EXCELLENCE IN MOUSE PHENOGENOMICS

# Genotyping protocol

Project Cd3e

(PHENOMIN-ICS reference IR00004766 / E4766)

This report has been **prepared** by: Amélie JEANBLANC

This report has been **validated** by: Sylvie Jacquot, PhD
Head of Genotyping Service

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**For any question, please contact:**

**PHENOMIN-ICS**
Email: genotypingrequest@igbmc.fr
Web site: http://www.ics-mci.fr/

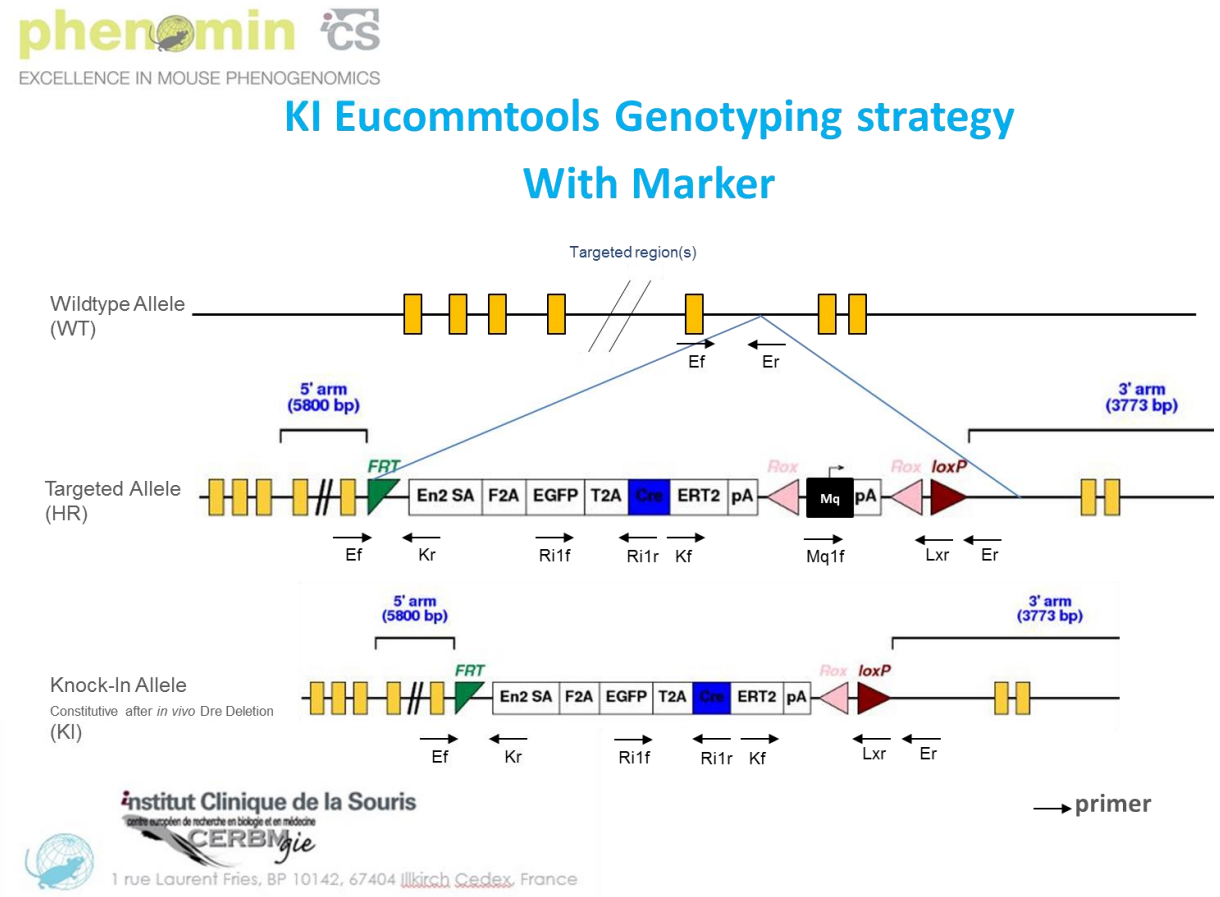
# Table of contents

# 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Cd3e** Eucommtools Knockin (KI E-Tool marker) 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 | 7482 | GTCACCTGGGGGGCTTATGGTT |
| Er | 7483 | CAGTAGACAGGGACTTGGGAAGGGAG |
| Lxr | 6957 | CTATACGAAGTTATGGCGCG |
| Kf | 5990 | CGGGCTCTACTTCATCGCATTCCTT |
| Kr | 3278 | GGGCAAGAACATAAAGTGACCCTCC |
| Mq1f | 7353 | GCTTCACCGTCACCGCCGA |
| Ri1f | 2344 | CGACCACTACCAGCAGAACACC |
| Ri1r | 5626 | GGTTCTTGCGAACCTCATCACTCGT |

PCR fragments expected size (bp):

| Region analyzed | Primers used | Position on the primer *(see the map above)* | Targeted allele (HR) | KI allele | WildType allele |
|---|---|---|---|---|---|
| Excision of the selection marker | 5990-6957 | Kf / Lxr | 2157* | 405** | --- |
| GFP-Cre | 2344-5626 | Ri1f / Ri1r | 317 | 317 | --- |
| WildType allele specific PCR (5' part of the targeted locus) **(with DMSO)** | 7482-7483 | Ef / Er | 6509* | 4757* | 340 |
| Cassette 5' | 7482-3278 | Ef / Kr | 366 | 366 | --- |
| 3' part of the selection marker | 7353-6957 | Mq1f / Lxr | 671 | --- | --- |

*: 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.