

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	37641
MMRRC Strain Name	B6J.129P2(Cg)- <i>Bhmt</i> ^{tm1.2Zei} /Mmnc
Gene Name(s)	betaine-homocysteine methyltransferase
Breeding Protocol(s)	Random Intra-strain Mating
Protocol Date	7/23/2025

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 (WT1+WT2 for wild-type and KO1+KO2 for mutant)

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: 327bp MUTANT: ~550bp

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

Bhmt(37641)WTF: GGGAGTGGGGCTTTGGAAT

Bhmt(37641)WTR: AGCCAGAGAGCCTTTATCGC

Bhmt(37641)KO1: AGAGGTTCTAGTTGTTGGTG

Bhmt(37641)KO2: AACACAGGTGCAATCTGGTAG