Ikzf4 cKO (IR00003147 / K596 ICS internal reference) mouse line genotyping protocol

Table of contents

Table of contents 1
1. Genotyping protocol and data 2
1.1. Genotyping strategy 2
1.2. PCR protocol 3
1.3. Picture of genotyping with various alleles 4

For any question, please contact:

Mouse Clinical Institute – Institut Clinique de la Souris (ICS)
ICS genotyping service
1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex France
Email: mutagenesis@igbmc.fr

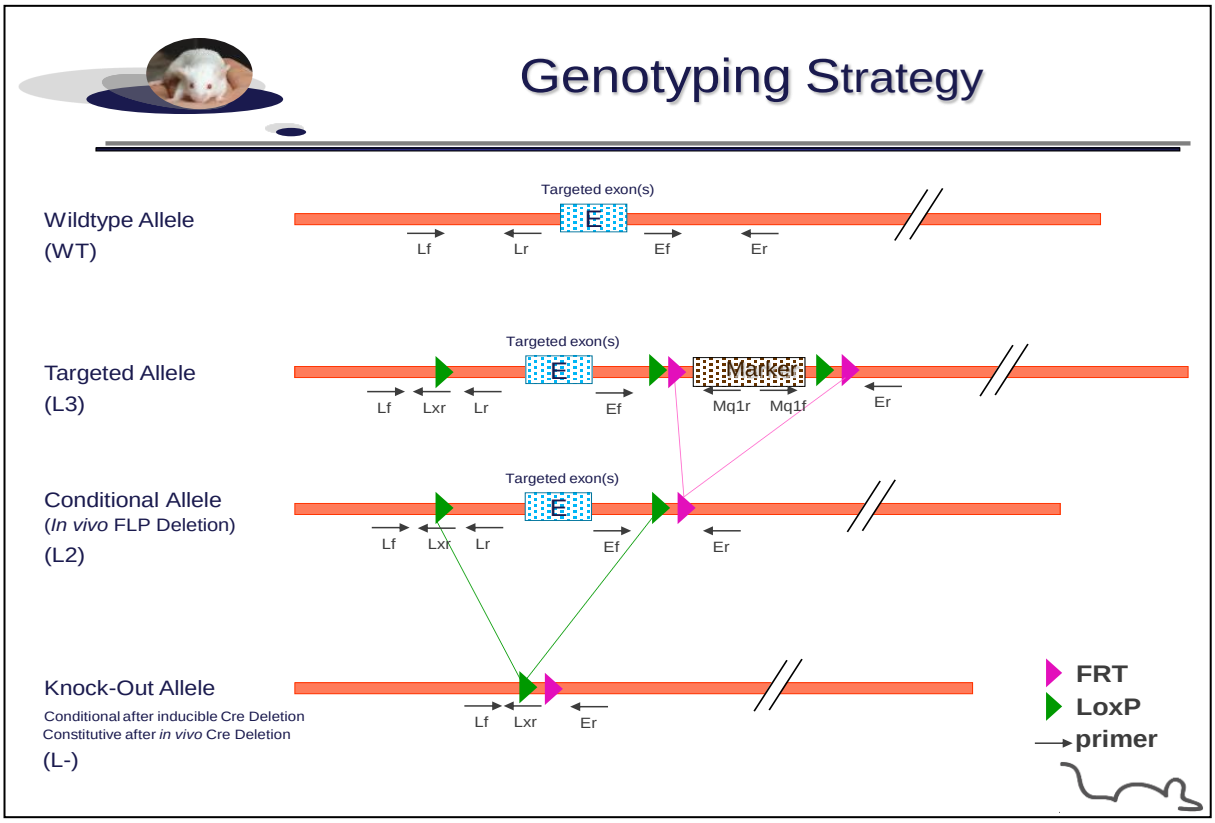
This protocol has been validated by Christelle Roth.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Ikzf4** Conditional Knockout (cKO) mouse line.

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	Sequence
Ef	GGACCCAACCCTGGGCTTTACATG
Ef	TCTCTACAGTCCCAGGTGACCTCACTT
Er	CGAGCTTTGCCATCCTTTGAAGAG
Er	CAAGGACTTCAGTCTACATGGGCTGAT
Lf	AGAAGGGAGCCATGGGCAGAAGAA
Lf	TAAGGGTGAGGATTAAAGCAAGAAGCC
Lr	TGTTACACCCTAAGGAGTTTTGCGTTG
Lxr	CGAAGTTATCTGCAGGTCGACCTTAAG
Mq1f	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mq1r	TGCTAAAGCGCATGCTCCAGACTGC

PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	Targeted allele (L3)	cKO allele (L2)	Wild Type allele (WT)
Presence of the distal loxP	Lf / Lr	337	337	259
Excision of the selection marker	Ef / Er	2207*	314	204
5' part of the selection marker	Ef / Mq1r	253	---	---
3' part of the selection marker	Mq1f / Er	368	---	---
LoxP specific PCR	Lf / Lxr	227	227	---

* This PCR product will not be observed using our PCR genotyping conditions (see description below)
--- No Amplicon should be obtained

NB: An aspecific band at 420pb is detected for the “Presence of the distal loxP” PCR on the L3/WT and L2/WT animals

An aspecific band at 564pb is detected for the “Excision of the selection marker” PCR on the L2/WT animals.

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:

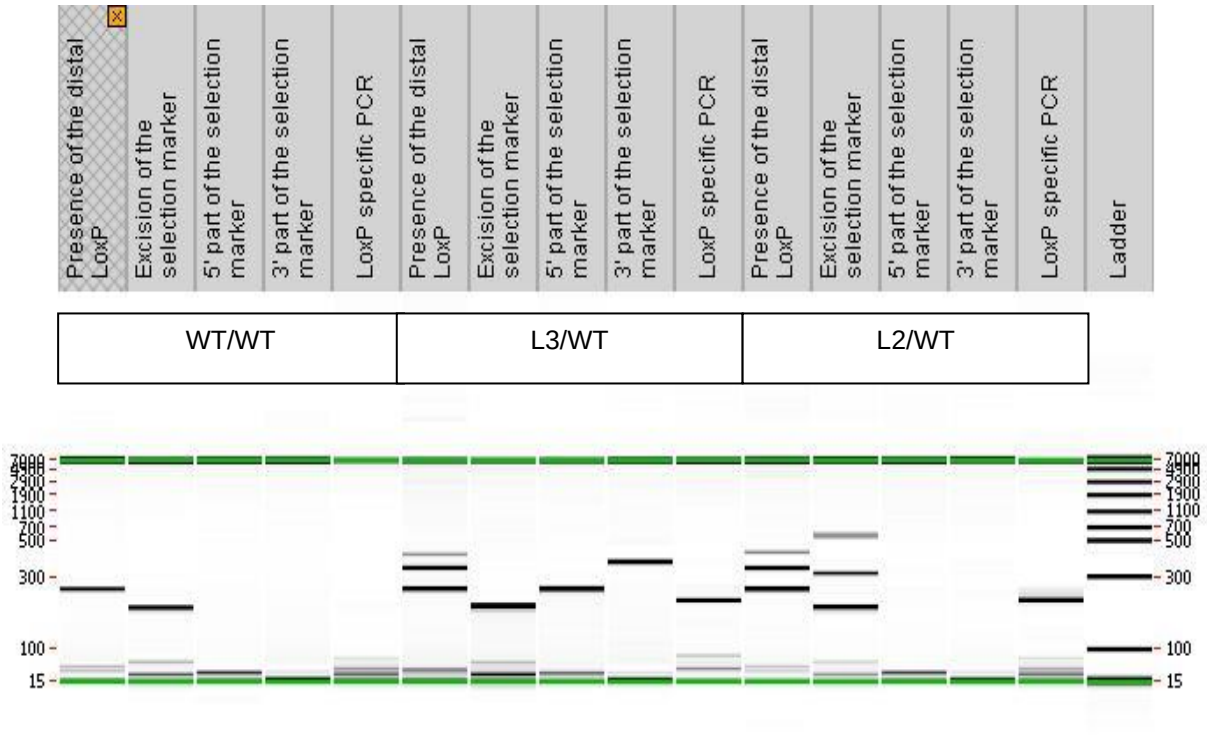
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

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.