EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Synonymous S293S PM in Shh

Shh$^{em1.1Ics}$

(PHENOMIN-ICS reference IR00007498 / Kos7498)

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Synonymous S293S PM in Shh - Shh$^{em1.1Ics}$

This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype the Synonymous S293S point mutation in Shh, Shhem1.1Ics.

1. PCR Genotyping protocol

1.1. Genotyping strategy

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

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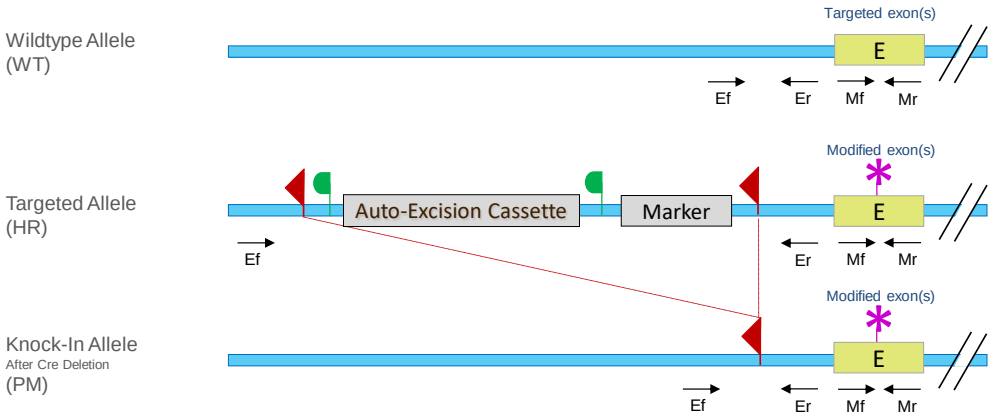
PM Genotyping strategy



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Sequence of primers used for genotyping:

Position	Sequence
Ef	GGAGAAAAGCTGGGAGTGTCC
Ef^{2}	GCGGAGCAATCTTGGAGGG
Er	GGGGATAAGGGTGGGAGTAGATC
Er^{2}	CTTTGGGGTGGAGAGACGGG
Mf	CGCCAAGAAGGTCTTCTACGTG
Mr	CGATGACAGCGTAGCACGAG

^{2}: for a selected position, a second primer was designed

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	PM allele	WildType allele
WildType / Mutated alleles (**with DMSO**)	Mf / Mr	317	317	317
Excision selection Marker	Ef / Er	4421*	266	187
Excision selection Marker (2)	Ef^{2} / Er^{2}	4379*	224	145

*: amplicon will not be observed using our genotyping conditions

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

If mentioned in table "PCR fragments expected size" add 5% of DMSO in the reaction mix

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	35
72°C	1min	
72°C	7min	1
14°C	---	---

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

2. Recommended papers:

2.1. Cre and Flp genotyping method

Highly-efficient, fluorescent, locus directed cre and FlpO deleter mice on a pure C57BL/6N genetic background.
Birling MC, Dierich A, Jacquot S, Hérault Y, Pavlovic G.
Genesis. 2012 Jun;50(6):482-9. doi: 10.1002/dvg.20826. Epub 2012 Mar 20.

2.1. Tips and tricks for optimizing your PCR genotyping procedures

Optimizing PCR for mouse genotyping: Recommendations for reliable, rapid, cost effective, robust and adaptable to high-throughput genotyping protocol for any type of mutation.
Jacquot, S, Chartoire, N, Piguet, F, Herault, Y, Pavlovic, G. (2019).
Current Protocols in Mouse Biology, 9, e65. doi: 10.1002/cpmo.65
Free copy of this paper can be accessed online though this link http://bit.ly/2sxxWvO