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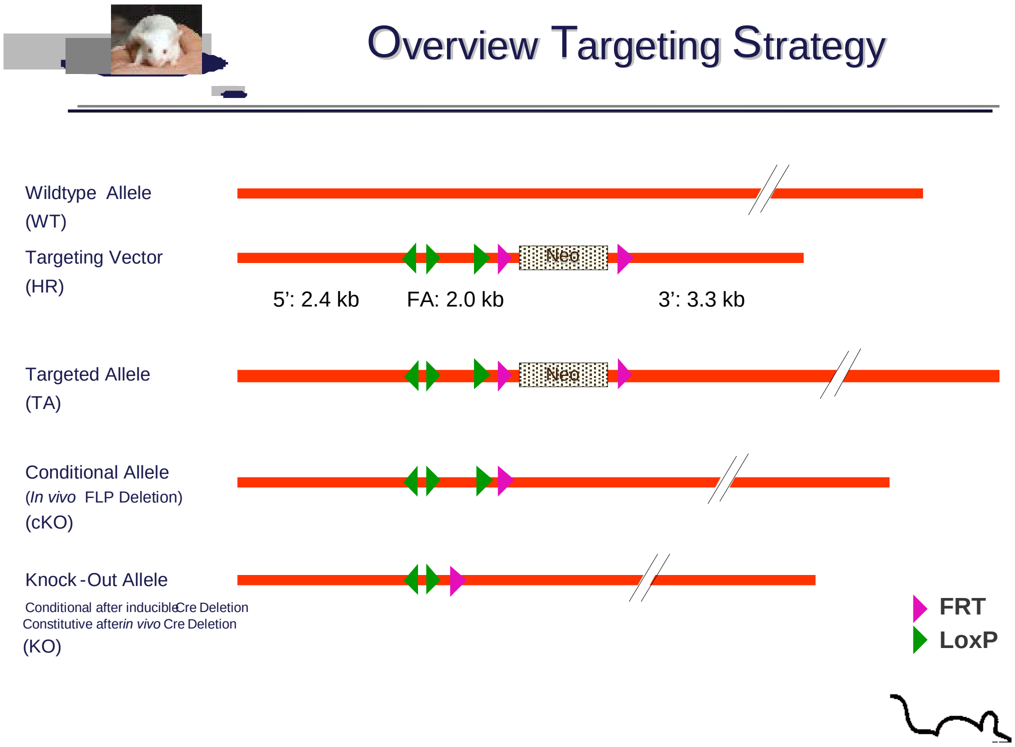
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

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:

Nr1h2 gene (also named LXRb) is a member of the nuclear receptor family. Additional information on this gene can be accessed at
http://www.informatics.jax.org/marker/MGI:1352463

1.2.1. Relevant informations

This section provides additional informations that can be useful for the comprehension of the strategy.

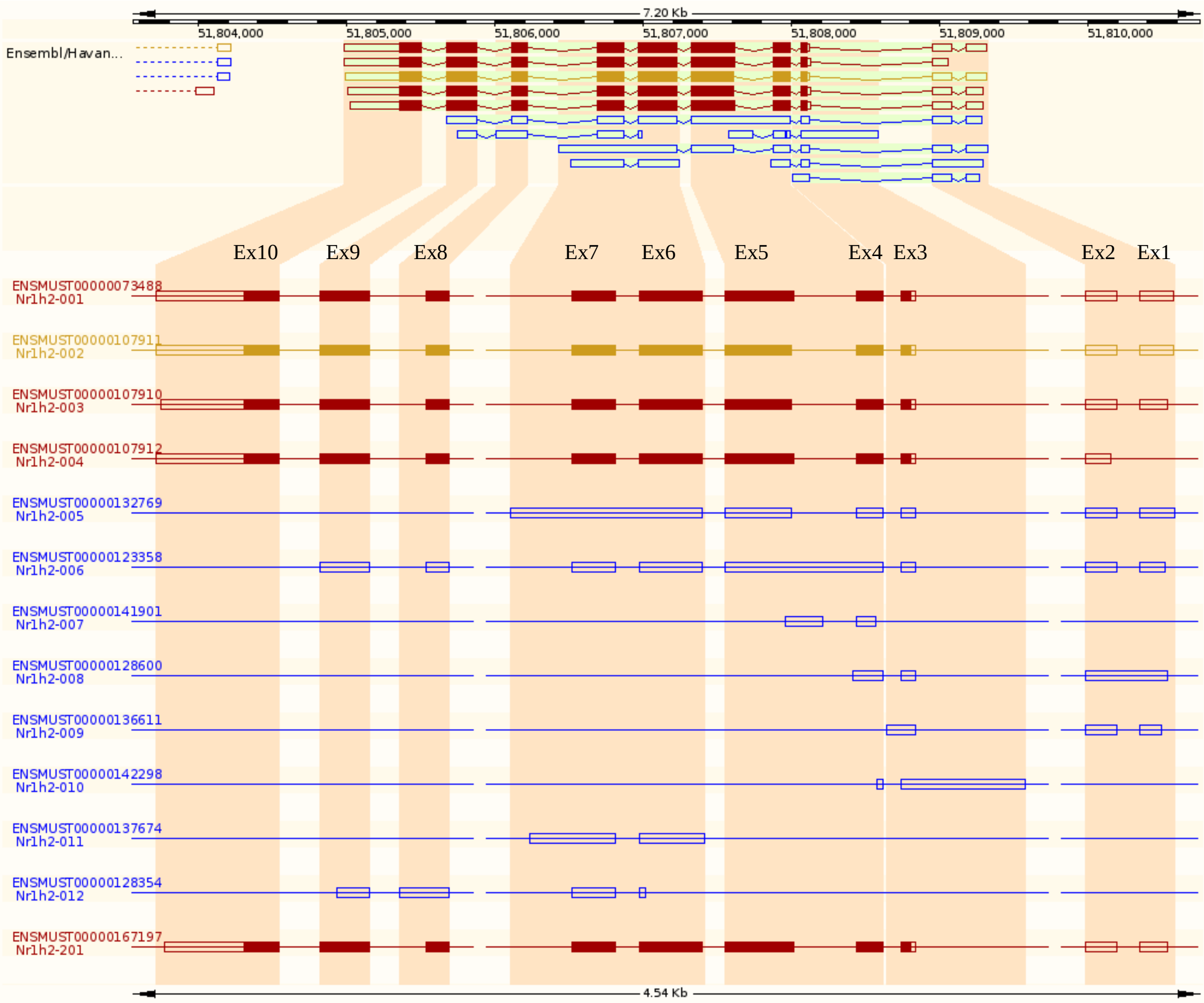
1.2.1.1. Know transcripts

Ensembl data (in May 2012) indicate that 13 splice variants are known for this gene (see below).

Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CCDS
Nr1h2-001	ENSMUST00000073488	1999	ENSMUSP00000073188	446	Protein coding[1]	CCDS21211
Nr1h2-002	ENSMUST00000107911	1988	ENSMUSP00000103544	443	Protein coding[1]	-
Nr1h2-003	ENSMUST00000107910	1948	ENSMUSP00000103543	443	Protein coding[1]	-
Nr1h2-004	ENSMUST00000107912	1839	ENSMUSP00000103545	446	Protein coding[1]	CCDS21211
Nr1h2-201	ENSMUST00000167197	1944	ENSMUSP00000126788	446	Protein coding[1]	CCDS21211
Nr1h2-005	ENSMUST00000132769	1530	No protein product	-	Retained intron[3]	-
Nr1h2-006	ENSMUST00000123358	1704	No protein product	-	Retained intron[3]	-
Nr1h2-007	ENSMUST00000141901	237	No protein product	-	Retained intron[3]	-
Nr1h2-008	ENSMUST00000128600	530	No protein product	-	Retained intron[3]	-
Nr1h2-009	ENSMUST00000136611	342	No protein product	-	Retained intron[3]	-
Nr1h2-010	ENSMUST00000142298	544	No protein product	-	Retained intron[3]	-
Nr1h2-011	ENSMUST00000137674	630	No protein product	-	Retained intron[3]	-
Nr1h2-012	ENSMUST00000128354	547	No protein product	-	Retained intron[3]	-

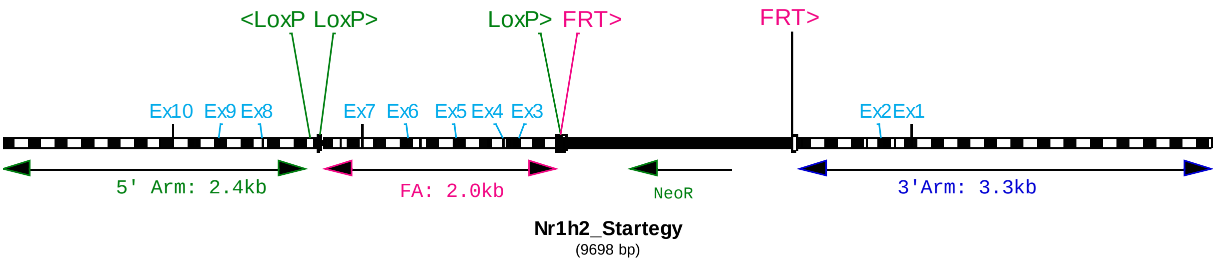
[1] A protein coding transcript is a spiced mRNA that leads to a protein product.
[2] Noncoding transcript containing intronic sequence.

1.2.1.2. Splice variants and exons nomenclature



Strategy is based on ENSMUST00000073488.

1.2.2.Strategy used to generate the conditional knock out model



2. Sequence of the targeted mouse line

Sequence of the targeted allele is presented.

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. 5' homology arm (4.4 kb)

CCGCCACAAGATACGCCGCCGTGACCCCTGAACCCTCTGCGCGCAGGCCCAGAGCTGCGGCGCGCCCGCTGGGTC
CCGCCCCTAGCCCGCTTGGGCGATCAAACCACGTCCCACTCTGGAGGACCCACAACCACGCCTCTCGCAAGTGGC
CCCGCCCCATGACGCTAACGGAACAAGGACACGCCCCCAGCATCCTGGCTCTTAGGGGTTTGGCCTGCGGTCCCC
GAGCGCCCACTGCAGGAGCGTTAGAAGACTGCCACAGTGGATGCTACCCTGATGCTTTGCTAGAAGCAAGCTTCT
TTTCTGTTTTCCTCAGAGTCCCTACACGCCCAGGTTCTTGTATAACTTCTGTTGCCCCACAAATCATTTCCTGAC
TTATGACACTCATGGCCAGATGCAGTCCTCAGCTTTTGAAAACCTGGCCCTAAGAAGTGTTCTTTTGGGCTGGTG
AGATGGCTCAGTGGGTAAGAGCACCCGACTGCTCTTCCAAAGGTCCGGAGTTCAAATCCCAGCAACCACATGGTG
GCTCACAACCACCCATAATGAGATCTGATGCCCTCTTCTGGTGTGTCTGAAGACAGCTACAGTGTACTTACATAT
AATAATAAAATAAATAAAATTAAAAAAAAAAAAAAAAAAAAAAAAAGAAGTGTTCTTTTGAGACCCTCCTCTTAAAG
TTCCTCTCCTTTTCCACTGGCCTCCATCCGCCTTAGTGTCTCAAACTATGCTGTATGAAGATAACCTGTCTTCGG
CGGAGCGACCCCTACCCCTGTGGCCTGAACCTAGACCAAGCGCAGTCACCTGGGCAGAGAGATGGCCCACCCTCT
CCACACTGGGCTCAGGAATGGCTTGTACTCAAACTTTATTTTTGTTTTTTTATTAAATAAAAGGGGCAGAAGAGA
GGGCCTGTGGTCTCCCAACTGTGGGGGGCCACCTGCCCTAACCTCTCTCCACTCAAGGTGCATGGTGTGGTAGGC
TGAGGTGTAAGCGTGGGTGGGAGGGCAGCTGGGAACGGTCAGAGGCTAGGACCTGTGACCCTCACCACTCTTGGA
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CACCTGCAAGCCTGCAAGAGCAGCCCCAGCCCAGCTTCACACCGGACCCTGGTCCCAGGGCACTCAAAGGGGAAG
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GGCCTCGCTAAACATGATCTTTCCTTGCCAGTGCCTGCCTGGCCTGGAGGGCCAACGGGGCAGAAACCCCAGGAC
CAAGTCAGTGCCTTCCACAGACCTGCACGGTGGAAGTCGTCCTTGCTGTAGGTGAAGTCCTTCAGGAACGTGATG
CATTCTGTCTCGTGGTTGTAGCGTCTGGCTGTTTCTAGCAACATGATCTGGAGGTGACAAGGTACAGGGCTCTTC
AGCTGCCCCAGAGTCAAATGTGCCAAGACCAGCAGGCTGCCACCCTTCTGCCAGGTAAGACATTCTTAACTTTCC
TTCCTTCAACTCTATCACCCACTGCCCCATCCAAGGCCACTAACCCCACATTACCGTGAGGCCCAGGCTAGAGGT
TTAATTCTTAGCATTCAGAACTCTGCA**CACAGTTAACTCTCAATACTTCATTATCCTTGATTTGGCTCCAATATC**
CATTCCTTTGGAGTCTTTGGGTCATTTTCCTGTTTAACAGCCCATCTGATATCCTCCGTTATTCTCTACCTCAGC
CCATTTCCCTTTTCTCCCTGGCTGGCTCT

2.3. Floxed fragment (2.0 kb)

ttaattaaataacttcgtatagcatacattatacgaagttatggccggccttattaagtttaaaccaccggtgcg
gccgctctagtataacttcgtataatgtatgctatacgaagttatcccgggctgcaggaattcgatatcaagctt
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TGCCTGTACCATGTTCAGCTCTGGTCTCGTCCTAGAGCCTAGTTATTACCTCAATGGTGGACGCCTTCAGGAGGG
CGATCTGGTCCTCCCGGCCCAACTGCAAGAACCCTGGCACCTGCTTGGCAAAGTCCACAATCTCCTGGACCGAGA
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GCTGCGCGGCAACTAACTGCTGGATCATCAGCTCCTGAGCCGCGGTCAGCTGGATGCCCTCTCCTTCCCCGGAGC

**CCTGGCTGCTTGCTTCCGAAGTGCCAGGGGAGGCCGCTGGCCGGCCTGAGCTGCTGGCTGCTGGCTCAGATGGGG
GTGGTGGCTGCTGCTGTTGCTGCTTCTGAATCCTTTTCTTCCGAATCTGCTCCTCAGAGAGCACGCCTGCGGGAA
AGCCGGCCTCAGAGGCGGGCCTCAGCCTCACTTACACCTCCCCTTGCAGGTGACCGGCTCTCCCTCTAGCCCCAC
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GACTTGGGGTGAGCAGATGCCATGAGAGATAGCGACAAAGAGAAACAGCAGCCGGGGAACGGGCCATGGGAGGTC
CCGAAGCGAGGGTGAGAGGACGGGGCTGCAACGCCAAGGGAAAGATCTGCAGGTCGGCGGGCTCCGGAGGACGAG
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CACCAGGCACGGGAGTGTCCAGAGAACTTGTGGGGGAAGACATAGTGGGTCACGAAGCAGCCTGCTGAACATAGG
GGAGCCAGCCAATTACAGGGAATAATAGGGGTGTCTCTTCTTCCAGTGCTCACGGACTCTTGAGACATGATCTCA
TACAGCTCAGGCTACCCGAACTATGTGGCTGAACAGCTTGAACCACCTCCAAAATCTTGGAACACACTACTCTAG
GCTGTCCTGGGATCTTCCGCACTCCTGCCTCGGCCTCTCAAGTGCTGCCATTACAAGACTGTGCCACACCTCAGT
GTTTTTAACTTCTATATTGAGCCCCCACTCCCAGCCCTAGTTCCTAATAAACcaccggtgataacttcgtataat
gtatgctatacgaagttat**

2.4. PGK-Neo region

**gcggccgggaagttcctattctctagaaagtataggaacttcgcggccaattctaccgggtaggggaggcgcttt
tcccaaggcagtctggagcatgcgctttagcagccccgctgggcacttggcgctacacaagtggcctctggcctc
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gattggagctacgggggtgggggtggggtgggattagataaatgcctgctctttactgaaggctctttactattg
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agtactgtggtttccaaatgtgtcagtttcatagcctgaagaacgagatcagcagcctctgttccacatacactt
cattctcagtattgtttgccaagttctaattccatcagaagctcgataccgtcgaggaagttcctattctctag
aaagtataggaacttcccgcggatccatcgacccctgcagg**

2.5. 3' homology arm (2.9 kb)

ATCACACACTTTAACATGCCCAACCCGGCCCACTGCTCCTTCCACCTCTCTACGAATCATGACCTCAGAGAGTTC
AACAACTCTATTCCTCTTCTGTGAGCACCAACTGTGCTCTTATCATGGGAGATCCTAACTTTGCCCCGCCTCTCC
CATCAGGGCGCAACCATTACCCTTCTACCATAGGCTCCTTCAAGGCTTTAACGCACCCCAATACAGACCCCAGAC
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CCTAGGCTCCGCCCCTCTCATTCCCCCAAAGCCAATCATAGGTCTTCACTGTGCCACGCCCTCCATGCCTCTGCC
CCTCCCAATCCCCCCCAACAGTGGTCTAGGCCCCGCCCCAAGAATCAAAAGCCTTTAATCACAGACTATCTACAG
CAGGCCTTCTGCCCATCTGTGTCCCGCCCCCGCCCACCAACCATAGGCTTTCCCTACGCCCCGCCCCGTAGAGTC

ACGCCCACCTTGTTCCAGGCCCCGCCCCTCTCCGTGCACCAGGGGGAGGCGGGGCTCATGACTGCACCGGCGATC
GGGAGGGGGGTCGCAGGGGGTCTCCGACTCTGTTGCCCTGGAAACGGCCGTGGGAGGCGAGAGCTGCGAGGAAAG
TGAGGGAAGGATGCGCGCGCTAAGCGGGACCCACGGCGATCCGCGTACCGGGCTCAGTCCCACCTGCCCGGCCGC
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GCCTCGGCCGGAAGTGGCACACCGGAAGTTTATTTCTTCTGGTAGTTCCCCTCTGGGACTCAGAAAAAAGTTCCG
GAAAGTTCTTTCGTTTCTTTTCGCAGGTGGCTAACGAAGCAGGTTTTAACCTTTTTGAGTAGTTTAAAGTTCTCT
ACTTTTGTAGCGGTGCTGCATGGCTGCTTTTTAAAAAAATTTTTGTCTTGATTTTGAGACAGGATCTCTTATTTC
AAGGGTGGCCTTAAACTTCTGAGCCTGTTGCCTTTACCTCCCCATTATTGCGGTAGCAGCTCCTCTTCCAGCCCT
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CTCTCCCATAAAAGTAATAATACCAGCTTTTTCACAAAAGATTGAAATACTTGTGTGAATCTCAGATGGAAAACA
TTCAGAGATTGTTTCTCCGCACTCCCCACTTATGGACAGTTGGGAATCTTTATTAAGCACAAATTCTTGCTTTTA
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GCCACTGTTGTTCTTGTTGATTTGTTTGTTTTGCAAGGCAGGGTTTCTTTGTGTAGCCATGGCTGTCCTAGAACT
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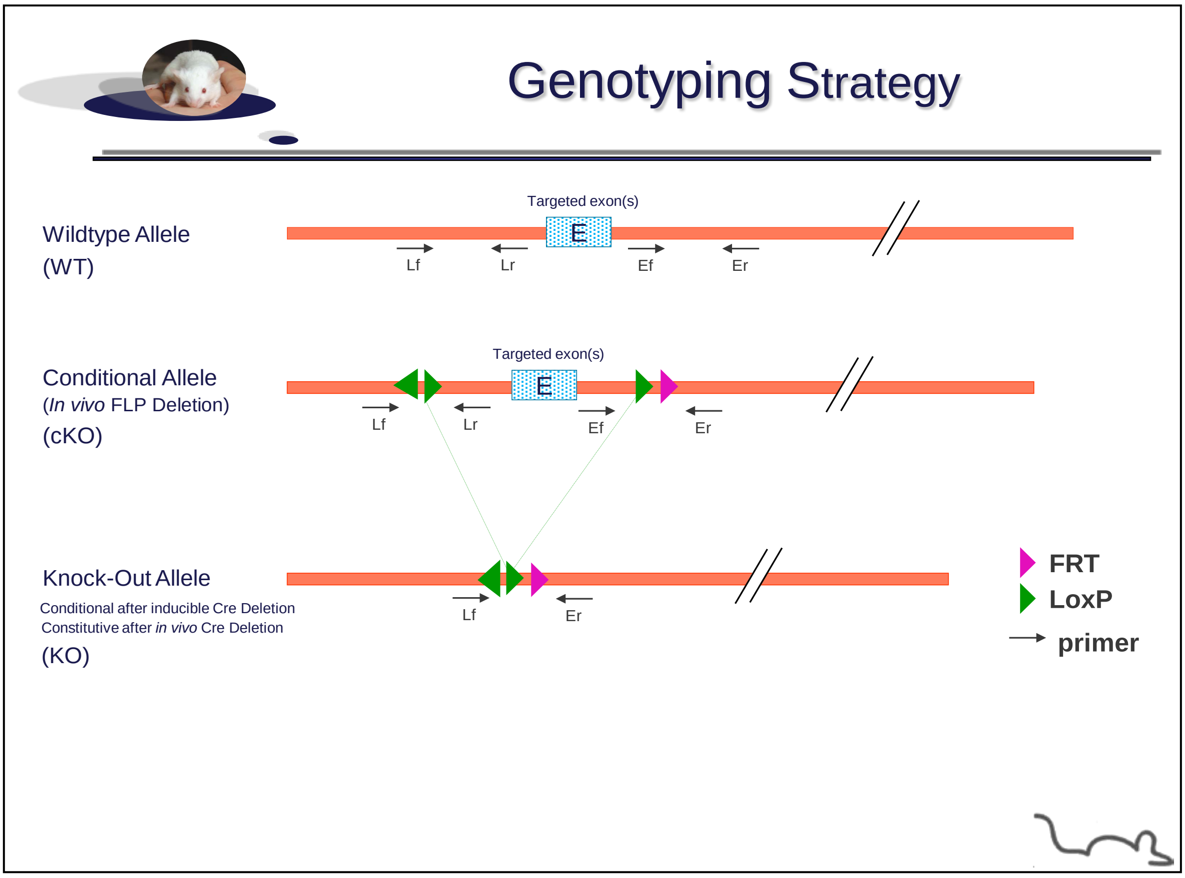
AGAACTCTATGTACACCAGGTTGGCTTCCAGGTCAGAGATAGATCCTCCTGCCTCTGCCTCATGAGTACTGGGAT
TAAAAGTGCCATGCTATGATAATCTAACAAATTCTTACAAAGTTGAGGCTGGAGCCTACGTTACCTCAGTCATGC
AACAAAATTGGTCCTGAGACCCAGCCACTAACCCATGCTTTTTTCAGAAATAAGTGATAGGTCTCAAAACAAACA
AACAAAAGCCAAAGCCTGGACGGATAGCTGAGGTGCATATTAATATGTGGACTCGGCGCCAGACCCTCTCAGTAT
CACCAATACCTGTTAGTGTATAGTTACCCCTACCCTCAGCCTTTTCCCCAAGATTCTCATTCCCACATAATCCAG
TTGTTTTGGCTACATGTCCCTTAGCTCTCTTGGTCCTCTTTGTGTCCTGGTTCCCCCATCCTCCCTCTCTCACCC
CTGACCCCATGGCCCGGTTTATTCTGTTGGCCCAGTTCAGTCTGGACTCTTACAAATGCCTCCGGATGGTCTCTT
CCCTATATCTACAATAAACCTCCTCTGCCATAACTGGGACCTGTCATGCGCTCACTGTTTTTCATTAAGTATGTA
TTTATACACTTATATATGTATGTGTGTTTATGTTTACATTTAATATACATTATATTAGTAAATTGTCACATACTG
TTTGAATTCACTGAGATGCTGGCCCTGGAAGCAGGGTTGCTCCCCACTCCAAACCTAAACATATGACTTAGTGTC
CTCTAGGAC

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

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

3.1. 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	1189	TCTAGGCTGTCCTGGGATCTTCC
Er	1191	CTCCCATGATAAGAGCACAGTTGGTG
Lf	1185	ACTAACCCCACATTACCGTGAGGC
Lr	1188	AGGTGCCAGGGTTCTTGCAGTTG

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 loxP	2095-2101	Lf / Lr	557	---	400
Excision of the selection marker	2097-2098	Ef / Er	367	---	258
Excision of the floxed exon(s), i.e. knock out	2095-2098	Lf / Er	2453*	529	2186*

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

3.2. PCR protocol

This section describes the composition of the mix and cycling conditions used for genotyping.

Reagents:	Volume:
- FastStart PCR Master (Roche)	7.5µl
- DNA (50ng/µl)	1.5µl
- 5' primer (100 µM)	0.06µl
- 3' primer (100 µM)	0.06µl
- Sterile H_2O	up to 15 µl

Cycling conditions:

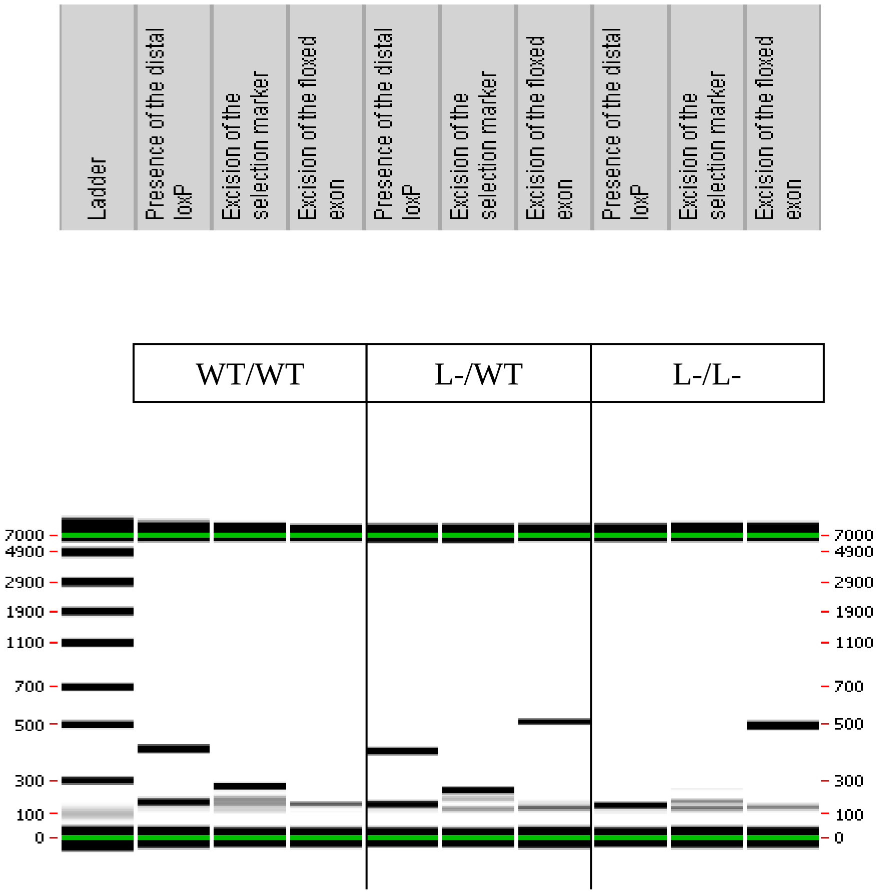
Temp	Time	#Cycles
95°C	4 min	1
94°C	30 s	
62°C	30 s	34
72°C	1 min	
72°C	7 min	1
20°C	5 min	1

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

3.3. 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.

<u>Representative genotyping picture</u>



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