

RMRC Genotyping Protocol-PCR

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|-----|-----------|-------------|--|
| No. | RMRC13363 | Strain Name | C57BL/6- <i>Gnb4</i> ^{tm1Fmj} /Narl |
|-----|-----------|-------------|--|

A. Primer

| | Primer name | Sequence |
|---|-------------|---------------------------------|
| 1 | CU | ATAGCGGCCGCGAAGGAGAGAATCAGTTCCA |
| 2 | Mut-D | TAGCGAGGTGGTCGCGCAGCGTGCGCCTT |

B. Reaction Conditions

| Component | Concentration | | Volume | | Step | Temperature, °C | Time, sec | Number of Cycles |
|------------------|---------------|-------------|--------|----|------|-----------------|-----------|------------------|
| | | | | | | | | |
| H ₂ O | -- | | 7.4 | μl | 1 | 94 | 300 | |
| 10X PCR buffer | 10x | | 2 | μl | 2 | 94 | 30 | 40 |
| dNTP | 10 | mM | 0.04 | μl | 3 | 55 | 30 | |
| CU | 10 | μM(ρmol/μl) | 0.02 | μl | 4 | 72 | 30 | |
| Mut-D | 10 | μM(ρmol/μl) | 0.02 | μl | 5 | 72 | 420 | |
| DNA | -- | | 0.5 | μl | 6 | | | |
| Taq | | U/μl | 0.02 | μl | 7 | | | |
| Total | 10 μl | | | | 8 | | | |

C. PCR Product

| Primer set | PCR product | | |
|------------|-------------|--------------------|-------------|
| | WT | mt/+ | mt/mt |
| CU+Mut-D | 584 bp (WT) | 584 bp+684 bp (WT) | 684 bp (WT) |

D. Gel Photo

