

## MMRRC UNC – Genotyping Protocol

|                             |                                                   |
|-----------------------------|---------------------------------------------------|
| <b>MMRRC Strain ID</b>      | 71553                                             |
| <b>MMRRC Strain Name</b>    | 129S6(Cg)- <i>Kras</i> <sup>tm6.1Cmco</sup> /Mmnc |
| <b>Gene Name(s)</b>         | Kirsten rat sarcoma viral oncogene homolog        |
| <b>Breeding Protocol(s)</b> | Backcross                                         |
| <b>Protocol Date</b>        | 9/26/2024                                         |

### PCR Reaction

|                        |                  |
|------------------------|------------------|
|                        | <b><u>1X</u></b> |
| ddH <sub>2</sub> O     | 12.5             |
| 5X Buffer              | 5                |
| 25mM MgCl <sub>2</sub> | 2                |
| 10mM dNTPs             | 0.5              |
| 10uM Primer 1          | 1                |
| 10uM Primer 2          | 1                |
| Taq                    | 1                |
| DNA                    | 2                |

#### **Thermal Cycler:**

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 60C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

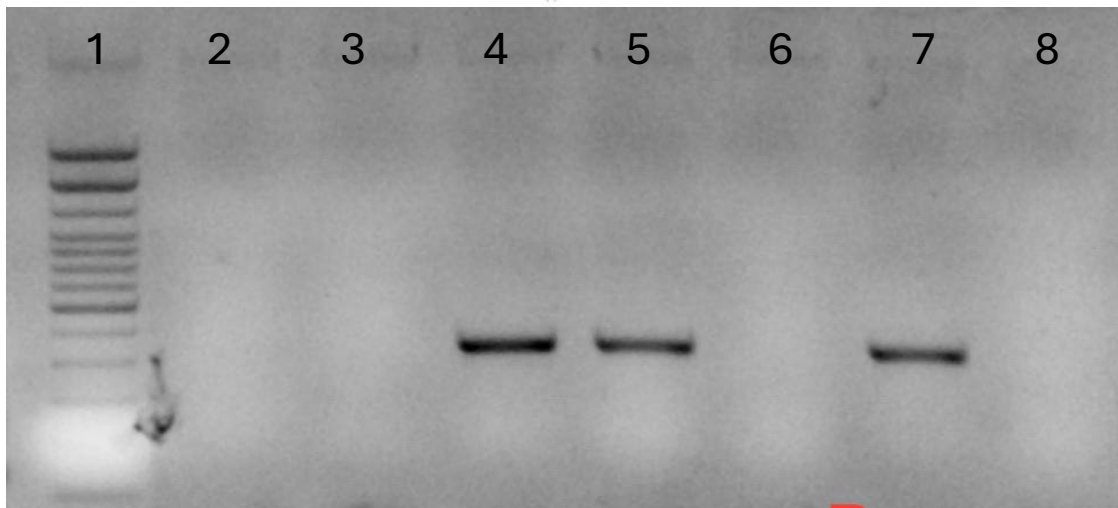
Taq: **Apex Taq**

Bands: WT: No Band MUTANT: 448bp

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

Kras(71553)F: CTTCCATTTGTCACGTCCTGC

Kras(71553)R: TCTTCGGTGGAAACAACGGT



1. 100bp Ladder
2. Wild-type Sample
3. Wild-type Sample
4. Mutant Sample
5. Mutant Sample
6. Wild-type Sample
7. Mutant Sample
8. Wild-type Control