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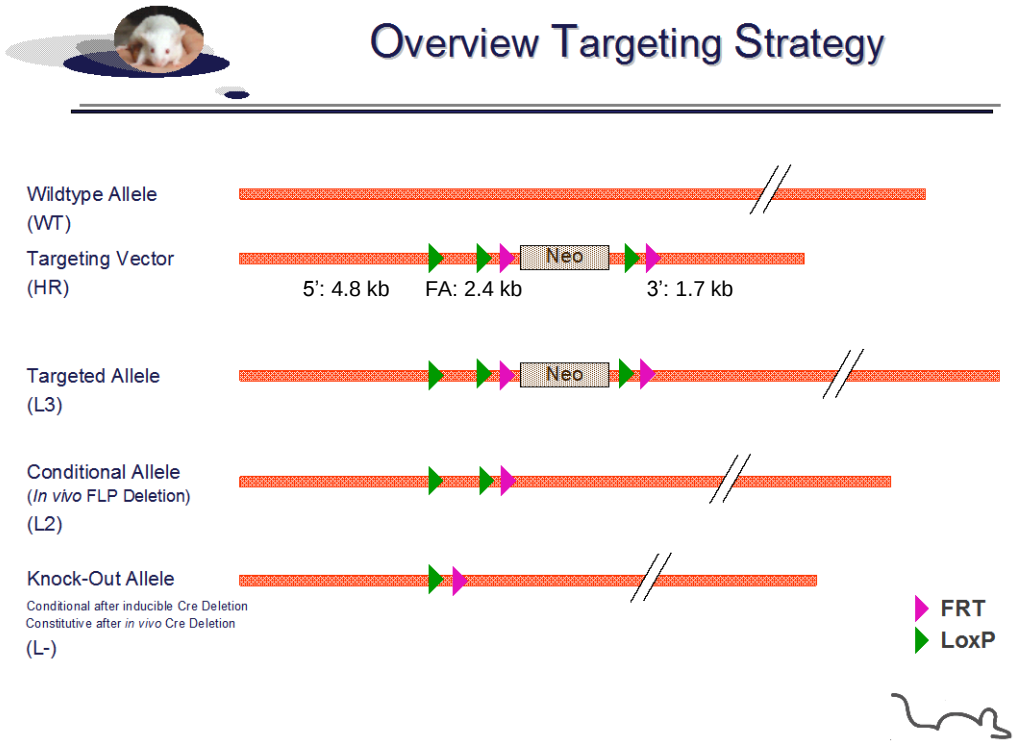
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

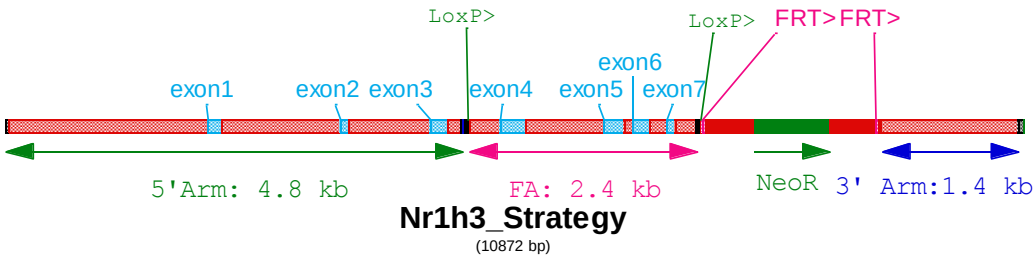


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen by MRL: flox of exons 4-7

Nr1h3 gene (also named LXRalpha) 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=45355

Strategy used to generate the conditional knock out model



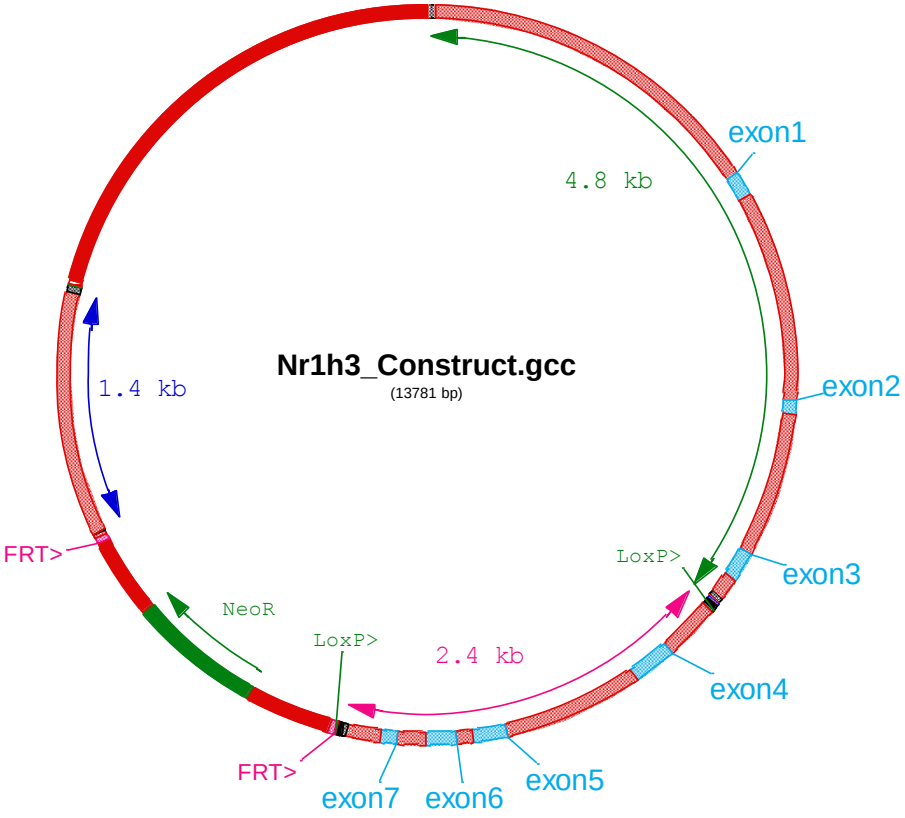
Nr1h3_Strategy
(10872 bp)

2. Construct used for homologous recombination in ES cells: Nr1h3 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



The sequencing of the exons from the targeting vector shows 1 mutation.

- In exon 3, an A>G mutation was detected. This mutation does not modify the Nr1h3 protein as GAA and GAG codons encode the same amino acid.

2.3. 5' homology arm (4.8 kb)

CCTTGAAATGAGGTGTGTCAGGGACATCTCTGAGGACCTATGATGCTGCAACTTGAGCAAGGGGAGAGAGCTGGA
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2.4. Floxed fragment (2.4 kb)

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2.5. PGK-Neo region

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2.6. 3' homology arm (1.7 kb)

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2.7. Vector backbone sequence

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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]

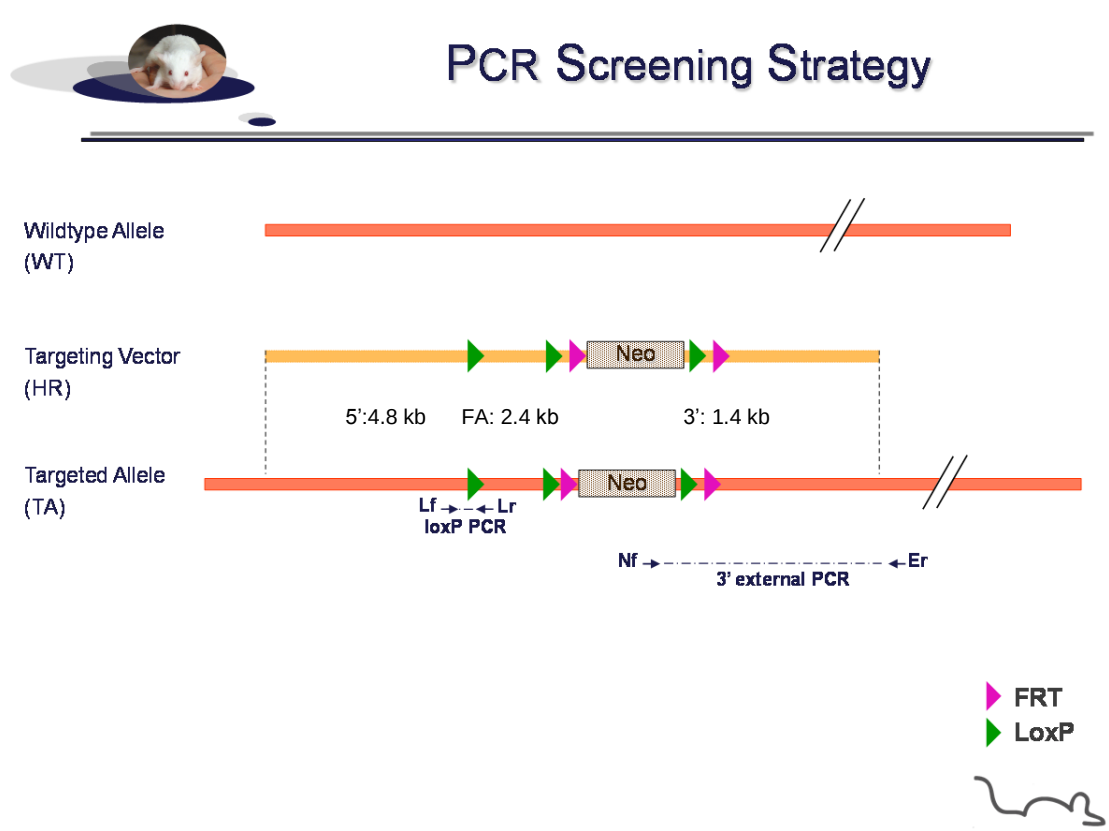
Number of clones screened: 372 x 2
Number of positive: 4

Reference of clone used to generate the mouse line:

- clone **K98P1-247**

3.2. PCR data on positive clone

3.2.1.PCR screening strategy



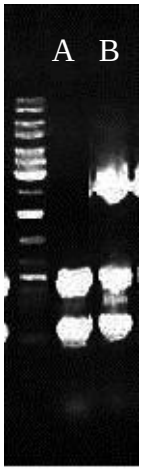
Primers used for PCR validation of ES clone

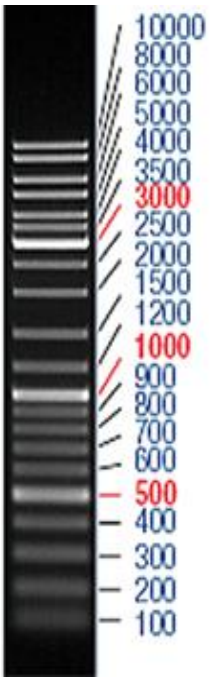
PCR	Primer Name	Primer sequences	PCR product size
3' external	Nf	AGGGGCTCGCGCCAGCCGAACTGTT	TA: 2.3 kb
	Er	GCTCCTGGGAGGGTCTGATAACACT	
LoxP	Lf	GTCCATATCTTAGGCGTTTCTTAGC	WT: 188 bp TA: 254 bp
	Lr	CTGGAATTACCAAGACGTGCCACAG	

3.2.2.Picture of PCR on positive clone

3' external PCR ladder

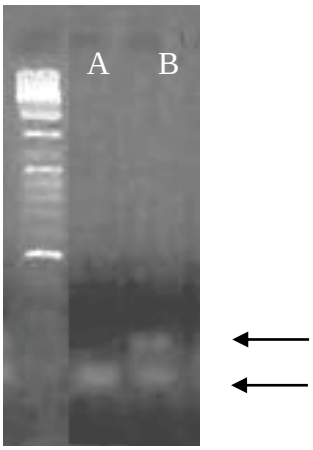
A: WT clone
B: positive clone

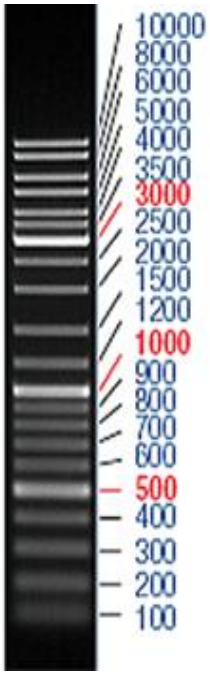




LoxP PCR ladder

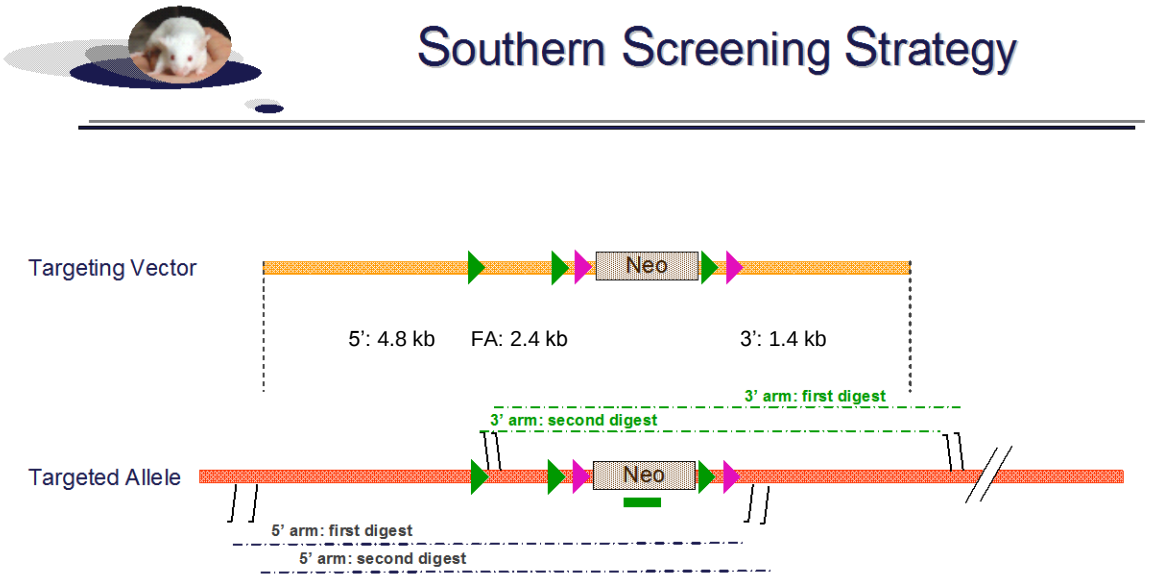
A: WT clone
B: positive clone





3.3. Southern data on positive clone

3.3.1.Neo Southern strategy



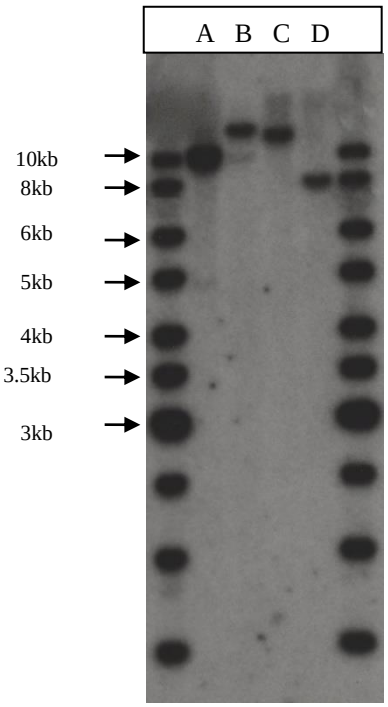
Digestions used to validate the 5' and 3' insertion

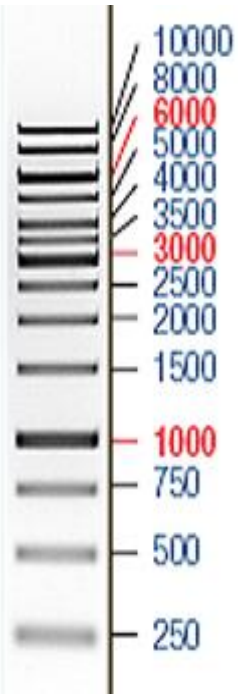
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	PshAI	/	10.7
	5' second digest	SspI	/	14.0
	3' arm first digest	NheI	/	13.2
	3' arm second digest	ApaI	/	8.0

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

3.3.2.Picture of Neo Southern

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





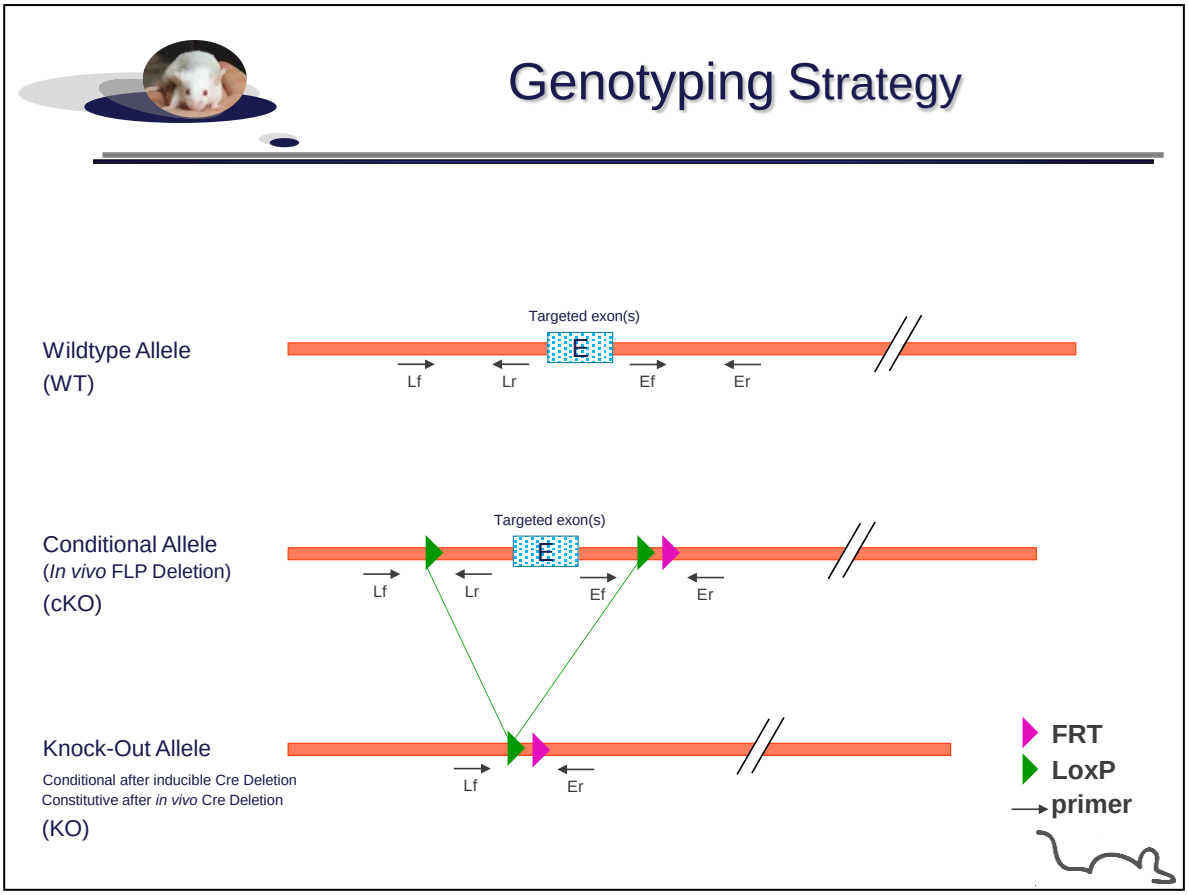
A: PshAI	10.7Kb
B: SspI	14.0Kb
C: NheI	13.2Kb
D: ApaI	8.0Kb

4. Data on conditional and knock-out animals: Genotyping protocol and data:

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

4.1. 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	563	GTCTCCTTGGTGGAGACCAGG
Er	565	ATGCCTGAAAAGGGCATCAGATGCC
Lf	319	GGATTTGGAGAAGGTAAAGTCTCCC
Lr	320	TGGACTCAAGTGATCTTGTCTCAGC

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	319-320	Lf / Lr	416	---	350
Excision of the selection marker	563-565	Ef / Er	343	---	242
Total Excision (excision of the floxed exon(s), i.e. knock out)	319-565	Lf / Er	2957*	456	2781*

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

4.2. 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:

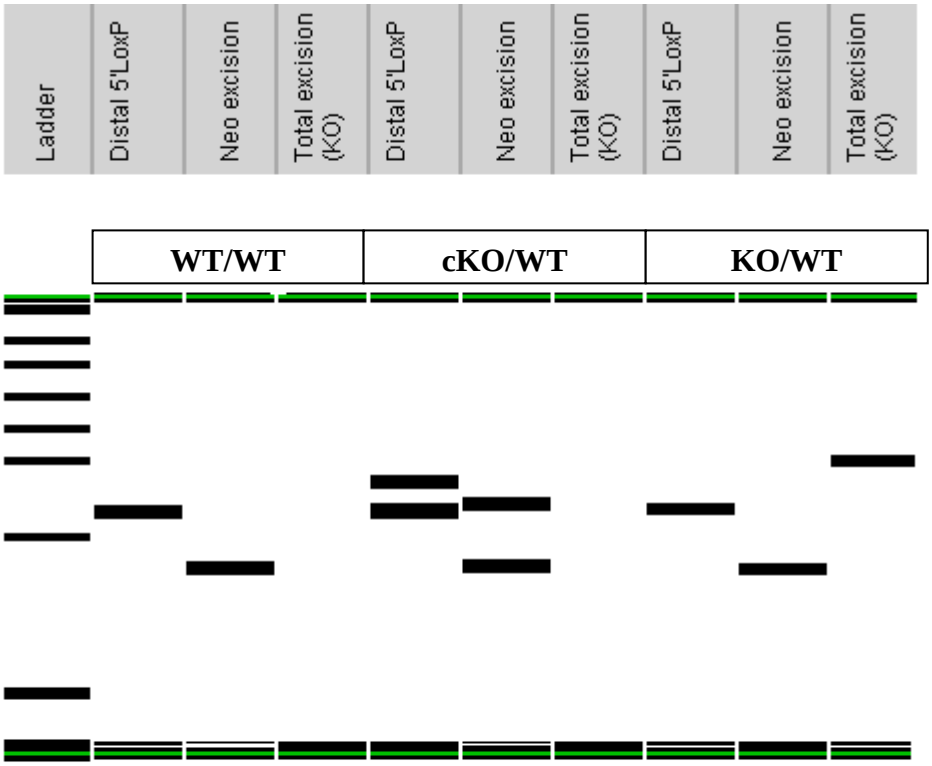
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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

4.3. 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.