Pik3r4 (Vps15, IR00001930 / K460 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.fr

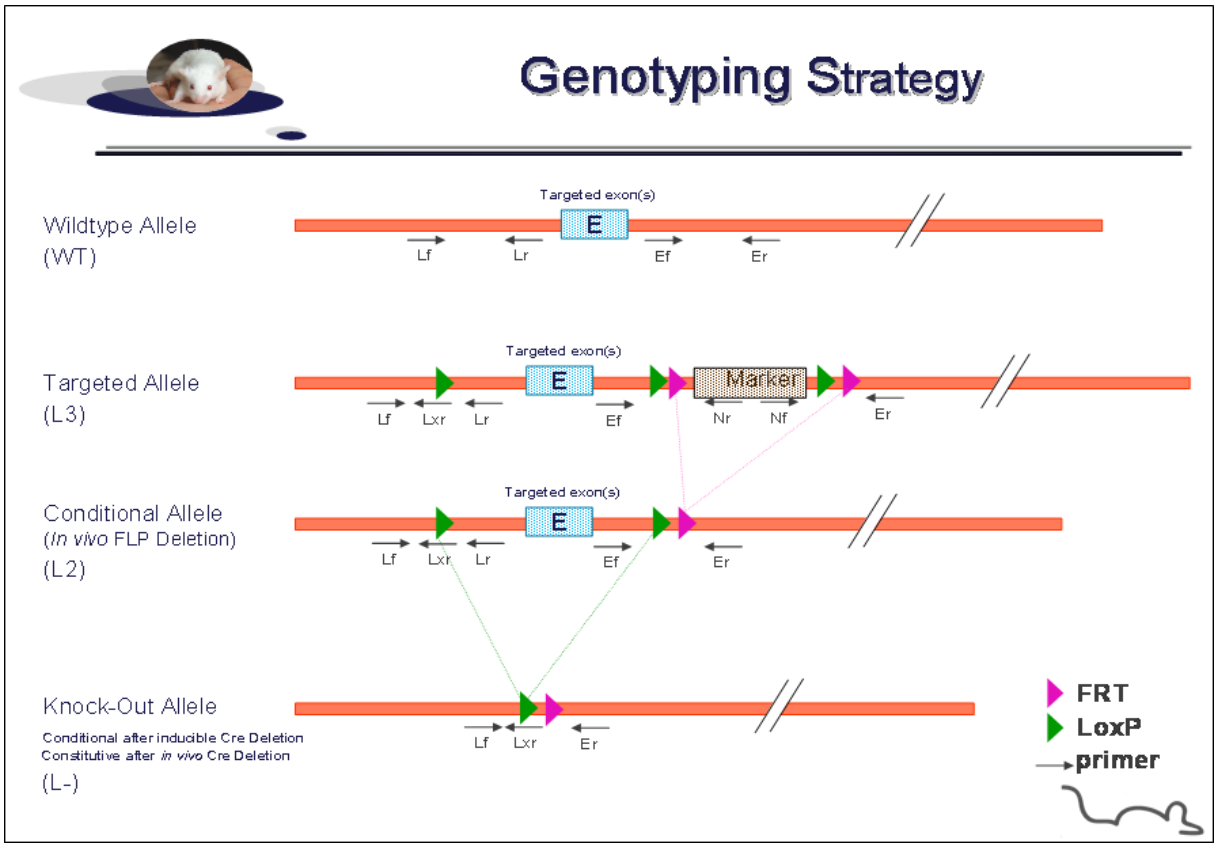
This protocol has been validated by Karim Essabri.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Vps15** 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	3690	GCTAGGCCCTCTTAGACGGTTTCAGAC
Ef	3692	GCACGGGGAGAGCTGAAGAGAGC
Er	3691	AGCTGTGTGCTTCTGTAGCAGCAACTG
Er	3693	CATGTAGCCTCCTCCCGTGCCG
Lf	3694	GACCGAGGCATACGGTACTTTTACG
Lr	3695	ACGTCATGTCATTCTTTCCAGCCGC
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	3694-3695	Lf / Lr	359	359	---	279
Excision of the selection marker	3690-3691	Ef / Er	2265*	372	---	258
5' part of the selection marker	3692-265	Ef / Nr	406	---	---	---
3' part of the selection marker	1219-3693	Nf / Er	356	---	---	---
Excision of the floxed exon(s), i.e. knock out	3694-3691	Lf / Er	3702*	1809*	315**	1615*

* 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:

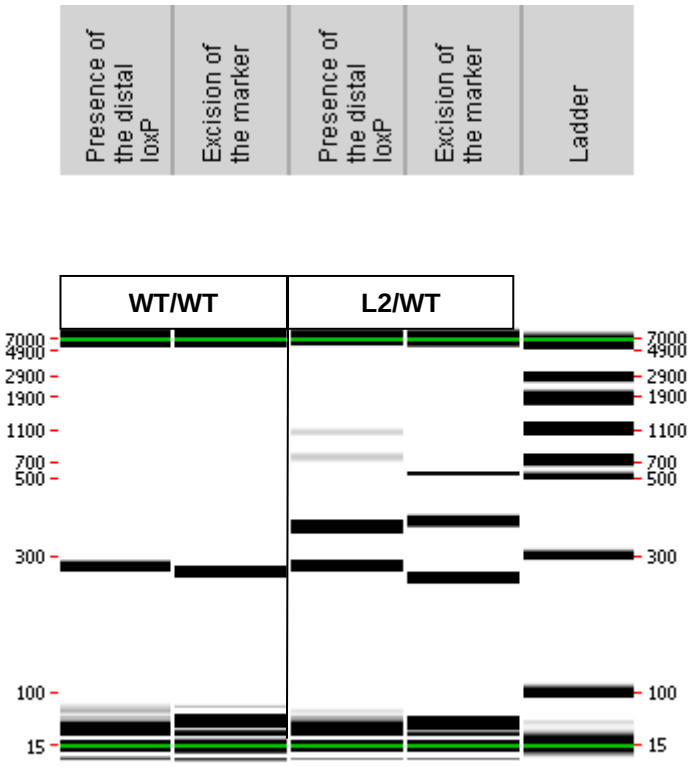
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

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.