



## Genotyping protocol

Ptch1

IR00002939 / E170

(ICS internal reference)

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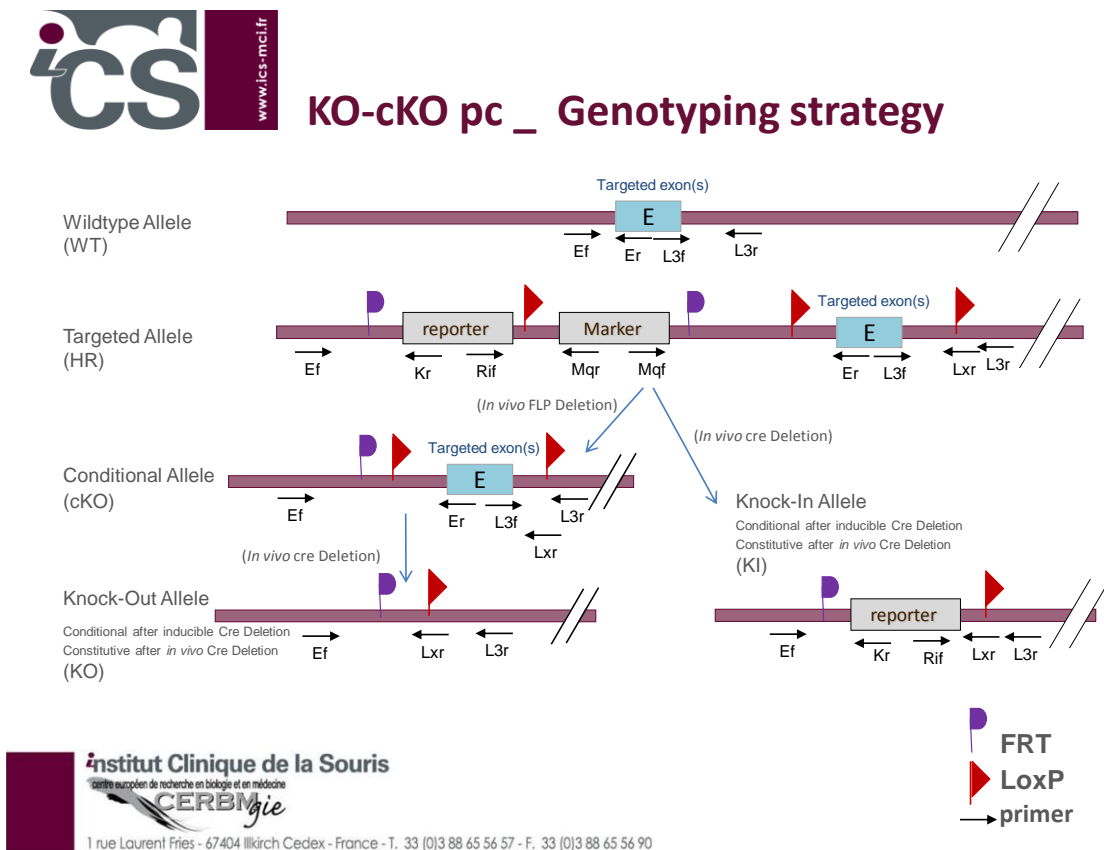
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### 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Ptch1** 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	5390	CTAGGAGCTTGCAGACACAGGC
Ef <sup>2</sup>	5389	CCTGACACCTGTGCATTCTGGATG
Er	6554	ACATCTCTGGGCACTGAGCGC
Kr	3278	GGGCAAGAACATAAAAGTGACCCTCC
L3f	5391	CCTGTGTCACCTGCACAGGTACAG
L3r	5393	GCTCGACATCTGAAAAGCGCAAG
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Ri1f	5966	GCACATGGCTGAATATCGACGGT

<sup>2</sup>: 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)	KO allele	WildType allele
5' part of the selection marker	5390-3278	Ef / Kr	338	---	338	---
Presence of the distal loxP	5391-5393	L3f / L3r	439	439	---	429
Distal loxP specific PCR	5391-3255	L3f / Lxr	257	257	---	---
Excision of the selection marker	5389-5392	Ef <sup>2</sup> / Er	7343*	387	---	255
Cre total excision	5966-3255	Ri1f / Lxr	3323*	---	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 <sub>2</sub> O	up to 15 µl

### Cycling conditions:

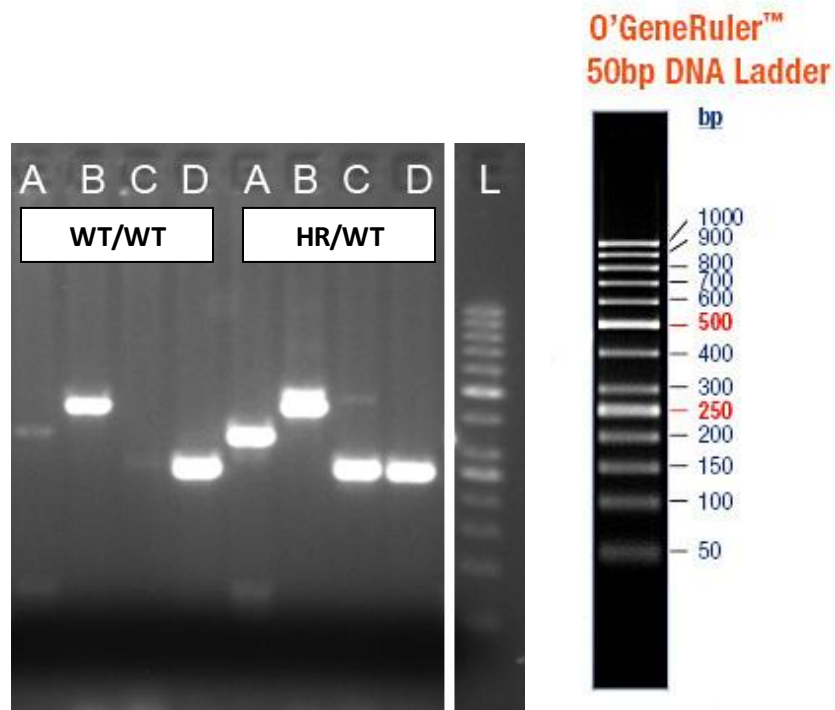
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5min	1

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

### 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



- A: 5' part of the selection marker
- B: Presence of the distal loxP
- C: Distal loxP specific PCR
- D: Excision of the selection marker
- L: O'GeneRuler 50bp DNA Ladder

## 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.