

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	11471
<b>MMRRC Strain Name</b>	STOCK <i>Spry4<sup>tm1.2Mrt</sup>/Mmnc</i>
<b>Gene Name(s)</b>	Sprouty homolog 4 ( <i>Spry4</i> )
<b>Breeding Protocol(s)</b>	Random Intra-Strain Mating
<b>Protocol Date</b>	12/2/13

### MMRRC# 11471 PCR Reaction

	<b><u>1X</u></b>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	1.0
20 uM Primer 1 & 2	1.0
Taq	1.0
ddH <sub>2</sub> O	12.5
25mM MgCl <sub>2</sub>	2.0
DMSO	2.5

**NOTE:** Two, 2-primer reactions – (A & B) and (A & C). Run separately. Try to load ~20ul, this is a somewhat weak reaction.

Denaturing: 94°C for 60 sec  
 Annealing Temp.: 57°C for 45 sec  
 Extension: 72°C for 60 sec  
 Cycles: 36

Taq: **Apex and Chromataq 10X Buffer**

**Bands:** WT (A&B): 300bp  
 Delta (A&C): 450bp

**Primer sequences 5' to 3':** Primers are 10uM with respect to each primer (20uM total).

**Primer A:** CAG GAC TTG GGA GTG CTT CCT TAG

**Primer B:** CCT CCT AGT ACC TTT TTG GGG AGA G

**Primer C:** TAC AGC AGG AAT GGC TAC GGT G

Run on 2% agarose gel in TAE.

