

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	32803
<b>MMRRC Strain Name</b>	C57BL/6J- <i>Spag9</i> <sup>m1B<sup>tlr</sup></sup> /Mmnc
<b>Gene Name(s)</b>	sperm associated antigen 9 (Spag9)
<b>Breeding Protocol(s)</b>	Random Intra-strain Mating
<b>Protocol Date</b>	7/16/13

### MMRRC #32803 PCR Reaction

	<u>1X</u>
ddH <sub>2</sub> O	13
5X Buffer	5
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10 μM Primer Forward	1
10 μM Primer Reverse	1
Apex Taq	0.5
DNA	2

**Note: This genotyping requires *ApoI* restriction digestion**

**Thermal Cycler:**

Step 1: 94°C for 5 min  
 Step 2: 94°C for 30 sec  
 Step 3: 61°C for 30 sec  
 Step 4: 72°C for 30 sec  
 Step 5: 35x from step 2 to step 4  
 Step 6: 72°C for 10 min

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

**Primer sequences 5' to 3':** Primers are 10 μM with respect to each primer.

SpaG9F (32803): CTGGATCTGTACCAGCACCCACTCA

SpaG9R (32803): CCTCCTCTATCAGCCAGCTCTCCCT

***ApoI* restriction digestion mix for each PCR sample**

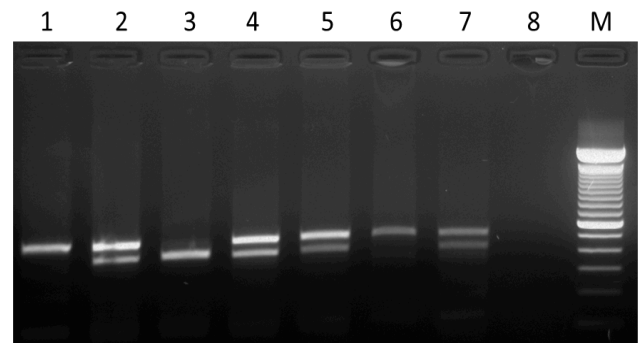
9.6 μl H<sub>2</sub>O  
 4.0 μl NEB Buffer 3, 10X  
 0.4 μl BSA, 100X  
 1.0 μl *ApoI*

**Add 15 μl Mix to each 25ul PCR product:** incubate at 50 °C for 3 hours - overnight. Then 80°C for 20 minutes

**Bands:** Mutant: 418 bp + 91 bp  
 Wild type: 509 bp

Run on a 2% agarose gel

32803



Lane 1, 6: WT; Lane 2, 4, 5 and 7: Het; Lane 3: Hom; Lane 8: H<sub>2</sub>O;  
 M: 100 bp DNA ladder