Calpastatin (IM00003130 / U129 ICS internal reference) mouse line genotyping protocol

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For any question, please contact:

Mouse Clinical Institute – Institut Clinique de la Souris (ICS)
ICS genotyping service
1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex France
Email: genotyping@igbmc.u-strasbg.fr

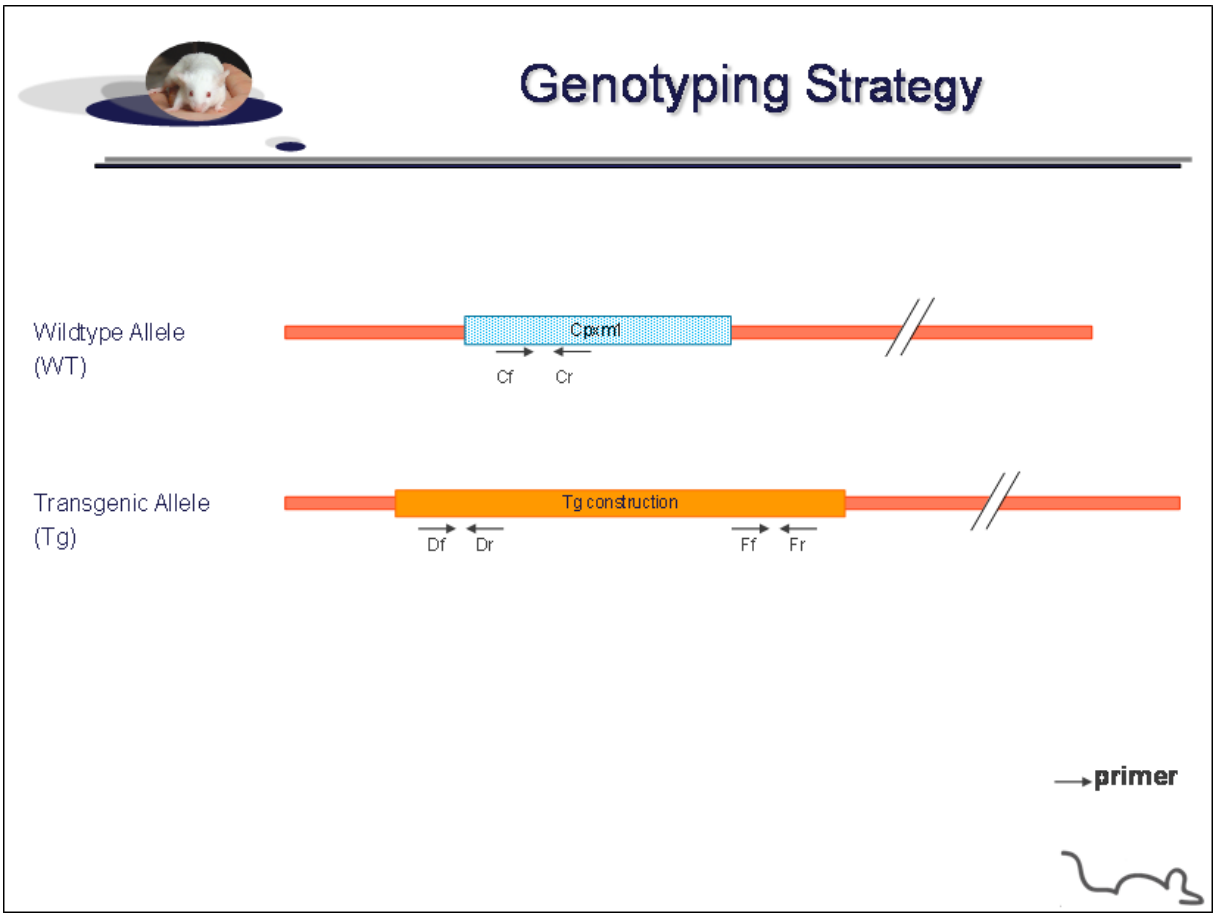
This protocol has been validated by Valérie Rousseau.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Calpastatin** Transgenic model, plasmid construct (Tg-P) 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
Df	5933	CAGCCGAATACATCTTTTCCAAAGGAG
Dr	5934	CACAGGTTGCTCACTTGATTGTGGTT
Ff	5935	TTGGATGAACTTTCTGACAGTCTTGGAC
Fr	5936	TCTTCTTGCTCTTCCCCTTTGCTG
Cf	4029	ACTGGGATCTTCGAACTCTTTGGAC
Cr	4030	GATGTTGGGGCACTGCTCATTCACC

PCR fragments expected size (bp):

Region analyzed	**Primers used**	Position on the primer (see the map above)	Transgenic gene detected (Tg)	No transgenic gene detected (WT)
5' part of the transgenic sequence	5933-5934	Df / Dr	397	---
3' part of the transgenic sequence	5935-5936	Ff / Fr	316	---
Control Assay	4029-4030	Cf/Cr	420	420

--- 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:

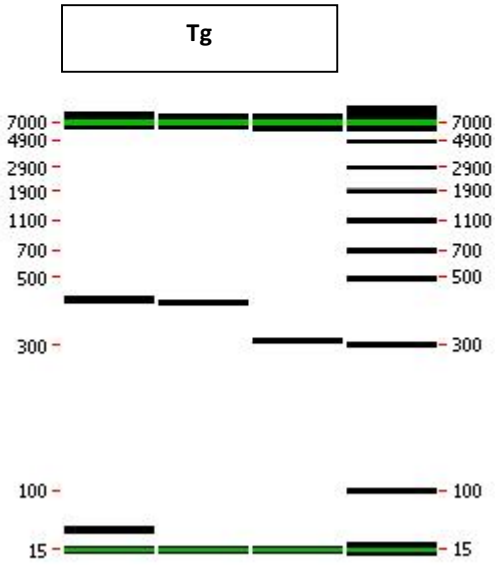
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

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.