

# Gnat2 Genotyping Protocol

## Primer sequences:

Reg-Gnat2-wtF: 5'-TCACATGCCCTCTTTCTACCCTTCC  
Reg-Gnat2-wtR: 5'-AATGGGAGCACGTTTTGAGAGTTGG

Reg-NeoF: 5'-GCAGCCTCTGTTCCACATACTTCA  
Reg-Gnat2-R: 5'-ATAGGACCAGTAAAACCACAGGGGC

\*stock primers are 200uM, 1:10 working dilution

## Reaction descriptions:

Reaction 1 - Amplification for WT: Reg-Gnat2-wtF and Reg-Gnat2-wtR yield a DNA fragment of 282 bp in wildtype animals

Reaction 2 - Amplification for KO: Reg-NeoF and Reg-Gnat2-R yield a DNA fragment of 566 in knockout animals

## Thermal Cycler settings:

### Reaction 1 – Gnat2 WT:

95°C for 5 minutes

35 cycles of:

1. Denaturation, 95°C for 30 seconds
2. Primer annealing, 60°C for 30 seconds
3. Primer extension, 72°C for 30 seconds

Final extension, 72°C for 7 minutes

Store at 4°C

### Reaction 2 – Gnat2 KO:

95°C for 10 minutes

35 cycles of:

1. Denaturation, 95°C for 30 seconds
2. Primer annealing, 61°C for 30 seconds
3. Primer extension, 72°C for 1 minute

Final extension, 72°C for 7 minutes

Store at 4°C