Genotyping protocol

CD7tm1a(KOMP)Wtsi

EPD0162_2_C11

This report has been prepared by: **Sylvie Jacquot**
genotyping@igbmc.fr

This report has been validated by: **Sylvie Jacquot, PhD, Head of Genotyping Service**
33 (0)3 88 65 57 44
genotyping @igbmc.fr

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For any question, please contact:

Institut Clinique de la Souris - ICS - Mouse Clinical Institute
1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex, France
Email: genotyping@igbmc.fr
Web site: http://www-mci.u-strasbg.fr/

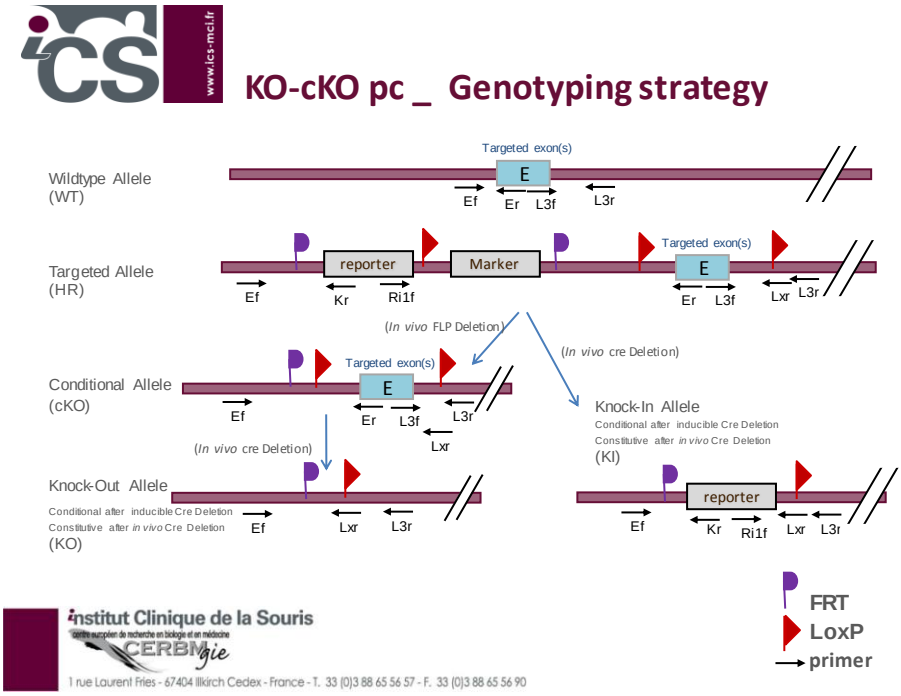
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Cd7** 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	7525	TTCTTGTGCAAAACCAGCATAGTGTTC
Er	7529	TGCATCTCAACACAAGGACTTCCCT
Kr	3277	CTCCTACATAGTTGGCAGTGTTTGGG
L3f	7526	CCCAGGAACCTCTGCAGACATCATTTT
L3f[2]	7527	AAGATACAGGTCAGTGTGAGCCTACGG
L3r	7528	GTTCCGAATTCCTACATTCACACAGGT
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	7525-3277	Ef / Kr	405	---	---	---
Presence of the distal loxP	7526-7528	L3f / L3r	389	389	---	376
Distal loxP specific PCR	7527-3255	L3f[2] / Lxr	236	236	---	---
Excision of the selection marker	7525-7529	Ef / Er	7431*	527	---	355
Cre total excision	5966-3255	Ri1f / Lxr	---*	---	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_2O	up to 15 µl

Cycling conditions:

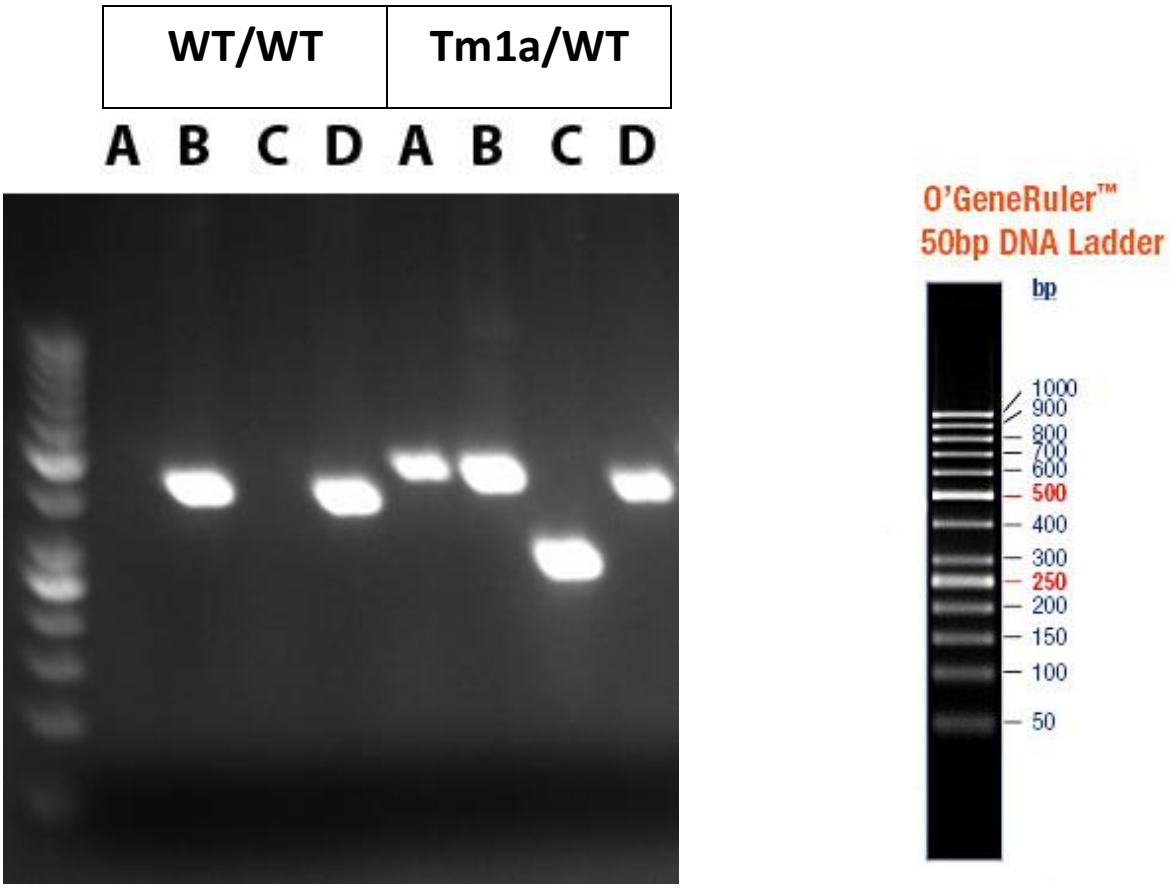
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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

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.