

Genotyping protocol

EMMA ID: EM:07460

Gene symbol: Hspg2

Strain designation: B6;129P2-Hspg2^{tm1Soin}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
Perl-intron 2	GTA GGG ACA CTT GTC ATC CT
Perl-WT 2	GTG CCA AGG CCA TCT GCA AG
MSRneo	AGG AGT AGA AGG TGG CGC GAA GG

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	Perl-intron 2	Perl-WT 2	300
Mutant	Perl-intron 2	MSRneo	450

Reaction mix:

Wild-type Component	Volume (µl)	Mutant Component	Volume (µl)
Genomic DNA (~50-100 ng)	2.0	Genomic DNA (~50-100 ng)	4.0
Primer 1 (100 ng/µl)	1.0	Primer 1 (100 ng/µl)	1.0
Primer 2 (100 ng/µl)	1.0	Primer 2 (100 ng/µl)	1.0
10x Buffer (15 mM MgCl ₂)	3.0	10x Buffer (15 mM MgCl ₂)	3.0
dNTPs (25 mM)	0.25	dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.05	DNA polymerase (5 U/µl)	0.05
H ₂ O	22.7	H ₂ O	20.7
Total	30.0	Total	30.0

PCR program:

Time	Temperature (+ °C)	Cycles
5 min	95	
1 min	94	30
1 min	57	
1 min	72	
7 min	72	
∞	4	