



Genotyping protocol

Tmem63b

IR00003291 / E227

(ICS internal reference)

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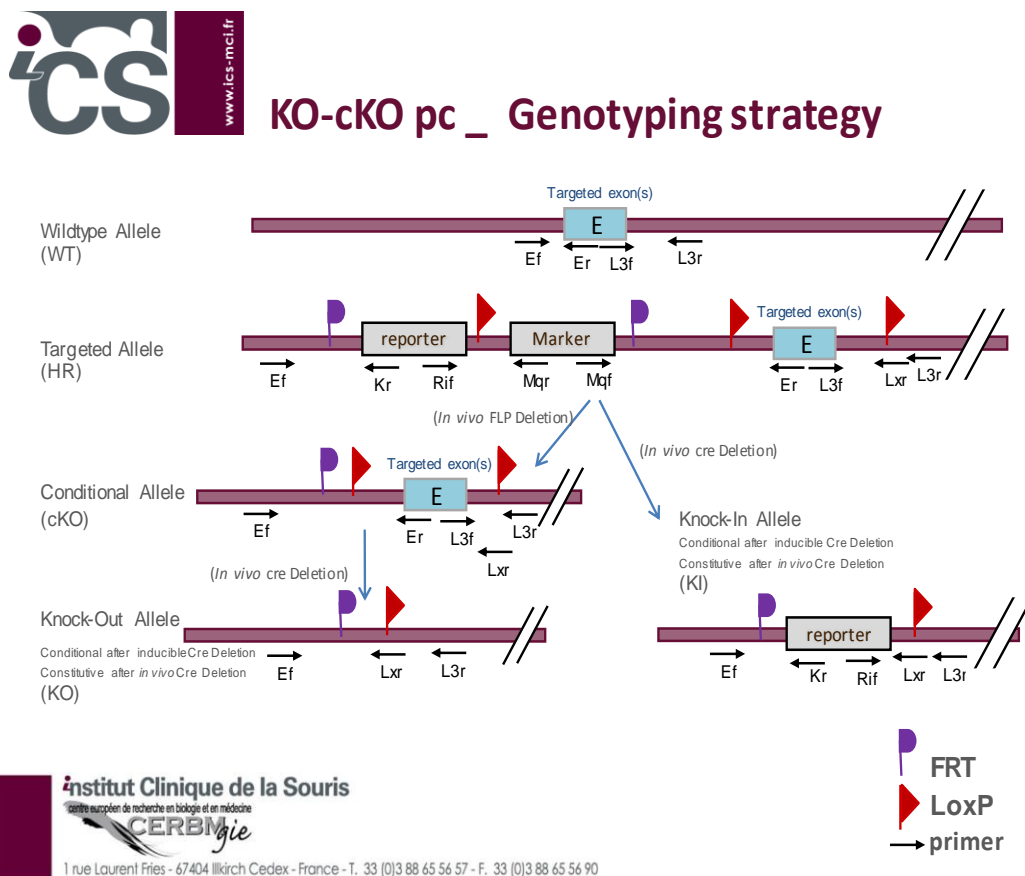
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1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Tmem63b** 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	6191	GTTCTTCATATTTTCAGGCTTCCTTGCTC
Er	6195	TGCAGTTCCAAAGATGACCAGCAG
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	6192	AGCTATGCATGGGGACAGTCACGAC
L3f ²	6193	AGCTCTGTGCGACTTTGACCAAAGGGA
L3r	6194	GCCACCATGTGGTTGCTGGGAT
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Ri1f	5966	GCACATGGCTGAATATCGACGGT

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)	conditional allele (cKO)	KI allele	WildType allele
5' part of the selection marker (with DMSO)	6191-3209 (with 5% DMSO)	Ef / Kr	336	---	---	---
Presence of the distal loxP (with DMSO)	6192-6194 (with 5% DMSO)	L3f / L3r	471	471	---	424
Distal loxP specific PCR	6193-3255	L3f ² / Lxr	296	296	---	---
Excision of the selection marker	6191-6195	Ef / Er	7379*	475	---	314
Cre total excision	5966-3255	Ri1f / Lxr	---*	---	471**	---

*: 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 ₂ O	up to 15 µl

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
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.