



Genotyping protocol

Pigu

IR00003393 / E244

(ICS internal reference)

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1. Genotyping protocol and data

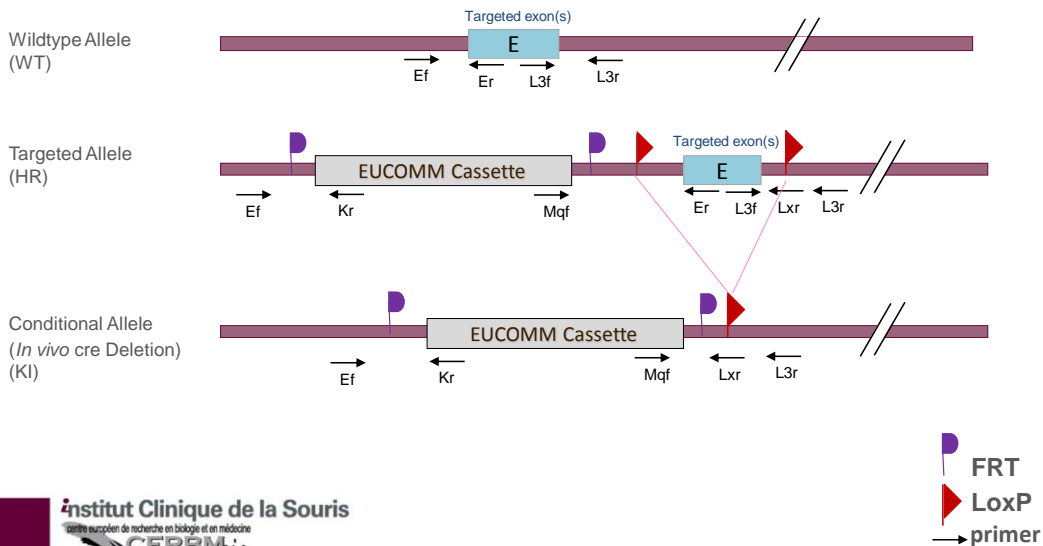
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Pigu** Constitutive Knockout / Conditional Knockout (KO-cKO) project.

1.1. Genotyping strategy

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



KO-cKO pl _ Genotyping strategy



Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	6689	CTGGTGGAGGTTGCAAGAAGGTAGAC
Ef ²	6688	GAATGCTTCAGAGCTGGTGGAGTTG
Er	6692	GAGAAATAGCGTGGCAACTAAGAAGTG
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	6690	GATACCAAAGCCTGAACTCTGTTTG
L3r	6691	GCATGTGGAGAACGGACCTCAACAATG
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Mq1f	4981	GGGATCTCATGCTGGAGTTCTTCG

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)	KI allele	WildType allele
5' part of the selection marker	6689-3209	Ef / Kr	270	270	---
Presence of the distal loxP	6690-6691	L3f / L3r	257	---	292
Distal loxP specific PCR	6690-3255	L3f / Lxr	194	---	---
Excision of the selection marker	6688-6692	Ef ² / Er	6342*	---	222
Exon excision	4981-3255	Mq1f / Lxr	---	424**	---

*: 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.