

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	36118
<b>MMRRC Strain Name</b>	B6;129S1(Cg)- <i>Mrgprd</i> <sup>tm1.1(cre)And</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>	6/27/13

### MMRRC #36118 PCR Reaction

**Thermal Cycler:**

Step 1: 94°C for 5 min  
 Step 2: 94°C for 30 sec  
 Step 3: 60°C for 30 sec  
 Step 4: 72°C for 45 sec  
 Step 5: Step 2 to 4 Cycles: 28  
 Step 6: 72°C for 7 min

Taq: **Apex and chromataq Buffer**

	<b>1X</b>
ddH <sub>2</sub> O	11
5X Buffer	5.0
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10 uM <i>MrgD</i> -2	1
10 uM EGFP2	1
<i>MrgD</i> -9	2
Taq	0.5
DNA	2

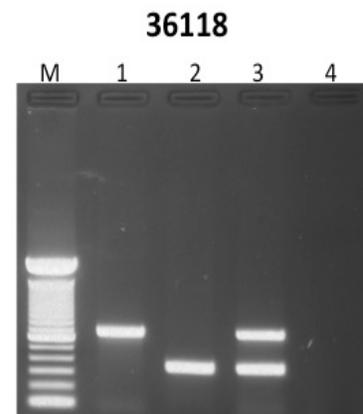
**Primer sequences 5' to 3', (Primers are 10uM with respect to each primer)**

*MrgD*-2(36112): GGATCATGACCGGCATGAAGATC;  
*MrgD*-9 (36112): GGTAGATTCAGGCACAGTGGGAG;  
 EGFP2 (36118): CCGTAGGTCAGGGTGGTCACGAGG

**Bands:**

*MrgD*-2 (36112) + *MrgD*-9(36112) amplify WT: 669 bp  
*MrgD*-9 (36112) + EGFP2 (36118) amplify MUTANT: 321 bp

Run on 1% agarose gel in TAE.



Lane 1: WT; Lane 2: Hom; Lane 3: Het; Lane 4: ddH<sub>2</sub>O;  
 M: 50 bp DNA ladder Invitrogen