Gt(ROSA)26Sortm20Ics/Ics (IR00002845 / K576 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 Christelle Roth.

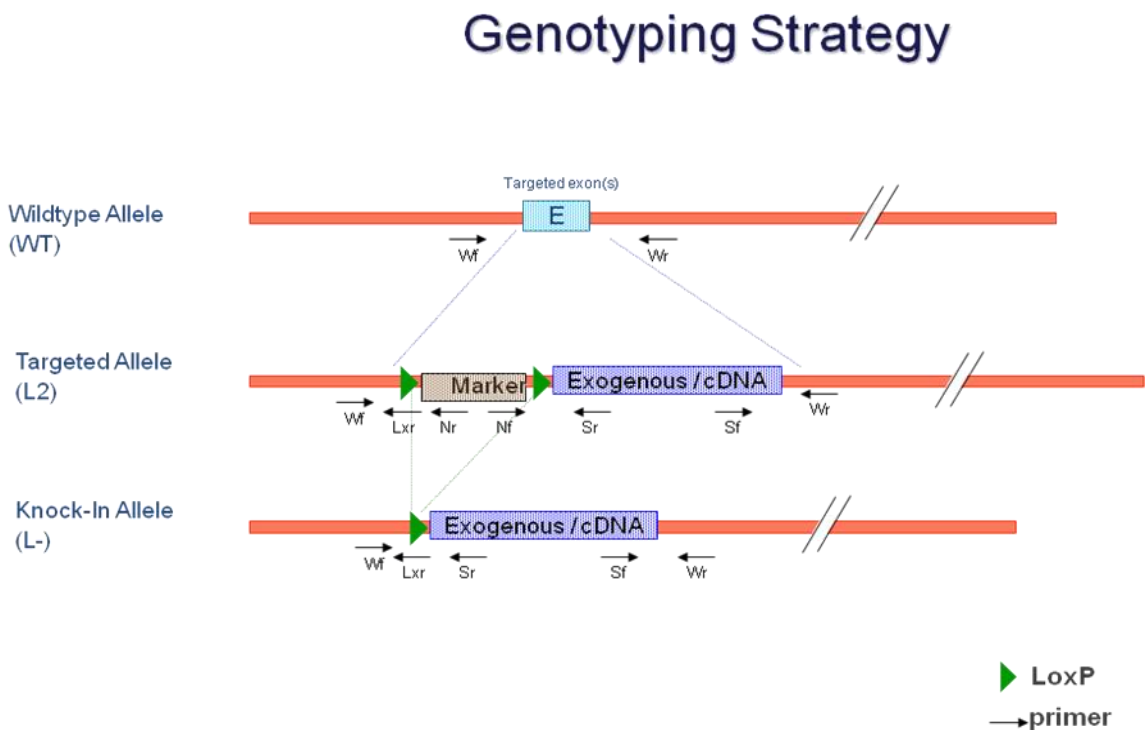
Genotyping protocol
Gt(ROSA)26Sortm20Ics/Ics (IR00002845 / K576 ICS internal reference)

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **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	Primers	Sequence
Wf	4036	AAAGTCGCTCTGAGTTGTTAT
Wr	4035	CCTTTAAGCCTGCCCAGAAG
Lxr	5196	GAAGTTATATTAAGGGTTCCGGATC
Nr	265	TGCTAAAGCGCATGCTCCAGACTGC
Nf	4040	TCCCCATCAAGCTGATCC
Sr	5193	AAAGCGCCGCCATAGTC
Sr	5194	ACCCGTTCACCACCACA
Sf	5195	CATCTACAAGGTGAAGCTGCGCG

PCR fragments expected size (bp):

Region analyzed	**Primers used**	Position on the primer (see the map above)	Targeted allele (L2)	KI allele (L-)	WildType allele (WT)
WildType allele specific PCR (5' part of the targeted locus)	4036-4035	Wf / Wr	---	---	239
Excision of the selection marker	4036-5194	Wf / Sr	3223*	554**	---
5' part of the selection marker	4036-265	Wf/ Nr	501	---	---
3' part of the selection marker	4040-5193	Nf / Sr	140	---	---
Exogenous/cDNA specific PCR	5195-4035	Sf / Wr	689	689	---
LoxP specific PCR	4036-5196	Wf / Lxr	396	396	---

* This PCR product will not be observed using our PCR genotyping conditions (see description below)
** This PCR is only verified if mice are generated
--- 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:

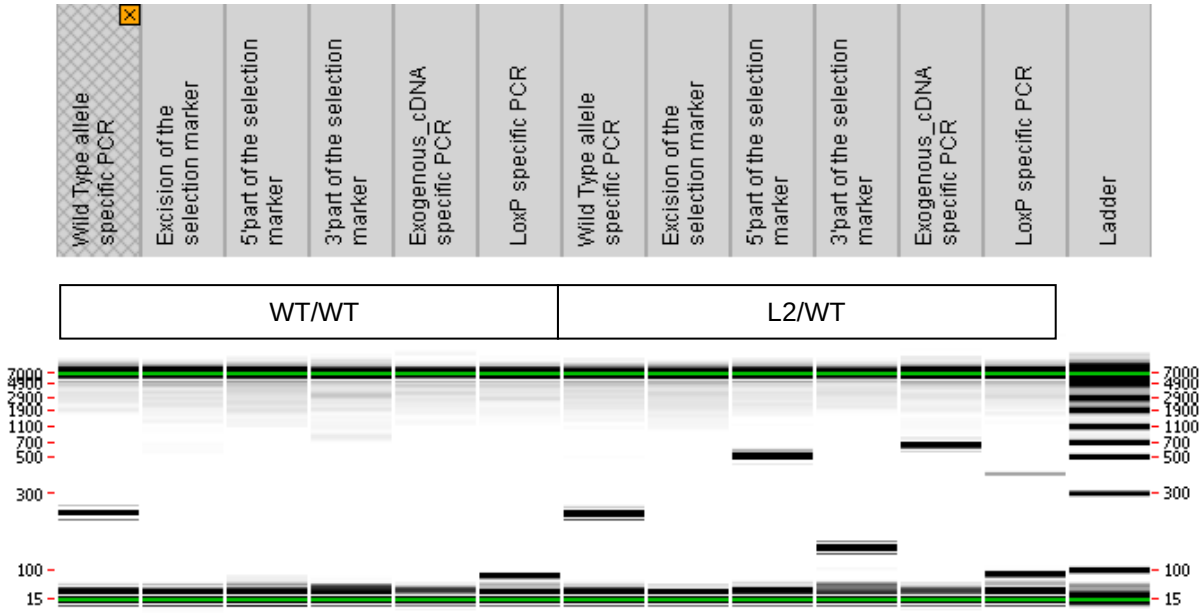
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

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

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was not done by gel electrophoresis but using LabChip® 90 microfluidic apparatus. PCR products were run on the HT DNA 5K LabChip® 90 Assay Kit.

Representative genotyping picture



Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.