EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project pCAG_lox_STOP_lox_Isthmin-1

Gt(ROSA)26Sor$^{tm24(CAG-Ism1)Ics}$/Ics

(PHENOMIN-ICS reference IR00006785 / Kos6785)

This report has been **prepared** by: Amélie Jeanblanc

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

The first version of this report was finalized the: 13 Feb 2019
The last update of this report was done the: 08 Nov 2020

For any question, please contact:

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

Table of contents

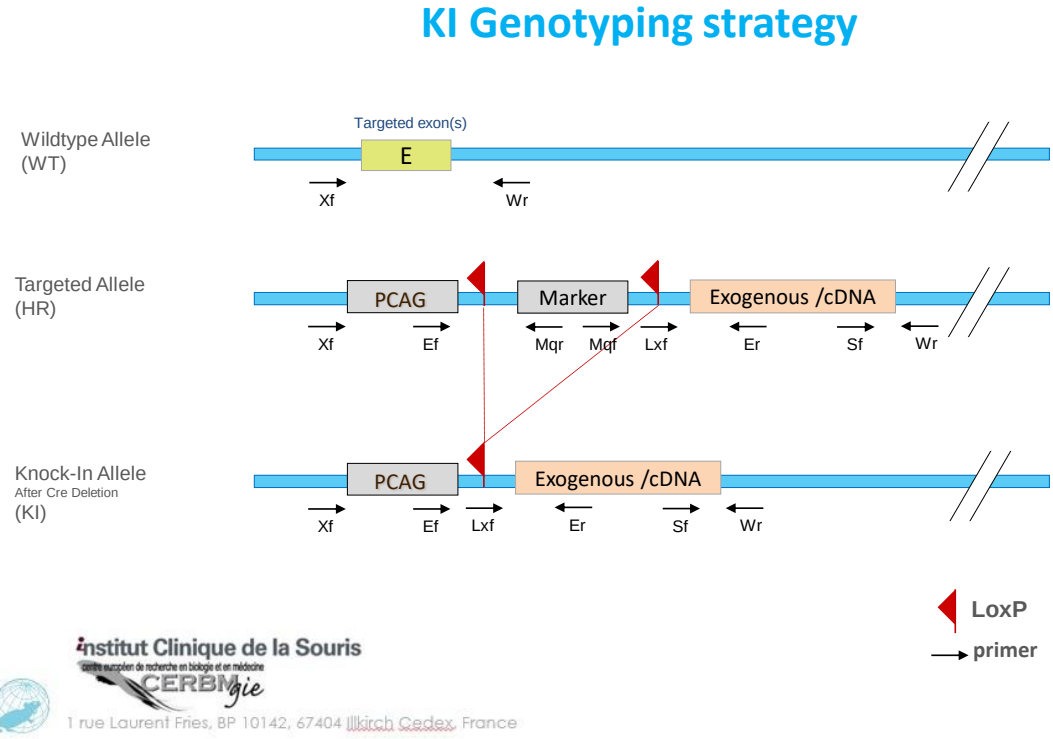
This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **pCAG_lox_STOP_lox_Isthmin-1** Knockin (KI) project.

1. PCR Genotyping protocol

1.1. Genotyping strategy

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

phenomin ics
EXCELLENCE IN MOUSE PHENOGENOMICS



Institut Clinique de la Souris
CERBMgie
1 rue Laurent Fries, BP 10142, 67404 Illkirch Cedex, France

Sequence of primers used for genotyping:

Position	Sequence
Ef	CGGCTCTAGAGCCTCTGCTAAC
Ef[2]	GCGGAGCCGAAATCTGGGAG
Er	CTGACGTGGAGTCACTCTCCAG
Er[2]	GTTATTCTGCAACTGGGACCCGC
Lxf	GAAGTTATCGGCGCGCCACC
Mq1f	GTTGTGGTTTGTCCAAACTCATCAATG
Mq1r	ATTCGCAGCGCATCGCCTTC
Sf	GGGGATCCACTAGTTCTAGAGCGG
Wr	CACACCAGGTTAGCCTTTAAGCC
Wr[2]	CCTTTAAGCCTGCCCAGAAG
Xf	CAGTAAGGGAGCTGCAGTGG

[2]: for a selected position, a second primer was designed

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	WildType allele
WildType allele specific PCR (5' part of the targeted locus)	Xf / Wr	6411*	3906*	231
Excision of the selection marker	Ef / Er	2843*	338**	---
Excision of the selection marker 2	Ef[2] / Er[2]	3300*	528**	---
5' part of the selection marker	Ef / Mq1r	262	---	---
3' part of the selection marker	Mq1f / Er[2]	256	---	---
cDNA 3'	Sf / Wr[2]	391	391	---
LoxP specific PCR	Lxf / Er	192	192	---

*: 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	35
72°C	1min	
72°C	7min	1
14°C	---	---

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

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.

2.1. 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