

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36772
MMRRC Strain Name	B6.129S1- <i>Mrgprd</i> ^{tm1Mjz} /Mmnc
Gene Name(s)	MAS-related GPR, member D
Breeding Protocol(s)	Backcross homo x homo
Protocol Date	8/14/2020

PCR Reaction

	<u>1X</u>
ddH ₂ O	12
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 30sec

Step 3: 58C, 30sec

Step 4: 72C, 30sec

Step 2 to 4 Cycles: 35

Step 5: 72C, 7min

Taq: Apex Taq and Chromataq 5X Buffer

Bands: WT: 981bp MUTANT: 516bp

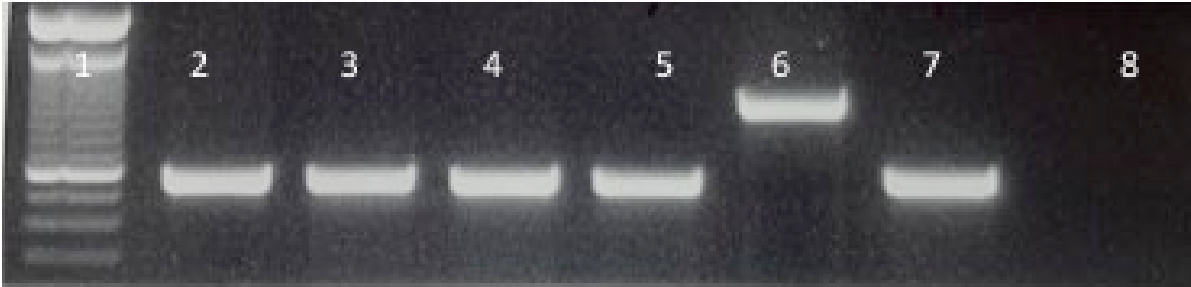
Primer sequences 5' to 3': Primers are 10uM with respect to each primer

MrgD-8(36772): CAT GAG ATG CTC TAT CCA TTG GG (Common Primer)

rtTA1(36772): GGA GAA ACA GCT AAA GTG CG (Mutant Primer)

MrgD-1(36772) : CTG CTC ATA GTC AAC ATT TCT GC (Wild-type Primer)

Run on 2% Agarose Gel



1. 100bp DNA Ladder (Invitrogen)
2. Homozygous Sample
3. Homozygous Sample
4. Homozygous Sample
5. Homozygous Sample
6. Wild-type Control
7. Positive Control
8. Negative Control