Ccp4/Agbl1 (IR00002131 / K490 ICS internal reference) mouse line genotyping protocol

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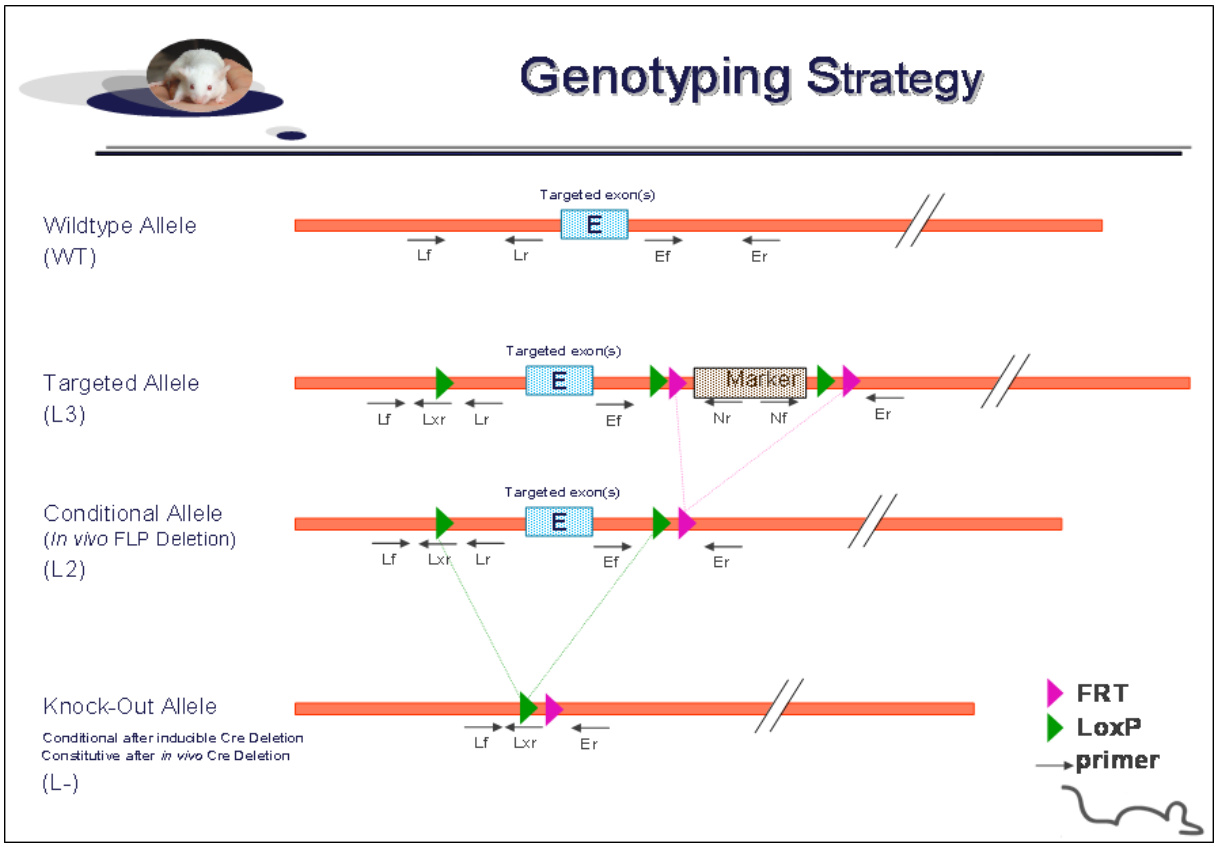
This protocol has been validated by Valérie Rousseau.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **CCP4** 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	4070	AGTGGCATGAGTGTAGAGGCCTTGG
Ef	4796	TCCCTTGGGCTCCATCTATCTGAACCA
Er	4071	GCCCTTCTGACAGGCCTCAGAGTT
Er	4072	AGTGCAGAAGCAGTTGTAGTCACCAGG
Lf	4068	GACTGCAATTGATTAAGGAGTGAGC
Lr	4069	CAAGACTCTGGGCTGTTATCAAAGC
Nf	3720	AGGGCCAGCTCATTCCTCCCACTC
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	4068-4069	Lf / Lr	426	426	---	355
Excision of the selection marker	4796-4071	Ef / Er	2626*	604	---	460
5' part of the selection marker	4070-265	Ef / Nr	525	---	---	---
3' part of the selection marker	3720-4072	Nf / Er	399	---	---	---
Excision of the floxed exon(s), i.e. knock out	4068-4071	Lf / Er	3540*	1518*	624	1305*

* 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:

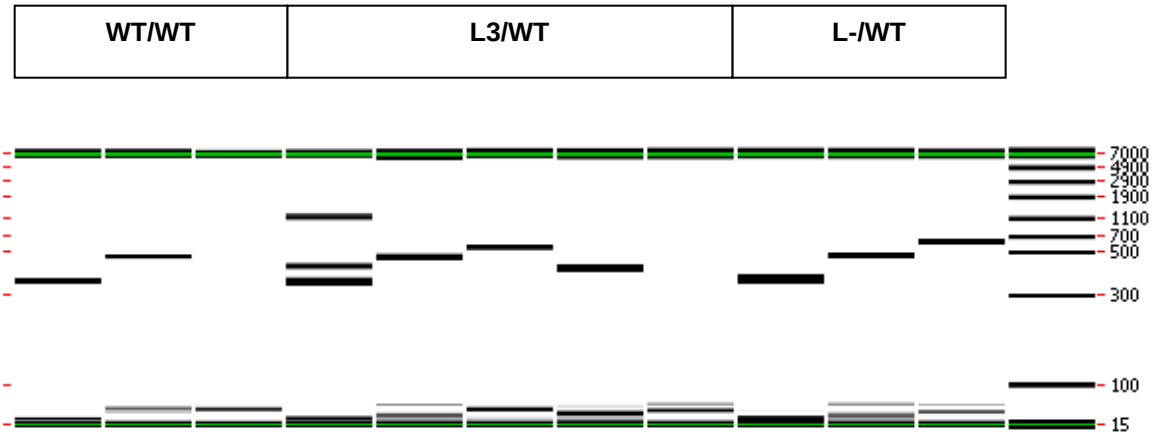
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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.