

Genotyping protocol

EMMA ID: EM:09865

Gene symbol: Grpel2

Strain designation: C57BL/6N-A^{tm1Brd} Grpel2^{tm1a(EUCOMM)Hmgu}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
Grpel2-F1	GAC CAG GAG TGA AAA TTC ATC CT
Grpel2-R1	CAG CAT TAT GTT CTA CAG ACA TGT
Cas_R1-Term	TCG TGG TAT CGT TAT GCG CC

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	Grpel2-F1	Grpel2-R1	677
Mutant	Grpel2-F1	Cas_R1-Term	394

Reaction mix:

Component	Volume (µl)
Genomic DNA (~50-100 ng)	2.0
Primer 1 (10 µM)	1.0
Primer 2 (10 µM)	1.0
10x Buffer (15 mM MgCl ₂)	3.0
MgCl ₂ (25 mM)	1.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.05
H ₂ O	21.7
Total	30.0

PCR program:

Time	Temperature (+ °C)	Cycles
5 min	95	
1 min	94	30
1 min	60	
1 min	72	
7 min	72	
∞	4	