

Genotyping protocol

Eml1 cKO

Eml1^{tm1.1Ics}

(PHENOMIN-ICS reference IR00004995 / K4995b)

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1. Genotyping protocol and data

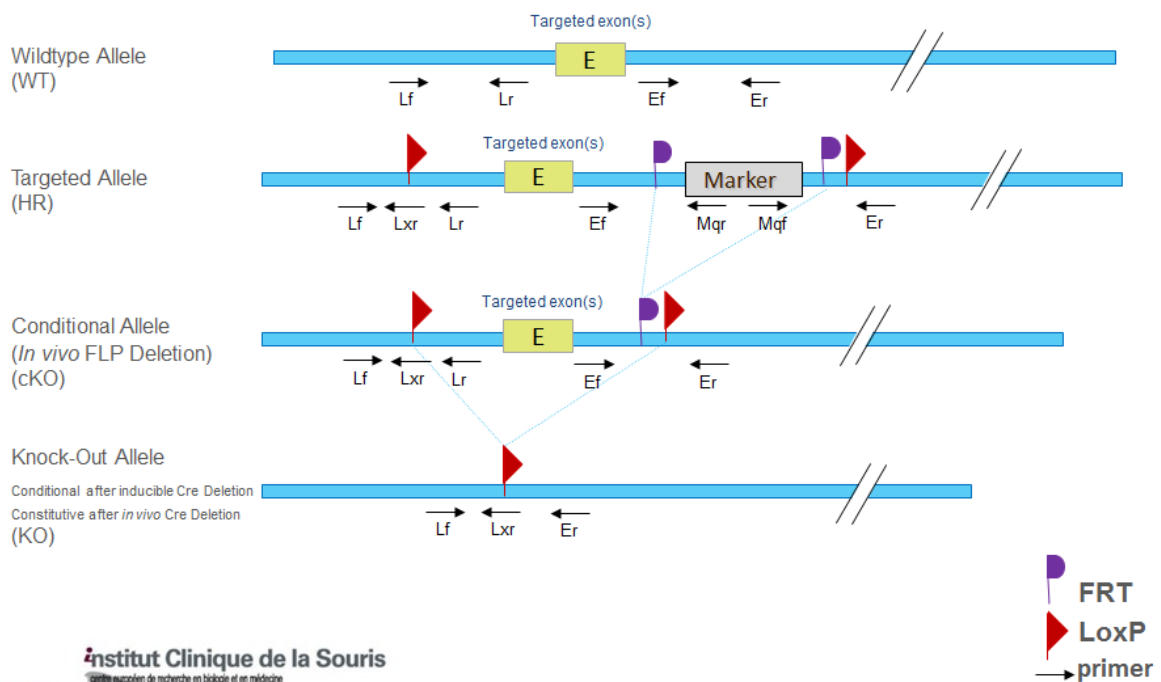
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Eml1** Conditional Knockout (cKO) mouse line.

1.1. Genotyping strategy

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



cKO Genotyping strategy



Sequence of primers used for genotyping:

Position	Sequence
Ef	CCCTGAGCTTCCTCATAACTTCAGC
Ef ²	CAGGTCTGTGGGCTCTGTAACAGG
Er	CACCCACTGAAGAAATGACTGGCAG
Er ²	CTTGTTAAAGCGTCTGCAGTCTGTCTG
Lf	GAAAACGTGCTTTGCTGTGTACATAGG
Lr	CGCCACCCTTTTCTACAAGTCATTTTTG
Lxr	CGAAGTTATCTGCAGGTCGACCTTAAG
Mqf	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mqr	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	245	245	---	165
Excision of the selection marker	Ef ² / Er ²	2268*	414	---	309
5' part of the selection marker	Ef / Mqr	327	---	---	---
3' part of the selection marker	Mqf / Er	309	---	---	---
LoxP specific PCR	Lf / Lxr	113	113	113	---
Excision of the floxed exon(s), i.e. knock out	Lf / Er	3029*	1175*	276**	990*

*: 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, Héroult Y, Pavlovic G.

Genesis. 2012 Jun;50(6):482-9. doi: 10.1002/dvg.20826. Epub 2012 Mar 20.

