Genotyping protocol

Mrpl54

IR00002731 / E98

(ICS internal reference)

This report has been prepared by: **Nathalie Chartoire**
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

The first version of this report was finalized the: 09 Nov 2012

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/

TABLE OF CONTENTS

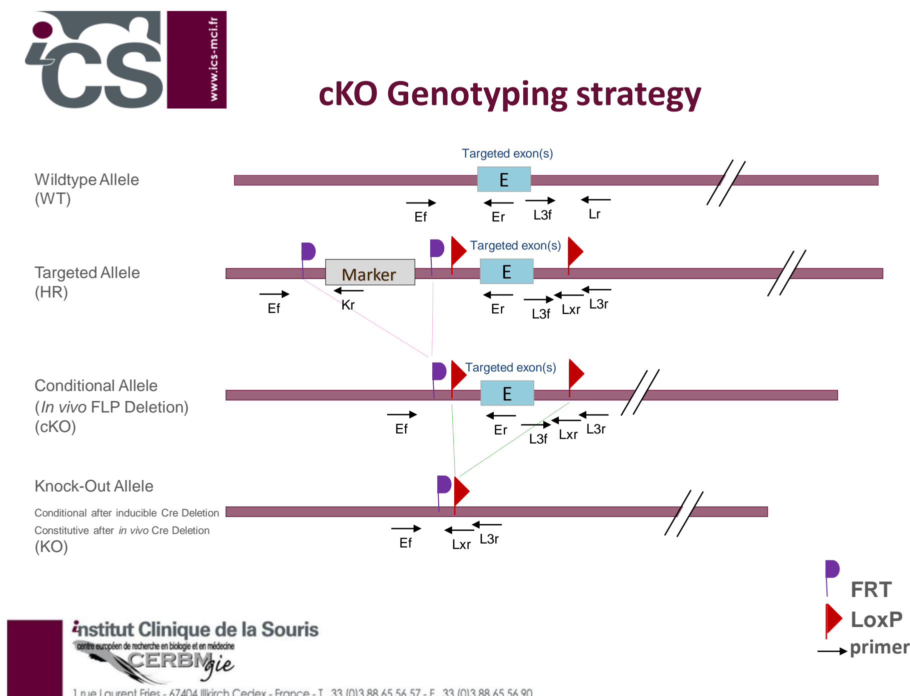
Table of contents **2**
1. Genotyping protocol and data **2**
1.1. Genotyping strategy 2
1.2. PCR protocol 5
1.3. Picture of genotyping with various alleles 6
2. Cre and Flp genotyping method **7**
2.1. Cre and Flp genotyping 7
2.2. PCR Protocol 8

1. Genotyping protocol and data

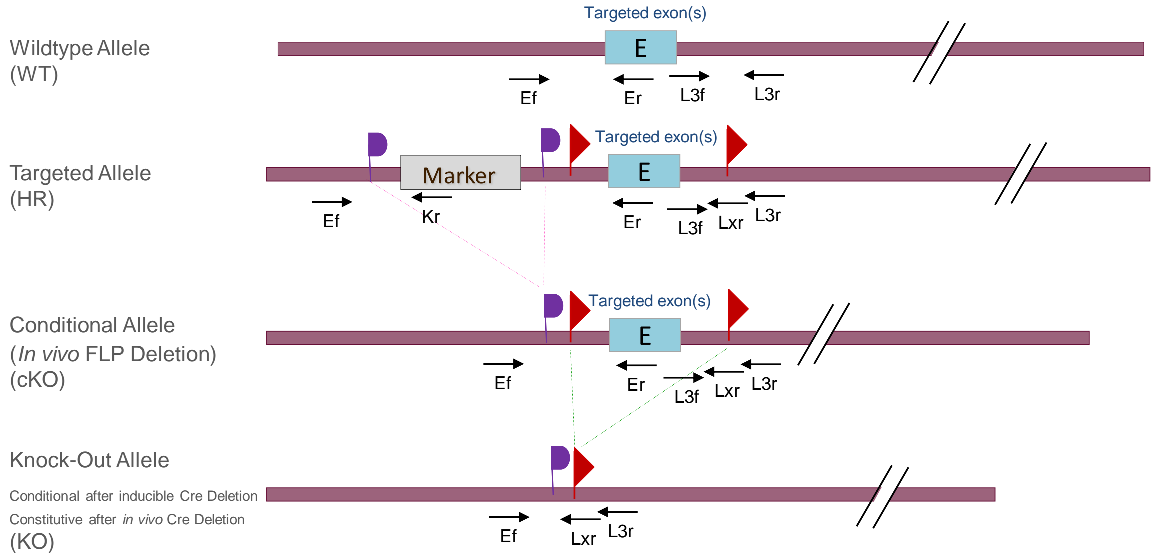
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Mrpl54** Constitutive Knockout / Conditional Knockout (KO-cKO) project.

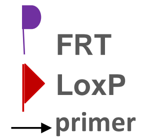
1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.



cKO Genotyping strategy





institut Clinique de la Souris
centre européen de recherche en biologie et en médecine
CERBM gie
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	4877	GACCCACATAAGCAGGGAAGGAGATG
Er	4883	TCTGAGAAGAGAGAGATGGCTCGGTAA
Kr	3210	CCTGTCCCTCTCACCTTCTACC
L3f	4882	CCACACTGCATAGGAATCCTGACACC
L3f[2]	4880	CATGGTGAGTGTACCACACTGCATAGG
L3r	4879	CAATCTCCTGAGAATGTAGCCCACCAT
Lxr	4881	GTATAATGTATGCTATACGAAGTTATCATTAATTG

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	4877-3210	Ef / Kr	439	---	---	---
Presence of the distal loxP	4882-4879	L3f / L3r	383	383	---	314
Distal loxP specific PCR	4880-4881	L3f[2] / Lxr	266	266	---	---
Excision of the selection marker	4877-4883	Ef / Er	7374*	470	---	320

*: 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:

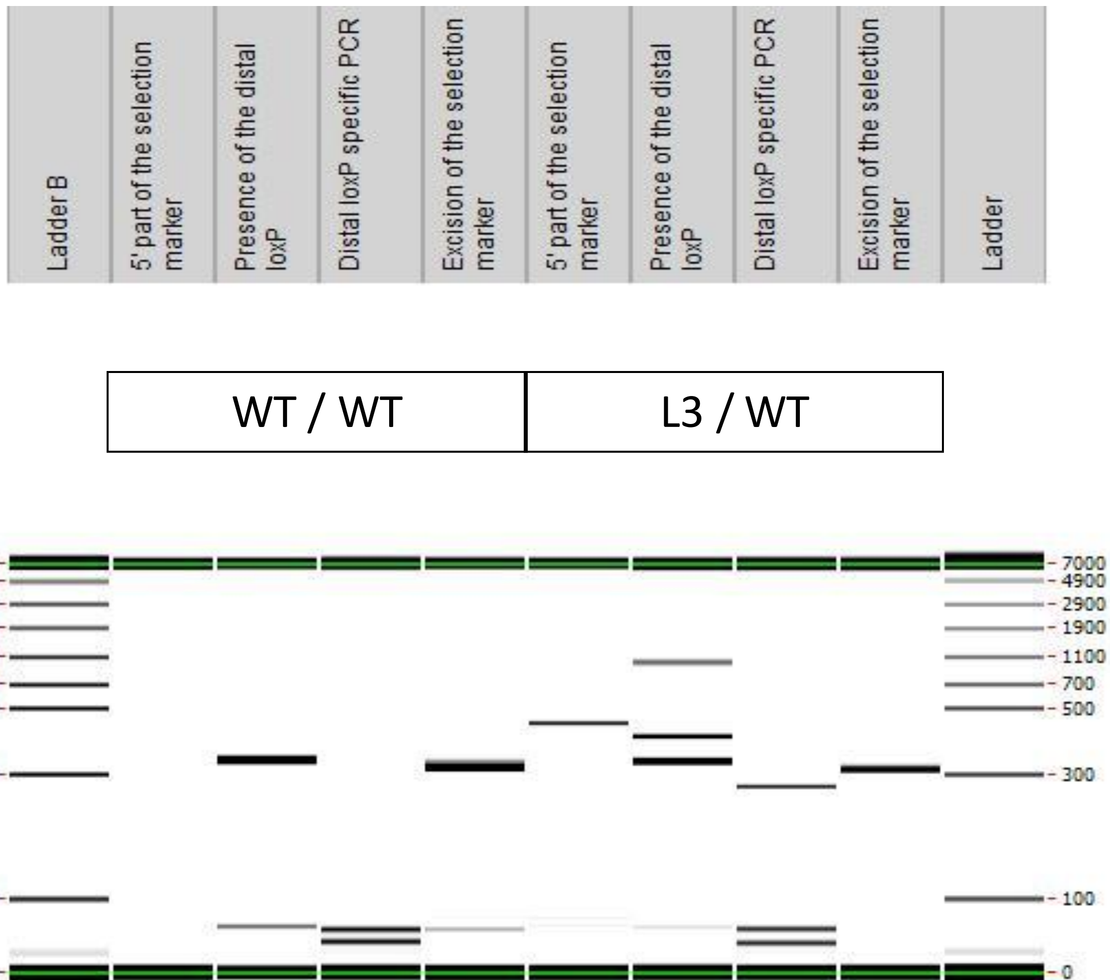
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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

<u>Representative genotyping picture</u>



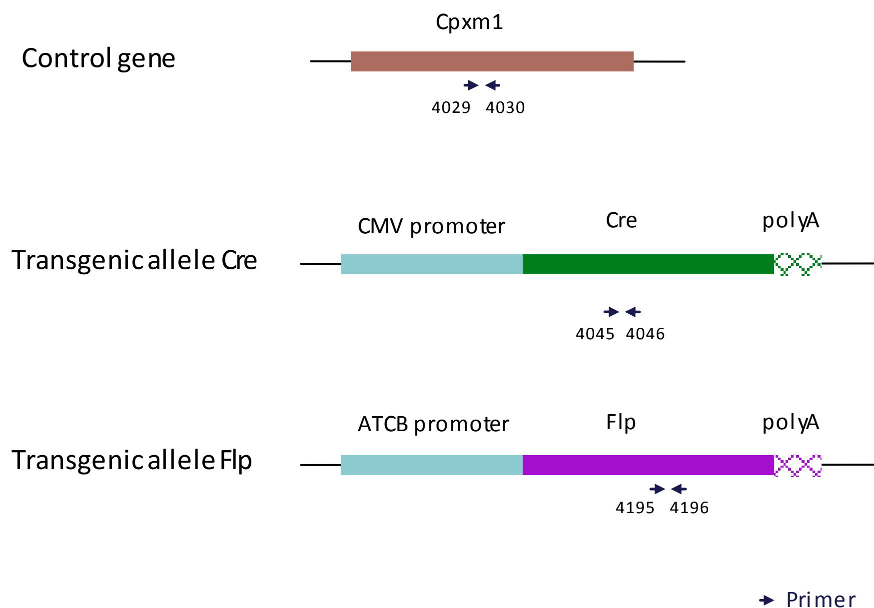
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

2. Cre and Flp genotyping method

The protocol used to segregate the cre and/or flp transgene is indicated below.
Detection of cre transgene and flp transgene is done using a multiplex assay: primer pairs were designed for each gene and for a positive control (Cpxm1 gene).

2.1. Cre and Flp genotyping

Schematic representation of the genotyping strategy



Sequence of primers used for genotyping:

Primers	Sequence
4029	ACTGGGATCTTCGAACTCTTTGGAC
4030	GATGTTGGGGCACTGCTCATTCACC
4045	CCATCTGCCACCAGCCAG
4046	TCGCCATCTTCCAGCAGG
4195	TCTTTAGCGCAAGGGGTAGGATCG
4196	GTCCTGGCCACGGCAGAAGC

PCR fragments expected size (bp):

Primer pair	4045-4046	4195-4196	4029-4030
Region analyzed	Middle part of Cre transgene	Middle part of Flp transgene	Cpxm1 control gene
Control gene	/	/	397
Tg allele	281	328	/

2.2. 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)	0.05µl
3’ primer (100 µM)	0.05µl
Sterile H_2O	up to 15 µl

Cycling conditions are identical to those described in chapter 1.2