renin receptor (IR00001219 / K342 ICS internal reference) mouse line genotyping protocol

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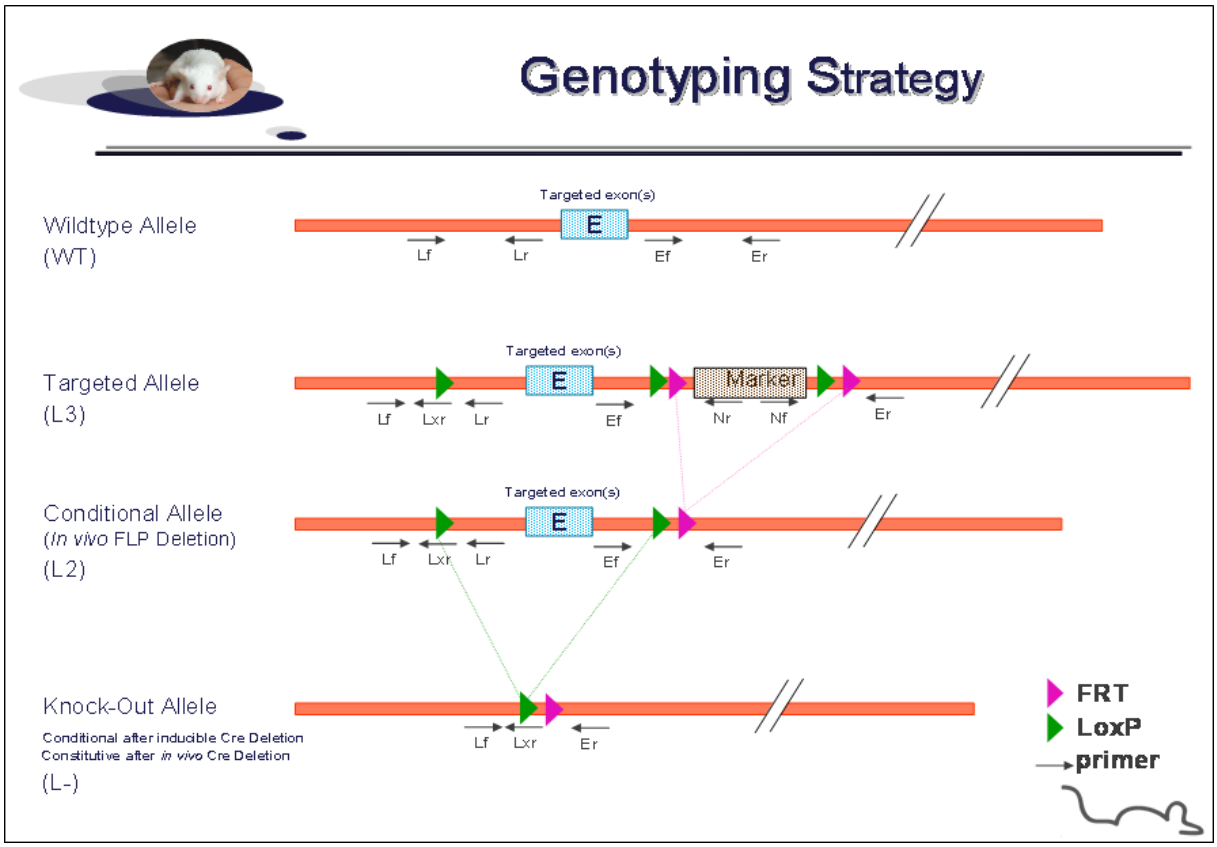
This protocol has been validated by Karim Essabri.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **renin receptor** 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	2275	CAGGCTGTGACCCATGGTAGTC
Er	2276	GCCCCTCTCTTACAGTTCTATCAGT
Er	2277	GCTGTCCAAAGAAACCAGAGCC
Lf	2271	AGCACTCTCTTCCAGGTATGTTGTG
Lr	2273	CTGGATCCCGGAGCATGGGTAAAGG
Nf	6	GAAGAACGAGATCAGCAGCCTCTGTTCC
Nr	237	CATCTGCACGAGACTAGTGAGACG

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	2271-2273	Lf / Lr	330	330	---	280
Excision of the selection marker	2275-2277	Ef / Er	2276*	388	---	278
5' part of the selection marker	2275-237	Ef / Nr	388	---	---	---
3' part of the selection marker	6-2276	Nf / Er	219	---	---	---
Excision of the floxed exon(s), i.e. knock out	2271-2276	Lf / Er	3528*	1640*	326**	1480*

* 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:

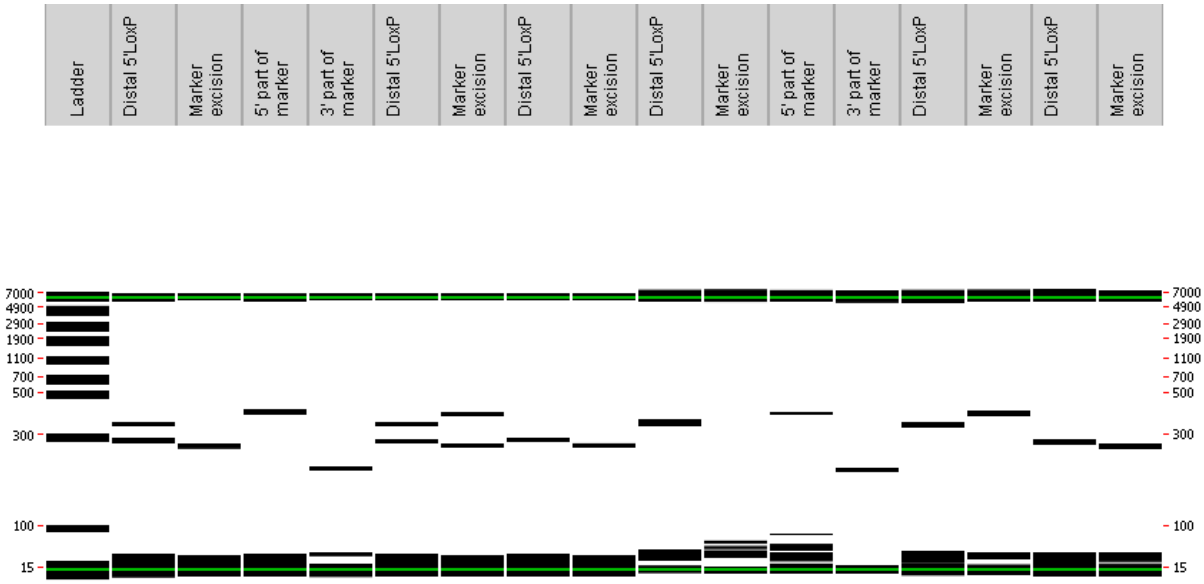
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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.