

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	29885
MMRRC Strain Name	B6;129S4- <i>Dnmt3a</i> ^{tm3.1Enl} <i>Dnmt3b</i> ^{tm5.1Enl} /Mmnc
Gene Name(s)	DNA methyltransferase 3A; DNA methyltransferase 3B
Breeding Protocol(s)	Random Intra-strain mating (homo x homo) Backcross to C57BL/6J
Protocol Date	8/3/2023

Dnmt3a 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

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 60C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: Apex Taq

Bands: WT: 160bp MUTANT: 220bp

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

Om142F: CTG TGG CAT CTC AGG GTG ATG AGC A

Om144R new: AGG TGG AGC ACT GAA CAG CAA G

Dnmt3b 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

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 65C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: **Apex Taq**

Bands: WT: 270bp MUTANT: 406bp

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

Forward 5'- AGA GCA CTG CAC CAC TAC TGC TGG A

Reverse 5'- CAG GTC AGA CCT CTC TGG TGA CAA G