

Genotyping protocol

Project Tti2

(PHENOMIN-ICS reference IR00005201 / G5201)

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The first version of this report was finalized the: 19 Jul 2021

The last update of this report was done the: 19 Jul 2021

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This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Tti2** Constitutive Knockout / Conditional Knockout (KO-cKO x Cre) project.

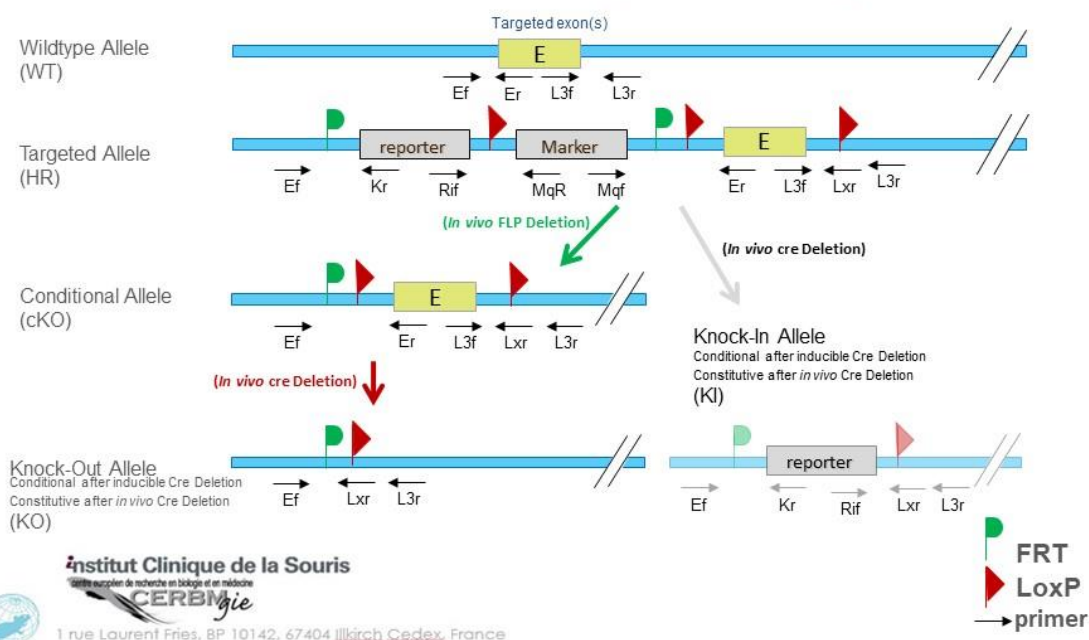
1. PCR Genotyping protocol

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.



KO-cKO Genotyping strategy



Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	7843	GTCAGATAGCATCCGTGAATCTTG
Ef2	7842	CTTCCTATGTAATCCATGTTGGCCTTG
Er	7846	GAAACTGCACATCTGACTCAACCTC
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	7844	GTTACTGTGGTAAGCTCCTCAGCTCTG
L3r	7845	GAGTACACAGAGACTCAGTAAAGC
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Mq1f	4981	GGGATCTCATGCTGGAGTTCTTCG
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)	KO allele	WildType allele
5' part of the selection marker	7843-3209	Ef / Kr	272	---	---	---
Presence of the distal loxP (with Betaine)	7844-7845	L3f / L3r	529	529	---	455
Distal loxP specific PCR	7844-3255	L3f / Lxr	270	270	---	---
Excision of the selection marker	7842-7846	Ef2 / Er	7353*	449	---	247
Exon excision	4981-3255	Mq1f / Lxr	---*	---	---	---
Cre total excision	5966-3255	Ri1f / Lxr	---*	---	---	---

*: 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 ₂ O	up to 15 µl

If mentioned in table "PCR fragments expected size" add 0.5% of Betaine (5% of DMSO) in the reaction mix

Cycling conditions:		
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	35
62°C	30s	
72°C	1min	
72°C	7min	1
14°C	---	---

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

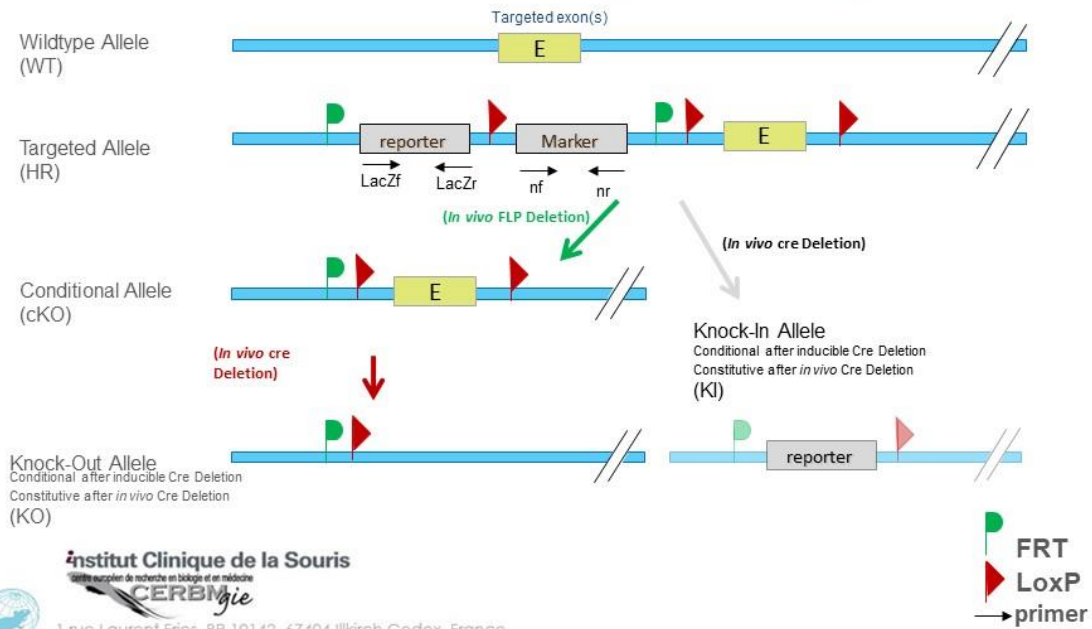
2. qPCR Genotyping protocol

2.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.



KO-cKO Genotyping strategy



Sequence of primers used for genotyping:

Position	Primers	Sequence
LacZf	7443	CTCGCCACTTCAACATCAAC
LacZr	7445	TTATCAGCCGGAAAACCTACC
Nf	Neo f1	TGAATGAACTGCAGGACGAG
Nr	Neo r1	TTCCCGCTTCAGTGACAAC

qPCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer (see the map above)	Targeted allele (HR)	Conditional allele (cKO)	Knock out allele (KO)
LacZ qPCR	7443 - 7445	LacZf / LacZr	73	---	---
Neomycine qPCR	Neof1 - Neor1	Nf / Nr	96	---	---

---: no Amplicon should be obtained

2.2. qPCR protocol

Reagents:

- EvaGreen (biorad)
- DNA (10ng/μl)
- Forward primer (100μM)
- Reverse primer (100μM)
- Sterile H2O

Volume:

- 3,5μl
- 3μl
- 0,06μl
- 0,06μl
- up to 7μl

Cycling conditions:

Temp	Time	#Cycles
98°C	2min	1
98°C	5s	45
60°C	20s	

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. Recommended papers:

3.1. Cre and Flp genotyping method

[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.

3.1. Tips and tricks for optimizing your PCR genotyping procedures

[Optimizing PCR for mouse genotyping: Recommendations for reliable, rapid, cost effective, robust and adaptable to high-throughput genotyping protocol for any type of mutation.](#)

Jacquot, S, Chartoire, N, Piguet, F, Herault, Y, Pavlovic, G. (2019).

Current Protocols in Mouse Biology, 9, e65. doi: 10.1002/cpmo.65

Free copy of this paper can be accessed online though this link <http://bit.ly/2sxxWvO>

