

RMRC Genotyping Protocol-PCR

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|-----|-----------|-------------|-----------------------------------------------------------------------|
| No. | RMRC13212 | Strain Name | C57BL/6- <i>Khsrp</i> ^{tm1a(GEMMS)<i>Narl</i>} / <i>Narl</i> |
|-----|-----------|-------------|-----------------------------------------------------------------------|

A. Primers

| No. | Primer name | Sequence |
|-----|-------------|--------------------------|
| 1 | KhsrpLF | TTGGGGGTGTGTGTGGATTG |
| 2 | KhsrpLR | CGAACTCCATATAGCTCAGGATGG |

B. Reaction Conditions

| Component | Concentration | Volume | Step | Temperature, °C | Time, sec | Number of Cycles |
|------------------|-----------------|---------|------|-----------------|-----------|------------------|
| H ₂ O | – | 10.8 μl | 1 | 94 | 180 | 1 |
| PCR buffer | 10 x | 1.5 μl | 2 | 94 | 30 | 35 |
| Formamide | – | 0.5 μl | 3 | 60 | 30 | 35 |
| dNTP | 10 mM | 0.3 μl | 4 | 72 | 30 | 35 |
| Primer 1 | 20 μM (μmol/μl) | 0.3 μl | 5 | 72 | 180 | 1 |
| Primer 2 | 20 μM (μmol/μl) | 0.3 μl | | | | |
| gDNA | 50-250 ng/μl | 1 μl | | | | |
| Taq | 5 U/μl | 0.3 μl | | | | |
| Total | | 15 μl | | | | |

C. PCR Product

| No. | Primer set | | Product size | Target region |
|-----|------------|---------|--------------|---------------|
| 1 | KhsrpLF | KhsrpLR | 440 bp | Wildtype |
| 2 | KhsrpLF | KhsrpLR | 570 bp | Mutant |

D. Gel Photo

