EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Trl7- IRES-eYFP-Cre

Trl7$^{tm1.1(Trl7,-eYFP/Cre)Ics}$

(PHENOMIN-ICS reference IR00007046 / Kos7046)

For any question, please contact:

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Table of contents

This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Tlr7-IRES-eYFP-Cre** 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.

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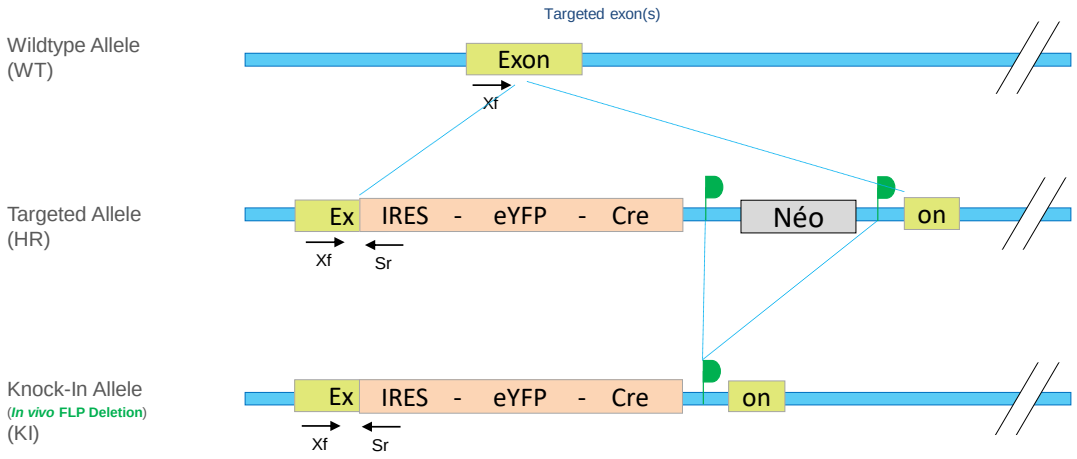
KI Genotyping strategy



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Sequence of primers used for genotyping:

Position	Primers	Sequence
Sr	5735	CAAGCGGCTTCGGCCAGTAACGTTAG
Xf	10132	GAGTGGCCTGCAAATCCACAG

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	WildType allele
Exogenous/cDNA specific PCR	10132-5735	Xf / Sr	195	195	---

---: 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.

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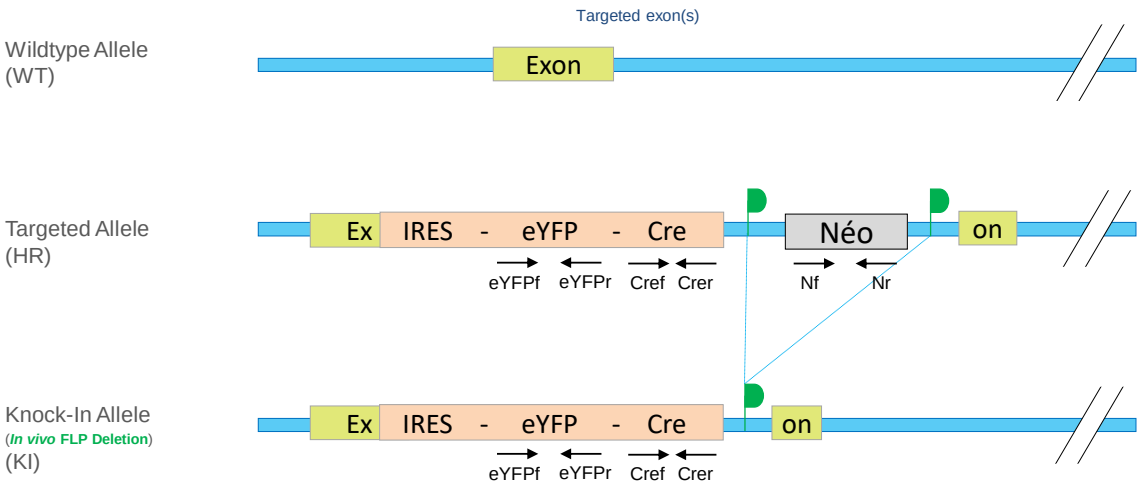
KI Genotyping strategy



FRT
primer

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Sequence of primers used for genotyping:

Position	Primers	Sequence
Cref	Cre f1	CGCAAGAACCTGATGGACATG
Crer	Cre r1	ACCGGCAAACGGACAGAA
eYFPf	YFP f1	AACCACTACCTGAGCTACCA
eYFPr	YFP r1	TCGTCCATGCCGAGAGT
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
Cre qPCR	Cre f1 – Cre r1	Cref / Crer	82	82	---
eYFP qPCR	eYFP f1 – eYFP r1	eYFPf / eYFPr	113	113	---
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.