



Genotyping protocol

Raf1

IR00003184 / E213

(ICS internal reference)

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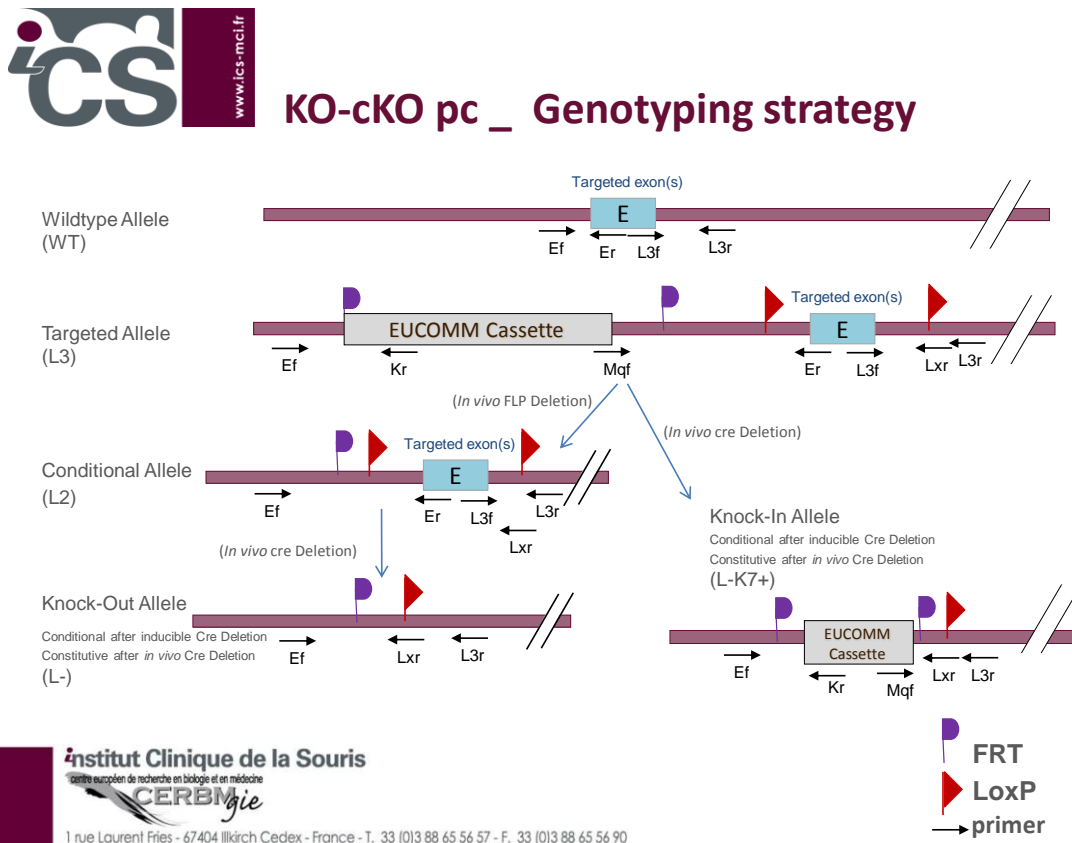
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1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Raf1** Constitutive Knockout/ Conditional Knockout (KO-cKO) 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	5516	TCACAAAGGAGAATGTAGCCTGCTTG
Ef ²	5517	ATGAACCTGACTGAGTCTCCCCTTCTG
Er	5522	TCCTCAAAGACTCGGGACCCCT
Kr	3277	CTCCTACATAGTTGGCAGTGTTTGGG
L3f	5519	GGGTCAGGTTCTTTGGCACTG
L3f ²	5518	AGAAGCCAGTGAGGTGATGCTGTCT
L3r	5521	GCACTAAGATGATCAGGAGCAGACAGC
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Mq1f	4981	GGGATCTCATGCTGGAGTTCTTCG

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 (KO-cKO)	KI allele	WildType allele
5' part of the selection marker	5516-3277	Ef / Kr	364	---	364	---
Presence of the distal loxP	5519-5521	L3f / L3r	407	407	---	408
Distal loxP specific PCR	5518-3255	L3f ² / Lxr	355	355	---	---
Excision of the selection marker	5517-5522	Ef ² / Er	5823*	423	---	246
Exon excision	4981-3255	Mq1f / Lxr	---	---	424	---

*: 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 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.