

Genotyping protocol

EMMA ID: EM:08331

Gene symbol: P4ha1

Strain designation: B6;129-P4ha1^{tm1Jomy}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
malintAGF	GCA TAG AAC ACA GAA GTA AGA GAA A
Ex2R	GCT AAA GAC TGA GGA AGT AGA ATC
LacZ 5'961R	ACC CTG CCA TAA AGA AAC TGT

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	malintAGF	Ex2R	1200
Mutant	malintAGF	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	