EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Prrx1 cKO

(PHENOMIN-ICS reference IR00006431 / Kos6431)

For any question, please contact:

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

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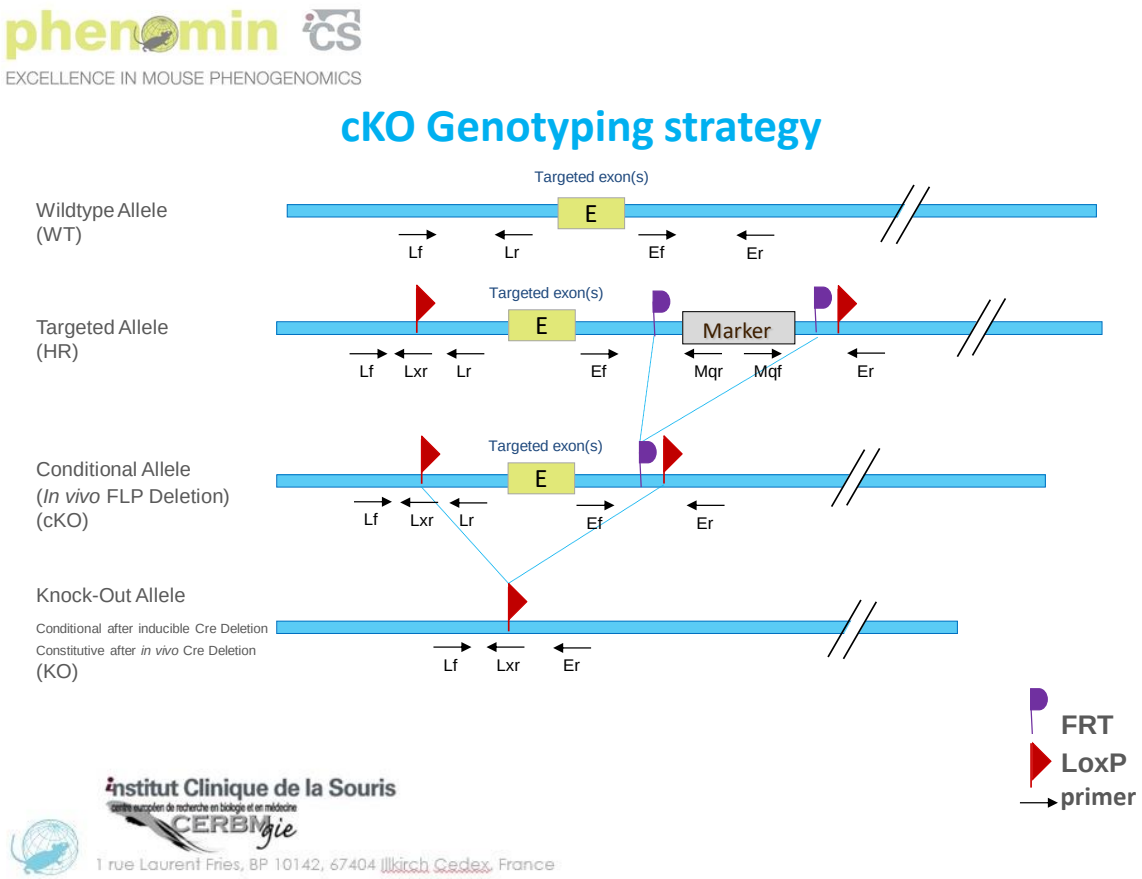
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Prrx1** Conditional Knockout (cKO) mouse line.

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	Sequence
Ef	GGAATATGCTGTAGGGAAGGGAAGGC
Er	GCTCAGGTGAGGAAGAGTTATCTATGGCC
Lf	CCCGTCTTTGGTGAAATGCAGAGAAC
Lr	GCCAAGAATCCTCCTCAGTCCTGG
Lxr	CGAAGTTATCTGCAGGTCGACCTTAAG
Mq1f	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mq1r	CATCTGCACGAGACTAGTGAGACG

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	cKO allele	KO allele	WildType allele
Presence of the distal loxP	Lf / Lr	254	254	---	174
Excision of the selection marker (with Betaine 0,5 %)	Ef / Er	2117*	263	---	158
5' part of the selection marker (with DMSO 5%)	Ef / Mq1r	424	---	---	---
3' part of the selection marker (with Betaine 0,5%)	Mq1f / Er	247	---	---	---
LoxP specific PCR	Lf / Lxr	138	138	138	---
Excision of the floxed exon(s), i.e. knock out (with Betaine 0,5%)	Lf / Er	2920*	1066*	239	881*

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)
---: 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.