

Genotyping protocol

EMMA ID: EM:09323

Gene symbol: Col13a1

Strain designation: B6.129S6-Col13a1^{tm4.1Pih}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
Nhe1for	CCA TGT CTC TAA TGC CCA
Nhe1rev	TGG AGG CCA CTA TCT GTG T

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	Nhe1for	Nhe1rev	328
Mutant	Nhe1for	Nhe1rev	420

Reaction mix:

Component	Volume (µl)
Genomic DNA (~50-100 ng)	2.0
Primer 1 (10 µM)	1.5
Primer 2 (10 µM)	1.5
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	20.65
Total	30.0

PCR program:

Time	Temperature (+ °C)	Cycles
10 min	95	
1 min	95	40
1 min	57	
30 sec	72	
5 min	72	
∞	10	