EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Cidea

(PHENOMIN-ICS reference IR00005841 / P5841)

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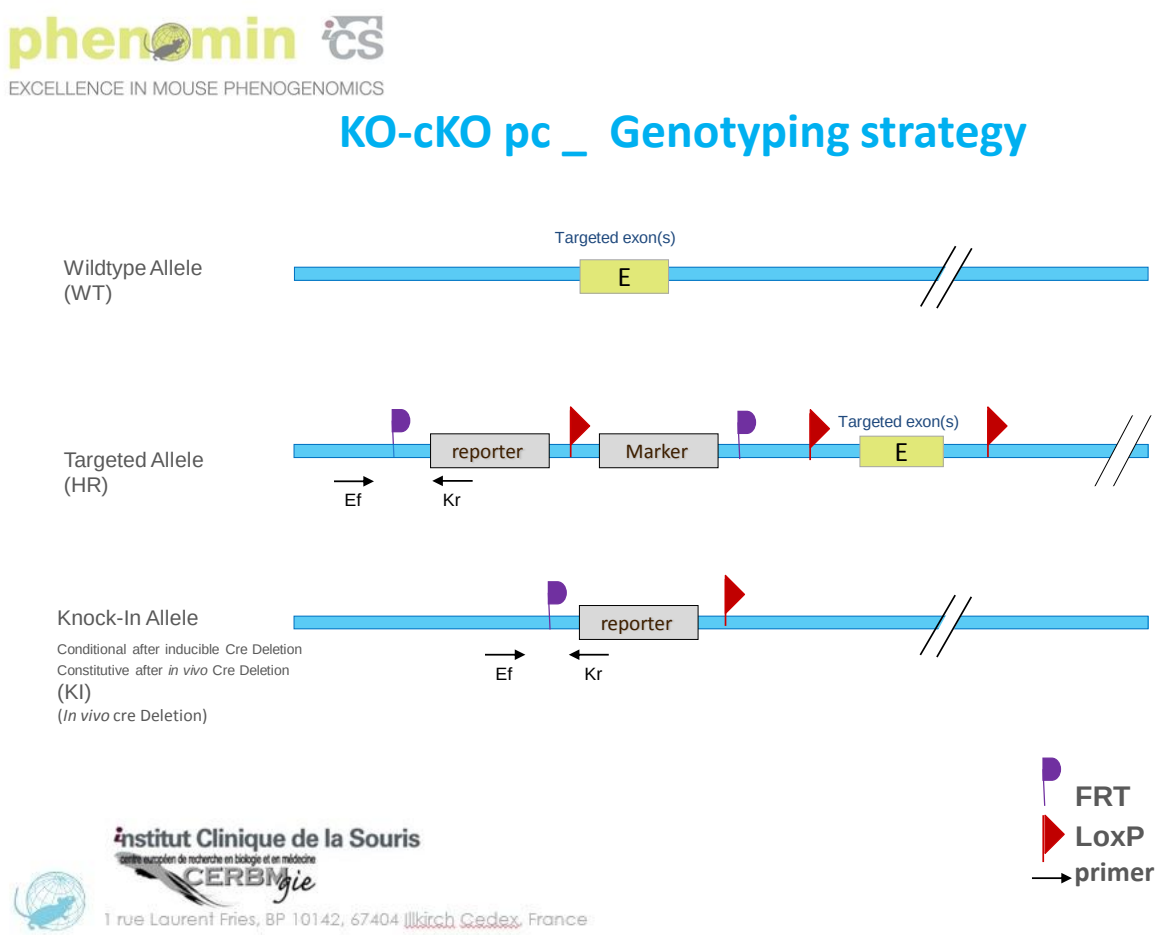
Table of contents

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Cidea** Constitutive Knockout / Conditional Knockout (KO-cKO x Cre) 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	8861	CTGGTCTTCTGATTGAGTTCTAGGACAGC
Kr	3209	CCAACAGCTTCCCCACAACGG

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

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	WildType allele
5' part of the selection marker	8861-3209	Ef / Kr	273	273	---

*: 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:

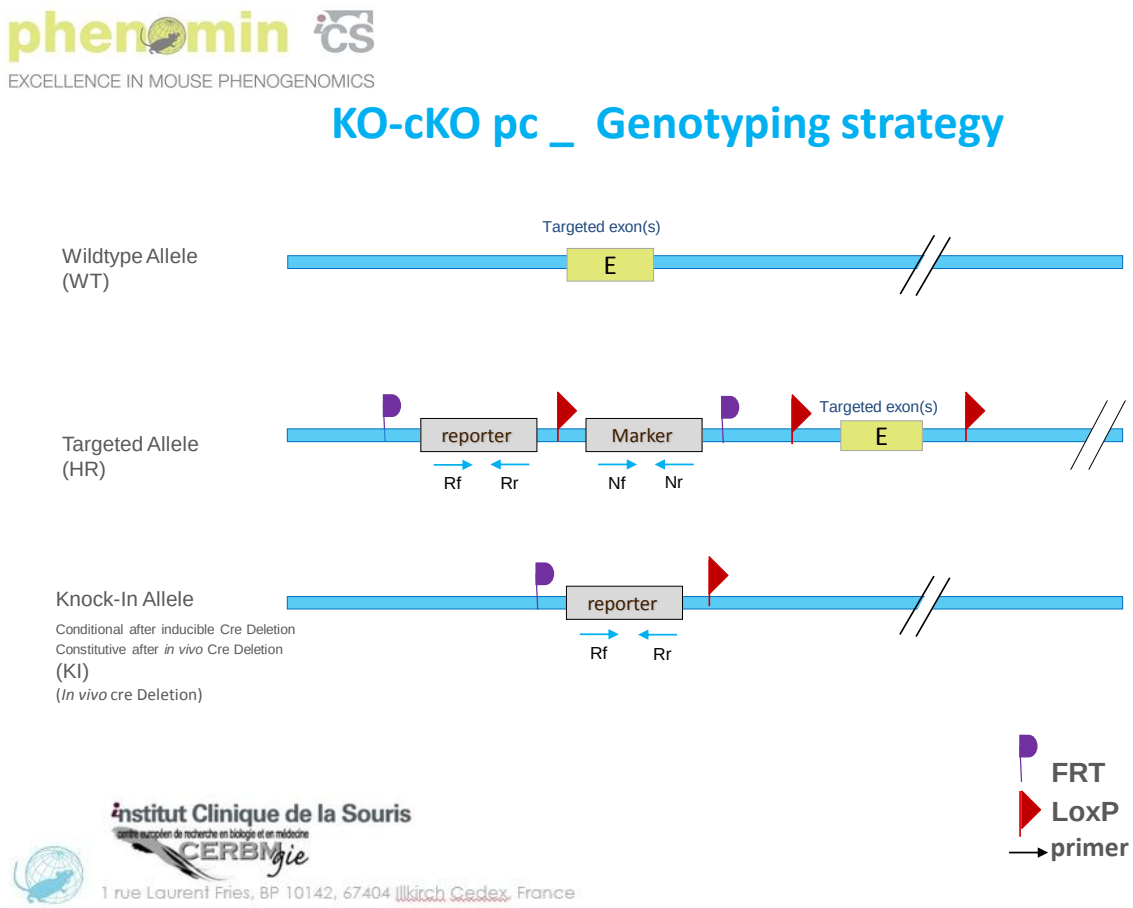
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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

2. qPCR Genotyping protocol and data

2.1. 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
Rf	7443	CTCGCCACTTCAACATCAAC
Rr	7445	TTATCAGCCGGAAAACCTACC
Nf	Neo f1	TGAATGAACTGCAGGACGAG
Nr	Neo r1	TTCCCGCTTCAGTGACAAC

2.2. qPCR protocol

Reagents:	Volume:
- EvaGreen (biorad)	3,5µl
- DNA (10ng/µl)	3µl
- Forward primer (100µM)	0,06µl
- Reverse primer (100µM)	0,06µl
- Sterile H2O	up to 7µl

Cycling conditions:

Temp	Time	#Cycles
95°C	10min	1
95°C	5s	
62°C	10s	34
95°C	15min	
Melting curve analysis		
65°c -> 95°c		

Follow manufacturer's protocol for programming the data acquisition of dsDNA product.

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

3. 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.