Genotyping protocol

tspan8

IR00003450 / K656

(ICS internal reference)

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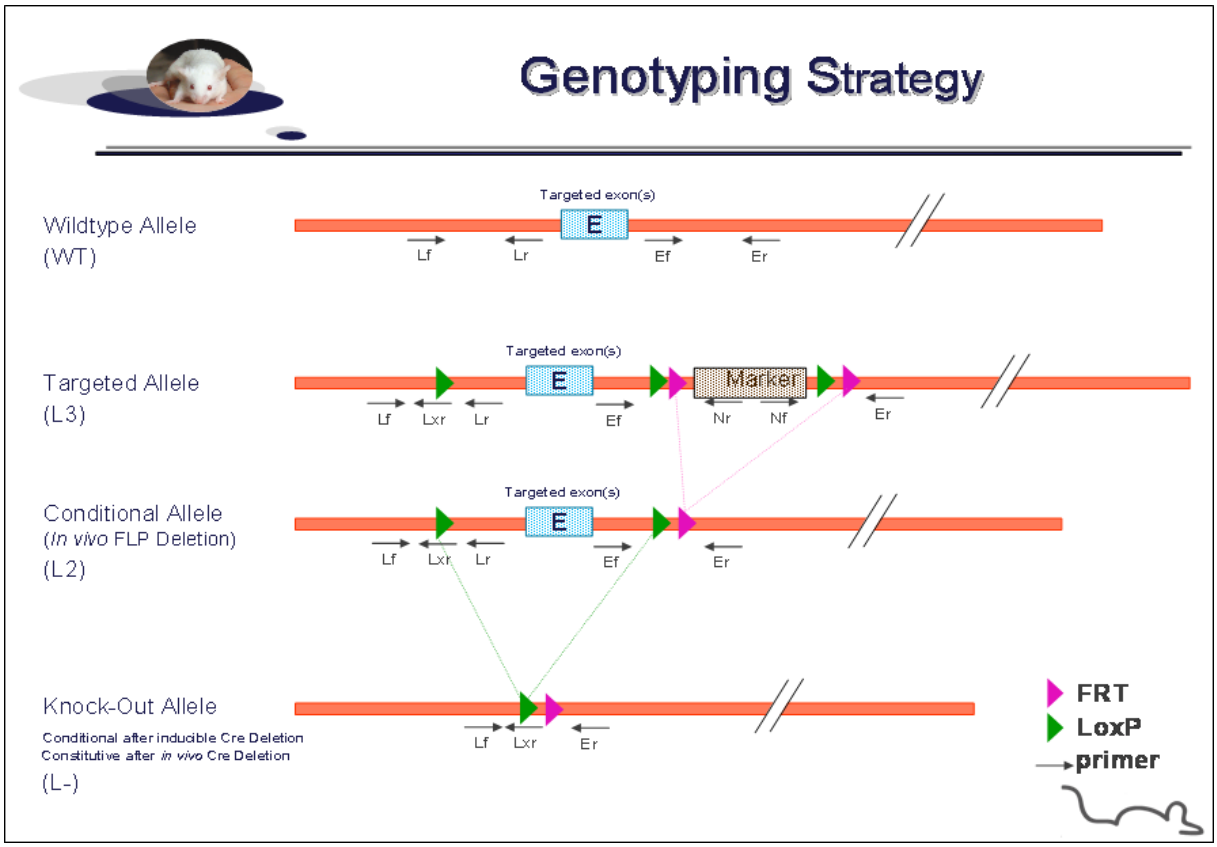
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **tspan8** 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	6057	GCCAATGACTCCAAAAAACCAAACC
Ef2	6055	GGCTGTTTGGGGAACTGAAAAGAAGC
Er	6058	CTAGATGTCAAAGGGAGGGAACCAGAAG
Er2	6056	CCACTAGTCAGTCACCTGTTTCAAACC
Lf	6053	CTGAGGAGATGAGGGCTCAGACAG
Lf2	6052	CAGCATCTAAGTCAAAGACAGGAGAGG
Lr	6054	GGTGAGAGCTAGGATTAGTTCGCC
Lxr	4724	CGAAGTTATCTGCAGGTCGACCTTAAG
Nf	1219	CAGCTCATTCCTCCCACTCATGATC
Nr	238	TGACTAGGGGAGGAGTAGAAGGTG

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	6053-6054	Lf / Lr	270	270	---	194
Excision of the selection marker	6057-6058	Ef / Er	2219*	365	---	262
5' part of the selection marker	6055-238	Ef2 / Nr	345	---	---	---
3' part of the selection marker	1219-6056	Nf / Er2	520	---	---	---
LoxP specific PCR	6052-4724	Lf2 / Lxr	93	93	93	---
Excision of the floxed exon(s), i.e. knock out	6053-6056	Lf / Er2	4174*	2320*	382	2141*

*: 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:
- 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:

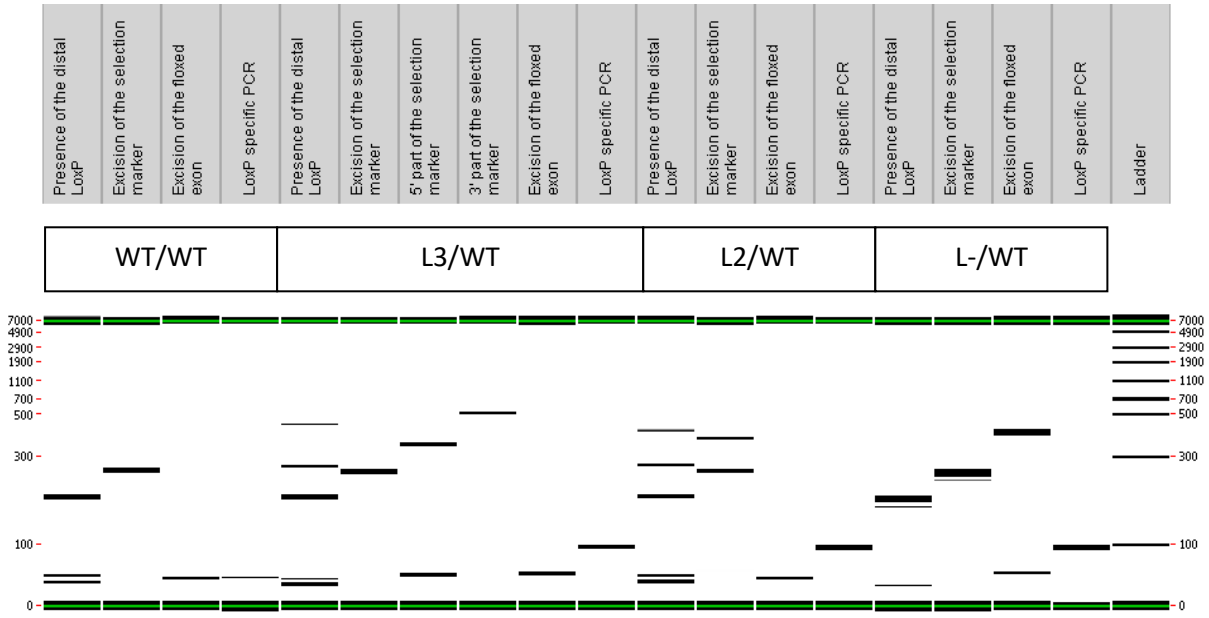
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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



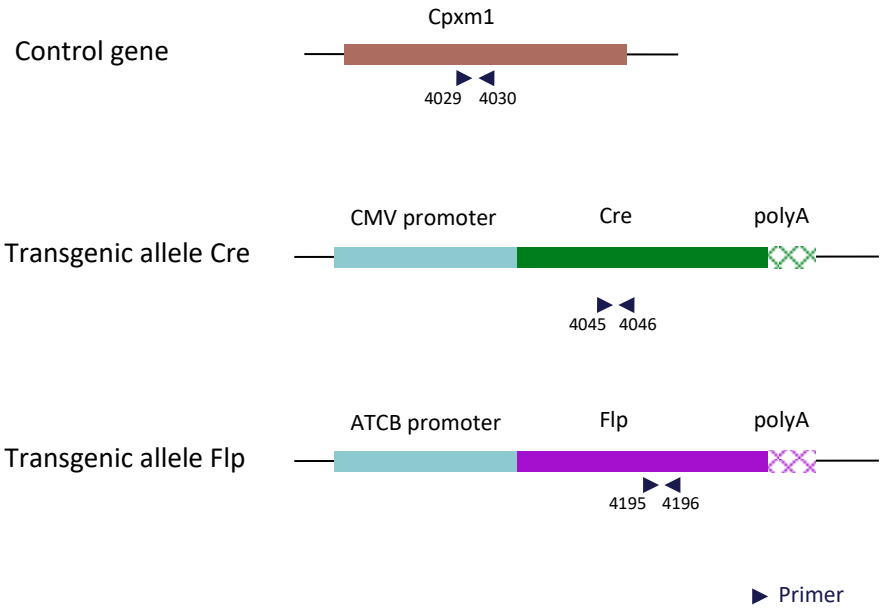
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

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	/	/	397
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

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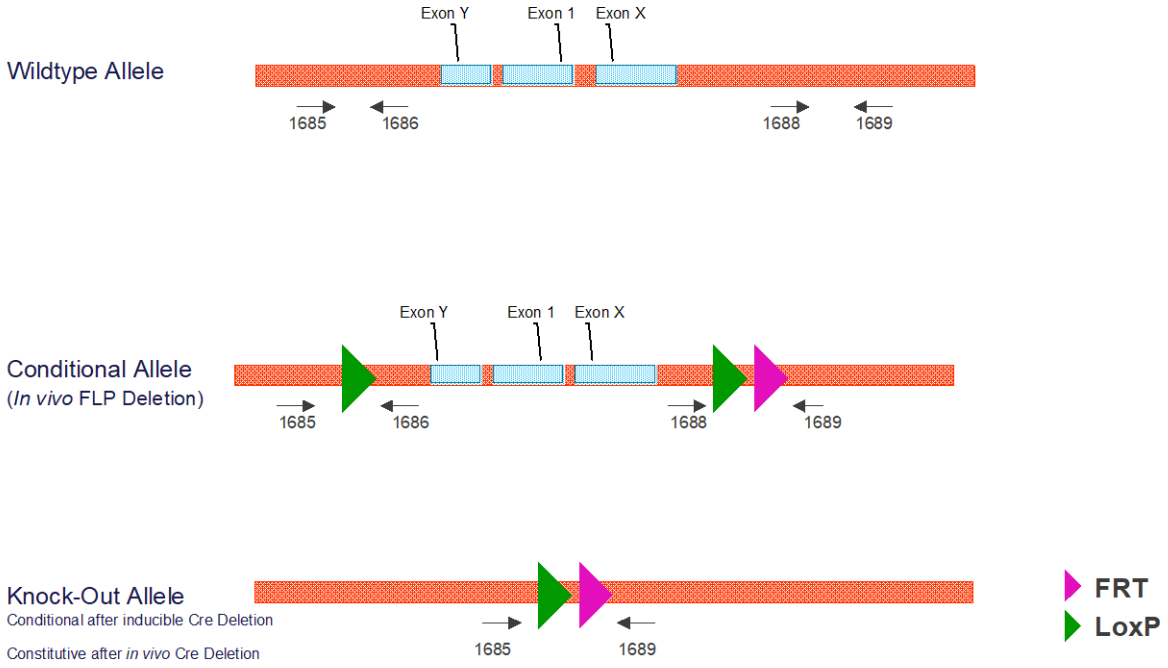
SL21(IR554/ICS internal reference K256) mouse line genotyping protocol

This protocol describes the strategy and the PCR conditions used for SL21 conditional knock out (cKO) line genotyping.

Genotyping Strategy



Primers sequence:

Primers	Sequence 5'-3'
1685	CAGTCCTCCTAGGTGCAGACTG
1686	GTTCCATTGCAGGGCTATAATCCAGC
1688	CATGGATTCCAGGGATCCATCTCTC
1689	GGGGCTTATGCCTGTGGCTATATG

PCR to be done (please see the scheme above):

- Characterisation of conditional allele animals: PCR 1685/1686; PCR 1688/1689

- Characterisation of conditional allele /Knock-out allele animals(after breeding with a Cre deleter mice[1]): PCR 1685/1686; PCR 1688/1689; PCR 1685/1689

- Characterisation of Knock-out allele animals: PCR 1685/1686; PCR 1688/1689; PCR 1685/1689

[1] Genotype Mosaïcism may be observed, ie conditional/Knock-out allele genotype in the same animal from breeding with a Cre deleter mice.

PCR fragments expected size:

Primer pair	1685/1686	1688/1689	1685/1689
WT allele	183	171	2014*
Conditional Allele	233	302	2195*
Knock-Out Allele	N/A	N/A	223

N/A: not applicable
*: no amplification products under standard PCR condition (see description below)

PCR protocol:

Reagents:	Volume:
-10x Thermopol Reaction Buffer (Biolabs)	2.5µl
-dNTPs 10mM (Amersham Biosciences)	0.5µl
-Taq DNA Polymerase (Sigma can be used)	0.2µl
-DNA (50ng/µl)	2µl
-Primer 1 (100 µM)	0.25µl
-Primer 2 (100 µM)	0.25µl
-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 optimised for high-throughput genotyping. Adaptation to small-scale may be required.

Director : Johan Auwerx