Genotyping protocol

Vps13b KO

Vps13b$^{tm1.2Ics}$

IR00003971 / K737

(ICS internal reference)

For any question, please contact:

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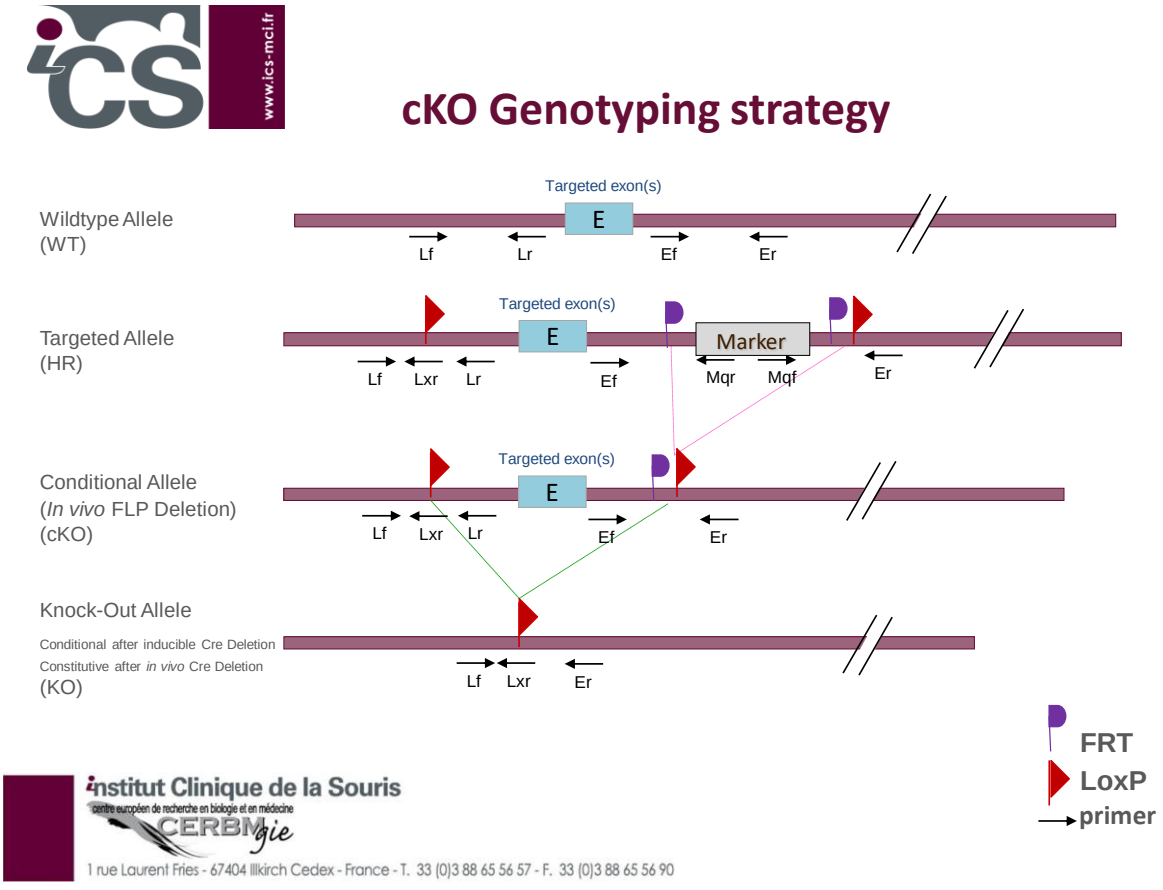
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Vps13b** Conditional Knockout (cKO, tm1.1) and KO (tm1.2) mouse lines.

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	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.