

MMRRC UNC – Genotyping Protocol

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|-----------------------------|---|
| MMRRC Strain ID | 11599 |
| MMRRC Strain Name | B6.129P2- <i>Adipor1</i> ^{tm1Dgen} /Mmnc |
| Gene Name(s) | Adiponectin receptor 1 (Adipor1) |
| Breeding Protocol(s) | Backcross to C57BL/6J |
| Protocol Date | 9/8/16 |

MMRRC #11599 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 45 sec

Step 3: 60°C for 45 sec

Step 4: 72°C for 90 sec

Step 2 to 4 Cycles: 36

Step 5: 72°C for 7 min

| | 1X |
|------------------------|-----------|
| DNA | 2.0 |
| 5X Buffer | 5.0 |
| 10mM dNTPs | 0.5 |
| 30 uM Primer F/R/Neo | 1.5 |
| Taq | 0.5 |
| ddH ₂ O | 13.5 |
| 25mM MgCl ₂ | 2.0 |

Taq: **Apex Taq and Chromataq 5X Buffer**

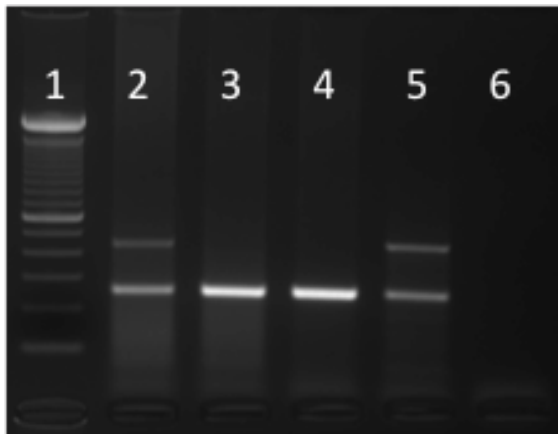
Bands: WT: 254bp MUTANT: 433bp

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

NIH-0062 GS1: TCCAAGTGTGTCAGCTTCTCTGTTAC

NIH-0062 GS2: AGGCAGGGTAAGCTGATTAGCTATG

NIH-0062 neo: GGGTGGGATTAGATAAATGCCTGCTCT



1. 100bp Marker (Invitrogen)
2. Heterozygous Sample
3. Wild-type Sample
4. Wild-type Control
5. Heterozygous Positive Control
6. Negative Control