

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	67375
<b>MMRRC Strain Name</b>	STOCK <i>Tagln<sup>tm1Liw</sup>/Mmnc</i>
<b>Gene Name(s)</b>	transgelin
<b>Breeding Protocol(s)</b>	Sib-mating
<b>Protocol Date</b>	8/16/2022

### 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 (WTF+WTR) for Wild-type and (MF+MR) for Mutant reactions**

#### **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 and Chromataq 5X Buffer**

Bands: WT: 259bp MUTANT: 352bp

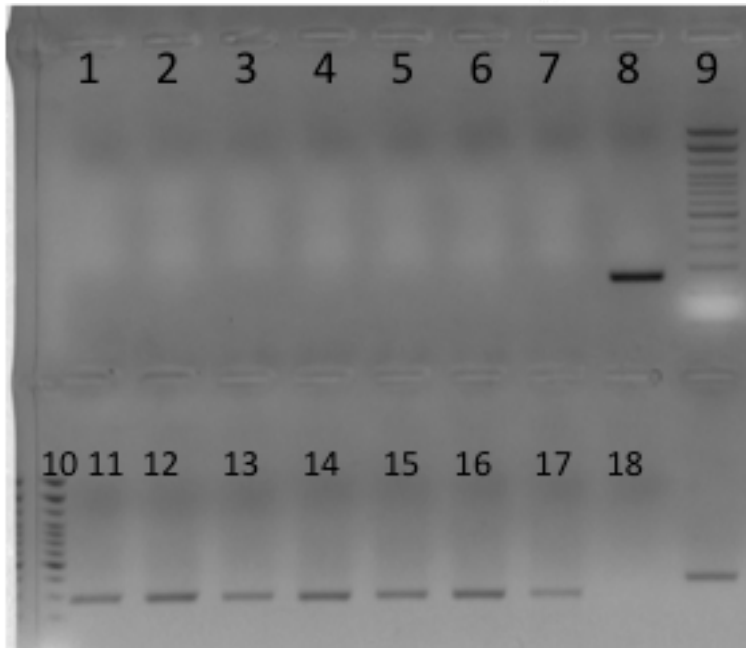
Primer sequences 5' to 3': Primers are 10uM with respect to each primer

SM22(67375)WTF CTCTTCTAGTTTGCTGGGTGGA

SM22(67375)WTR TGCTTCCAGAACTTAGCCCC

SM22(67375)MF TACCACAGCGGATGGTTCGG

SM22(67375)MR GTGGTGGTTATGCCGATCGC



#### Wild-type Reaction

1. Homozygous Sample
2. Homozygous Sample
3. Homozygous Sample
4. Homozygous Sample
5. Homozygous Sample
6. Homozygous Sample
7. Homozygous Sample
8. Wild-type Control
9. 100bp Ladder (Invitrogen)

#### Mutant Reaction

10. 100bp Ladder (Invitrogen)
11. Homozygous Sample
12. Homozygous Sample
13. Homozygous Sample
14. Homozygous Sample
15. Homozygous Sample
16. Homozygous Sample
17. Homozygous Sample
18. Wild-type Control