



Genotyping protocol

Orc4l

IR00002421 / E32

(ICS internal reference)

This report has been prepared by: **Valérie Rousseau**
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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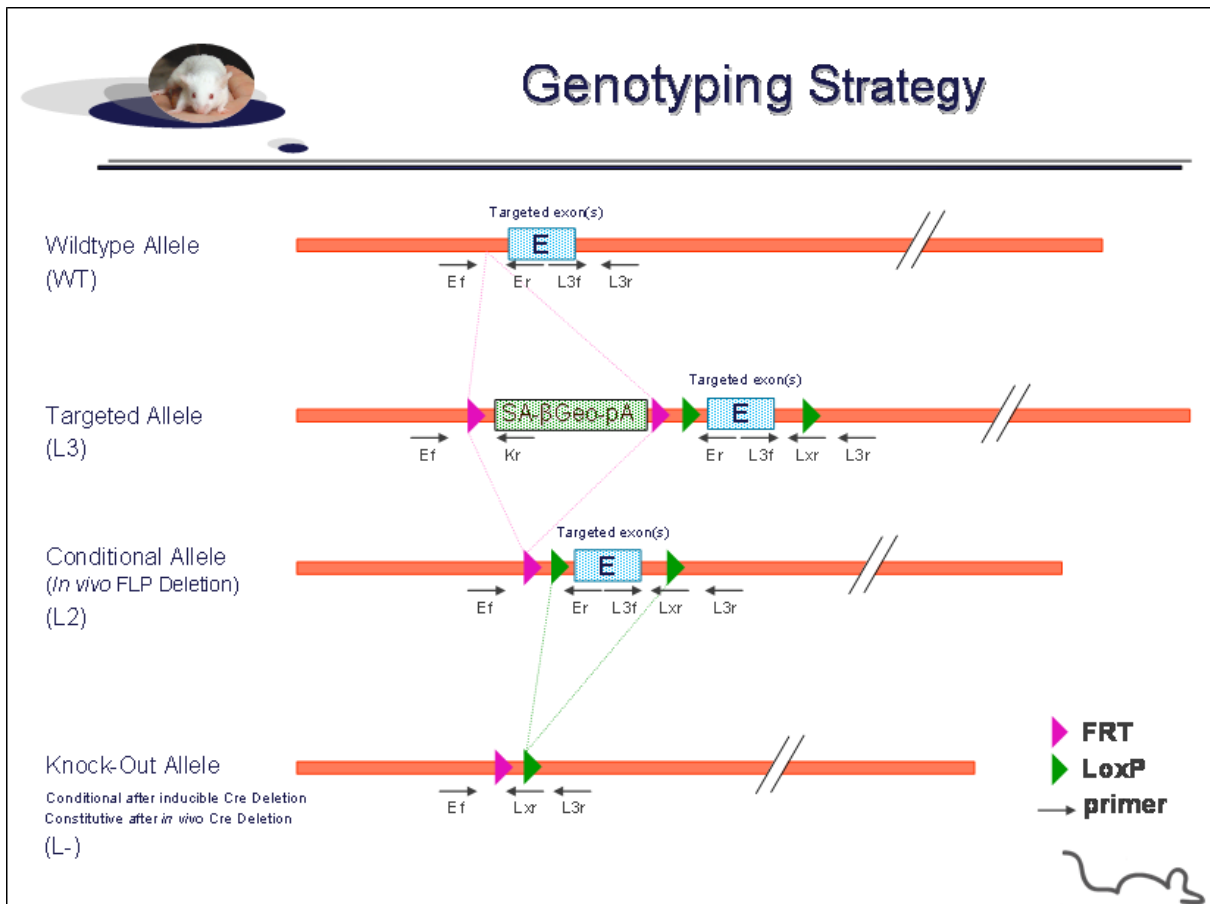
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1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Orc4l** Constitutive Knockout / Conditional Knockout (KO-cKO) 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	3795	CATACCCACATGTGGACAAACATTC
Er	3796	GCTGAGGACTAGAAATATATTCCAC
Kr	3278	GGCAAGAACATAAAGTGACCCTCC
L3f	6296	GTGATCTAATGGCCACTGTAATG
L3r	3797	GCTCTAGTGTGTATGGTGAGC
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer (see the map above)	Targeted allele (KO allele) (L3)	cKO allele (L2)	KO allele (L-)	WildType allele (WT)
5' part of the selection marker	3795-3278	Ef / Kr	340	---	---	---
Presence of the distal loxP	6296-3797	L3f / L3r	189	189	---	189
Distal loxP specific PCR	6296-3255	L3f / Lxr	130	130	---	---
Excision of the selection marker	3795-3796	Ef / Er	5772*	372**	---	275
Excision of the floxed exon(s), i.e. knock out	3795-3797	Ef / L3r	6431*	1031*	325**	939**

*: 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:

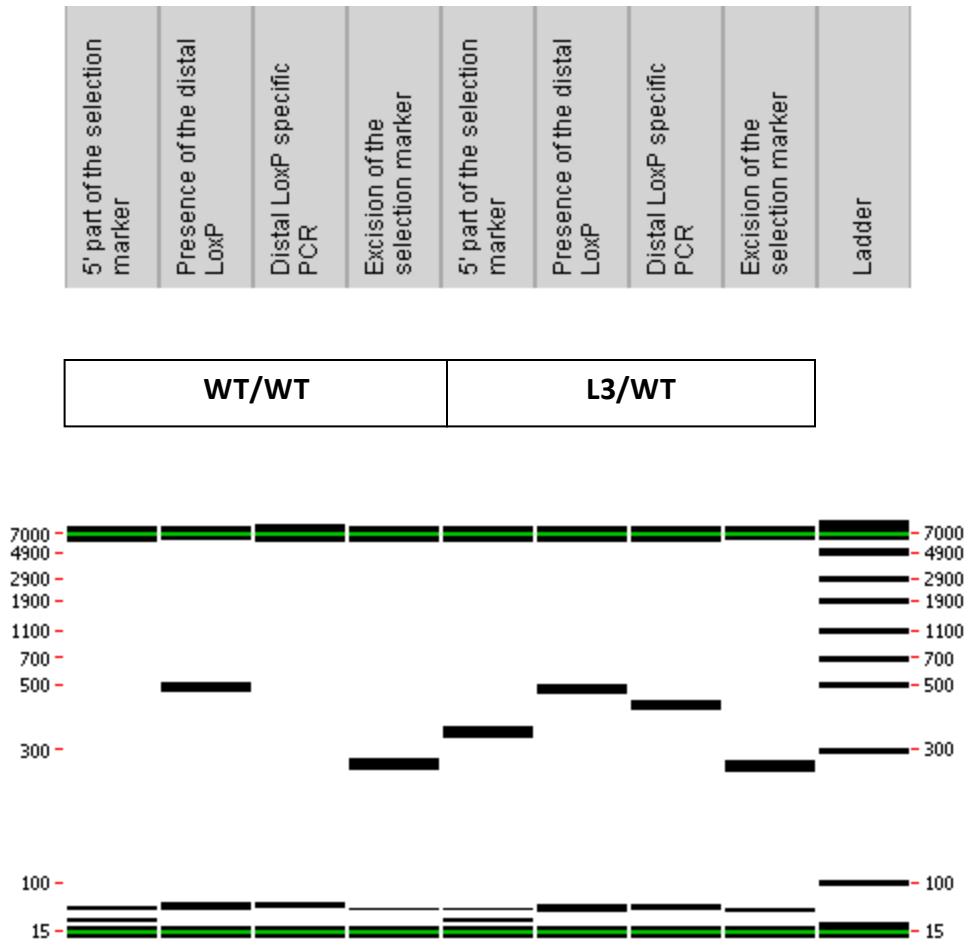
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5min	1

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

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was not done by gel electrophoresis but using LabChip® 90 microfluidic apparatus. PCR products were run on the HT DNA 5K LabChip® 90 Assay Kit.

Representative genotyping picture



Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

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.