EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project LincPint cKO

Lncpint$^{tm1.1Ics}$

(PHENOMIN-ICS reference IR00007657 / Kos7657)

For any question, please contact:

PHENOMIN-ICS
Email: mutagenesis@igbmc.fr
Web site: http://www.ics-mci.fr/

Table of contents

This protocol describes the condition used at the Institut Clinique de la Souris (ICS) to genotype the **Lncpint** Conditional Knockout (cKO) mouse line.

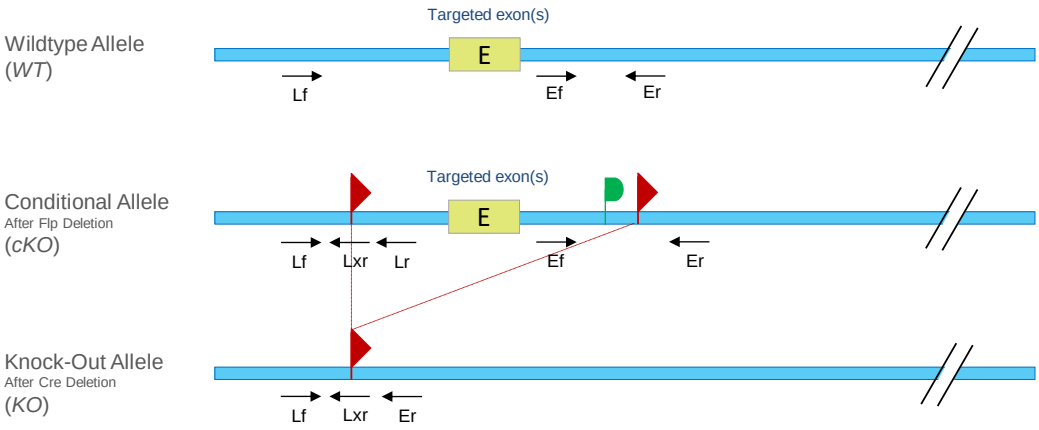
1. Genotyping protocol and data

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.

phenomin ics
EXCELLENCE IN MOUSE PHENOGENOMICS

cKO Genotyping strategy



Institut Clinique de la Souris
CERBMgie
1 rue Laurent Fries, BP 10142, 67404 Illkirch Cedex, France

Sequence of primers used for genotyping:

Position	Sequence
Ef	GGCTTATTCGTGTCTAGTGATGTATGG
Er	GGTCCTCTCCCATCCCACTCC
Er^2	GAATGTTGGTCCTCTCCCATCCC
Lf	GAGTTGCCTCTAGGTTCGGGATG
Lf^2	GGGATGTTGGTCTCCTCTGAGTG
Lr	CAGACGAAGAGGAGGAGGAGG
Lxr	CATACATTATACGAAGTTATCTGCAG

^{2}: for a selected position, a second primer was designed

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	cKO allele	KO allele	WildType allele
Presence of the distal loxP ***(with Betaine)***	Lf / Lr	182	---	---
Excision of the selection marker ***(with DMSO)***	Ef / Er	407	---	301
LoxP specific PCR ***(with Betaine)***	Lf / Lxr	104	104	---
Excision of the floxed exon(s), i.e. knock out ***(with DMSO)***	Lf / Er	2399*	308**	2244*
Excision of the floxed exon(s), i.e. knock out 2 ***(with DMSO)***	Lf^2 / Er^2	2389*	298**	2234*

*: amplicon will not be observed using our genotyping conditions
**: 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

If mentioned in table "PCR fragments expected size" add 0.5% of Betaine or 5% of DMSO in the reaction mix

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	35
72°C	1min	
72°C	7min	1
14°C	---	---

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