Genotyping protocol

Gmfg

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(ICS internal reference)

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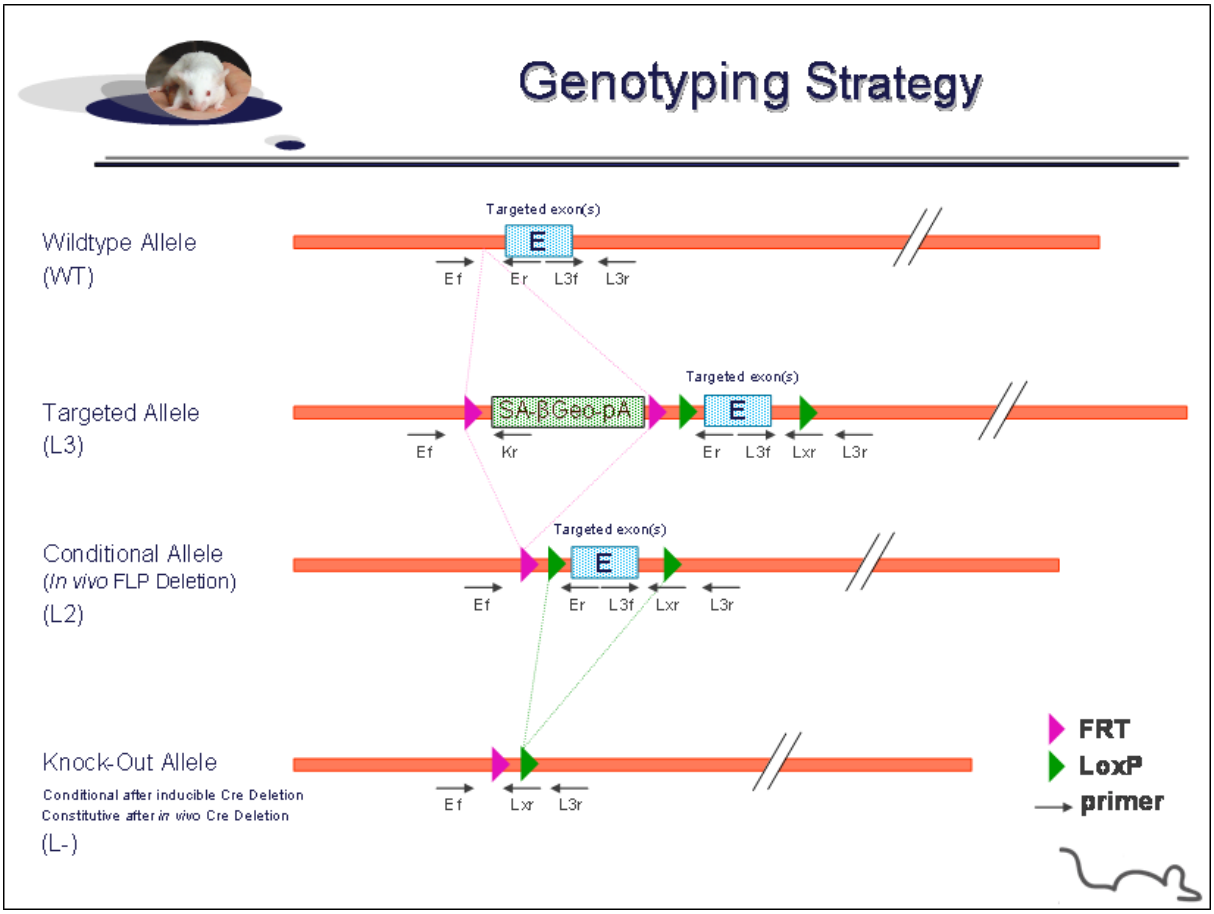
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Mat2a** 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.



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Sequence of primers used for genotyping

Position	Primers	Sequence
Ef	5421	TGGCTTAGAGCACACTTGAAAGTTTCC
Ef[2]	5423	AATTGGATATGGCTTAGAGC
Er	5422	CACCTGCCTCTGCCCTTCAGATTAA
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	5424	ATTTGTTATTGGTGGGCCTCAGGTAA
L3f[2]	5425	AGATTTGTTATTGGTGGGCCTCAGG
L3r	5426	ACCTGTGTTTTTTCCTGCCTCCTAAGT
Lxr	3254	TTATCATTAATTGCGTTGCGCCATC

[2]. For a selected position a second primer was designed

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	5421-3209	Ef / Kr	419	---	---	---
Presence of the distal loxP	5425-5426	L3f[2] / L3r	389	389	---	346
Distal loxP specific PCR	5424-3254	L3f / Lxr	165	165	---	---
Excision of the selection marker	5421-5422	Ef / Er	7418*	514**	---	391
Excision of the floxed exon(s), i.e. knock out	5423-5426	Ef[2] / L3r	9694*	2790*	555**	2624**

* 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

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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:

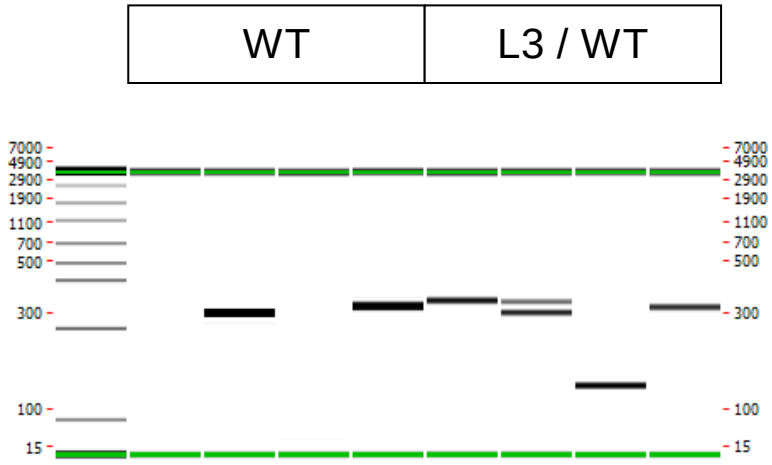
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

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



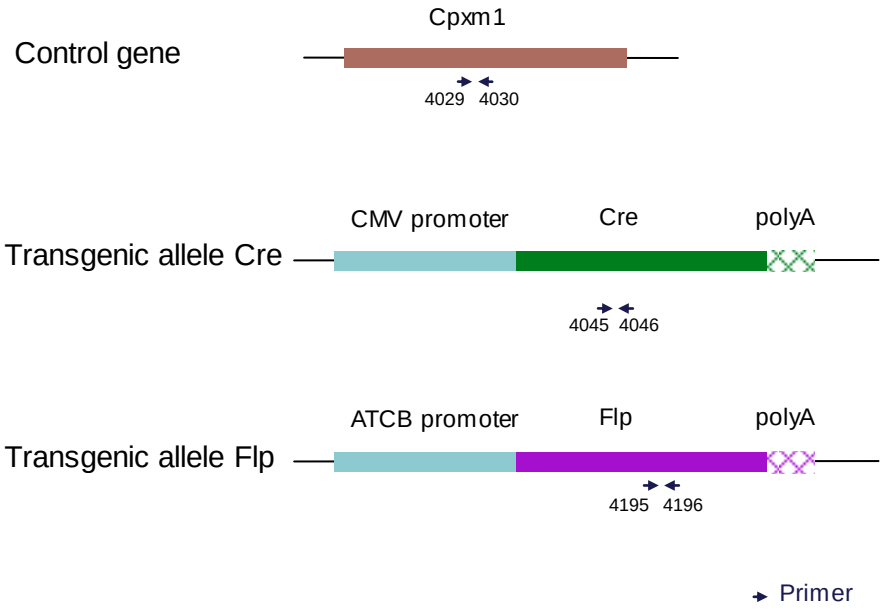
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

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.