Genotyping protocol

Gzmk

IR00004149 / P4149

(ICS internal reference)

This report has been prepared by: **Nathalie Chartoire**
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/

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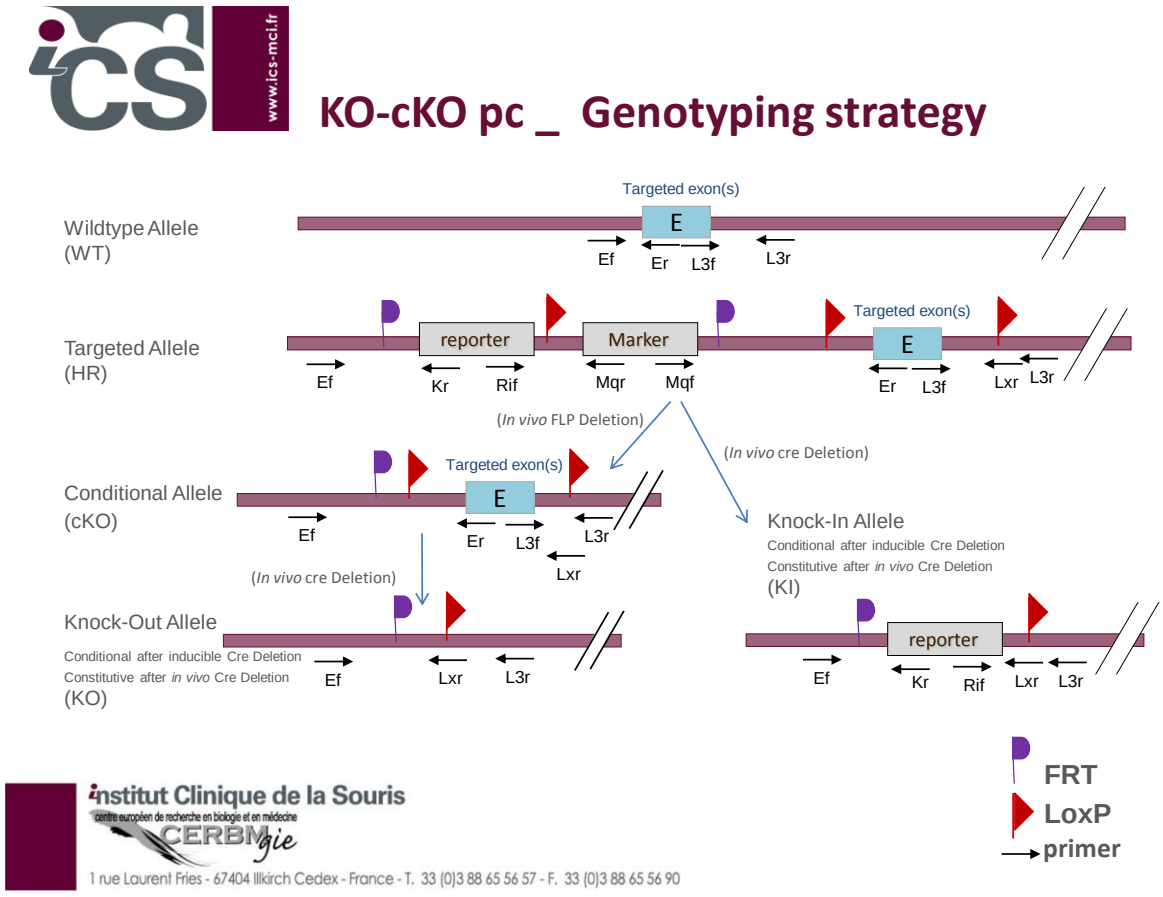
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Gzmk** 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	7146	TCTGCCTTTGTTTTTCATGGTGGGG
Er	7149	GCAGCCTGTTGAAACCATTTGCATG
Kr	3277	CTCCTACATAGTTGGCAGTGTTTGGG
L3f	7147	CCACTGAGGGAATTTCGGTAGCACC
L3r	7148	GCTGGATAGGCCCCTGGAGTATGCT
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Ri1f	5966	GCACATGGCTGAATATCGACGGT

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (KO allele) (HR)	cKO allele (cKO)	KI allele (KI)	WildType allele (WT)
5' part of the selection marker	7146-3277	Ef / Kr	392	---	---	---
Presence of the distal loxP	7147-7148	L3f / L3r	417	417	---	375
Distal loxP specific PCR	7147-3255	L3f / Lxr	229	229	---	---
Excision of the selection marker	7146-7149	Ef / Er	7398*	494	---	299
Cre total excision	5966-3255	Ri1f / Lxr	3267*	---	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:

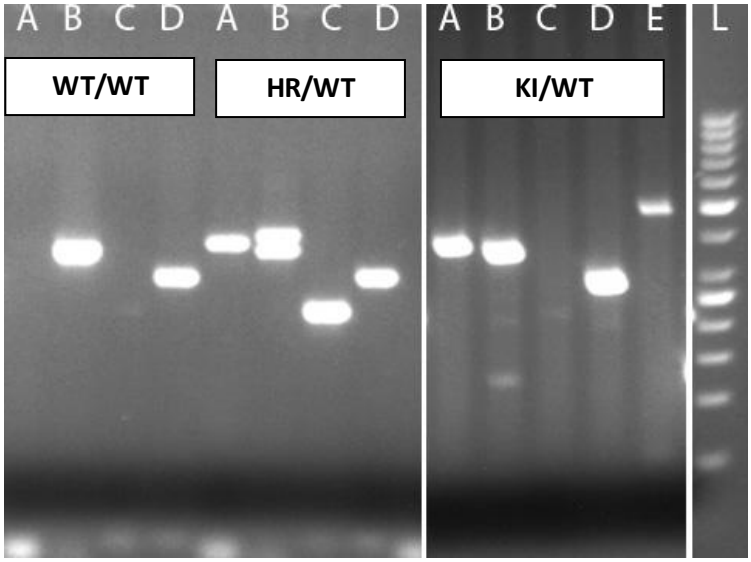
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

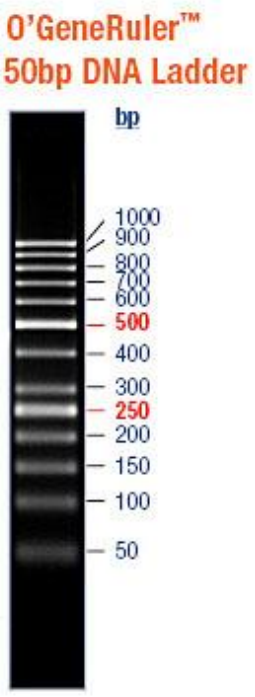
NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

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: 0'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.