Genotyping protocol

Ftx cKO

$Ftx^{tm1.1Ics}$

IR00003714 / K665-K680

(ICS internal reference)

For any question, please contact:

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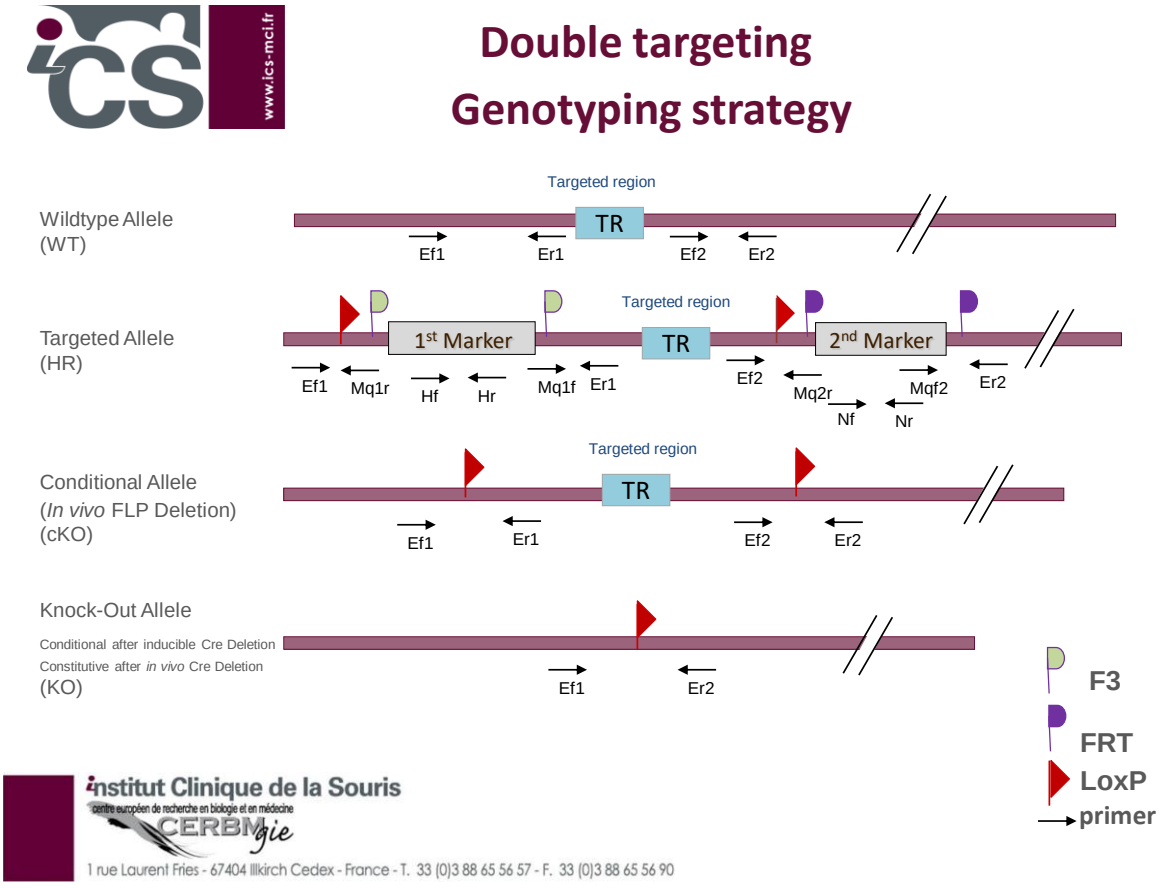
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Ftx** Conditional Knockout (cKO) project.

1.1. Genotyping strategy

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



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Sequence of primers used for genotyping:

Position	Sequence
Ef1	GGCTAAGTCATTAGGCAGGTCCAATC
Ef1[2]	GGTCTTACATGTCATGCAGCTCTC
Er1	GCATTAAAAAACGACAACTTTAGAGTAC
Er1[2]	CAATAAGACTCTGTCTCACTGGGC
Ef2	GCATGGGTTTGAAGCTAGCCTGG
Er2	CTACAGCAGACACTAGCTCAGAAGTG
Er2[2]	GTACAGTGCCCATGCCTGCAATC
Lxr	CGAAGTTATCTGCAGGTCGACCTTAAG
Mq1f	CCGATAGTGGAAACCGACGCC
Mq1r	GACAGACGTCGCGGTGAGTTCAGGC
Hf	GCTATGACTGGGCACAACAGACAATC
Hr	CAAGGTGAGATGACAGGAGATCCTG
Nf	TCCGGAAGTGCTTGACATTGGG
Nr	GGCCCAAAGCATCAGCTCATCG
Mq2f	CAGCTCATTCCTCCCACTCATGATC
Mq2r	TGCTAAAGCGCATGCTCCAGACTGC

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

PCR fragments expected size (bp):

Region analyzed	*Position on the primer (see the map above)*	*Targeted allele (HR)*	*cKO allele*	*KO allele*	*WildType allele*
Excision of the selection marker 1	Ef1 / Er1	2161*	355	---	209
5' part of the selection marker 1	Ef1[2] / Mq1r	817	---	---	---
3' part of the selection marker 1	Mq1f / Er1[2]	591	---	---	---
internal marker1	Hf/ Hr	250	---	---	---
Excision of the selection marker 2	Ef2 / Er2	2321*	468	---	350
5' part of the selection marker 2	Ef2 / Mq2r	417	---	---	---
3' part of the selection marker 2	Mq2f / Er2[2]	451	---	---	---
internal marker2	Nf /Nr	334	---	---	---
LoxP specific PCR	Ef1[2] / Lxr	169	169	169	---
Excision of the floxed exon(s), i.e. knock out	Ef1[2] / Er2	16706*	13047*	360	12783*

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

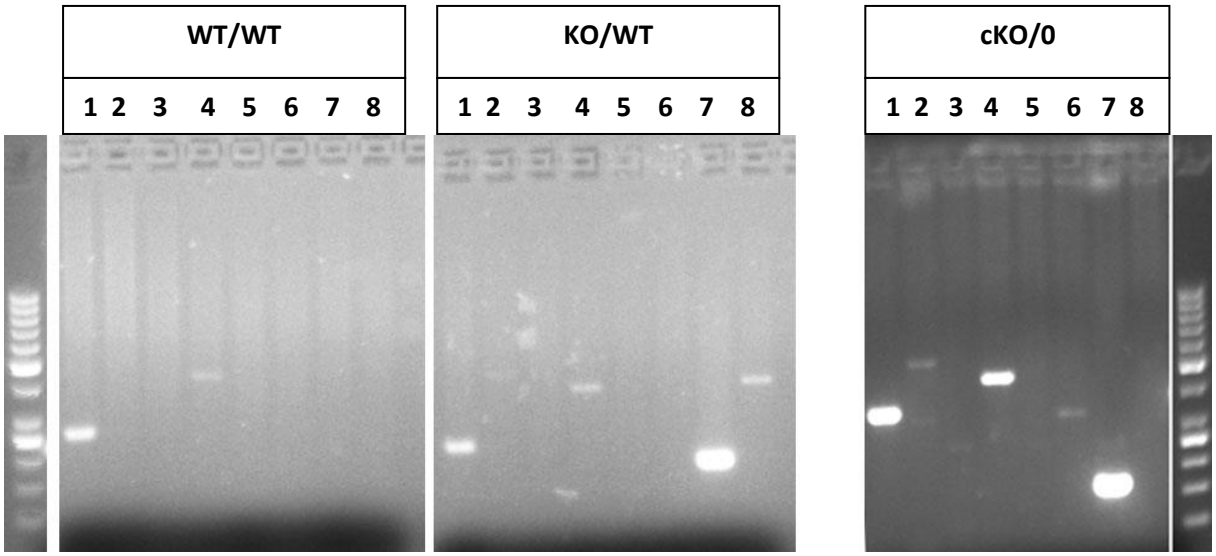
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was done by gel electrophoresis 2% agarose (SB buffer).

Representative genotyping picture



1 Excision of the selection marker 1
2 Multiplex (5' part of the selection marker 1 +3' part of the selection marker 1)
3 Multiplex (internal marker1 + internal marker2)
4 Excision of the selection marker 2
5 5' part of the selection marker 2
6 3' part of the selection marker 2
7 LoxP specific PCR
8 Excision of the floxed exon(s), i.e. knock out