

***Stmn4* cKO and KO (IR00001349 / K376 ICS internal reference) mouse line genotyping protocol**

Table of contents

Table of contents	1
1. Genotyping protocol and data.....	2
1.1. Genotyping strategy	2
1.2. PCR protocol.....	3
1.3. Picture of genotyping with various alleles	4

For any question, please contact:

Mouse Clinical Institute – Institut Clinique de la Souris (ICS)

ICS genotyping service

1 rue Laurent Fries, BP 10142

67404 Illkirch Cedex France

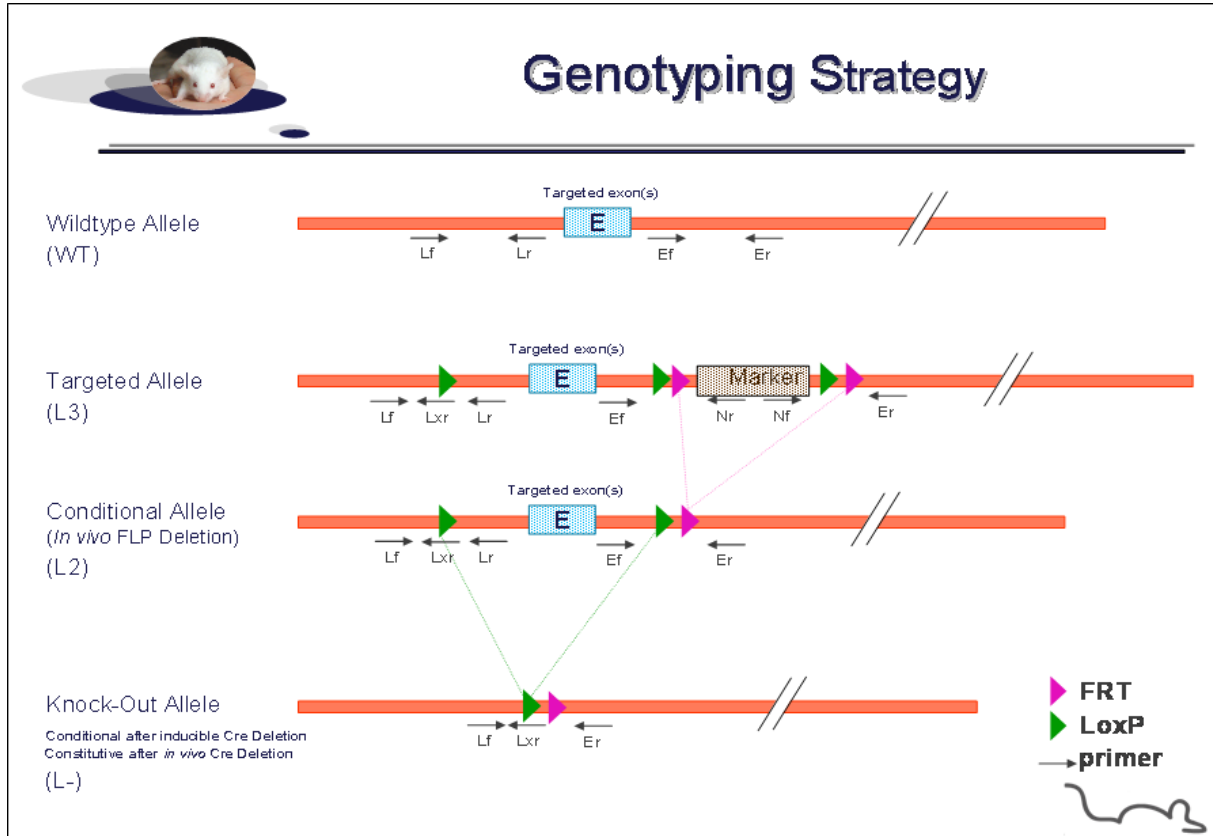
Email: mutagenesis@igbmc.fr

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the *Stmn4* conditional knock-out (cKO) and KO mouse line.

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

Position	Sequence
Ef	CACTGGTGCAGACTAAGACACCAC
Er	GGGAAGAAGGAAGCTGCATACAAAGC
Lf	GCATTTGGTAGTAACAGAGGATAGA
Lr	CCGAGCCCCCACTTACCTTCATATT
Nf	CCTTCTTGACGAGTTCTTCTGAGGG
Nr	TGACTAGGGGAGGAGTAGAAGGTG

PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	Targeted allele (L3)	cKO allele (L2)	KO allele (L-)	Wild-type allele (WT)
Presence of the distal loxP	Lf / Lr	354	354	---	274
Excision of the selection marker	Ef / Er	2113*	243	---	133
5' part of the selection marker	Ef / Nr	349	---	---	---
3' part of the selection marker	Nf / Er	557	---	---	---
Excision of the floxed exon(s), i.e. knock out	Lf / Er	*	*	227	*

* This PCR product will not be observed using our PCR genotyping conditions (see description below)

--- No Amplicon should be obtained

1.2. PCR protocol

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

Reagents:

- FastStart PCR Master (Roche)
- DNA (50ng/μl)
- 5' primer (100 μM)
- 3' primer (100 μM)
- Sterile H₂O

Volume:

- 7.5μl
- 1.5μl
- 0.06μl
- 0.06μl
- up to 15 μl

Cycling conditions:

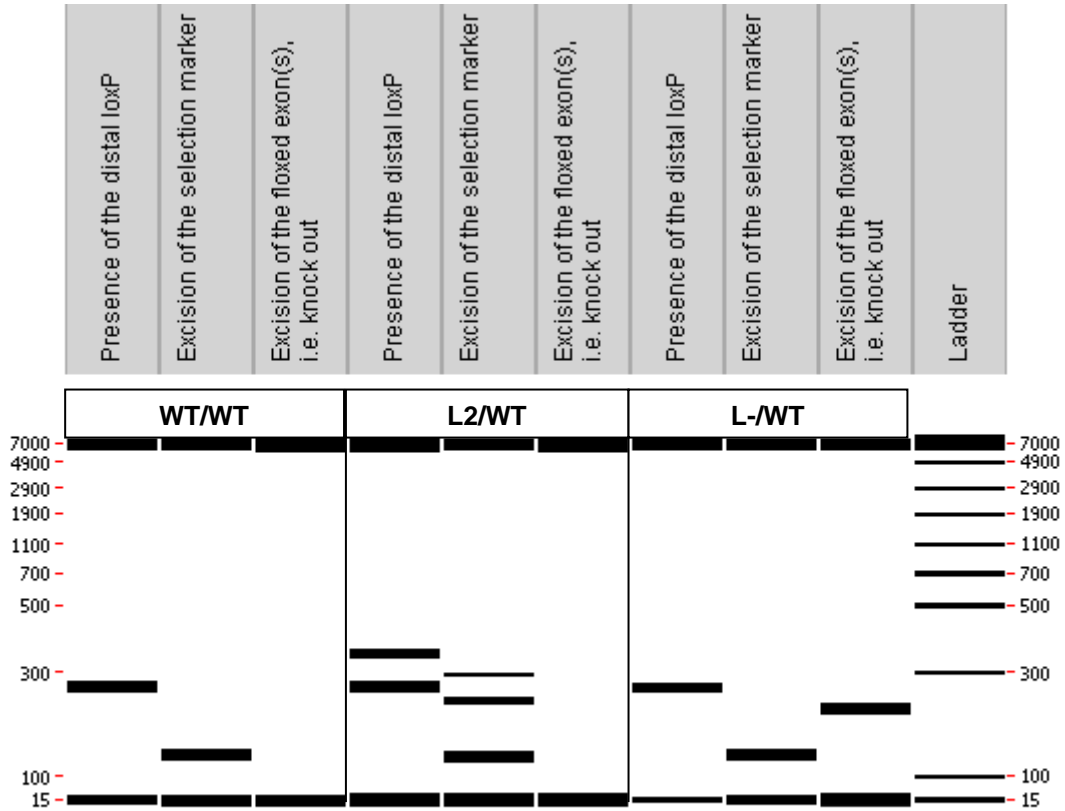
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5 min	1

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

1.3. Picture of genotyping with various alleles

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.