



## Genotyping protocol

Cstb

/ P5378

(ICS internal reference)

This report has been prepared by: **Christelle Roth & Valérie Rousseau**  
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: 26 Jun 2015

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](mailto:genotyping@igbmc.fr)  
Web site: <http://www-mci.u-strasbg.fr/>

## TABLE OF CONTENTS

**Table of contents** .....2

**1. Genotyping protocol and data** .....2

    1.1. Genotyping strategy .....2

    1.2. PCR protocol .....4

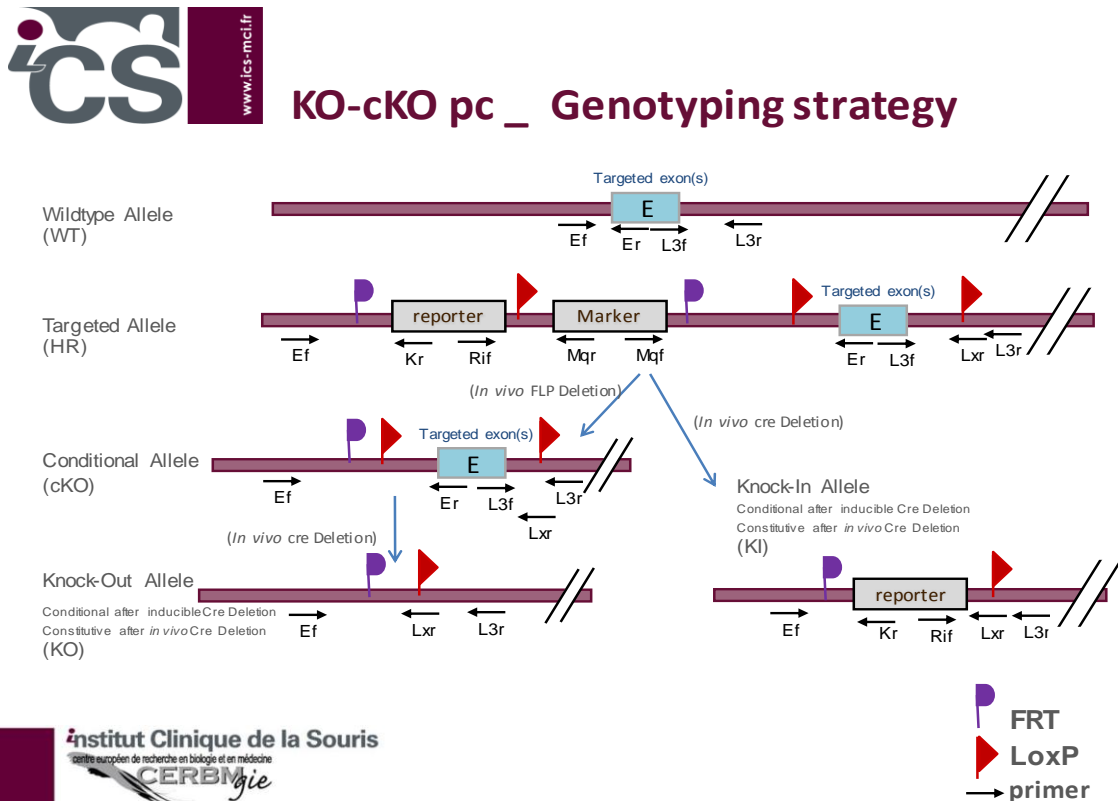
**2. Cre and Flp genotyping method** .....5

### 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Cstb** 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	8522	GAACTTGCTTGTGCCTAGTCTGAAACGAG
Er	8525	CTTGCCTGGCCAGTGTGTCTGTAAC
Kr	3209	CCAACAGCTCCCCACAACGG
L3f	8523	AGTGGTGGCTCATGCCTTTAATCCC
L3r	8524	CTCCAGCAGGTTGTCCTCGGAC
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Rif	5966	GCACATGGCTGAATATCGACGGT

<sup>2</sup>: 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 (cKO)	KI allele	WildType allele
5' part of the selection marker	8522-3209	Ef / Kr	343	---	---	---
Presence of the distal loxP	8523-8524	L3f / L3r	342	342	---	264
Distal loxP specific PCR	8523-3255	L3f / Lxr	230	230	---	---
Excision of the selection marker	8522-8525	Ef / Er	7318*	414	---	306
Cre total excision	5966-3255	Rif / Lxr	3768*	---	471**	---

\*: 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 <sub>2</sub> O	up to 15 µl

### Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
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.