Genotyping protocol

Fkbp10

IR00002730 / E97

(ICS internal reference)

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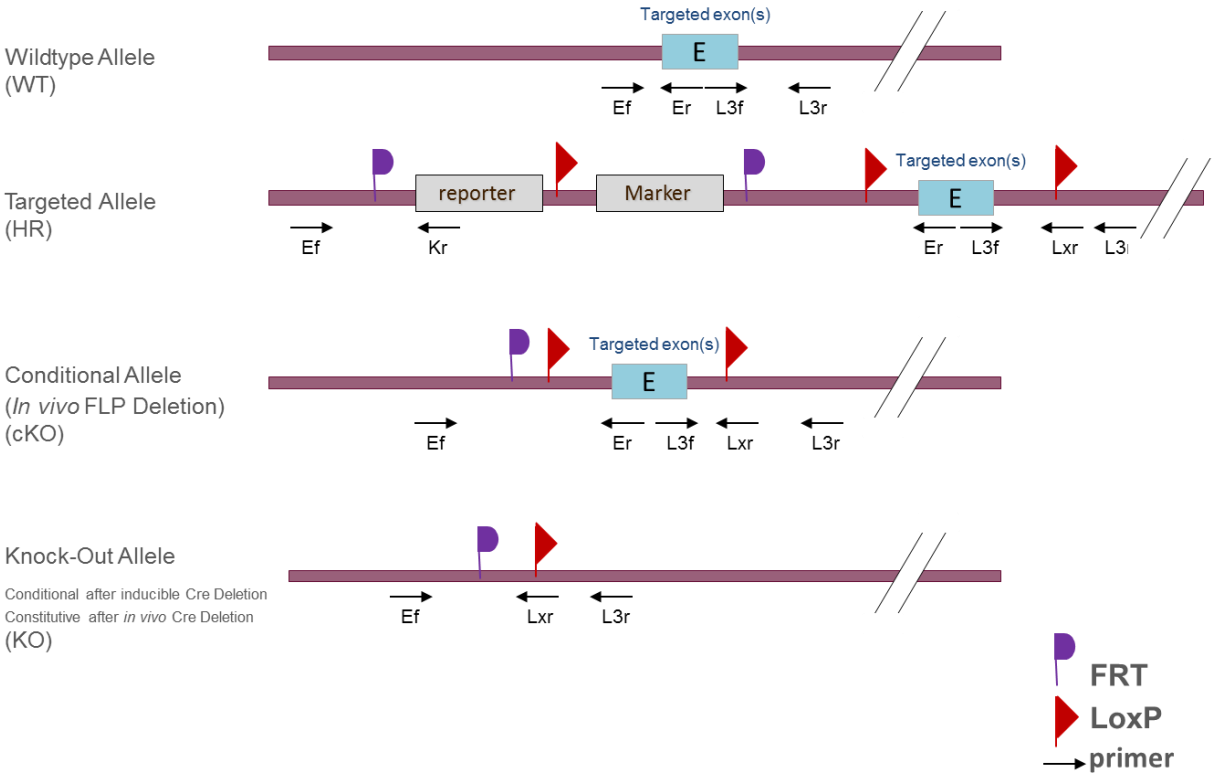
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Fkbp10** Constitutive Knockout / Conditional Knockout (KO-cKO) 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	5527	CCGCAAAGCCAAACAGAACAGC
Er	6554	ACATCTCTTGGGCACTGAGCGC
Er[2]	1936	GTGGATGTGGAATGTGTGCGAGG
Er[3]	5533	CCAGAGGAGGCCAGGAGCATTTA
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	5529	TGCCTACGGGGAGAATGGGACA
L3f[2]	5531	CCCCAGGCTACCCTTATGCTCCTCT
L3r	5530	CCAACAACTTGCAGGCTCCCA
Lxf	6295	TTATGTTTAAACGGCGCGCCC
Lxf[2]	6013	TCATGTCTGGATCCGGAATAACTTCGTA
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC

[2] or [3]: for a selected position, a second or third primer was designed

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	conditional allele (KO-cKO)	KO allele	WildType allele
Lox interne K7 Eur (with DMSO)	6295-6554 (with 5% DMSO)	Lxf/ Er	119	---	---	---
Lox interne K7 Eur (with DMSO)	6013-1936 (with 5% DMSO)	Lxf[2] / Er[2]	199	---	---	---
5' part of the selection marker	5527-3209	Ef / Kr	448	---	---	---
Presence of the distal loxP	5529-5530	L3f / L3r	416	416	---	359
Distal loxP specific PCR	5531-3255	L3f[2] / Lxr	277	277	---	---
Excision of the selection marker	5527-5533	Ef / Er[3]	7379*	475	---	307

*: 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.

2. Cre and Flp genotyping method

You will find the genotyping protocol in the publication:

Highly-efficient, fluorescent, locus directed cre and FlpO deleter mice on a pure C57BL/6N genetic background.
Birling MC, Dierich A, Jacquot S, Hérault Y, Pavlovic G.
Genesis. 2012 Jun;50(6):482-9. doi: 10.1002/dvg.20826. Epub 2012 Mar 20.