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EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

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

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

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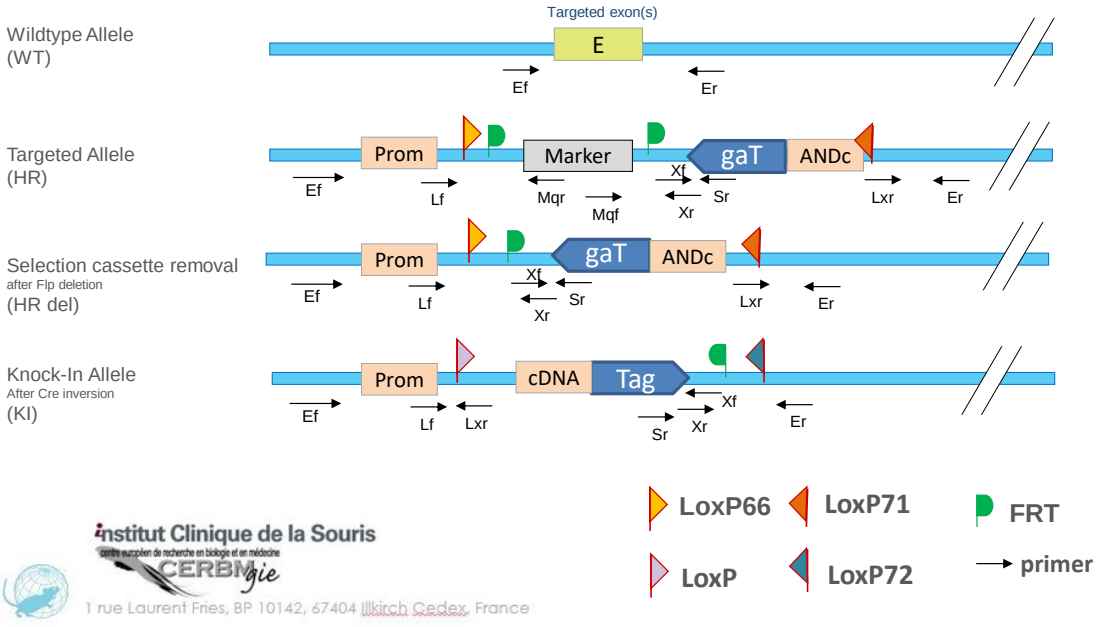
KI _FLEX Genotyping strategy



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

conditional overexpression of 5X HA-C4b-T2A-mScarlett in Rosa - Gt(ROSA)26Sor$^{tm30(CAG-C4b,-mScarlet)Ics}$/Ics

Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	10398	GCACTTGCTCTCCCAAAGTCGC
Er	10399	CCGAGGCGGATCACAAGC
Lf	10400	GGCAACGTGCTGGTTA
Lxr	10402	GGCCGAATTCATCGATATAACTTCG
Sr	10401	GGCATGGACGAGCTGTACAAG
Mq1f	3720	AGGGCCAGCTCATTCCTCCCACTC
Mq1r	265	TGCTAAAGCGCATGCTCCAGACTGC
Xf	2861	CATTGATGAGTTTGGACAAACCAC
Xr	2687	CTGCATTCTAGTTGTGGTTTGTC

PCR fragments expected size (bp):

Region analyzed	Primers used	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)	10398-10399	Ef / Er	10354*	8501*	8535*	449
Excision of the selection marker	10400-10401	Lf / Sr	2272*	419	---	---
5' part of the selection marker	10400-265	Lf / Mq1r	190	---	---	---
3' part of the selection marker	3720-2687	Mq1f / Xr	199	---	---	---
Exogenous/cDNA specific PCR	2861-10401	Xf/ Sr	261	261	261	---
LoxP specific PCR	10400-10402	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.