



Genotyping protocol

Y1698C in Fbn1

Fbn1^{tm1.1lcs}

IR00003809 / K699

(ICS internal reference)

For any question, please contact:

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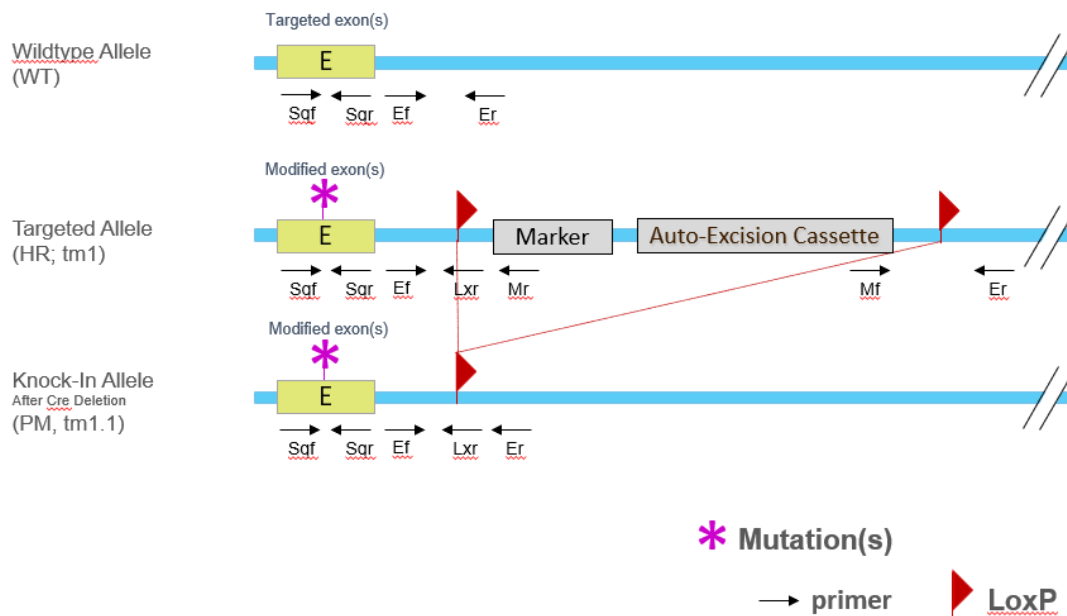
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Fbn1^{tm1.1ics}** mouse line (MGI allele ID 8280978).

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.

PM Genotyping strategy PCR



Sequence of primers used for genotyping:

Position	Sequence
Ef	GAGAAAACCTTGACCTACCAAGGTGAG
Er	GAACACAGAGCCTTCTCCCTGGC
Er2	CCAGTGTTTTAATTATGTGGTGGG
Lxr	AGTTATACTAGAGCGGCCGTTACCG
Sqf	CTTTGAGTCTCCTATTGTGCTCTGCTG
Mf	GATAGTGAAACAGGGGCAATGGTGC
Mr	TGCTAAAGCGCATGCTCCAGACTGC
Sqr	CTGCCGATGTTGTAGGAGCAACAG

PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	Targeted allele (L2)	PM allele (L-)	WildType allele (WT)
WildType / Mutated alleles	Sqf / Sqr	195	195	195
Excision of the selection marker	Ef / Er	4540*	385	290
5' part of the selection marker	Ef / Mr	300	---	---
3' part of the selection marker	Mf / Er2	591	---	---
LoxP specific PCR	Ef / Lxr	206	206	---

*: 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 ₂ O	up to 15 µl

Cycling conditions:

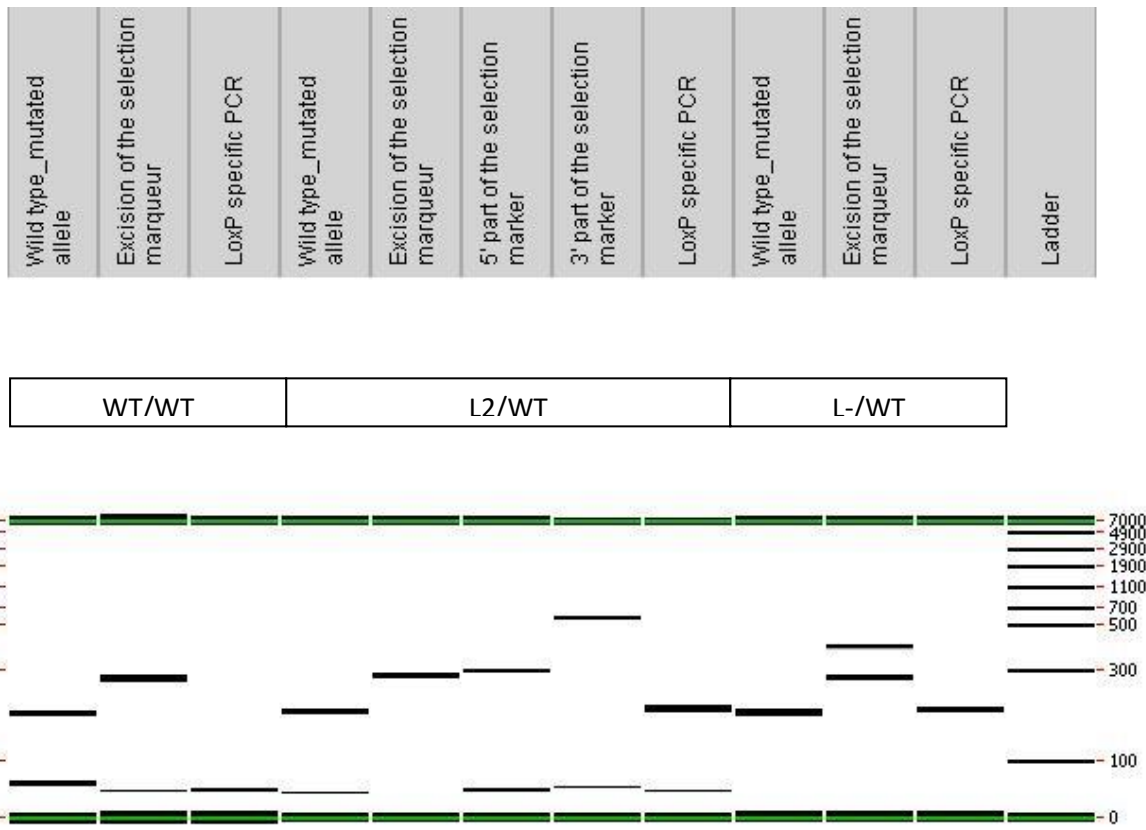
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5min	1

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.