Genotyping protocol

Cdk8

IR00003723 / G13

(ICS internal reference)

This report has been prepared by: **Pauline Cayrou**
33 (0)3 88 65 56 55
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/

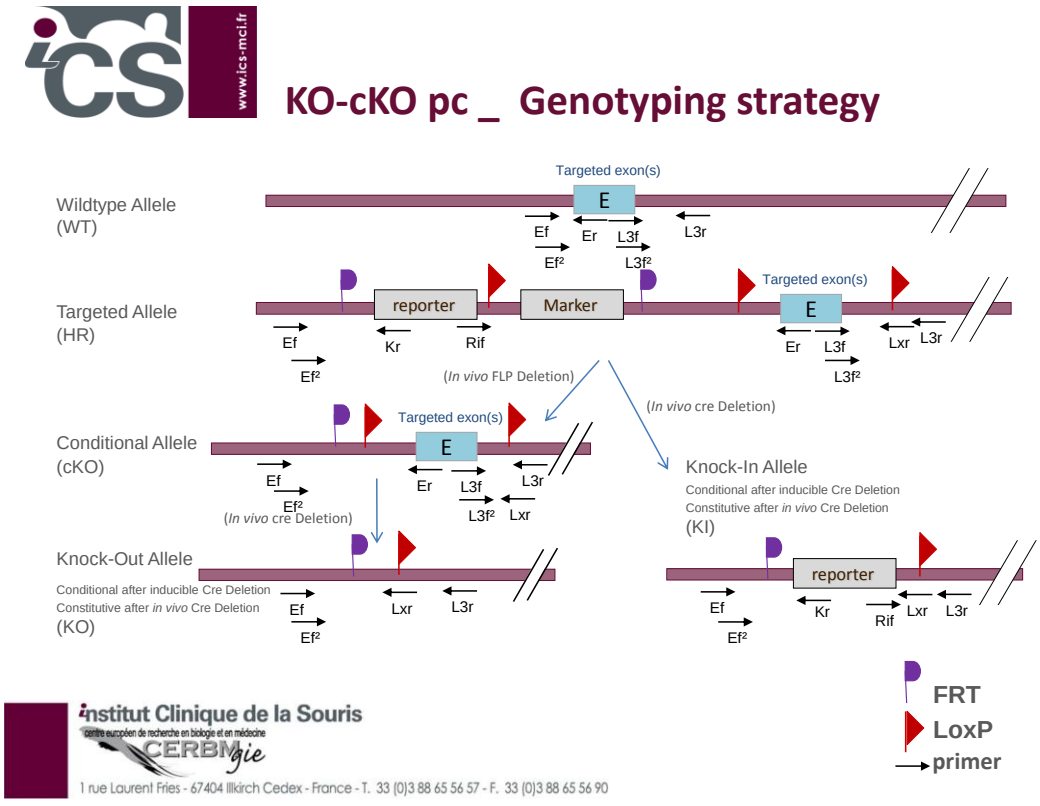
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Cdk8** Constitutive Knockout / Conditional Knockout (KO-cKO pc) 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
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Kr	3278	GGGCAAGAACATAAAGTGACCCTCC
Rif	5966	GCACATGGCTGAATATCGACGGT
Ef	6108	GCTTCCAAAAAAAGAGTACCAAAGGCG
Ef[2]	6109	CGTAGGTAGCAATCTGGTCGGGGT
L3f	6110	TTATGGGTGAAGGTCCTGAGCGA
L3f[2]	6111	ACCTTCCCCCTGTCTCAGACAGTCCTT
L3r	6112	GGAAAACGACAATGGAAGCAGCAG
Er	6113	CAGGTACACAGGCTGGATTTGCAC

[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)	cKO allele	KI allele	WildType allele (WT)
5' part of the selection marker	6108-3278	Ef / Kr	262	---	---	---
Presence of the distal loxP	6111-6112	L3f[2] / L3r	499	499	---	446
Distal loxP specific PCR	6110-3255	L3f / Lxr	472	472	---	---
Excision of the selection marker	6109-6113	Ef[2] / Er	7501*	597	---	414
Cre total excision	5966-3255	Ri1f / Lxr	3472*	---	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:

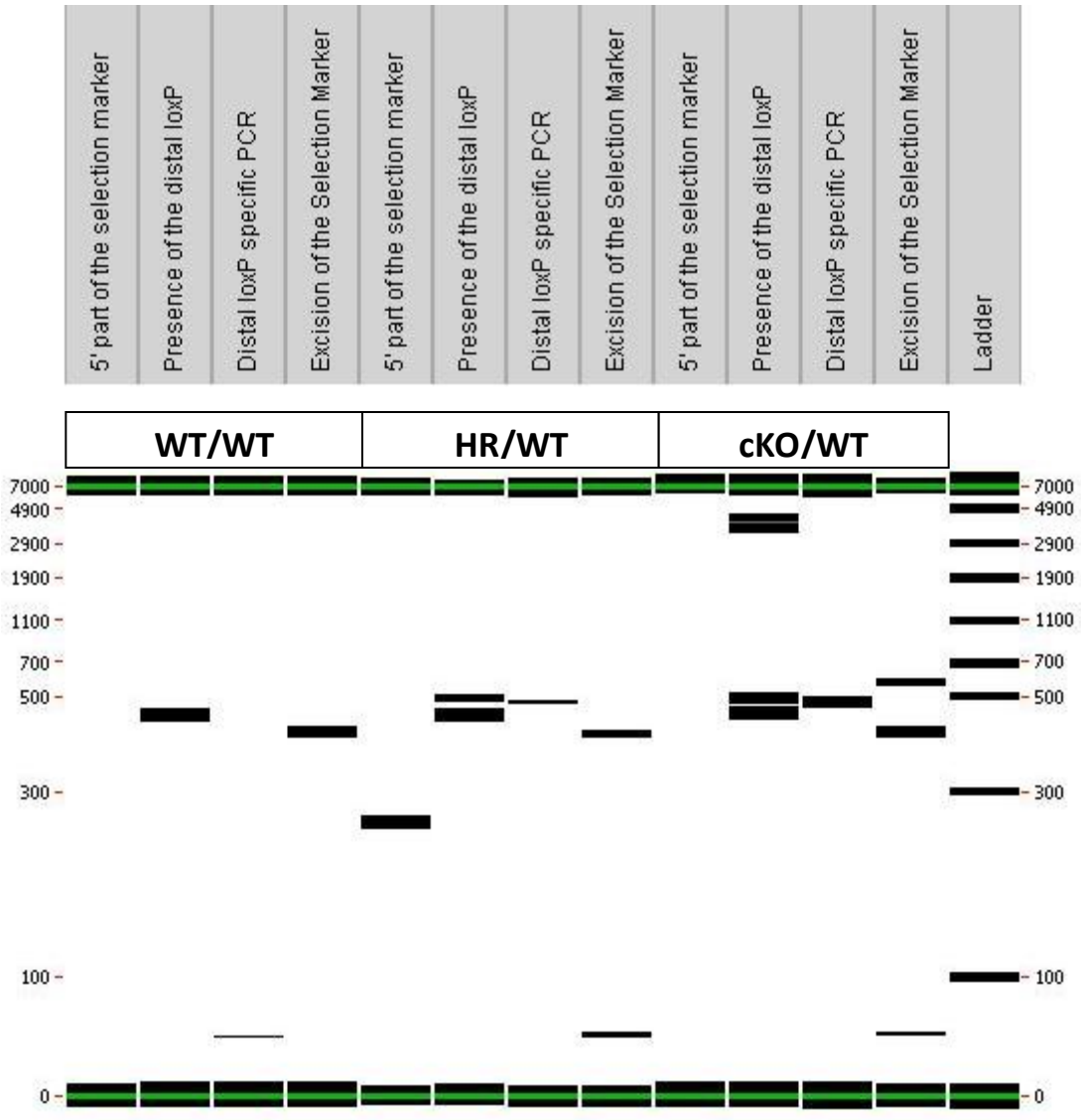
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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 not done by gel electrophoresis but using LabChip® 90 microfluidic apparatus. PCR products were run on the HT DNA 5K LabChip® 90 Assay Kit.

Representative genotyping picture



Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the 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.