

Genotyping protocol

EMMA ID: EM:10424

Gene symbol: Col13a1

Strain designation: FVB.129(B6)-Col13a1^{tm3.1Pih}/Oulu

Primers:

| Primer name | Primer sequence (5'- 3') |
|----------------|---------------------------------|
| EM09878 wtfor | GTGCTGCCTGGTTTTATGGTCACATT |
| EM09878 rev | CAGGATTCCCCGAGCAAAGACA |
| EM09878 mutfor | CTATACGAAGTTATTAGGTGAGTGAGGCCCT |

Products:

| Allele | Primer 1 | Primer 2 | Product size (bp) |
|-----------|----------------|-------------|-------------------|
| Wild-type | EM09878 wtfor | EM09878 rev | 318 |
| Mutant | EM09878 mutfor | EM09878 rev | 224 |

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) | 0.1 |
| H ₂ O | 21.65 |
| 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 | |
| ∞ | 10 | |