RDC1 (IR00001247 / K349 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

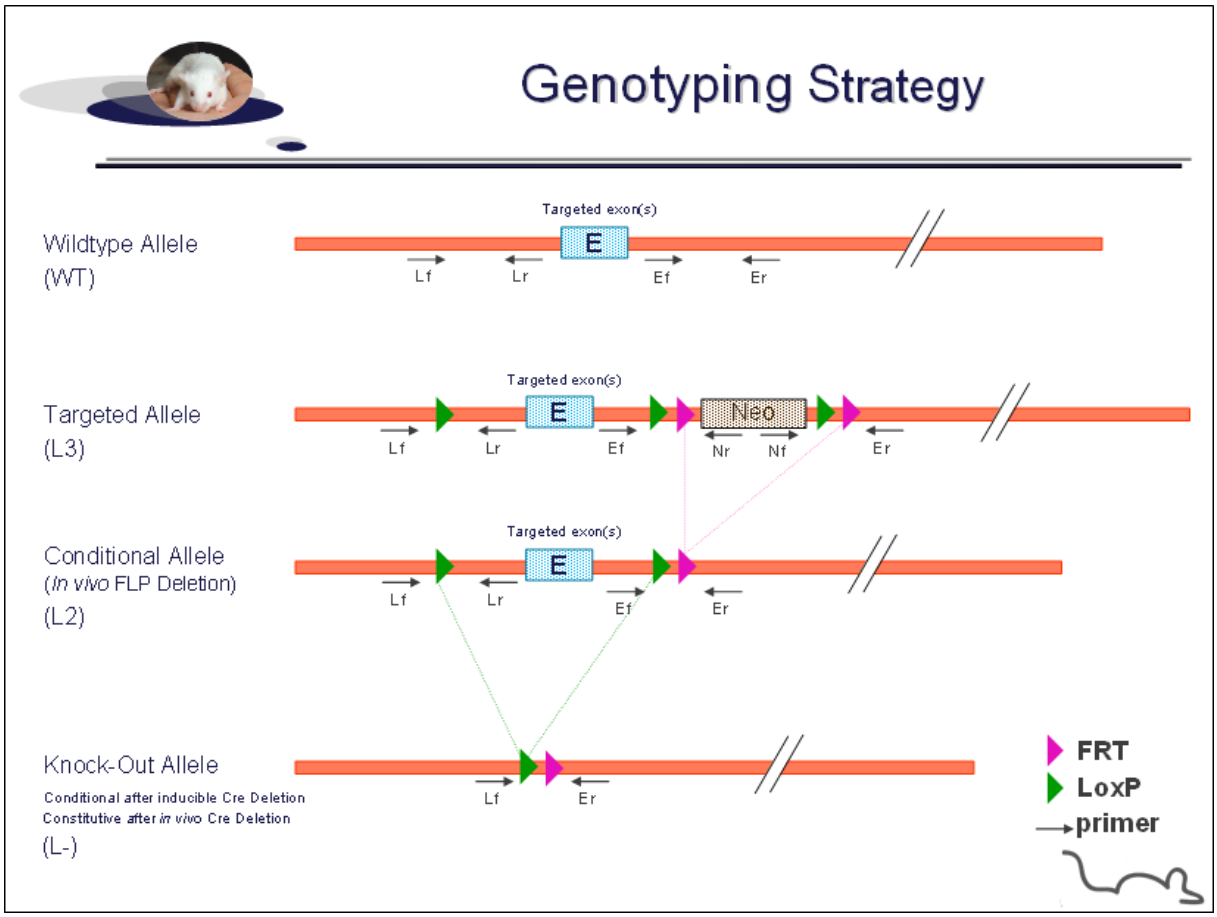
This protocol has been validated by Karim Essabri.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **RDC1** Conditional Knockout (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	3163	CCTTTGCAATATCCATCTGCCAACC
Er	3164	GAGTCAATTGAGTGGGCAAGGAATG
Lf	3159	CCTGGTGCTGGCTTTGATACGCAGC
Lr	3161	CTGGTTGCTTGAGTGGTATGAAGAG
Nf	239	GAAGGGTGAGAACAGAGTACCTAC
Nr	238	TGACTAGGGGAGGAGTAGAAGGTG

PCR fragments expected size (bp):

Region analyzed	**Primers used**	Position on the primer (see the map above)	Targeted allele (L3)	Conditional allele (L2)	Knock-Out allele (L-)	WT allele (WT)
Presence of the distal loxP	3159-3161	Lf / Lr	291	291	---	211
Excision of the selection marker	3163-3164	Ef / Er	NA*	324	---	214
5' part of the selection marker	3163-238	Ef / Nr	303	---	---	---
3' part of the selection marker	239-3164	Nf / Er	684	---	---	---
Excision of the floxed exon(s), i.e. knock out	3159-3164	Lf / Er	NA*	NA*	516	2141*

*NA: Not Applicable. This PCR product will not be observed using our PCR genotyping conditions (see description below)
--- 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:
-10x Buffer (Roche)	2.5µl
-dNTPs 10mM (Amersham Biosciences)	0.5µl
-Taq DNA Polymerase (Roche)	0.2µl
-DNA (50ng/µl)	3µl
-5' primer (100 µM)	0.125µl
-3' primer (100 µM)	0.125µl
-Sterile H2O	up to 25 µl

Cycling conditions:

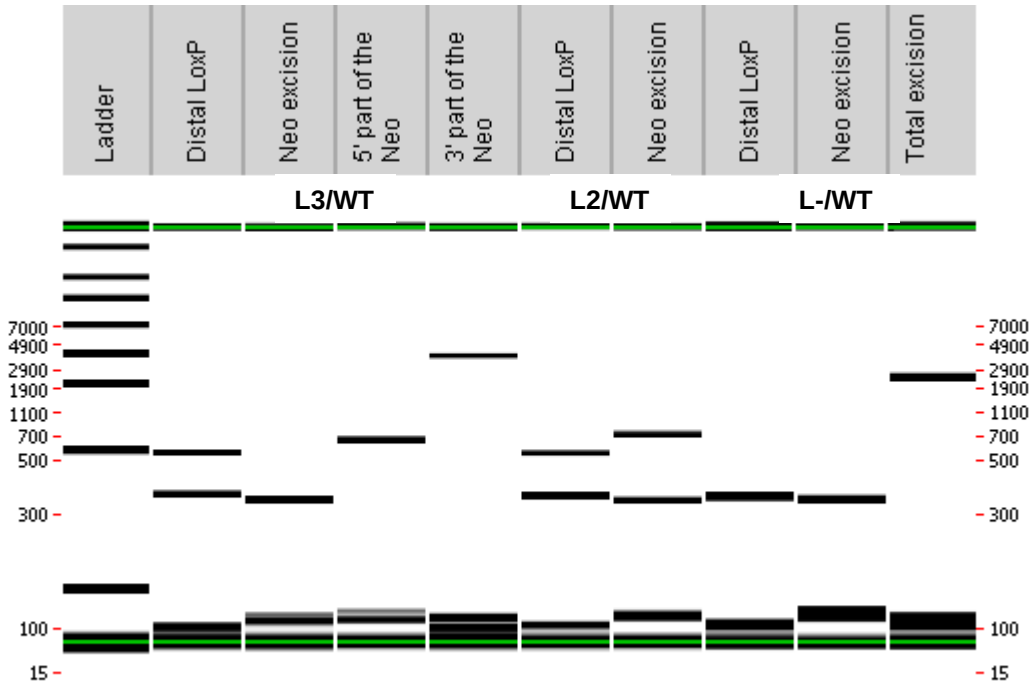
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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.