



Genotyping protocol

Prdm10

IR00003182 / E211

(ICS internal reference)

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The first version of this report was generated the: 08 Apr 2014

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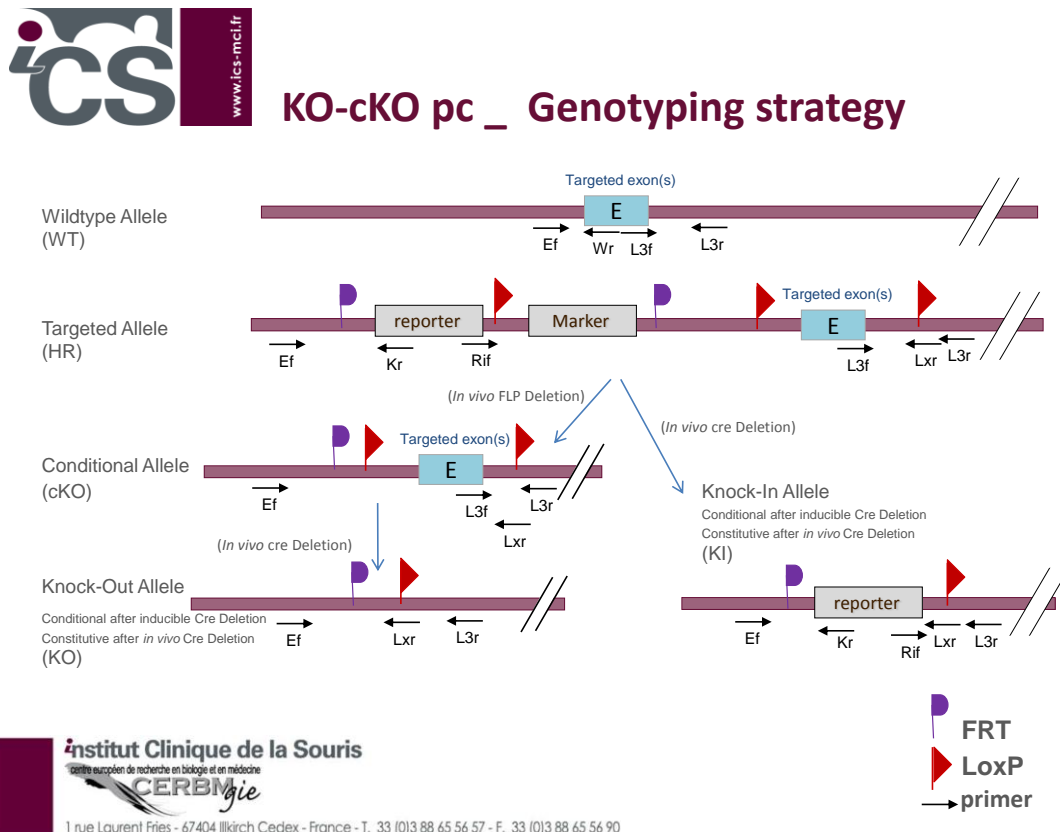
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1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Prdm10** 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	6067	AGTGCTAGAACATTTGCCTCGTATGCA
Ef ²	6121	TAAGAAGGACAGGGTTTAATACG
Wr	6122	GACTACTCATTCCCCTTAGTG
Kr	3210	CCTGTCCCTCTCACCTTCTACC
L3f	6120	GGCCTTTTGACCCCATGACTTGT
L3r	6068	TCAGCTCTCCAAGGCACTCAAAGTTA
Lxr	5079	GGCGAGCTCAGACCATAACTTCGTATA
Lxr ²	3255	ACTGATGGCGAGCTCAGACCATAAC
Rif	5966	GCACATGGCTGAATATCGACGGT

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

PCR fragments expected size (bp):

PCR	Region analyzed	Primers used	Position on the primer (see the map above)	Targeted allele (HR)	KI allele	WildType allele
A	5' part of the selection marker	6067-3210	Ef / Kr	345	345	---
B	Presence of the distal loxP	6120-6068	L3f / L3r	432	---	438
C	Distal loxP specific PCR	6120-5079	L3f / Lxr	287	---	---
D	WildType specific PCR	6121-6122	Ef ² / Wr	---	---	425
E	Cre total excision	5966-3255	Rif / Lxr ²	4258*	471	---

*: 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:

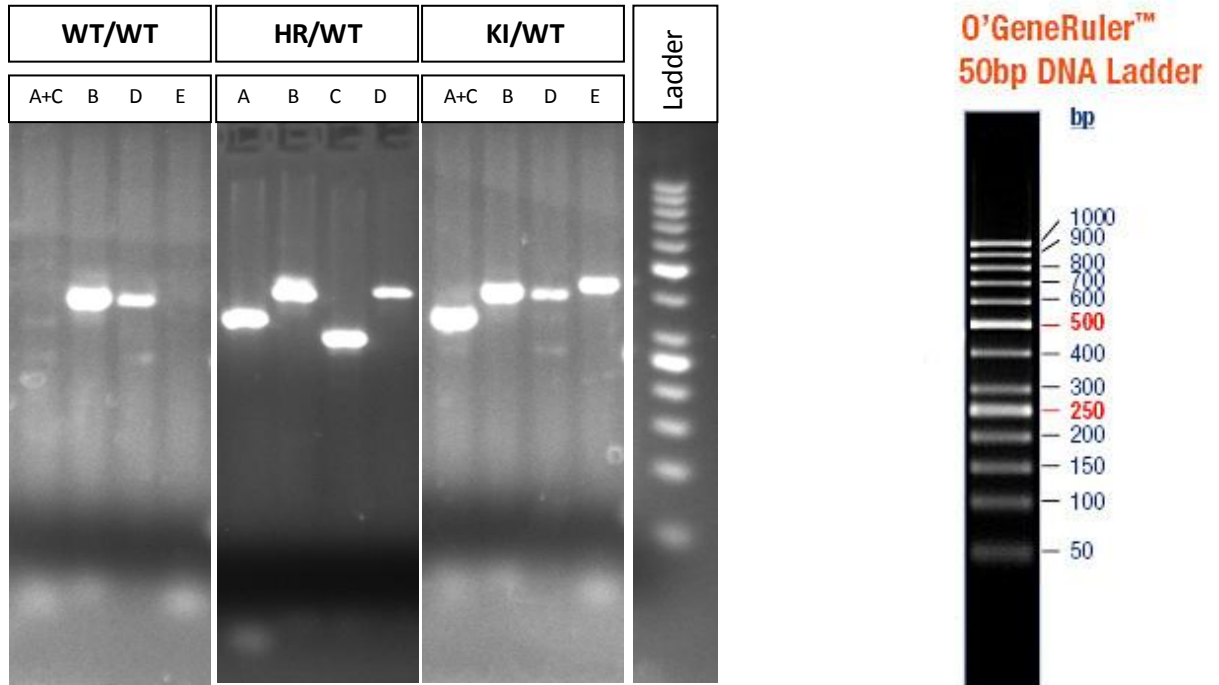
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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



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.