

## MMRRC UNC – Genotyping Protocol

|                             |   |
|-----------------------------|---|
| <b>MMRRC Strain ID</b>      | 32019   |
| <b>MMRRC Strain Name</b>    | 129S6/Svev(B6)- <i>Map3k4</i> <sup>tm1Gjl</sup> /Mmnc     |
| <b>Gene Name(s)</b>         | Mitogen-activated protein kinase kinase kinase 4 (Map3k4) |
| <b>Breeding Protocol(s)</b> | Sib-mating (heterozygous x heterozygous)                  |
| <b>Protocol Date</b>        | 2/26/13   |

### MMRRC# 32019 PCR Reaction

2,2 Primer reactions (M41168F + 22R1) for wild-type and (NeoR + 2003R) for mutant reaction.  
**Note:** In heterozygous samples, light 1.6 and 2.3 kb bands may be seen in the wild-type reaction

**Thermal Cycler:**

94°C for 30 sec  
 61°C for 1 min  
 72°C for 3 min  
 32x

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

**Bands:** MUTANT: ~600bp  
 WT: ~1kb

|                        | <b>1X</b> |
|------------------------|-----------|
| ddH <sub>2</sub> O     | 13        |
| 5X Buffer              | 5.0       |
| 25mM MgCl <sub>2</sub> | 2         |
| 10mM dNTPs             | 0.5       |
| 10 uM Primer F         | 1         |
| 10 uM Primer R         | 1         |
| Taq                    | 0.5       |
| DNA                    | 2         |

**Primer sequences 5' to 3':**

M41168F: CTG CAT CAG TGT TGA CAC AGG GGA  
 22R1: GTG AAG CTC CAC GCC AAA ATA CCG  
 NeoR 5082: CCG ACT GCA TCT GCG TGT TCG  
 2003R: GGG ATG TGA GCG ACT CTG GCC  
 TTA

Run on 2.0% agarose gel in TAE.

