Genotyping protocol

Sdsl

IR00002871 / E142

(ICS internal reference)

This report has been prepared by: **Christelle Roth**
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: 20 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/

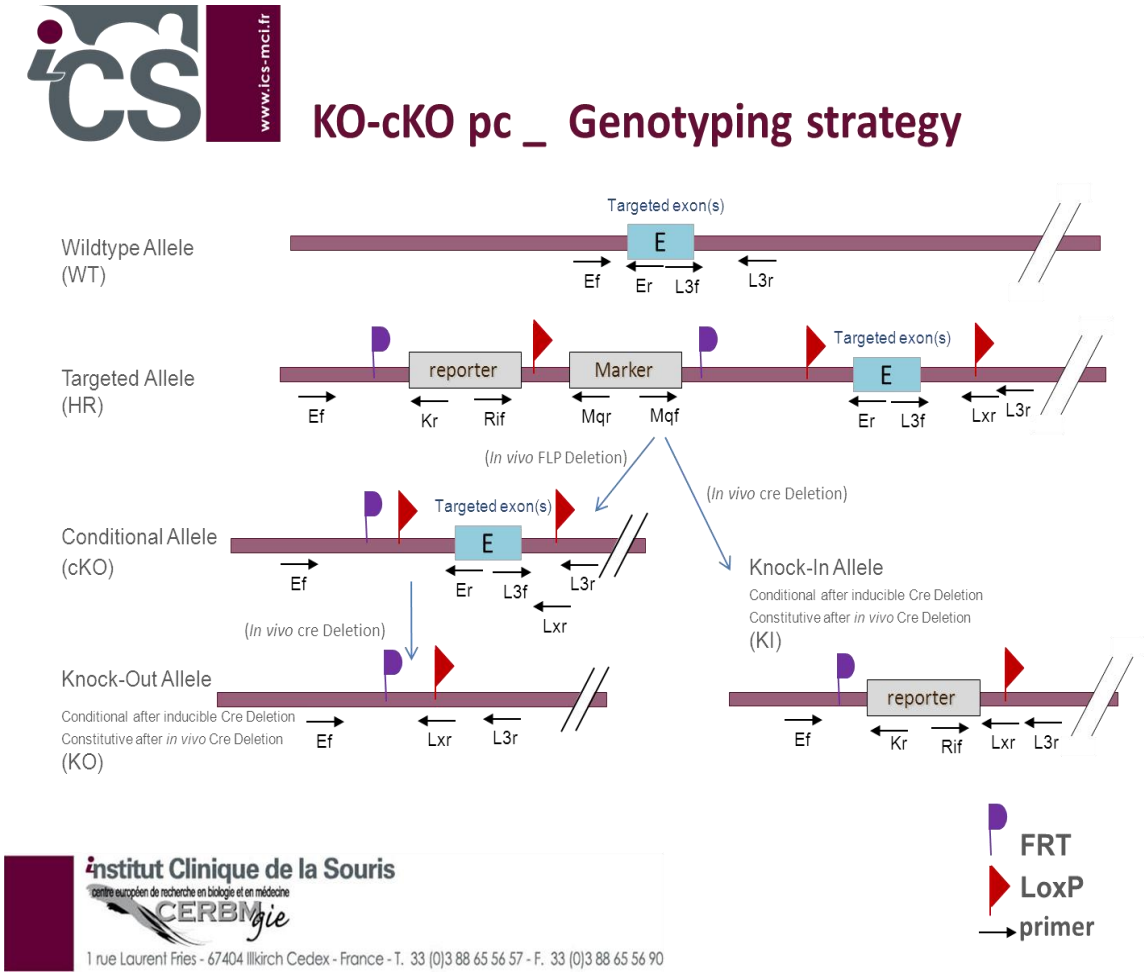
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Sdsl** 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.



institut Clinique de la Souris
centre européen de recherche en biologie et en médecine
CERBMgie
1 rue Laurent Fries - 67404 Illkirch Cedex - France - T. 33 (0)3 88 65 56 57 - F. 33 (0)3 88 65 56 90

Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	5315	GCTTGGCTTTCCCTTCCTTCCTTC
Er	5318	AAGAGTGTTCAGAACTTGGAGCCTCTG
Kr	3210	CCTGTCCCTCTCACCTTCTACC
L3f	5319	CGAGGTCCAGCTGACTGGGAAA
L3f[2]	5320	GGGCACGAGAAGTCTGAGGAACTCC
L3r	5316	CGACTTCAAGGGAGGTCCCGTTATT
Lxr	5079	GGCGAGCTCAGACCATAACTTCGTATA
Lxr[2]	3255	ACTGATGGCGAGCTCAGACCATAAC
Rif	5966	GCACATGGCTGAATATCGACGGT

[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 (cKO)	KI allele	WildType allele
5' part of the selection marker (with DMSO)	5315-3210 (with 5% DMSO)	Ef / Kr	531	---	---	---
Presence of the distal loxP	5319-5316	L3f / L3r	351	351	---	405
Distal loxP specific PCR	5320-5079	L3f[2] / Lxr	410	410	---	---
Excision of the selection marker	5315-5318	Ef / Er	7449*	545	---	330
Cre total excision	5966-3255	Ri1f / Lxr[2]	*		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_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.