

Genotyping protocol

EM:14795

C57BL/6N-Hmgcr<em1Lclem>/Kctt Primers

F5 5' GGT GTT TCA GTG CGT GTT CAT TTA C 3'

R5 5' AAG CCT CAT GAA ACC CAG ATT GAC 3'

PCR profile

95 °C, 5 min

94 °C, 30 s

58 °C, 30 s

35 cycles

72 °C, 30 s

72 °C, 10 min

4 °C, ∞

PCR mix

| | |
|--|-----------------|
| 10 x PCR Buffer II (Life technologies) | 2.0 µl |
| MgCl ₂ (25 mM) | 1.2 µl |
| dNTP mix (25 mM of each nucleotide) | 0.16 µl |
| F5 (100 µM) | 0.1 µl |
| R5 (100 µM) | 0.1 µl |
| AmpliTaq Gold (5 U/µl) | 0.13 µl |
| DNA template (~ 0.5 µg tail DNA) | 1.0 µl |
| H ₂ O | <u>15.31 µl</u> |
| | 20 µl |

Post-PCR analysis

2% agarose gel.

Expected pattern is: mut:217bp, wt: 157bp