Tnfrsf1b (IR00002580 / E67 ICS internal reference) mouse line genotyping protocol

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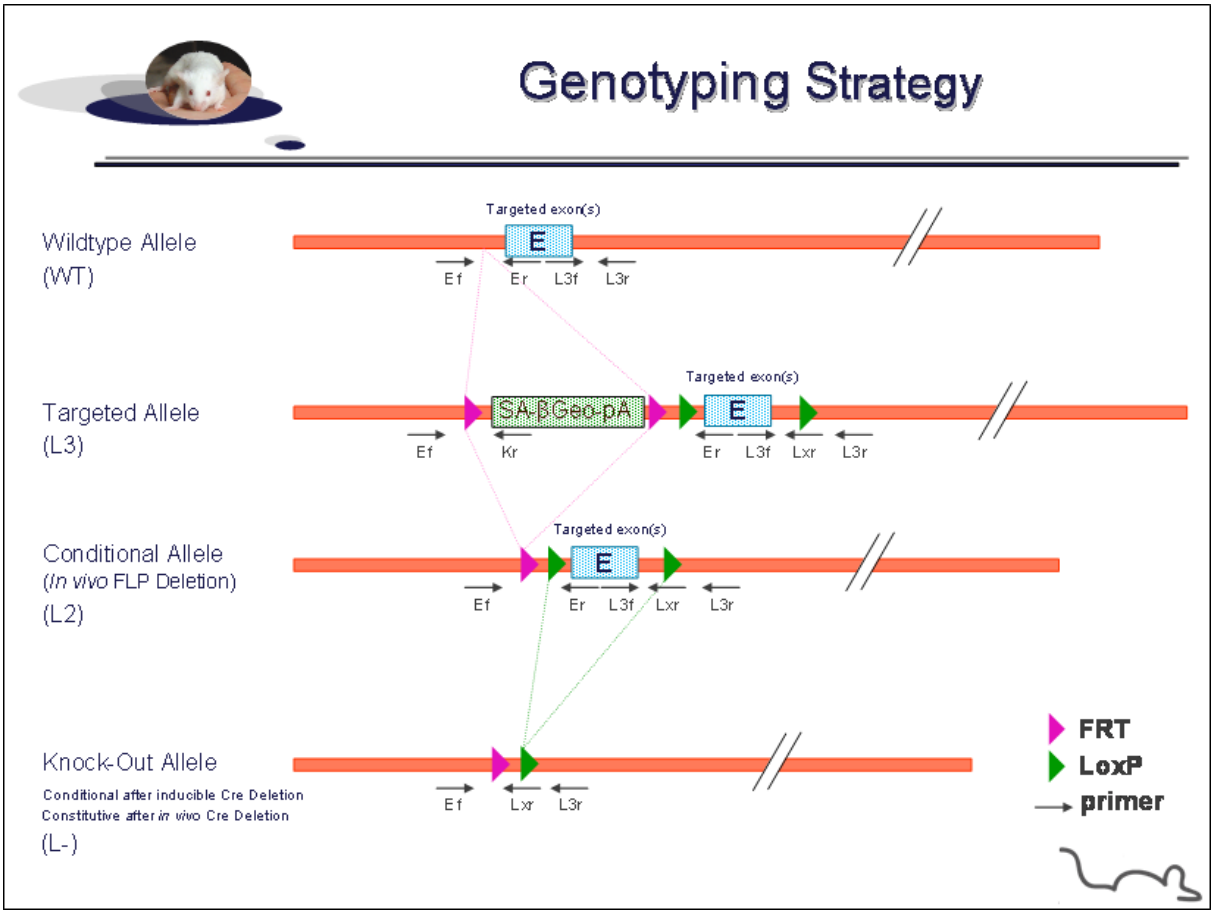
This protocol has been validated by Christelle Roth.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Tnfrsf1b** Constitutive Knockout / Conditional Knockout (KO-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	4710	TTGGGTCTAGAGGTGGCGCAGC
Ef	4712	TGTGAGTGCAAGGACACACGGTGC
Er	4716	GGCCAGGAAGTGGGTTACTTTAGGGC
Kr	3210	CCTGTCCCTCTCACCTTCTACC
L3f	4714	GGCTGCCTCCCTTGTTCCCTTCC
L3f	4715	GAGGCAGCTGCTGGGGCTTTGG
L3r	4711	GGGGCCAGCTACTCAGTCCTCG
Lxr	4713	GAAGTTATCATTAATTGCGTTGC

PCR fragments expected size (bp):

Region analyzed	**Primers used**	Position on the primer (see the map above)	Targeted allele (KO allele) (L3)	cKO allele (L2)	KO allele (L-)	WildType allele (WT)
5' part of the selection marker	4710-3210	Ef / Kr	469	---	---	---
Presence of the distal loxP	4715-4711	L3f / L3r	481	481	---	448
Distal loxP specific PCR	4714-4713	L3f / Lxr	368	368	---	---
Excision of the selection marker	4712-4716	Ef / Er	7406*	483	---	323
Excision of the floxed exon(s), i.e. knock out	4710-4711	Ef / L3r	8263*	1359*	547**	1147**

* 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:

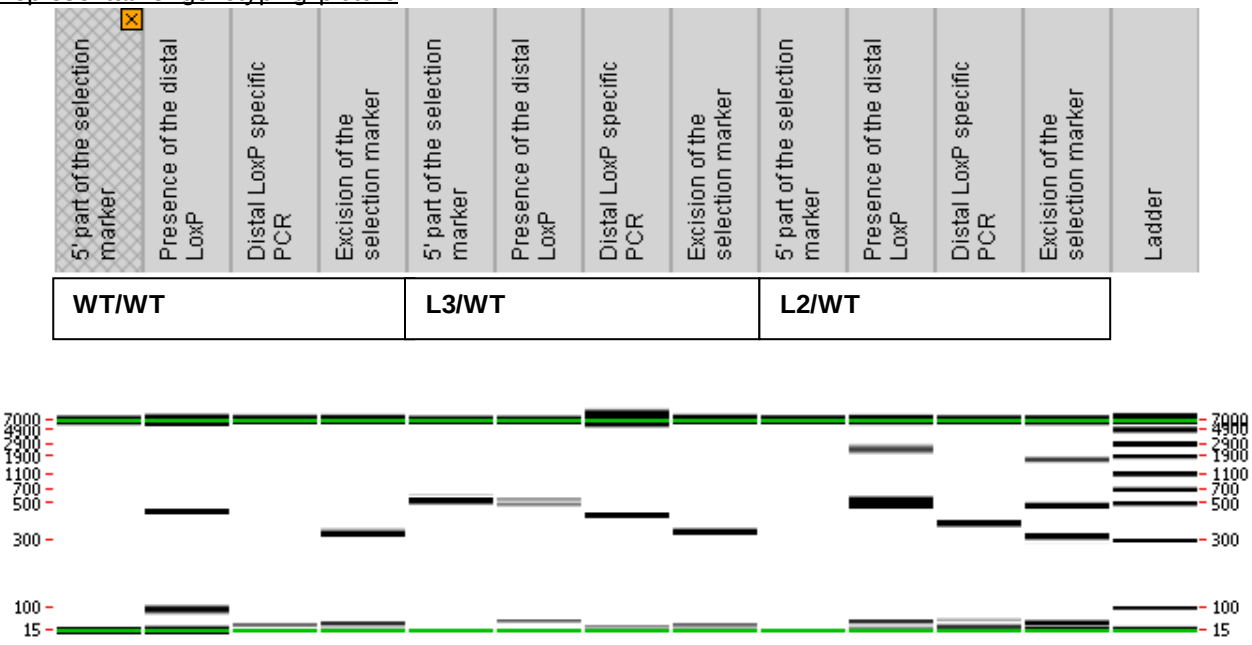
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

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



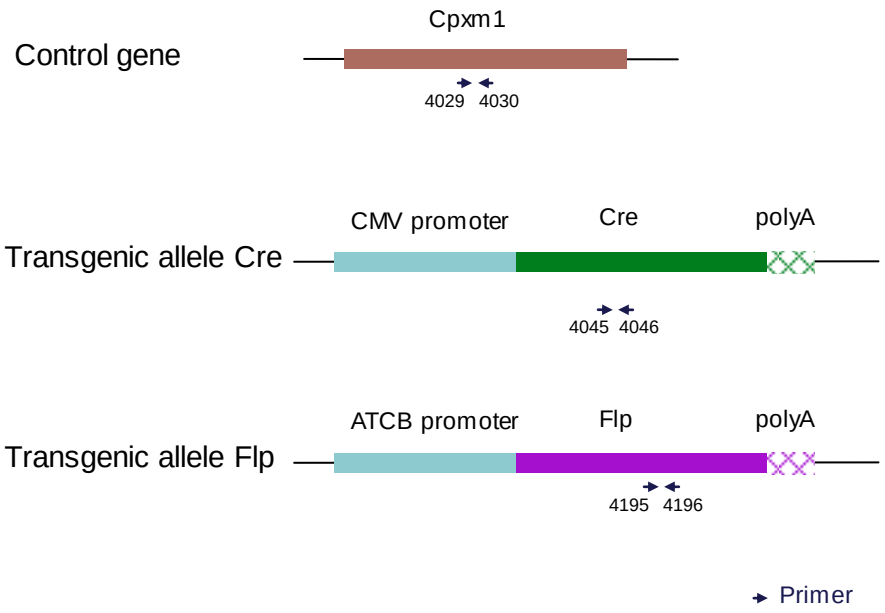
An aspecific band is detected on PCR "Presence of the distal LoxP" and "Excision of the selection marker" for the L2/WT animal.
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

2. Cre and Flp genotyping method

The protocol used to segregate the cre and/or flp transgene is indicated below.
Detection of cre transgene and flp transgene is done using a multiplex assay: primer pairs were designed for each gene and for a positive control (Cpxm1 gene).

2.1. Cre and Flp genotyping

Schematic representation of the genotyping strategy



Sequence of primers used for genotyping:

Primers	Sequence
4029	ACTGGGATCTTCGAACTCTTTGGAC
4030	GATGTTGGGGCACTGCTCATTCACC
4045	CCATCTGCCACCAGCCAG
4046	TCGCCATCTTCCAGCAGG
4195	TCTTTAGCGCAAGGGGTAGGATCG
4196	GTCCTGGCCACGGCAGAAGC

PCR fragments expected size (bp):

Primer pair	4045-4046	4195-4196	4029-4030
Region analyzed	Middle part of Cre transgene	Middle part of Flp transgene	Cpxm1 control gene
Control gene	/	/	446
Tg allele	281	328	/

2.2. 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)	0.05µl
- 3' primer (100 µM)	0.05µl
- Sterile H_2O	up to 15 µl

Cycling conditions are identical to those described in chapter 1.2

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.