

Genotyping protocol

EMMA ID: EM:08330

Gene symbol: Plod3

Strain designation: B6.129-Plod3^{tm1Soin}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
mLH3-Sal	GTC AAC CCA GGT GAG AAC CCG
LH3-pex6R	GAG TTG GTT AAG TTA GCA TAC TGG TGA G
LH2-LacZF	TTA TCG ATG AGC GTG GTG GTT ATG C
LH2-LacZR	GCG CGT ACA TCG GGC AAA TAA TAT C

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	mLH3-Sal	LH3-pex6R	1500
Mutant	LH2-LacZF	LH2-LacZR	750

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
DMSO	1.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.1
H ₂ O	15.65
Total	30.0

PCR program:

Wild-type

Time	Temperature (+ °C)	Cycles
2 min	95	
1 min	95	30
1 min	56	
2 min	72	
10 min	72	
∞	10	

Mutant

Time	Temperature (+ °C)	Cycles
4 min	95	
30 sec	95	40
30 sec	68	
1 min	72	
5 min	72	
∞	10	