

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36112
MMRRC Strain Name	B6;129SP2- ^{Mrgprdtm4.1(COP4)^{Mjz}} /Mmnc
Gene Name(s)	MAS-related GPR, member D (Mrgprd)
Breeding Protocol(s)	Intra-Strain Mating (homozygous x homozygous)
Protocol Date	2/26/13

MMRRC #36112 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 2 to 4 Cycles: 28

Step 5: 72°C for 7 min

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

Bands: MUTANT: 500bp

WT: 669bp

Primer sequences 5' to 3':

MrgD-2: GGATCATGACCGGCATGAAGATC;

MrgD-9: GGTAGATTCAGGCACAGTGGGAG

Arg2: GTGAGGTTGCTCAGGCGGATAAGGATGACAG

	<u>1X</u>
ddH ₂ O	11
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10 mM MrgD-2	1
10 mM Arg2	1
10 mM MrgD-9	2
Taq	0.5
DNA	2

Run on 2% agarose gel in TAE.