Genotyping protocol

Lmna

IR00004163 / P4163

(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/

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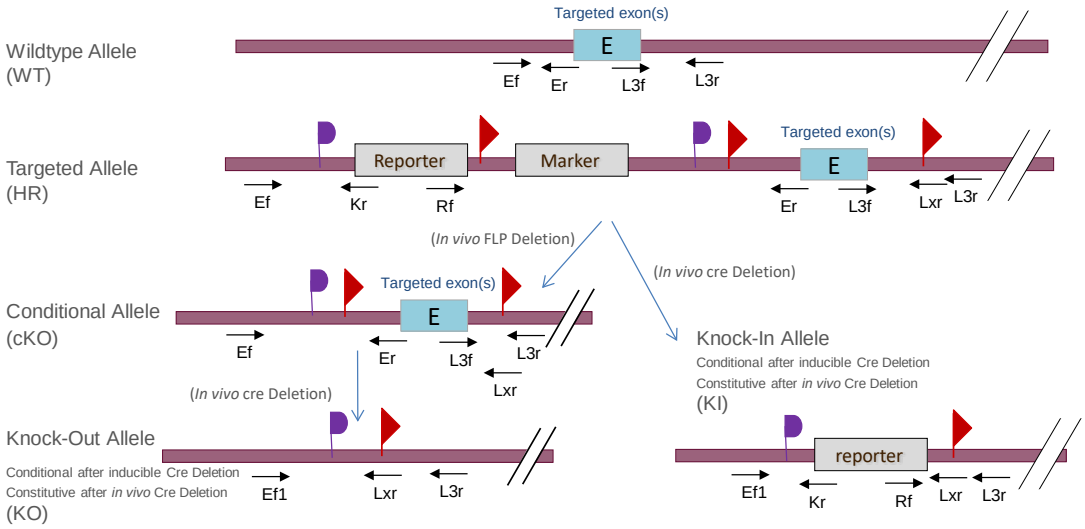
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Lmna** Constitutive Knockout / Conditional Knockout (KO-cKO) project.

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.

KO-cKO pc _ Genotyping strategy



Institut Clinique de la Souris
CERBMgie
1 rue Laurent Fries - 67404 Illkirch Cedex - France - T. 33 (0)3 88 65 56 57 - F. 33 (0)3 88 65 56 90

Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	6500	GGGAGGATTCCTCTGTTGTGTGG
Ef[2]	6501	CAGATGCCAGAAAGCCAAGCAGTAG
Er	6506	GCCCCATCTCCAGTTGGAGTTTC
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	6503	CAGTCTAGAACACTGCTCATCCTAAAGC
L3f[2]	6502	GACATATCATTCGGTCCCATTTGCCTG
L3r	6505	GTGGCTCACAATCATCTGTAATGGG
L3r[2]	6504	CTTCCTCAAGGTCCTGAGTTCAATTCC
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Rif	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 allele)	cKO allele	KO allele	KI allele	WildType allele (WT)
A	5' part of the selection marker	6500-3209	Ef / Kr	300	---	---	300	---
B	Presence of the distal loxP	6503-6505	L3f/ L3r	210	210	---	---	253
C	Distal loxP specific PCR	6502-3255	L3f[2] / Lxr	192	192	---	---	---
D	Excision of the selection marker	6501-6506	Ef[2] / Er	7297*	393	---	---	233
E	Total excision with Cre, i.e. knock in	5966-3255	Rif / Lxr	3292*	---	---	471	---
F	Excision of the floxed exon(s), i.e. knock out	6500-6504	Ef / L3r[2]	8170*	1266*	356**	---	1149*

*: 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

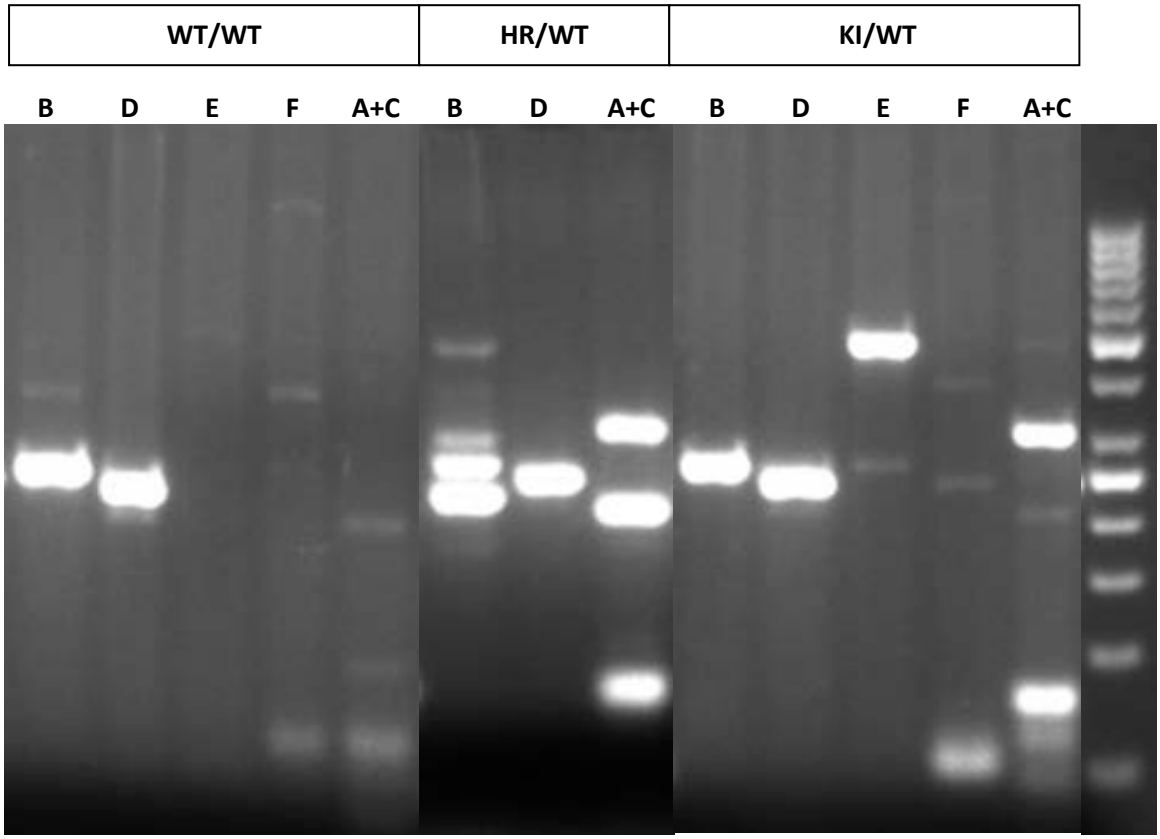
Cycling conditions:

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 agarose gel 3% electrophoresis
Representative genotyping picture



2. Recommended papers:

2.1. Cre and Flp genotyping method

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.

2.2. Tips and tricks for optimizing your PCR genotyping procedures

Optimizing PCR for mouse genotyping: Recommendations for reliable, rapid, cost effective, robust and adaptable to high-throughput genotyping protocol for any type of mutation.
Jacquot, S, Chartoire, N, Piguet, F, Herault, Y, Pavlovic, G. (2019).
Current Protocols in Mouse Biology, 9, e65. doi: 10.1002/cpmo.65
Free copy of this paper can be accessed online though this link http://bit.ly/2sxxWvO