

## MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11653
MMRRC Strain Name	NIH-1286
Gene Name(s)	B6;129S5- <i>Rgs11</i> <sup>tm1Lex</sup> /Mmnc
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	4/9/15

### MMRRC #11653 PCR Reaction

	<b>1X</b>
DNA	2.0
5X Buffer	5.0
10mM dNTPs	0.5
10 uM Primer F/R	1/1
Taq	0.5
ddH <sub>2</sub> O	13.0
25mM MgCl <sub>2</sub>	2.0

**Note: Run 2,2 primer reactions  
(P3 + P4 for wild-type) and (P1 + P2 for mutant)**

#### Thermal Cycler:

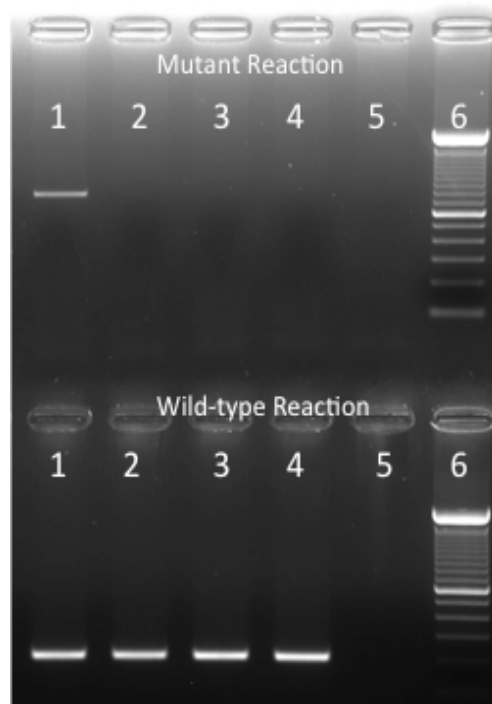
- Step1: 94°C for 5min
- Step 2: 94°C for 15 sec
- Step 3: 65°C for 30 sec, Decrease 1°C/cycle
- Step 4: 72°C for 40 Sec
- Step 5: go to step 2, 10 Cycles
- Step 6: 94°C for 15 sec
- Step 7: 55°C for 30 sec
- Step 8: 72°C for 40 sec
- Step 9: go to step 6 to 8, 30 Cycles
- Step 10: 72°C for 7 min

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

**Bands:** MUTANT: 753bp WT: 203bp

#### Primer sequences 5' to 3':

NIH1286(11653)P1: GCAGCGCATCGCCTTCTATC  
 NIH1286(11653)P2: CTTCCAATATCCACCCTAGC  
 NIH1286(11653)P3: AGTTAAGGGCATTGGAGACCGT  
 NIH1286(11653)P4: CCAAAGAAACCGAAAGTGTGTTAGGG



1. Heterozygous Sample
2. Wild-type Sample
3. Wild-type Sample
4. Wild-type Control
5. Negative Control
6. 100bp Marker

Run on 2% agarose gel.