# Genotyping protocol

## Pik3c3

## IR00002476 / E42

(ICS internal reference)


This report has been prepared by: **Karim Essabri**
genotyping@igbmc.fr

This report has been validated by: **Sylvie Jacquot, PhD, Head of Genotyping Service**
33 (0)3 88 65 57 44
genotyping @igbmc.fr


The first version of this report was generated the: 08 Jul 2010

For any question, please contact:


**Institut Clinique de la Souris - ICS - Mouse Clinical Institute**
1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex, France
Email: genotyping@igbmc.fr
Web site: http://www-mci.u-strasbg.fr/

# TABLE OF CONTENTS

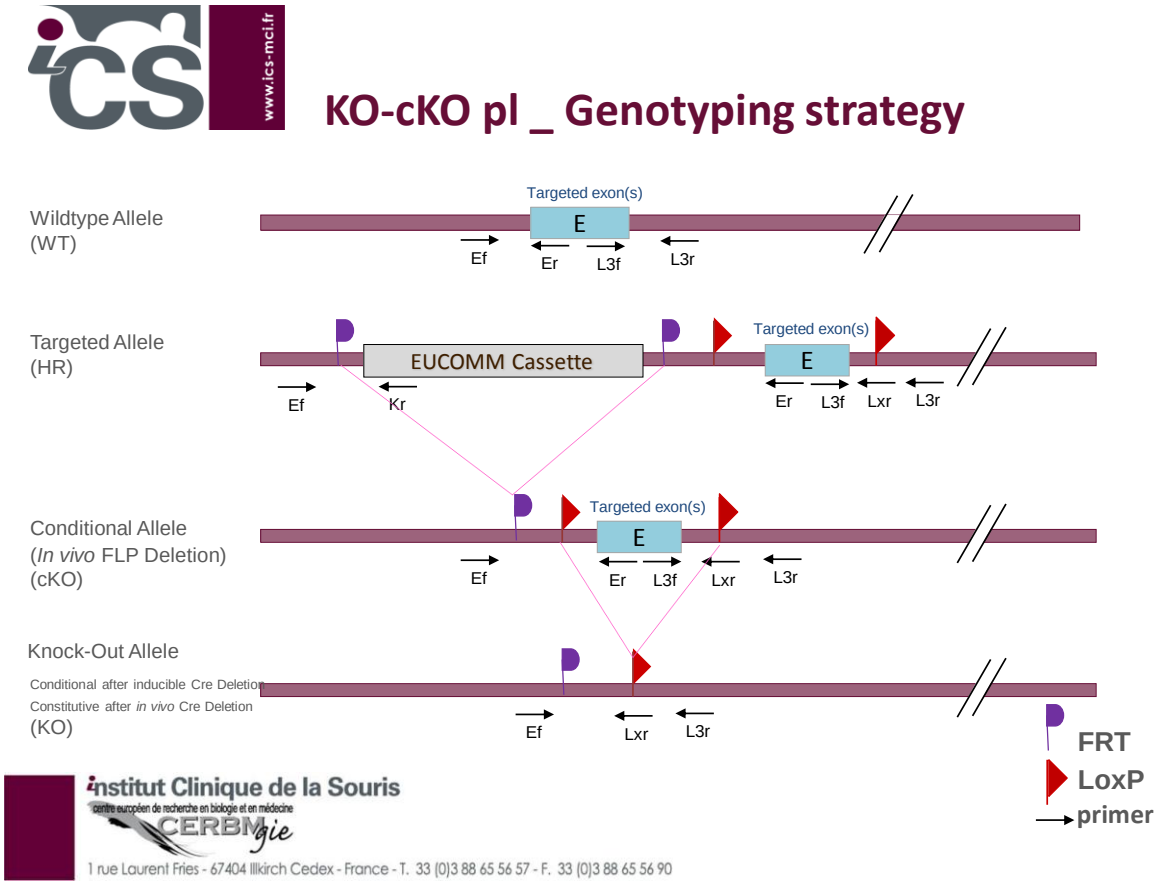


# 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Pik3c3** 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 | 4395 | ATTTATGGAATGGATCCCCGAGTGGAG |
| Ef[2] | 4396 | CTTGCTGGCATATGCAATAGTGTCTGG |
| Er | 5163 | AACTCAAGAAGAAGGAAGCCCAAAGTG |
| Kr | 3209 | CCAACAGCTTCCCCACAACGG |
| L3f | 4399 | GGGTACTTGATGTTGTGAGTCC |
| L3f[2] | 4398 | CCGCCTCGCCAAGGTACACAGG |
| L3r | 4397 | AAATACCAGAGACCCACATTTC |
| Lxr | 3255 | ACTGATGGCGAGCTCAGACCATAAC |

[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 (HR) | conditional allele (KO-cKO) | WildType allele |
|---|---|---|---|---|---|
| 5' part of the selection marker | 4395-3209 | Ef / Kr | 257 | --- | --- |
| Presence of the distal loxP | 4399-4397 | L3f / L3r | 491 | 491 | 505 |
| Distal loxP specific PCR | 4398-3255 | L3f2 / Lxr | 245 | 245 | --- |
| Excision of the selection marker | 4396-5163 | Ef[2] / Er | 5752* | 345 | 196 |

*: 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:

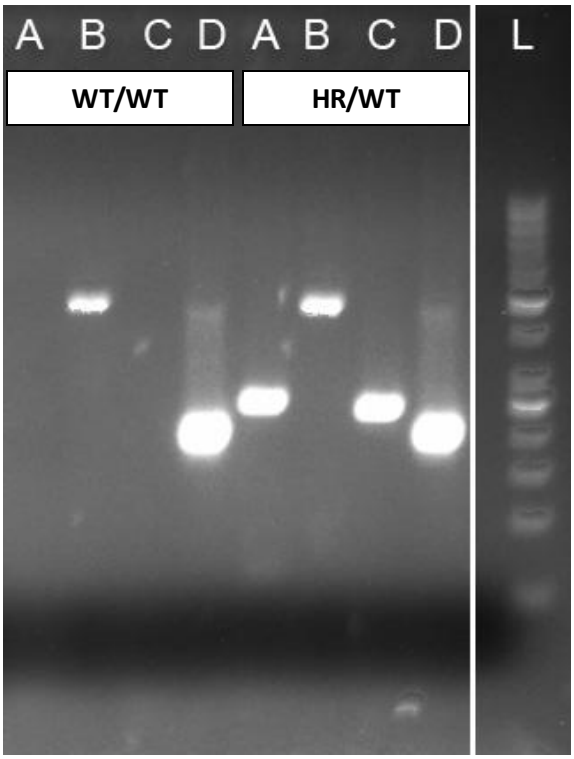
| Temp | Time | #Cycles |
|---|---|---|
| 95°C | 4min | 1 |
| 94°C | 30s | |
| 62°C | 30s | 34 |
| 72°C | 1min | |
| 72°C | 7min | 1 |
| 20°C | 5min | 1 |

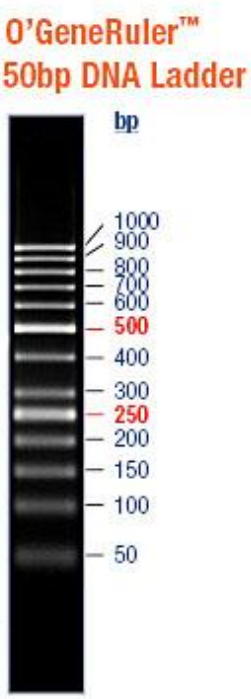
**NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.**

### 1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was done by gel electrophoresis 2% agarose (SB buffer).

Representative genotyping picture




A: 5' part of the selection marker
B: Presence of the distal loxP
C: Distal loxP specific PCR
D: Excision of the selection marker
L: 0'GeneRuler 50bp DNA Ladder

## 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.