Genotyping protocol

Fus WT first with conditional delta NLS

$Fus^{tm2.1Ics}$

IR00004993 / K4993

(ICS internal reference)

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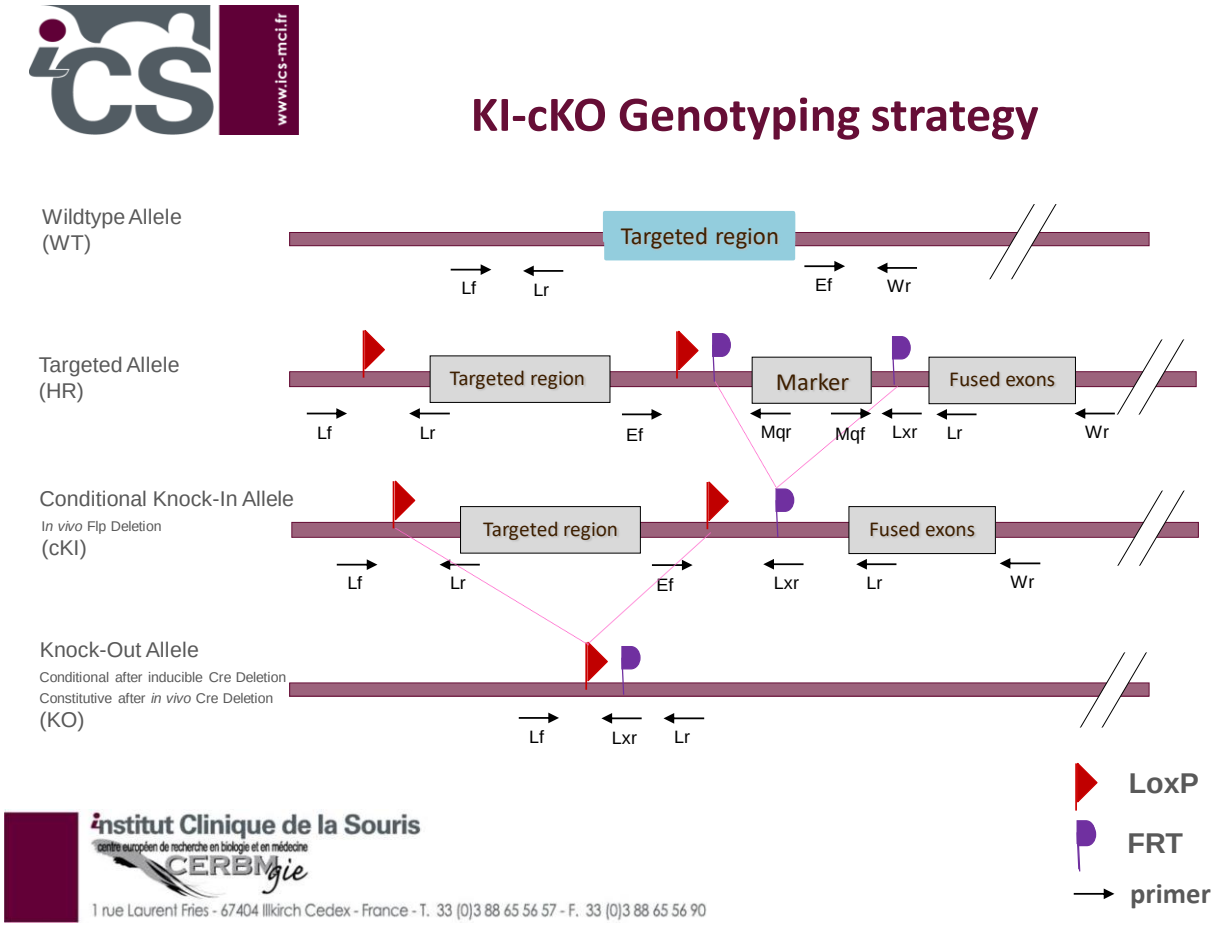
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **fus/als** 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	GGATCACATCTTGCCTAAATCCAGC
Lf	GCAGTAACTGAGGAGAGAGAACTGG
Lf^2	CTAGCCCACCTCCTAATCCTCATCAC
Lr	CCACTGTTATCTACACATGATCACCC
Lr^2	CCACACTTTAGGTTAGTCACAGATCAGC
Lxr	GGATCCGCGGGAAGTTCCTATAC
Mqf	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mqr	TGCTAAAGCGCATGCTCCAGACTGC
Wr	CCAGTTTCCCTTTATCGTTCAACTTCC

^{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)	cKI allele	KO allele	WildType allele
WildType allele specific PCR (5' part of the targeted locus)	Ef / Wr	3478*	1625*	---	212
Presence of the distal loxP	Lf / Lr	315	315	375	236
Excision of the selection marker	Ef / Lr	2187*	334	---	---
5' part of the selection marker	Ef / Mqr	317	---	---	---
3' part of the selection marker	Mqf / Lr^2	286	---	---	---
LoxP specific PCR	Lf^2 / Lxr	3846*	1993	260	---
Excision of the floxed exon(s), i.e. knock out	Lf^2 / Lr^2	347	347	407	268

*: 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.