

MMRRC UNC – Genotyping Protocol

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|-----------------------------|---|
| MMRRC Strain ID | 259 |
| MMRRC Strain Name | STOCK Tg(Chrn4-EGFP)CL200Gsat/Mmnc |
| Gene Name(s) | cholinergic receptor, nicotinic, beta polypeptide 4 |
| Breeding Protocol(s) | Backcross, Sib-mating or Outcross to Swiss Webster |
| Protocol Date | 9/20/23 |

PCR Reaction

| | 1X |
|------------------------|-----------|
| ddH ₂ O | 11.5 |
| 5X Buffer | 5.0 |
| 25mM MgCl ₂ | 2 |
| 10mM dNTPs | 0.5 |
| 10uM Primer 1 | 1 |
| 10uM Primer 2 | 1 |
| 10uM Primer 3 | 1 |
| Taq | 1 |
| DNA | 2 |

Thermal Cycler:

- Step 1: 94°C, 5 min
- Step 2: 94°C for 30 s
- Step 3: 60°C for 30 s
- Step 4: 72°C for 30 s
- Step 5: 35x from step 2 to step 4
- Step 6: 72°C for 7 min

Taq: Apex

Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

Com-F (259): AGCACAGGCTCCATCGGGTCCTAGT

WT-R (259): ACTCCTCCCACACTGCGACCCTTGT

Mut-R (259): TAGGTGAAGGTGGTCACGAGGGTGG

Bands expected: WT: 217bp Mutant: 311bp