

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	43505
<b>MMRRC Strain Name</b>	129/Sv-Sp4 <sup>tm3(Sp4)<sup>Krc</sup></sup> /Mmnc
<b>Gene Name(s)</b>	trans-acting transcription factor 4
<b>Breeding Protocol(s)</b>	Sib-mating and outcross
<b>Protocol Date</b>	3/10/2023

### PCR Reaction

	<b><u>1X</u></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

### **2,2 Primer Reactions (CF+WR) for wild-type and (CF+lac) for the mutant reaction**

#### **Thermal Cycler:**

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 55C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 35

Step 5: 72C, 7min

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

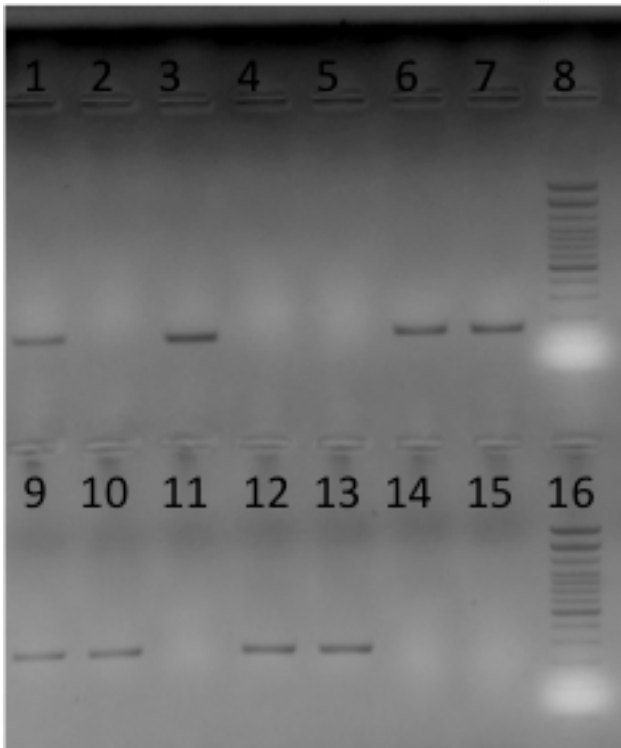
Bands: WT: 250bp MUTANT: ~400bp

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

Sp4(43505)CF: GGG TTT CAA ACC ACG TTT CGT GAG

Sp4(43505)WR: TTT TCC CTC CTT CTG TAG CCA T

Sp4(43505)lac: GCG CTT CTT CTT AGG AGG GAT CTT GG



### Wild-type Reaction

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

### Mutant Reaction

9. Heterzygous Sample
10. Homozygous Sample
11. Wild-type Sample
12. Homozygous Sample
13. Homozygous Sample
14. Wild-type Sample
15. Wild-type Control
16. 100bp Ladder