EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Fat1 isoforms double cKO (Dre et LoxP)

Fat1$^{em1.1Ics}$

(PHENOMIN-ICS reference IR00005811 / K5811)

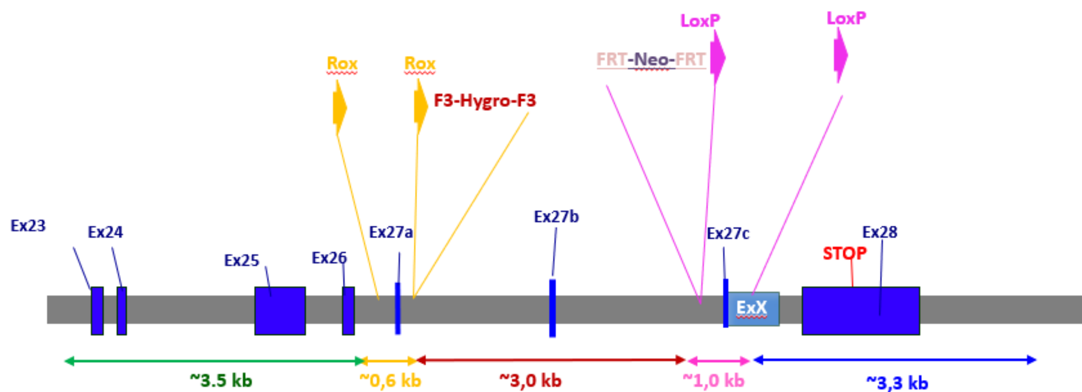
For any question, please contact:

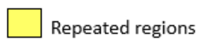
PHENOMIN-ICS
Email: mutagenesis@igbmc.fr
Web site: http://www.ics-mci.fr/

Table of contents

1. Genotyping protocol and data ... 3
1.1. Genotyping strategy ... 3
1.2. PCR protocol ... **Erreur ! Signet non défini.**
2. Cre and Flp genotyping method ... 5

Strategy proposed





ExX : part of isoform Fat1-007 ENSMUST00000189367 (non coding potential mRNA)

- After Flp mediated excision: one F3 remaining and 1 FRT remaining
- Ex27a removed after Dre excision
- Ex27c removed after Cre excision

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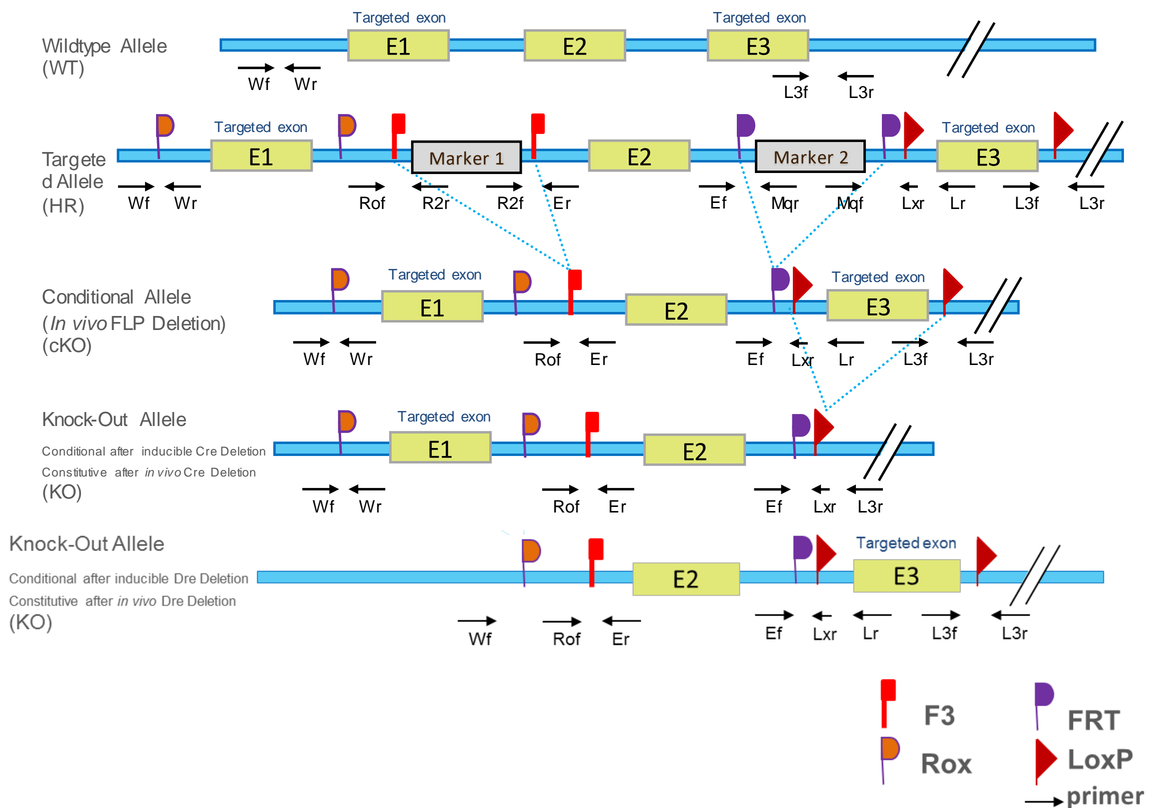
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Fat1 double cKO (Dre et Cre)** Conditional Knockout (cKO) project.

1.1. Genotyping strategy

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

cKO Genotyping strategy



Sequence of primers used for genotyping:

Position	Sequence
Ef	CCACAGTTCTACTGTGACTGACTGC
Er	TGGTCATGGATCTTAAGGAAGTTCC
L3f	CACCAAAAGAATCTTTGGCTGC
L3r	GTGGTTTCATTGGCCTCATTAGC
Lr	CACAGATTATTGAAGACAACGTGGG
Lxr	CATTATACGAAGTTATCCGGCCG
Mq1f	ATTCGCAGCGCATCGCCTTC
Mq1r	TGCTAAAGCGCATGCTCCAGACTGC
R2r	GACAGACGTCGCGGTGAGTTCAGGC
Rof	CTACCACTTCCAAAATAACATGGGC
Wf	AGTGCTGCTGTCATTCCTTAGGC
Wr	TGTTATGAGTGTGCACTGCATGG

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	cKO allele	KO allele (Cre)	KO allele (Dre)	WildType allele
WildType allele specific PCR (5' part of the targeted locus)	Wf / Wr	444	444	444	---	412
5' part of the selection marker 1 (**with DMSO** 5%)	Rof / R2r	662	---	---	---	---
3' part of the selection marker 1	R2f / Er	316	---	---	---	---
Excision of the selection marker 1	Rof / Er	1921*	93**	93**	93**	---
5' part of the selection marker 2	Ef / Mq1r	162	---	---	---	---
3' part of the selection marker 2	Mq1f / Lxr	592	---	---	---	---
Excision of the selection marker 2	Ef / Lr	2068*	215	---	215	93
LoxP specific PCR	Ef / Lxr	1974*	121**	121**	121**	---
Presence of the distal loxP (**with Betaine** 0,5%)	L3f / L3r	471	471	---	471	437
Excision of the floxed exon(s), i.e. knock out (CRE)	Ef / L3r	3238*	1385*	207**	1385*	---
Excision of the floxed exon(s), i.e. knock out (DRE)	Wf / Er	1015*	1015*	1015*	500	---

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)
**:This PCR 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.