STMN4-KO

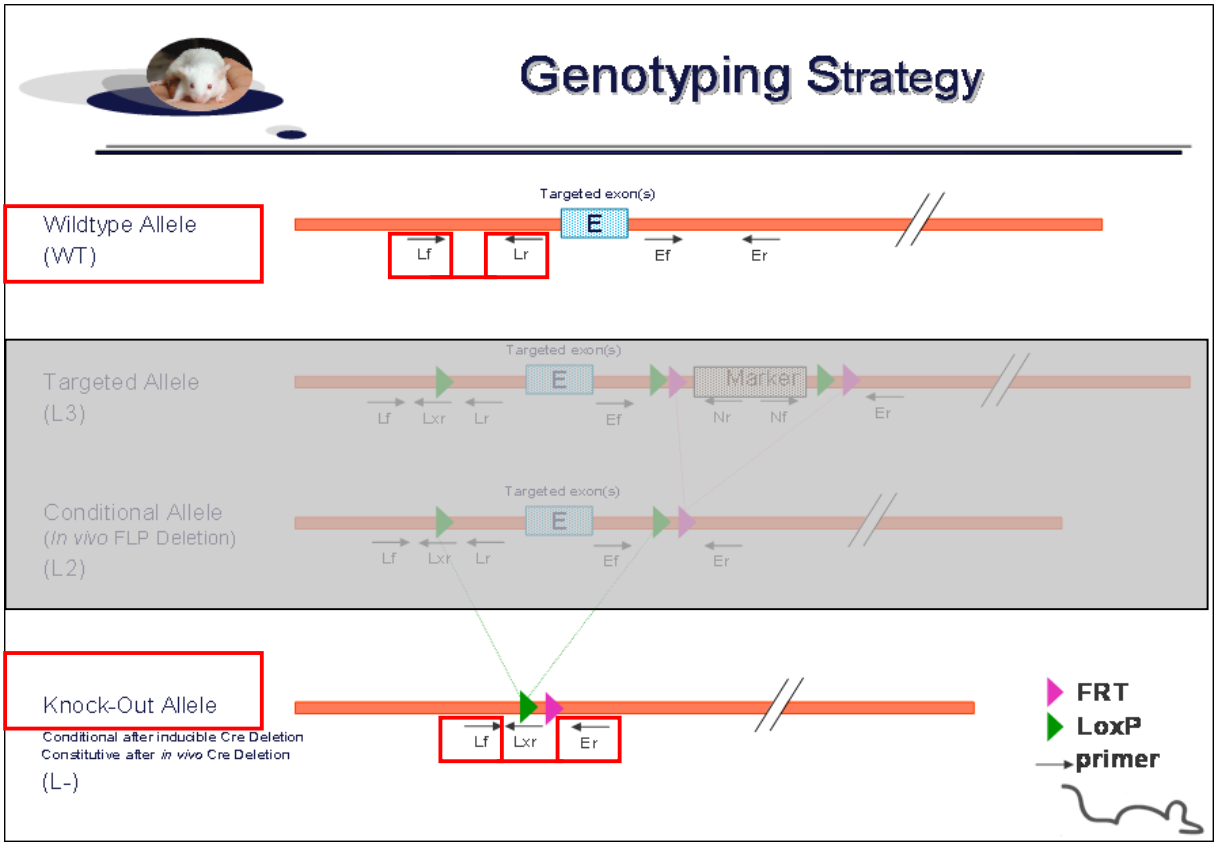
Protocole de génotypage extrait des recommandations de l'ICS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **STMN4** Conditional Knockout (cKO) project.

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	Primers	Sequence
Er	3194	GGGAAGAAGGAAGCTGCATACAAAGC
Lf	3189	GCATTTGGTAGTAACAGAGGATAGA
Lr	3191	CCGAGCCCCCACTTACCTTCATATT

PCR fragments expected size (bp):

Region analyzed	**Primers used**	Position on the primer (see the map above)	Targeted allele (L3)	cKO allele (L2)	**KO allele** (L-)	**WildType** allele (WT)
Presence of the distal loxP	3189-3191	Lf / Lr	354	354	---	**274**
Excision of the floxed exon(s), i.e. knock out	3189-3194	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:	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.

N.B. Au laboratoire, nous mélangeons les amorces Lf, Lr et Er dans une même PCR, ce qui permet de voir sur le gel une bande (homozygote KO : 227 / WT : 274) ou deux (hétérozygote).