# Stk30 (IR00002808 / E126 ICS internal reference) mouse line genotyping protocol

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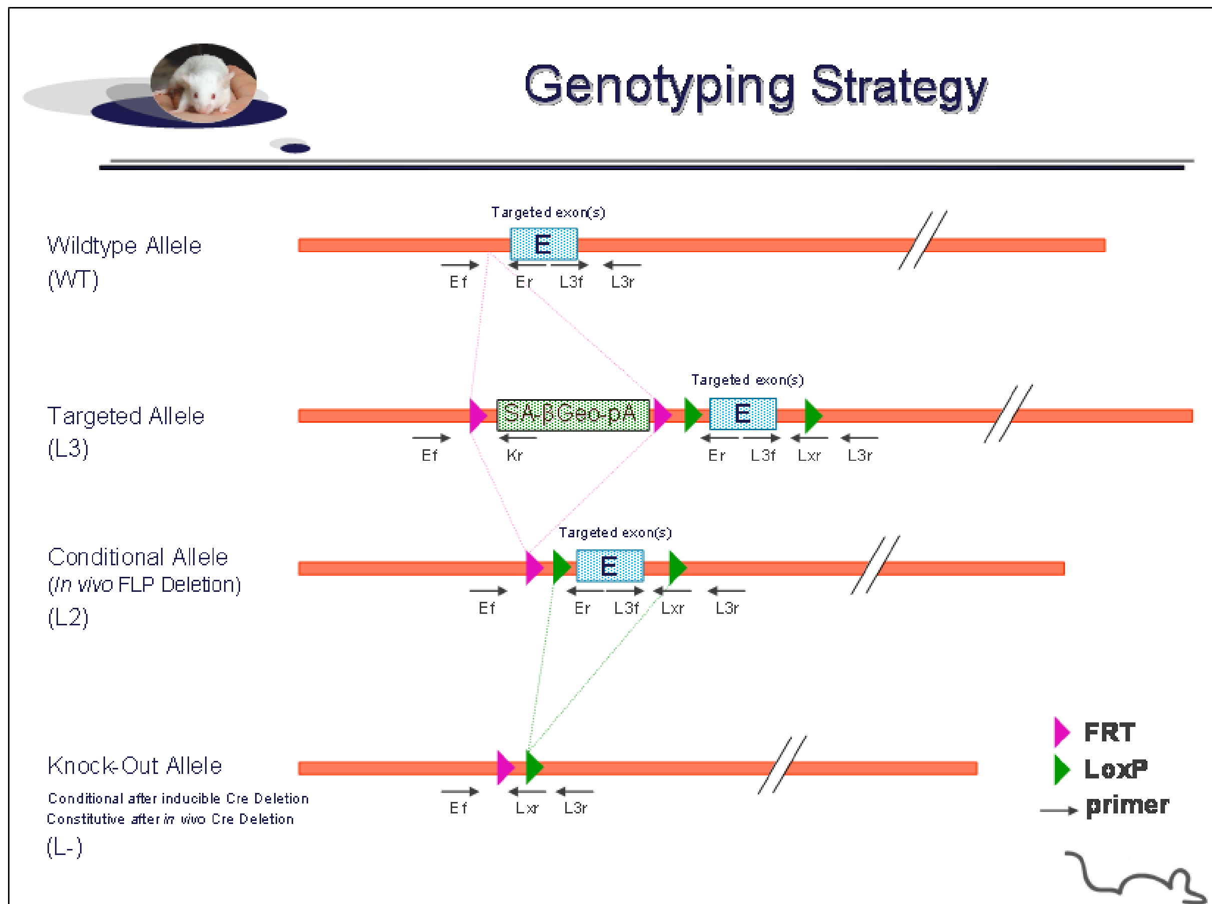
This protocol has been validated by Valérie Rousseau.

## 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Stk30** Constitutive Knockout / Conditional Knockout (KO-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 |
|---|---|---|
| Ef | 5092 | AGCAACACAGATCTCAGAGGAGTGTCC |
| Er | 5094 | TAGTTAGTTAGGGGAGGGCACCGGC |
| Kr | 3277 | CTCCTACATAGTTGGCAGTGTTTGGG |
| L3f | 5095 | CGAGGCGTAGGTGCGAGTCATT |
| L3r | 5093 | TGGCCAAGGACTCAGGTTCAGTTC |
| Lxr | 3254 | TTATCATTAATTGCGTTGCGCCATC |

PCR fragments expected size (bp):

| Region analyzed | Primers used | Position on the primer (see the map above) | Targeted allele (KO allele) (L3) | cKO allele (L2) | KO allele (L-) | WildType allele (WT) |
|---|---|---|---|---|---|---|
| 5' part of the selection marker | 5092-3277 | Ef / Kr | 321 | --- | --- | --- |
| Presence of the distal loxP | 5095-5093 | L3f / L3r | 378 | 378 | --- | 367 |
| Distal loxP specific PCR | 5095-3254 | L3f / Lxr | 301 | 301 | --- | --- |
| Excision of the selection marker | 5092-5094 | Ef / Er | 7356* | 375 | --- | 301 |
| Excision of the floxed exon(s), i.e. knock out | 5092-5093 | Ef / L3r | 8210* | 1254* | 350** | 1144** |

* This PCR product will not be observed using our PCR genotyping conditions (see description below)
** This PCR is only verified if mice are generated
--- 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:

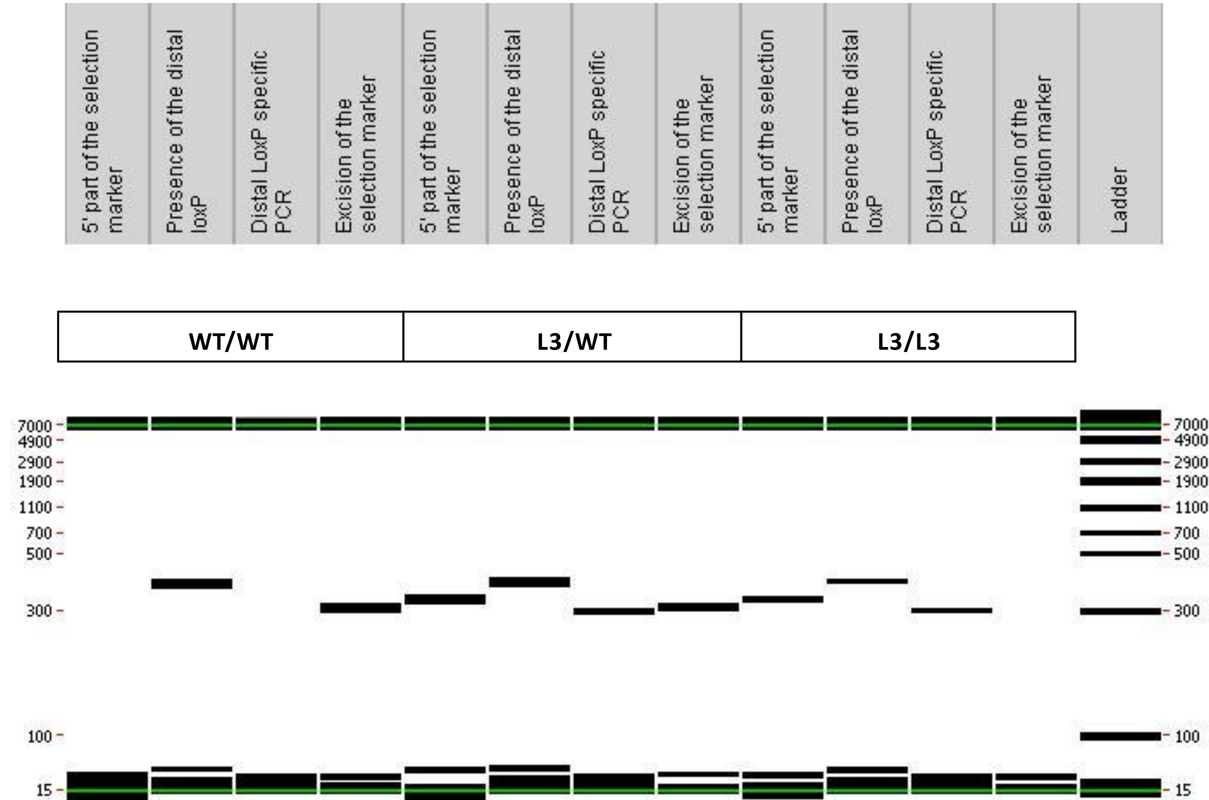
| Temp | Time | #Cycles |
|---|---|---|
| 95°C | 4min | 1 |
| 94°C | 30s | |
| 62°C | 30s | 34 |
| 72°C | 1min | |
| 72°C | 7min | 1 |
| 20°C | 5 min | 1 |

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

### 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



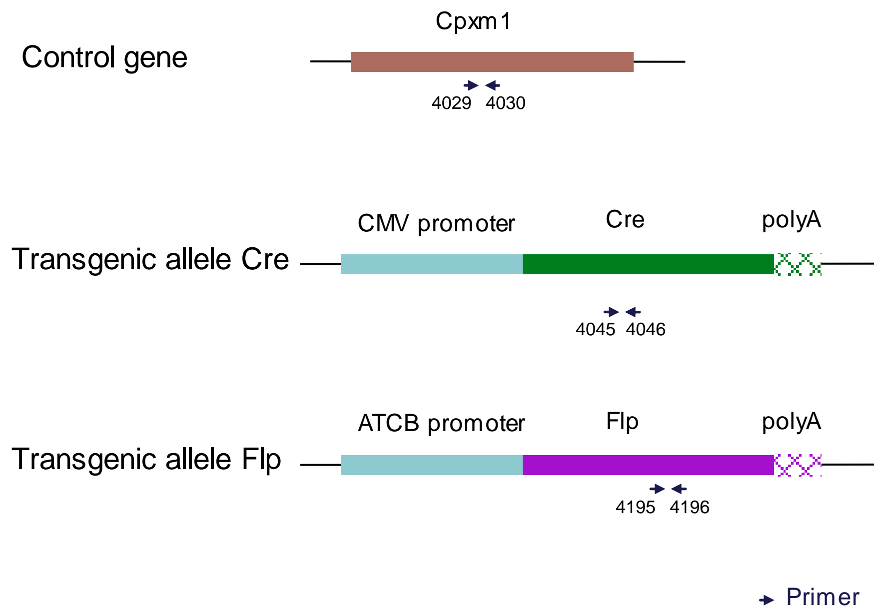

Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

## 2. Cre and Flp genotyping method

The protocol used to segregate the cre and/or flp transgene is indicated below.
Detection of cre transgene and flp transgene is done using a multiplex assay: primer pairs were designed for each gene and for a positive control (Cpxm1 gene).

### 2.1. Cre and Flp genotyping

Schematic representation of the genotyping strategy




Sequence of primers used for genotyping:

| Primers | Sequence |
|---|---|
| 4029 | ACTGGGATCTTCGAACTCTTTGGAC |
| 4030 | GATGTTGGGGCACTGCTCATTCACC |
| 4045 | CCATCTGCCACCAGCCAG |
| 4046 | TCGCCATCTTCCAGCAGG |
| 4195 | TCTTTAGCGCAAGGGGTAGGATCG |
| 4196 | GTCCTGGCCACGGCAGAAGC |

PCR fragments expected size (bp):

| Primer pair | 4045-4046 | 4195-4196 | 4029-4030 |
|---|---|---|---|
| Region analyzed | Middle part of Cre transgene | Middle part of Flp transgene | Cpxm1 control gene |
| Control gene | / | / | 446 |
| Tg allele | 281 | 328 | / |

### 2.2. 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) | 0.05µl |
| - 3' primer (100 µM) | 0.05µl |
| - Sterile $H_2O$ | up to 15 µl |

Cycling conditions are identical to those described in chapter 1.2

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