EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Gnal cKO

Gnal$^{tm1.1Ics}$

(PHENOMIN-ICS reference IR00006505 / Kos6505)

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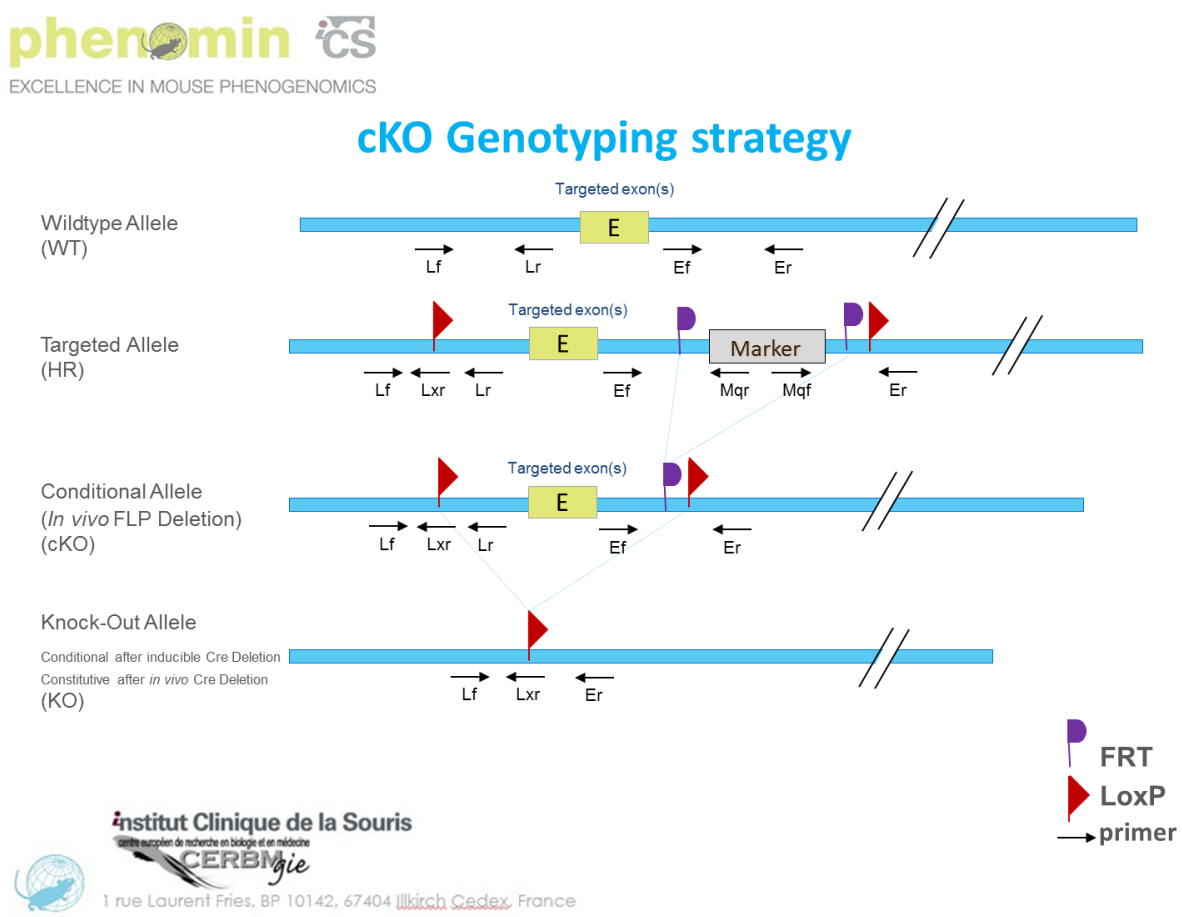
Table of contents

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Gnal** 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	GGAAAGCAGCCTGAATTCTCTAACCC
Ef^2	GCCCCTATCACTGACTTTGAATATTCCC
Er	GGTCTGTGTTAACTTACATCATGCACGC
Er^2	CATACAGGAAAAAGCGTCGCATTTCTG
Lf	GCTAGTTGAATCCACAGTGTCAAACTGCC
Lf^2	CCACAGTGTCAAACTGCCTGTATCGC
Lr	CAAGGCATCTGCCAAGTTTTTACCC
Lxr	CGAAGTTATCTGCAGGTCGACCTTAAG
Mqf	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mqr	CATCTGCACGAGACTAGTGAGACG

^{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	Lf^2 / Lr	367	367	---	287
Excision of the selection marker	Ef^2 / Er^2	2273*	419	---	293
5' part of the selection marker	Ef / Mqr	456	---	---	---
3' part of the selection marker	Mqf / Er^2	269	---	---	---
LoxP specific PCR	Lf / Lxr	197	197	197	---
Excision of the floxed exon(s), i.e. knock out	Lf / Er	3238*	1384*	474**	1178*
Excision of the floxed exon(s), i.e. knock out 2	Lf^2 / Er^2	3073*	1219*	309**	1013*

*: 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.