Conditional overexpression of cDNA of a mutated CPT1A (IR00002684 / K566 ICS internal reference) mouse line genotyping protocol

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This protocol has been validated by Christelle Roth.

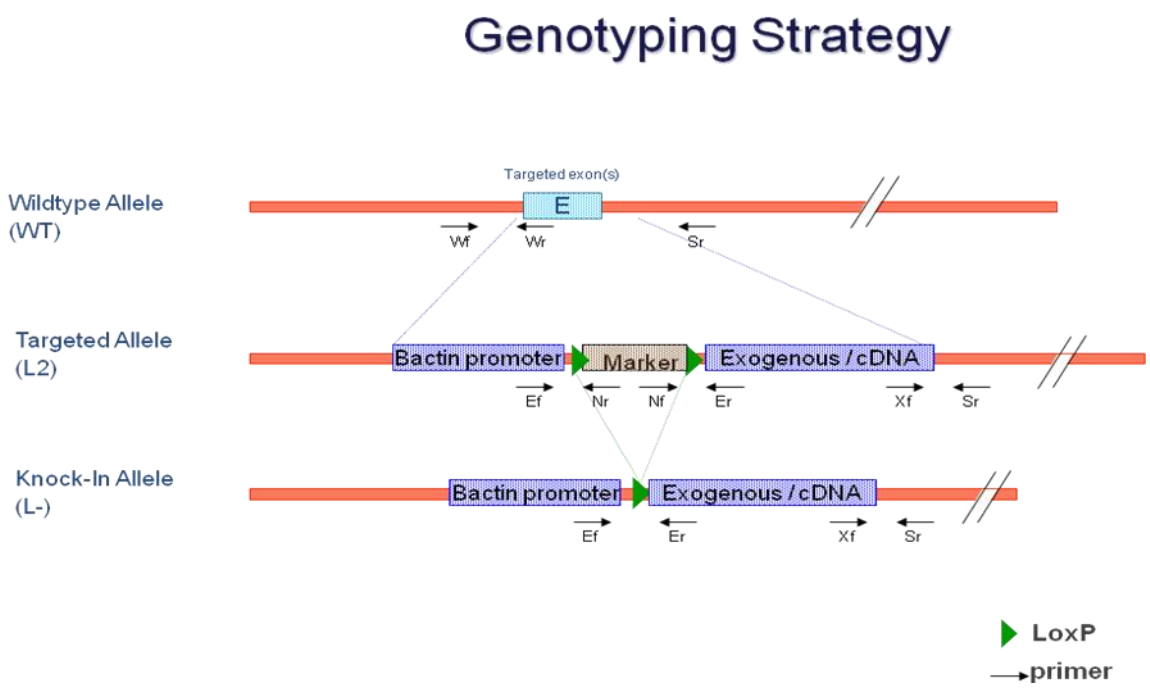
Genotyping protocol
cDNA of a mutated CPT1A - Gt(ROSA)26Sor $^{tm3(CAG-Cpt1a*)Ics}$/Ics
(IR00002684 / K566 ICS internal reference)

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **cDNA of a mutated CPT1A** Knockin (KI) 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
Nf	3300	GTGGTTTGTCCAAACTCATCA
Ef	5696	GCCTTCTTCTTTTTCCTACAGCTC
Wr	3003	CCCACACACCAGGTTAGCCTTTAAG
Er	5697	GGGGTGACGGTGAACTGGAAG
Er	5698	CCAGTGATGATGCCATTCTTGAACC
Nr	265	TGCTAAAGCGCATGCTCCAGACTGC
Sr	5695	GAAAATCTGTGGGAAGTCTTGTCCCTC
Wf	5693	GGAAGCACTTGCTCTCCCAAAGTCG
Xf	5694	CCTTCCTTGACCCTGGAAGGTG

PCR fragments expected size (bp):

Region analyzed	**Primers used**	Position on the primer (see the map above)	Targeted allele (L2)	KI allele (L-)	WildType allele (WT)
WildType allele specific PCR (5' part of the targeted locus)	5693-3003	Wf / Wr	---	---	274
Excision of the selection marker	5696-5697	Ef / Er	2865*	215**	---
5' part of the selection marker	5696-265	Ef / Nr	169	---	---
3' part of the selection marker	3300-5698	Nf / Er	324	---	---
Exogenous/cDNA specific PCR	5694-5695	Xf / Sr	307	307	---

* 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:

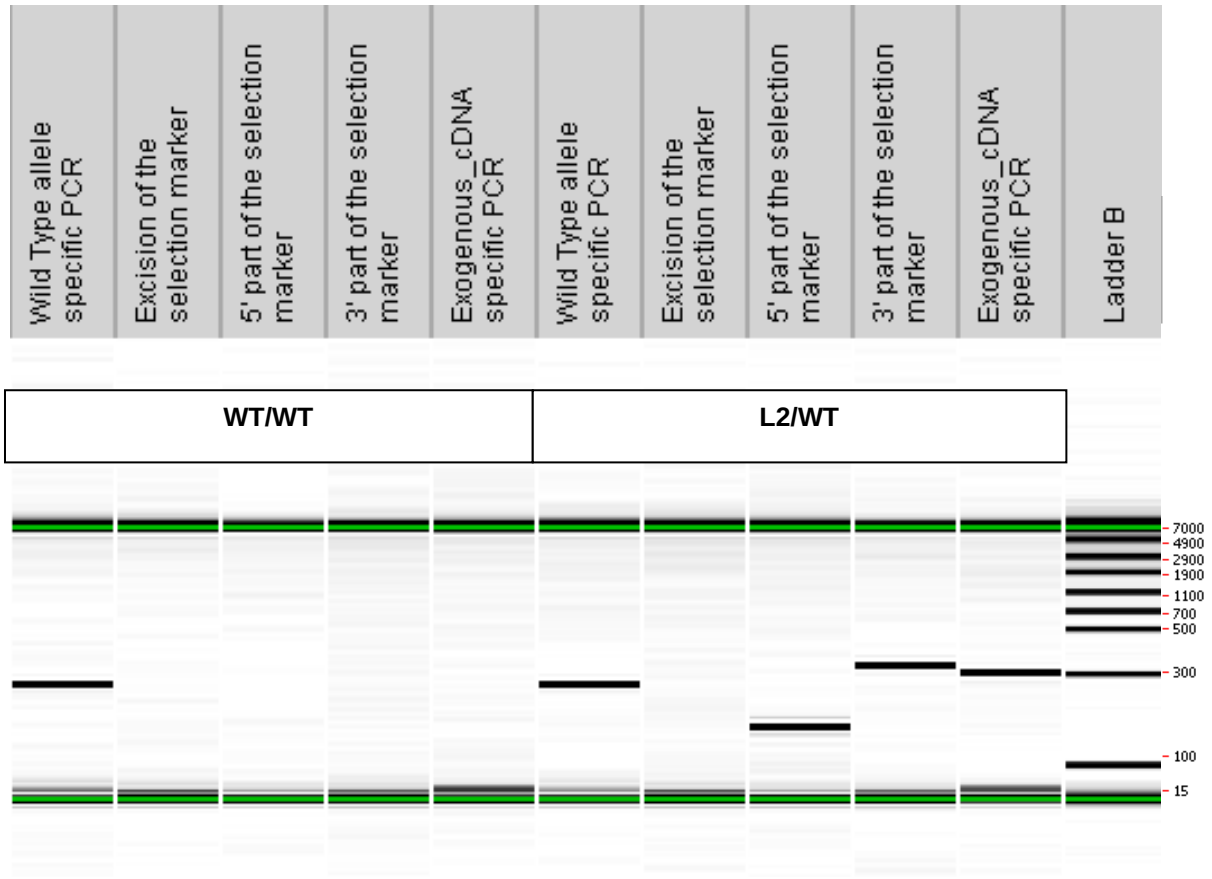
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

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.