

Genotyping protocol

EMMA ID: EM:09864

Gene symbol: *Ndnf*

Strain designation: C57BL/6N-*Ndnf*^{tm1(NCOM)Mfgc}/Oulu

Primers:

| Primer name | Primer sequence (5'- 3') |
|------------------|---------------------------------|
| NDNF F1 | ACA ATT GTT CAC TCT GCT CAC CAG |
| NDNF R1 | GTA GTT CTG GGT CAC CTG AAG AG |
| Construct primer | TCG TGG TAT CGT TAT GCG CC |

Products:

| Allele | Primer 1 | Primer 2 | Product size (bp) |
|-----------|----------|------------------|-------------------|
| Wild-type | NDNF F1 | NDNF R1 | 744 |
| Mutant | NDNF F1 | Construct primer | 300 |

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 | |