Gt(ROSA)26Sortm1(CAG-Trib1) (IR00003210 / K624 ICS internal reference) mouse line genotyping protocol

Table of contents

For any question, please contact:

Mouse Clinical Institute – Institut Clinique de la Souris (ICS)
ICS genotyping service
1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex France
Email: Mutagenesis@igbmc.fr

This protocol has been validated by Valérie Rousseau.

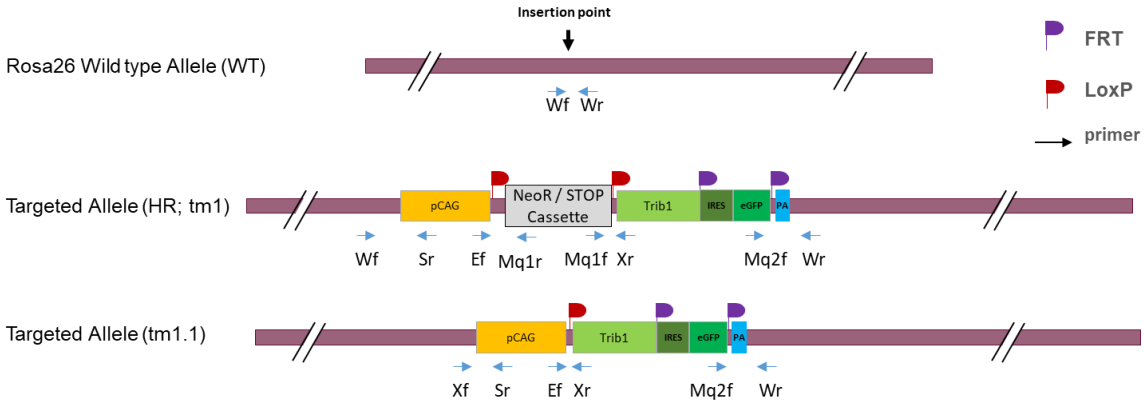
Genotyping protocol
Gt(ROSA)26Sortm1(CAG-Trib1) (IR00003210 / K624 ICS internal reference)

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the Gt(ROSA)26Sor$^{tm1(CAG-Trib1)}$

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
Ef	GCGGCTCTAGAGCCTCTGCTAACCAT
Wr	CCGAAAATCTGTGGGAAGTCTTGTCC
Mq1f	GTGGTTTGTCCAAACTCATC
Mq1r	CAAGCGGCTTCGGCCAGTAACGTTAG
Mq2f	CACATGGTCCTGCTGGAGTTCGT
Wf	GGGAGGGGAGTGTTGCAATACCTTTCT
Sr	TGGCGTTACTATGGGAACATACGTCAT
Xr	GTCGGGGGGACTCGAGCACTC

PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	Targeted allele (HR; tm1 allele) (L3)	Tm1.1 allele	WildType allele (WT)
5' part of the targeted construct	Wf / Sr	424	424	---
Wild type allele specific PCR	Wf / Wr	7674*	5138*	244
5' part of the selection marker (Neo)	Ef / Mq1r	263	---	---
3' part of the selection marker (Neo)	Mq1f / Dr	330	---	---
Excision of the NeoR cassette	Ef-Dr	2894*	388	
3' part of the targeting construct marker (GFP)	Mq2f / Wr	634		---

* 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	5 min	1

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