

Genotyping protocol

Project Spi1-eGFP-CreER^{T2}

(PHENOMIN-ICS reference IR00005013 / E5013)

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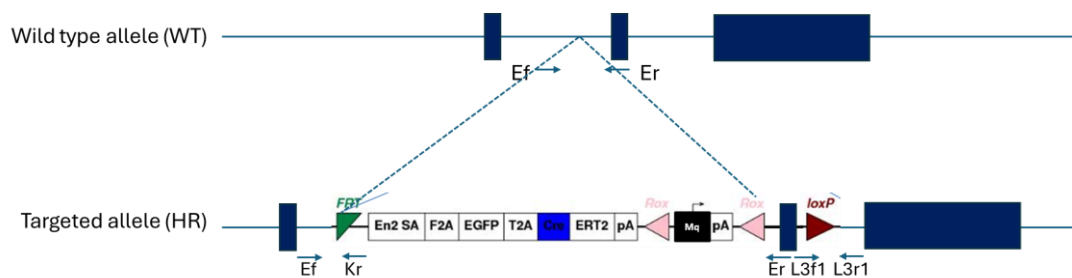


1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Spi1** Eucommtools Knockin (KI E-Tool marker) 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:

primers	Sequence
Ef	CCCATTTTCTGTCTCTCCATGTACCCA
Er	CAACTGTGGCCTCTGGTTTAGGGAA
Kr	GGGCAAGAACATAAAGTGACCCTCC
L3f1	CCAGCAGCCATGTTTCAGAGGTG
L3r1	CATGACCTCACACTCACAGTCCTG

PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	Targeted allele (HR)	WildType allele
WildType allele specific PCR (5' part of the targeted locus)	Ef / Er	6538*	330
Cassette 5'	Ef / Kr	360	---
3' LoxP	L3f1/L3r1	315	216

*: 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 ₂ 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.

