Genotyping protocol

Lhx1

IR00002733 / E100

(ICS internal reference)

This report has been prepared by: **Valérie Rousseau**
33 (0)3 88 65 56 55
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: 11 Apr 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/

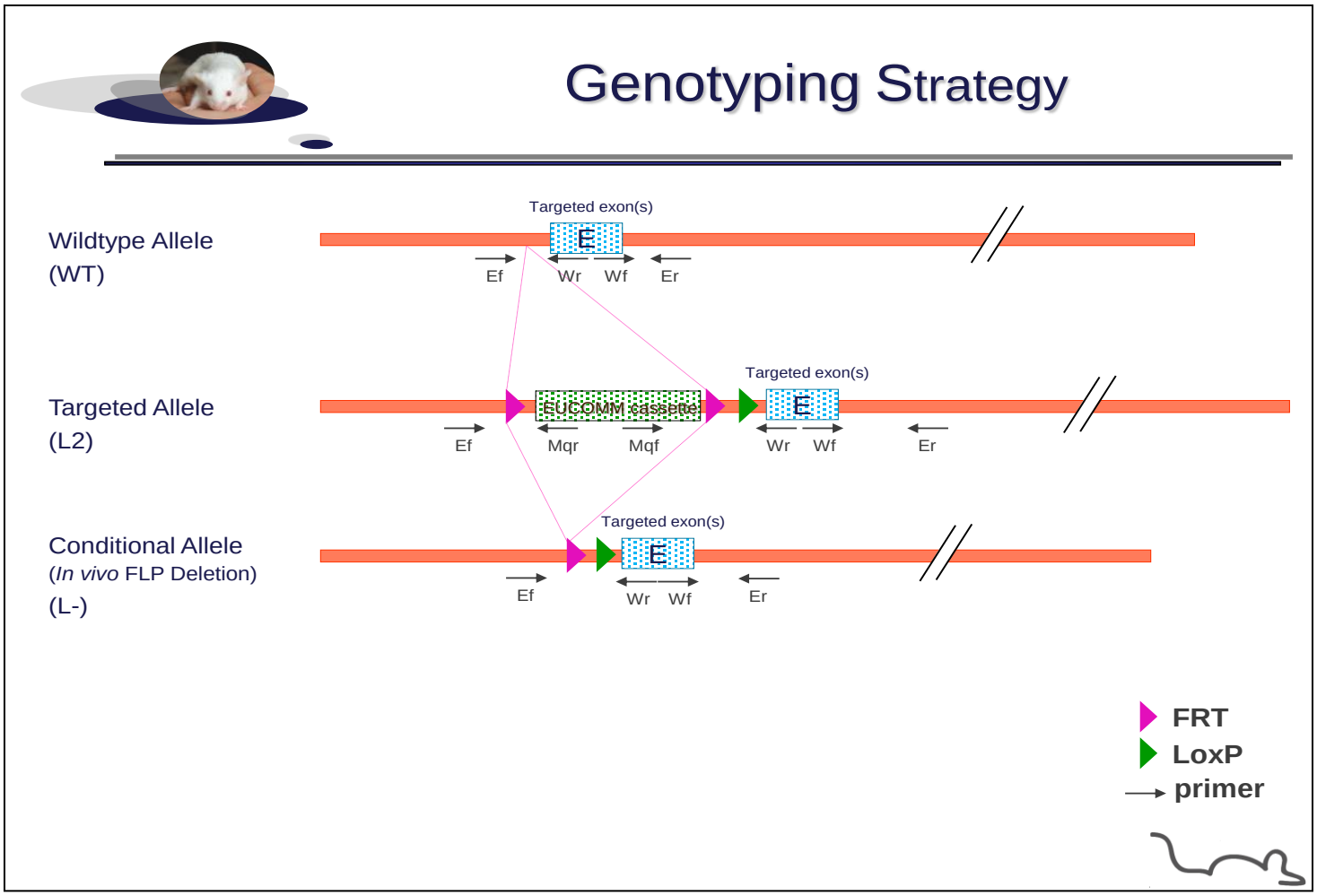
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Lhx1** Conventional or Constitutive Knockout (KO) 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	5113	CTTGAGGCCTCGCTAACCGCAA
Er	5116	CCACAGCCAGCTTCTAAAGGGTGG
Er[2]	5117	CGAGAGCATAAGGGGAAACAGCACAT
Mq1f	2687	CTGCATTCTAGTTGTGGTTTGTC
Mq1r	3210	CCTGTCCCTCTCACCTTCTACC
Wf	5115	TGAGCAACTGGCCCAGGAGACT
Wr	5114	CGAGAGCATAAGGGGAAACAGCACAT

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 (L2)	KO allele (L-)	WildType allele (WT)
WildType allele specific PCR (5' part of the targeted locus)	5113-5114	Ef / Wr	---	---	332
WildType allele specific PCR (3' part of the targeted locus)	5115-5116	Wf / Er	---	---	369
Excision of the selection marker	5113-5117	Ef / Er[2]	7378*	474**	332
5' part of the selection marker	5113-3210	Ef / Mq1r	469	---	---
3' part of the selection marker	2687-5117	Mq1f / Er[2]	326	---	---

*: 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:

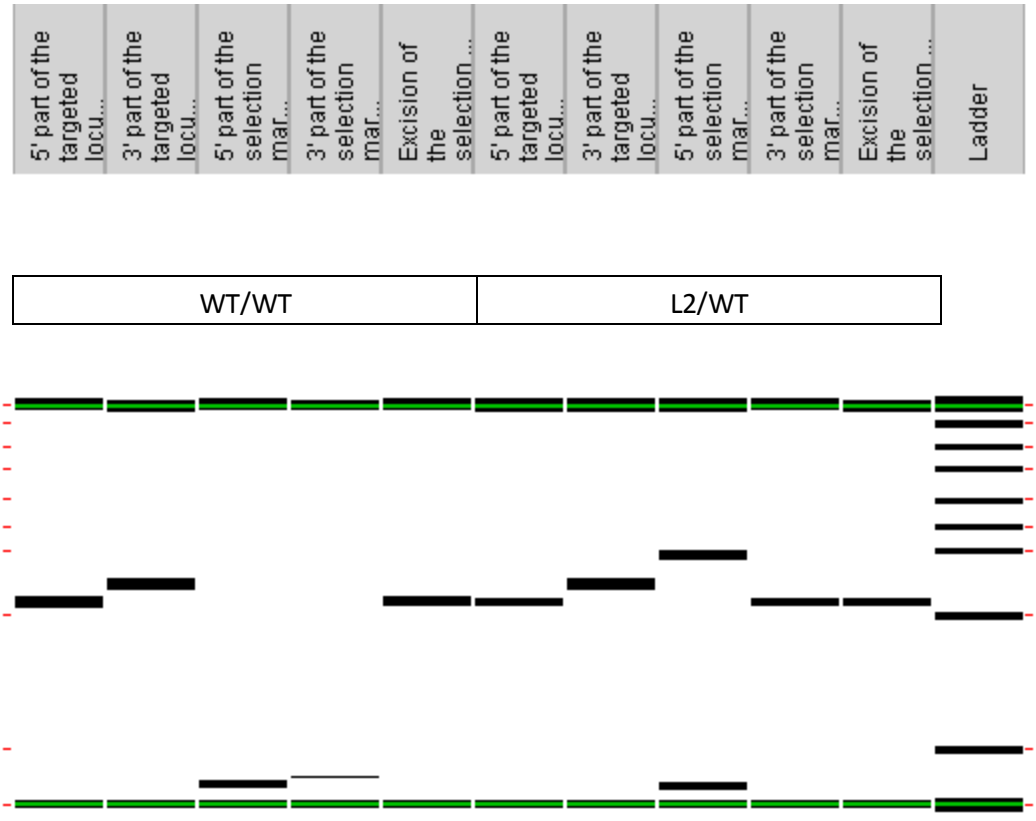
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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.

Representative genotyping picture



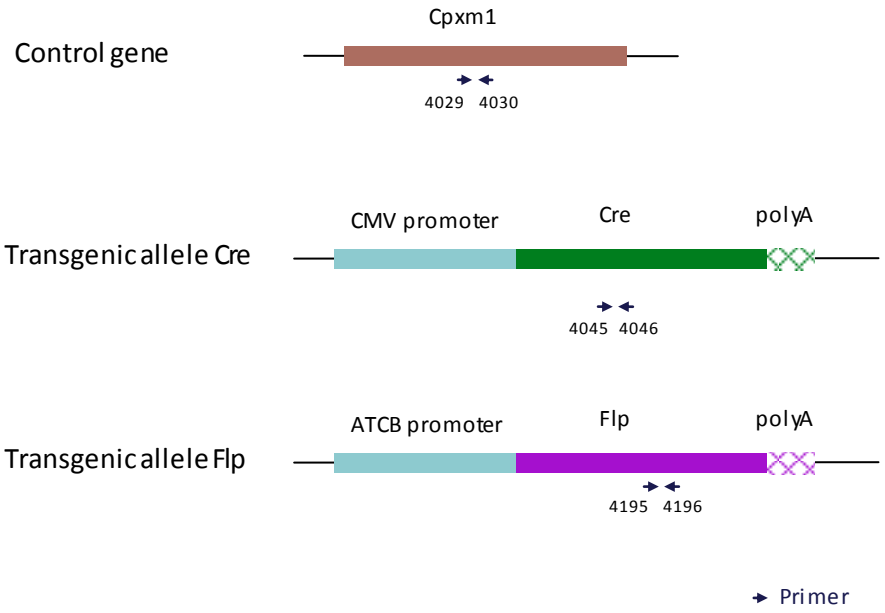
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

2. Cre and Flp genotyping method

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	/	/	446
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