



Genotyping protocol

Ncs1

IR00002596 / E71

(ICS internal reference)

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TABLE OF CONTENTS

Table of contents2

1. Genotyping protocol and data2

 1.1. Genotyping strategy2

 1.2. PCR protocol4

 1.3. Picture of genotyping with various alleles5

2. Cre and Flp genotyping method6

 2.1. Cre and Flp genotyping6

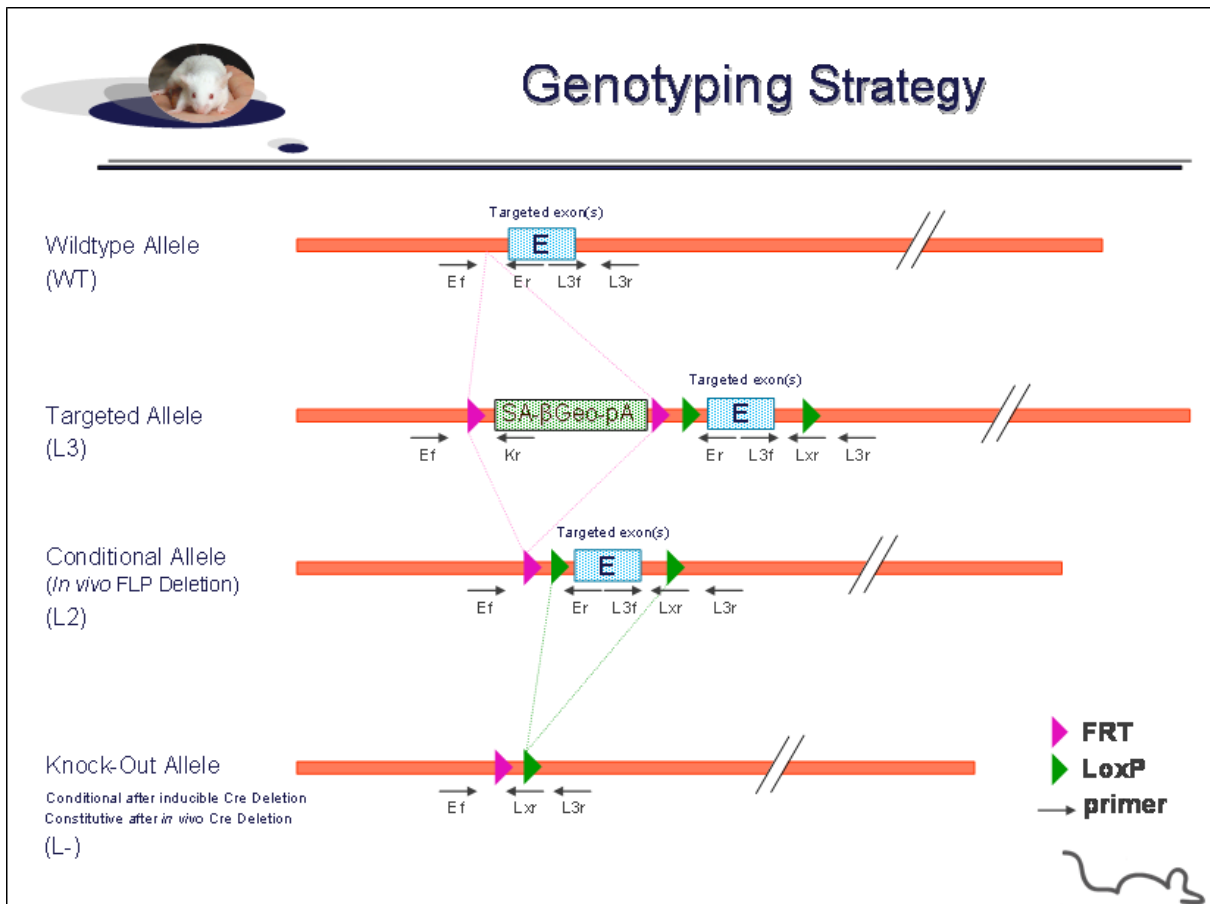
 2.2. PCR Protocol7

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Ncs1** 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	4578	GGTCTTGTGCTGGGCACTGGGG
Er	4581	CGGTCCCCAGGAAGCCTCCTCA
Kr	3277	CTCCTACATAGTTGGCAGTGTGGG
L3f	4579	GGCTGCGTCCCAGTTCAGTCC
L3r	4580	GCAACGCCAACCCACAGATGG
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	4578-3277	Ef / Kr	235	---	---	---
Presence of the distal loxP	4579-4580	L3f / L3r	400	400**	---	431
Distal loxP specific PCR	4579-3255	L3f / Lxr	192	192**	---	---
Excision of the selection marker	4578-4581	Ef / Er	7219*	315**	---	168
Excision of the floxed exon(s), i.e. knock out	4578-4580	Ef / L3r	8122*	1218*	445**	1102*

*: 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:

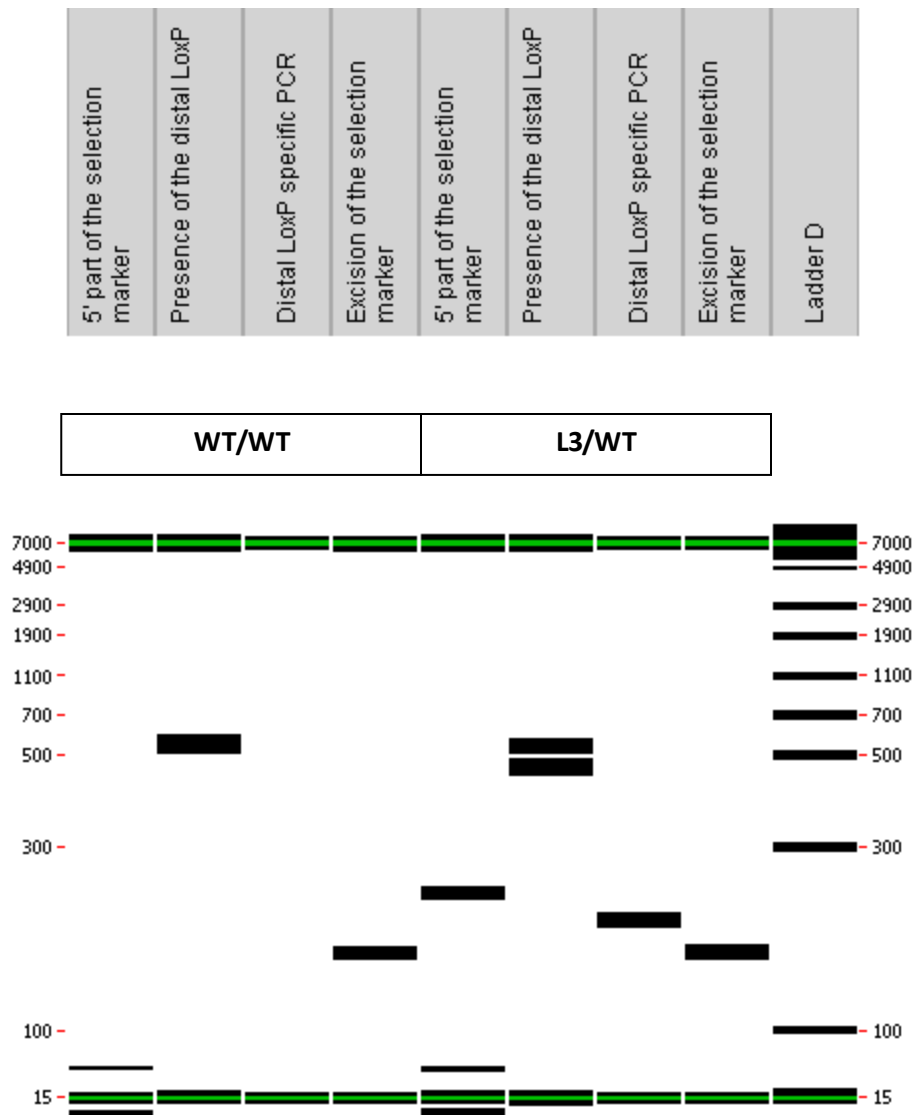
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5min	1

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.

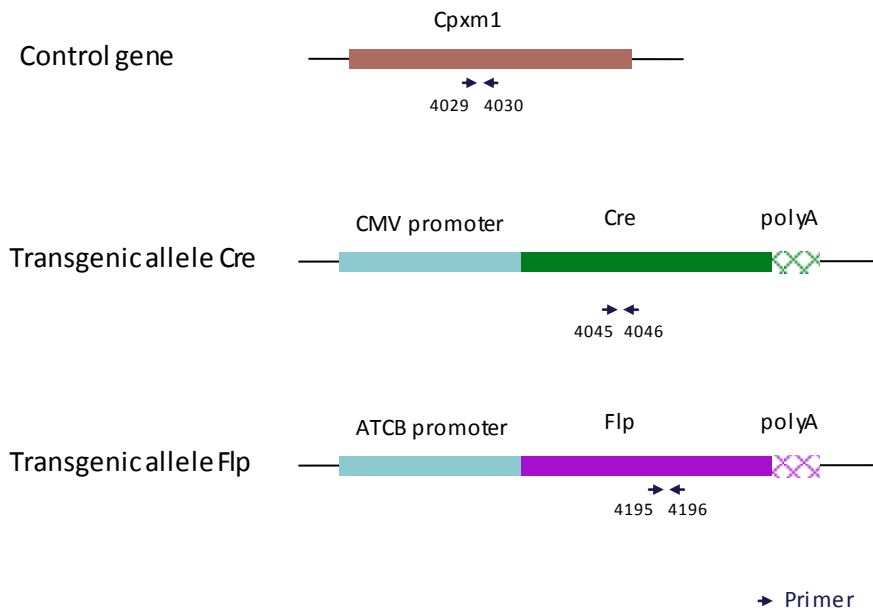
2. Cre and Flp genotyping method

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	GATGTTGGGGCACTGCTCATTACCC
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 ₂ O	up to 15 µl

Cycling conditions are identical to those described in chapter 1.2