

MMRRC UNC – Genotyping Protocol

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|-----------------------------|---|
| MMRRC Strain ID | 36724 |
| MMRRC Strain Name | C57BL/6J- <i>Ruvbl2</i> ^{M1B^{tr}} /Mmnc |
| Gene Name(s) | RuvB-like protein 2 (Ruvbl2) |
| Breeding Protocol(s) | Backcross to C57BL/6J |
| Protocol Date | 8/12/13 |

MMRRC #36724 PCR Reaction

| | 1X |
|-------------------------|-----------|
| ddH ₂ O | 13 |
| 5X Buffer | 5.0 |
| 25 mM MgCl ₂ | 2 |
| 10 mM dNTPs | 0.5 |
| 10 μM Primer F | 1 |
| 10 μM Primer R | 1 |
| Taq | 0.5 |
| DNA | 2 |

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94 °C for 30 sec
 Step 3: 63 °C for 30 sec
 Step 4: 72 °C for 45 sec
 Step 5: Step 2 to 4; Cycles: 30
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

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

WT-F (36724): GATGTGACAAGAATCGAGCGAATCGcT

R-Com (36724): AGGTGGTGAGCGAAGCTTCCTACACAG

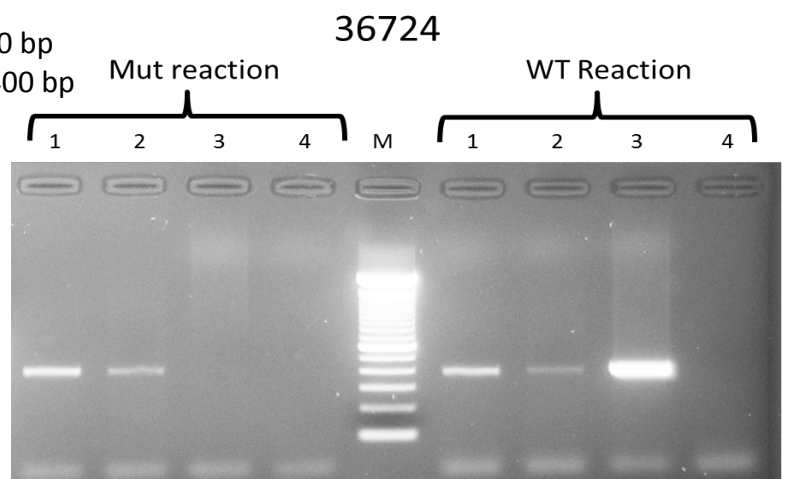
Mut-F (36724): GATGTGACAAGAATCGAGCGAATCGcA

Bands expected:

WT [primers WT-F (36724) + R-Com (36724)] 400 bp

Mut [primers Mut-F (36724) + R-Com (36724)] 400 bp

Run on 2% agarose gel in TAE.



Lane 1 and 2: Het, :Lan3: B6; lane 4: H2O; M: 100 bp ladder