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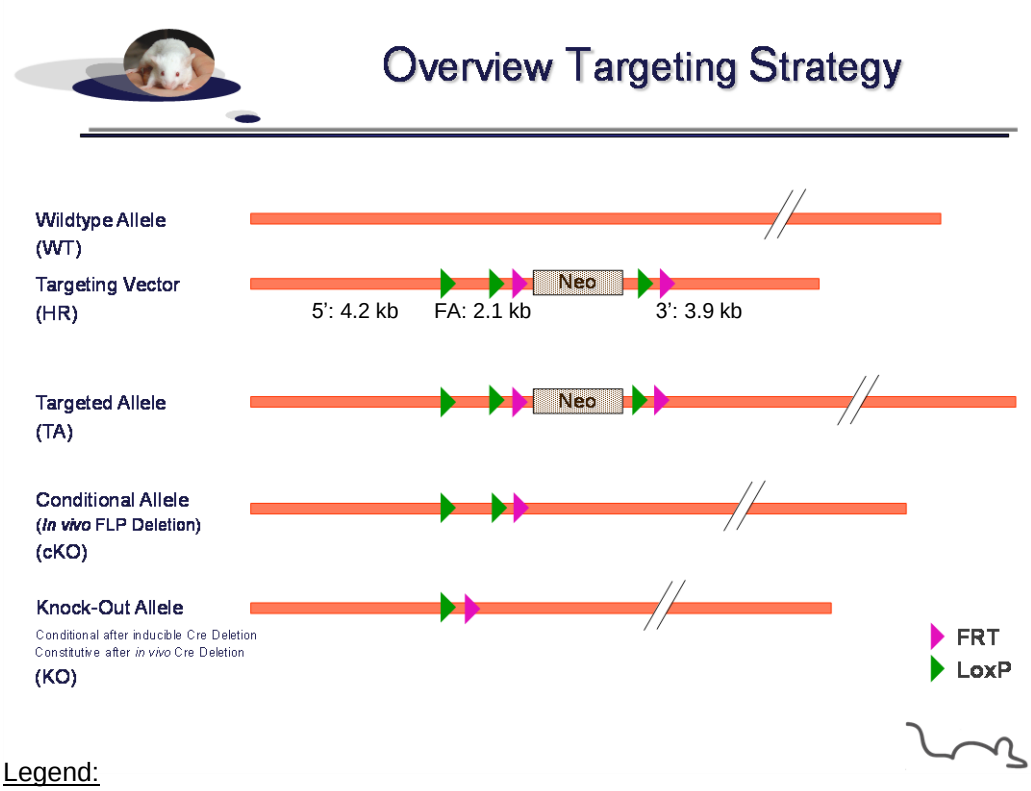
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview

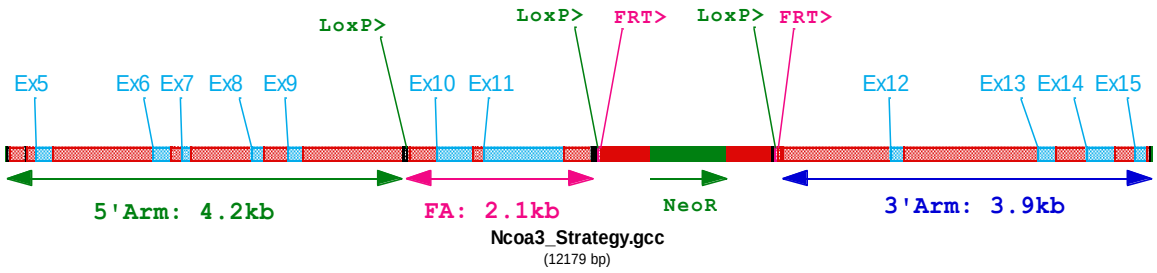


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exons 10-11

Ncoa3 gene (also named Src3) is a member of the nuclear receptor coactivator family. Additional information on this gene can be accessed at
http://www.informatics.jax.org/javawi2/servlet/WIFetch?page=markerDetail&key=36790

Strategy used to generate the conditional knock out model

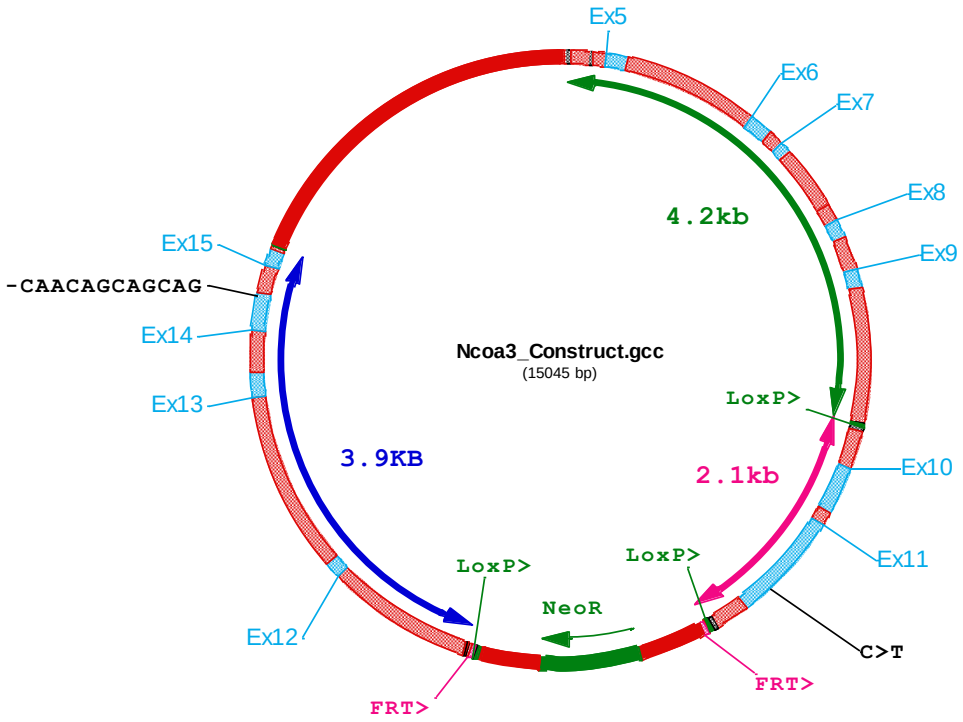


2. Construct used for homologous recombination in ES cells: Ncoa3 project

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 2 variations against C57BL/6J strain. The C>T located in the exon 11 and the missing CAACAGCAGCAG sequence located in the exon 14 are found in 129Sv/Pas strain.

2.3. 5' homology arm (4.2 kb)

TGAGGGTGTTGGCAGGGTTGCTTTCTCCTGAGTCCTCTCTCCTTGGCTCGCTATGTGTCTTAATAAGGCCTTTTT
CCCTGAGCATGCCTCCCCTGTGTATTTTTAATCTGTCCTTATAAGGATACCAGTTGTATTGGATAGGGTCCTCTT
TAATACTATTGACCCTTTAAATGCTCTATTTTGCCAGTATAACCCTAACCTGAACCCTAACCCAAACCCTGGCAA
AAATGGGGGGGAGGGGGTTTAATGCTTTTGTGGGGTTATGATTTTCATAATATTATTAACACATTCCACTGTAGG
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ATGCCATGTGTTACTGCAGTGTCATGGGGGCCAGAAGAGGACATTATCTGAAGCAGGAGTTGAAGGTGCTTATGA
GCTGCCTGACTCTGGTCCTCGGGAAGAGCCAACTAAGTGCTCTTAACCAGTGAGCC**GCCCTTGCAGCCCCTAGCT**
TGAATTTTTAAAAATTCTGTATATATAATGAAAAGTTTGCTTGCATGCATGTATGTGCATGAGGTGTGCACCAAA
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CTAAGTAAACGCTAAGTATAGTCAAA**TACCCTTCCTAGCACCAGCGCATTATAAAAGGCTCATCCGGTGCAGAGG**
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CTGTAAAGCACTCACTGTGTGCTCAGTCCTCGTCCTGCTCTTTGGTGGTTG**TGGGAGAGGG**AGTGGTTTCTGAGC
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CTGTTCCTGTCTGCCTTGGTCTAGTCTCAGATGCTAGTGACAGTGTGCTTCC**CCCTCCGTAGCTTATGTTCATGG**
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2.4. Floxed fragment (2.1 kb)

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CACACACACACACACACAGACAAAATACCAG**TGTACACGAAATATGTCTATAAGCTAGAAAAGCAAGGCAGAGTA**
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2.5. PGK-Neo region

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gctatacgaagttatgaattc**gaagttcctattctctagaaagtataggaacttc**ccgcggatccatcgaccccc
tgcagg

2.6. 3' homology arm (3.9 kb)

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AGTGCAGTGTGGGCC**AGCAGAGCCCAGGGATTTATCGAAATGGCTATCCTTGCAGTGAAGGTGGAGATAGTGTGT**
GTGTGTGTGTGTATGTGTGTGTCCCAAGACAGCAGCCTCTGTCCTGGTCATTGTTATCTCTGGTAATGGCTTGTG
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TCACTCTACCACTGAGCTAAATCCCCAACCCTTATTTTTGTTTTTGTTTGTTTGCTTGCTTTGTTGTTGGTCTGT
TTTCTTTGG**AAAGGGAGAGTCTCACTGTCTGTCTCTGGCTGTCCCGGAACTTAGTTTGCTGGGATTCAATGTGCG**
CACTACCAGTCGTTCTTTTTTTTTTTTTTTGGCTGATGCCTTGATAGCTGCATCTGGTGTTACATACAGAATAAT
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CTGGA

2.7. Vector backbone sequence

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ggggggcggagcctatggaaaaacgccagcaacgcggccttttacggttcctggccttttgctggccttttgct
cacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgct
cgccgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcccaatacgcaaaccgcct
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aacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttg
tgtggaattgtgagcggataacaatttcacacaggaaacagctatgaccatgattacgccaagcgcgcaattaac
cctcactaaagggaacaaaagctggagctcgcggccgcggcgcgcc

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]

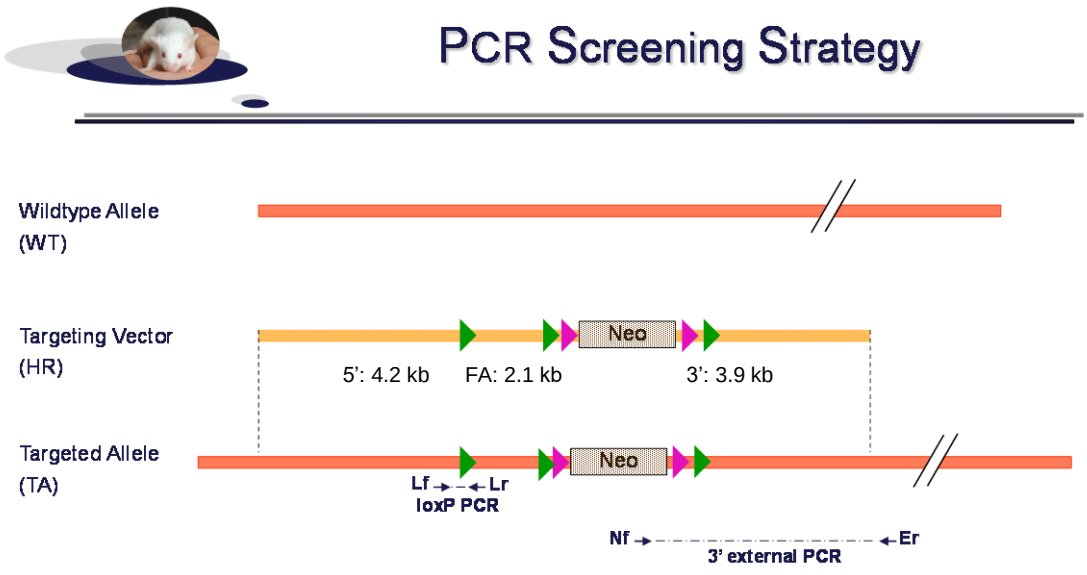
Number of clones screened: 240
Number of positive: 2

Reference of clone used to generate the mouse line:

- clone **K285-70**

3.2. PCR data on positive clone:

3.2.1.PCR screening strategy



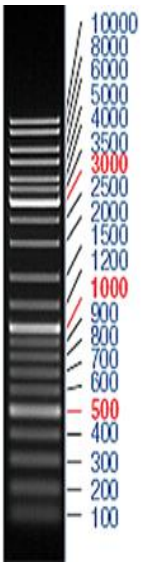
Primers used for PCR validation of ES clone

PCR	Primer Name	Primer sequences	PCR product size
LoxP	Lf	CAGCAGCAGGACCTCAAGTTCTG	WT: 0.31kb TA: 0.36kb
	Lr	GGTCAGAAGGCGGCATTGGATCC	
3' external	Nf	AGGGGCTCGCGCCAGCCGAACTGTT	TA:5.2Kb
	Er	GCACAAGAAGGCCCAAGATGCACTC	

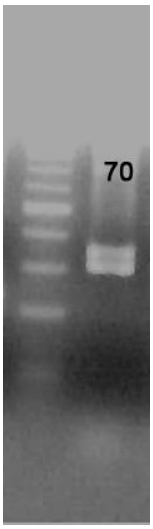
3.2.2.Picture of PCR on positive clone

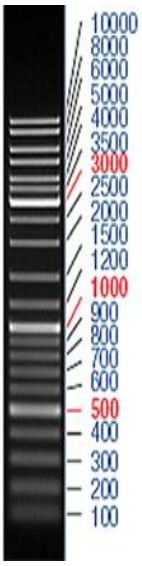
3' external PCR (5.2kb) ladder

A: WT clone
B: clone #70



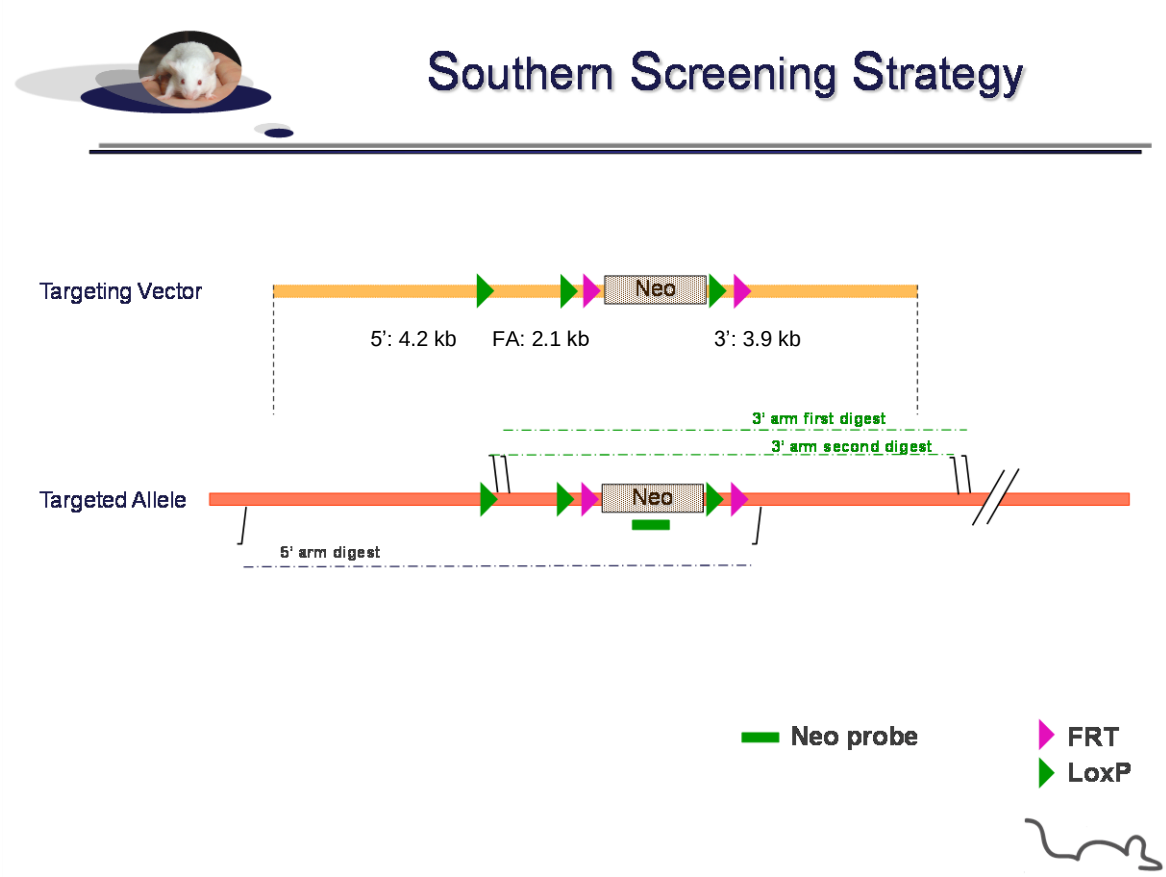
LoxP PCR (WT: 0.31kb/TA: 0.36kb) ladder





3.3. Southern data on positive clone:

3.3.1.Neo Southern strategy



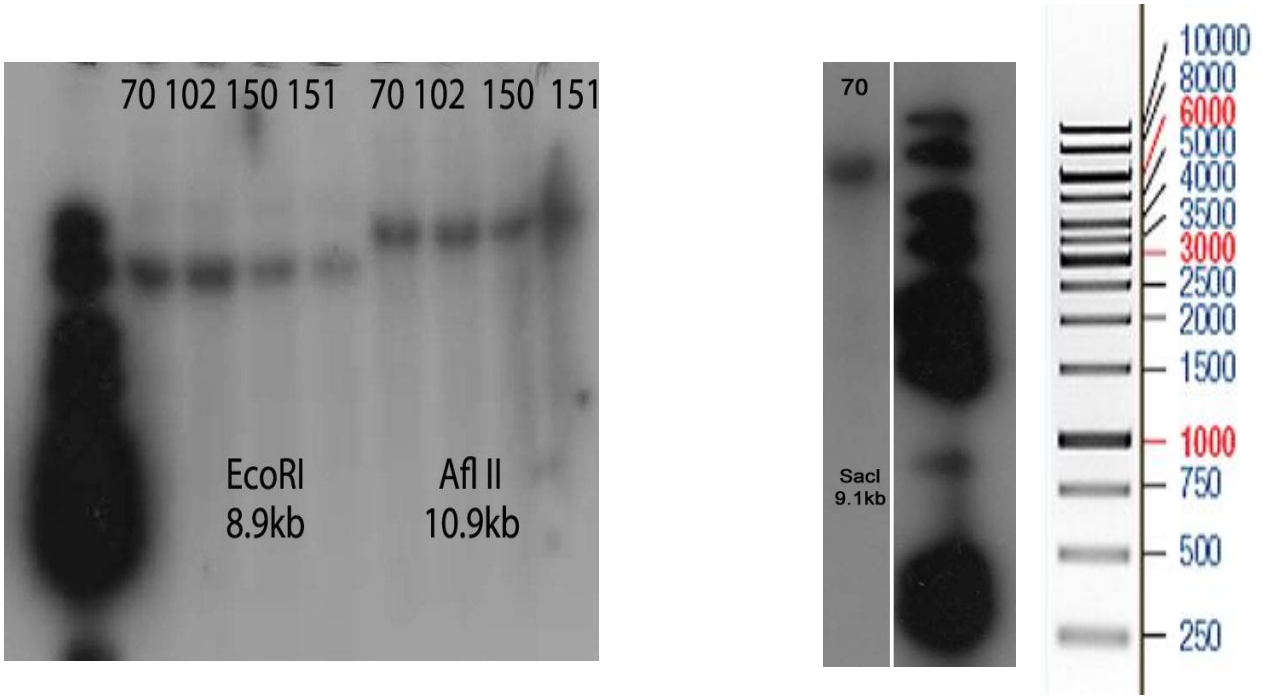
Digestions used to validate the 5' and 3' insertion

Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm digest	EcoRI	/	8.9
	3' arm first digest	AflII	/	10.9
	3' arm second digest	SacI	/	9.1

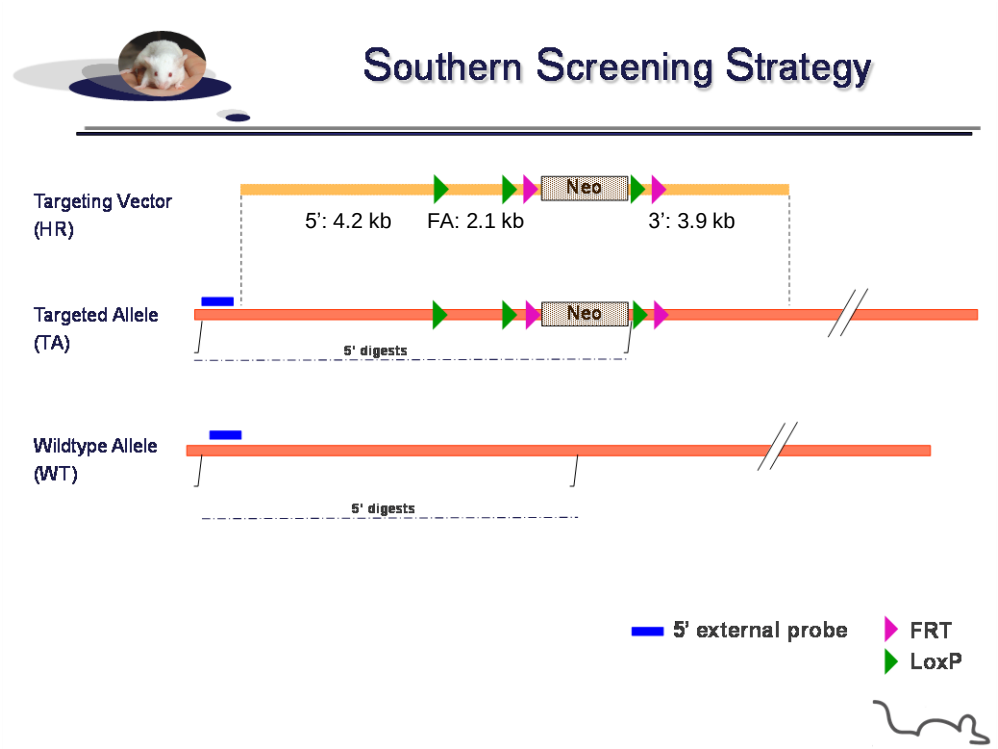
Three different digests are used to validate correct HR event. One digest validates the 5' insertion, 2 other digests validate the 3' insertion

3.3.2.Picture of Neo Southern

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



3.3.3.External probes Southern



Digestion used to validate with 5'

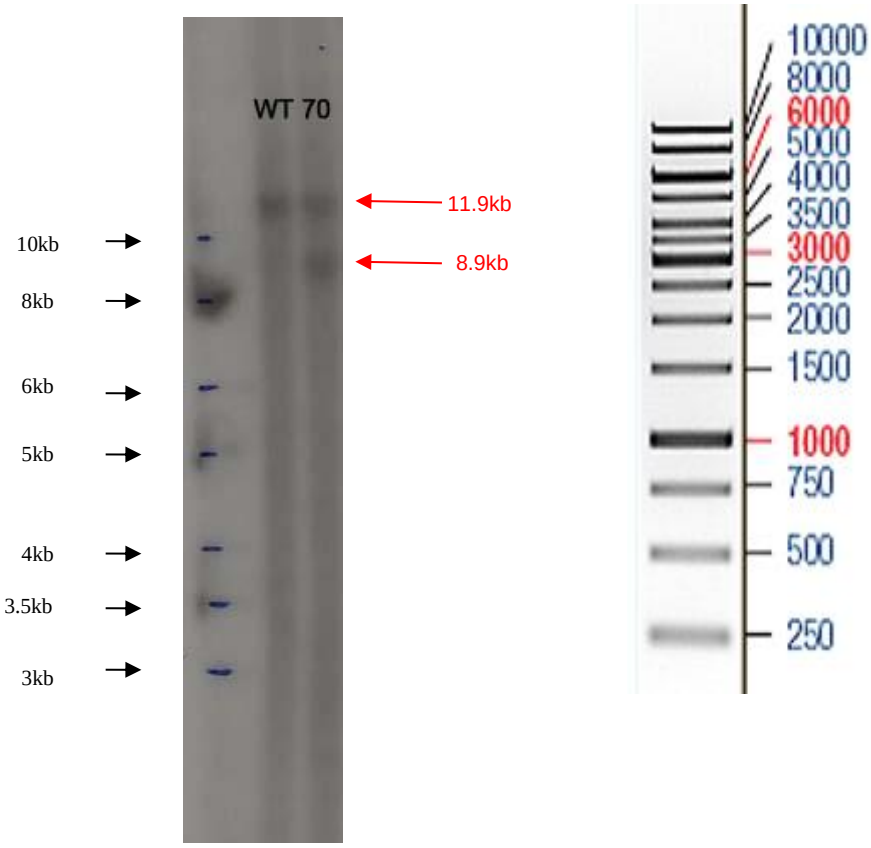
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
5' external	5'digest	EcoRI	11.9	8.9

Primers for probe synthesis:

5' probe
TGAGACGTTGTAATGTTAGCACAGG
AACACCCTCATCTTGGGCTCCAGTC

3.3.4.Picture of Southern with external 5' probe

5' external probe ladder

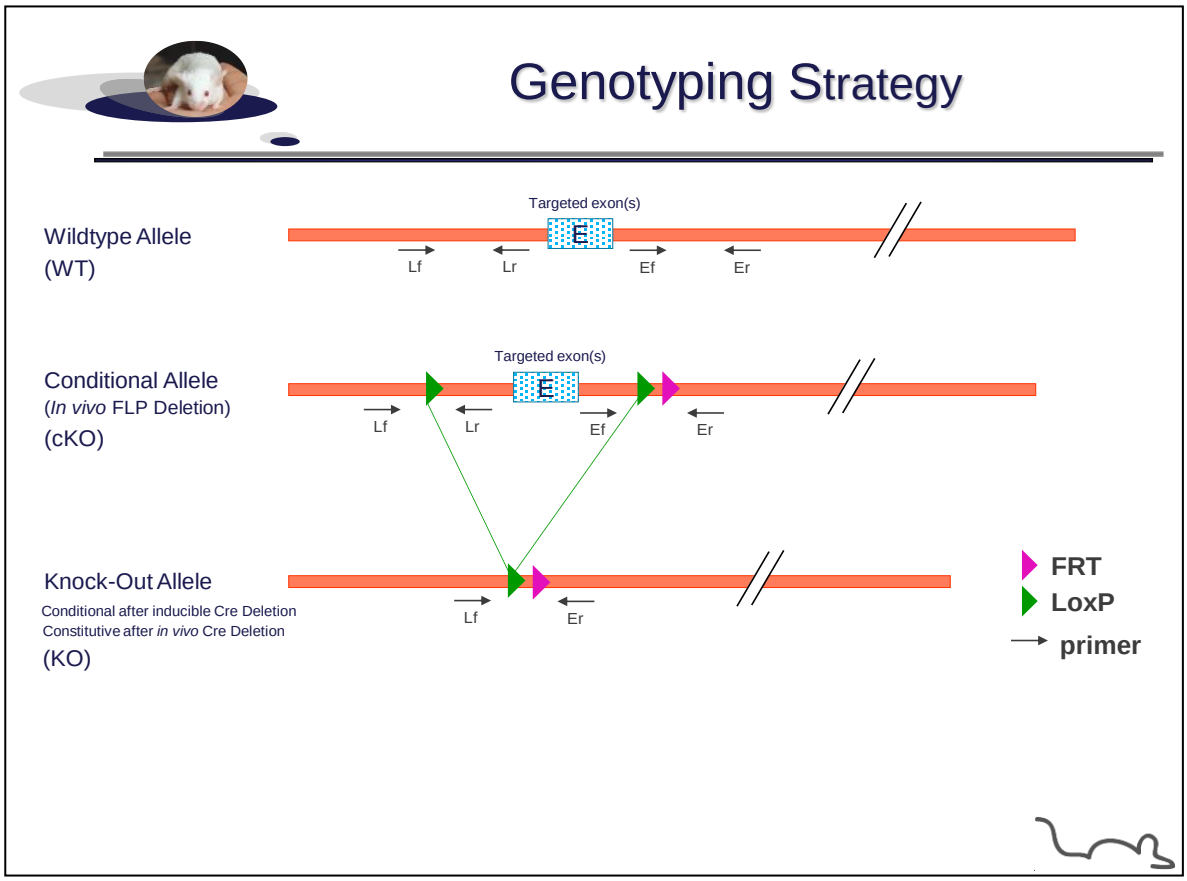


4. Genotyping protocol and data on conditional and knock-out animals

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
Ef	2042	GCCAAGAGAAAGACCCCAAAATTAAGACC
Er	2043	CCACTTCCATGAAAATACTTCTAACTGGCC
Lf	2039	CTCAGTACTGGAGAGCTTGCTTAGC
Lf	2040	CAGCAGCAGGACCTCAAGTTCTG
Lr	2041	GGTCAGAAGGCGGCATTGGATCC

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	2040-2041	Lf / Lr	361	---	311
Excision of the selection marker	2042-2043	Ef / Er	581	---	450
Excision of the floxed exon(s), i.e. knock out	2039-2043	Lf / Er	2397*	365	2216*

* 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:
- 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	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

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

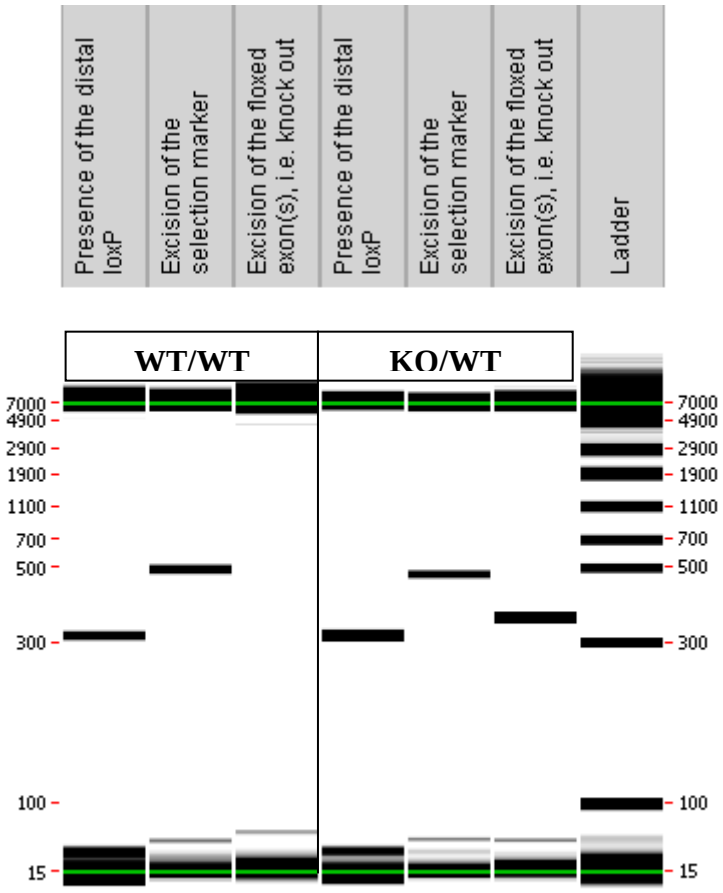
- Picture of genotyping with conditional knock-out (cKO) allele

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.

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.