



Genotyping protocol

Rasal3

/ 1460 Rasal3

(ICS internal reference)

This report has been prepared by: **David Moolaert**
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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 1.3. Picture of genotyping with various alleles **Erreur ! Signet non défini.**

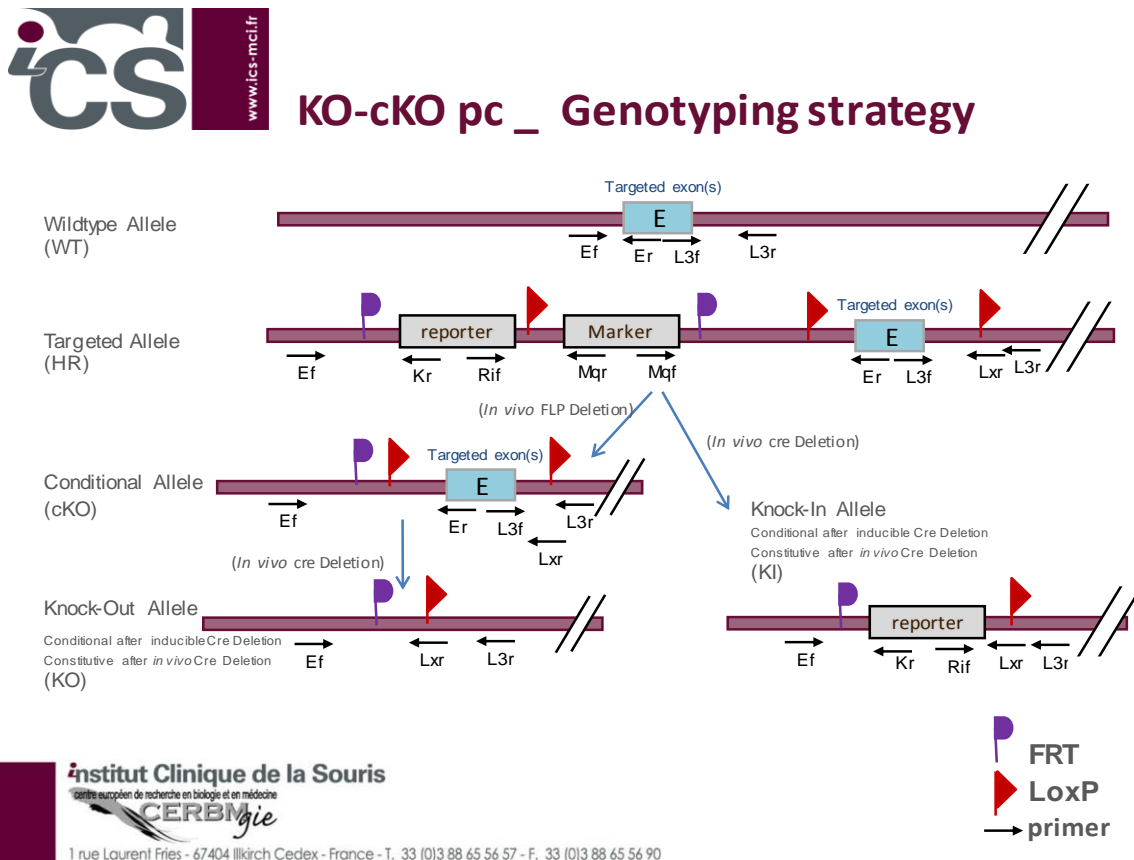
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1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Rasal3** 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	8555	CAAACCTGGCCTGAAACTCATCCTCC
Er	8558	AGGCAGAGGCAGGTGAATTTCTGAG
Kr	3277	CTCCTACATAGTTGGCAGTGTGGG
L3f	8556	GTCCACAATGTTCCGGGTATGGCAG
L3r	8557	GCCCAGAGCGTCAGAGAAATCATG
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Rif	5966	GCACATGGCTGAATATCGACGGT

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	8555-3277	Ef / Kr	289	---	289	---
Presence of the distal loxP	8556-8557	L3f / L3r	493	493	---	419
Distal loxP specific PCR	8556-3255	L3f / Lxr	307	307	---	---
Excision of the selection marker	8555-8558	Ef / Er	7385*	429	---	326
Cre total excision	5966-3255	Rif / Lxr	3647*	---	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.