EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project conditional overexpression of pHsensor in Rosa

Gt(ROSA)26Sor^{tm33Ics}/Ics

(PHENOMIN-ICS reference IR00005742 / R5742)

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The first version of this report was finalized the: 31 Jan 2018
The last update of this report was done the: 31 Jan 2018

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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 overexpression of pHsensor 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. The KI allele corresponds to the tm33.1 allele

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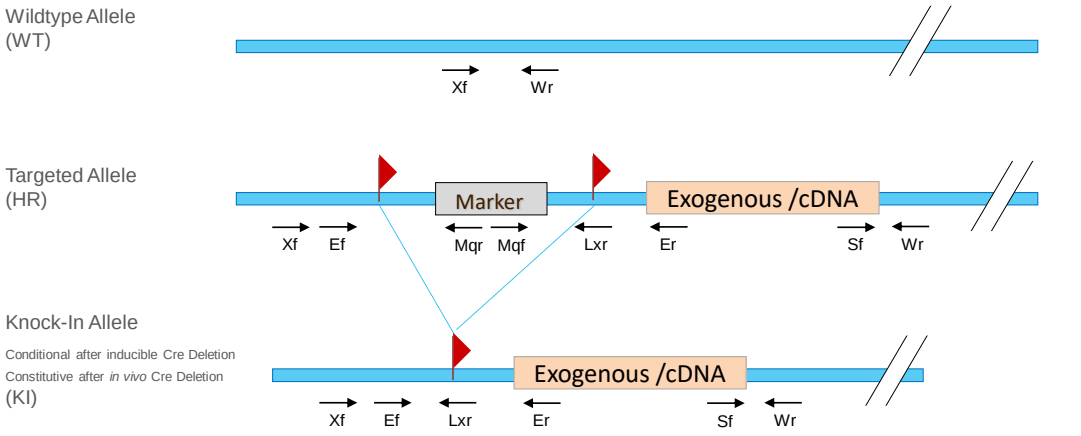
KI Genotyping strategy



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Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	8761	CGCAGTAGTCCAGGGTTTCCTTGATG
Ef^2	8759	CCTCCTGGCTTCTGAGGACCG
Er	8763	GTTGCATCACCTTCACCCTCTCCAC
Er^2	8789	CCATGGAACAGGTAGTTTTCCAGTAGTG
Lxr	8765	GCAGGTCGAGGGACCTAATAACTTCG
Mq1f	2687	CTGCATTCTAGTTGTGGTTTGTC
Mq1r	265	TGCTAAAGCGCATGCTCCAGACTGC
Sf	8764	CAATCTGCCCTTTCGAAAGATCCC
Wr	8762	ACCTGTTCAATTCCCCTGCAGGA
Wr^2	4035	CCTTTAAGCCTGCCCAGAAG
Xf	8760	CCCTCGTGATCTGCAACTCCAGTC

^{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)	KI allele	WildType allele
WildType allele specific PCR (5' part of the targeted locus)	8760-8762	Xf / Wr	---	---	111
Excision of the selection marker	8761-8763	Ef / Er	3034*	365	---
Excision of the selection marker 2	8759-8789	Ef^2 / Er^2	3199*	530	---
5' part of the selection marker	8759-265	Ef^2 / Mq1r	381	---	---
3' part of the selection marker	2687-8763	Mq1f / Er	289	---	---
cDNA 3'	8764-4035	Sf / Wr^2	454	454	---
LoxP specific PCR	8761-8765	Ef / Lxr	212	212	---

*: 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.