Genotyping protocol

Rab38 conditional knock-out

IR00003875 / K3875

(ICS internal reference)

This report has been prepared by: **Christelle Roth**

This report has been validated by: **Sylvie Jacquot, PhD, Head of Genotyping Service**

The first version of this report was finalized the: 01 Aug 2013

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: mutagenesis@igbmc.fr
Web site: http://www-mci.u-strasbg.fr/

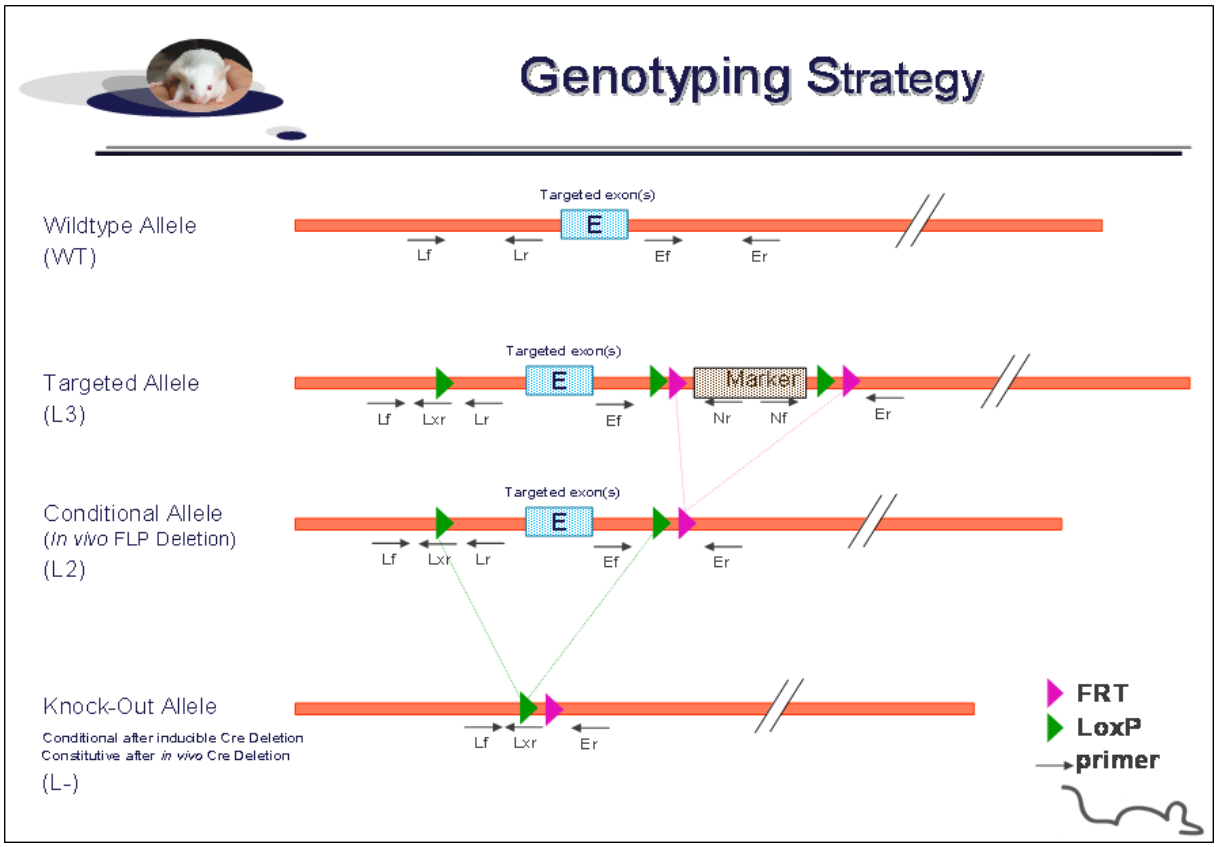
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Rab38** Conditional Knockout (cKO) 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	6772	TAGTGACAATAATGTTTCTGTCCAG
Er	6773	GTGCAAGAATGATTTTAAAACATGCCAG
Lf	6770	TGCACACACAAATACACAAGCACGTA
Lr	6771	CCCCAAATGATCAGGTGATGCAATA
Lxr	4724	CGAAGTTATCTGCAGGTCGACCTTAAG
Nf	6	GAAGAACGAGATCAGCAGCCTCTGTTCC
Nr	238	TGACTAGGGGAGGAGTAGAAGGTG

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (L3)	cKO allele (L2)	KO allele (L-)	WildType allele (WT)
Presence of the distal loxP (with DMSO)	6770-6771 (with 5% DMSO)	Lf / Lr	395	395	---	315
Excision of the selection marker (with DMSO)	6772-6773 (with 5% DMSO)	Ef / Er	2327*	473	---	369
5' part of the selection marker	6772-238	Ef / Nr	400	---	---	---
3' part of the selection marker	6-6773	Nf / Er	398	---	---	---
LoxP specific PCR	6770-4724	Lf / Lxr	207	207	207	---
Excision of the floxed exon(s), i.e. knock out	6770-6773	Lf / Er	3160*	1306*	459**	1122*

*: 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:

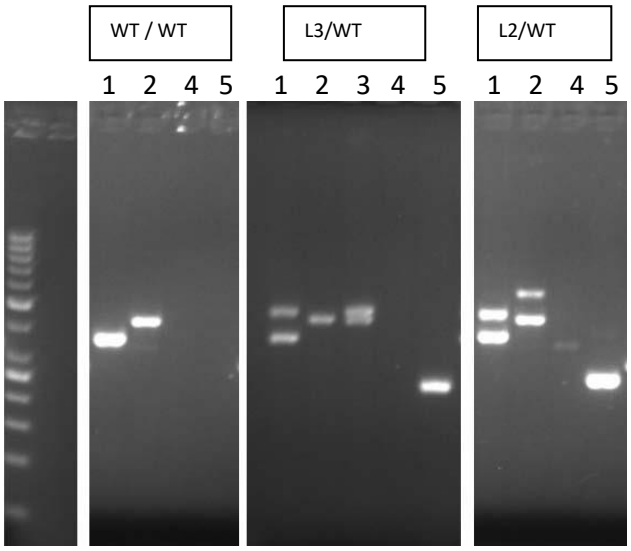
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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

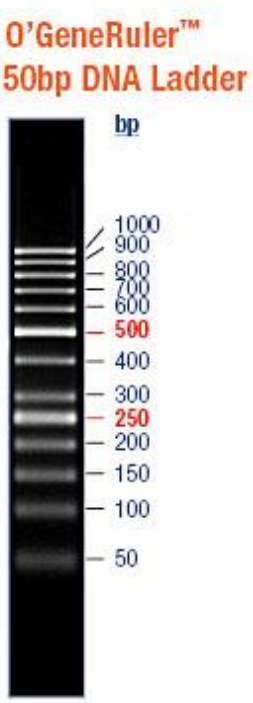
1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was done by gel electrophoresis 2% agarose (SB buffer).

Representative genotyping picture



PCRs numbers:
1: Presence of the distal loxP (with DMSO)
2: Excision of the selection marker (with DMSO)
3: 5' part of the selection marker + 3' part of the selection marker
4: Excision of the floxed exon(s), i.e. knock out
5: LoxP specific PCR



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.