Genotyping protocol

Epx

/ Epx EUCOMM

(ICS internal reference)

This report has been prepared by: **Christelle Roth**
genotyping@igbmc.fr

This report has been validated by: **Sylvie Jacquot, PhD, Head of Genotyping Service**
33 (0)3 88 65 57 44
genotyping @igbmc.fr

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For any question, please contact:

Institut Clinique de la Souris - ICS - Mouse Clinical Institute
1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex, France
Email: genotyping@igbmc.fr
Web site: http://www-mci.u-strasbg.fr/

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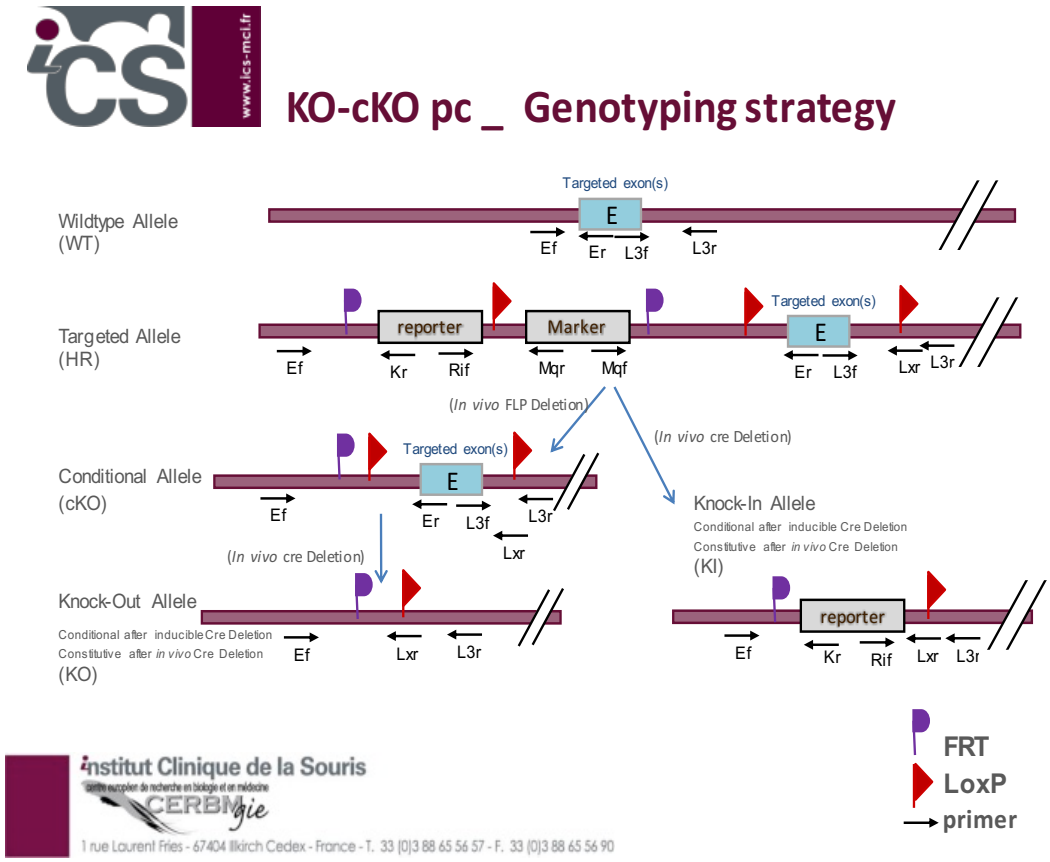
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Epx** 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	Ef1	CGCTAAATATAAAGAGCCATGCACCTGTG
Ef	Ef2	TTCCCAGGGCTGGGATTATAGCC
Kr	3277	CTCCTACATAGTTGGCAGTGTTTGGG
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	L3f1	CACAGGATAGAGACCCTCAGCATGG
L3f	L3f2	CAGTCTACAGAGGAGTCGTCCCAGG
L3r	L3r1	GGAGCAATGGGCAGATACTTAAGAGGG
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Er	Er1	GGCAGGAGGATTGTAAATTCAGGGC
Er	Er2	CAACAAGTGTGAGGAGGCTGGCC

[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	Ef1-3209	Ef / Kr	378			
5' part of the selection marker	Ef2-3277	Ef / Kr	284			
Presence of the distal loxP	L3f1-L3r1	L3f/L3r	242	242		212
Presence of the distal loxP	L3f2-L3r1	L3f/L3r	324	324		294
Distal loxP specific PCR	L3f1-3255	L3f/Lxr	170	170		
Distal loxP specific PCR	L3f2-3255	L3f/Lxr	252	252		
Excision of the selection marker	Ef1/Er1	Ef/Er	7406	502		422
Excision of the selection marker	Ef2/Er2	Ef/Er	7377	473		393
Cre total excision	5966-3255	Ri1f / Lxr	3391*	---	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_2O	up to 15 µl

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

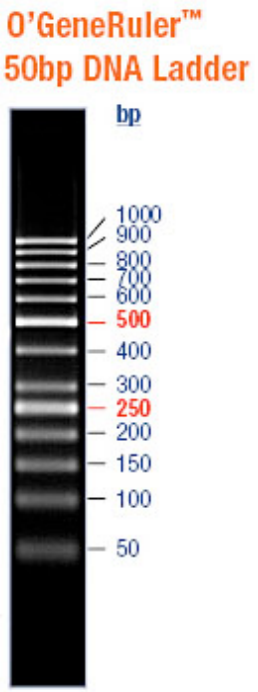
NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was done by gel electrophoresis 2% agarose (SB buffer).

Representative genotyping picture

_ADD_THE_PHOTO_



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.