Molecular Biology Data
Nr3c2 conditional knock out model
ICS references K179/DG41/EUMO14

Table of contents

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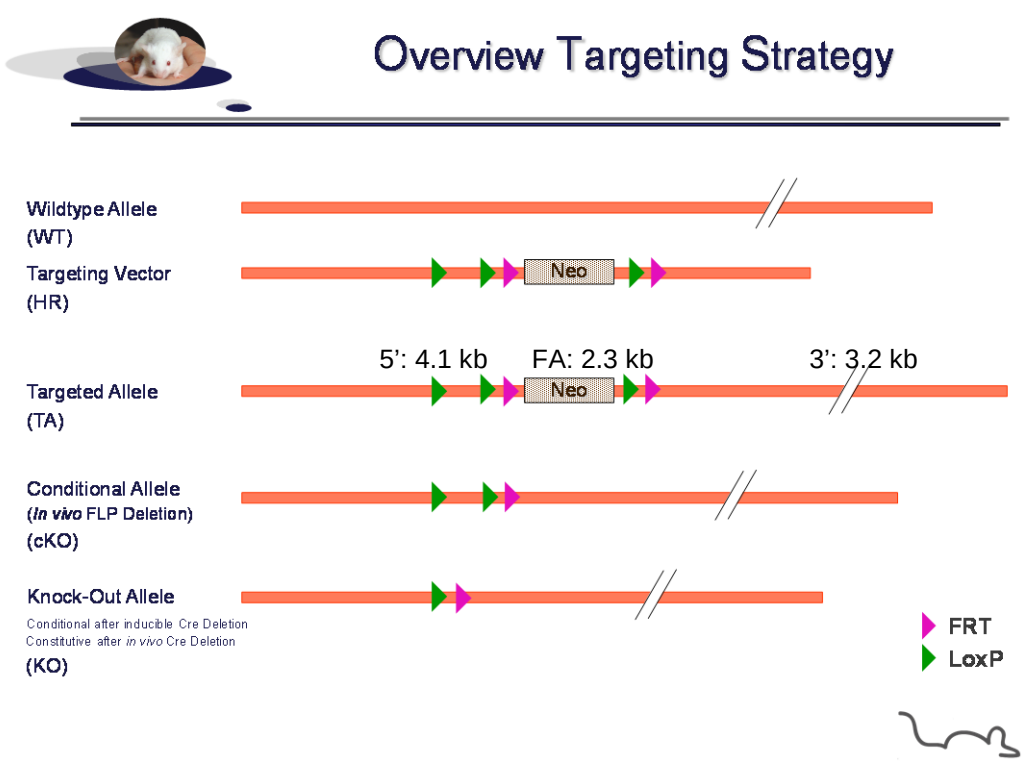
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

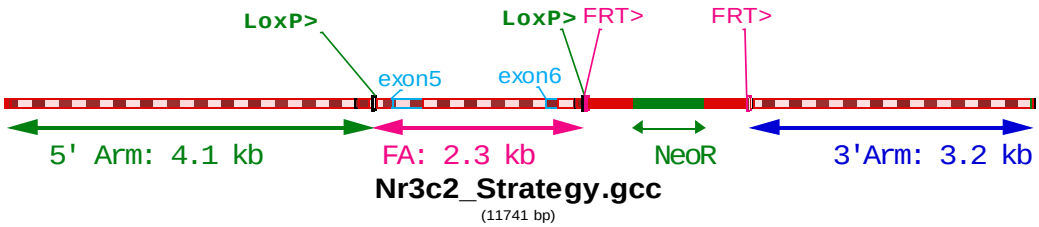


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exons 5 and 6

Nr3c2 gene (also named MR) 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=15265

Strategy used to generate the conditional knock out model

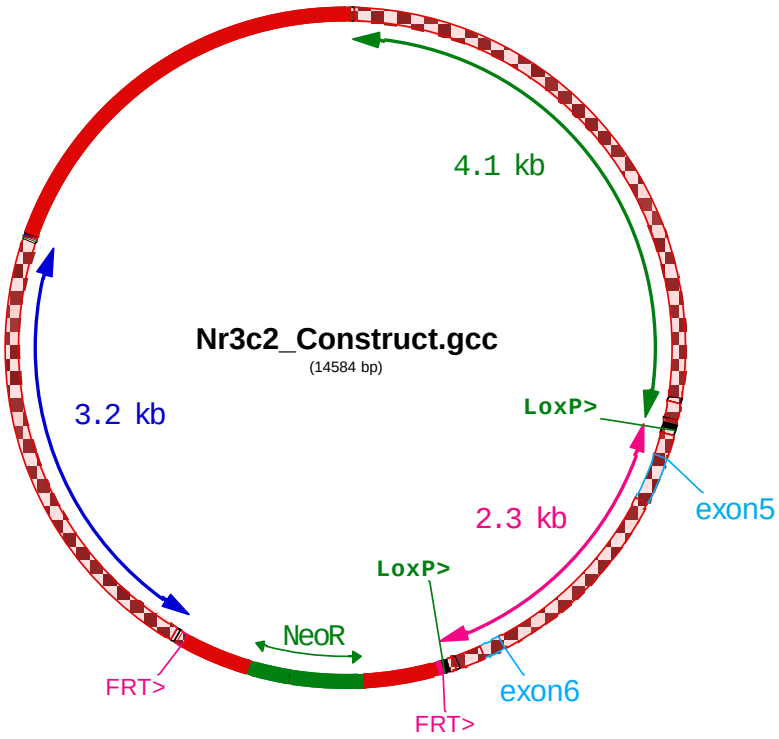


2. Construct used for homologous recombination in ES cells: Nr3c2 project

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

2.2. Map of targeting vector plasmid



2.3. 5' homology arm (4.1 kb)

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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]

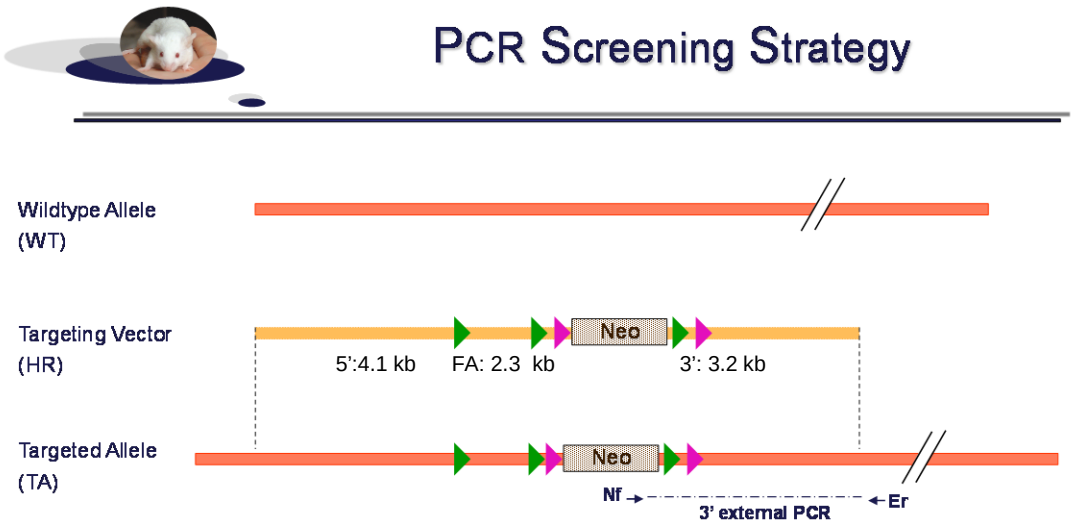
Number of clones screened: 372
Number of positives: 3

Reference of clone used to generate the mouse line:

- clone **K179-30**

3.2. PCR data on positive clone

3.2.1.PCR screening strategy



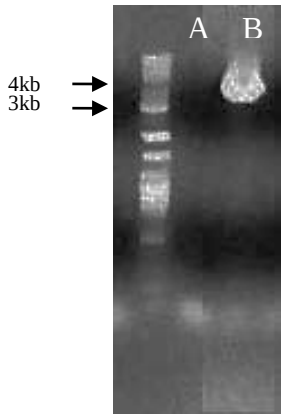
Primers used for PCR validation of ES clone

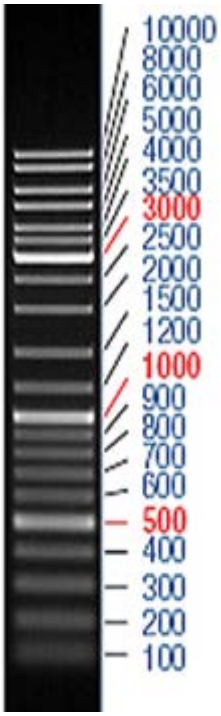
PCR	Primer Name	Primer sequences	PCR product size
3' external	Nf	CACGAGTTAACTTCATGTGTGAACC	4.0Kb
	Er	AGGGGCTCGCGCCAGCCGAACTGTT	

3.2.1.Picture of PCR on positive clone

3' external PCR ladder

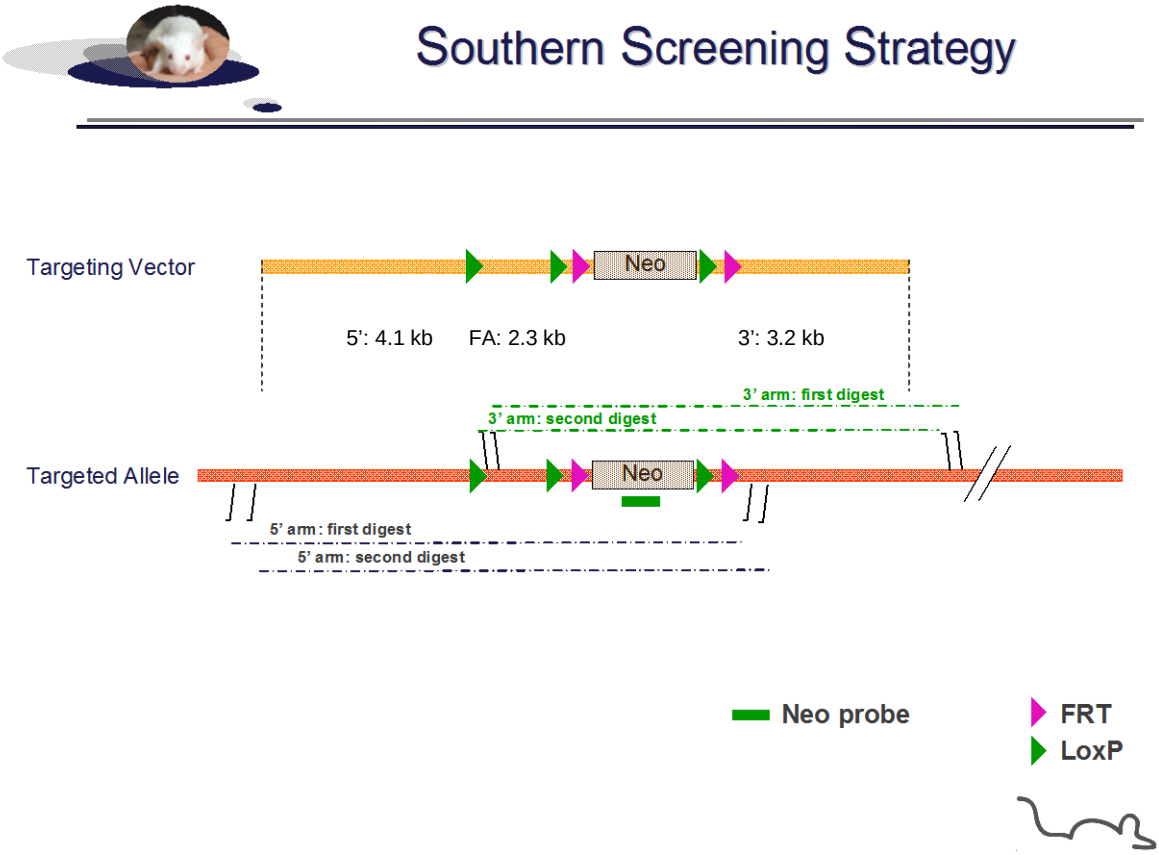
A: WT clone
B: positive clone





3.3. Southern data on positive clone

3.3.1.Neo Southern strategy



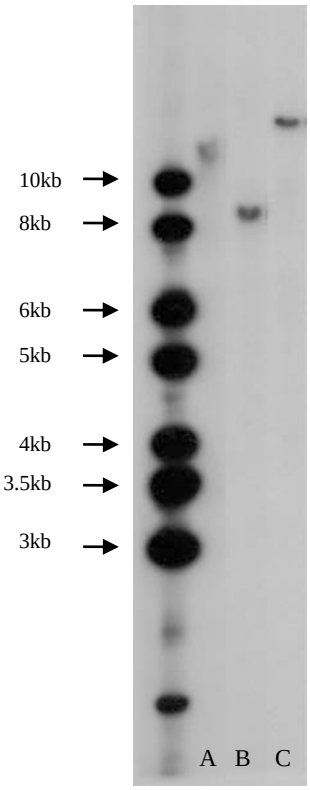
Digestions used to validate the insertion

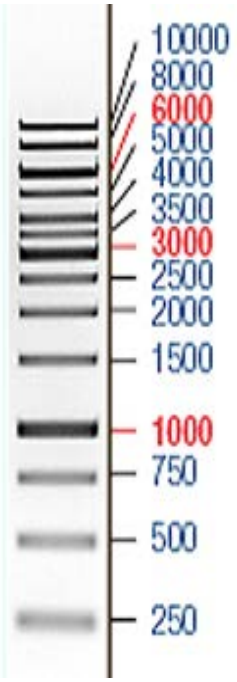
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	3' arm first digest	KpnI	/	12.4
	3' arm second digest	Afl II	/	8.7
	3' arm third digest	Hind III	/	14

3.3.2.Picture of Neo Southern

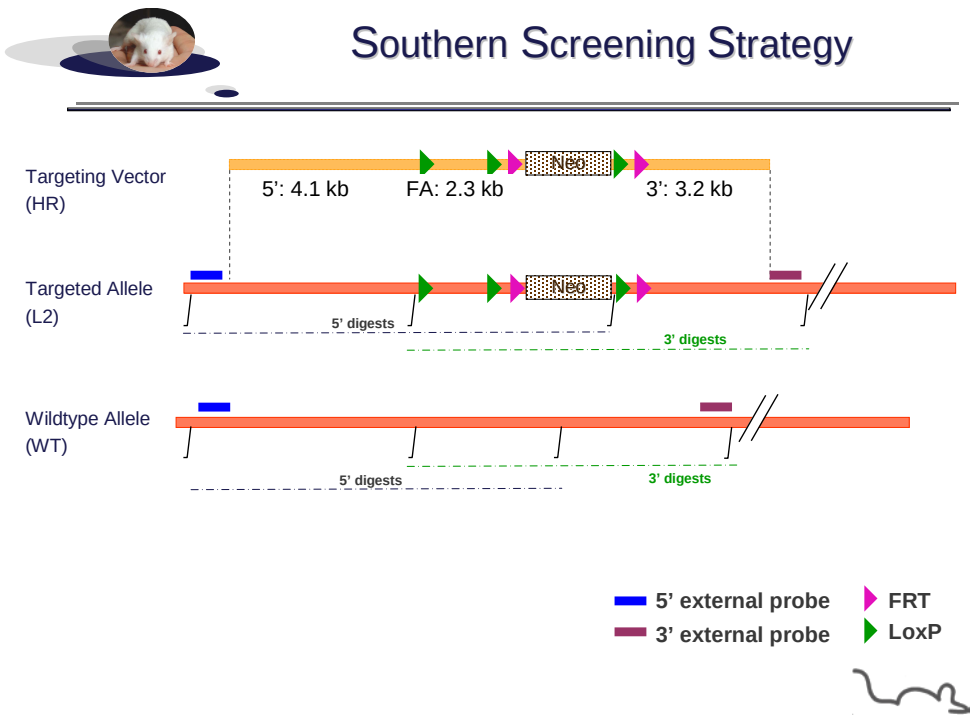
Neo southern blot: 3' arm validation ladder

A: Kpn 12.4kb
B: AflII 8.7kb
C: HindIII 14kb





3.3.3.External Southern strategy



Digestions used to validate with 5' probe

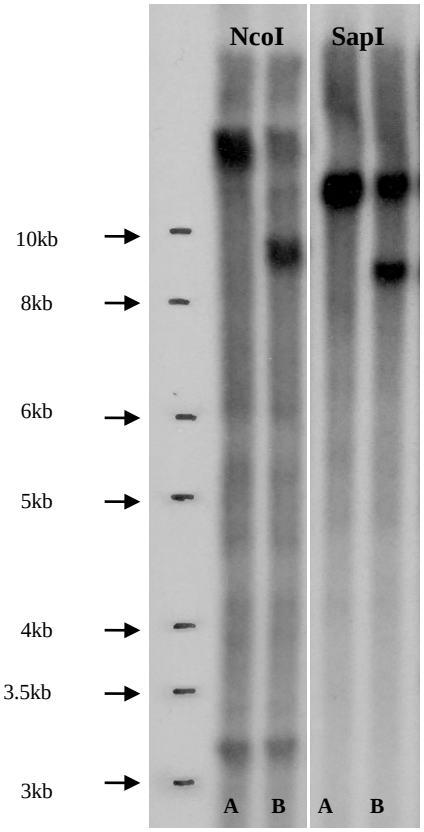
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
5' external	first digest	Nco I	15.6	9.6
	second digest	Sap I	12.7	8.9

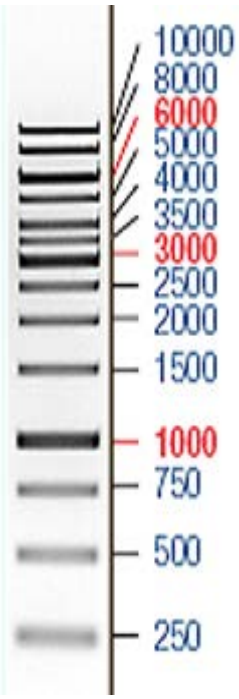
Primers for the 5'probe synthesis:
CAGAACTGTGGCCTAGTCCACTTCCA
GCATCTCCCACTACAGTTTCAGGTGT

3.3.4.Picture of Southern with external 5’

5’ external probe ladder

A: Wt clone
B: positive clone



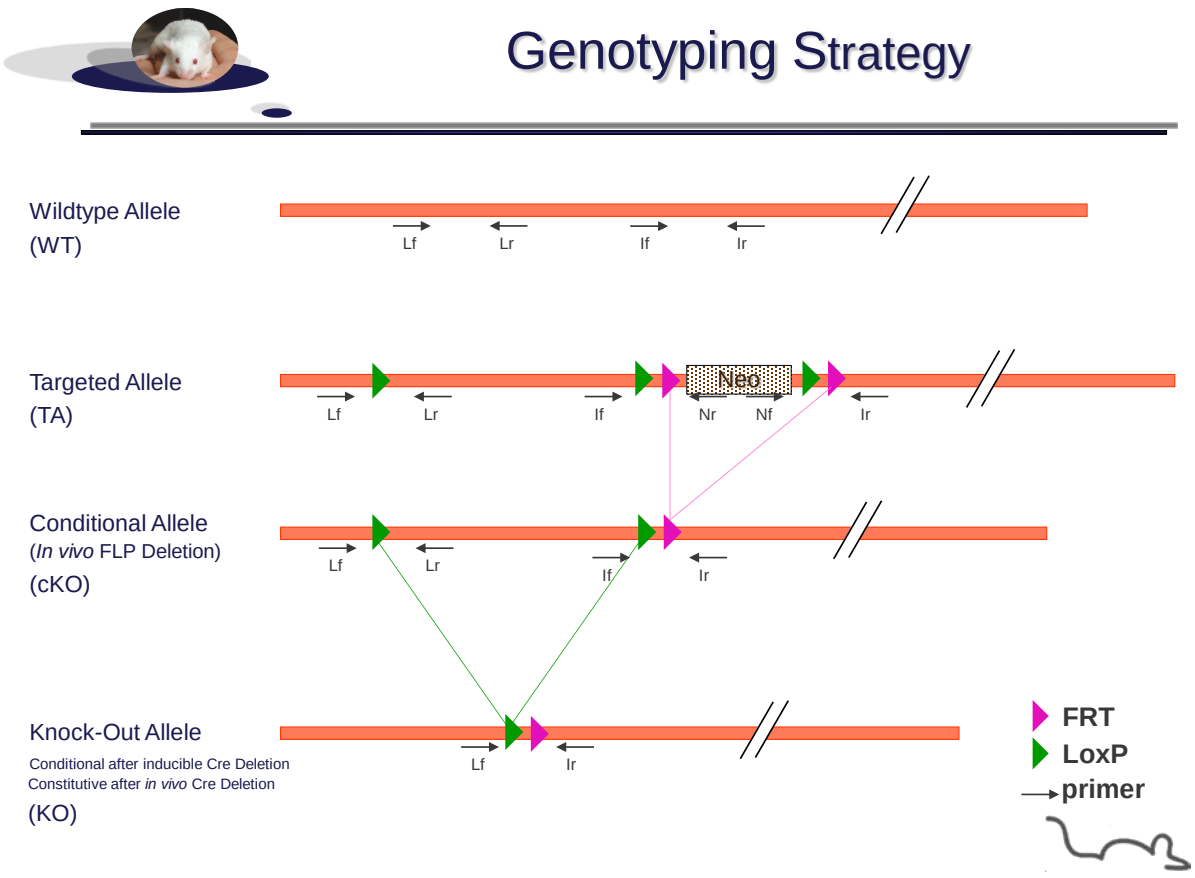


4. Data on conditional and knock-out animals

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

4.1. Genotyping protocol and data

4.1.1. Genotyping strategy



Sequence of primers used for genotyping

Position	Primers	Sequence
Lf	783	CCACTTGTATCGGCAATACAGTTTAGTGTC
Lr	785	CACATTGCATGGGGACAACTGACTTC
Ef	786	GGAGATCGTACAAACATACGAACAGC
Er	788	CTGTGATGCGCTCGGAAACGG

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	783-785	Lf / Lr	358	---	308
Excision of the selection marker	786-788	Ef / Er	565	---	464
Total Excision (excision of the floxed exon(s), i.e. knock out)	783-788	Lf / Er	2812*	454	2652*

* 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:

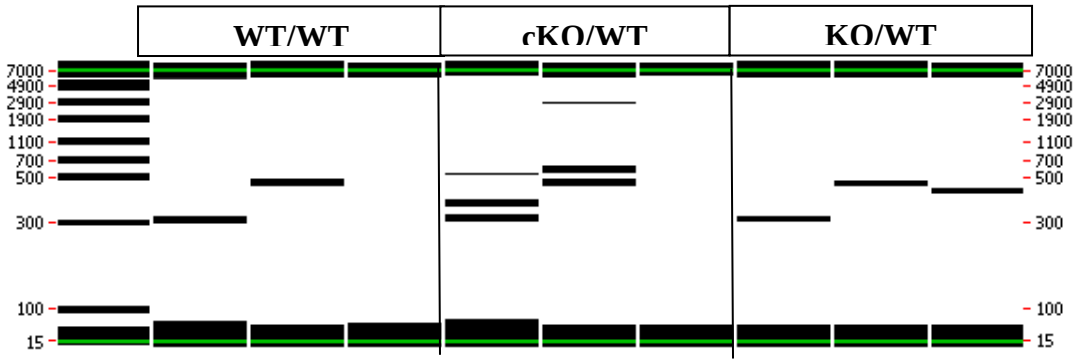
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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	24	33	1	**58**
Experimental Ratio	41,4%	56,9%	1,7%	**100%**
Theoretical Ratio	25%	50%	25%	**100%**
Theoretical Ratio if KO/KO are not viable	33%	66%	0%	**100%**

The Nr3c2a1 knock-out homozygotes are subviable.

Legend:

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