Genotyping protocol

Smc5

IR00004495 / P4495

(ICS internal reference)

This report has been prepared by: **David Moulaert**
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
centre européen de recherche en biologie et en médecine
CERBMgie

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

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1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Smc5** 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.

KO-cKO pc _ Genotyping strategy
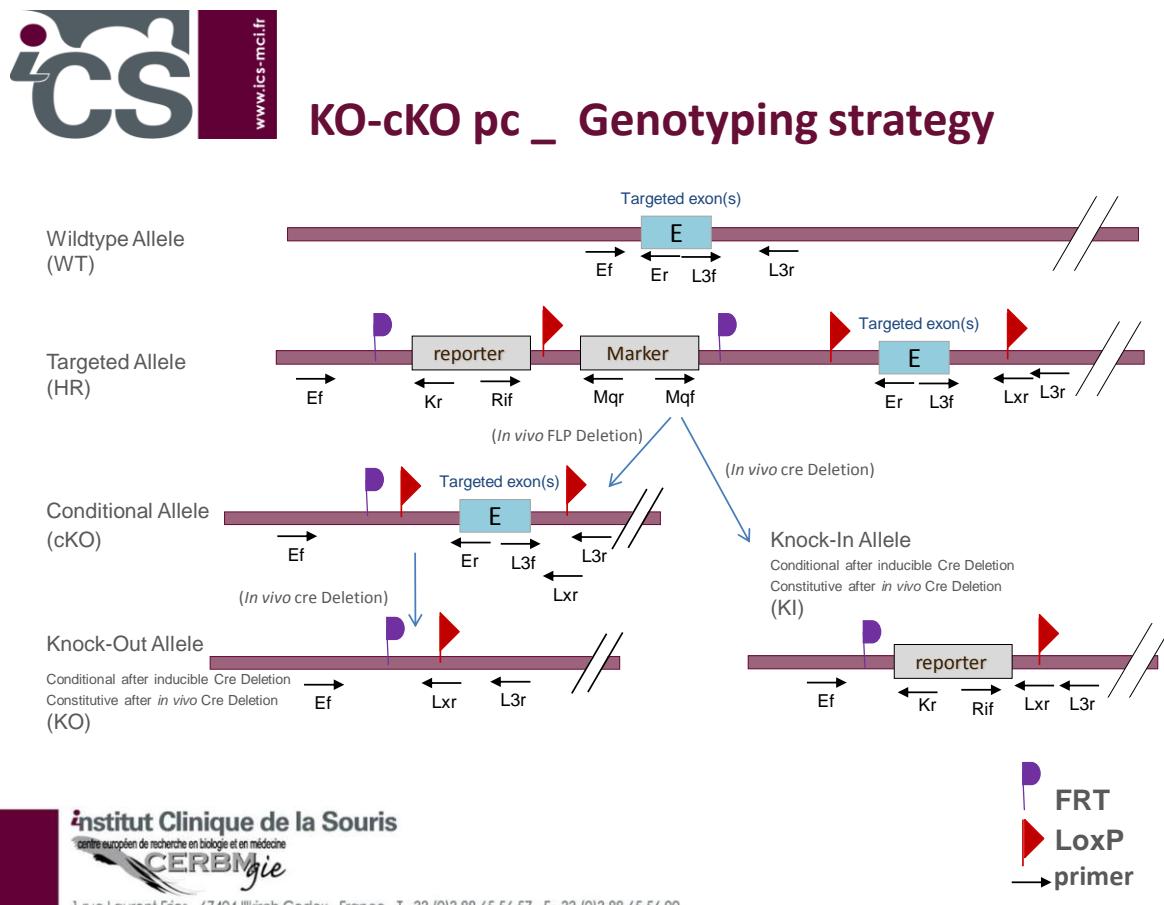
Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	7272	TAGCAGTTACTCAGTCTCACACGGCAA
Er	7274	GCCACAGTGGCTATGGATTCCAG
Kr	3278	GGGCAAGAACATAAAGTGACCCTCC
L3f	7275	GGGGAGACCTCCAAACATGGG
L3f^2	7273	ATCTGTGCCAGTTCTTGCCTCAGGTC
L3r	7276	AAAAATTAGAGTGCTGTGCTGAATGCAA
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Ri1f	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)	cKO allele	KI allele	WildType allele (WT)
5' part of the selection marker	7272-3278	Ef / Kr	272	---	272	---
Presence of the distal loxP	7275-7276	L3f / L3r	253	253	---	214
Distal loxP specific PCR	7273-3255	L3f^2 / Lxr	293	293	---	---
Excision of the selection marker	7272-7274	Ef / Er	7277*	373**	---	222
Cre total excision	5966-3255	Ri1f / Lxr	3277*	---	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.

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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.