# Genotyping protocol

## Klhl29

## IR00003008 / E182

(ICS internal reference)


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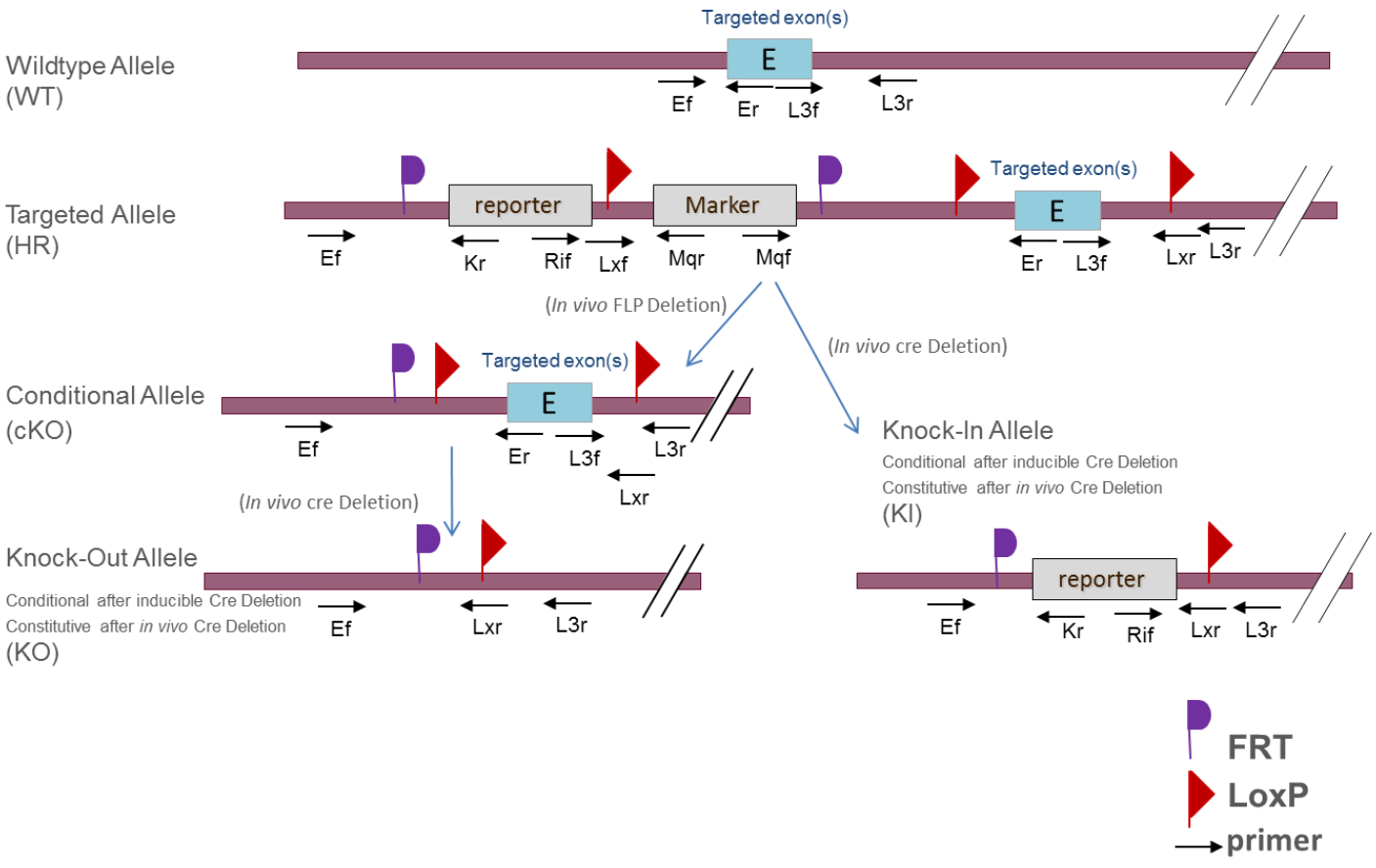
# 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Klhl29** Constitutive Knockout / Conditional Knockout (KO-cKO x Cre) 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 | 5380 | TACCCCAGAAGTCTGTGAAAGCCTG |
| Er | 6554 | ACATCTCTTGGGCACTGAGCGC |
| Er[2] | 1936 | GTGGATGTGGAATGTGTGCGAGG |
| Er[3] | 5383 | ACTGAAAGATTGGGGAGTAGGCTGG |
| Kr | 3209 | CCAACAGCTTCCCCACAACGG |
| L3f | 5381 | CAGCAACGCAGAGCGAAAGCA |
| L3r | 5382 | CCTGAGGGATGGAGTAGTGTGTAATGG |
| Lxf | 6295 | TTATGTTTAAACGGCGCGCCC |
| Lxf[2] | 6013 | TCATGTCTGGATCCGGAATAACTTCGTA |
| Lxr | 3255 | ACTGATGGCGAGCTCAGACCATAAC |
| Rif | 5966 | GCACATGGCTGAATATCGACGGT |

[2]: for a selected position, a second primer was designed
[3]: for a selected position, a third primer was designed

PCR fragments expected size (bp):

| Region analyzed | Primers used | Position on the primer *(see the map above)* | Targeted allele (HR) | conditional allele (cKO) | KO allele | WildType allele |
|---|---|---|---|---|---|---|
| Lox interne K7 Eur (with DMSO) | 6295-6554 (with 5% DMSO) | Lxf / Er | 119 | --- | --- | --- |
| Lox interne K7 Eur (with DMSO) | 6013-1936 (with 5% DMSO) | Lxf[2] / Er[2] | 199 | --- | --- | --- |
| 5' part of the selection marker | 5380-3209 | Ef / Kr | 383 | --- | --- | --- |
| Presence of the distal loxP | 5381-5382 | L3f / L3r | 485 | 485 | --- | 538 |
| Distal loxP specific PCR | 5381-3255 | L3f / Lxr | 351 | 351 | --- | --- |
| Excision of the selection marker | 5380-5383 | Ef / Er[3] | 7448* | 492 | --- | 359 |
| Cre total excision | 5966-3255 | Rif / Lxr | * | | | |

*: 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 | 5min | 1 |

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

## 2. Cre and Flp genotyping method

You will find the genotyping protocol in the publication:

Highly-efficient, fluorescent, locus directed cre and FlpO deleter mice on a pure C57BL/6N genetic background.
Birling MC, Dierich A, Jacquot S, Hérault Y, Pavlovic G.
Genesis. 2012 Jun;50(6):482-9. doi: 10.1002/dvg.20826. Epub 2012 Mar 20.