Rab4b (IR00001209 / K350 ICS internal reference) mouse line genotyping protocol

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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.u-strasbg.fr

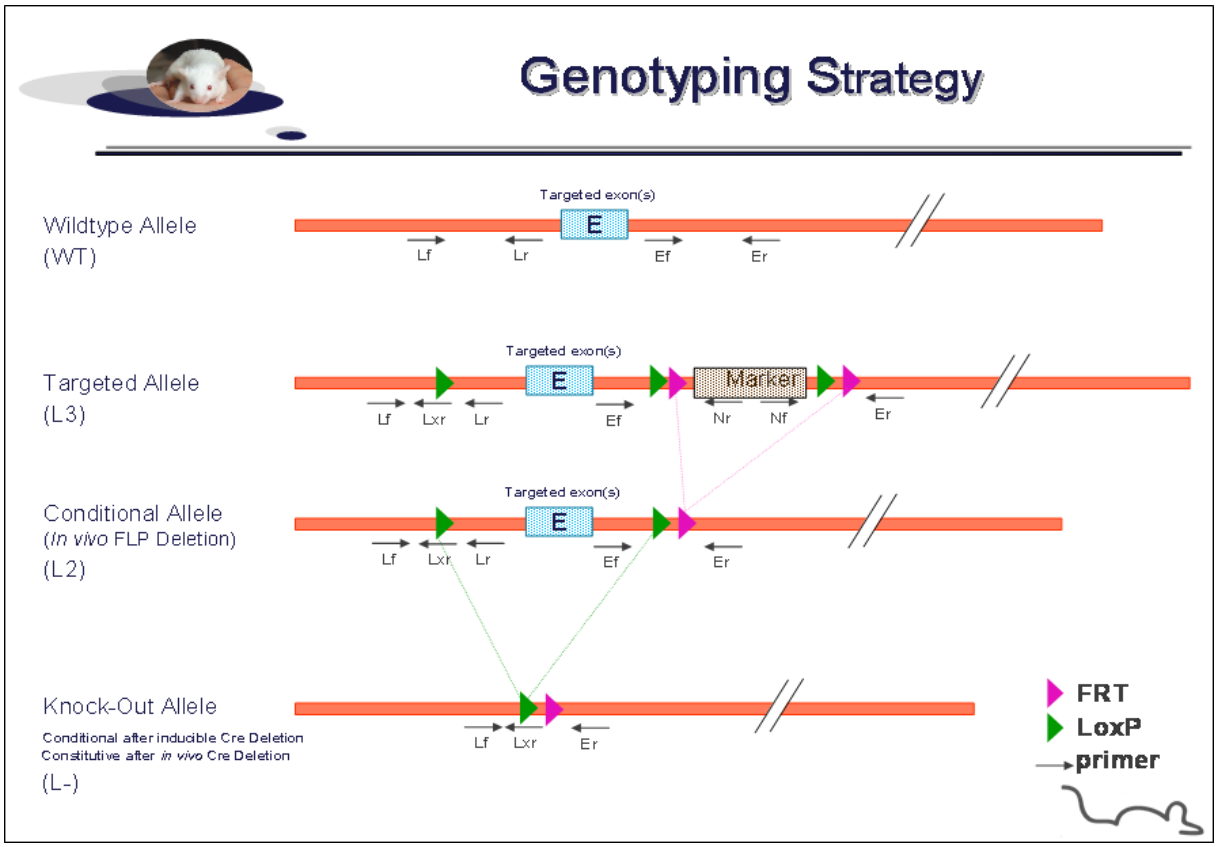
This protocol has been validated by Valérie Rousseau.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Rab4b** 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	4565	AGCCTGGGTTCCCAAGGAGGGG
Er	4228	CCTGACCAAGTGCTATACAACC
Er	4566	TGTCTGAGGCAGGCCAACTCCC
Lf	4224	TGGCACTTCCAGCAGTGGGT
Lf	4226	TTTTAGAGGCTGAGGTAGGA
Lr	4225	TTCCCCTGCCTCTTCTGCCC
Nf	1219	CAGCTCATTCCTCCCACTCATGATC
Nr	265	TGCTAAAGCGCATGCTCCAGACTGC

PCR fragments expected size (bp):

Region analyzed	**Primers used**	Position on the primer (see the map above)	Targeted allele (L3)	cKO allele (L2)	KO allele (L-)	WildType allele (WT)
Presence of the distal loxP	4224-4225	Lf / Lr	467	467	---	413
Excision of the selection marker	4565-4566	Ef / Er	2341*	447	---	337
5' part of the selection marker	4565-265	Ef / Nr	431	---	---	---
3' part of the selection marker	1219-4228	Nf / Er	415	---	---	---
Excision of the floxed exon(s), i.e. knock out	4226-4228	Lf / Er	3642*	1750*	268**	1768*

* 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:
-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:

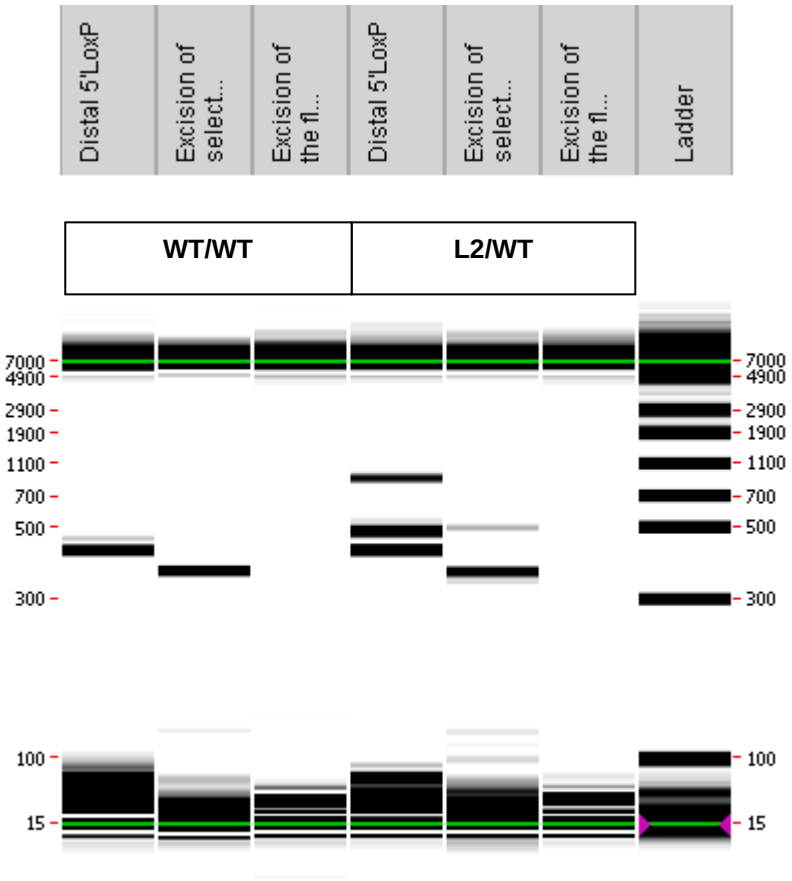
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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.