EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Fignl1

(PHENOMIN-ICS reference IR00007215 / Kos7215)

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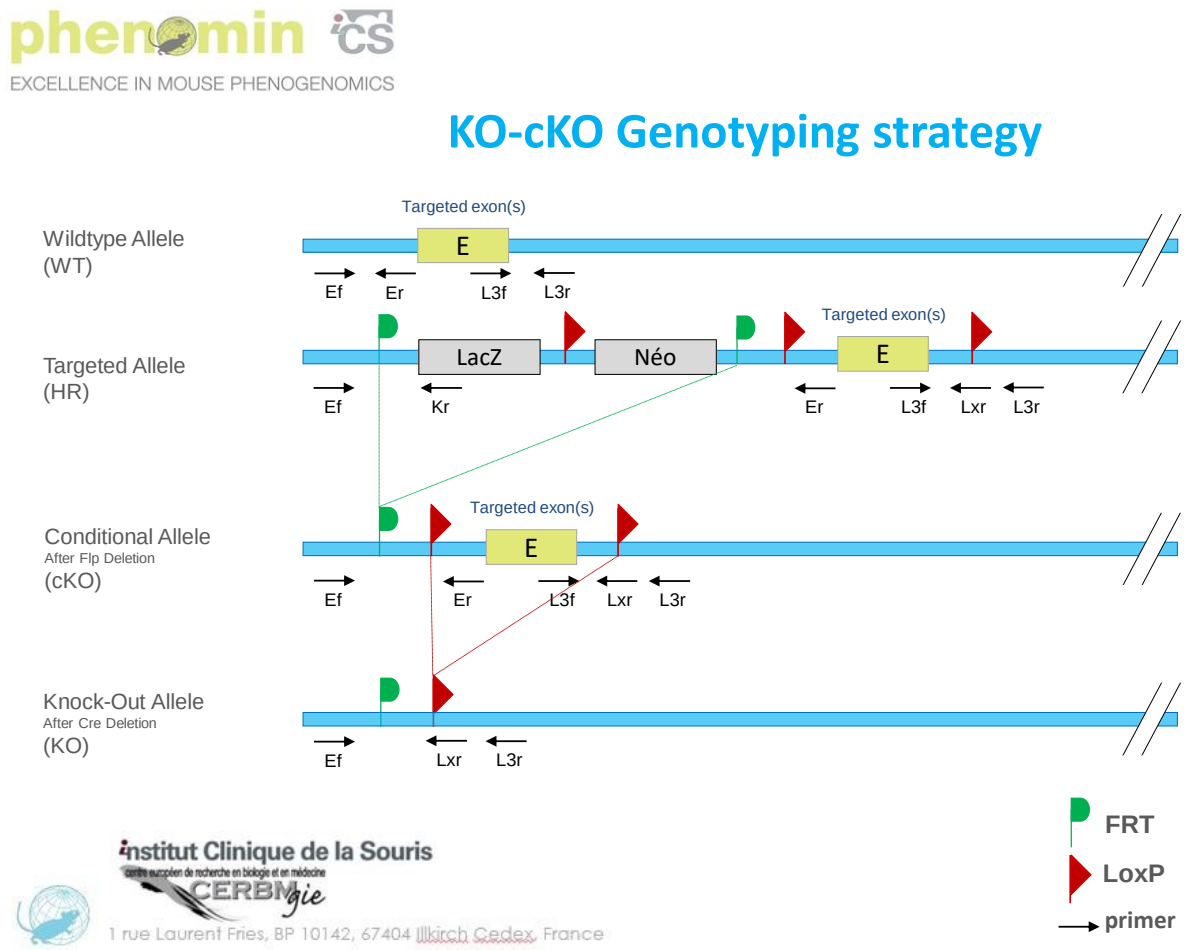
This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Fignl1** Constitutive Knockout / Conditional Knockout (KO-cKO) project.

1. PCR Genotyping protocol

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	Primers	Sequence
Ef	10176	GGGATCAAACACTAGGGTTCAGGC
Er	10180	GATACAGTCTTCAAGATTAAGGACAACC
Kr	10177	CTTGGGCAAGAACATAAAGTGACCC
L3f	10178	CTCGGAAGCTTGCTGTGCTAC
L3r	10179	CGGGTTACGGTAGTTTACTCCC
Lxr	5079	GGCGAGCTCAGACCATAACTTCGTATA

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	conditional allele (cKO)	KO allele	WildType allele
5' part of the selection marker	10176-10177	Ef / Kr	367	---	---	---
Presence of the distal loxP (with Betaine)	10178-10179	L3f / L3r	326	326	---	332
Distal loxP specific PCR (with Betaine)	10178-5079	L3f / Lxr	203	203	---	---
Excision of the selection marker	10176-10180	Ef / Er	7304*	400	---	200
Excision of the floxed exon(s), i.e. knock out	10176-10179	Ef / L3r	9925*	3021*	412**	2817*

*: 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

If mentioned in table "PCR fragments expected size" add 0.5% of Betaine 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.

2. Recommended papers:

2.1. Cre and Flp genotyping method

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.

2.1. Tips and tricks for optimizing your PCR genotyping procedures

Optimizing PCR for mouse genotyping: Recommendations for reliable, rapid, cost effective, robust and adaptable to high-throughput genotyping protocol for any type of mutation.
Jacquot, S, Chartoire, N, Piguet, F, Herault, Y, Pavlovic, G. (2019).
Current Protocols in Mouse Biology, 9, e65. doi: 10.1002/cpmo.65
Free copy of this paper can be accessed online though this link http://bit.ly/2sxxWvO