Molecular Biology Data
Nr1c1 conditional knock out model
ICS reference K127/EUMO19

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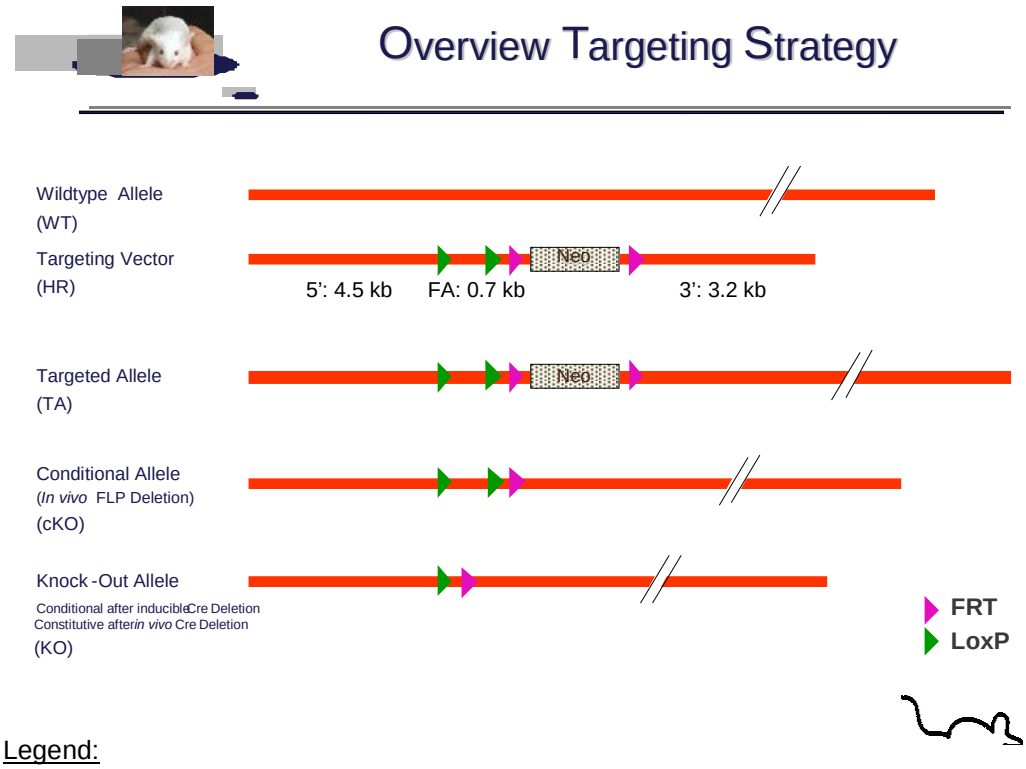
This protocol has been prepared by Alban Roudaut, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

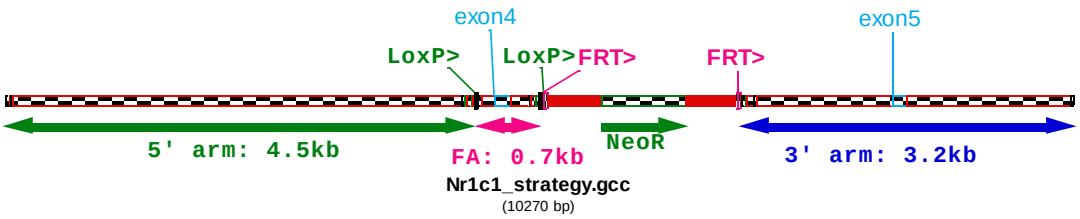


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: Flox exon 4

Nr1c1 gene (also named PPARa) 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=25000

Strategy used to generate the conditional knock out model

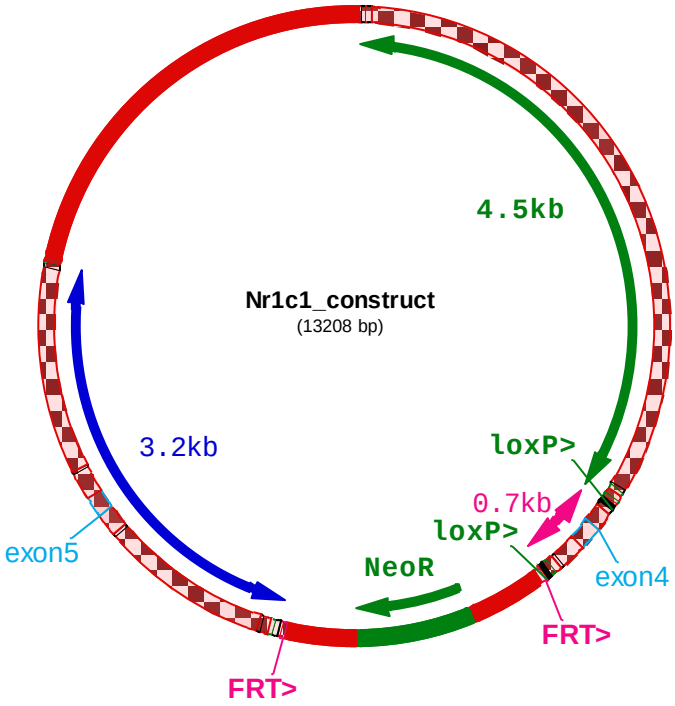


2. Construct used for homologous recombinaison in ES: Nr1c1 project

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 (4.5 kb)

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CA

2.4. Floxed fragment (0.7 kb)

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AATGACTCTTCCCATGGTACGAAGTATAGcaccggtg**ataacttcgtataatgtatgctatacgaagttat**gcgg
ccgg

2.5. PGK-Neo region

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2.6. 3’ homology arm (3.2 kb)

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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]

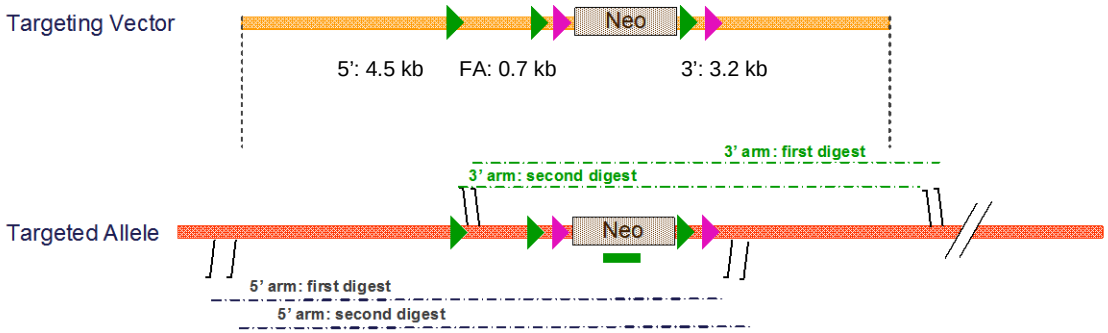
Number of clones screened: ~400
Number of positives: 2

Reference of clone used to generate the mouse line:

- clone **K127-289**

3.2. Southern data on positive clone

3.2.1. Neo Southern strategy



Digestions used to validate the 5' and 3' insertion

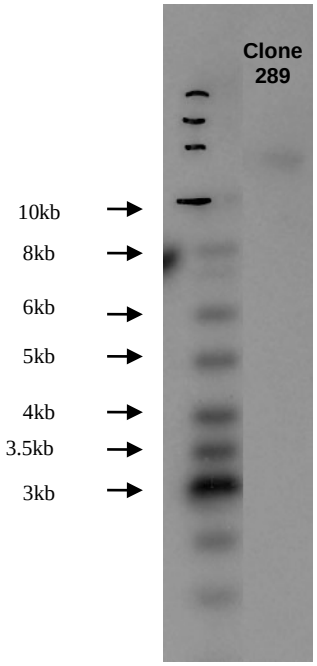
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm digest	SexAI	/	13.1
	3' arm first digest	XcmI	/	10.0
	3' arm second digest	NdeI	/	12.2

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

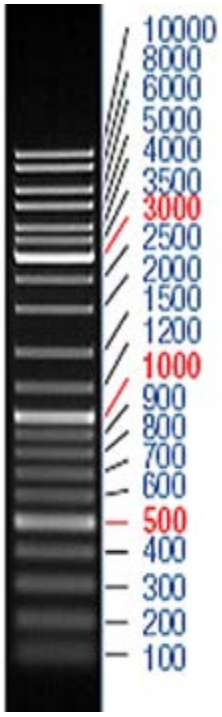
3.2.2.Picture of Neo Southern

Neo southern blot: 5' arm validation

SexAI : 13.1kb

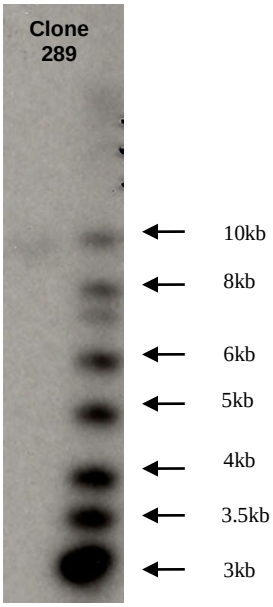


Ladder

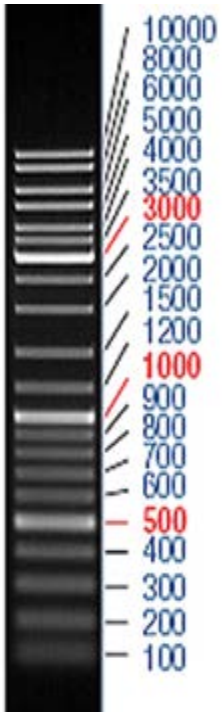


Neo southern blot: 3' arm first digest validation

XcmI : 10.0kb

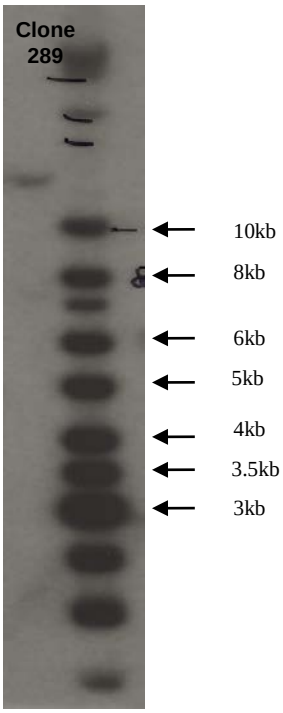


Ladder

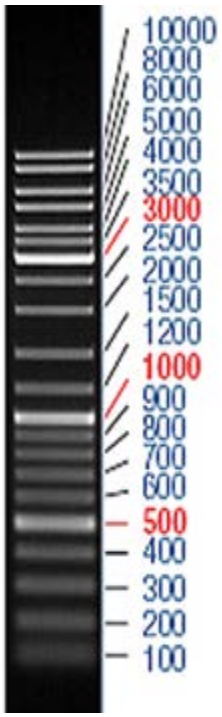


Neo southern blot: 3' arm second digest validation

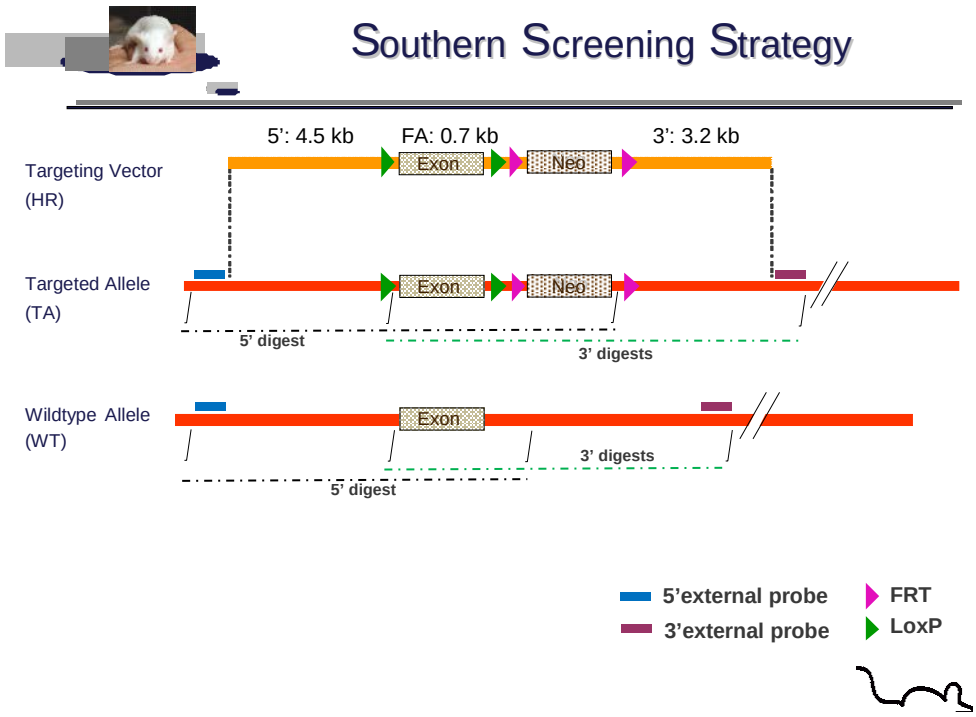
NdeI: 12.2kb



Ladder



3.2.3.External probes Southern



Digestions used to validate with 5' and 3' probes

Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
5' external	5' digest	NsiI	9.0	11.0
3' external	3' first digest	HindIII	11.6	13.7
	3' second digest	NdeI	9.9	12.0

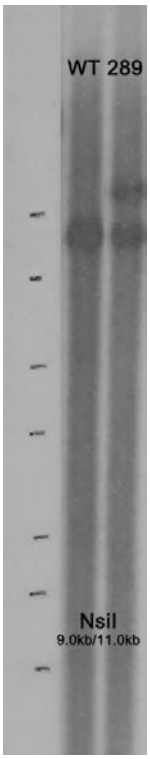
Primers for probe synthesis:

5' probe
AATGTTAGACAGGAATGGCAATGCC
CTCTGTGTACAGCTGTCTTTTGAAC

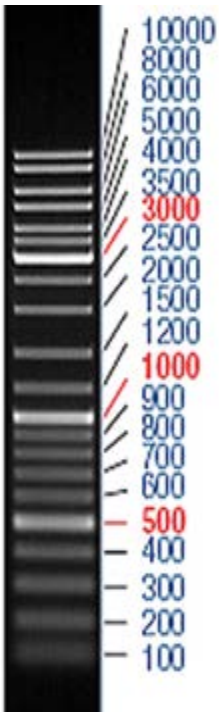
3' probe
CTACTGCCCTTGGTACCTTGAAATG
CCTACCGTCTTTGTTACCTTCTTGC

3.2.4.Pictures of Southern with 5' and 3' external probes

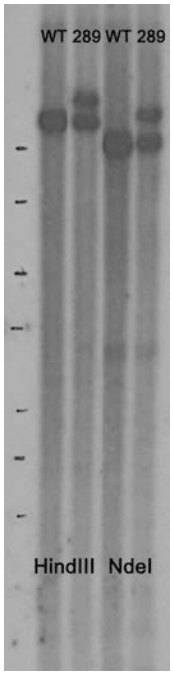
Southern blot with external 5' probe



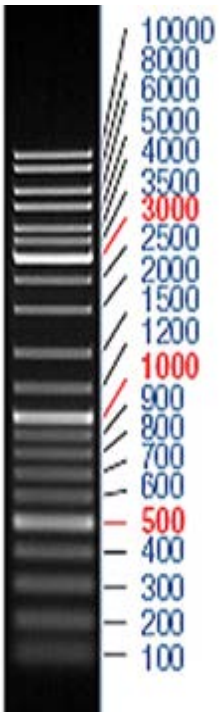
Ladder



Southern blot with external 3' probe



Ladder

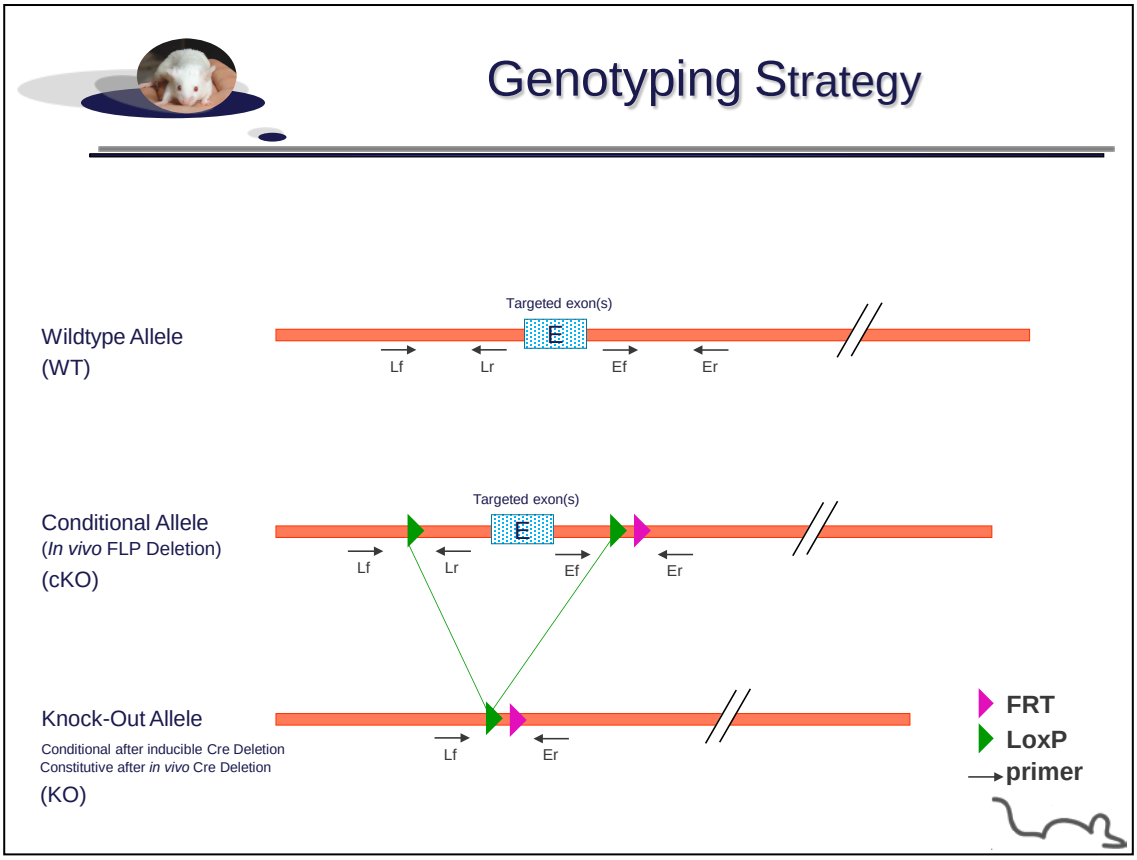


4. Genotyping protocol and data on conditional and knock-out animals

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	821	CTGTACTTTGTAGACATCTGAGAGGCG
Er	823	TAGGTACCGTGGACTCAGAGCTAG
Lf	824	AAAGCAGCCAGCTCTGTGTTGAGC
Lr	825	GGGAAGATACCTTTTCGTAAGAGGGG

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	821-823	Lf / Lr	296	---	246
Excision of the selection marker	824-825	Ef / Er	390	---	280
Excision of the floxed exon(s), i.e. knock out	821-825	Lf / Er	1074*	445	914

* 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

Representative genotyping picture