Genotyping protocol

Pnpla1

IR00004147 / P4147

(ICS internal reference)

This report has been prepared by: **Pauline Cayrou**
33 (0)3 88 65 56 55
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: 31 May 2013

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/

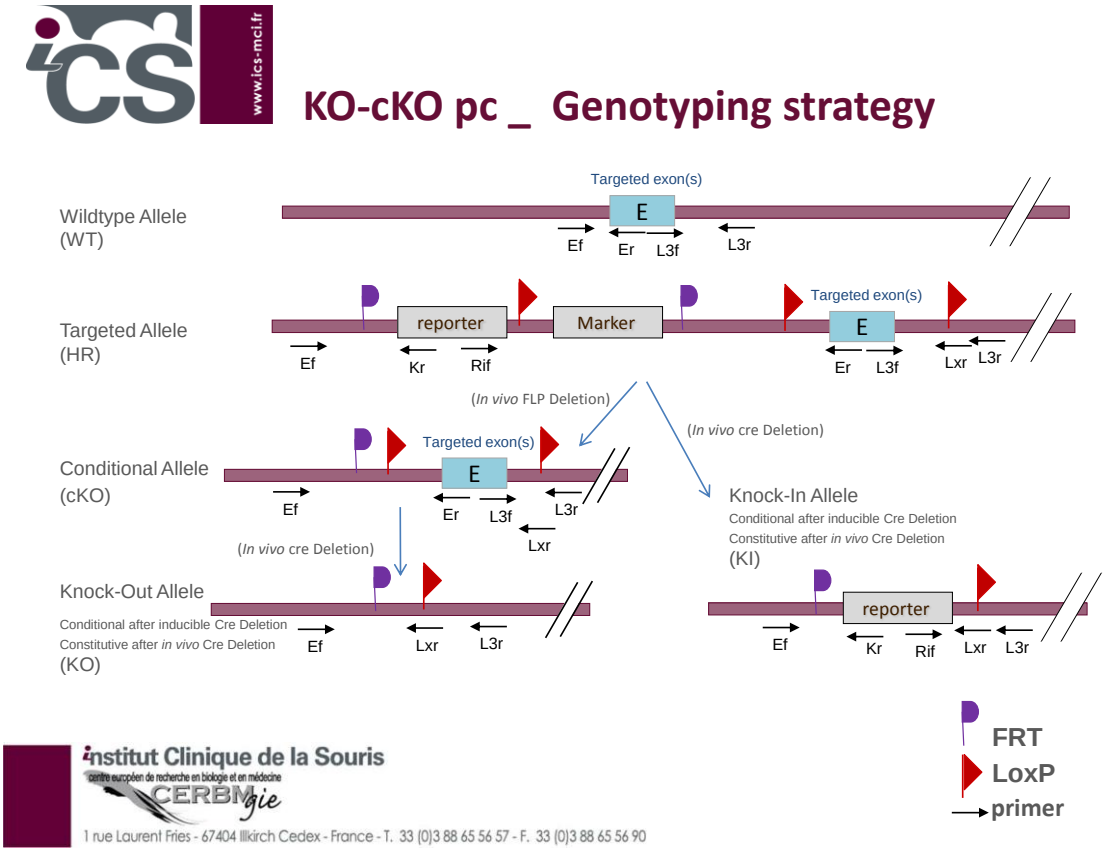
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Pnpla1** 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.



Institut Clinique de la Souris
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	6891	TGGCACCGCATAAACCAAGCAT
Er	6894	TGCACCTGAGGTCTGCCACCAA
Kr	3278	GGGCAAGAACATAAAGTGACCCTCC
L3f	6892	GGGGCACCTTAAGGTGAGGATCGTT
L3r	6893	GAAAGCCTGTGACCACTGACCCAG
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Mqf	4981	GGGATCTCATGCTGGAGTTCTTCG
Rif	5966	GCACATGGCTGAATATCGACGGT

PCR fragments expected size (bp):

N° of PCR	Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR allele)	cKO allele	KO allele	KI allele	WildType allele (WT)
1	5' part of the selection cassette	6891-3278	Ef / Kr	419	---	---	419	---
2	Presence of the distal loxP	6892-6893	L3f / L3r	297	297	---	---	247
3	Excision of the selection cassette	6891-6894	Ef / Er3	7337*	433**	---	---	230
4	Distal loxP specific PCR	6892-3255	L3f / Lxr	246	246	---	---	---
	Exon and Marker excision	5966-3255	Mq1f / Lxr	3692*	---	---	471**	---
	Total excision	6891-6893	Ef / L3r	8615*	---	401**	---	1458*

*: 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:

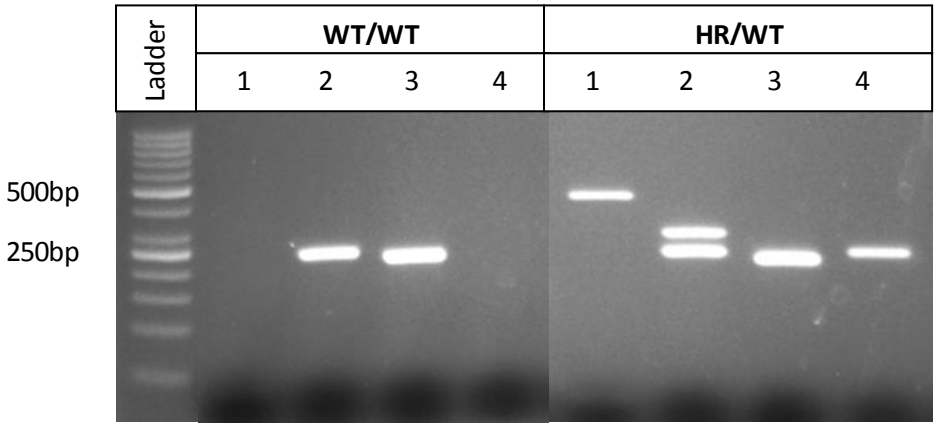
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was done by gel electrophoresis

Representative genotyping picture



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.