Genotyping protocol

Plscr1

IR00002917 / E159

(ICS internal reference)

This report has been prepared by: **Nathalie Chartoire**
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: 13 May 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/

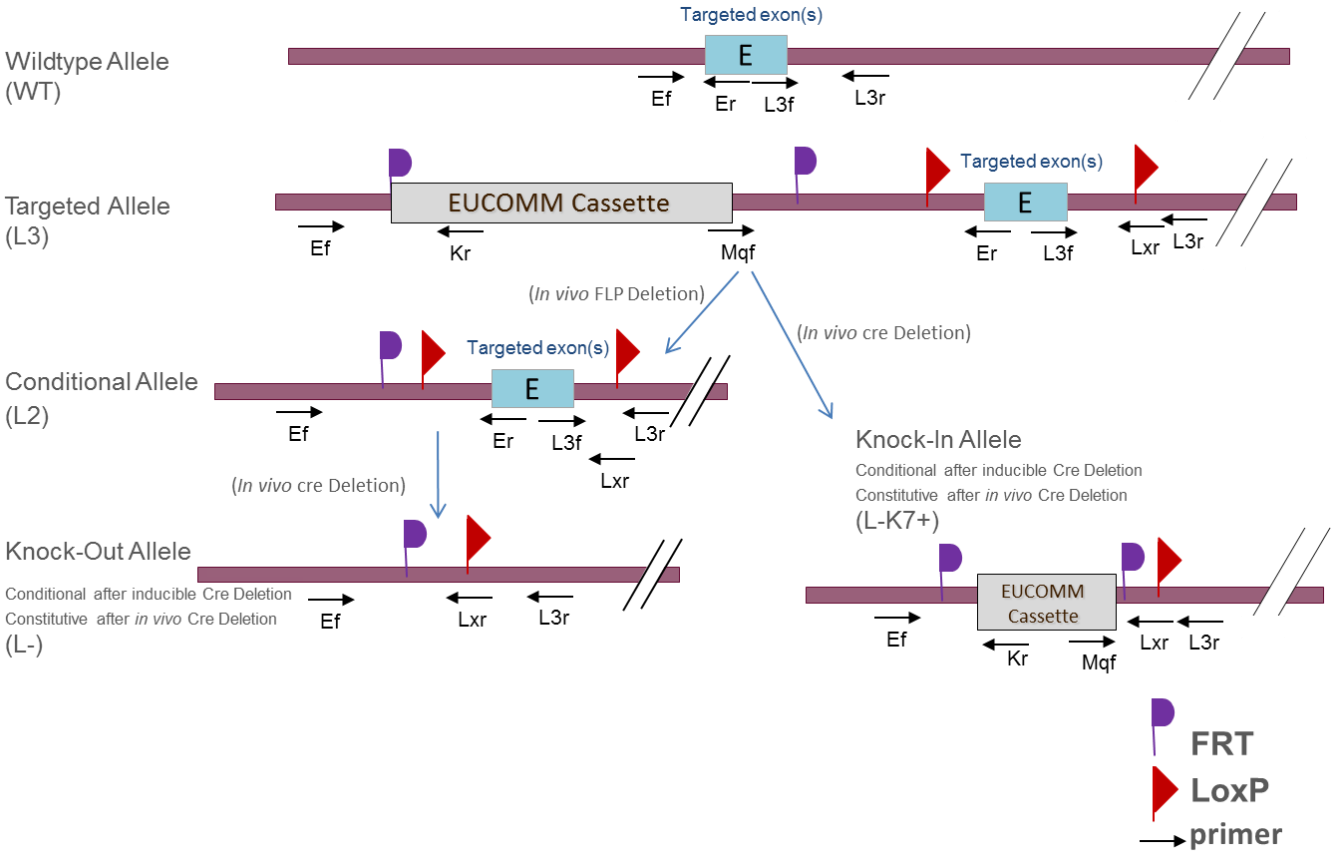
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Plscr1** 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	5298	AGACACAGAGCTGCTAAGAGCCTCTTG
Er	6554	ACATCTCTTGGGCACTGAGCGC
Er[2]	1936	GTGGATGTGGAATGTGTGCGAGG
Er[3]	5303	GGGTGTGAAAAAGGAGAGTGAAAAGTG
Kr	3278	GGGCAAGAACATAAAGTGACCCTCC
L3f	5302	ATGTCTGCCCAAGTTCACTCTCCAA
L3f[2]	5301	GCTGCTGTTCCGACATTGACTTTG
L3r	5300	ACAGTGAGCAACTGTCAACCGAGC
Lxf	6295	TTATGTTTAAACGGCGCGCCC
Lxf[2]	6013	TCATGTCTGGATCCGGAATAACTTCGTA
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Mqf	4981	GGGATCTCATGCTGGAGTTCTTCG

[2]: for a selected position, a second primer was designed
[3]: for a selected position, a third 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)	KO allele	WildType allele
Lox interne K7 Eur (with DMSO)	6295-6554 (with 5% DMSO)	Lxf / Er	119	---	---	---
Lox interne K7 Eur (with DMSO)	6013-1936 (with 5% DMSO)	Lxf[2] / Er[2]	199	---	---	---
5' part of the selection marker	5298-3278	Ef / Kr	324	---	---	---
Presence of the distal loxP	5302-5300	L3f / L3r	427	427	---	383
Distal loxP specific PCR	5301-3255	L3f[2] / Lxr	290	290	---	---
Excision of the selection marker	5298-5303	Ef / Er[3]	5749*	335	---	162
Exon excision	4981-3255	Mqf / Lxr				

*: 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.