EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Rbpjl

(PHENOMIN-ICS reference IR00005014 / E5014)

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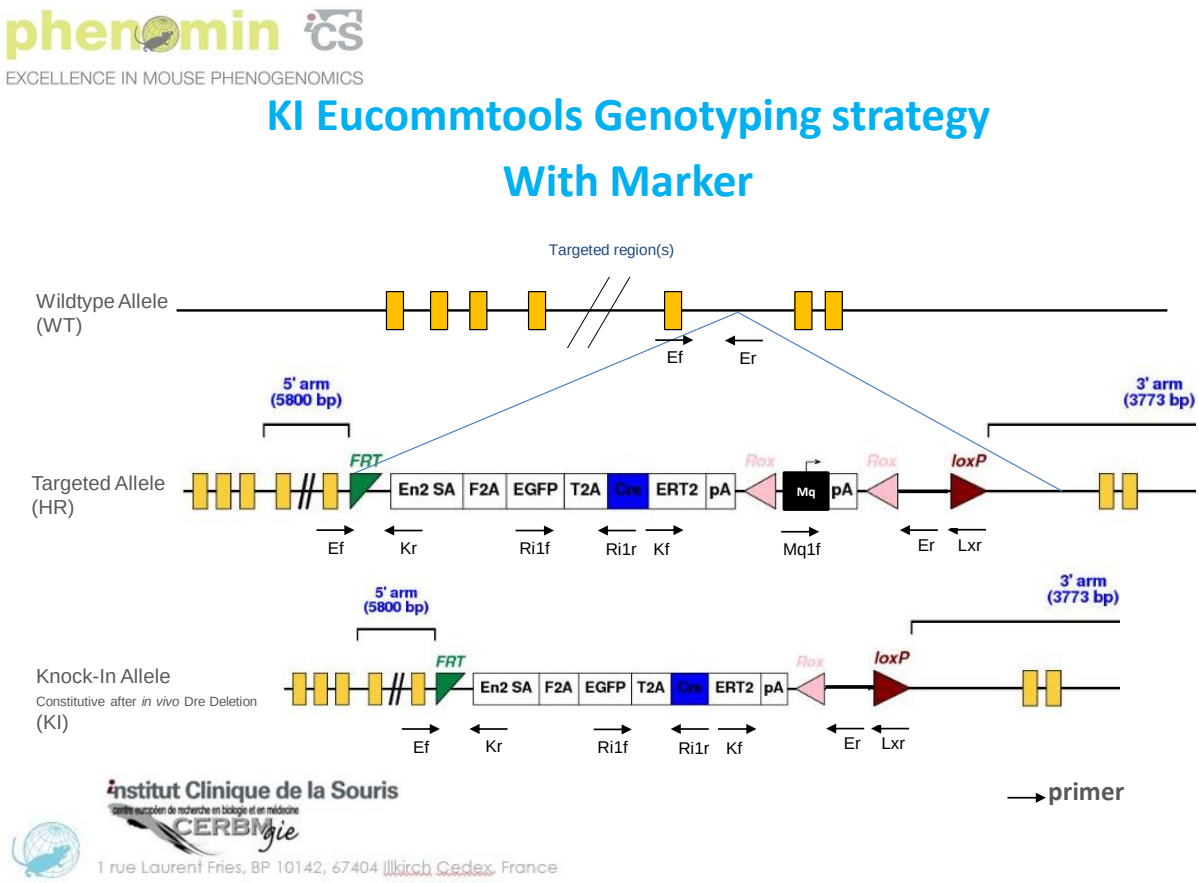
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Rbpjl** 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:

Position	Primers	Sequence
Ef	8588	CTTAACTGCTGAGCCGTCTCTCCTACC
Er	8589	GCCTGCATGGCAGAAGAGACC
Lxr	6957	CTATACGAAGTTATGGCGCG
Kf	5990	CGGGCTCTACTTCATCGCATTCCTT
Kr	3277	CTCCTACATAGTTGGCAGTGTTTGGG
Mq1f	7353	GCTTCACCGTCACCGCCGA
Ri1f	2344	CGACCACTACCAGCAGAACACC
Ri1r	5626	GGTTCTTGCGAACCTCATCACTCGT

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	WildType allele
Excision of the selection marker	5990-6957	Kf / Lxr	2157*	405**	---
GFP-Cre	2344-5626	Ri1f / Ri1r	317	317	---
WildType allele specific PCR (5' part of the targeted locus)	8588-8589	Ef / Er	6576*	4824*	421
Cassette 5'	8588-3277	Ef / Kr	457	457	---
3' part of the selection marker	7353-6957	Mq1f / Lxr	671	---	---

*: 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.