Molecular Biology Data
K175-Ncor2 conditional knock out model
- ICS references K175/DG34/EUMO15

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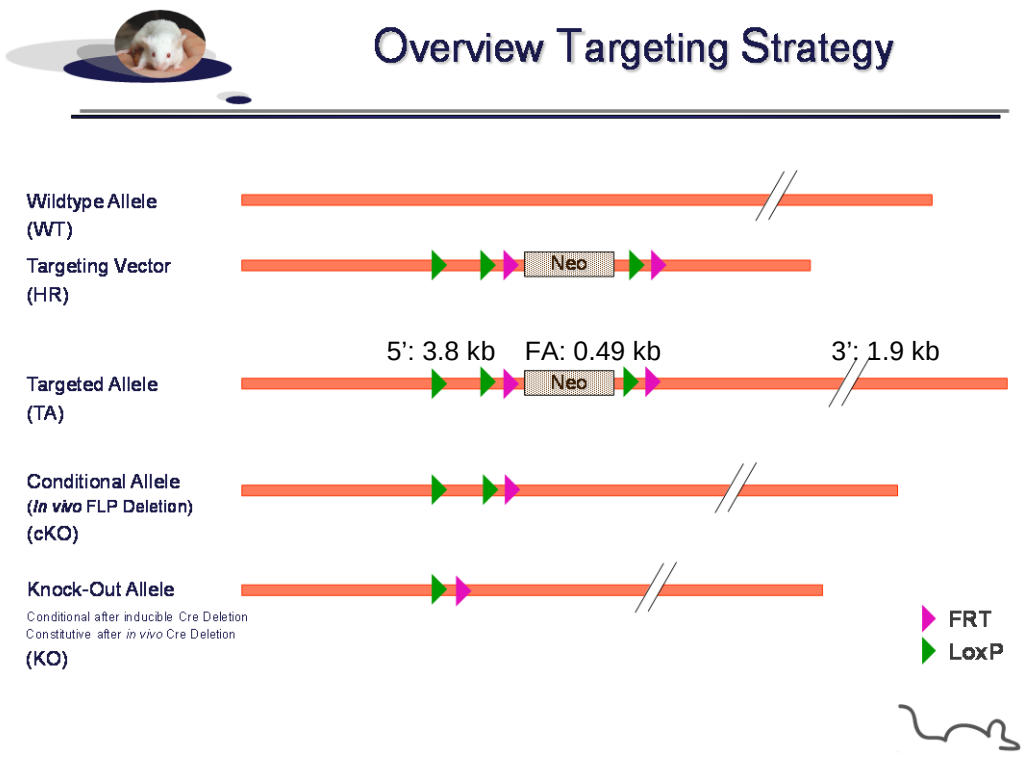
This protocol has been prepared by Loic Lindner, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

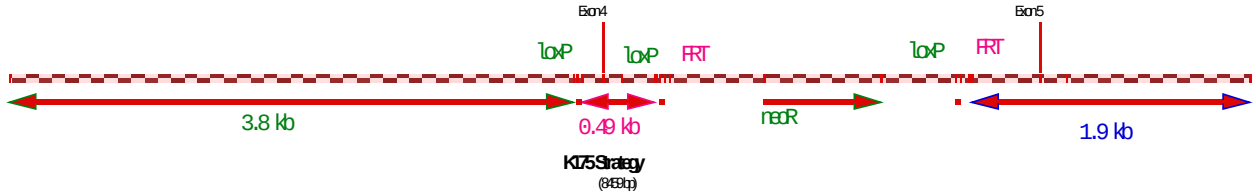


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exons 4

Ncor2 gene 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=40530

Strategy used to generate the conditional knock out model



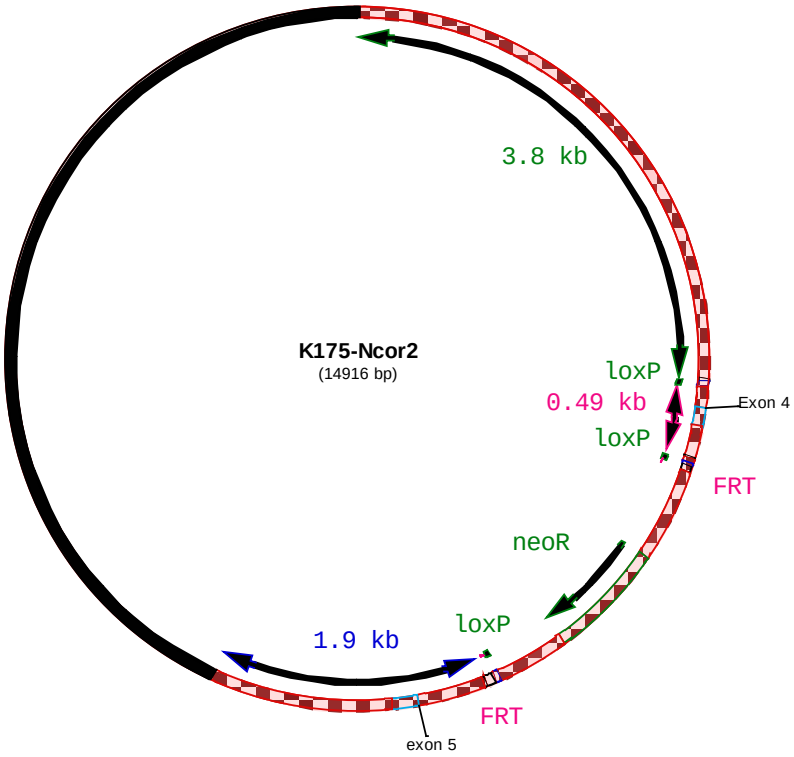
2. Construct used for homologous recombination in ES cells: Ncor2 project

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

We use the nomenclature of the Ensembl reference: ENSMUST00000111398



2.3. 5' homology arm (3.8 kb)

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2.4. Floxed fragment (0.49 kb)

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2.5. PGK-Neo region

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2.6. 3' homology arm (1.9 kb)

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2.7. Vector backbone sequence

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acgagagaggatgctcacgatacgggttactgatgatgaacatgcccggttactggaacgttgtgagggtaaaca
actggcggtatggatgcggcgggaccagagaaaaatcactcagggtcaatgccagcgcttcgttaatacagatgt
aggtgttccacagggtagccagcagcatcctgcgatgcagatccggaacataatggtgcagggcgctgacttccg
cgtttccagactttacgaaacacggaaaccgaagaccattcatgttgttgctcaggtcgcagacgttttgcagca
gcagtcgcttcacgttcgctcgcgtatcggtgattcattctgctaaccagtaaggcaaccccgccagcctagccg
ggtcctcaacgacaggagcacgatcatgcgcacccgtggccaggacccaacgctgcccgagatgcgccgcgtgcg
gctgctggagatggcggacgcgatggatatgttctgccaaggcgtttaaacttaattaagtcgacggccggccct
cgaggcc

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]

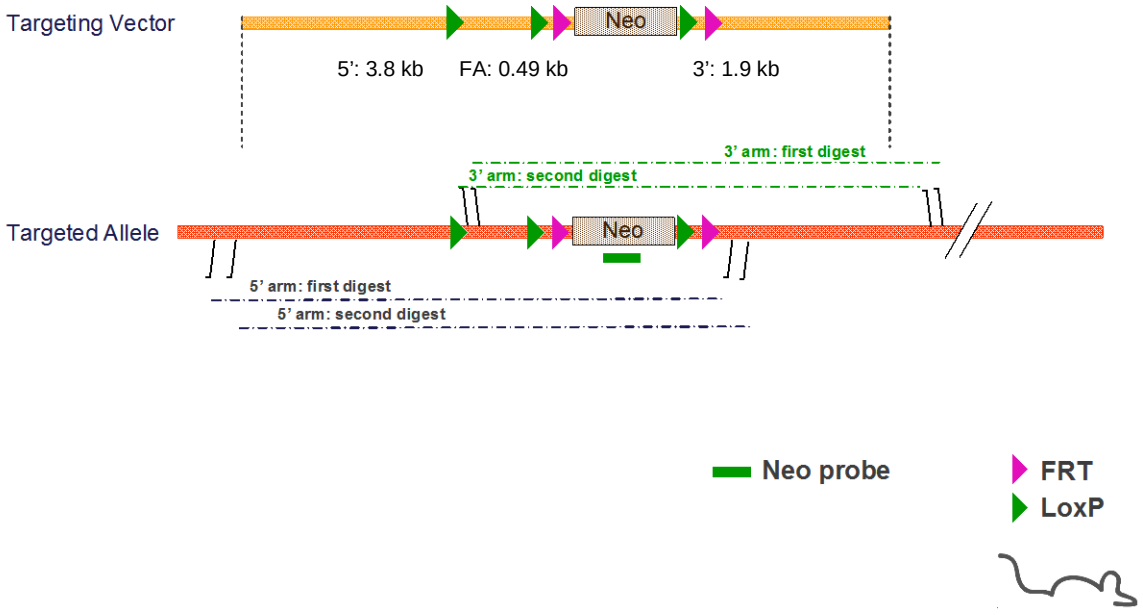
Number of clones screened: 372
Number of positive: 2

Reference of clone used to generate the mouse line:

- clone **K175-78**

3.2. Southern data on positive clone

3.2.1.Neo Southern strategy



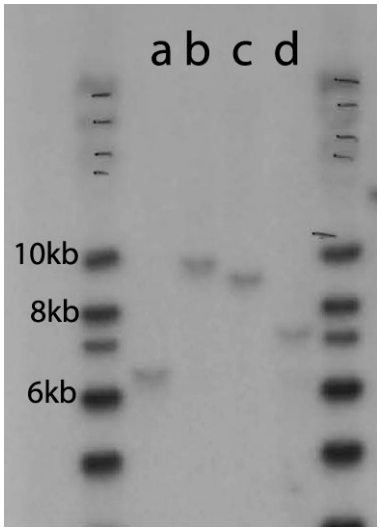
Digestions used to validate the 5' and 3' insertion

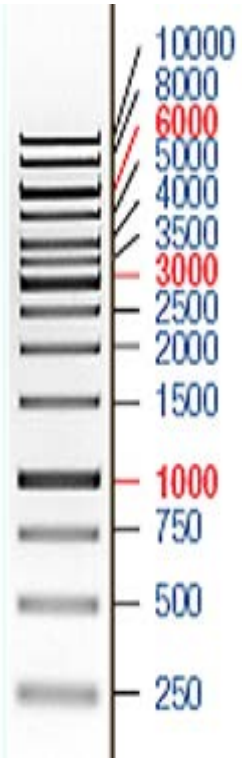
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	XmnI	/	6.5
	5' second digest	ScaI	/	9.5
	3' arm first digest	NheI	/	9
	3' arm second digest	NdeI	/	7.4

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

3.3. Picture of Neo Southern

Neo southern blot: 5' and 3' arm validation ladder





a: Xmn I; b: ScaI; c: Nhe I; d: NdeI

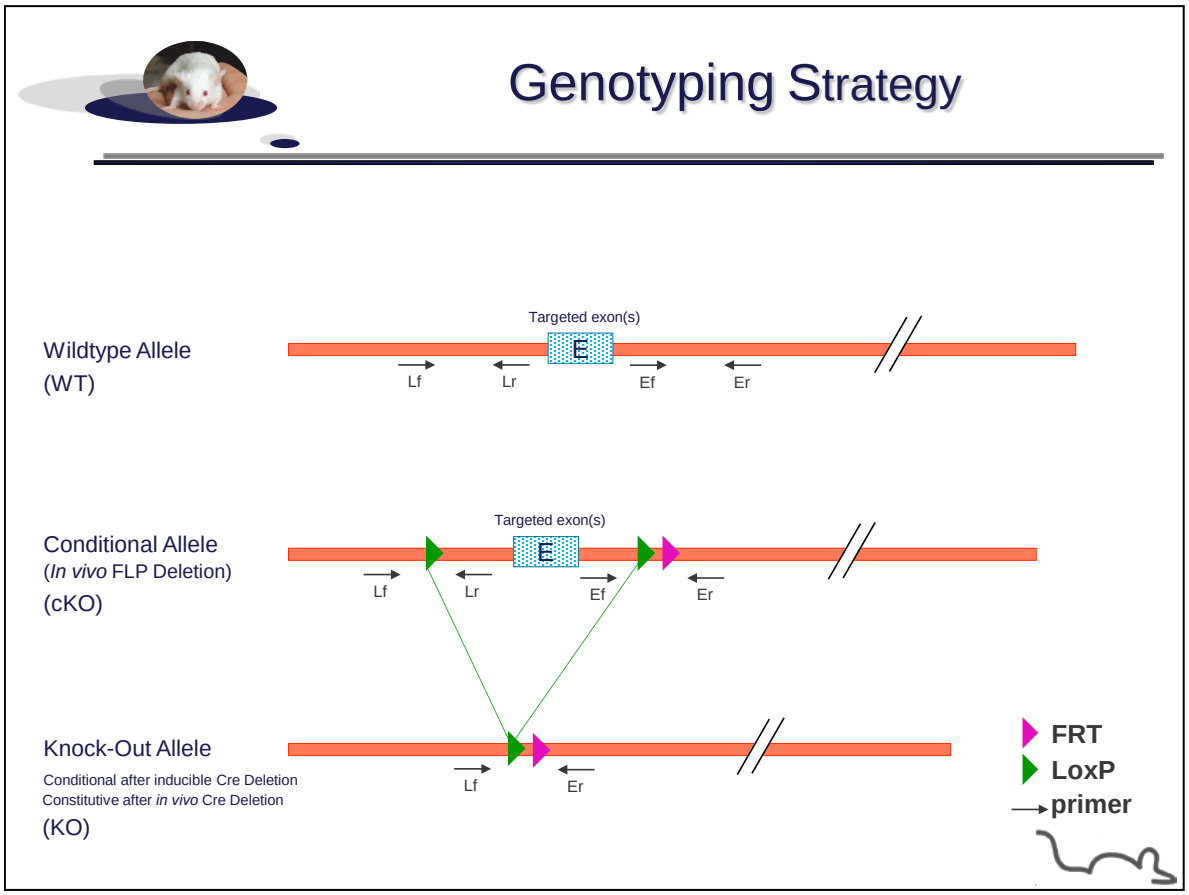
4. Data on conditional and knock-out animals

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	637	CCAGACTTTAGCCCTTCCTAGTGC
Er	638	TCACTAGCTGATCACAGCACTCCTG
Lf	634	AACACCACAGGTCCCAGAGAAGC
Lr	635	CTACAATTGCCACCACCATGGTCC

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	634-635	Lf / Lr	187	---	140
Excision of the selection marker	637-638	Ef / Er	296	---	215
Total Excision (excision of the floxed exon(s), i.e. knock out)	634-638	Lf / Er	837*	280	673*

* 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 □M)		0.125µl
-3' primer (100 □M)		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

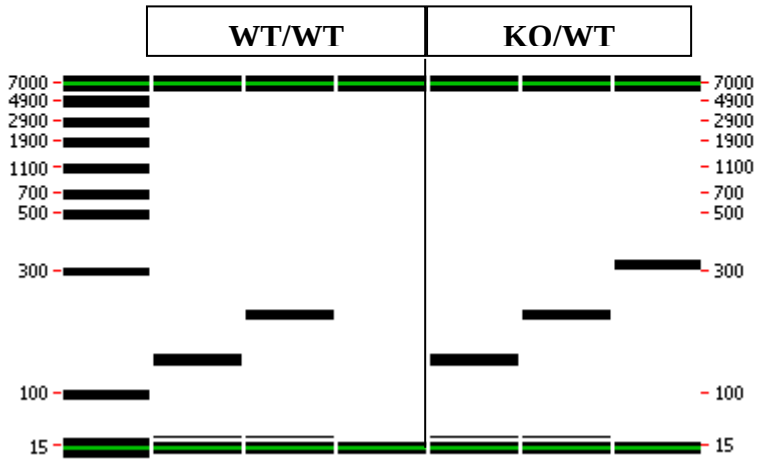
Picture of genotyping with conditional knock-out (cKO) allele

Data not shown

Picture of genotyping with knock-out (KO) 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.

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	53	81	22	**156**
Experimental Ratio	34,0%	51,9%	14,1%	**100%**
Theoretical Ratio	25%	50%	25%	**100%**
Theoretical Ratio if KO/KO are not viable	33%	66%	0%	**100%**
Homozygote Viability	Viable			

The Ncor2 knock-out homozygotes are viable.

Legend:

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