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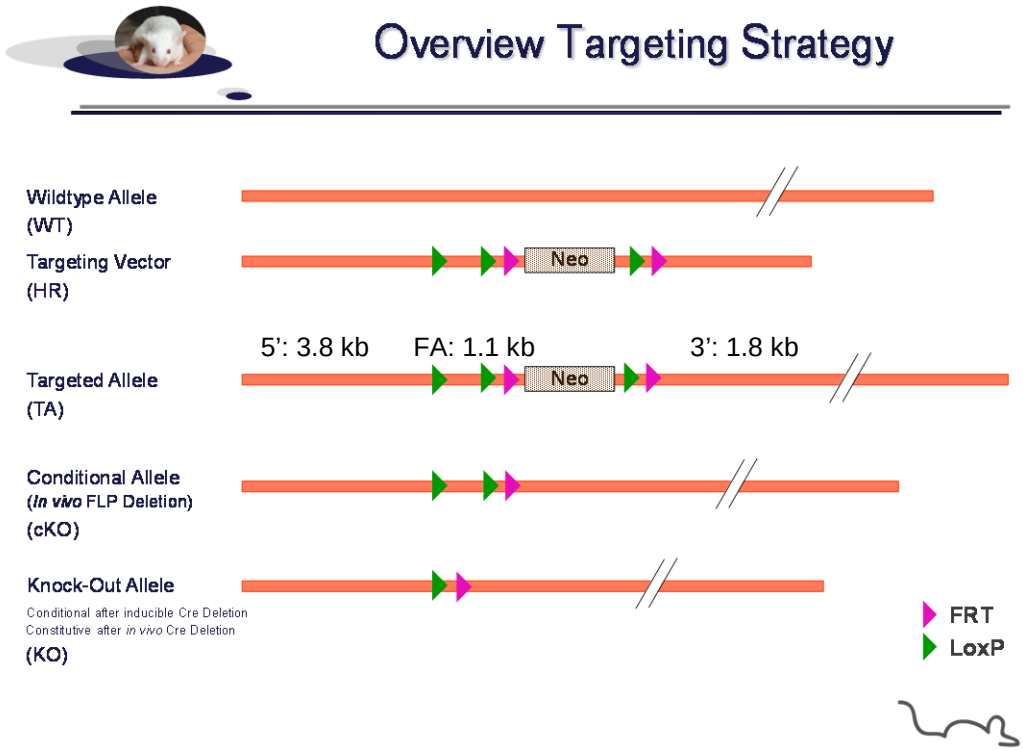
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

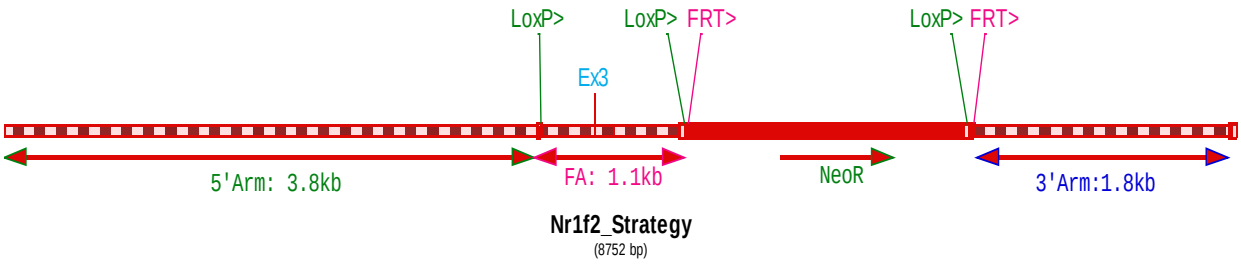


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exon 4

Nr1f2 gene (also named Rorb) is a member of the nuclear receptor family. Additional information on this gene can be accessed at
http://www.informatics.jax.org/marker/MGI:1343464

Strategy used to generate the conditional knock out model



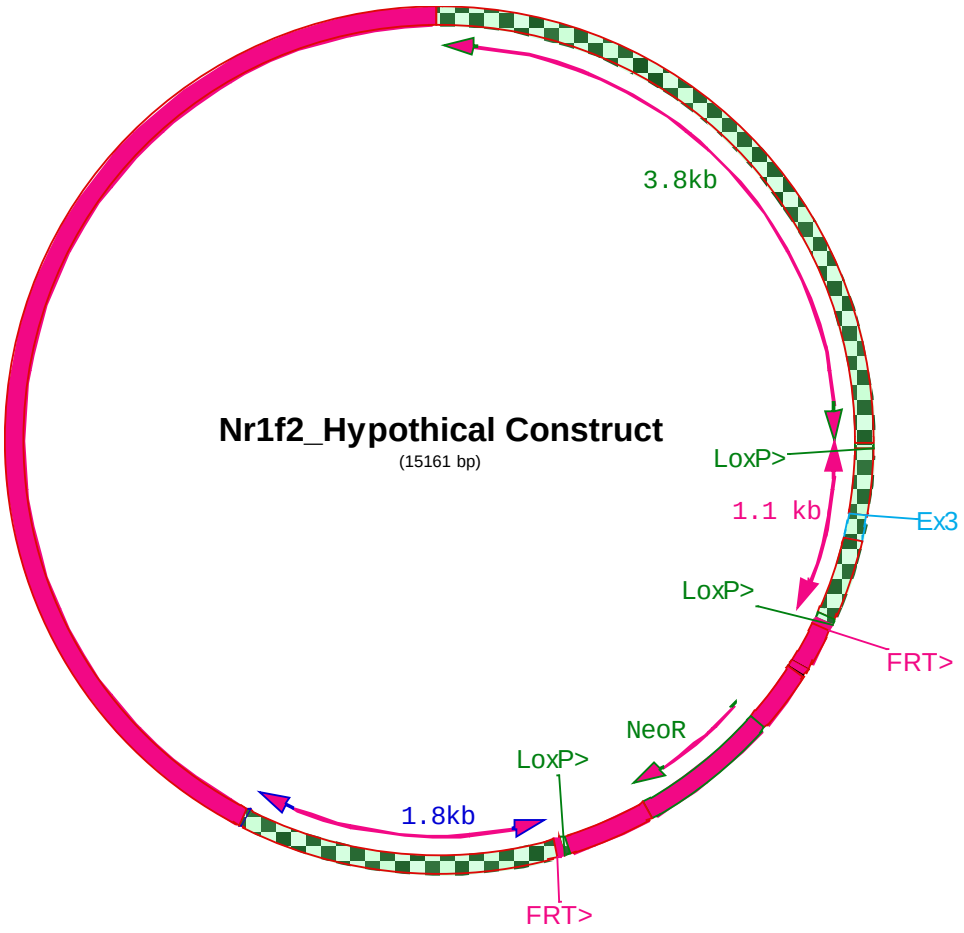
2. Construct used for homologous recombination in ES cells: Nr1f1 project

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.

2.2. Map of targeting vector plasmid

This plasmid was constructed at Deltagen USA. A theoretical map of targeting vector plasmid was established thanks to the available information.



2.3. Theoretical 5' homology arm (3.8 kb)

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2.4. Theoretical floxed fragment (1.1 kb)

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2.5. PGK-Neo region

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2.6. Theoretical 3' homology arm (1.8 kb)

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2.7. Theoretical vector backbone sequence

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gcggctgctggagatggcggacgcgatggatatgttctgccaagtcagcgtttaaacttaattaagtcgacggcc
ggccctcgaggcc

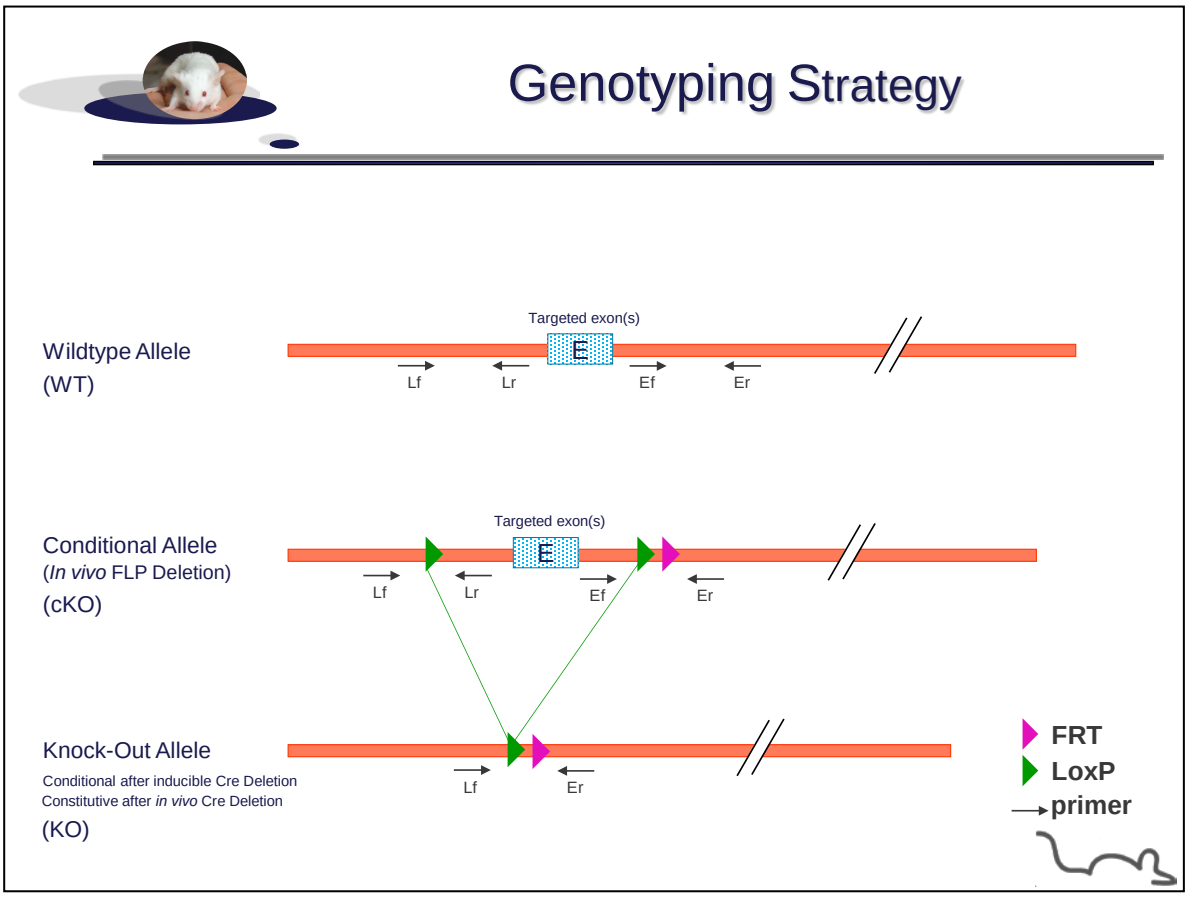
3. Data on conditional and knock-out animals

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

3.1. Genotyping protocol

3.1.1. 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	AACACCCAGCTGCAGATGTCAAGG
Lr	TGCTTCTTAGAGGGCTTCTAGGTGG
Ef	ATGAAGATCCTAGGAACAGAACACG
Er	AAGCAATTGTTTGGGTATGAAAGAG

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	60-61	Lf / Lr	534	---	487
Excision of the selection marker	62-63	Ef / Er	302	---	187
Excision of the floxed exon(s), i.e. knock out	60-63	Lf / Er	---*	485	---*

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

3.1.2.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	∞	

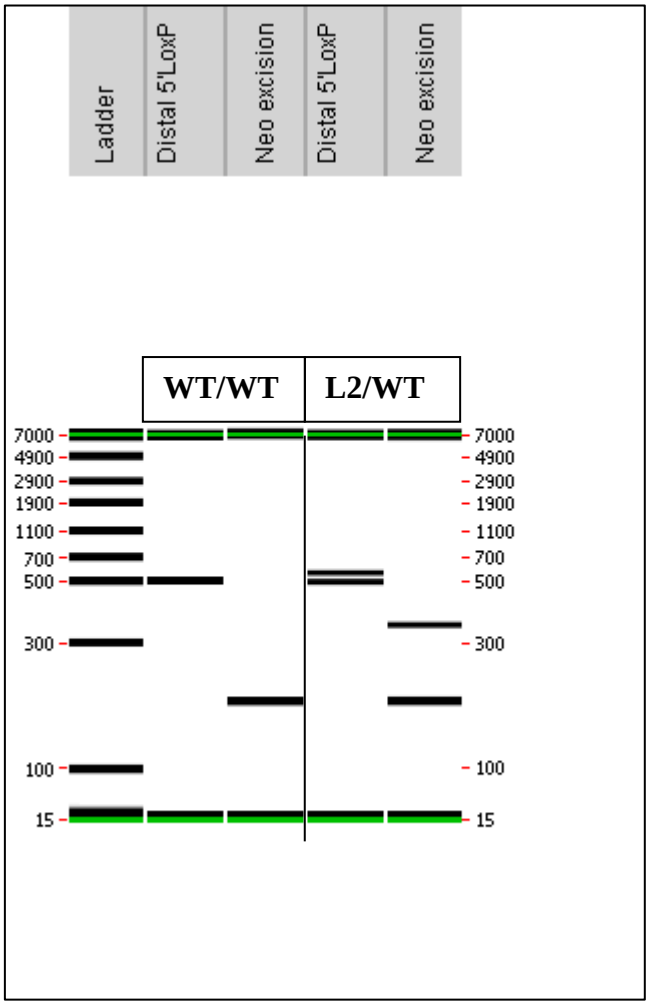
NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

3.2. Genotyping data

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