Molecular Biology Data
Med1 conditional knock out model
ICS references DG32

Table of contents

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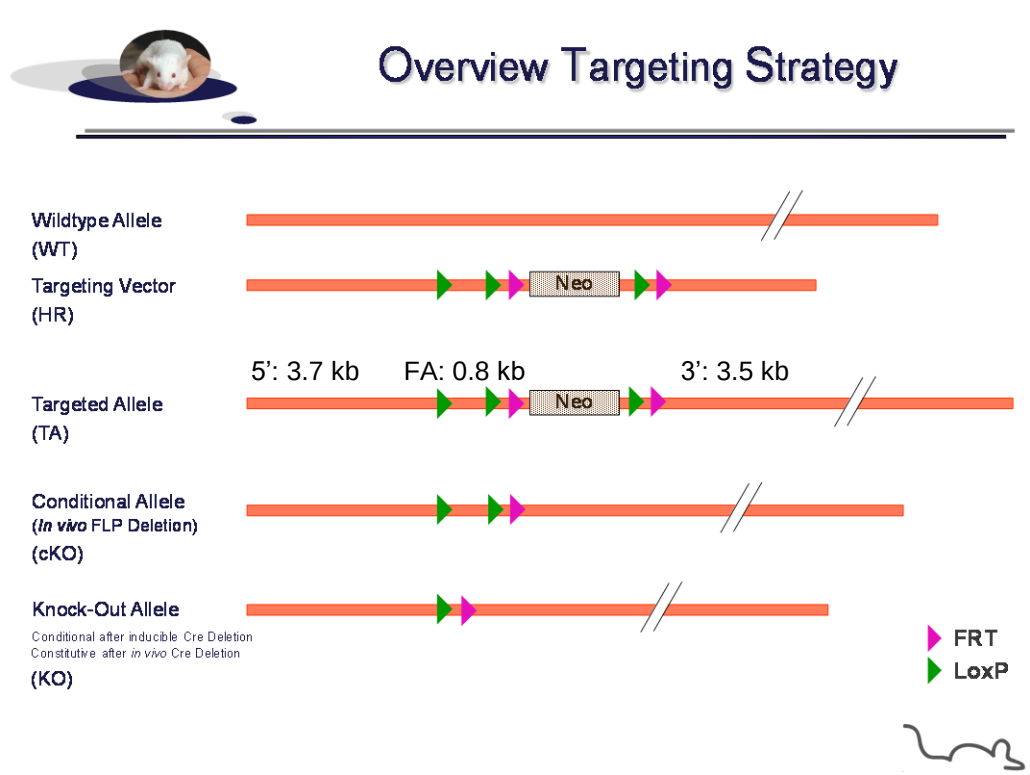
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

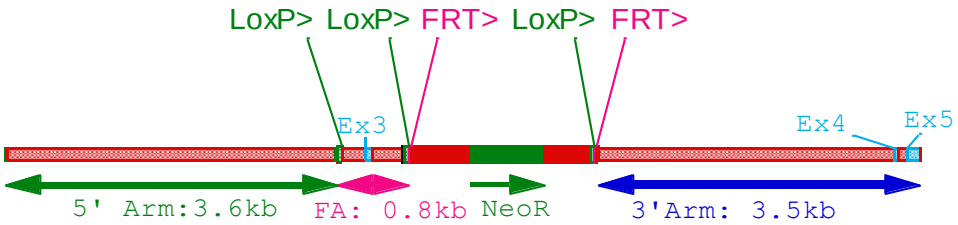


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exon 3

Med1 gene (also named TRAP220) is a member of the nuclear receptor family. Additional information on this gene can be accessed at
http://www.informatics.jax.org/marker/MGI:1100846

Strategy used to generate the conditional knock out model



Med1_Strategy.gcc
(9958 bp)

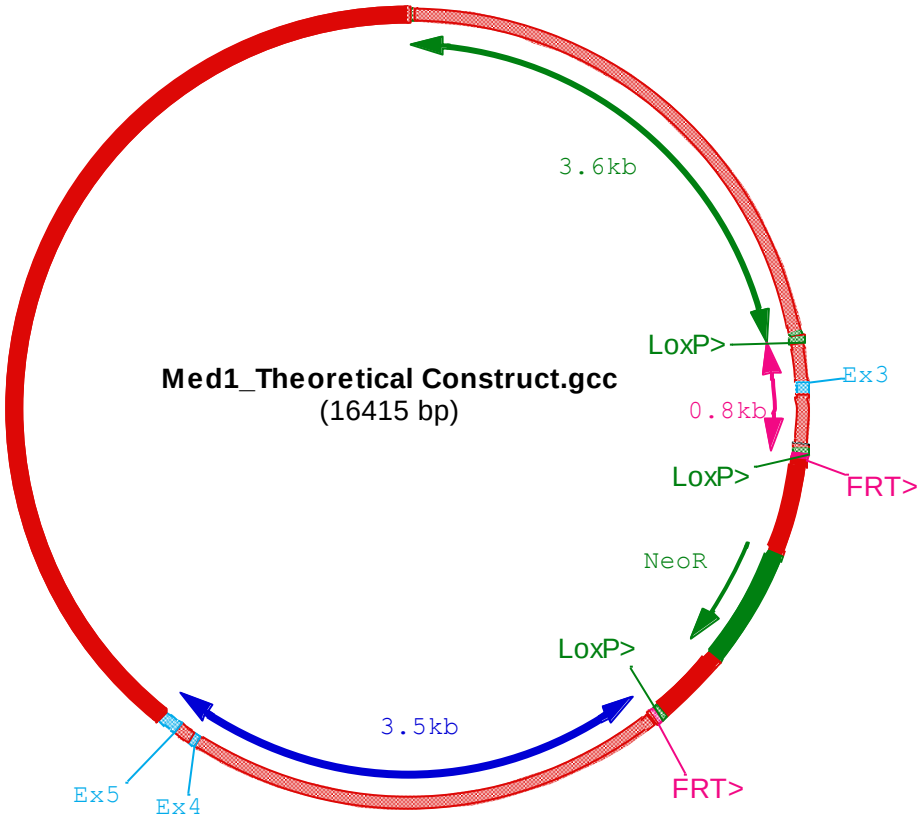
2. Construct used for homologous recombination in ES cells: Med1 project

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 came from Deltagen and the extremities are not clearly defined (a difference of some nucleotides can exist). A theoretical map of targeting vector plasmid was established thanks to the collected information.



2.3. Theoretical 5' homology arm (3.7 kb)

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2.4. Theoretical floxed fragment (0.8kb)

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2.5. PGK-Neo region

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2.6. Theoretical 3' homology arm (3.5 kb)

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2.7. Theoretical vector backbone sequence

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cgaggcc

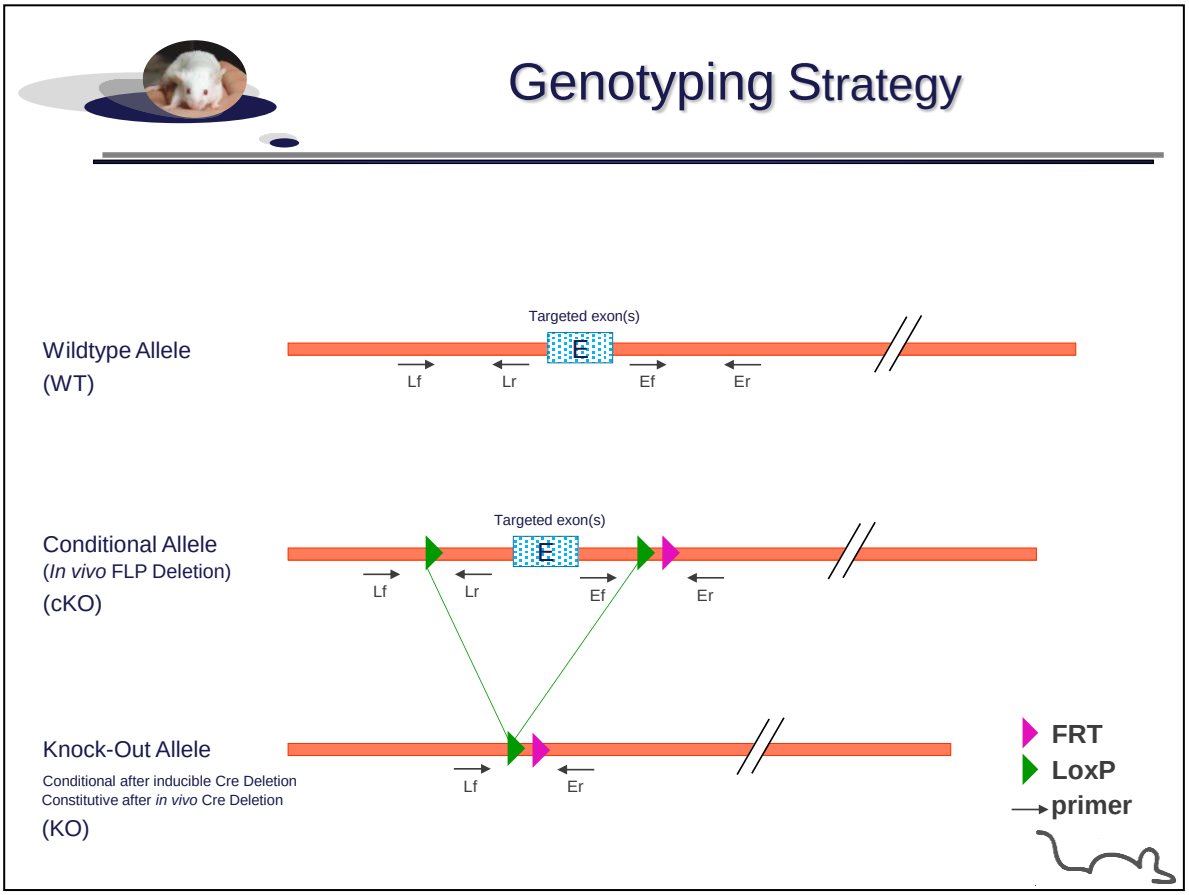
3. Data on conditional and knock-out animals

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

3.1. Genotyping protocol and data

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	TTGCTGCCAAGAGTTGTGGCCTGAG
Lr	ATGCCCTCCAGAACTCATTACGACC
Ef	CATGGAGGTTAGAAATCGCTTATGAG
Er	ATTGTTTCAAGCCAGGCTTGGTGGC

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	80-81	Lf / Lr	421	---	374
Excision of the selection marker	82-83	Ef / Er	414	---	298
Total Excision (excision of the floxed exon(s), i.e. knock out)	80-83	Lf / Er	1152*	417	989*

* 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:

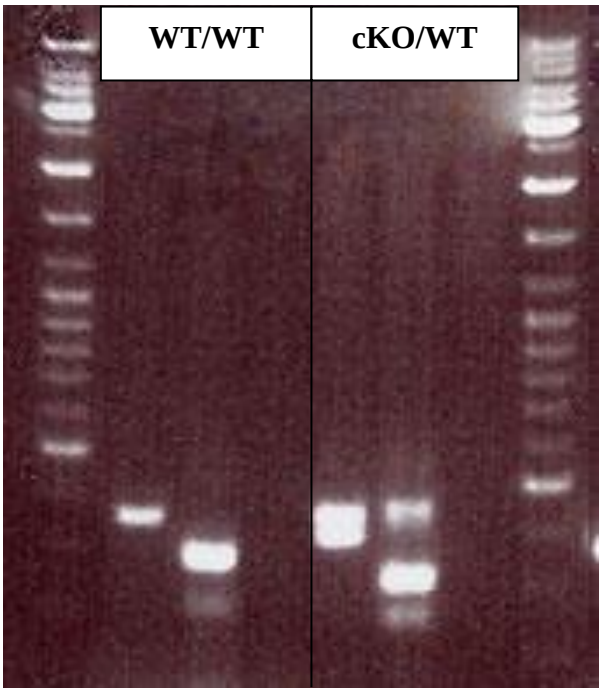
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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

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

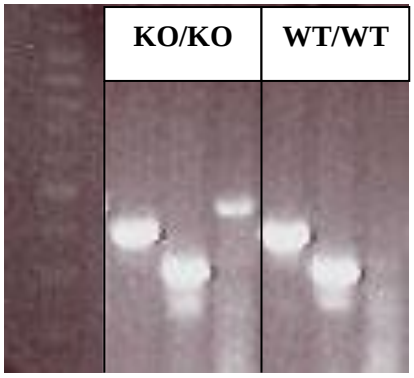
- Picture of genotyping with conditional knock-out (cKO) allele

Representative genotyping picture



- Picture of genotyping with knock-out (KO) allele

Representative genotyping picture



3.2. 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	32	27	1	**60**
Experimental Ratio	53,3%	45,0%	1,7%	**100%**
Theoretical Ratio	25%	50%	25%	**100%**
Theoretical Ratio if L-/L- are not viable	33%	66%	0%	**100%**

The Med1 knock-out homozygotes seem subviable but the number of pups obtained is not enough.

Legend:

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