

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	36050
<b>MMRRC Strain Name</b>	B6.129S1- <i>Mrgprd</i> <sup>tm5Mjz</sup> /Mmnc
<b>Gene Name(s)</b>	MAS-related GPR, member D ( <i>Mrgprd</i> )
<b>Breeding Protocol(s)</b>	Intra-strain Mating
<b>Protocol Date</b>	2/26/13

### MMRRC #36050 PCR Reaction

**Thermal Cycler:**

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 58°C for 30 sec

Step 4: 72°C for 90 sec

Step 2 to 4 Cycles: 30

Step 5: 72°C for 7 min

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

	<u>1X</u>
ddH <sub>2</sub> O	11
5X Buffer	5
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10 mM MrgD-8	1
10 mM rtTA1	1
10 mM MrgD-1	2
Taq	0.5
DNA	2

**Bands:** MUTANT: 516bp

WT: 981bp

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

MrgD-8: CAT GAG ATG CTC TAT CCA TTG GG

rtTA1: GGA GAA ACA GCT AAA GTG CG

MrgD-1: CTG CTC ATA GTC AAC ATT TCT GC

Run on 2% agarose gel in TAE.