Molecular Biology Data
Nr3c3 conditional knock out model
ICS references K180/DG40/Eumo18

Table of contents

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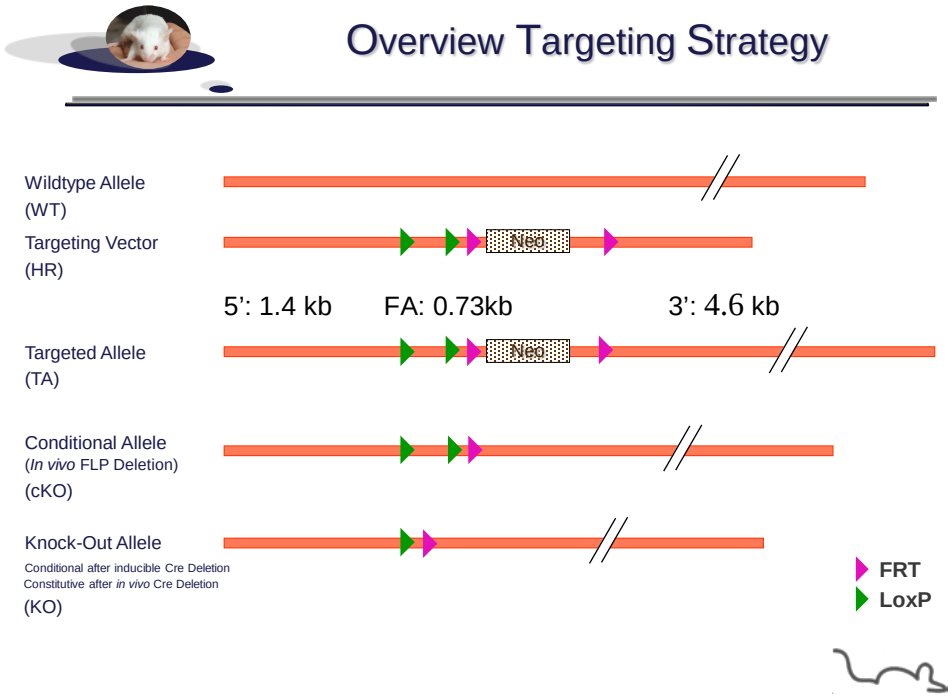
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

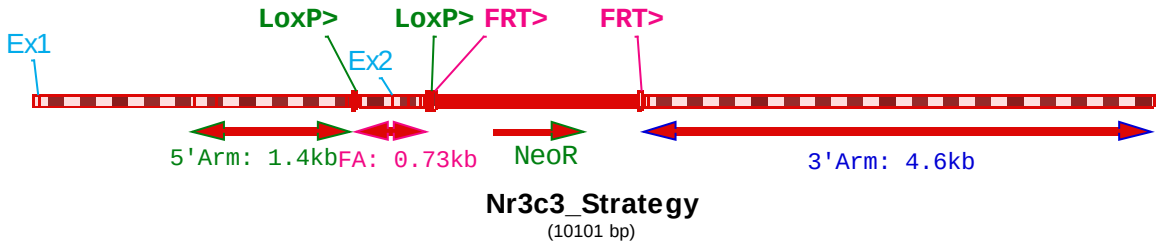


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exon 2

Nr3c3 gene (also named Pgr) 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=12309

Strategy used to generate the conditional knock out model



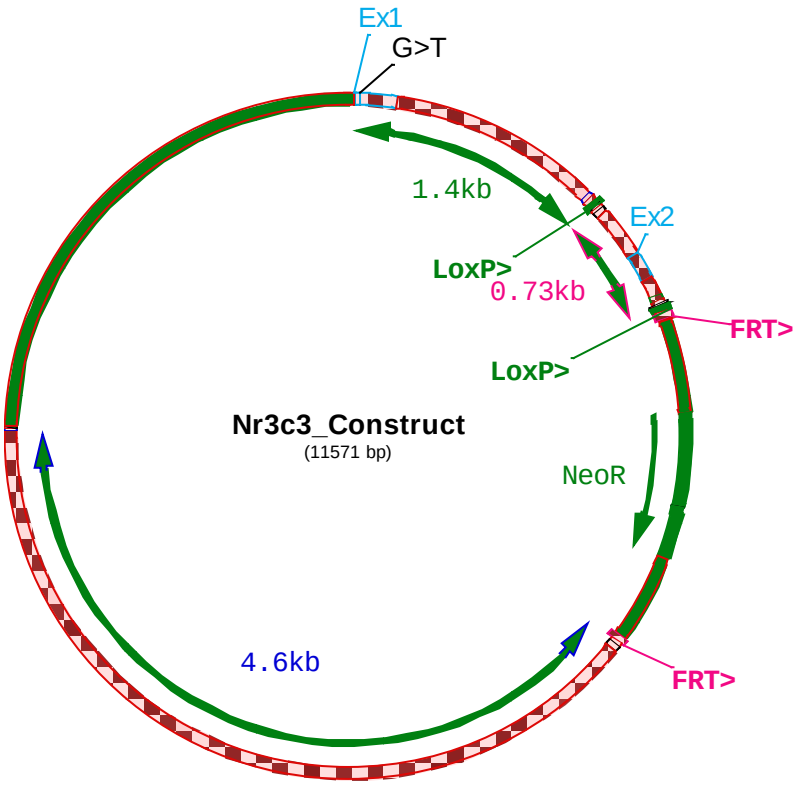
Nr3c3_Strategy
(10101 bp)

2. Construct used for homologous recombination in ES cells: Nr3c3 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 one mutation against C57BL/6J strain. The G>T located in the exon 1 doesn't change the amino acid 471 (Ser).

2.3. 5' homology arm (1.4 kb)

GCCCACGCAGGGTTCTTTCGCGCCACTGCCGTGCAAGCCCCCAGCCGCCGGCTCCTGCCTACTACCCCGGGACAG
CCTGCCGGCCGCCCCGGCCACCGCCGCAGCACCCGCCATCTACCAGCCGCTCGGCCTCAATGGGCTCCCGCAGCT
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ACCAAGTTTAGTGCACTGAAATTCAAATATGTGTGTATACGTATGTATGTGTATATGTGTGTATGCATGTAAGTA
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2.4. Floxed fragment (0.73 kb)

ggccggccataacttcgtataatgtatgctatacgaagttattaattaaCTTT**GCTTACCTACCATACACTGAG**
CCCAGAGTTCTGTCCCCAGCACCTTAGAAACTGGGAATGGTGGCAAATGCTTCTGTTTCCAGCACTTGGAAGCTG
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AGGAAGCTTCCTCCTCATTCTCCCTTCCCTCTCATTGTCATGGAGACCATCCAATCCTTTATCCACTTAGTTATG
CTCGTTTCTAAGAAATGATGGCCTTTCTTTCCACCTGAAATTATGATGCTCTTTTAACCAGTTGCATTGCCTACA
GTACCCAcaccggtgataacttcgtataatgtatgctatacgaagttat

2.5. PGK-Neo region

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2.6. 3' homology arm (4.6 kb)

AGAGTGAACGTGGTTTGCTATTATAAGTGTATGCTGTGACTCACACTTTCAACAGTACTCAAGCCATGACACT**GG**
CGAGAGATTCTCTTCCTCTCAACAGCTTTACTCTAGTGATGTGTTCATATATATCTCCTGAATAAACAAAACACT
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ATTCCTTCCAAGTAGTGACATCCCTGATGACTAAGCATTCAAAGATATGCGCCTC**TGTGAGCTATGCC

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]

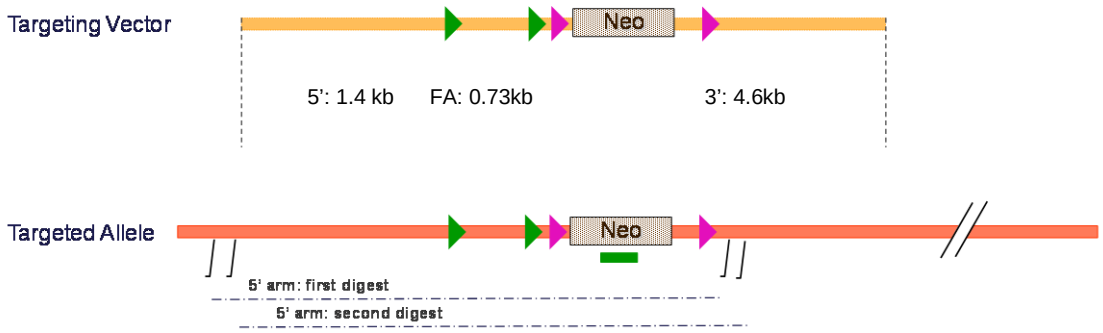
Number of clones screened: ~700
Number of positives: 1

Reference of clone used to generate the mouse line:

- clone **K180-305**

3.2. Southern data on positive clone

3.2.1.Neo Southern strategy



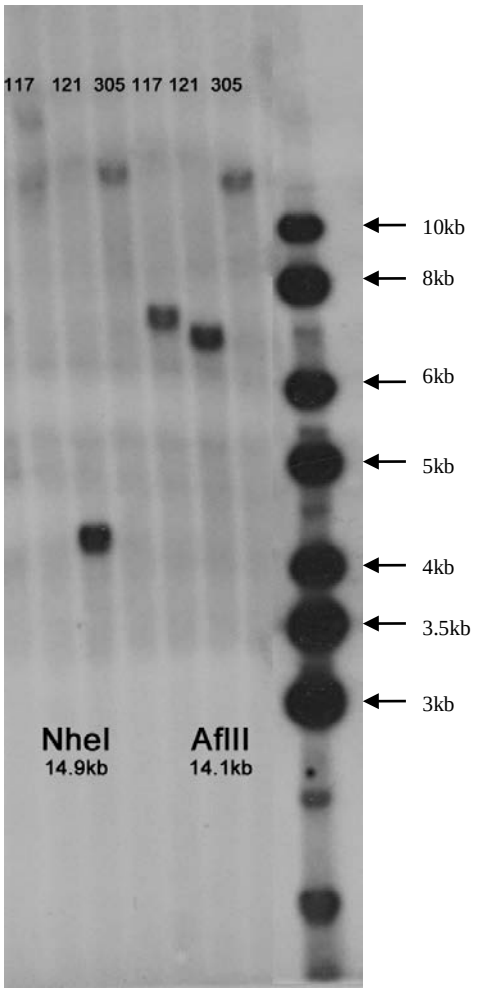
Digestions used to validate the insertion

Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	NheI	/	14.9
	5' arm second digest	AflII	/	14.1

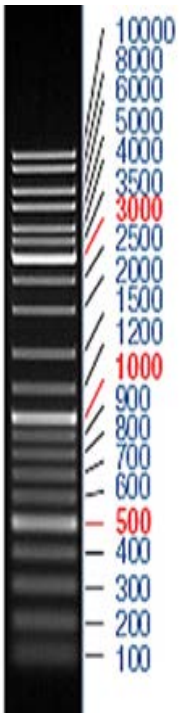
Two different digests are used to validate correct HR event.

3.2.2.Picture of Neo Southern

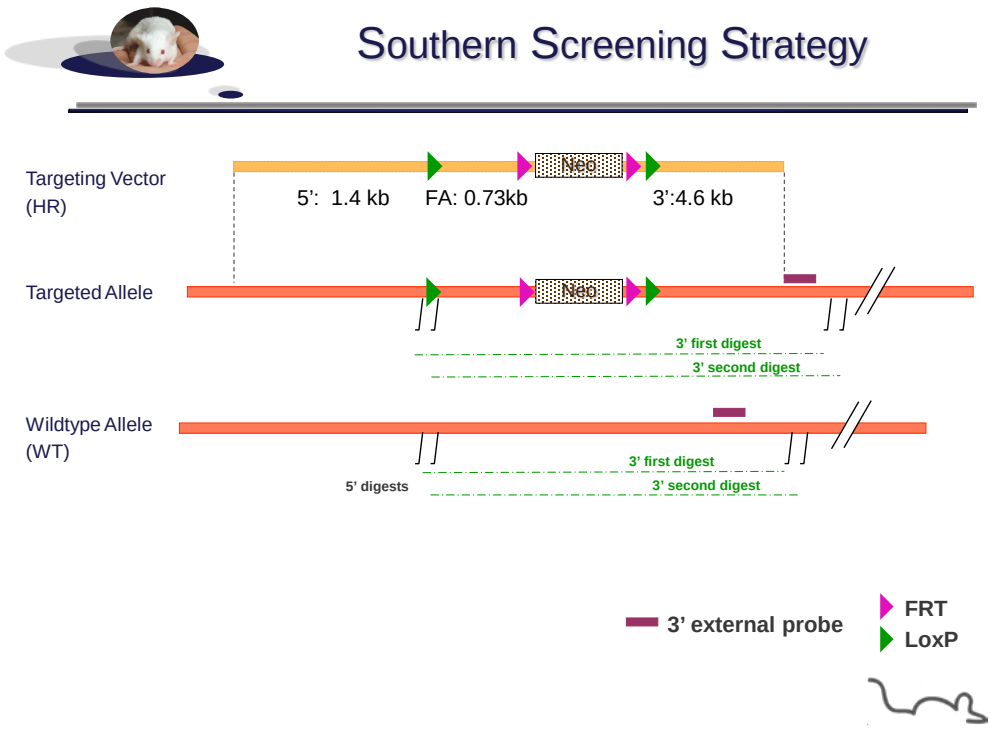
Neo southern blot



ladder



3.2.3.External probes Southern



Digestions used to validate with 3' probes

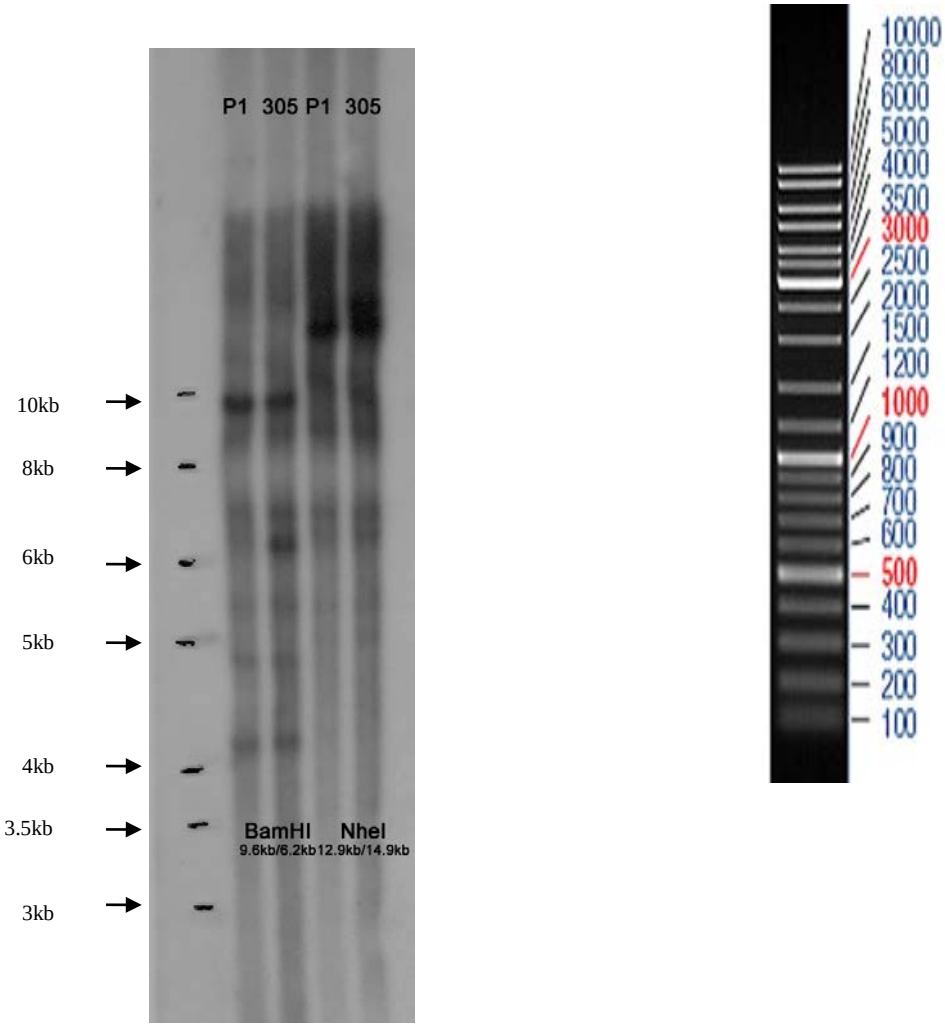
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
3' external	first digest	BamHI	9.6	6.2
	second digest	NheI	12.9	14.9

Primers for probe synthesis:

3' probe
GTCAGGACGGATGTAGGATGTCCAA
CCACCACAATCACTCAATTGAGACGT

3.2.4. Picture of Southern with external 5’ probe

Southern blot with external 3’ probe ladder

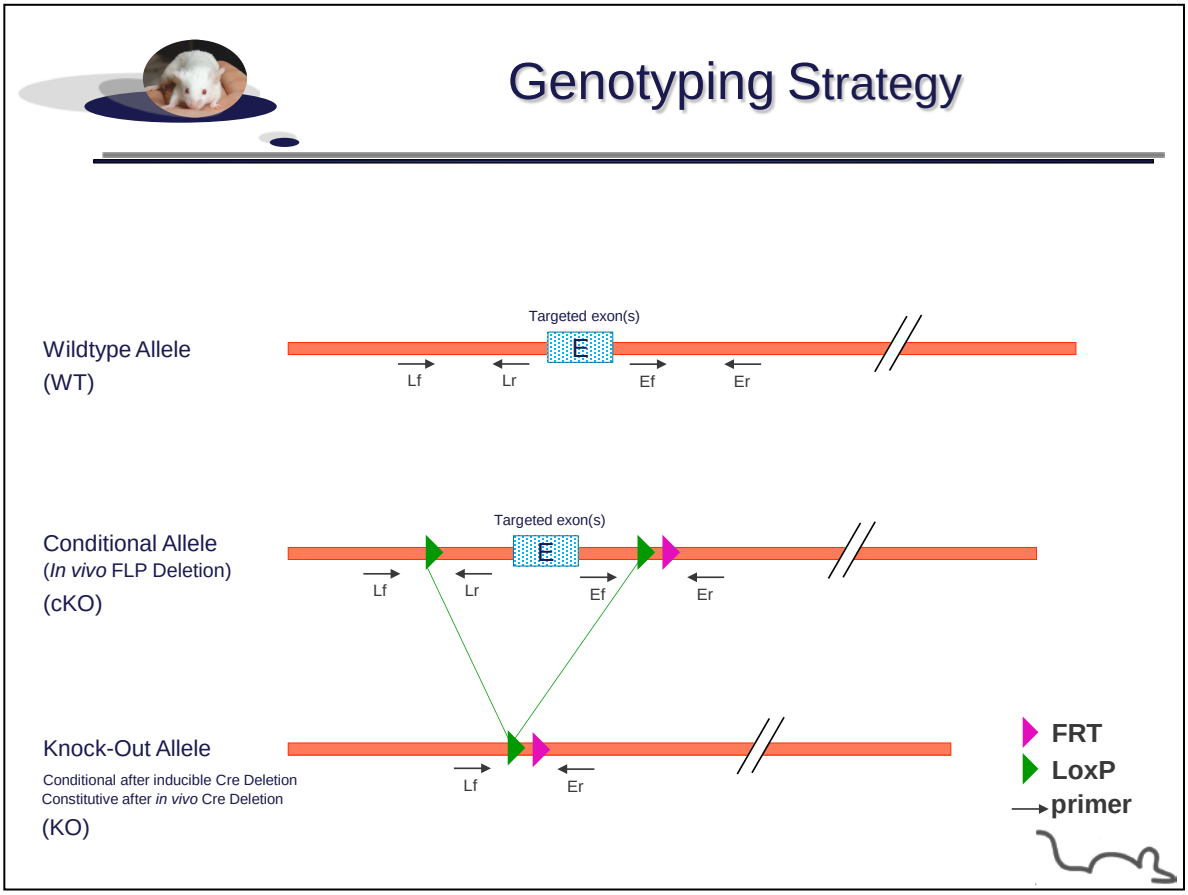


4. Genotyping protocol and data on conditional and knock-out animals

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
Lf	833	AACATGGAAGGATGCACCTGTTCCC
Lr	835	GCCTTGAACTCATGAATTCCCAGCTTC
Ef	836	CCTTCCCTCTCATTGTCATGGAGAC
Er	838	AGAGAATCTCTCGCCAGTGTCATGG

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	833-835	Lf / Lr	409	---	344
Excision of the selection marker	836-838	Ef / Er	332	---	222
Total Excision (excision of the floxed exon(s), i.e. knock out)	833-838	Lf / Er	1119*	437	945*

* 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.

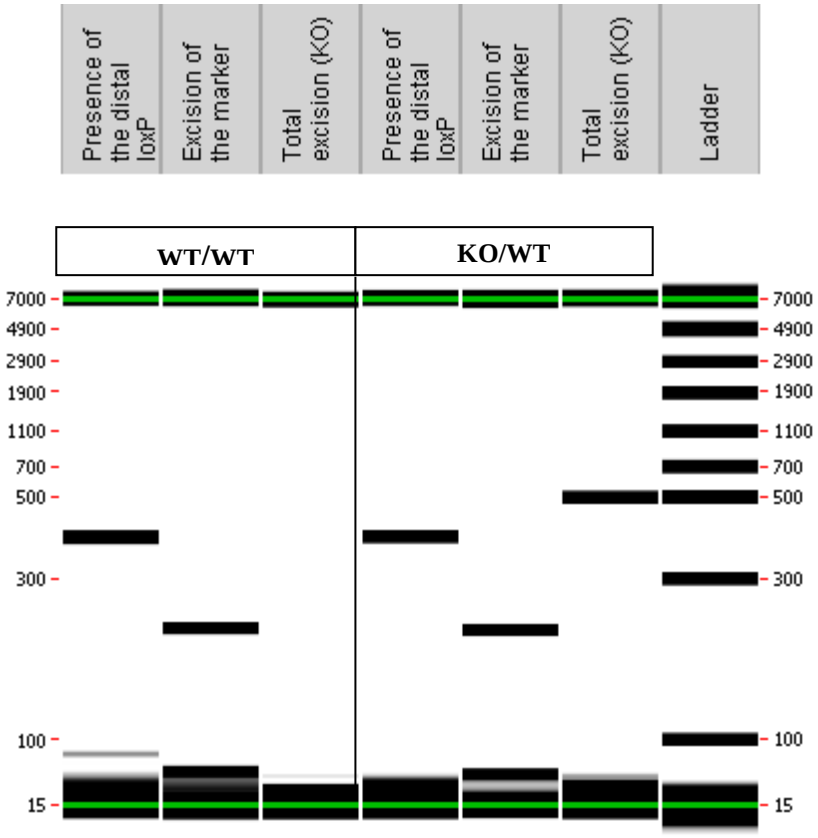
4.3. Picture of genotyping with various alleles

- 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.



Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.