Genotyping protocol

Mmp9

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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 **Mmp9** 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.

imgstrat

Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	6806	GTGGATAGCTGAGAAGGGCTG
Er	6554	ACATCTCTTGGGCACTGAGCGC
Er2	1936	GTGGATGTGGAATGTGTGCGAGG
Er3	6809	CTGCGCCATGTCACAACCCTT
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	6807	GATGTGGCTCAGCTGTAGGAAGAG
L3r	6808	GCTGACCCTTCTTGCTGAGGAG
Lxf	6295	TTATGTTTAAACGGCGCGCCC
Lxf2	6013	TCATGTCTGGATCCGGAATAACTTCGTA
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Ri1f	5966	GCACATGGCTGAATATCGACGGT

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)
Lox interne K7 Eur (with DMSO)	6295-6554 (with 5% DMSO)	Lxf / Er	119	---	---	---
Lox interne K7 Eur (with DMSO)	6013-1936 (with 5% DMSO)	Lxf2 / Er2	199	---	---	---
5' part of the selection marker	6806-3209	Ef / Kr	332	---	---	---
Presence of the distal loxP	6807-6808	L3f / L3r	254	254	---	220
Distal loxP specific PCR	6807-3255	L3f / Lxr	164	164	---	---
Excision of the selection marker	6806-6809	Ef / Er3	7282*	378	---	205
Cre total excision	5966-3255	Ri1f / Lxr	---*	---	---	---

*: 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	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

_ADD_THE_PHOTO_

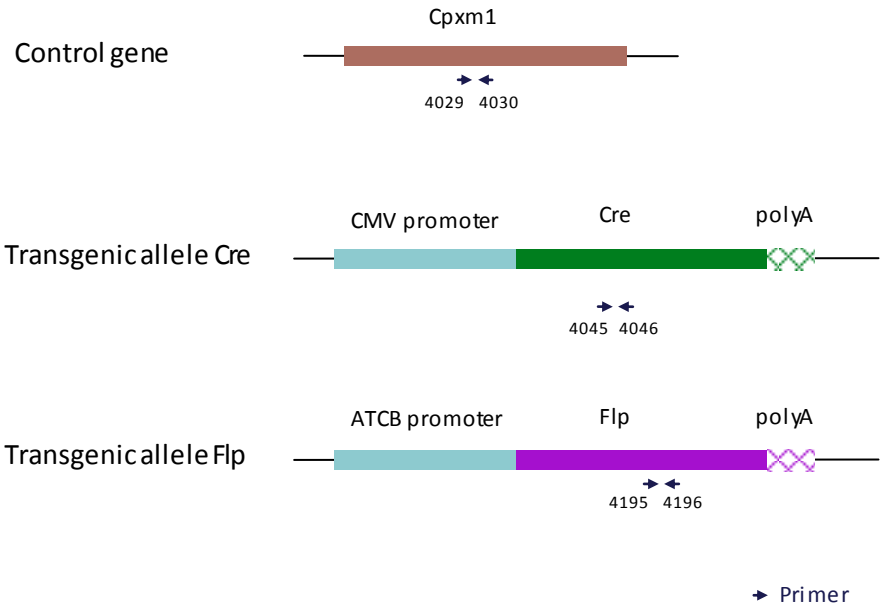
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

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