



Genotyping protocol

Ptchd1

IR00003728 / G14

(ICS internal reference)

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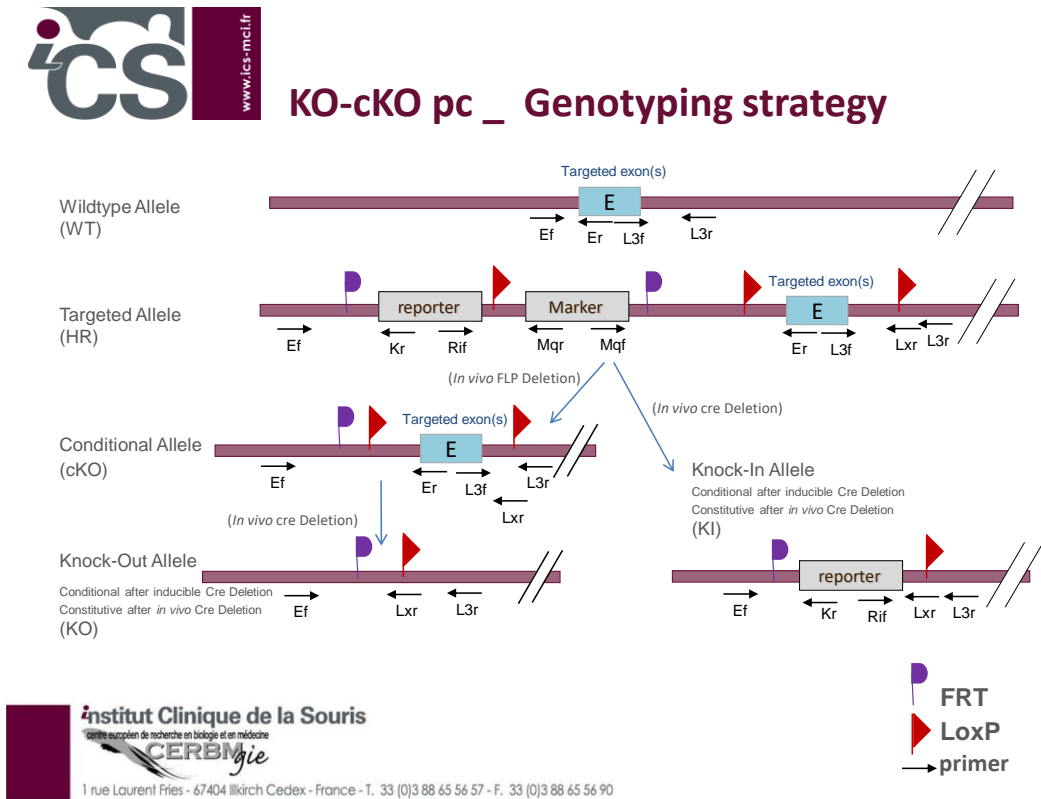
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1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Ptchd1** 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	6348	GCTTTGGAGAGACTGCTTACTGG
Ef ²	6347	CCAGGGATGGTACCACCTACAATATG
Er	6353	GGGGGATTCTGATGGTGCTAACAG
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	6349	CCTTTCGTCATGCTAGGTAAGTACTGC
L3f ²	6350	GCAGCCGGAATCATCAATCTCACTG
L3r	6351	CCTCTTCGACCCCACTACTGGTG
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)	conditional allele (cKO)	KI allele	WildType allele
5' part of the selection marker	6348-3209	Ef / Kr	382	---	382	---
Presence of the distal loxP	6349-6351	L3f / L3r	337	337	---	396
Distal loxP specific PCR	6350-3255	L3f ² / Lxr	300	300	---	---
Excision of the selection marker	6347-6353	Ef ² / Er	7247*	343	---	250
Cre total excision	5966-3255	Ri1f / Lxr	3678*	---	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:

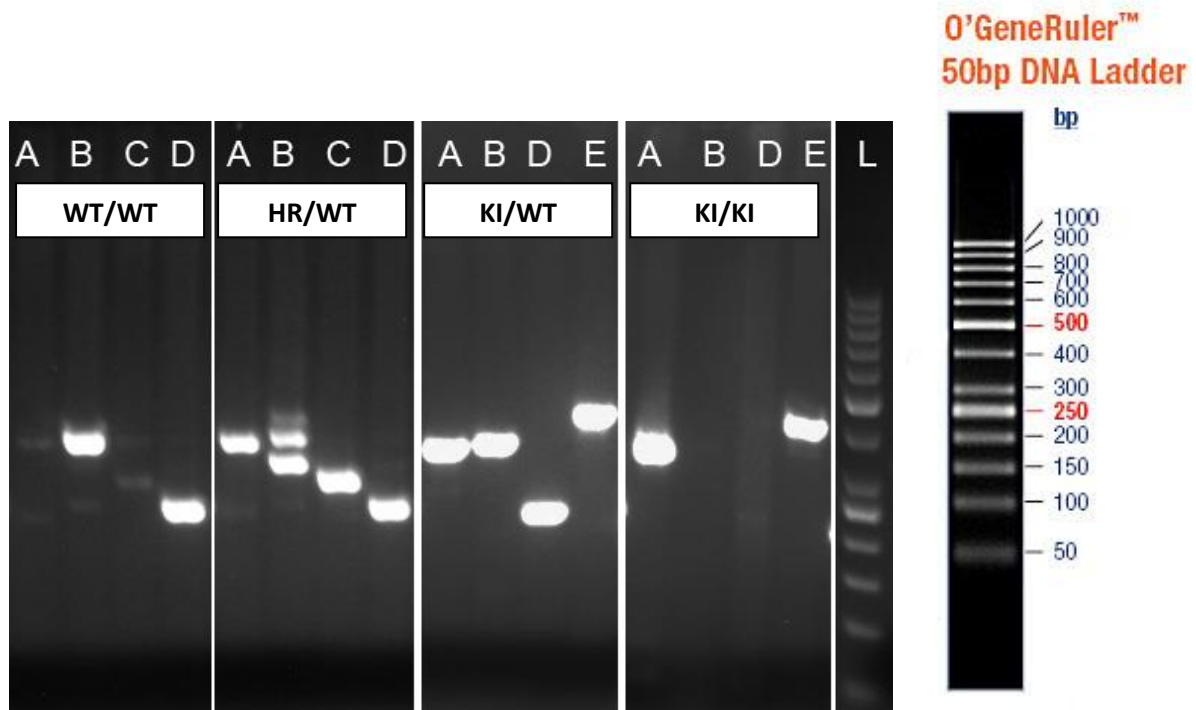
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5min	1

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

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
- E: Cre total excision
- 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.