



Ccp2 / Agbl2 (IR00002129 / K488 ICS internal reference) mouse line genotyping protocol

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For any question, please contact:

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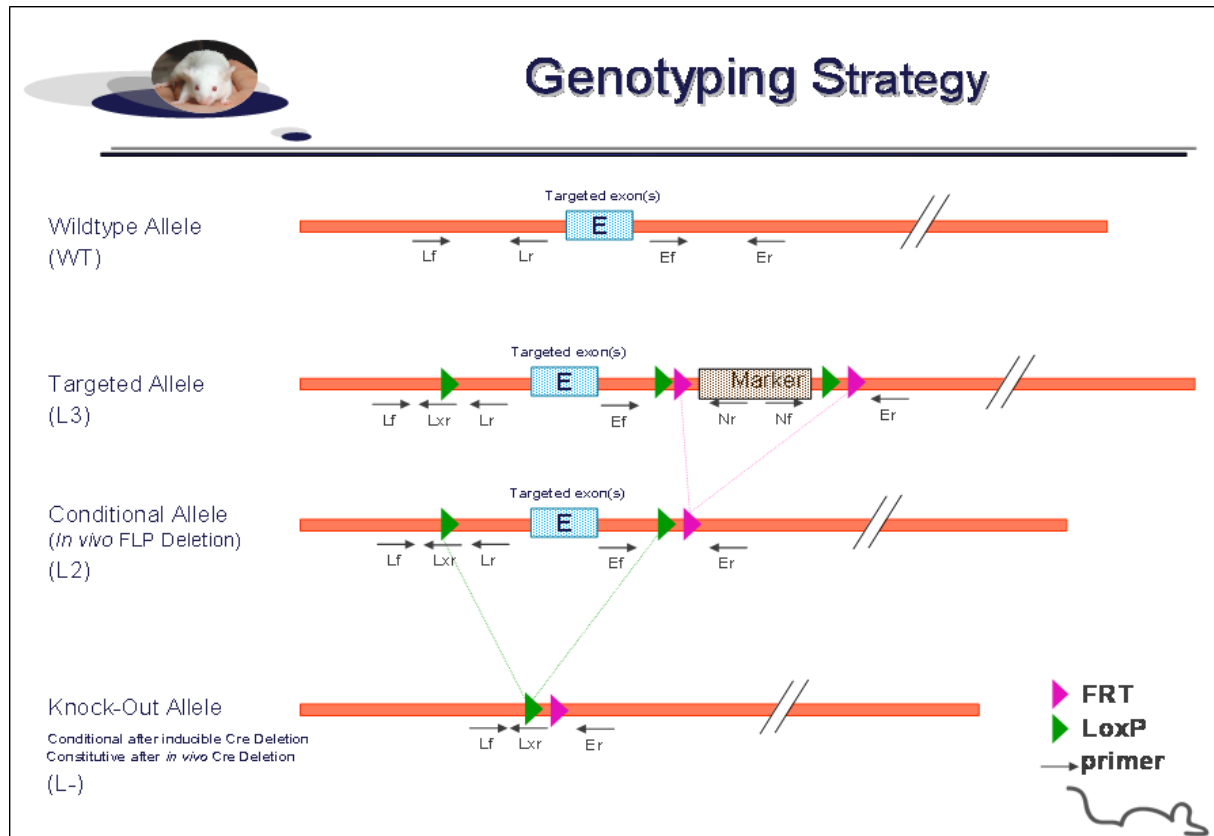
This protocol has been validated by Christelle Roth.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **CCP2** 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	4270	CTTAGCAACTCTCCCGATGC
Er	4271	TGTGTGTGAAATGGCTGACA
Lf	4268	CCACGAGCGACCTTCCAAACCTACC
Lr	4269	AGCTGCCTGCTACAGCAAACGGG
Nf	1219	CAGCTCATTCTCCCACTCATGATC
Nr	265	TGCTAAAGCGCATGCTCCAGACTGC



Genotyping protocol

CCP2 (IR00002129 / K488 ICS internal reference)

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	4268-4269	Lf / Lr	488	488	---	392
Excision of the selection marker	4270-4271	Ef / Er	2411*	518	---	408
5' part of the selection marker	4270-265	Ef / Nr	469	---	---	---
3' part of the selection marker	1219-4271	Nf / Er	408	---	---	---
Excision of the floxed exon(s), i.e. knock out	4268-4271	Lf / Er	3600*	1707*	480**	1501*

* 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:

-10x Buffer (Roche)
 -dNTPs 10mM (Amersham Biosciences)
 -Taq DNA Polymerase (Roche)
 -DNA (50ng/μl)
 -5' primer (100 μM)
 -3' primer (100 μM)
 -Sterile H2O

Volume:

2.5μl
 0.5μl
 0.2μl
 3μl
 0.125μl
 0.125μl
 up to 25 μl

Cycling conditions:

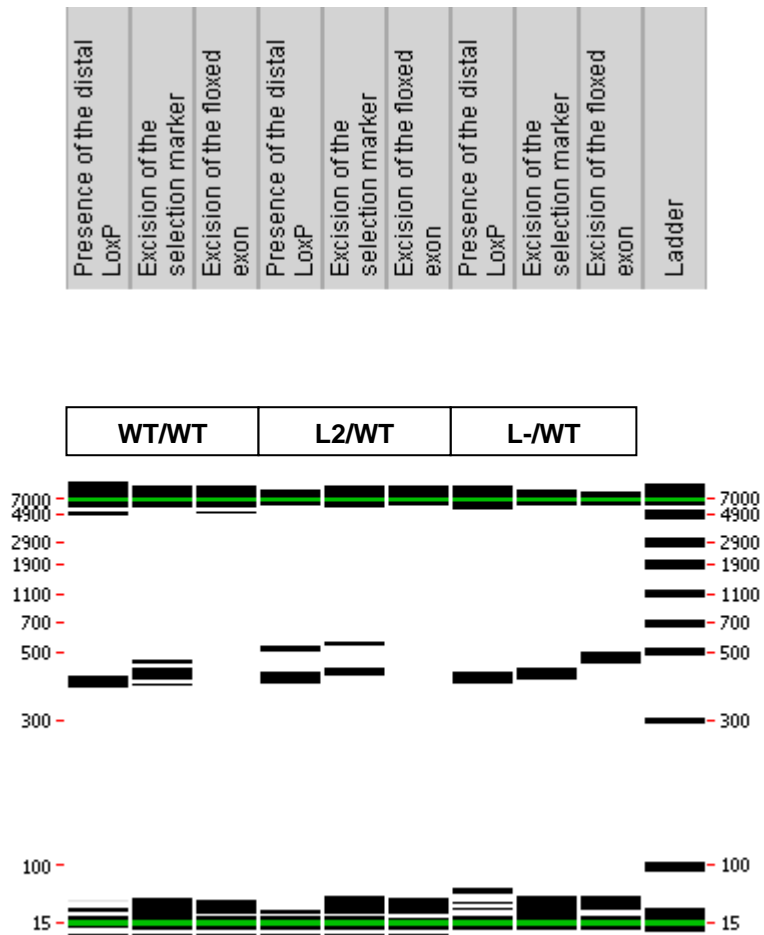
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	2
62°C	1min	
72°C	1min	
94°C	30s	30
62°C	30s	
72°C	30s	
72°C	3min	1
4°C	∞	

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.