EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project mEos4 KI in Glrb

(PHENOMIN-ICS reference IR00006068 / Kos6068)

This report has been **prepared** by: David MOULAERT

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

The first version of this report was finalized the: 07 Apr 2017
The last update of this report was done the: 07 Apr 2017

For any question, please contact:

PHENOMIN-ICS
Email: mutagenesis@igbmc.fr
Web site: http://www.ics-mci.fr/

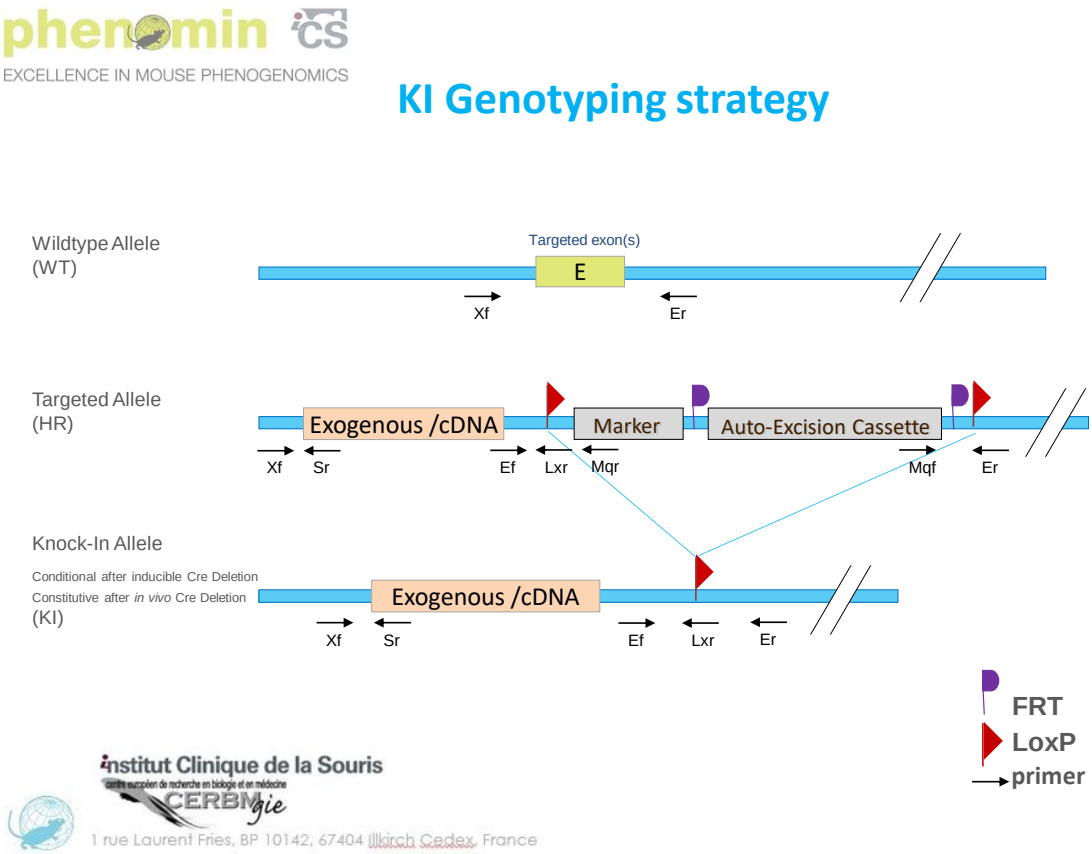
Table of contents

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **mEos4 KI in Glrb** Knockin (KI) 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	9202	TGCCCACCTACAGCACTGTTTGCAT
Er	9203	GCCCACAGCATCCAATTAAAGTTGC
Lxr	4774	GAAGTTATACTAGAGCGGCCGTTCAC
Mq1f	1219	CAGCTCATTCCTCCCACTCATGATC
Mq1r	265	TGCTAAAGCGCATGCTCCAGACTGC
Sr	9205	CGGAGTTTGATCCTCATGTCTGGCTT
Xf	9204	AGGACACTCAAACGTTCAGTGATGTAGGAT

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	WildType allele
Excision of the selection marker	9202-9203	Ef / Er	4445*	290	211
5' part of the selection marker	9202-265	Ef / Mq1r	297	---	---
3' part of the selection marker	1219-9203	Mq1f / Er	355	---	---
Exogenous/cDNA specific PCR	9204-9205	Xf / Sr	282	282	---
LoxP specific PCR	9202-4774	Ef / Lxr	205	205	---

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)
---: 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.

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.