



Genotyping protocol

Hrh4

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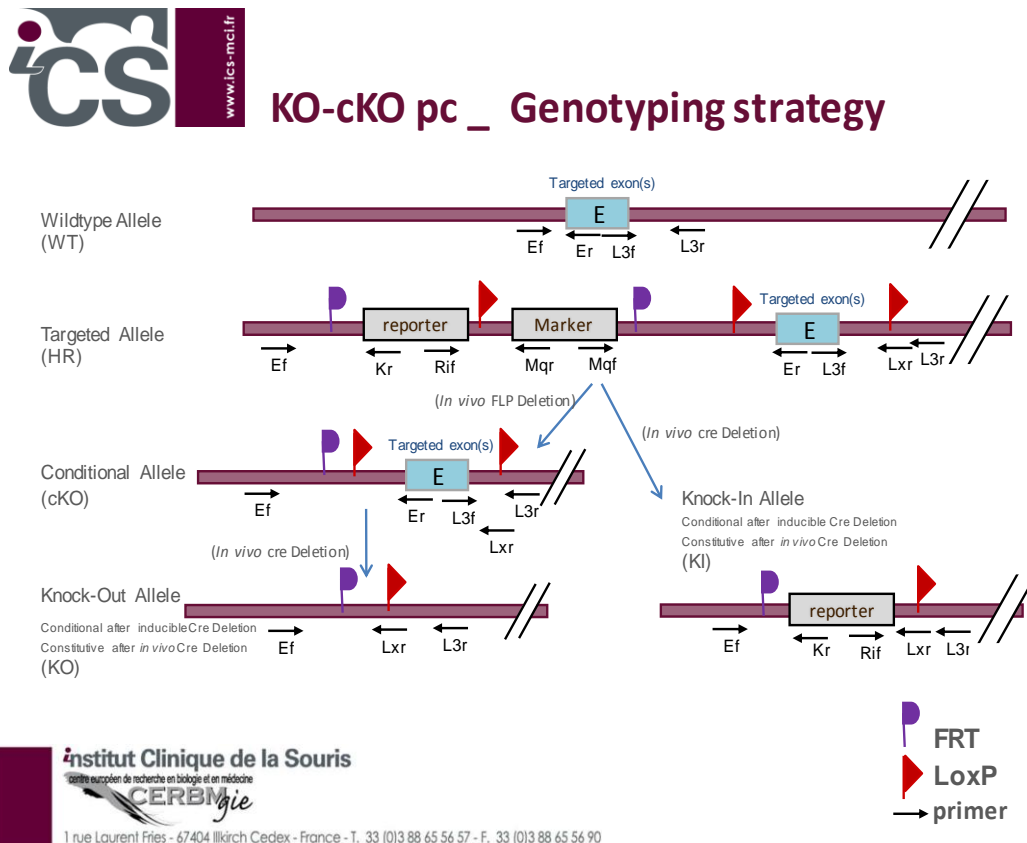
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1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Hrh4** 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	8505	CATTGCTTTGGCTCCTGATGGTGTTAG
Ef ²	8506	CACCTGGCTCCATCTCTACAGGTCC
Er	8509	GGCATGTCCAAATCCACAGAATATAGC
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	8507	GTACTGGGAATGTTGGGGATGGGTC
L3r	8508	CGATTTTCTCACCCATTCACTAAGGG
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Rif	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	8505-3209	Ef / Kr	386	---	---	---
Presence of the distal loxP	8507-8508	L3f / L3r	290	290	---	277
Distal loxP specific PCR	8507-3255	L3f / Lxr	206	206	---	---
Excision of the selection marker	8506-8509	Ef ² / Er	7205*	301	---	140
Cre total excision	5966-3255	Rif / Lxr	3249*	---	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.