

Genotyping protocol

EMMA ID: EM:10975

Gene symbol: Hspg2

Strain designation: C57BL/6-Hspg2^{tm2Soin}/Oulu

Primers:

| Primer name | Primer sequence (5'- 3') |
|-------------|-------------------------------------|
| EM10975 for | GCG GGC GAA TAT GAG TTC CAG TGC |
| EM10975 wt | GCC TTC AGG GTG CCA GTC TGA CTC CAC |
| EM10975 rev | GTG TGG CCA CAT CT GGG GCA CCA C |

Products:

| Allele | Primer 1 | Primer 2 | Product size (bp) |
|-----------|-------------|-------------|-------------------|
| Wild-type | EM10975 for | EM10975 wt | 400 |
| Mutant | EM10975 for | EM10975 rev | 650 |

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 |
|--------|--------------------|--------|
| 5 min | 95 | |
| 1 min | 94 | 35 |
| 30 sec | 65 | |
| 45 sec | 72 | |
| 10 min | 72 | |
| ∞ | 10 | |