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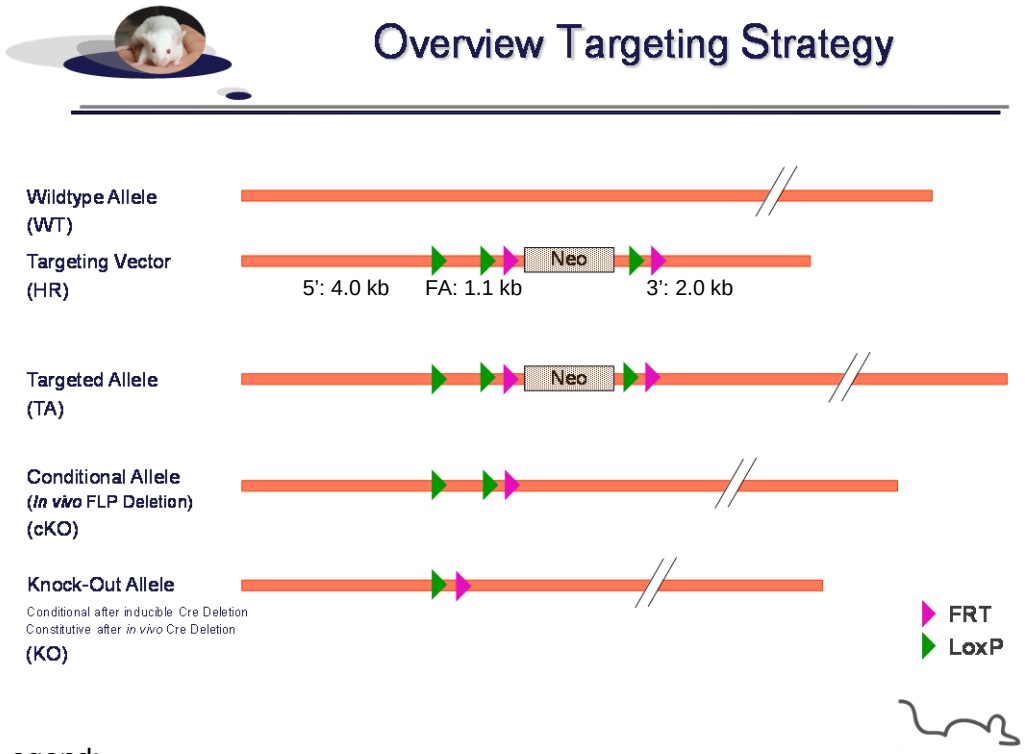
This protocol has been prepared by Claudia Caradec, Scientific Report Editor
This protocol has been validated by Monika Jagla-Eberlin Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

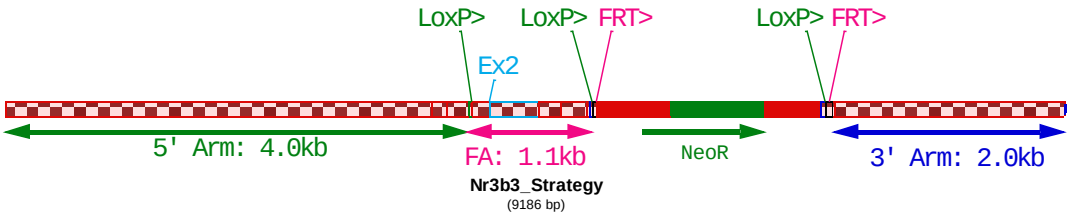


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exon 2

Nr3b3 gene (also named Esrrg) 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=43437

Strategy used to generate the conditional knock out model

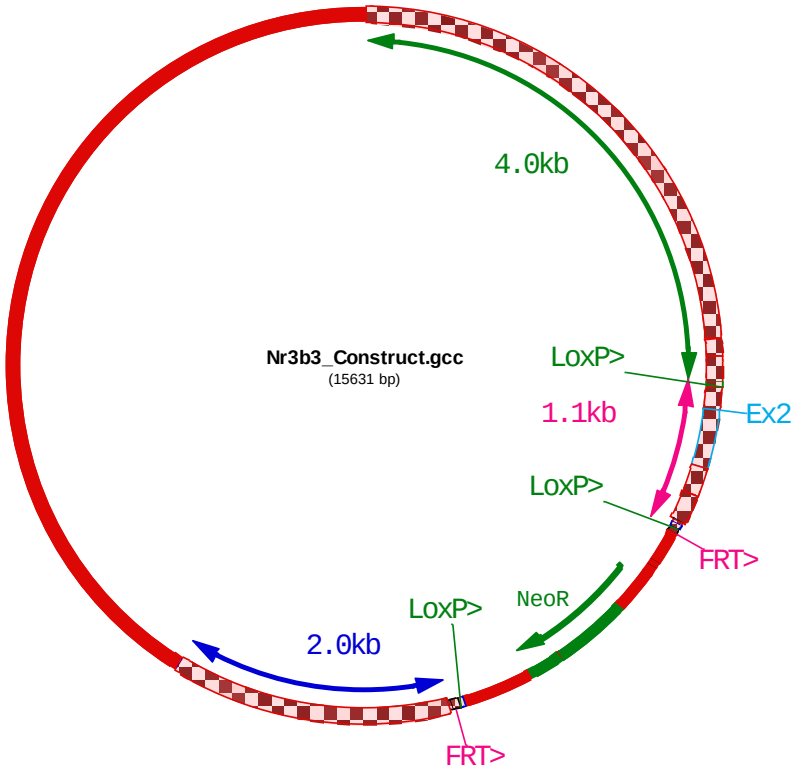


2. Construct used for homologous recombination in ES cells: Nr3b3 project

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 (4.0 kb)

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2.4. Floxed fragment (1.1 kb)

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2.5. PGK-Neo region

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2.6. 3’ homology arm (2.0 kb)

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2.7. Vector backbone sequence

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gcggctgctggagatggcggacgcgatggatatgttctgccaagtcagcgtttaaacttaattaagtcgacggcc
ggccctcgaggcc

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]

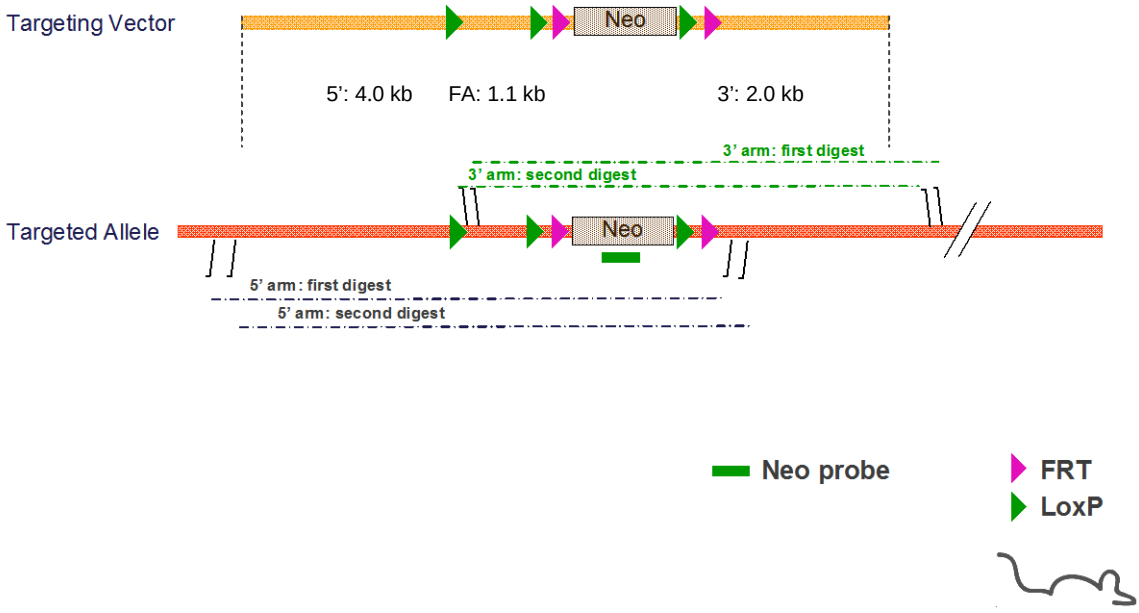
Number of clones screened: ~ 400
Number of positive: 2

Reference of clone used to generate the mouse line:

- clone **DG42 -249**

3.2. Southern data on positive clone

3.2.1.Neo Southern strategy



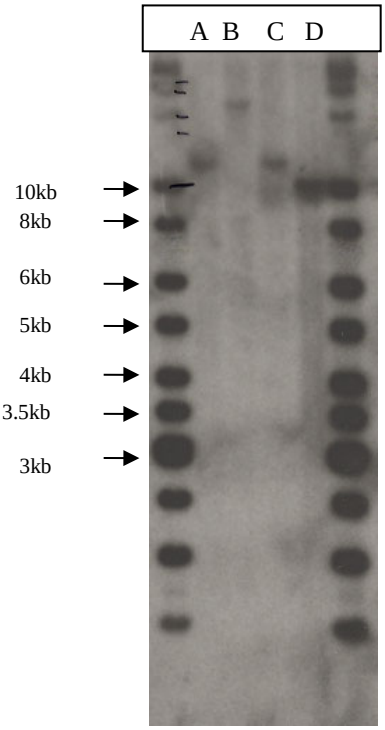
Digestions used to validate the 5' and 3' insertion

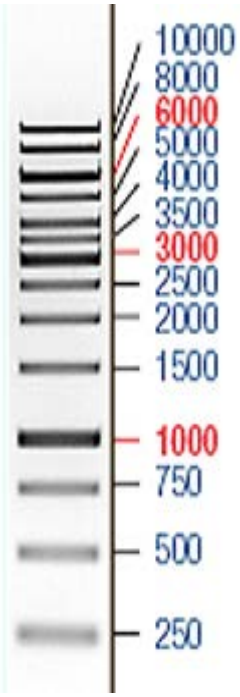
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	ScaI	/	11.7
	5' second digest	PshAI	/	19.8
	3' arm first digest	ApaI	/	11.9
	3' arm second digest	AseI	/	10.4

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: ScaI 11.7kb
B: PshAI 19.8kb
C: ApaI 11.9kb
D: AseI 10.4kb

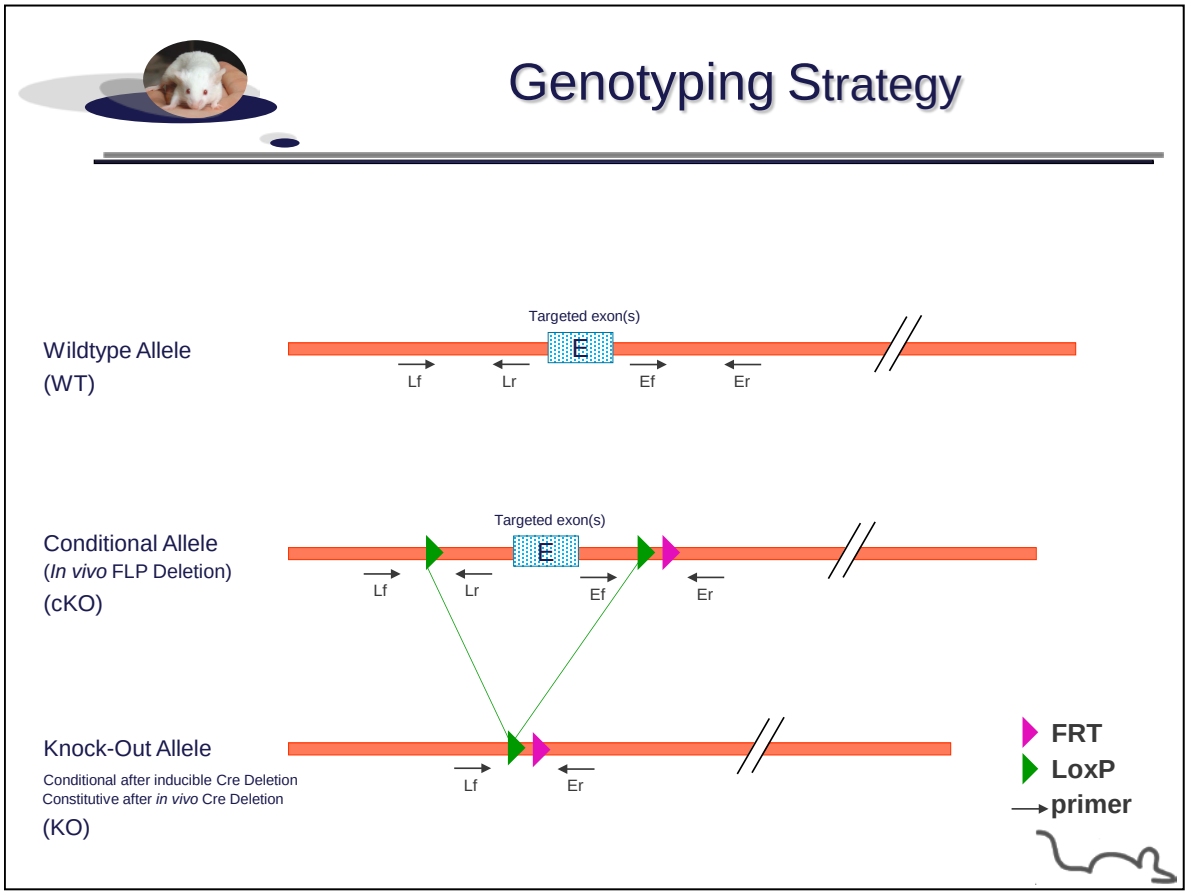
4. Data on conditional and knock-out animals

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
Lf	87	CCCTTATGCTGATTACCTTCTTGTA
Lr	88	CAACAATGTAGACACAAAGACATGG
Ef	610	GTTTTAAAGGCCCTTGGTGATCTCGC
Er	612	CTGCAACCCTTGGACTGCCAGAAC

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	87-88	Lf / Lr	208	/	161
Excision of the selection marker	610-612	Ef / Er	288	/	149
Total Excision (excision of the floxed exon(s), i.e. knock out)	87-612	Lf / Er	1276*	232	967*

* 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:

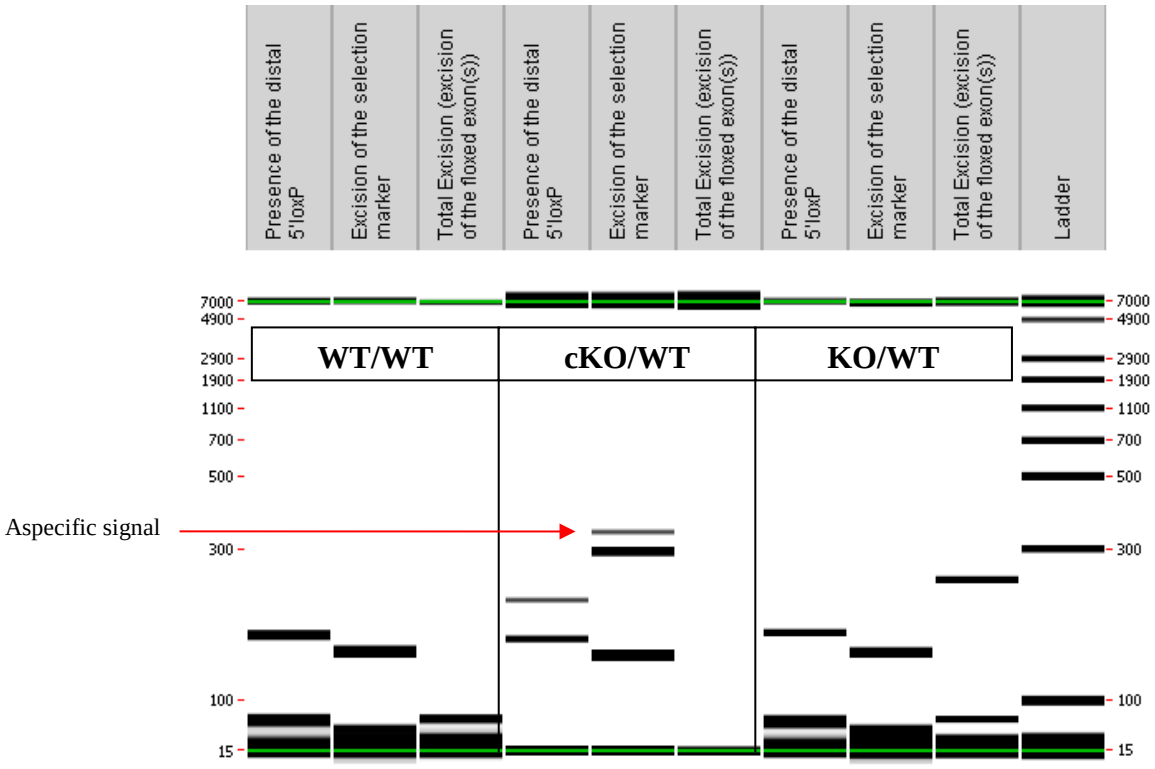
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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

4.1.3.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.

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	41	43	0	**84**
Experimental Ratio	49%	51%	0%	**100%**
Theoretical Ratio	25%	50%	25%	**100%**
Theoretical Ratio if KO/KO are not viable	33%	66%	0%	**100%**

The Nr3b3 knock-out homozygotes are not viable.

Legend:

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