

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	29887
MMRRC Strain Name	B6.129S4(Cg)- <i>Dnmt3b</i> ^{tm5.1Enl} /Mmnc
Gene Name(s)	DNA methyltransferase 3B (Dnmt3b)
Breeding Protocol(s)	Intra-Strain Mating
Protocol Date	12/16/13

MMRRC# 29887 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 3 min
 Step 2: 94°C for 30 sec
 Step 3: 65°C for 45 sec
 Step 4: 72°C for 45 sec
 Steps 2-4 35x

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

	<u>1X</u>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	0.5
20 uM Primer F/R	1.0
Taq	0.5
ddH ₂ O	15.0
25mM MgCl ₂	2.0

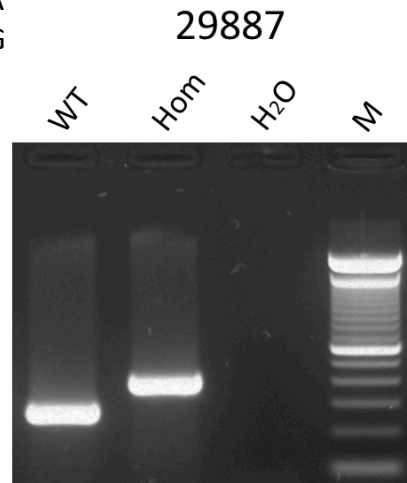
Bands: MUTANT: 406 bp
 WT: 270 bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

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

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

Run on 2.0% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)