phenomin ICS

EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Tlr7-IRES-mCherry

Trl7$^{tm2.1(Trl7,-mCherry)Ics}$

(PHENOMIN-ICS reference IR00007047 / Kos7047)

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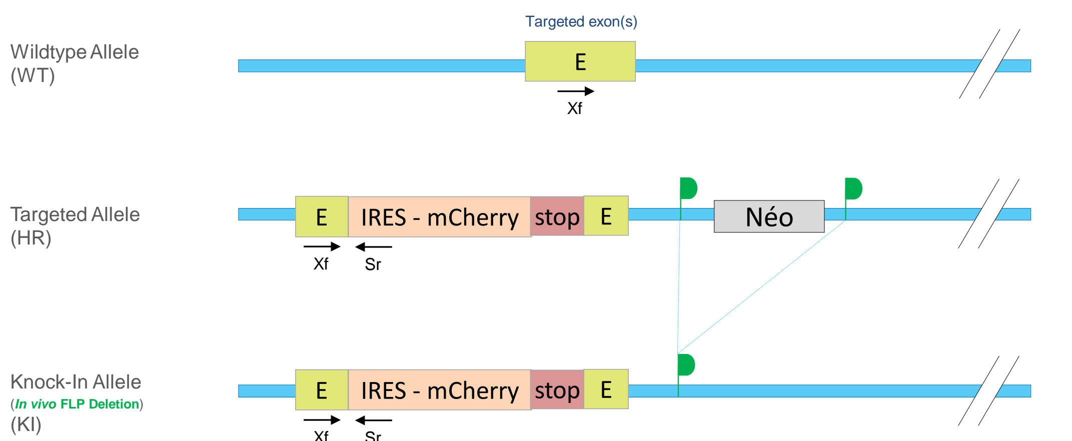
This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Tlr7-IRES-mCherry** Knockin (KI) mouse line.

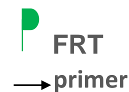
1. PCR Genotyping protocol

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.

KI Genotyping strategy





Sequence of primers used for genotyping:

Position	Sequence
Sr	CAAGCGGCTTCGGCCAGTAACGTTAG
Xf	GCTCAGGAAGAGACTCTGCAGG

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	WildType allele
Exogenous/cDNA specific PCR	Xf / Sr	229	229	---

---: 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:

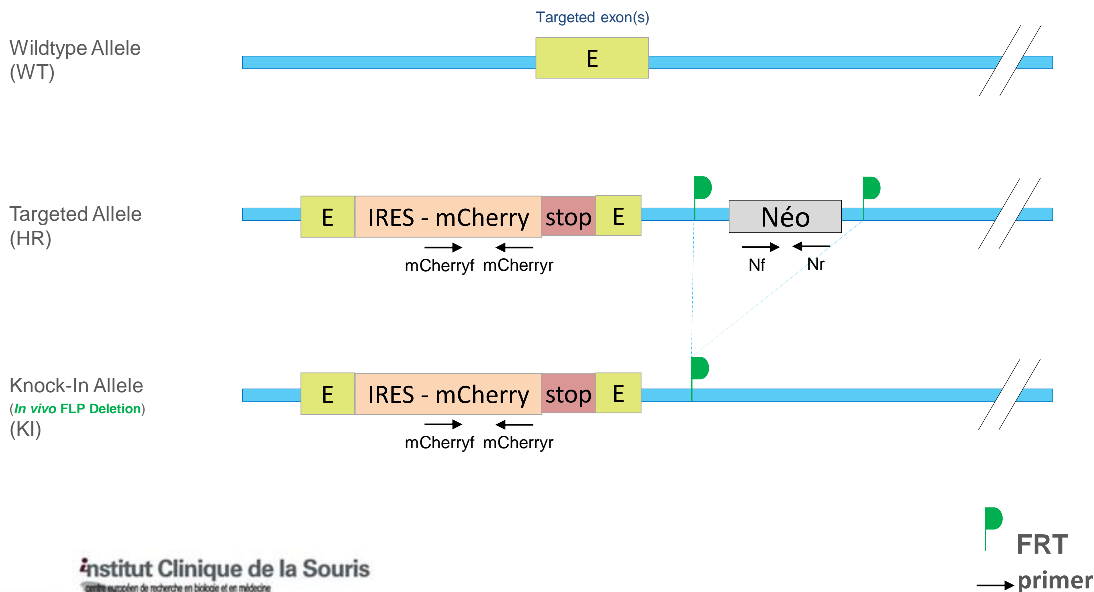
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	35
72°C	1min	
72°C	7min	1
14°C	---	---

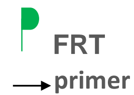
NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

2. qPCR Genotyping protocol

2.1. 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
mCherryf	mCherry f1	TGAGGTCAAGACCACCTACA
mCherryr	mCherry r1	CTGTTCCACGATGGTGTAGTC
Nf	Neo f1	TGAATGAACTGCAGGACGAG
Nr	Neo r1	TTCCCGCTTCAGTGACAAC

qPCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	WildType allele
mCherry qPCR	mCherry f1 – mCherry r1	mCherryf / mCherryr	115	115	---
Neomycine qPCR	Neo f1 – Neo r1	Nf / Nr	96	---	---

---: no Amplicon should be obtained

2.2. qPCR protocol

Reagents:	Volume:
- EvaGreen (biorad)	3,5µl
- DNA (10ng/µl)	3µl
- Forward primer (100µM)	0,06µl
- Reverse primer (100µM)	0,06µl
- Sterile H2O	up to 7µl

Cycling conditions:

Temp	Time	#Cycles
98°C	2min	1
98°C	5s	
60°C	20s	45
Melting curve analysis 65°c -> 95°c		

Follow manufacturer's protocol for programming the data acquisition of dsDNA product.

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