Genotyping protocol

Gt(ROSA)26Sortm21(CAG-Phox2b,-tdTomato)Ics/Ics

IR00003528 / K668

(ICS internal reference)

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1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **ROSA locus** Conditional Knockin / Knockout (KI-cKO) project.

1.1. Genotyping strategy

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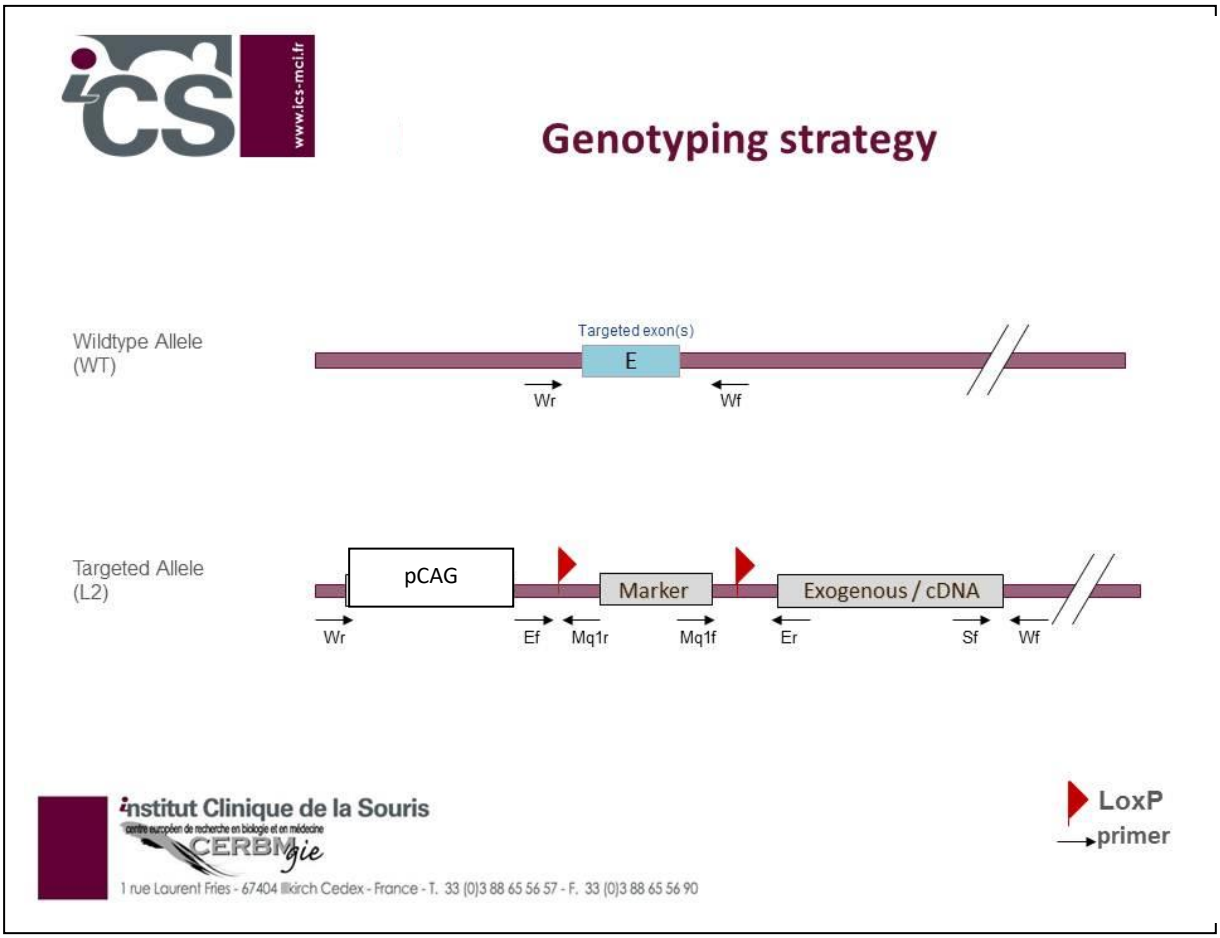


Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	2434	CCCCTCTGCTAACCATGTTCATGC
Er	6507	GTGGCCCCAAAAGTGGTCCTTATC
Mq1f	3300	GTGGTTTGTCCAAACTCATCA
Mq1r	265	TGCTAAAGCGCATGCTCCAGACTGC
Sf	6508	GCATGGACGAGCTGTACAAGTAAGC
Wf	4036	AAAGTCGCTCTGAGTTGTTAT
Wr	4035	CCTTTAAGCCTGCCCAGAAG

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	KI allele (L2)	KO 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	2434-6507	Ef / Er	2993	342	---
5' part of the selection marker	2434-265	Ef / Mq1r	191	---	---
3' part of the selection marker	3300-6507	Mq1f / Er	317	---	---
cDNA 3'	6508-4035	Sf / Wr	385	385	---

*: 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:

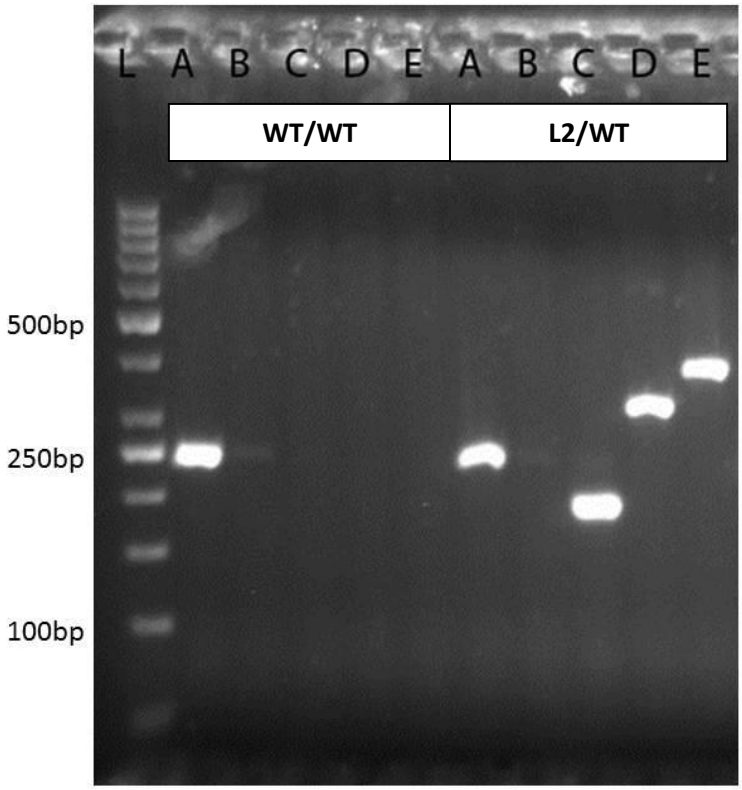
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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 done by gel electrophoresis.

Representative genotyping picture



L: Ladder (Thermo Generuler 50bp DNA ladder SM0372)
A: WildType allele specific PCR
B: Excision of the selection marker
C: 5' part of the selection marker
D: 3' part of the selection marker
E: cDNA 3'