Genotyping protocol

Vps13b cKO

Vps13b$^{tm1.1Ics}$

IR00003971 / K737

(ICS internal reference)

For any question, please contact:

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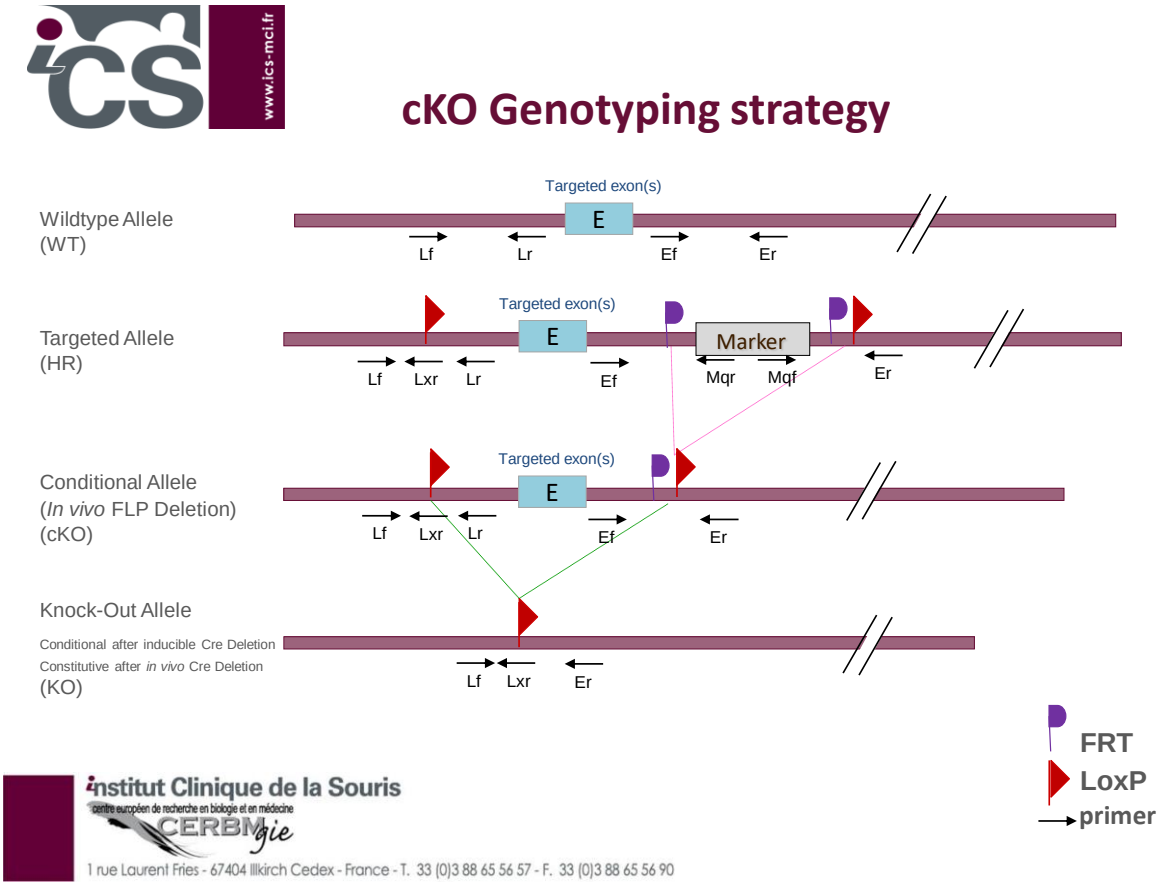
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Vps13b** 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
Ef	CTTCAGAACTTGTCAGGTACAGAGCCATCG
Er	CTAACAGTTGACTGAGGAAGCAGCAATG
Er[2]	CTAAGGTTTCCTTCAGCTACAGC
Lf	GCTAGATTGGCTGTCATGAAGCAC
Lr	CCAAACATGGTGCTGCACACAC
Lxr	CATACATTATACGAAGTTATCTGCAG
Mq1f	AATGCCTGCTCTTTACTGAAGGCTC
Mq1r	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
Presence of the distal loxP	Lf / Lr	170	170	---	90
Excision of the selection marker	Ef / Er	2314*	461	---	355
5' part of the selection marker	Ef / Mq1r	286	---	---	---
3' part of the selection marker	Mq1f / Er2	437	---	---	---
LoxP specific PCR	Lf / Lxr	95	95	95	---
Excision of the floxed exon(s), i.e. knock out	Lf / Er	2962*	1109*	307**	923*

*: 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.