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This protocol has been prepared by Claudia Caradec, Engineer

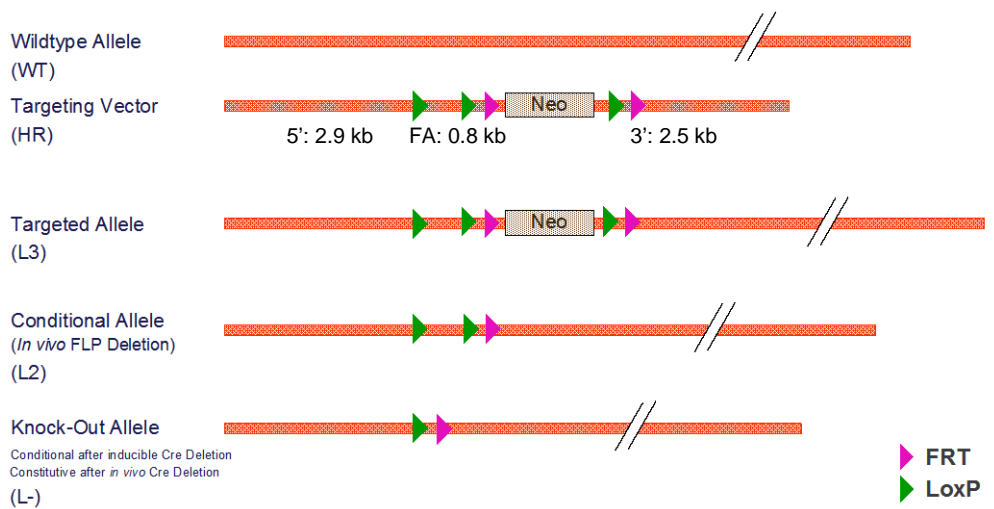
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview



## Overview Targeting Strategy



Legend:

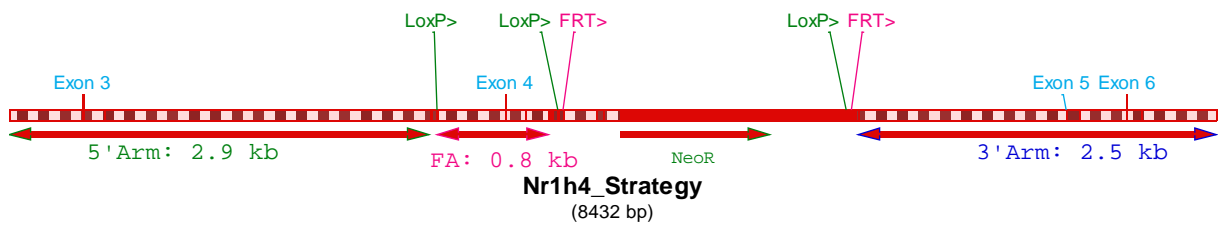
5': 5' homology arm;    FA: floxed fragment;    3': 3' homology arm  
 This schematic representation is not on scale

**1.2. Strategy chosen: flox of exon 4**

Nr1h4 gene (also named FXR alpha) 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=45357>

**Strategy used to generate the conditional knock out model**



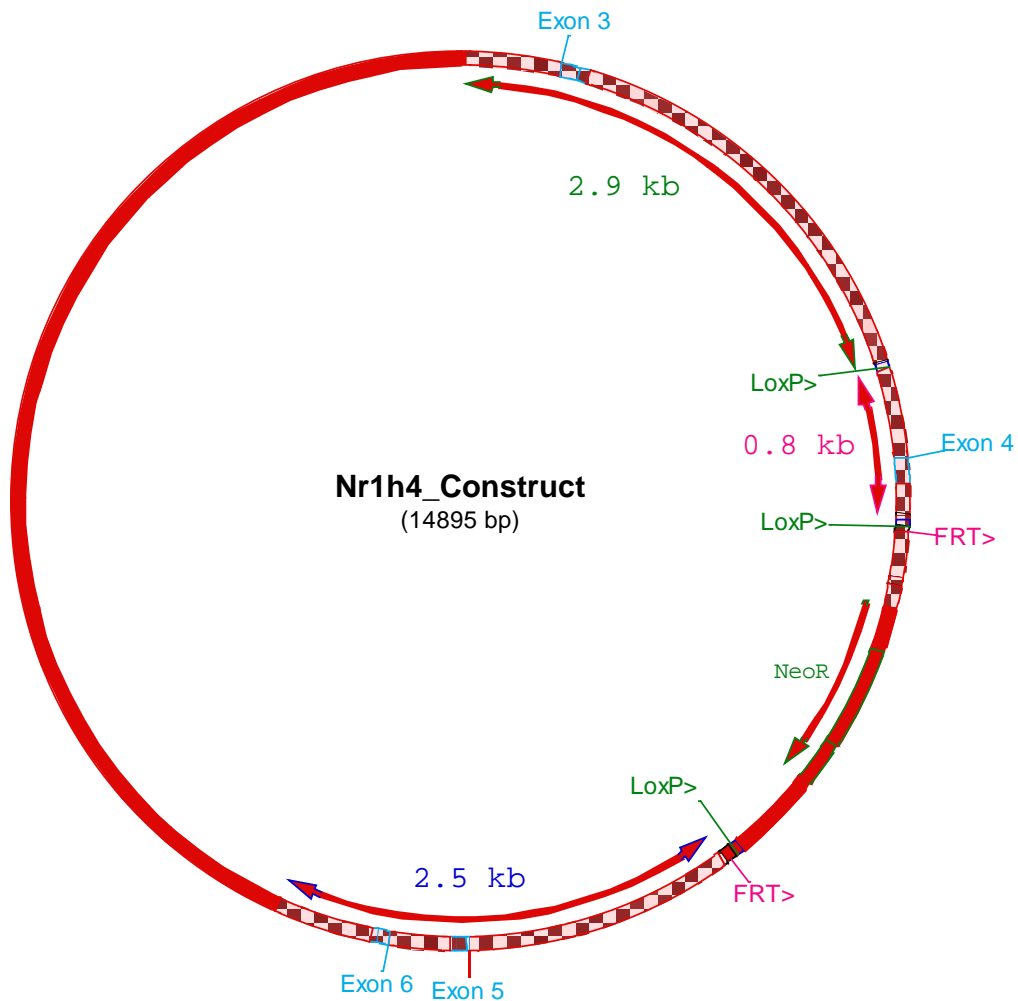
2. Construct used for homologous recombination in ES cells: Nr1h4 project

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 (on knock-out animals) are indicated in bolds.

2.2. Map of targeting vector plasmid



**2.3. 5' homology arm (2.9 kb)**

GCTCTTGATTCCAGTGCCAAATCTCTAACCCCTCACTTGCTATATTAACAGTTCCCTGCATGCGTCTGCCTCAG  
 CACAGCGTCCAACAGTATCCACACGAACCCTACTCTTTCTTGTGGTTATTTGCATGCATTTGCTTTTGGTCA  
 ACCTTTAAACTCTGCGAGGGCAGGGACGGATTGTTTGCCTTTATAGCGTCAGCACATAGCACTTACTGGCCATA  
 ATTGTCCCCCAATAAAGATAAAATGGAAGCAATATATTCTTTAAAAAACAACCTGATTTTTTCCCCTGAG  
 AGTCCCTAATTTCCACTTGTTATTGTTCTAGAGAGATAAAATTTAAATAGCTCCCTTTTCTATAACAGCGCGCG  
 TGAAATGTCCATTGCTACATCCCCTTGGCTCAAGGCTCATCCGGGTGATGCCAGCGGTTCTTGAGAACTTTTTG  
 ACTCCACATATATATCCTGAAAACCTTGTGGGGACCTTCTTACAGTTTCCAATGTTTCTCCCACAGGTTTCTTCC  
 GAAGAAGCATTACCAAGAACGCCGTGTACAAGTGTAAAGAACGGGGGCAACTGCGTGATGGACATGTACATGCGCA  
 GGAAGTGCCAGGAGTGCCGGCTAAGGAAGTGCAAAGAGATGGGGATGTTGGCTGAATGTATGTATACAGGTATCC  
 ACCTCAAGCAATTAACCTCCACAAAACCTCCTAAGGAGGCAGGCGCCAGGTAACCTCAGACCACCCCATGTTAAG  
 GACAGGATCTGGCGAGCTAGCTGTCTCAGTTCTTGAACGGGGTGCTGGACCGCTTACTTCTTAAACTGCCGTCA  
 GAAGAGCAACTTATTTCTCTAAAAGAAATGACGGATTTGTTTGGGGATTGTGTTTCCGTTGTTGCGAGGACTTT  
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 CATCTAAATTTGATTTTAACTACTCCTTATTTATTGCTTAATTACCATAAAAAGTCATAGCGGTGGACAGTAA  
 ATGCCTGTGAATTATCTAAATAGGGTTTTAAACTAAGTCATTTTTATTAAGAATTCGTCTCCCCTCAGTGCAGCT  
 GTGAGCCAATTTGTATAGGTTTACACAGGAAATTAACGACCCTGAGTTTTAATATAATTTTGTCTTTCGATCTGA  
 TAAGGGGACACTGTATTAACACTGGCCCTATGAGGAACCACCGCTTCATCAGAAAATTAGAATGCATGTTAGTA  
 TAACATTATTAATTTTTTATCCTACAGCCCGCTCAGATTTAGAGATATGCCTTGTTCCTCCTCCAAACAAACGGA  
 CAAACAAATGTCACCAAGGGGATGACAACATTCAGGGAGGGAATGCCACGTGAAATAAAAAGCCCATGGAAGGTGA  
 CCCTAGCTCCATTGTTCTGAACTGTGTGGGTGACTGGTACGCTCCAGAGAAACAATGTGTTTCAAGAGGGAGAA  
 AAATCGGCCTGTATAAATAAATAATCCATCCCTGGATGGATGAAAGTTTTATACAGGCTGATCACAATCTCATT  
 TAGCAAACATGAAATAAGAACCTGACCGGGGCAGCGGCTGCAACCCAATCTTGCAGCGGTAATCCTTTGACACTG  
 GCGGTTTTCATGTTGCTCATGACTTAAATATGAACTTGTTCATCTGAGCTCACTTGGTAAGAGACCCCTCCCACA  
 GAAGTCAGTAGCTTTATTTATAAGCACAAACATCTTCCCACAGGGACGTAGCTGTTTCATAAGTCTTTTGTACA  
 GTGTACCTCCCGGAGCCTTGTTCACCATCGCTGAGAGAAGAACAAGAAATCCAAAATACATAGAAGGCAGCAG  
 GTCTTGTACTTGTTCAGCACAAAGCATAATCAATGATAACTACACGCTCGCTAAAAAAGATTTCAGACACTTTG  
 TGTTTGTGGGCTTTTATCAATATCTAAAAAATACCCGAGAGAATTATTAAGAGGACACATTTTCATTTCCGGTCC  
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 TAGCACAAAAGCTTCCAGGTCTCATGGCCTGGAAGAAAAGGACCCGAGGAAGAAGCTGGGGTCCACAGTCCCA  
 TCTGAGGGCATCCACAGATCTGGGCCCTCCTCAGGGCTCCCAACTCTCGATAGCACAAATAGAACAACA  
 ATAGGGCTTTTGTCTTTAACTTGGAAACCAAGCAATGGCTGGCAATCCTTAAGTATTGTAACCTTTGATTTTTTA  
 AATAATTTGCATAGATTAGTGAGTGGGGCTTATTTTTTTTTCTGAAAGGAGTTCACGTTTGTCTTACTCAACAAG  
 TTTTAATATTATAAACTGAATATACTTTTTTATTGAACAGTATTAATATAGGTGAAAAACACTTGGATGTTTTAAA  
 AGTAATTGTATGTTCTGAAGGCAGCTTCCCTGGTCTCCTAGGGAAACAGGGAGTGTGTCAATTTGCGATACCATTGA  
 CTAACATCTTCTGCTTTGTATGATCCTTTAGCATCACAAATTATTTATATATAAAGCCAGTGTTTTTTTTTAA  
 TACCTAATCACATCTATGCAAACCTCTGTGGCAAATATTTGTGTTCCACAAATGAAGAAGCACACTCACAGATGT  
 CAAATGCCTCACTCAAAATCAAATCTTAAAAAAGAAAATTAGCTTTTTTATTTTTCTTAAAGATTTATTTTTTT  
 GTCCAAGTGTACGTGTTTGTGTGGATGTATATCATGTATACAGGATAACCAAGGAGGCCAGGAGAAGGCATGGGA  
 CCCCTAGAAGTGAATCACAGGTGGTTGTGCCCCCCCCCCCCACAGGTGGTGTGGGTGCAACCAATCTCTCC

**2.4. Floxed fragment (0.8 kb)**

ggccgggatggccataacttcgtataatgtatgctatacgaagttatCGCAGCCTTCTGCAAGATCTGCAACTAC  
 TCTTAACCACAAAGCCATTCTCCATCTGGCCTTCTCCTTTTAAATGCCAGACGAGCCAGAATTAGCTCTGCTTA  
 TCACCTGAGCTGACCACCTAACAGCATCTTCAAGGACCATAACCCAATAGGATTTTTTAAAATTAGATATTTTC  
 TTTATTTACACTTTAAATGTTATCCCCTTCTTAGTTTTCCCTCTGAACACCCCCCTATTCTCCCCCCCCCCCCCT  
 GCTACCAACCCACCCACTCCTGCTTCTTAGCCCTGGCATTCCCCTATACTGGGGCATAGAGCCTTTCAGAGACC  
 AAGGGCCTCTCTCCACTGATGAAGGACTAAGCCATCCTCTGCTACACATGCAGCTGGTGCCGTGAATCCCACC  
 ATGATGTGTTTTCTTTGGTTGGGGTTTTATCCTTATTTTTCTTTCTTTCCCCCAACCCCCAACCCCCCCCC  
 CCAAGGTTTTGTTAACTGAAATCCAGTGTAAATTTAAACGGCTAAGGAAAAATGTGAAGCAGCACGCTGATCAGA  
 CAGCTAATGAGGACGACAGCGAAGGGCGTGACTTGCACAAGTGACCTCCACAACCAAGTTTTGAGGGTAACAG  
 TGCGAGGGCGAGGGCGGGGGGGGGGGGGGGGGGGGGGCAAGCTGGTGTGAATGGGCTTCTGGTTGAACCTG  
 TCAGTCTGGCTGGGAGGTTGTCTGAGTAGAGCCTTGCCATGGCCAAATTTTCATCTCAGTTGGCTTTGGAACAG  
 AGTCTTGGCCTGTGTGGCCATCGCGGACTAATGGCCataacttcgtataatgtatgctatacgaagttat



**2.5. PGK-Neo region**

gaagttcctattctctagaaagtataggaacttcgctagctcataaaaaatttatttggctttcaggaaaaattttt  
 ctgtataatagattcataaaatttgagagaggaggcgccgaattcctgcaggattcgagggccctgcaggtca  
 attctaccgggtaggggagggcgcttttcccaaggcagctctggagcatgcgcttagcagccccgctgggcacttg  
 gcgctacacaagtggcctctggcctcgcacacattccacatccaccggtaggcgccaaccggctccgttctttgg  
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 gacgtgacaaatggaagtagcacgtctcactagtctcgtgcagatggacagcaccgctgagcaatggaagcgggt  
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 cgggggcgggctcaggggcgggttcaggggcggggcgggcgcaaggctcctattgtgagcgcctcacaatcccggc  
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 ttctctattctctagaaagtataggaacttcaaggctcctcgcctctgtgtccgcttgagctggccagctaggcc

**2.6. 3' homology arm (2.5 kb)**

CCTGCCAGAGGGAACAATGTAATCTTCACGATGGAGTTTTTCACTTGAGGTTTTTTGGTTTTTTGTTTTGTTTTT  
 GTTTTTTGTTTTTCTGAGACGGGATCACAATGTGCGGCCATGTCTAGCTTAGAACACATTATTTAGACCAGAATA  
 GCCTCAAAGTTACAACGATCCCCCTGCTTCTGCCTTCCAAGTGCTGGGACTTGCCTCCAGAATTTCTGGAATTA  
 AGCATAACAGGGCCATGCCTGGTCTCCACGCAAGTTTTTGATGGCTATATCCCAATTTCCCACTCCCTCTCTTGCC  
 CTTAAGAAGGAAAGTTCCAACCTCAGGAAGTCTGGACCCTGCAGAGGTTGTGGATTGGTCCATGTCAACTAGGAAT  
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 ACTTCCCTCCCCTCTTAGGTTTTTAATCTTATTGTTTTGTTTTGATTTTCGGGAAAAACAAATCTTATGAAAAAGAATC  
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 AAAAAAATCCTTCGTCATTAGTTTTGTTTTAGATCAGTGCACATACTGGAATATGCCTTTGACCGCCCTCCCTAA  
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 AAGTGATTGTTTTCTGAGACTTCAAGTTATATGTCCAAACTTTTTATCAGCATTAACAATTTGAAAAGGAAGTCT  
 CAGGCTATCTTGTAAATGGTATTTTCCAATATGACCTGTGTCTTAAAAATTCATTATTTCAAGTCAGGTT  
 TTTGTCCTCTAACAAATTTTATGTTGGGTTATTTCTTTTCTGCTGCTATTTTATTAATTAATTTACTTTTTCTTAAAT  
 TAGTTAAAAGAAGAATTTAGTGCAGAAGAAAAATTTCTCATATTAACAGAAATGGCAAAATGGCAACAGTCATGTACAGAT  
 CTCGTAGAATTCACAAAAAGCTTCCAGGTATTTTTTTTTTAAATAATAAAAAATTAATGTTCTTGAAATATGTAAAG





**Molecular Biology Data for Mouse Trap**  
**Nr1h4 conditional knock out model**  
- ICS references K97/DG56/EUMO20

gtgcacgagtggttacatcgaactggatctcaacagcggtaagatccttgagagtttgcgccccgaagaacggtt  
ttccaatgatgagcacttttaagttctgctatgtggcgcggtattatcccgtgttgacgcgccccgaagagcaac  
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gcaaactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatggagggcggataaag  
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cgttatcccctgattctgtggataaccgattaccgcctttgagtgagctgataccgctcgcgcgagccgaacga  
ccgagcgcagcagtgagtgagcaggaagcgggaagagcgcctgatgaggatTTTTCTCCTTACGCATCTGTGCG  
gtatttcacaccgcatatgggtgactctcagtacaatctgctctgatgcccagatagtttaagccagtatacactcc  
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ttcatccgcgtccagctcgttgagtttctccagaagcgttaatgtctggcttctgataaagcggggccatgttaag  
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gaaacgagagaggatgctcacgatacgggttactgatgatgaacatgcccggttactggaaacgttgtgagggtaa  
acaactggcggatggatgccccgggaccagagaaaaatcactcaggggtcaatgccagcgttctgttaatacaga  
tgtaggtgttccacagggtagccagcagcatcctgcatgagatccggaaacataatgggtgcagggcgctgactt  
ccgcgtttccagactttacgaaacacggaaaccgaagaccattcatgttgggtcaggtcgcagacgttttgca  
gcagcagtcgcttccagttcgcctcgcgtatcgggtgattcattctgctaaccagtaaggcaaccccgcagcctag  
ccgggtcctcaacgacaggagcagcatcatgccccgctggccaggaccaacgctgcccagatgcgcccgcgt  
gcccgtgctggagatggcggacgcgatggatattgttctgccaagtcagcgtttaaacttaattaagtgcagcggcc  
ggcctcgaggcc



3. ES cell lines targeted:

3.1. ES cell lines targeted

The targeting vector was electroporated in P1 ES cells [MCI-129Sv/Pas background]

Number of clones screened: 372

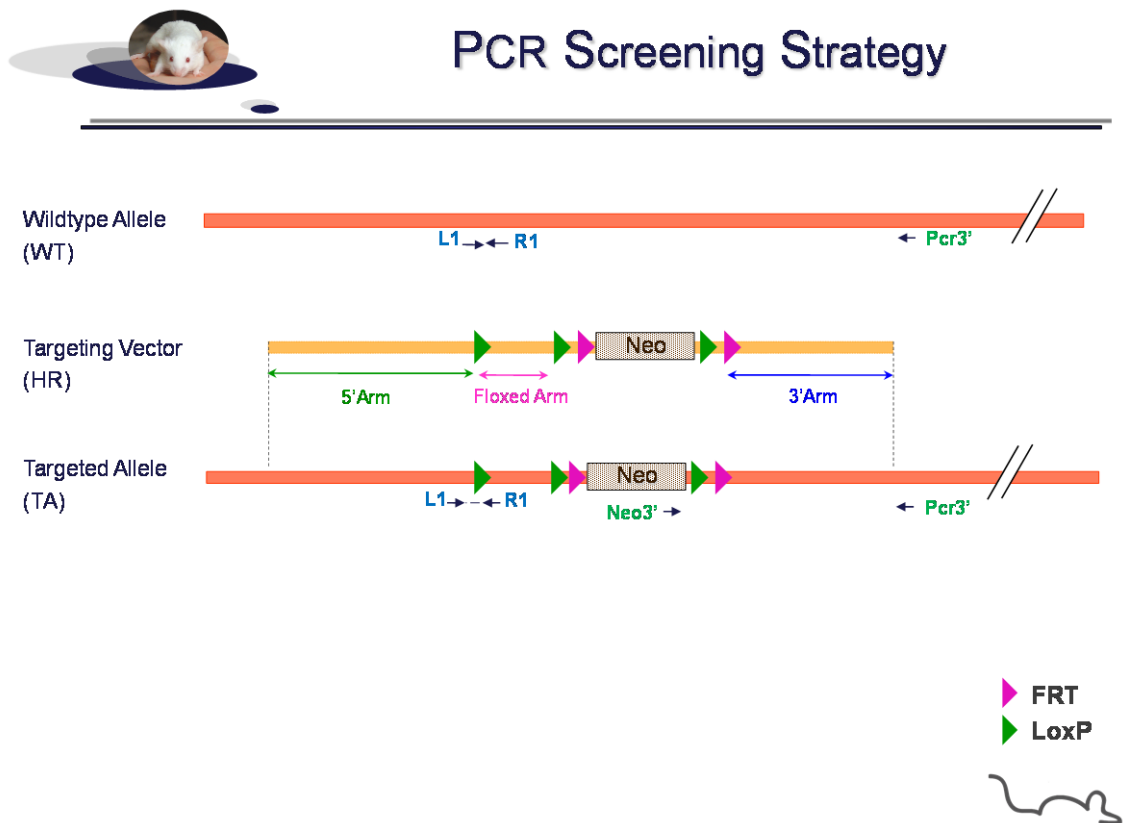
Number of positives: 1

Reference of clone used to generate the mouse line:

- clone **K97P1-171**

3.1. PCR data on positive clone

3.1.1. PCR screening strategy



Primers used for PCR validation of ES clone

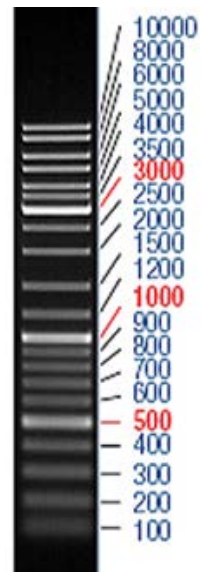
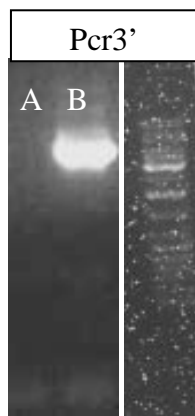
PCR	Primer Name	Primer sequences	PCR product size
LoxP	L1	TATACAGGATACCCAAGGAGGCCAG	0.218 kb WT 0.274 kb TA
	R1	AGCAGAGCTAATTCTGGCTCGTCTG	
3' external	Pcr3'	ACTACCCTTCTTGAAGGGAACAGCC	3.7kb en TA
	Neo3'	AGGGGCTCGCGCCAGCCGAACTGTT	

### 3.1.2. Picture of PCR

PCR: 3'arm validation

ladder

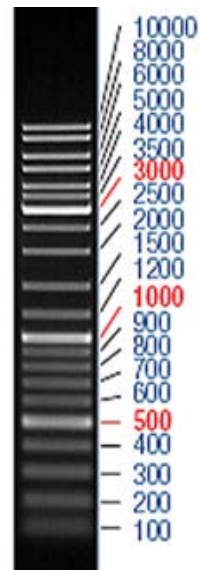
**A:** negative clone  
**B:** clone **K97P1-171**



PCR: LoxP validation

ladder

**A:** negative clone  
**B:** clone **K97P1-171**

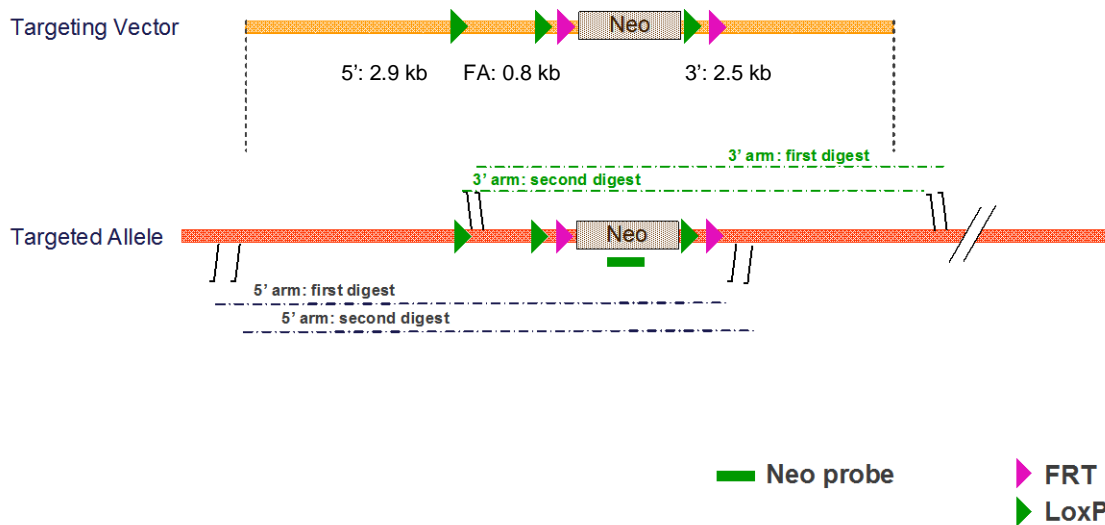


### 3.2. Southern data on positive clone

#### 3.2.1. Neo Southern strategy



## Southern Screening Strategy



Digestions used to validate the 5' and 3' insertion

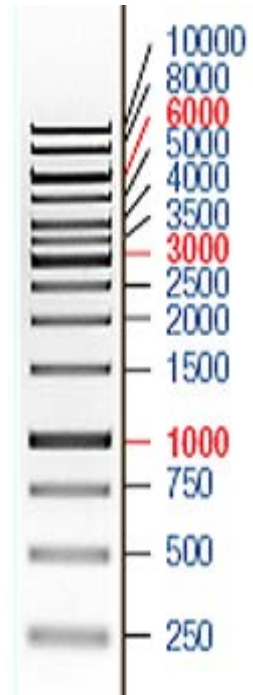
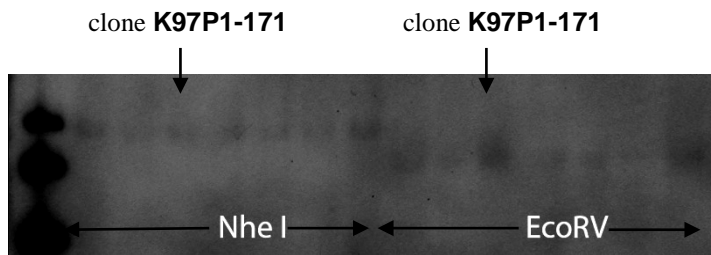
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	EcoRV	/	8.5
	3' arm first digest	Nhe I	/	10.2

Two different digests are used to validate correct HR event. One digest validate the 5' insertion, one other digest validate the 3' insertion

**3.2.2. Picture of Neo Southern**

Neo southern blot: 5' and 3' arm validation

ladder



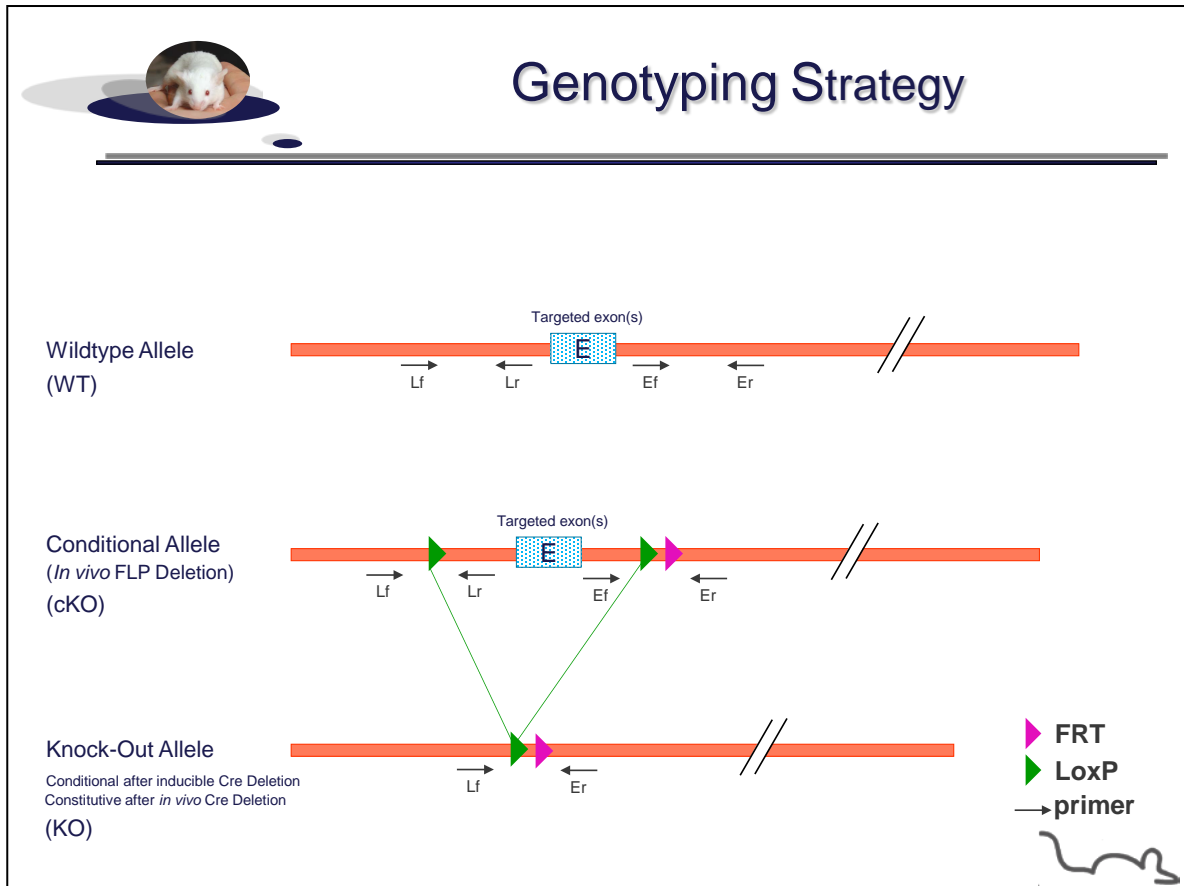
4. Data on conditional and knock-out animals

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

Primers	Sequence
Lf	GAAGCACACTCACAGATGTCAAATGC
Lr	AGATGCTGTTAGGTGGTCAGCTCAG
Ef	AGGTTGTCTGAGTAGAGCCTTGGC
Er	ATGTGTTCTAAGCTAGACATGGCCG



**Molecular Biology Data for Mouse Trap**  
**Nr1h4 conditional knock out model**  
- ICS references K97/DG56/EUMO20

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	566-568	Lf / Lr	429	---	379
Excision of the selection marker	569-570	Ef / Er	280	---	200
Total Excision (excision of the floxed exon(s), i.e. knock out)	566-570	Lf / Er	1358*	500	1163*

\* 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

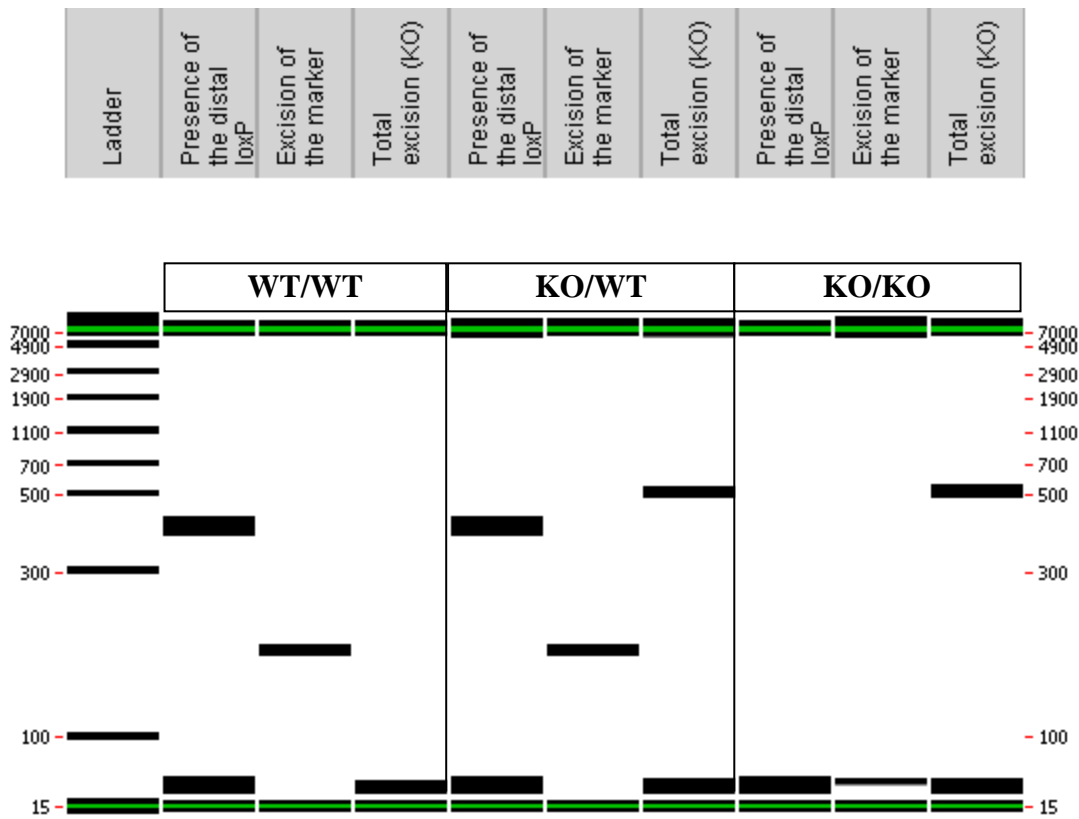
- Picture of genotyping with conditional knock-out (cKO) allele

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
<b>Number of pups obtained</b>	15	39	22	<b>76</b>
<b>Experimental Ratio</b>	20%	51%	29%	<b>100%</b>
<b>Theoretical Ratio</b>	25%	50%	25%	<b>100%</b>
<b>Theoretical Ratio if KO/KO are not viable</b>	33%	66%	0%	<b>100%</b>

The Nr1h4 knock-out homozygotes are viable.

#### Legend:

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