

Genotyping protocol

EMMA ID: EM:08332

Gene symbol: P4ha2

Strain designation: B6.Cg-P4ha2^{tm1Jomy}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
mall132F	CTG CCC TTT CCA GTT ATG AAG CTC CAG
mallint2BR	AGG GCA TTG GTT TTC TAA GGG CGC
mallint10F	TGA GCC ATT CCG AGA TTT GGT TTA
LacZ 5'961R	ACC CTG CCA TAA AGA AAC TGT

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	mall132F	mallint2BR	600
Mutant	mallint10F	LacZ 5'961R	2000

Reaction mix:

Component	Volume (µl)
Genomic DNA (~50-100 ng)	1.0
Primer 1 (10 µM)	4.0
Primer 2 (10 µM)	4.0
10x Buffer (15 mM MgCl ₂)	3.0
MgCl ₂ (25 mM)	1.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.1
H ₂ O	16.65
Total	30.0

PCR program:

Time	Temperature (+ °C)	Cycles
5 min	95	
1 min	94	30
1 min	60	
3 min	72	
5 min	72	
∞	10	