EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Lrba cKO

Lrba$^{tm1.1Ics}$

(PHENOMIN-ICS reference IR00005807 / K5807)

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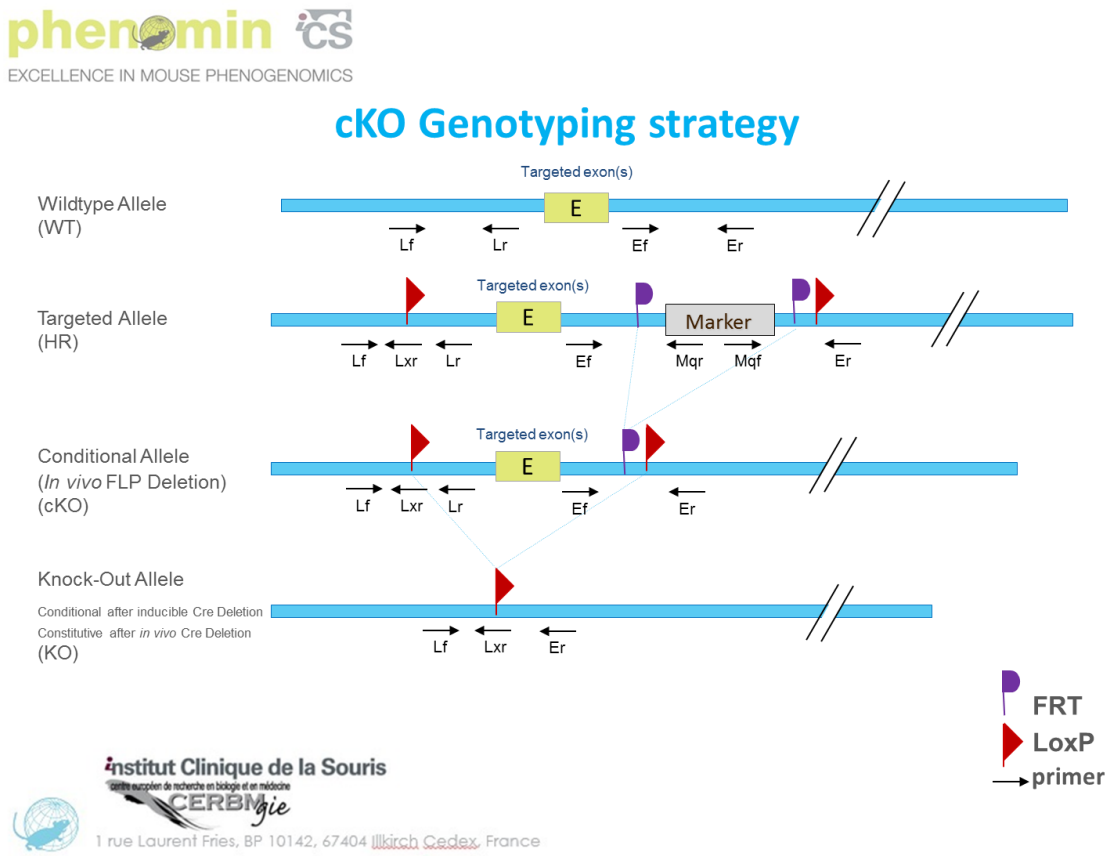
Table of contents

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Lrba** 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	GCCTGAGGCAGCCACAGTGTG
Er	CCACTTAAAATAAGCCATTGCCCACC
Er[2]	CTGCCGGGAATAAAACAGTCTAACCTC
Lf	GGTGAGGAGGAACTTTGGACCTCAG
Lf[2]	CGAGCGCTGAGGAGAATCACCG
Lr	CTGACAACAACGCCAAAGCCTATTG
Lxr	CGAAGTTATCTGCAGGTCGACCTTAAG
Mqf	CAGCTCATTCCTCCCACTCATGATC
Mqr	TGCTAAAGCGCATGCTCCAGACTGC

[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)*	Targeted allele (HR)	cKO allele	KO allele	WildType allele
Presence of the distal loxP (with DMSO)	Lf / Lr	415	415	---	334
Excision of the selection marker (with Betaine)	Ef / Er	2079*	225	---	120
5' part of the selection marker	Ef / Mqr	187	---	---	---
3' part of the selection marker (with Betaine)	Mqf / Er	358	---	---	---
LoxP specific PCR (with DMSO)	Lf / Lxr	206	206	206	---
Excision of the floxed exon(s), i.e. knock out	Lf / Er[2]	3525*	1671*	293	1486*
Excision of the floxed exon(s), i.e. knock out (bis)	Lf[2] / Er	3904*	2050*	672	1865*

*: 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.

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.