# Genotyping protocol

## Bloc1s1

## IR00002901 / E154

(ICS internal reference)


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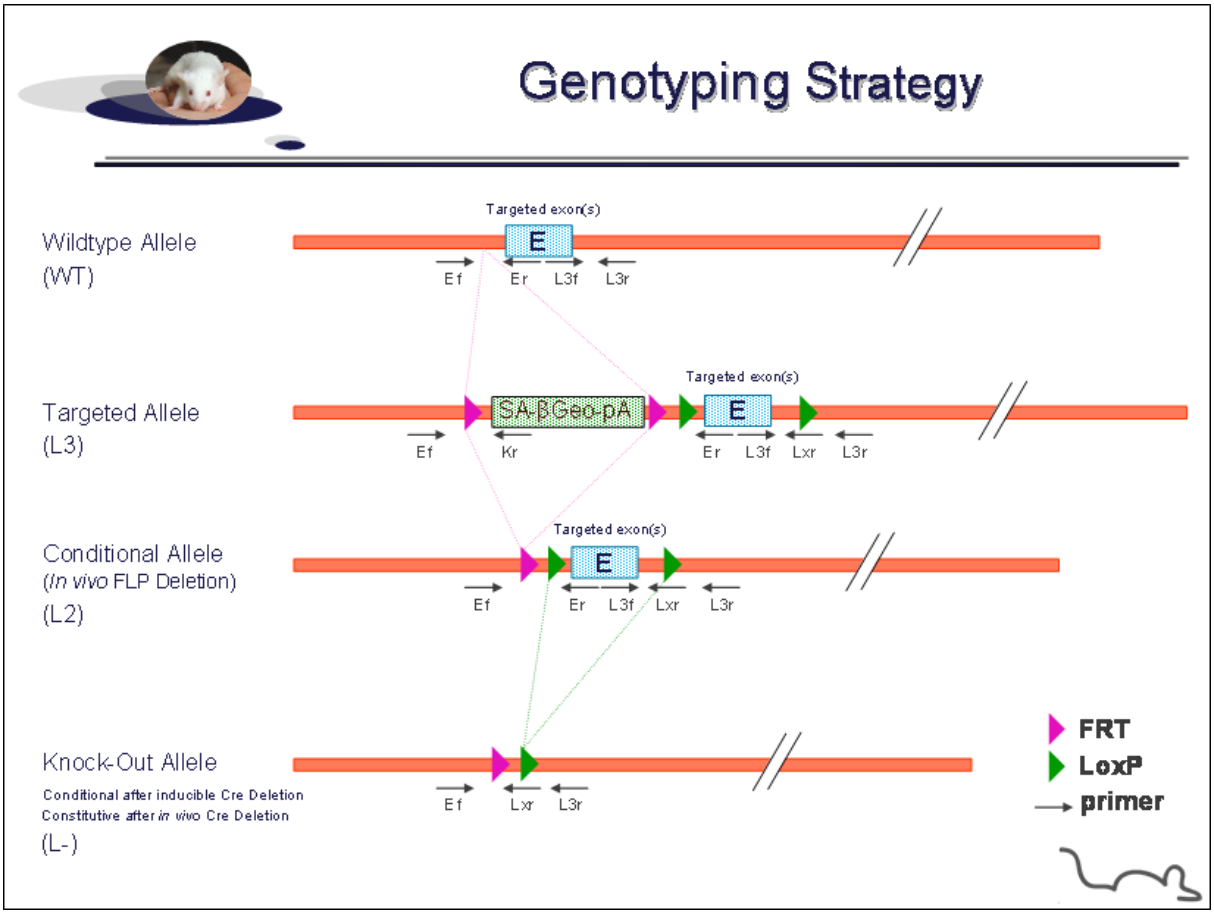
# 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Bloc1s1** 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 | 5266 | TGTTCACTCATCTGTGTGGGTGGG |
| Ef[2] | 5267 | CTTCATCCACATAGCGAGTTTGGGG |
| Er | 5271 | GGGAAACAGCCTATAACAGTCGTGGG |
| Kr | 3209 | CCAACAGCTTCCCCACAACGG |
| L3f | 5270 | AAGGTTTCCTCAGTCCTCCCTCCTG |
| L3r | 5268 | TGGTCCATGTACCATACTCAGGGTTTT |
| L3r[2] | 5269 | GGCCCTAAACATGTCACCGAGCTATAT |
| Lxr | 5086 | GAAGTTATCATTAATTGCGTTGCGCC |

[2]. For a selected position a second primer was designed

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 | 5266-3209 | Ef / Kr | 435 | --- | --- | --- |
| Presence of the distal loxP | 5270-5269 | L3f / L3r[2] | 509 | 509 | --- | 447 |
| Distal loxP specific PCR | 5270-5086 | L3f / Lxr | 413 | 413 | --- | --- |
| Excision of the selection marker | 5267-5271 | Ef[2] / Er | 7511* | 607** | --- | 508 |
| Excision of the floxed exon(s), i.e. knock out | 5266-5268 | Ef / L3r | 8139* | 1235* | 518** | 1074** |

*: 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

An aspecific band is detected on PCR “Presence of the distal loxP” at 884 bp for the mutant mices.

## 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:

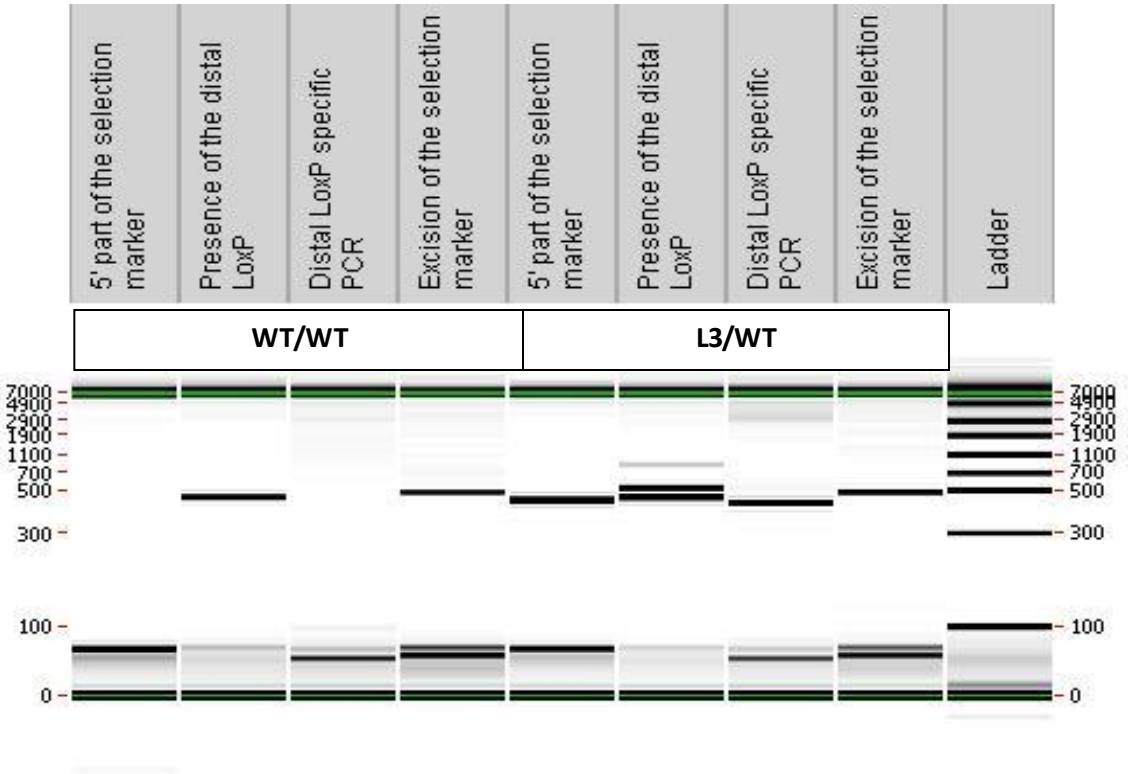
| Temp | Time | #Cycles |
|---|---|---|
| 95°C | 4min | 1 |
| 94°C | 30s | |
| 62°C | 30s | 34 |
| 72°C | 1min | |
| 72°C | 7min | 1 |
| 20°C | 5min | 1 |

**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



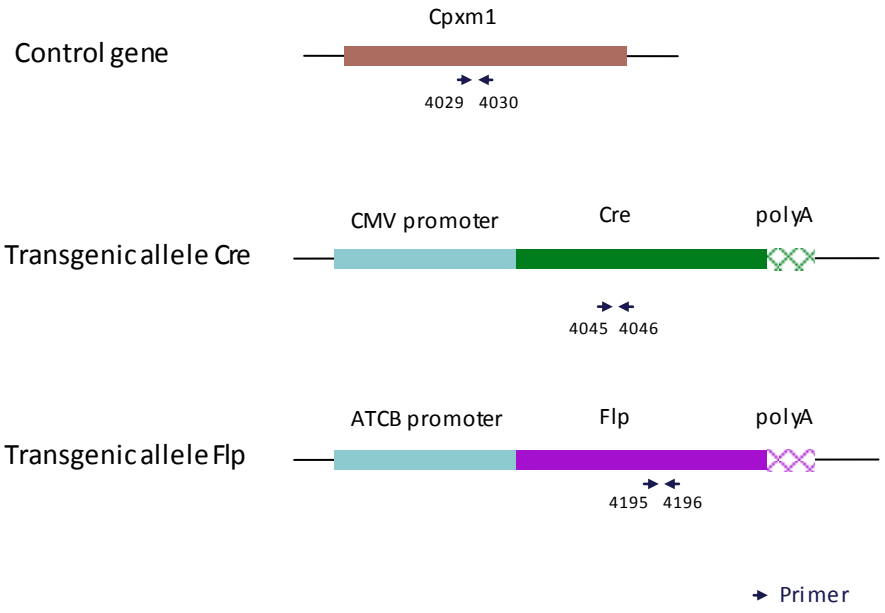

Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

# 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