

Genotyping protocol

EMMA ID: EM:08328

Gene symbol: Plod1

Strain designation: B6.129P2-Plod1^{Gt(RST531)Byg}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
mLH1-ex11f-2	CTG TGC CGT CAG GAC CAA AC
mLH1-ex12R-2	ACA CAC GAC GGC CCT GCA C
LH1-LacZF	TTA TCG ATG AGC GTG GTG GTT
LH1-LacZR	GCG CGT ACA TCG GGC AAA TAA

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	mLH1-ex11f-2	mLH1-ex12R-2	500
Mutant	LH1-LacZF	LH1-LacZR	800

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:

Wild-type

Time	Temperature (+ °C)	Cycles
5 min	95	
1 min	95	30
1 min	68	
2 min	72	
10 min	72	
∞	10	

Mutant

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