



Genotyping protocol

Tmod3

IR00003011 / E185

(ICS internal reference)

This report has been prepared by: **David MOULAERT**
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: 24 Apr 2014

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

Table of contents2

1. Genotyping protocol and data2

 1.1. Genotyping strategy2

 1.2. PCR protocol4

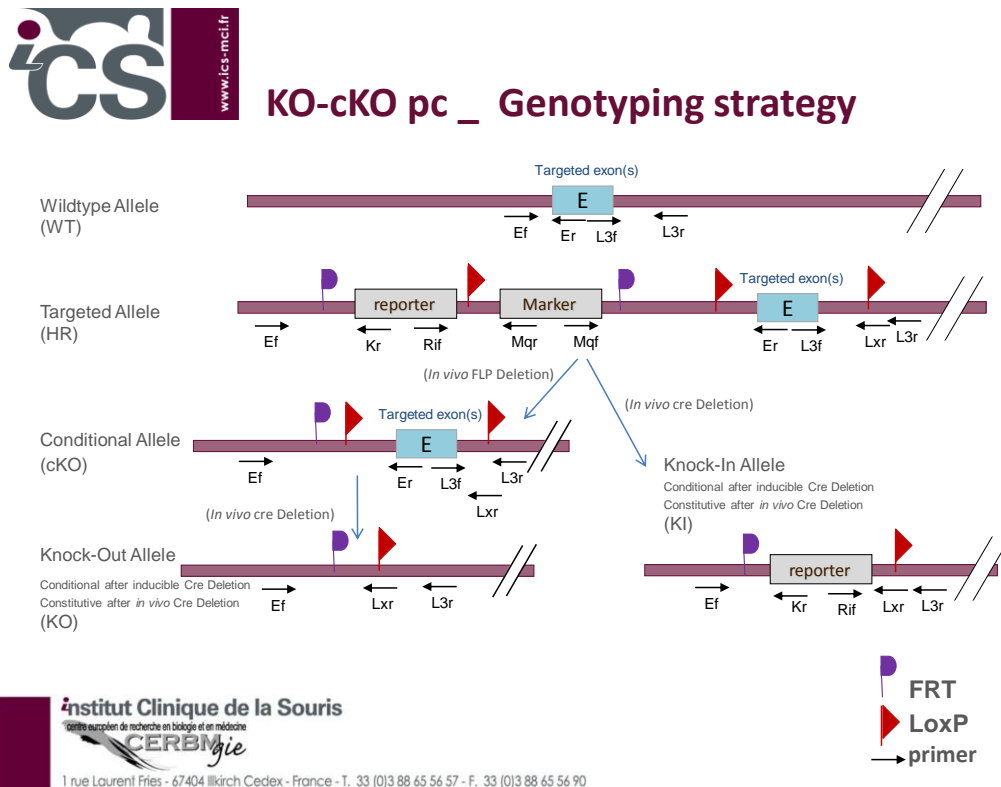
2. Cre and Flp genotyping method5

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Tmod3** 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	5395	GACTTGTCGGCAGCAATGACACA
Ef ²	5394	AAATGCTGGTGGCTGTTTCCTGC
Er	5399	TTGGTTCCTTCCTTCTACTGGGATCTT
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	5397	AGTAGTTCTGGGTTCTTTTGGGAATGG
L3r	6283	GGAAAGAGCACTGCTTTGCTGTC
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	5395-3209	Ef / Kr	258	---	---
Presence of the distal loxP (with DMSO)	5397-6283 (with 5% DMSO)	L3f / L3r	331	331	301
Distal loxP specific PCR	5397-3255	L3f / Lxr	251	251	---
Excision of the selection marker (with DMSO)	5394-5399 (with 5% DMSO)	Ef ² / Er	7369*	465	353

*: 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 ₂ 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.