EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Conditional overexpression of TGM2
Targeted transgenesis in Rosa26

(PHENOMIN-ICS reference IR00005648 / K5648)

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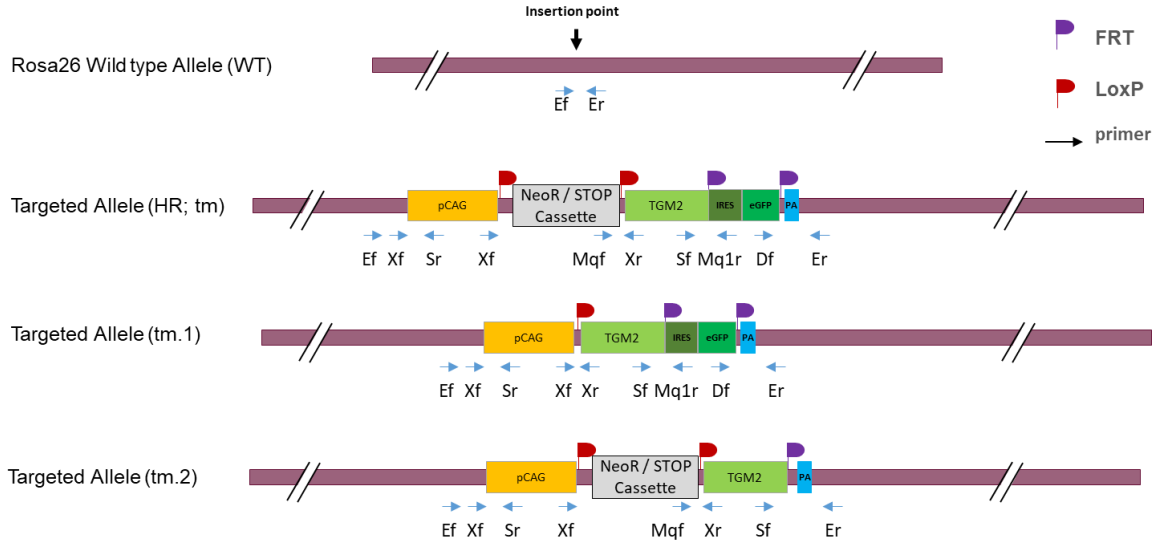
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Conditional overexpression of TGM2 Targeted transgenesis in Rosa26** 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	Sequence
Df	CCTGAGCAAAGACCCCAACG
Ef	AAAGTCGCTCTGAGTTGTTAT
Ef[2]	GGGAGGGGAGTGTTGCAATACCTTTCT
Er	CCTTTAAGCCTGCCCAGAAG
Sf	ACAAGCTGAAGGCTGTGAAGGGCTT
Sr	TGGCGTTACTATGGGAACATACGTCAT
Mqf	GTGGTTTGTCCAAACTCATCA
Xf	GCGGAGCCGAAATCTGGGAG
Xr	CATCTCTTAGTGGAAAACGGGCCTT
Mq1r	CAAGCGGCTTCGGCCAGTAACGTTAG

[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)	Tm.2 allele	Tm.1 allele	WildType allele
Excision of the selection marker	Xf / Xr	3133*	3133*	628	---
5' part of the marker	Ef[2]/Sr	422	422	422	---
3' part of the reporter	Df/Er[2]	584	---	---	---
Excision of the Floxed reporter	Sf/Er[2]	1937*	544	544	---
5' part of Exogenous/cDNA specific PCR	Mqf / Xr	353	353	---	---
3' part of Exogenous/cDNA specific PCR	Sf/Mq1r	192	---	---	---
WildType allele specific PCR (5' part of the targeted locus)	Ef / Er	8566*	7173*	4668*	239

*: 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.