



## Genotyping protocol

Mtrf1

IR00002804 / E122

(ICS internal reference)

This report has been prepared by: **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 finalized the: 05 Dec 2012

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

    2.1. Cre and Flp genotyping .....5

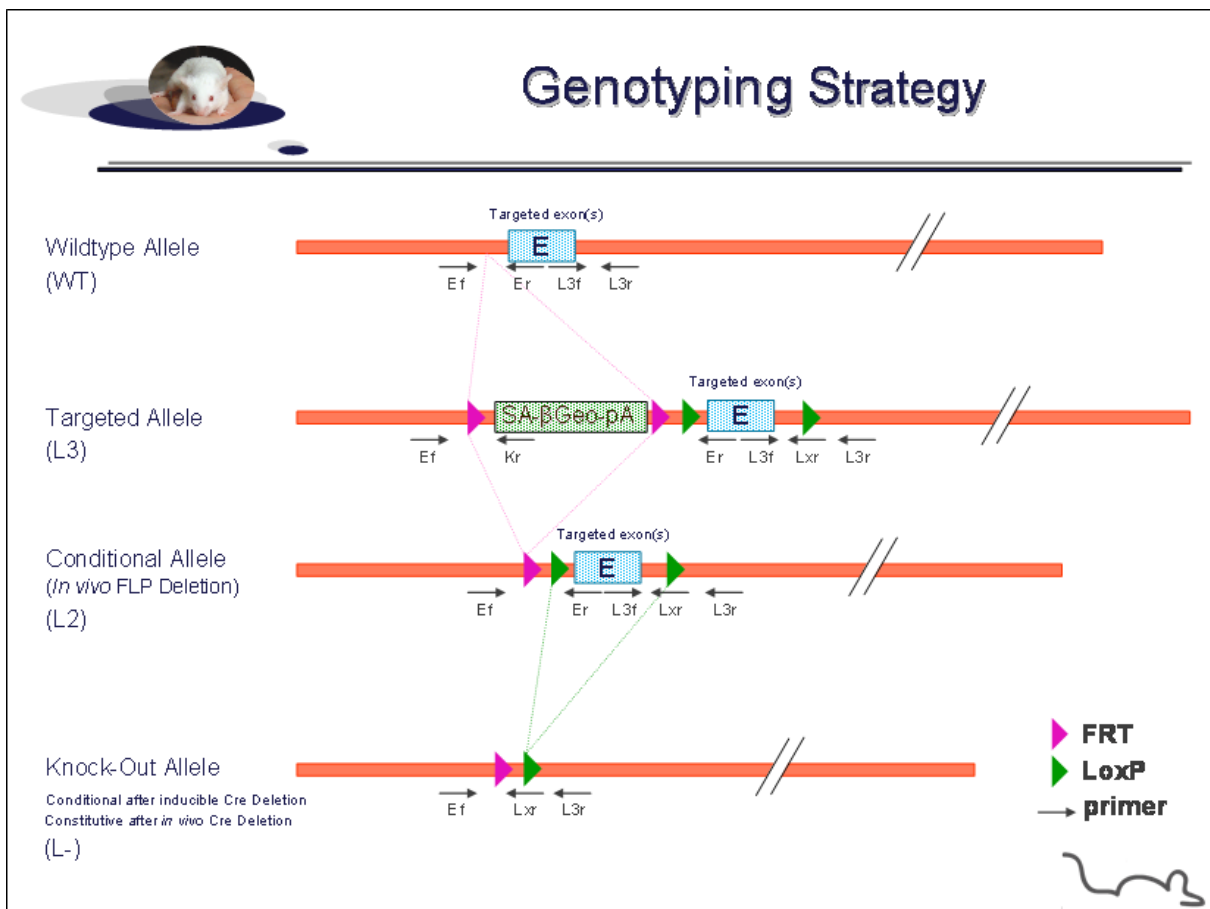
    2.2. PCR Protocol .....6

### 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Mtrf1** 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	4965	ATGGTTCCTCTCCATCCAAGGGAG
Ef <sup>2</sup>	4964	ACCACCACCCGGTGA ACTCTTG
Er	4969	CACCACCAGGCCAGTGTATTTTAGACA
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	4970	TGAAAAGCAGTTGCAAGAACTTGTGTC
L3f <sup>2</sup>	4966	TGTCTAAAATACACTGGCCTGGTGGTG
L3r	4968	CTAACTTTCCTTACTACAGGGTCA TGCTG
L3r <sup>2</sup>	4967	CATCAGAACTCAGGTTGTAGGACAGG
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC

<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 (KO allele) (L3)	cKO allele (L2)	KO allele (L-)	WildType allele (WT)
5' part of the selection marker	4965-3209	Ef / Kr	282	---	---	---
Presence of the distal loxP (with DMSO)	4970-4968 (with 5% DMSO)	L3f / L3r	386	386	---	344
Distal loxP specific PCR (with DMSO)	4966-3255 (with 5% DMSO)	L3f <sup>2</sup> / Lxr	667	667	---	---
Excision of the selection marker	4965-4969	Ef / Er	7308*	352	---	264
Excision of the floxed exon(s), i.e. knock out	4964-4967	Ef <sup>2</sup> / L3r <sup>2</sup>	8290*	1334*	579**	1204**

\*: 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.**

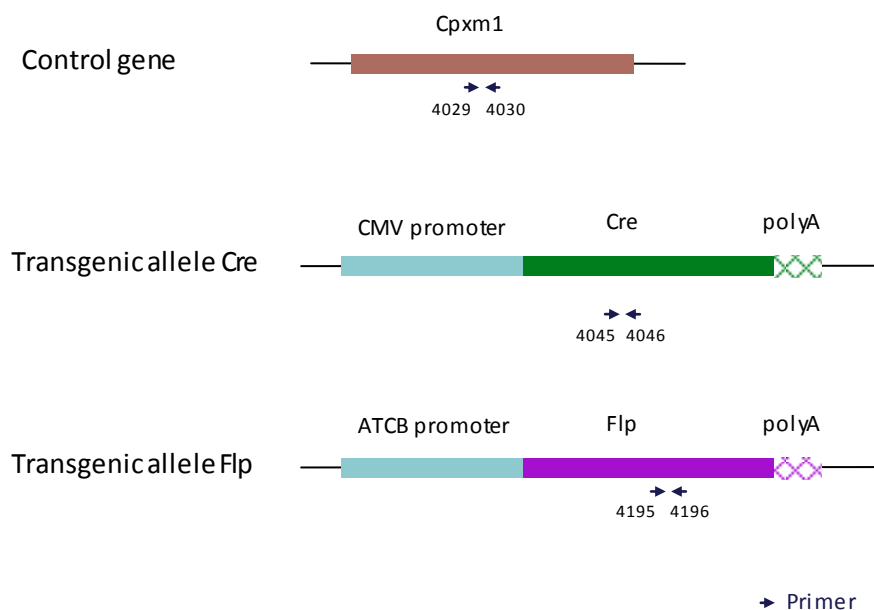
## 2. Cre and Flp genotyping method

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

Detection of cre transgene and flp transgene is done using a multiplex assay: primer pairs were designed for each gene and for a positive control (Cpxm1 gene).

### 2.1. Cre and Flp genotyping

Schematic representation of the genotyping strategy



Sequence of primers used for genotyping:

Primers	Sequence
4029	ACTGGGATCTTCGAACTCTTTGGAC
4030	GATGTTGGGGCACTGCTCATTACCC
4045	CCATCTGCCACCAGCCAG
4046	TCGCCATCTTCCAGCAGG
4195	TCTTTAGCGCAAGGGGTAGGATCG
4196	GTCCTGGCCACGGCAGAAGC

PCR fragments expected size (bp):

Primer pair	4045-4046	4195-4196	4029-4030
Region analyzed	Middle part of Cre transgene	Middle part of Flp transgene	Cpxm1 control gene
Control gene	/	/	397
Tg allele	281	328	/

## 2.2. 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)	0.05µl
3' primer (100 µM)	0.05µl
Sterile H <sub>2</sub> O	up to 15 µl

Cycling conditions are identical to those described in chapter 1.2