# STMN2-KO

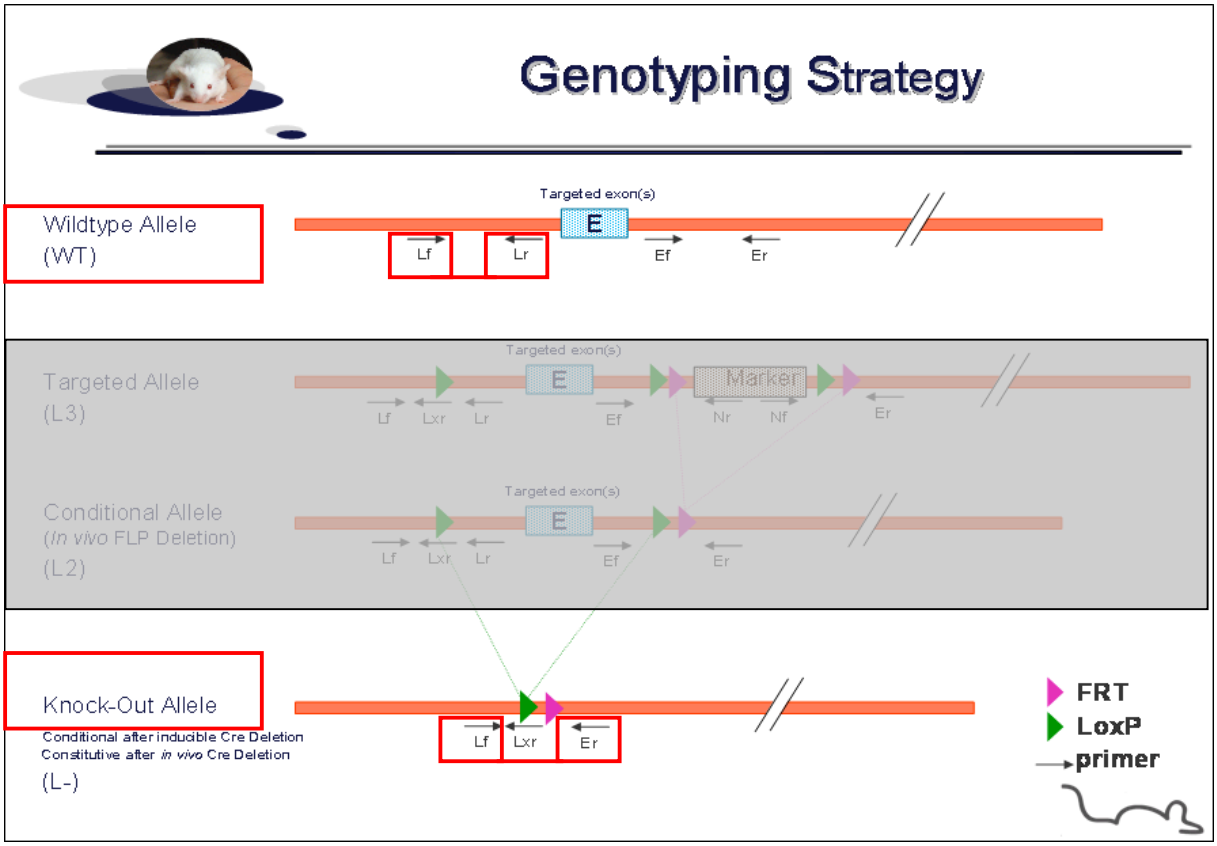
***Protocole de génotypage extrait des recommandations de l'ICS***

## 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **STMN2** 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 | 3198 | GATGCTTGGAATGGAACTTG |
| | | |
| Lf | 3195 | ATCCAACATTTTGTACAATGAGCAC |
| Lr | 3197 | CCGGCCTCCTTTCTGTGCTTTGCCC |
| | | |
| | | |

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 | 3195-3197 | Lf / Lr | 378 | 378 | --- | **298** |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| Excision of the floxed exon(s), i.e. knock out | 3195-3198 | Lf / Er | * | * | **555** | 1469* |

* 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.**