

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	291
<b>MMRRC Strain Name</b>	STOCK Tg(Sparcl1-EGFP)BE2Gsat/Mmnc
<b>Gene Name(s)</b>	SPARC-like 1 (Sparcl1)
<b>Breeding Protocol(s)</b>	Outcross to SW/Tac
<b>Protocol Date</b>	7/5/2016

### MMRRC #291 PCR Reaction

**Thermal Cycler:**

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 57°C for 30 sec

Step 4: 72°C for 30 sec

Step 2 to 4 Cycles: 30

Step 5: 72°C for 7 min

Taq: **Denville (5U/ul) and Chromataq 5X Buffer**

	<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

**Bands:** MUTANT: ~800bp

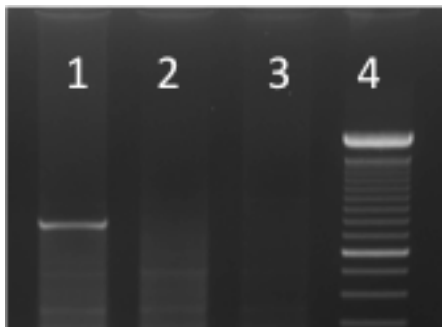
WT: No Band

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

Sparcl(291)5': GGCGGCCTCACAGATATATCCTCTGTGTCCCCTC

Sparcl(291)EGFP: TAGCGGCTGAAGCACTGCA

Run on 2.0% agarose gel in TAE.



1. EGFP+ Sample
2. Wild-type Control
3. Negative Control
4. 100bp Ladder (Invitrogen)