

## MMRRC UNC – Genotyping Protocol

|                             |   |
|-----------------------------|---|
| <b>MMRRC Strain ID</b>      | 65288   |
| <b>MMRRC Strain Name</b>    | B6J.129P2(Cg)- <i>Nras</i> <sup>em1(Q61K)<i>Burd</i></sup> Tg(Tyr-cre/ERT2)13Bos/Mmnc |
| <b>Gene Name(s)</b>         | neuroblastoma ras oncogene; tyrosinase; cre tamoxifen-dependent recombinase           |
| <b>Breeding Protocol(s)</b> | Sib-mating  |
| <b>Protocol Date</b>        | 11/2/23   |

### PCR Reaction

|                        |           |
|------------------------|-----------|
|                        | <b>1X</b> |
| 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         |

### 4, 2 Primer Reactions

#### Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 30sec

Step 3: 60C(Nras and Q61K) or 57C(Tyr-Cre), 30sec

Step 4: 72C, 30sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

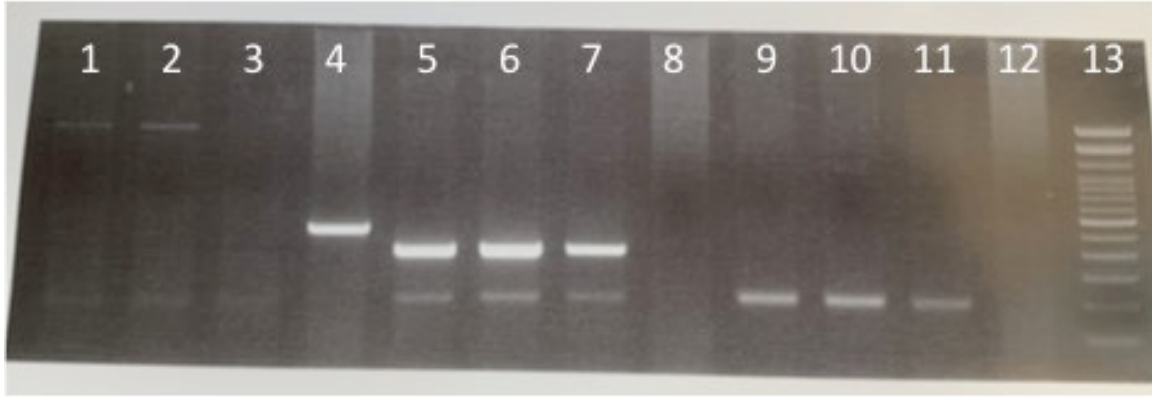
Bands: MUT: 371bp (Nras1 P1+P3), ~180bp (Tyr-Cre 1+IVSR), 453bp (SF+SR)  
WT: 487bp (Nras1 P2+P3), No Band (Tyr-Cre 1+IVSR), 453bp (SF+SR)

Primer sequences 5' to 3':

Nras(65288)P1 GCAAGAGGCCCGGCAGTACCTA  
Nras(65288)P2 AGACGCGGAGACTTGGCGAGC  
Nras(65288)P3 GCTGGATCGTCAAGGCGCTTTTCC  
TyrCre(65288)1 TGATAGTCACTCCAGGGGTTG  
TyrCre(65288)IVSR CGA TCC GGA GCT TTT TG  
Nras(65288)SF CACATAGGTAGGCGTATTCATCCTGG

Nras(65288)SR GGCACATATCTGCTGTTGCACTTG

Run on 2.0% agarose gel in TAE.



Nras-tm1 Wild-type Reaction

1. Homozygous Sample
2. Homozygous Sample
3. Homozygous Sample
4. Wild-type Control

Nras-tm1 Mutant Reaction

5. Homozygous Sample
6. Homozygous Sample
7. Homozygous Sample
8. Wild-type Control

Tyr-Cre Reaction

9. Tyr-Cre Positive Sample
10. Tyr-Cre Positive Sample
11. Tyr-Cre Positive Sample
12. Wild-type Control

13. 100bp Marker (Invitrogen)

**Q61K (Primers SF+SR): Amplicon must be sequenced to determine genotype.**

**Perform PCR Reaction**

**Sequence with SF primer.**

**Mutations are around 160bp in the sequence**

|                |                  |               |
|----------------|------------------|---------------|
| <b>WT:</b>     | <b>TGGACAAGA</b> | <b>CAA: Q</b> |
| <b>MUTANT:</b> | <b>TGGAAAGGA</b> | <b>AAG: K</b> |