# PCR Protocol

## Protocol for MMRRC: 0118 at Donor's Laboratory

**Reagents:**

| Reagent / Constituent | Volume (µL) |
|---|---|
| DNA Sample (mouse tail genomic DNA) | 1.0 |
| 10X buffer (Qiagen containing 15 mM MgCl2)) | 2.0 |
| Qiagen 5X Q solution | 4.0 |
| Primer Mix: (each primer at 0.333 µg/µl) | 0.2 |
| 10 mM dNTPs (Qiagen) | 0.4 |
| Water (dH2O) | 12.3 |
| AmpliTag at 5 U/µl | 0.1 |
| **Total Volume of Reaction:** | 20.0 µL |

**Primers:**

| # | Primer Name | Nucleotide Sequence (5' - 3') |
|---|---|---|
| 1 | 1 | TCC ACA GAG AAG AAC TGC TG |
| 2 | 2 | CAG GAC ATA GCG TTG GCT AC |
| 3 | 3 | TTG CTG TAC TGT GTG TCC AG |

**Cycling:**

| Step | Temp(°C) | Time |
|---|---|---|
| 1. | 94 | 5 min |
| 2. | 94 | 15 sec |
| 3. | 65-55 (decrease temp1°/cycle) | 30 sec |
| 4. | 72 | 45 sec |
| 5. | Go to 2: 10 cycles | |
| 6. | 94 | 15 sec |
| 7. | 55 | 30 sec |
| 8. | 72 | 45 sec |
| 9. | Go to 6: 30 cycles | |
| 10. | 72 | 1 min |
| 11. | 4 | hold |

**Electrophoresis:**

2.5% **Agarose gel**

| Band # | bp | Genotype |
|---|---|---|
| 1 | ~140 | WT |
| 2 | ~250 | KO |