EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Tnfsf4 FP-KI/cKO

(PHENOMIN-ICS reference IR00005907 / K5907)

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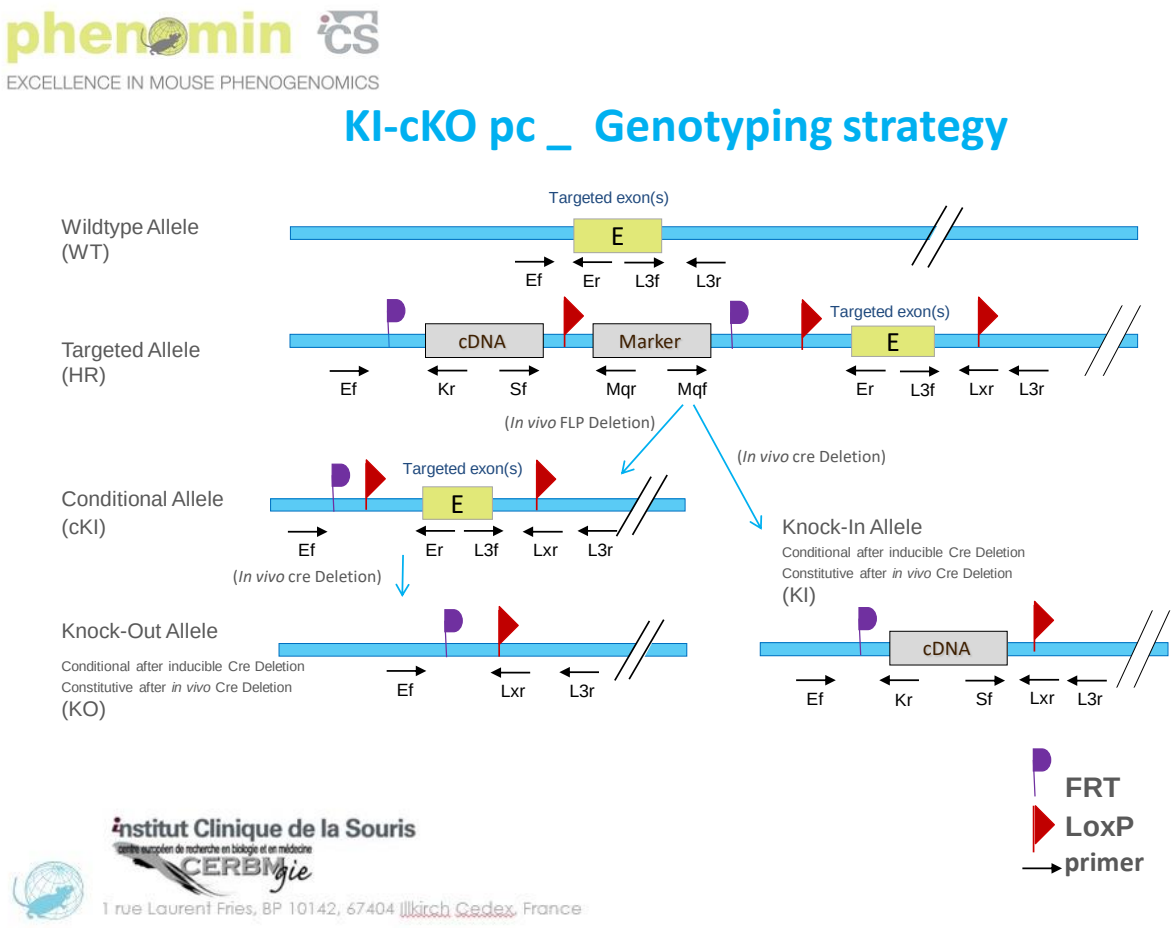
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Tnfsf4 FP-KI/cKO** Conditional Knockin / Knockout (KI-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	Sequence
Ef	GTGAGTCTGATTCCCAGGGGCAT
Er	GGTAGAGGGAAAGGCAGAGCCAA
Kr	GGGCAAGAACATAAAGTGACCCTCC
L3f	AAACATTTCCAGATGGTTTCATATCCCC
L3r	TGTTAGTCATGCACATTGCACATCCAT
Lxr	ACTGATGGCGAGCTCAGACCATAAC
Sf	CAGATACTGCGACCTCCCTAGCAAA

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	cKI allele	KO allele	WildType allele
Cre total excision	Sf / L3r	4484*	---	588	---
WildType allele specific PCR (5' part of the targeted locus)	Ef / Er	4488*	507	---	366
Cassette 5'	Ef / Kr	310	---	---	---
Presence of the distal loxP	L3f / L3r	532	532	---	507
Distal loxP specific PCR	L3f / Lxr	324	---	---	---
Excision of the floxed exon(s), i.e. knock out	Ef / L3r	6414*	2433*	449	2267

*: 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.