

Genotyping protocol

EMMA ID: EM:10964

Gene symbol: Pxmp2

Strain designation: B6.129X1-Pxmp2^{tm1Jkh}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
EM:10964 for	GAG AAG CGG TTG TTT ACA GGG CAC
EM:10964 rev	GCT TCT CTG ATG CTT CTT AAA CAA
EM:10964 lacZrev	GCG GGC CTC TTC GCT ATT ACG

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	EM:10964 for	EM:10964 rev	1700
Mutant	EM:10964 for	EM:10964 lacZrev	1500

Reaction mix:

Component	Volume (μl)
Genomic DNA (~100-250 ng)	2.0
Primer 1 (100 μM)	0.5
Primer 2 (100 μM)	0.5
10x long PCR buffer (w/o MgCl ₂)	5.0
MgCl ₂ (25 mM)	4.0
DMSO	1.0
dNTPs (25 mM)	0.4
Long DNA polymerase (5 U/μl)	0.25
H ₂ O	36.35
Total	50.0

PCR program:

Time	Temperature (+ °C)	Cycles
2 min	94	
30 sec	94	10
30 sec	60	
5 min	68	
1 min	94	25
1 min 30 sec	60	
6 min	68	
10 min	68	
∞	4	