



## Genotyping protocol

Gt(ROSA)26Sortm22(CAG-DCLRE1C,-  
EGFP)<sup>Ics/Ics</sup>

IR00003637 / K686b

(ICS internal reference)

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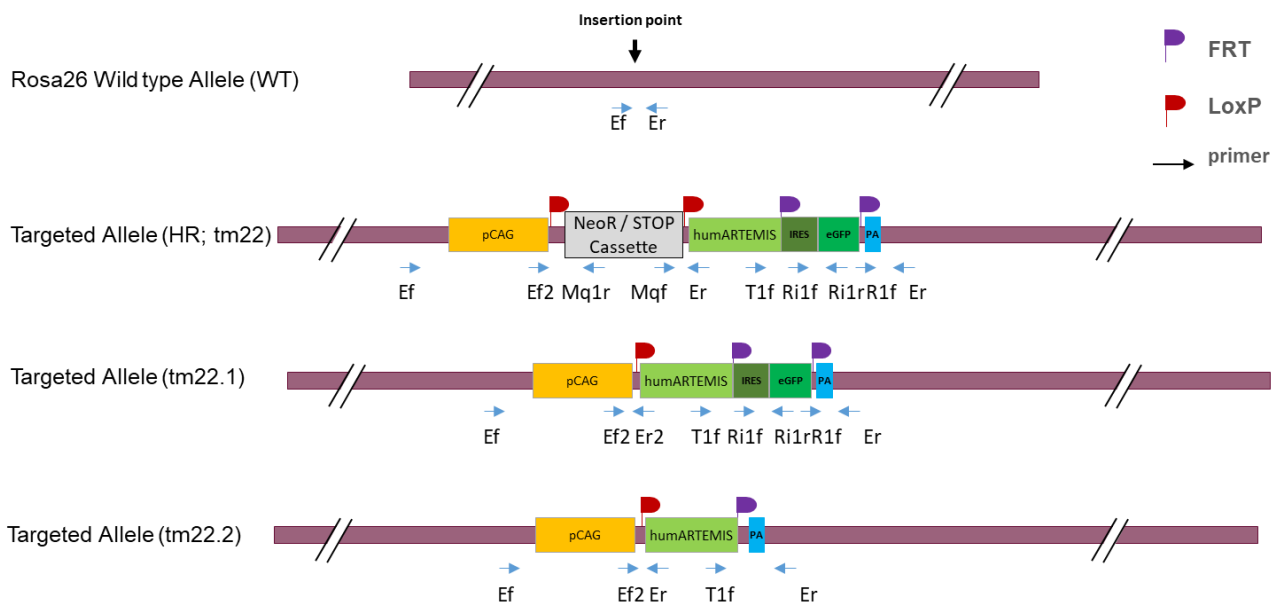
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### 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **pCAG-loxSTOPlox-hum Artemis 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
Ef	AAAGTCGCTCTGAGTTGTTAT
Er	GCCTTTAAGCCTGCCAAGA
Ef2	GCGGCTCTAGAGCCTCTGCTAACCAT
Er2	AATCCTTTCATGTGATCTTTGTGGCAG
Mq1r	ATTCGACGCGCATCGCCTTC
R1f	CTCGGCATGGACGAGCTGTACAAG
Ri1f	GCTGAAGGATGCCAAGAAGGTAC
Ri1r	GTGAACAGCTCCTCGCCCTTG
T1f	AAGCTGGCAACTGGTGAGAGTATAGCAG

### PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer (see the map above)	Tm22 (HR)	Tm22.1 KI allele GFP+	Tm22.2KI allele GFP-	WT allele
WildType allele specific PCR (5' part of the targeted locus)	4036-4035	Ef / Er	8627	6121	4728	239
Excision of the selection marker	6474-6473	Ef2 / Er	2798	292	292	---
5' part of the selection marker	6474-1255	Ef2 / Mq1r	263	---	---	---
3' reporter	2242-4035	R1f / Er	509	509	---	---
Internal reporter	1877-2936	Ri1f / Ri1r	193	193	---	---
Excision of the floxed fragment	6472-4035	T1f / Er	1990	1990	597	---

\*: 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 <sub>2</sub> O	up to 15 µl

### Cycling conditions:

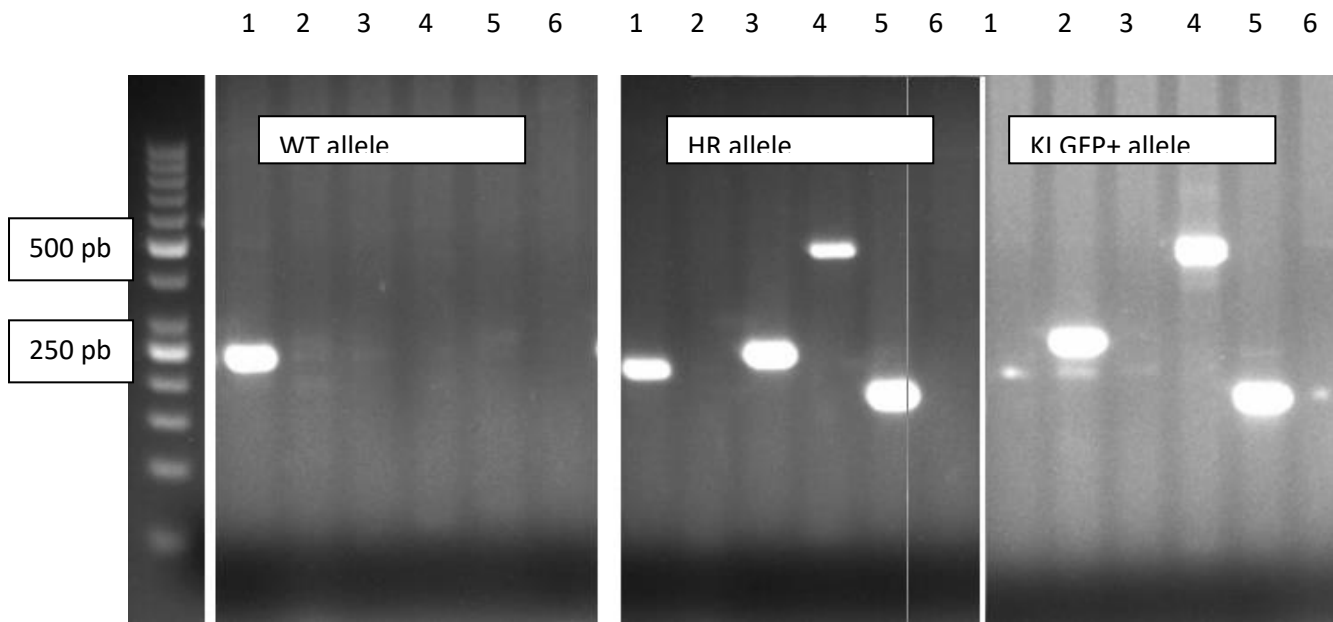
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5min	1

**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 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



PCR numbers:

1. WildType allele specific PCR
2. Excision of the selection marker
3. 5' part of the selection marker
4. 3' reporter
5. Internal reporter
6. Excision of the floxed fragment

