Genotyping protocol

Rltpr tm1a (KOMP)Mbp
DEPD00524_2_G07

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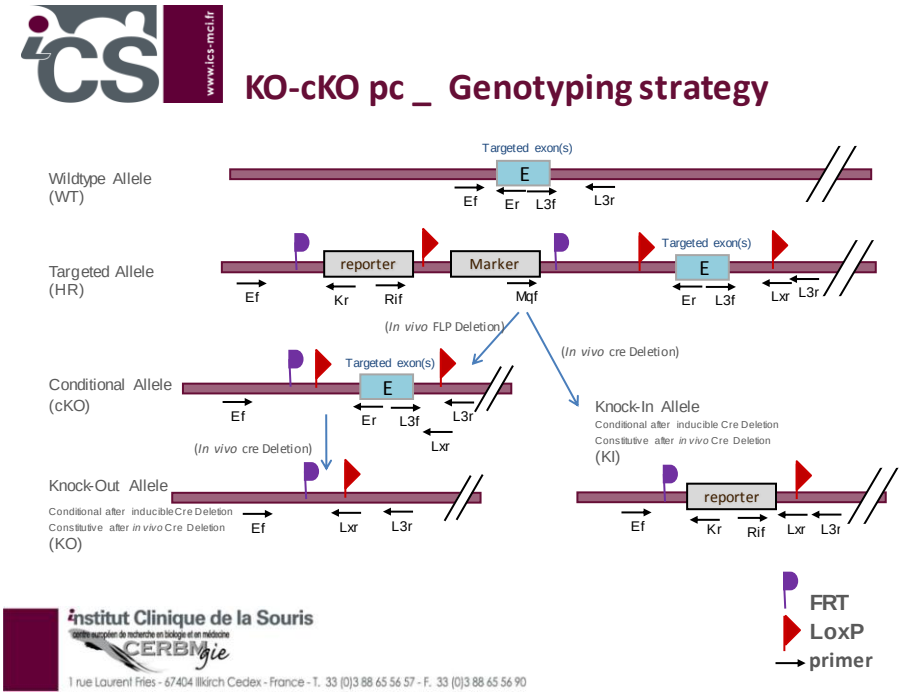
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Rltpr** 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	7229	GTCACCGTTCTTGAAACAGCTTCC
Ef[2]	7228	CTTCTCTCTGCCCTTTTGTGTGTGG
Er	7232	GAATTGAGGCTTGGGAATGCAGAGG
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	7230	CCACCAGGATGAGAGTTCTTCATG
L3r	7231	GTAGGGAAGGAGGTGTCTTAG
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 (KO allele) (L3)	cKO allele (L2)	KO allele (L-LacZ+)	WildType allele (WT)
5' part of the selection marker	7229-3209	Ef / Kr	381	---	---	---
Presence of the distal loxP	7230-7231	L3f / L3r	311	311	---	268
Distal loxP specific PCR	7230-3255	L3f / Lxr	263	263	---	---
Excision of the selection marker	7228-7232	Ef[2] / Er	7596*	692	---	496
Cre total excision	5966-3255	Ri1f / Lxr	---*	---	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_2O	up to 15 µl

Cycling conditions:

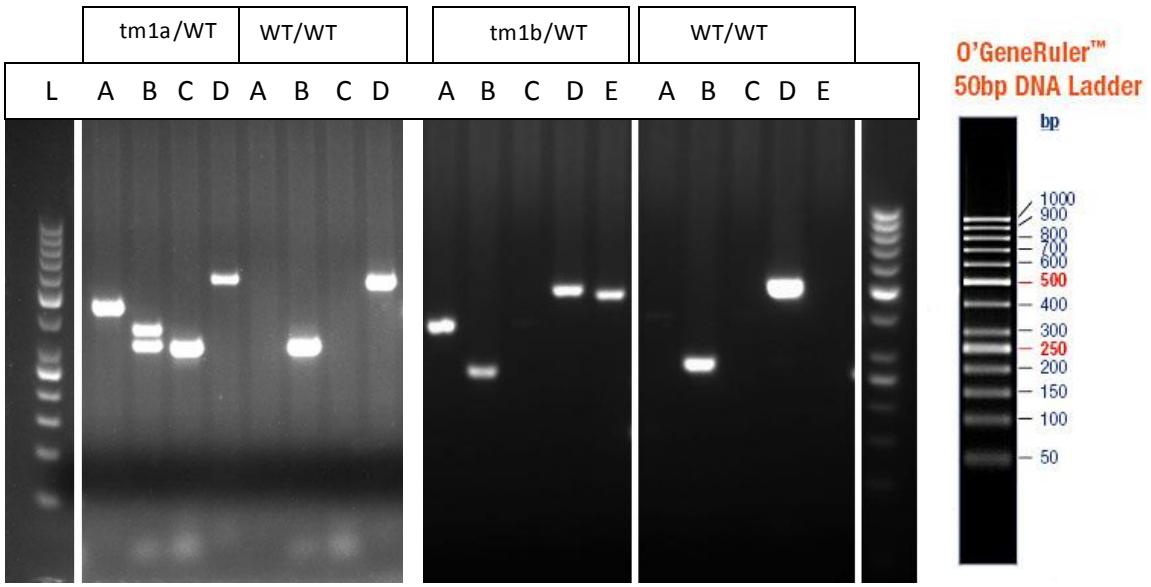
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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



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: 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.