



## Genotyping protocol

NOX1

IR00004999 / P4999

(ICS internal reference)

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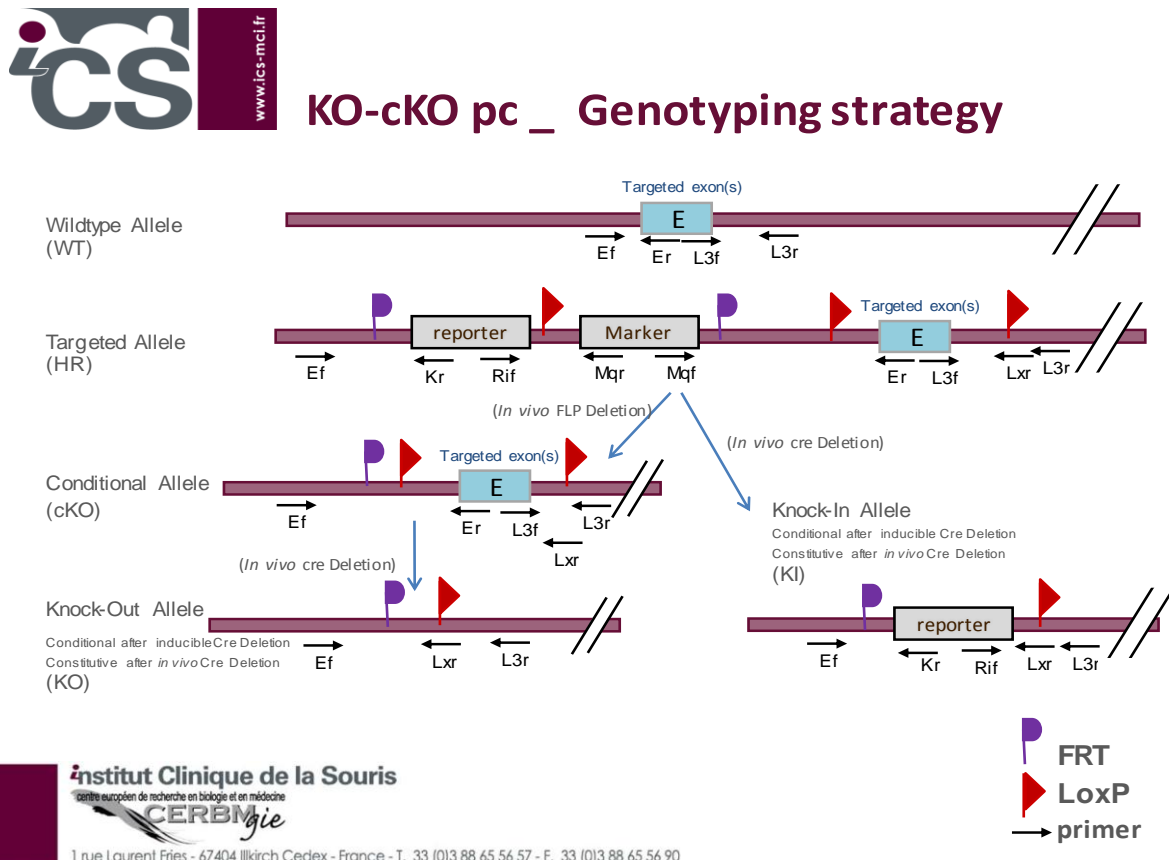
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### 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **NOX1** 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	8125	GCAATGGACCCTGTGTACATGC
Ef <sup>2</sup>	8126	CAAGGGTTCCATTTTAAATATGTCTTGC
Er	8129	CCATGAGAGTGGTTTGCAGTTGTTGG
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	8127	GGAGGAGGAAACATGCTCTGTGTCTC
L3r	8128	GCTACAAGATCAGTCGCAGAGCC
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Rif	5966	GCACATGGCTGAATATCGACGGT

<sup>2</sup>: 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	8125-3209	Ef / Kr	446	---	446	---
Presence of the distal loxP	8127-8128	L3f / L3r	280	280	---	238
Distal loxP specific PCR	8127-3255	L3f / Lxr	156	156	---	---
Excision of the selection marker	8126-8129	Ef <sup>2</sup> / Er	7421*	517	---	354
Cre total excision	5966-3255	Rif / Lxr	6535*	---	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 <sub>2</sub> 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.