EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project conditional overexpression of 5X HA-C4b-T2A-mScarlett in Rosa

Gt(ROSA)26Sor$^{tm30.1(CAG-C4b,-mScarlet)Ics}$/Ics

(PHENOMIN-ICS reference IR00007413 / Kos7413)

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:

PHENOMIN-ICS
Email: mutagenesis@igbmc.fr
Web site: http://www.ics-mci.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 overexpression of 5X HA-C4b-T2A-mScarlett in Rosa** Conditional Knock In (Flex strategy) (KI-Flex) project.

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele. HR del allele corresponds to tm30.1

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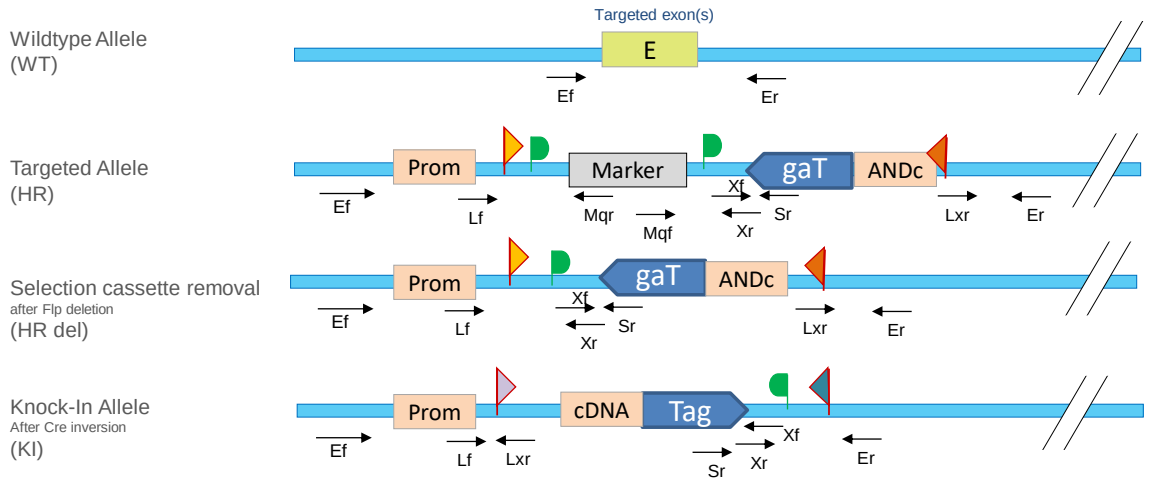
KI _FLEX Genotyping strategy



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

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Sequence of primers used for genotyping:

Position	Sequence
Ef	GCACTTGCTCTCCCAAAGTCGC
Er	CCGAGGCGGATCACAAGC
Lf	GGCAACGTGCTGGTTA
Lxr	GGCCGAATTCATCGATATAACTTCG
Sr	GGCATGGACGAGCTGTACAAG
Mq1f	AGGGCCAGCTCATTCCTCCCACTC
Mq1r	TGCTAAAGCGCATGCTCCAGACTGC
Xf	CATTGATGAGTTTGGACAAACCAC
Xr	CTGCATTCTAGTTGTGGTTTGTC

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	HR del allele	KI allele	WildType allele
WildType allele specific PCR (3' part of the targeted locus)	Ef / Er	10354*	8501*	8535*	449
Excision of the selection marker	Lf / Sr	2272*	419	---	---
5' part of the selection marker	Lf / Mq1r	190	---	---	---
3' part of the selection marker	Mq1f / Xr	199	---	---	---
Exogenous/cDNA specific PCR	Xf/ Sr	261	261	261	---
LoxP specific PCR	Lf / Lxr	---	---	100	---

*: 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.