Genotyping protocol

conditional miR-142 in Rosa Gt(ROSA)26Sor$^{tm1(CAG-Mir142)Sbel}$/Ics

IR00004618 / K4618

(ICS internal reference)

This report has been prepared by: **Valérie Rousseau**

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

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

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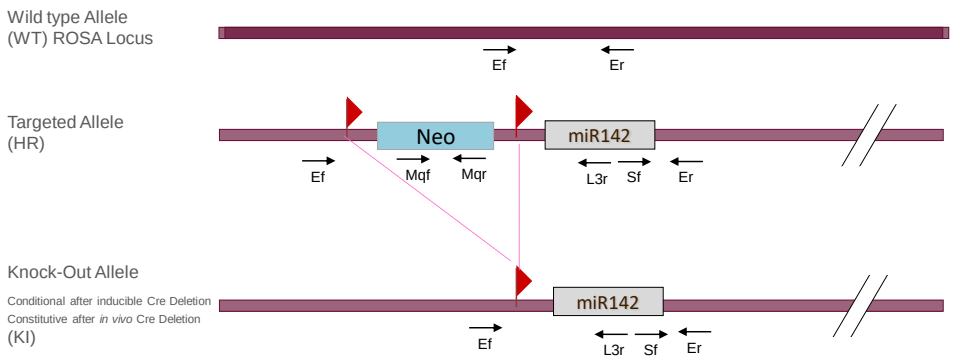
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **conditional miR-142 in Rosa** Knockin (KI) project.

1.1. Genotyping strategy

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

KI Genotyping strategy



LoxP
primer

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	7401	AGCACGTTTCCGACTTGAGTTGCC
Er	7402	CACACACCAGGTTAGCCTTTAAGCCT
L3r	7404	CATAAAGTAGGAAACAC
Mqf	4122	GCTATGACTGGGCACAACAGACAATC
Mqr	548	CCAGACTGCCTTGGGAAAAG
Mqr^2	4123	CAAGGTGAGATGACAGGAGATCCTG
Sf	7403	AAGCACTACTAACAGCACTGGAGGGTG

^{2}: for a selected position, a second primer was designed

PCR fragments expected size (bp):

PCR number	Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	WT allele
1	WildType allele specific PCR (5' part of the targeted locus)	7401-7402	Ef / Er	3695	1028	386
2	5' part of the selection marker	7401-548	Ef / Mqr	619	---	---
3	internal marker	4122-4123	Mqf / Mqr^2	261	---	---
4	cDNA 3'	7403-7402	Sf / Er	411	411	---
5	Excision of the floxed exon(s), i.e. knock out	7401-7404	Ef / L3r	3330	663**	---

*: 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:

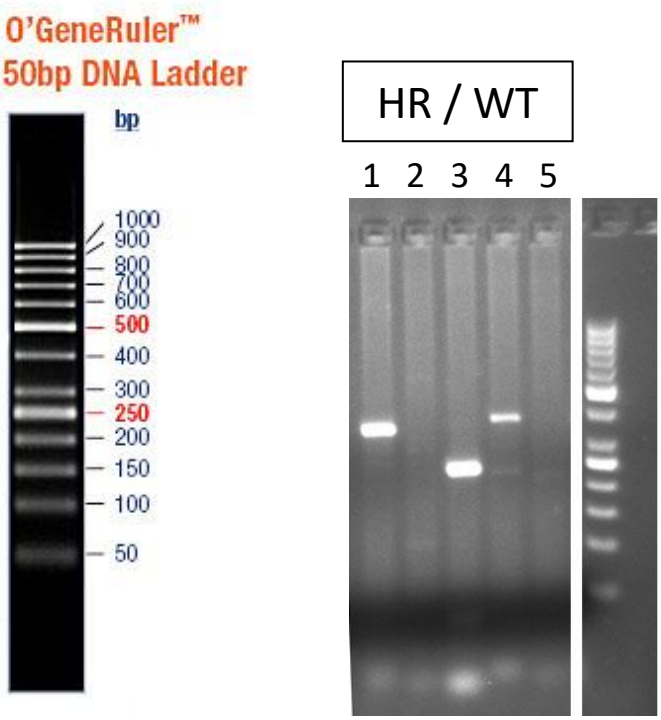
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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



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.