

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	37644
<b>MMRRC Strain Name</b>	B6.Cg- <i>Mdm2</i> <sup>tm1.1Ypz</sup> /Mmnc
<b>Gene Name(s)</b>	abhydrolase domain containing 8 (Abhd8)
<b>Breeding Protocol(s)</b>	Backcross to C57BL/6J
<b>Protocol Date</b>	11/5/15

### MMRRC #37644 PCR Reaction

**2,2 primer Reaction: (P1+P2) for WT and (P3+P4) for mutant**

**Thermal Cycler:**

Step 1: 94°C for 5 min  
 Step 2: 94°C for 45 sec  
 Step 3: 58°C for 45 sec  
 Step 4: 72°C for 60 sec  
 Step 2 to 4 Cycles: 35  
 Step 5: 72°C for 7 min

	<u>1X</u>
ddH <sub>2</sub> O	13
5X Buffer	5
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	0.5
DNA	2

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

**Bands:** MUTANT: 340bp  
 WT: 450bp

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

Mdm2(37644)P1      TTATTGAAGGACTATTGGAAGGTACCTCA  
 Mdm2(37644)P2      ACGTGAAACATGACATGAGGTGTCCAG  
 Mdm2(37644)P3      GTCTTTTTTATTGAAGGACTATTGGAAG TTTACTAGT  
 Mdm2(37644)P4      TTCAACTCTTCACGCTTTCTTGGCTGC

Wild-type Reaction

1. 100bp Marker
2. Heterozygous Sample
3. Heterozygous Sample
4. Heterozygous Sample
5. Heterozygous Sample
6. Heterozygous Sample
7. Wild-type Control
8. Negative Control

Mutant Reaction

9. 100bp Marker
10. Heterozygous Sample
11. Heterozygous Sample
12. Heterozygous Sample
13. Heterozygous Sample
14. Heterozygous Sample
15. Wild-type Control
16. Negative Control

