Genotyping protocol

Rbck1

/ Rbck1 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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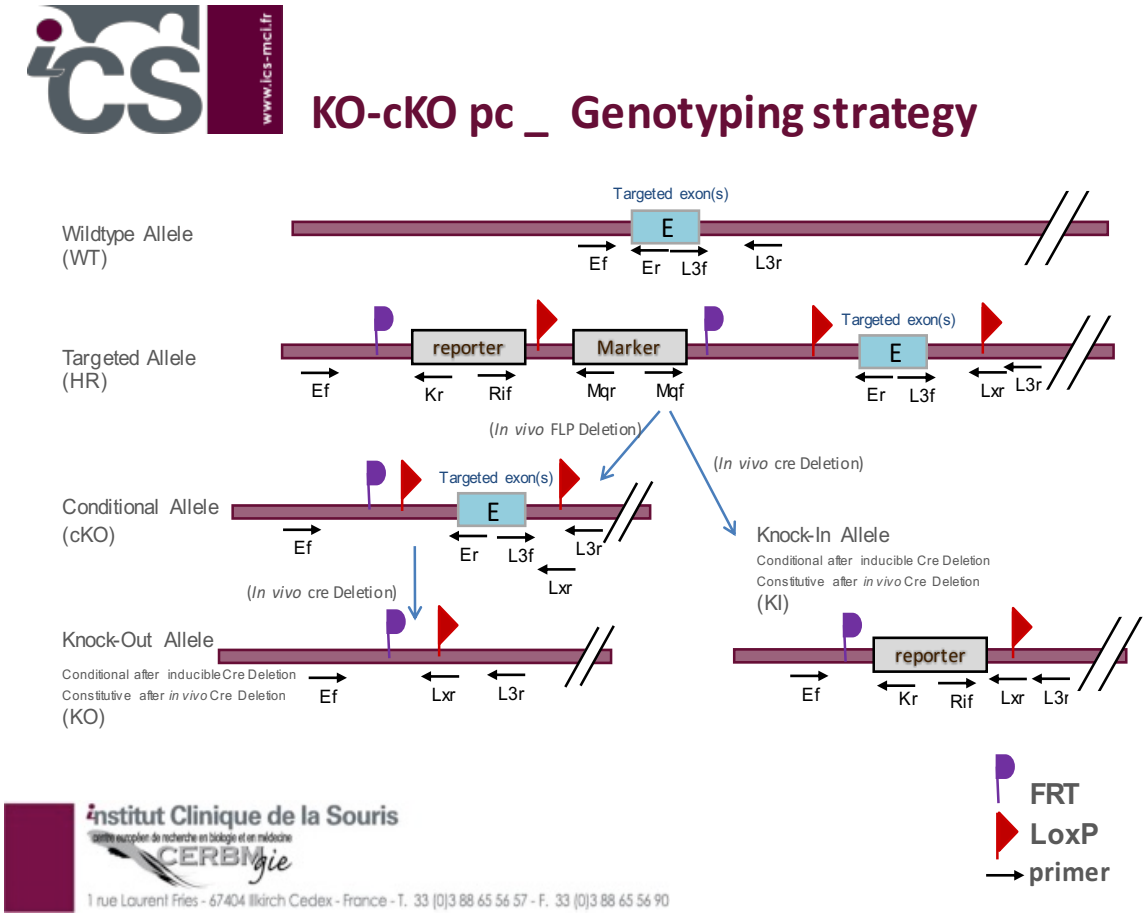
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Rbck1** 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.



Institut Clinique de la Souris
1 rue Laurent Fries - 67404 Illkirch Cedex - France - T. 33 (0)3 88 65 56 57 - F. 33 (0)3 88 65 56 90

Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	Ef1	CCACTGAGCCCTCTCTCTGGTTGAC
Ef	Ef2	CAGTTGTCCAACCAGTGTCCTTGGTG
Kr	3277	CTCCTACATAGTTGGCAGTGTTTGGG
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	L3f1	CTCCTGGCCATTGCCACTCACC
L3f	L3f2	GCCTGAGCCAGAAAGGGAACCAC
L3r	L3r1	CATGGGAGGAGGGGGTGTCAAAG
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Er	Er1	GTGCTGAAATGCCCACCAAGGAG

[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	Ef1-3277	Ef / Kr	310			
5' part of the selection marker	Ef2-3209	Ef / Kr	294			
Presence of the distal loxP	L3f1-L3r1	L3f/L3r	228	228		177
Presence of the distal loxP	L3f2-L3r1	L3f/L3r	265	265		215
Distal loxP specific PCR	L3f1-3255	L3f/Lxr	145	145		
Distal loxP specific PCR	L3f2-3255	L3f/Lxr	183	183		
Excision of the selection marker	Ef1/Er1	Ef/Er	7309	405		200
Excision of the selection marker	Ef2/Er1	Ef/Er	7246	342		137
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

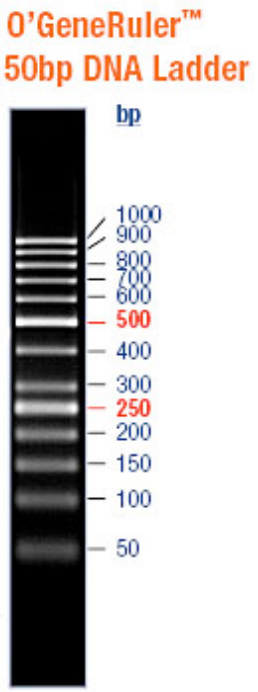
NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

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.