

Genotyping protocol

EMMA ID: EM:10575

Gene symbol: Col13a1

Strain designation: B6.129-Col13a1^{tm1Pih}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
EM09878 wtfor	GTGCTGCCTGGTTTTATGGTCACATT
EM09878 rev	CAGGATTCCCCGAGCAAAGACA
EM10575 mutfor	CGAAGTTATTAGGTCCCTCGAAGCTTATCGT

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	EM09878 wtfor	EM09878 rev	318
Mutant	EM10575 mutfor	EM09878 rev	291

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
10 min	95	
1 min	94	30
1 min	66	
1 min 30 sec	72	
10 min	72	
∞	4	