# Bspry (IR00002551 / E60 ICS internal reference) mouse line genotyping protocol

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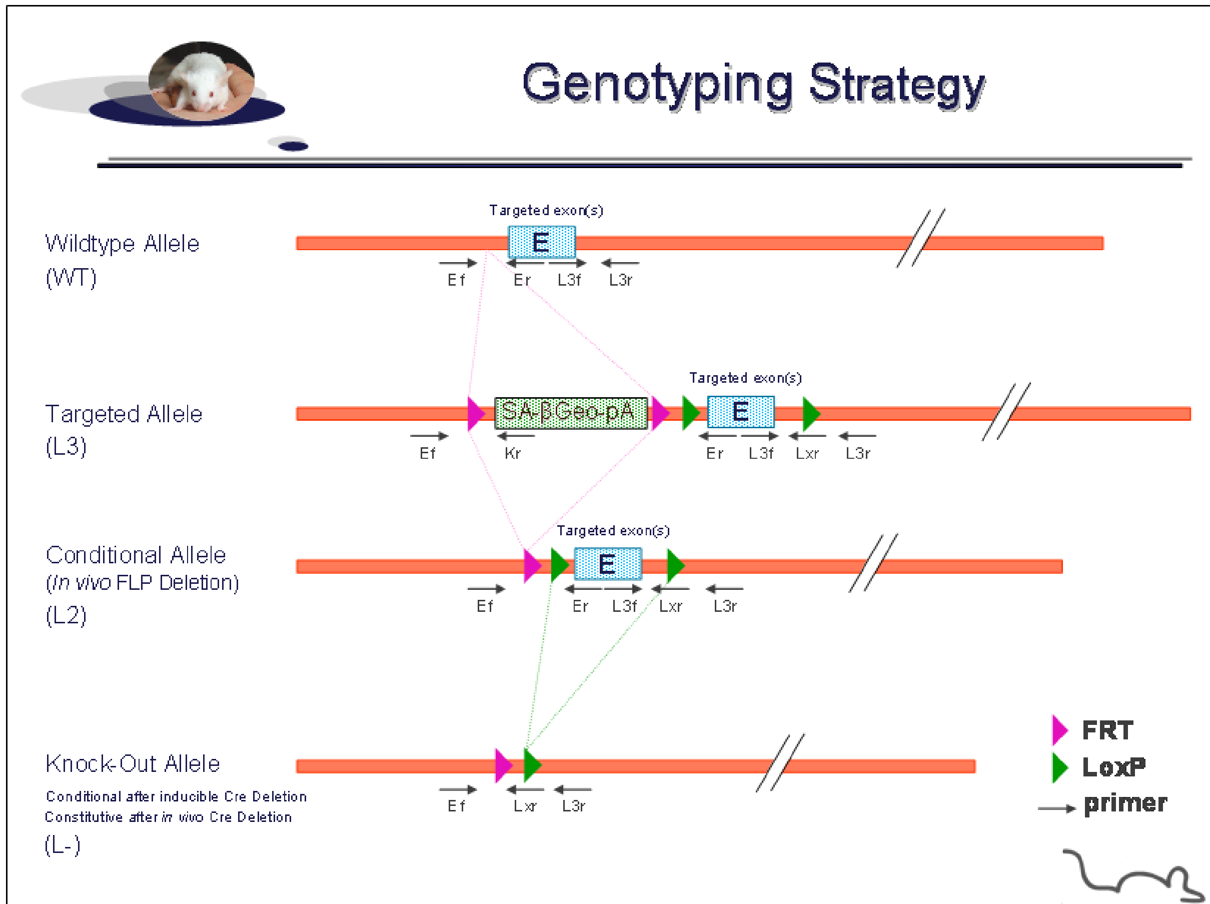
This protocol has been validated by Karim Essabri.

## 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Bspry** 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 | 4561 | TGAGCAACACTGCAGAGTGGAGGG |
| Ef | 4564 | ACAGGAAGAAGGCCGCGCTTGG |
| Er | 4563 | CCCATTGCCACCTACCCTGAGGC |
| Kr | 3277 | CTCCTACATAGTTGGCAGTGTTTGGG |
| L3f | 4562 | GCCCACTTGCTGGGAGTGCTGC |
| L3r | 4560 | GGTACCAAGTGACTCCAGGCCCC |
| Lxr | 3254 | TTATCATTAATTGCGTTGCGCCATC |

Genotyping 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) |
|---|---|---|---|---|---|---|
| Excision of the floxed exon(s), i.e. knock out | 4561-4560 | Ef / L3r | 6345* | 945* | 249** | 835** |
| 5' part of the selection marker | 4564-3277 | Ef / Kr | 360 | --- | --- | --- |
| 3' loxP specific PCR | 4562-3254 | L3f / Lxr | 301 | 301 | --- | --- |
| Presence of the distal loxP | 4562-4560 | L3f / L3r | 404 | 404 | --- | 391 |
| Excision of the selection marker | 4561-4563 | Ef / Er | 5800* | 400** | --- | 303 |

* 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: |
|---|---|
| -10x Buffer (Roche) | 2.5µl |
| -dNTPs 10mM (Amersham Biosciences) | 0.5µl |
| -Taq DNA Polymerase (Roche) | 0.2µl |
| -DNA (50ng/µl) | 3µl |
| -5' primer (100 µM) | 0.125µl |
| -3' primer (100 µM) | 0.125µl |
| -Sterile H2O | up to 25 µl |

Cycling conditions:

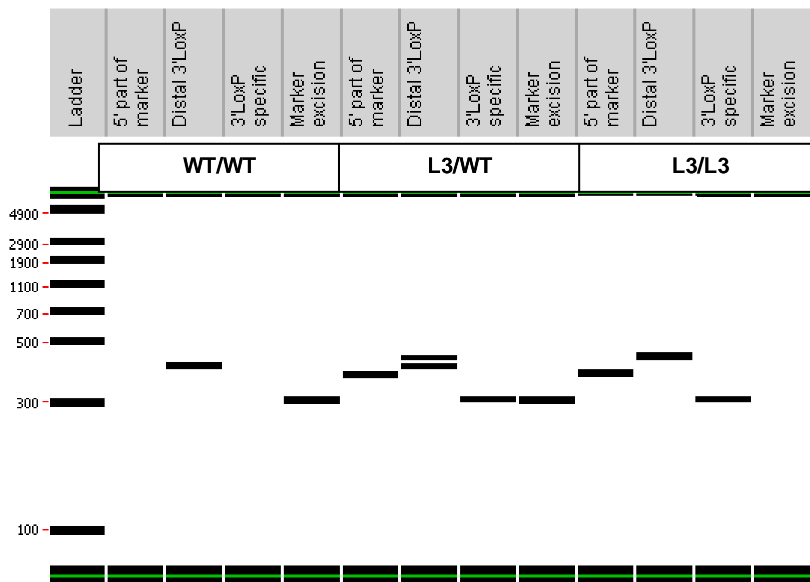
| Temp | Time | #Cycles |
|---|---|---|
| 94°C | 3min | 1 |
| 94°C | 1min | |
| 62°C | 1min | 2 |
| 72°C | 1min | |
| 94°C | 30s | |
| 62°C | 30s | 30 |
| 72°C | 30s | |
| 72°C | 3min | 1 |
| 4°C | ∞ | |

**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**



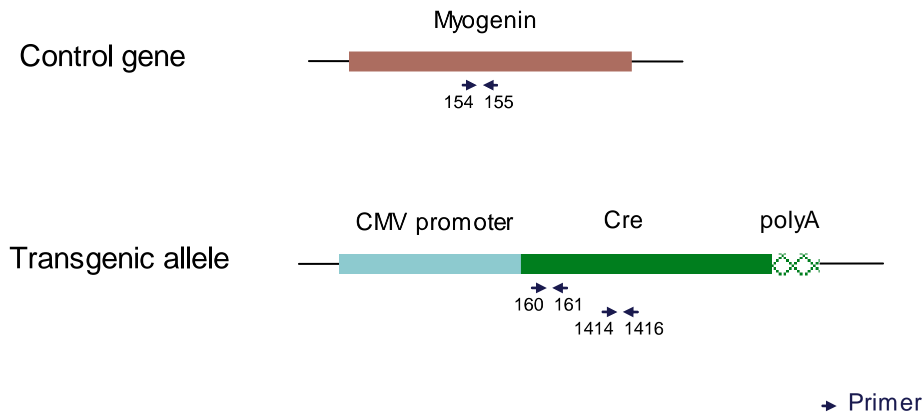

Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

## 2. Cre and Flp genotyping method

The protocol used to segregate the cre and/or flp transgene is indicated below.

### 2.1. Cre genotyping

Schematic representation of the genotyping strategy




Sequence of primers used for genotyping

| Primers | Sequence |
|---|---|
| 154 | ACTCCCTTACGTCCATCGTG |
| 155 | ACCCAGCCTGACAGACAATC |
| 160 | GAACCTGATGGACATGTTCAGG |
| 161 | AGTGCGTTCGAACGCTAGAGCCTGT |
| 1414 | CGTACTGACGGTGGGAGAAT |
| 1416 | CCCGGCAAAACAGGTAGTTA |

PCR fragments expected size (bp):

| Primer pair | 160-161 | 1414-1416 | 154-155 |
|---|---|---|---|
| Region analyzed | 5' part of Cre transgene | Middle of Cre transgene | Myogenin control gene |
| Control gene | / | / | 99 |
| Tg allele | 345 | 165 | / |

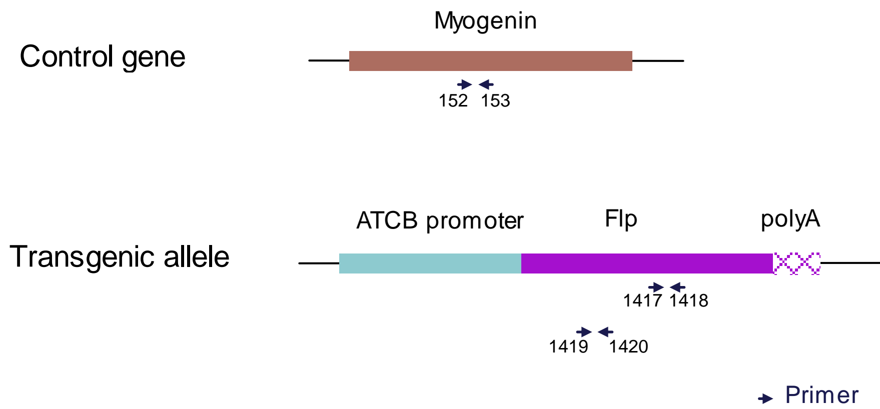
Cycling conditions:

| Temp | Time | #Cycles |
|---|---|---|
| 95°C | 3min | 1 |
| 95°C | 10s | |
| 62°C | 20s | 35 |
| 72°C | 20s | |
| 95°C | 5s | |
| 62°C | 30s | 1 (melting curve generation) |
| 72°C | 72s | |
| 37°C | 30s | 1 |
| 4°C | ∞ | |

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

### 2.2. Flp genotyping

Schematic representation of the genotyping strategy




Sequence of primers used for genotyping

| Primers | Sequence |
|---|---|
| 152 | TTACGTCCATCGTGGACAGC |
| 153 | TGGGCTGGGTGTTAGCCTTA |
| 1417 | TTCTTTAGCGCAAGGGGTAG |
| 1418 | GCTCCAATTTCCCACAACAT |
| 1419 | TGGGAAATTGGAGCGATAAG |
| 1420 | CTGCCACTCCTCAATTGGAT |

PCR fragments expected size (bp):

| Primer pair | 1417-1418 | 1419-1420 | 152-153 |
|---|---|---|---|
| Region analyzed | Middle part of Flp transgene | 5' of Flp transgene | Myogenin control gene |
| Control gene | / | / | 245 |
| Tg allele | 299 | 175 | / |

PCR protocol and cycling conditions are identical to those described in chapter 1.2