



Genotyping protocol

Il18rap

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(ICS internal reference)

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TABLE OF CONTENTS

Table of contents2

1. Genotyping protocol and data2

 1.1. Genotyping strategy2

 1.2. PCR protocol4

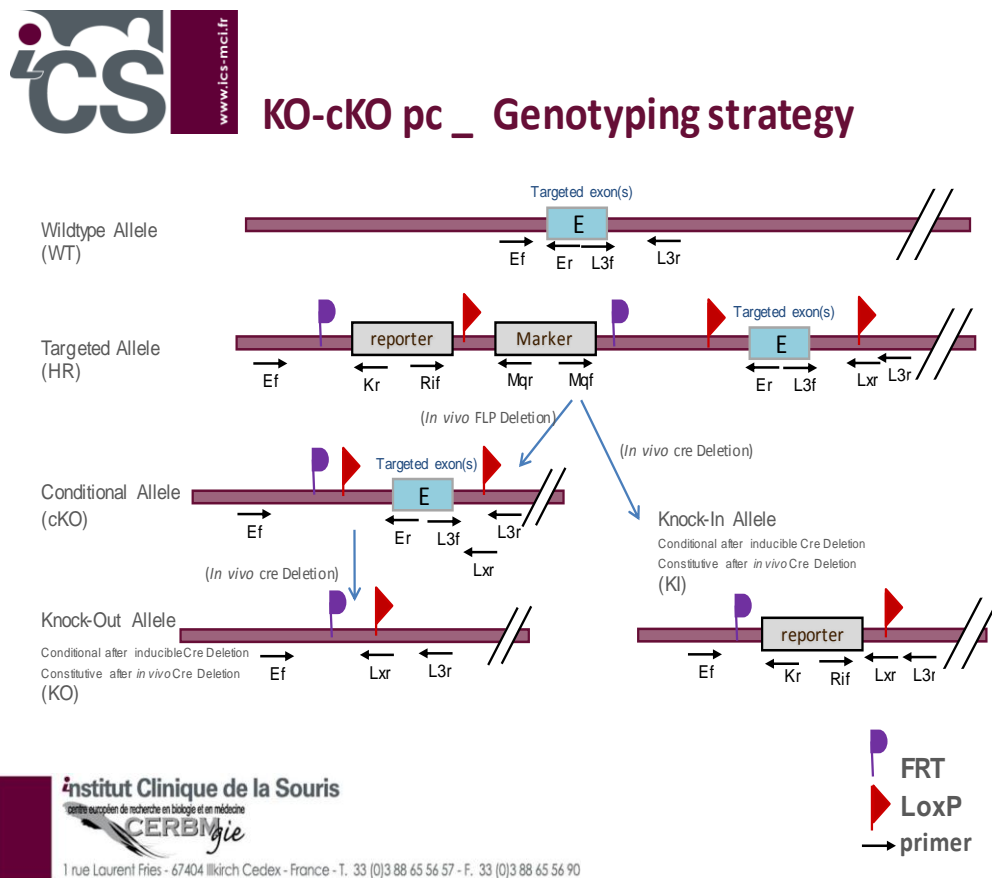
2. Cre and Flp genotyping method5

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Il18rap** Constitutive Knockout / Conditional Knockout (KO-cKO x Cre) project.

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	7957	GGAGAAGGGACTCATGTTTACTCCC
Er	7961	GCTCAGAGGCAATCAGCTGTTATGG
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	7958	CACACACCGGCAACCATTAAAGTGTTG
L3f ²	7959	GAGCTGTGGTTAAAGTGAGAACCATTGG
L3r	7960	GGGGCACAGATCTTAAGCTGTAGC
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Rif	5966	GCACATGGCTGAATATCGACGGT

²: for a selected position, a second primer was designed

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer (see the map above)	Targeted allele (HR)	conditional allele (cKO)	KI allele	WildType allele
5' part of the selection marker	7957-3209	Ef / Kr	280	---	280	---
Presence of the distal loxP	7958-7960	L3f / L3r	302	302	---	304
Distal loxP specific PCR	7959-3255	L3f2 / Lxr	265	265	---	---
Excision of the selection marker	7957-7961	Ef / Er	7239*	335	---	182
Cre total excision	5966-3255	Rif / Lxr	3207*	---	471**	---

*: 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 ₂ O	up to 15 µl

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
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.