Genotyping protocol

Rnf144b

IR00003112 / E203

(ICS internal reference)

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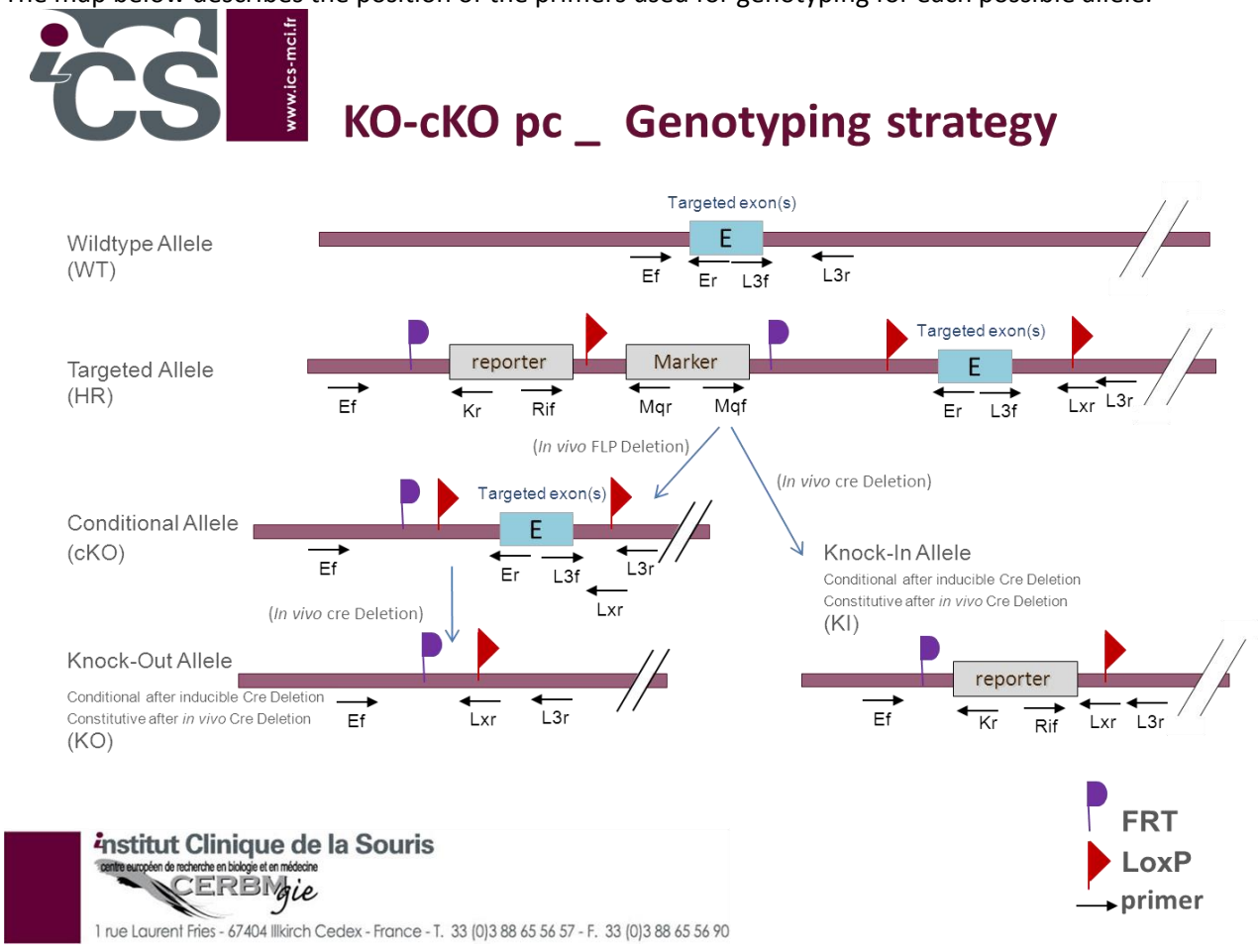
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Rnf144b** 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.



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Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	5507	GCTGTCTGAAGAGGACACAGGATGC
Ef[2]	5506	ACCGCATCACGTGTCATGTGGT
Er	5510	TCAGTGATGAAGTTCCCTGATTGCC
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	5508	TCTCTCACTGTCTTTGTTGCAGATTGC
L3r	5509	GCCTCGGCTGGTTAATGTTTGCA
Lxf	6295	TTATGTTTAAACGGCGCGCCC
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Ri1f	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)	cKO allele	KI allele	WildType allele (WT)
5' part of the selection marker	5507-3209	Ef / Kr	339	---	---	---
Presence of the distal loxP	5508-5509	L3f / L3r	443	443	---	491
Distal loxP specific PCR	5508-3255	L3f / Lxr	313	313	---	---
Excision of the selection marker	5506-5510	Ef[2] / Er	7421*	465	---	298
Cre total excision	5966-3255	Ri1f / Lxr	3140*	---	474	---

*: 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:

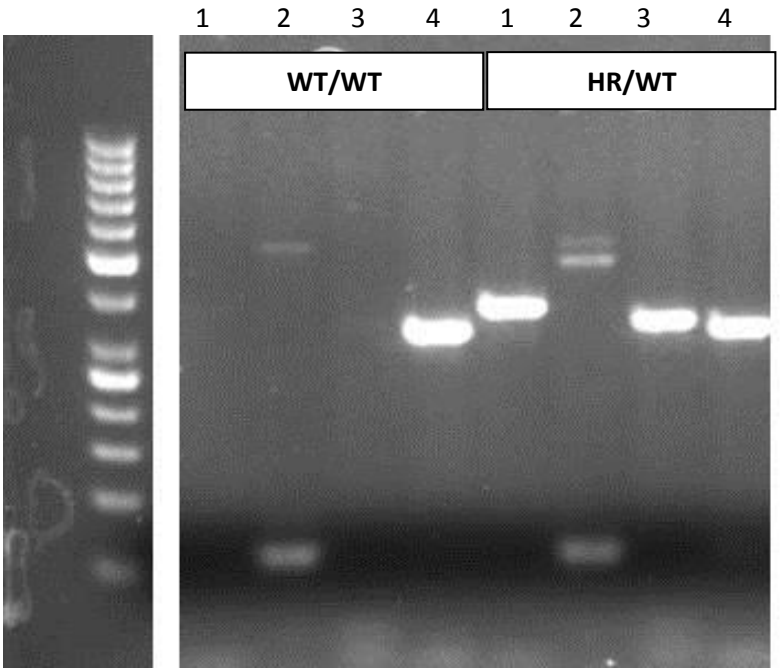
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

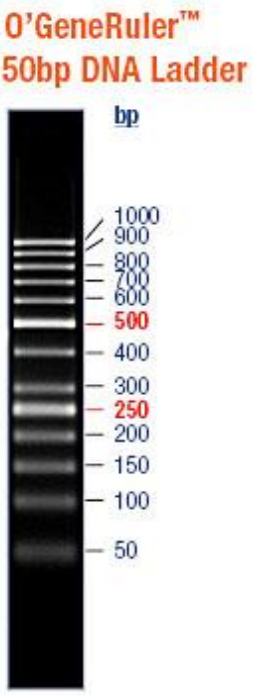
1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was done by gel electrophoresis 2% agarose (SB buffer).

Representative genotyping picture



PCRs numbers:
1: 5' part of the selection marker
2: Presence of the distal loxP
3: Distal loxP specific PCR
4: Excision of the selection marker



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.