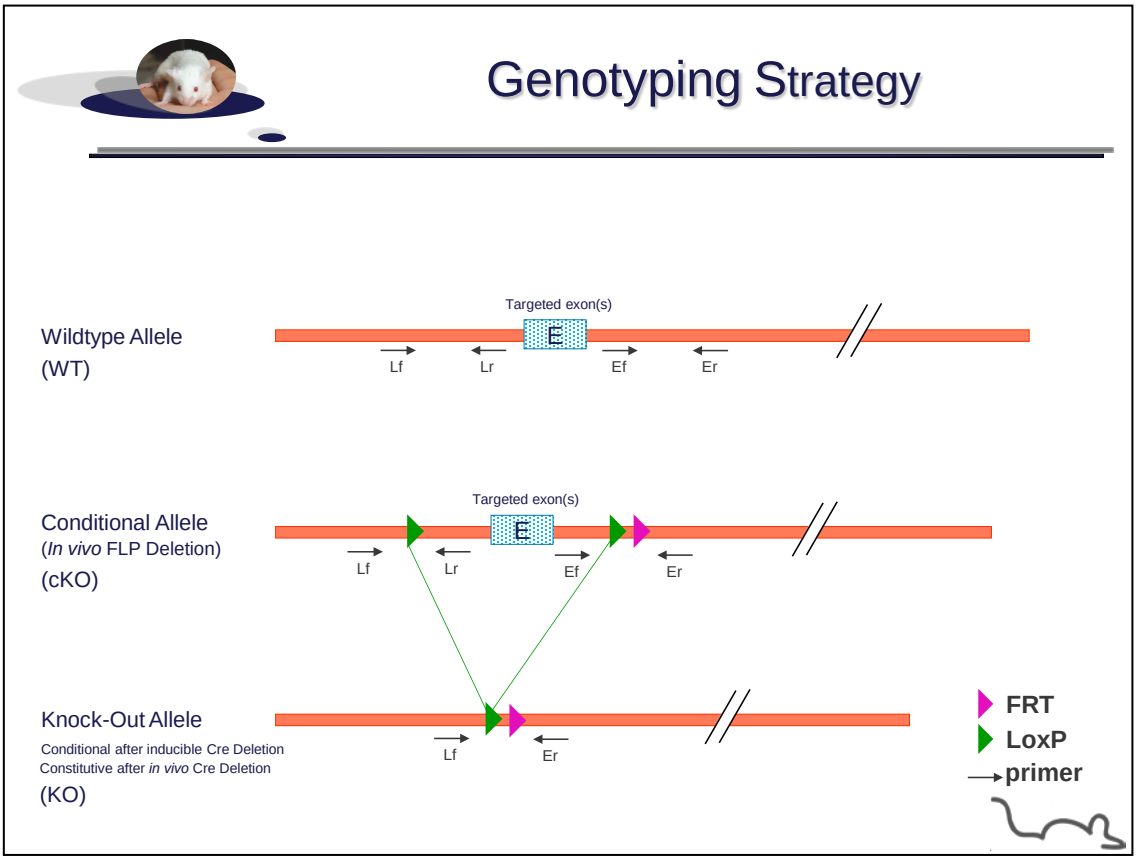
4. Data on conditional and knock-out animals

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

Position	Primers	Sequence
Ef	3	TCACCTGCATATATGTCTAAGTACC
Er	4	CCTTGAAAGAGATTGGGATGGGGAG
Lf	1	ATCATTGCCACACGAGGGTTCC
Lr	2	AACATGCAGGTGGGTCTAAGAGCAC

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	1-2	Lf / Lr	211	---	161
Excision of the selection marker	3-4	Ef / Er	317	---	207
Total Excision (excision of the floxed exon(s), i.e. knock out)	1-4	Lf / Er	---*	309	---*

* 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:
- 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

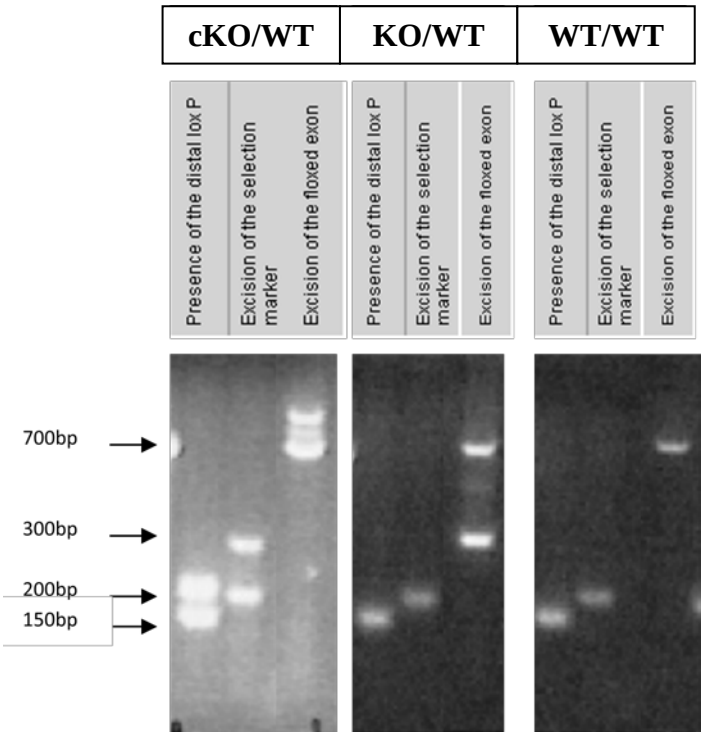
Cycling conditions:

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.

4.1.3. Picture of genotyping with various alleles

Representative genotyping picture



4.2. 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	19	27	15	**61**
Experimental Ratio	31%	44%	25%	**100%**
Theoretical Ratio	25%	50%	25%	**100%**
Theoretical Ratio if KO/KO are not viable	33%	66%	0%	**100%**

The Nr1i3 knock-out homozygotes are viable.

Legend:

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

0% = Lethal