EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Chmp2b (truncated Chmp2b-IRES-eYFP with WT potential

Chmp2b$^{tm1.1Ics}$

(PHENOMIN-ICS reference IR00005808 / Kos5808)

For any question, please contact:

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

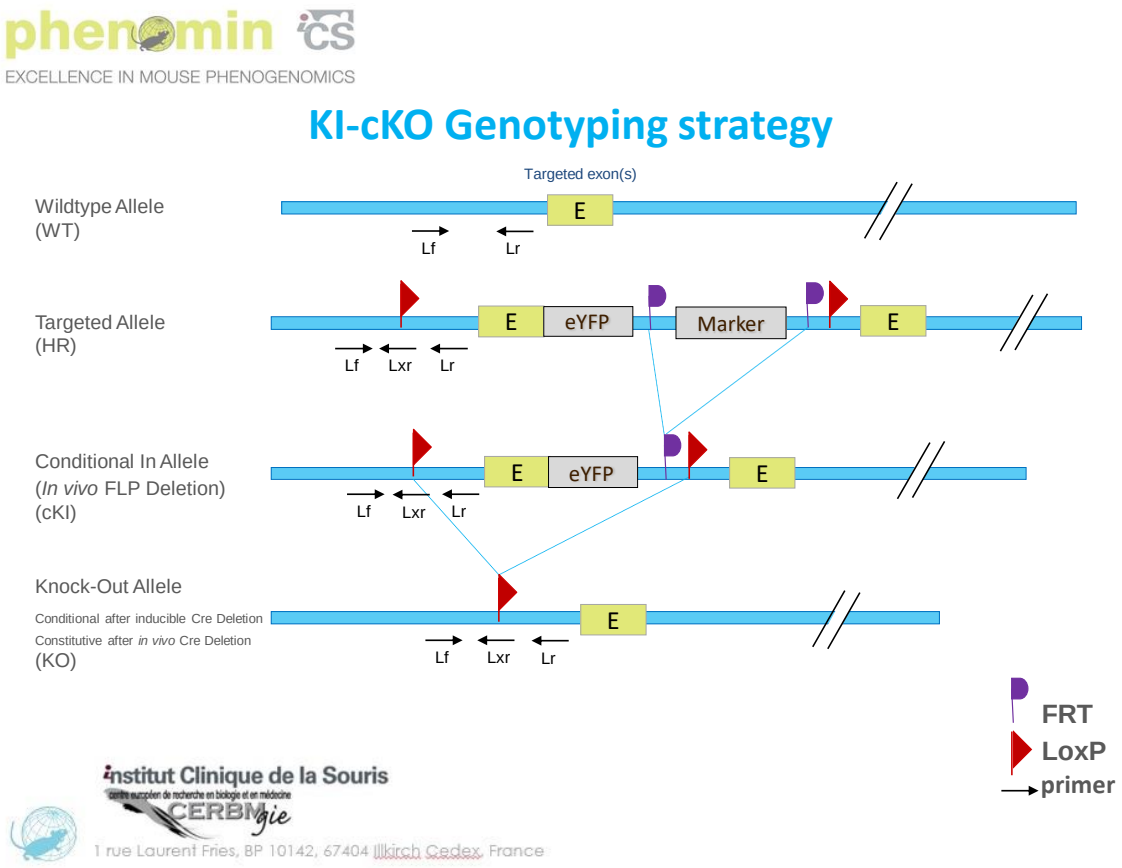
Table of contents

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Chmp2b** Conditional Knockin / Knockout (KI-cKO) mouse line.

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
Lf	TGGTGCTGACTCTTGAATTCAGG
Lf[2]	CTTCCTTCCTTCCTCATATTCTGG
Lr	CGAACTACAAAACTCAGGTTTCACC
Lxr	GTATAGCATACATTATACGAAGTTATCTGCAG

[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
Presence of the distal loxP	Lf / Lr	264	264	264	170
LoxP specific PCR	Lf[2] / Lxr	179	179	179	---

---: 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:

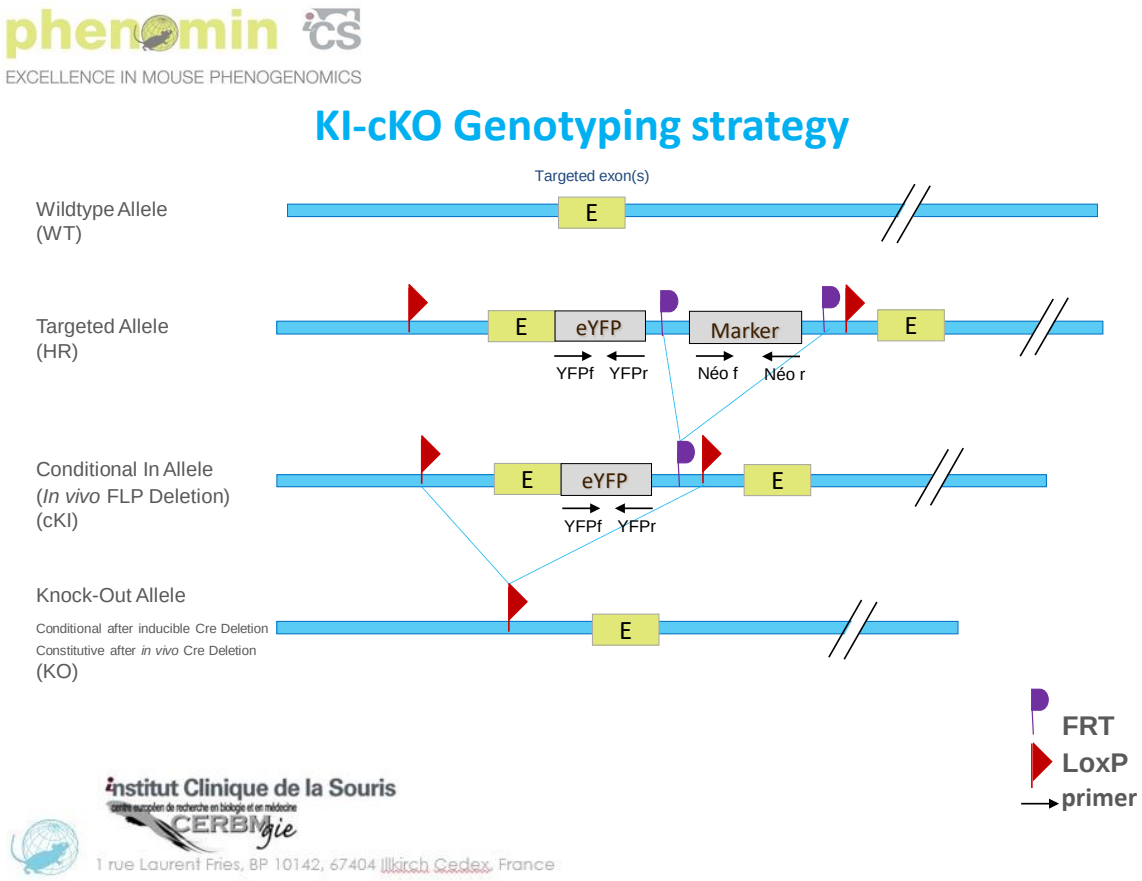
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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
YFPf	Yfp f1	AACCACTACCTGAGCTACCA
YFPr	Yfp r1	TCGTCCATGCCGAGAGT
Nf	Neo f1	TGAATGAACTGCAGGACGAG
Nr	Neo r1	TTCCCGCTTCAGTGACAAC

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	WildType allele
YFP qPCR	Yfp f1 – Yfp r1	YFPf / YFPr	113	113	---
Neomycine qPCR	Neof1 - Neor1	Nf / Nr	96	---	---

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.