

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	69604
MMRRC Strain Name	B6N.129S6-Is(HSA10;19)1Pmv/Mmunc
Gene Name(s)	arsenite methyltransferase; BLOC-1 related complex subunit 7
Breeding Protocol(s)	Sib-mating
Protocol Date	11/22/2022

PCR Reaction

	<u>1X</u>
ddH ₂ O	12.5
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	1
DNA	2

2,2 Primer Reactions (WF+WR for Wild-type and MF+MR for the mutant reaction)

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 55C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: Apex Taq

Bands: WT: 236bp MUTANT: ~500bp

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

As(69604)WF: GGTAGAGCATGTGAGTTAGC

As(69604)WR: TTCCTCAACTGCACACATAG

As(69604)MF: TTTACCCCAATTTCACTATGAAG

As(69604)MR: GCTCCATCTGCTAAATCTACATCC



Wild-type Reaction

1. 100bp Ladder (Invitrogen)
2. Homozygous Sample
3. Homozygous Sample
4. Homozygous Sample
5. Homozygous Sample
6. Wild-type Control

Mutant Reaction

7. 100bp Ladder (Invitrogen)
8. Homozygous Sample
9. Homozygous Sample
10. Homozygous Sample
11. Homozygous Sample
12. Wild-type Control