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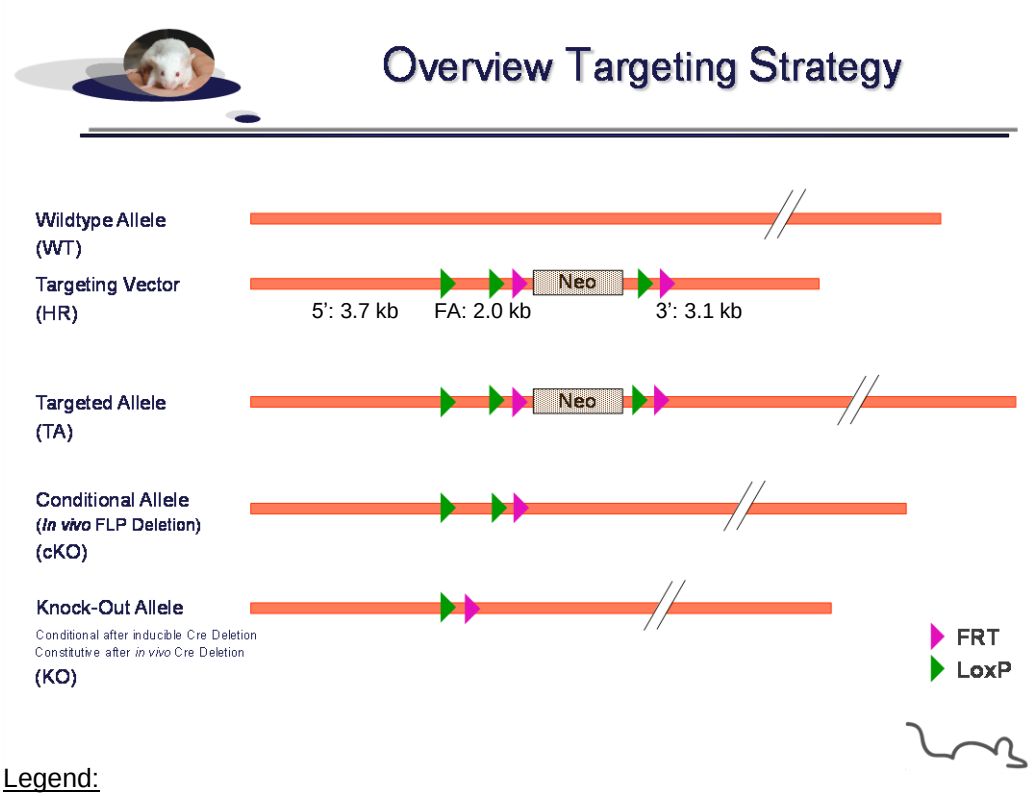
Web site: http://www.phenomin.fr/en-us//

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

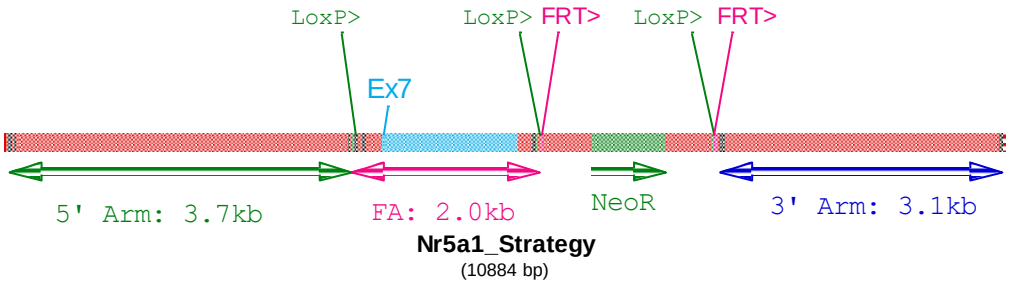


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exon 7

Nr5a1 gene (also named SF1) 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=43304

Strategy used to generate the conditional knock out model



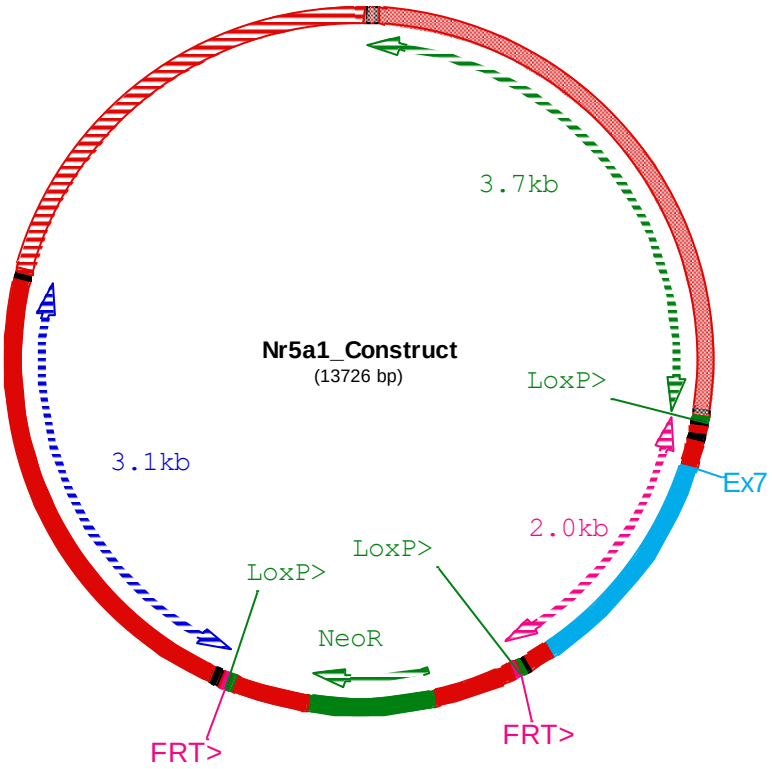
Nr5a1_Strategy
(10884 bp)

2. Construct used for homologous recombination in ES cells: Nr5a1 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



2.3. 5' homology arm (3.7kb)

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2.4. Floxed fragment (2.0kb)

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2.5. PGK-Neo region

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2.6. 3’ homology arm (3.1kb)

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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]

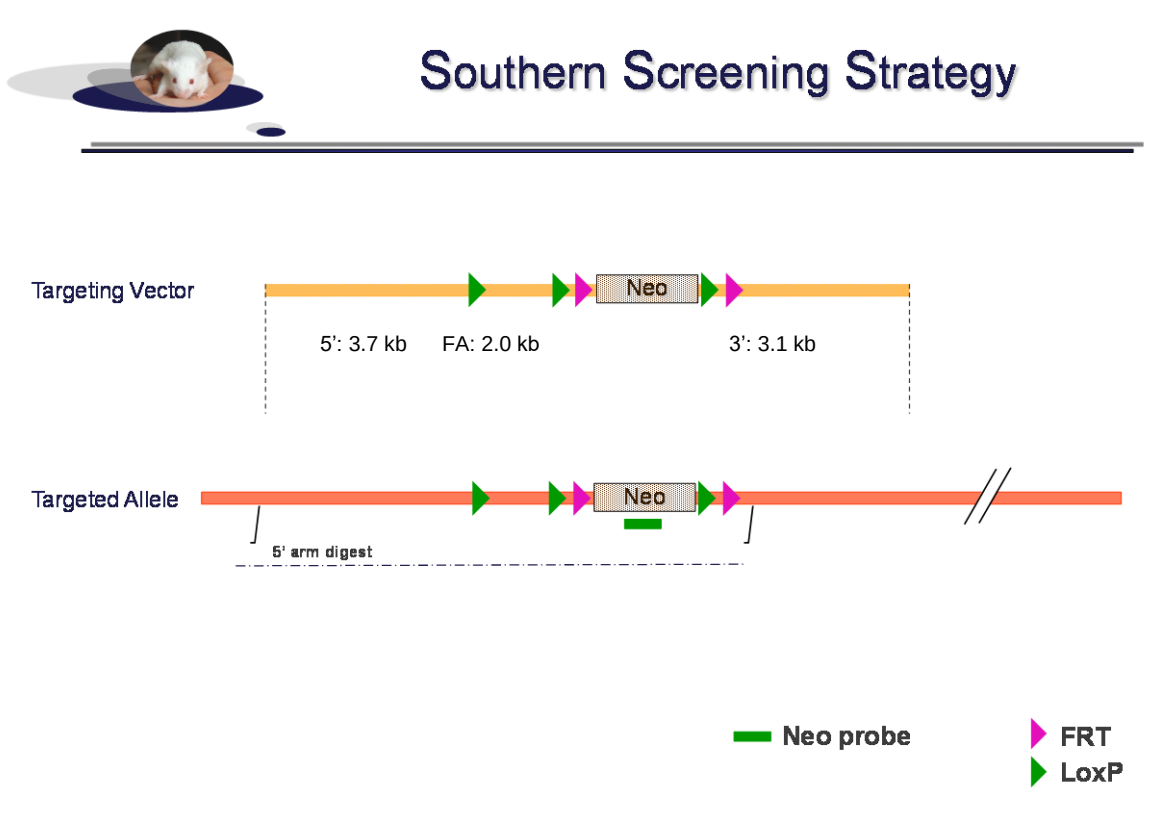
Number of clones screened: ~ 400
Number of positives: 4

Reference of clone used to generate the mouse line:

- clone **K168P1-67**

3.2. Southern data on positive clone

3.2.1.Neo Southern strategy

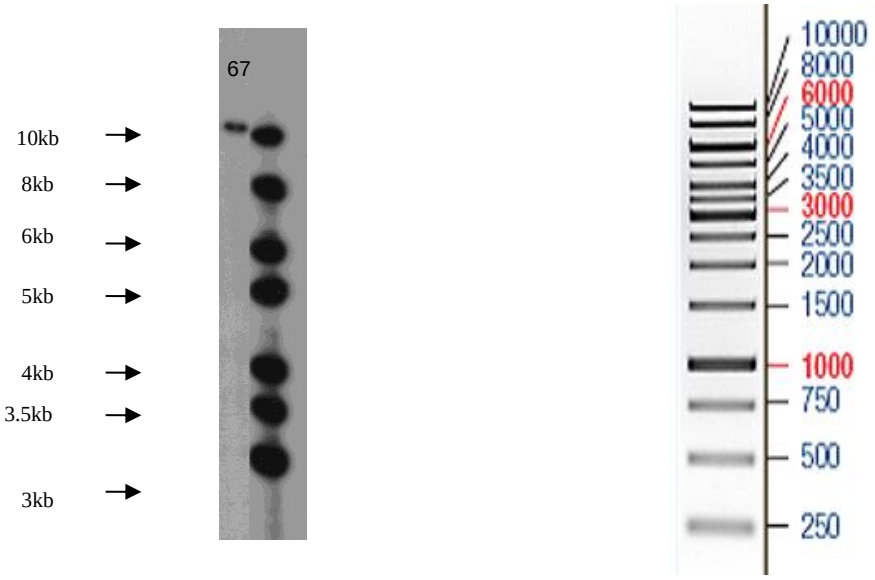


Digestion used to validate the insertion

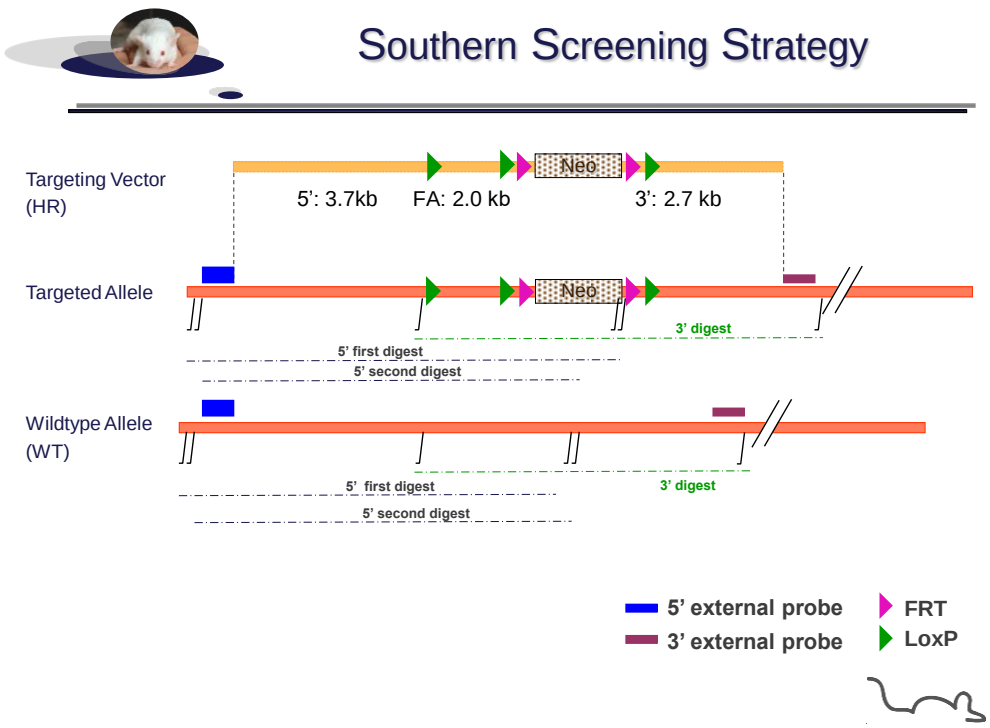
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm digest	AvrII	/	10.3

3.2.2.Picture of Neo Southern

Neo southern blot validation: AvrII digest ladder



3.2.3.External probes Southern



Digestions used to validate with 5' and 3' probes

Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
5' external	5' arm first digest	AvrII	8.3	10.3
	5' arm second digest	AflIII	9.2	11.2
3' external	3' arm digest	XbaI	10.0	6.5

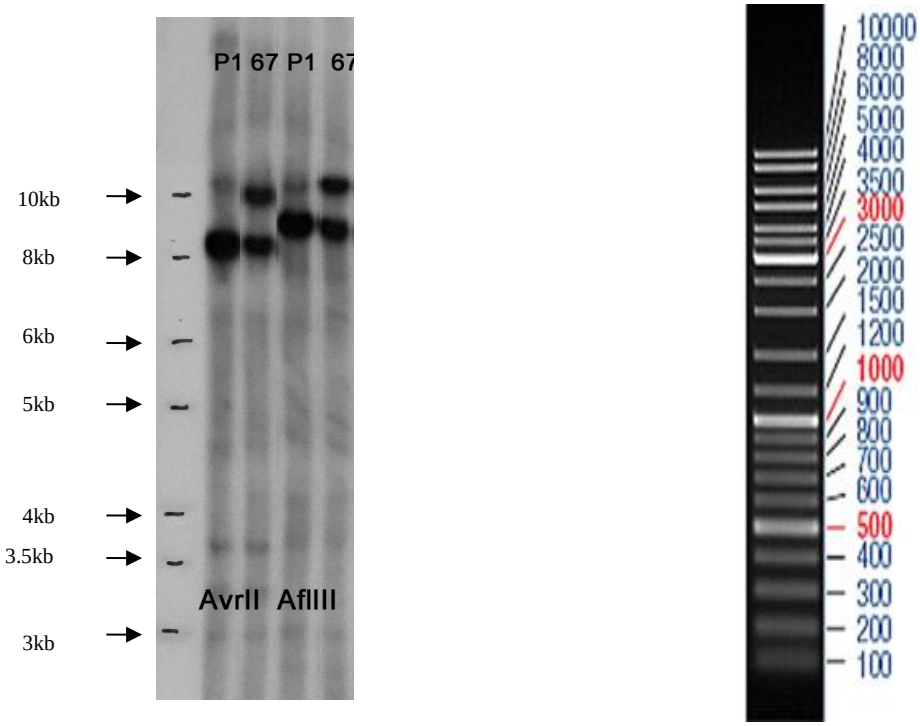
Primers for probe synthesis:

5' probe
CCTAACTCCCTGAACTACAGGGACCC
CAGGCTGAGAGTCCCAGGCCCTTGTC

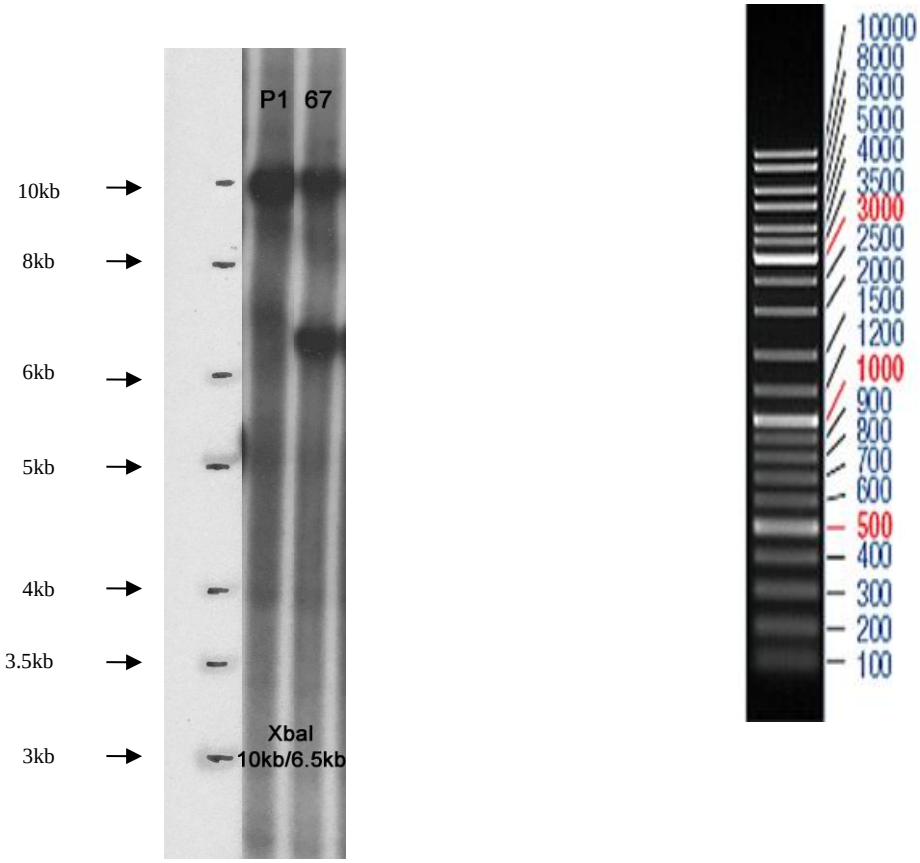
3' probe
GAGGAAAACCCTGCACATACGTGCC
CCGCAAGCACAACCGTCTGCGAGAAC

3.2.4. Picture of Southern with external probes

Southern blot with external 5' probe ladder



Southern blot with external 3' probe ladder

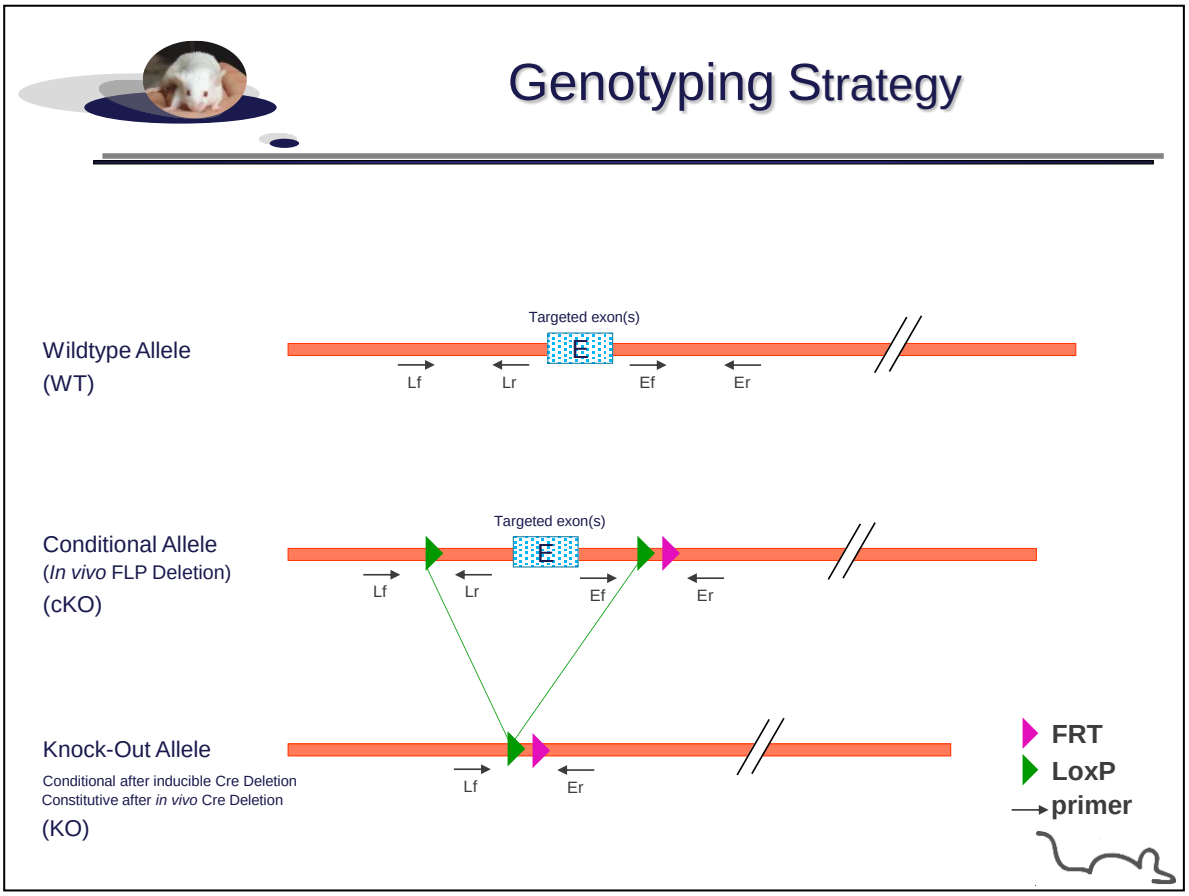


4. Genotyping protocol and data on conditional and knock-out animals

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

Primers	Sequence
Lf	GTGGCACATGCATTAGTCCACTTGG
Lr	GAGACTGCTATGAGCCTCTCAATGG
Ef	CTGTCTCCTGTCTTCTACTACCCTG
Er	AGCCATTTCAACAGTGCCCCTTCC

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	762-763	Lf / Lr	277	---	228
Excision of the selection marker	764-766	Ef / Er	397	---	289
Total Excision (excision of the floxed exon(s), i.e. knock out)	762-766	Lf / Er	2246*	240	2086*

* 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

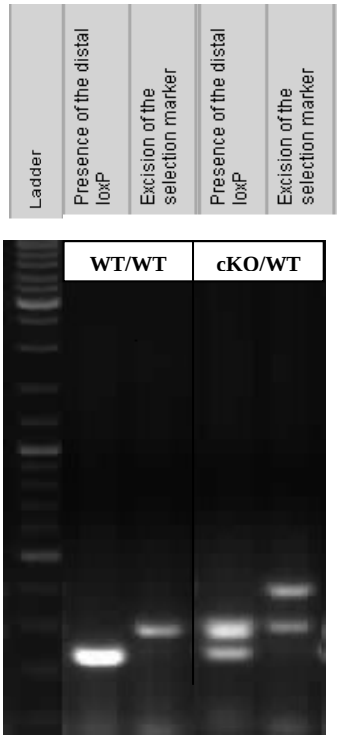
Cycling conditions:

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



- Picture of genotyping with knock-out (KO) allele