

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	29884
MMRRC Strain Name	B6.129S4- <i>Dnmt3a</i> ^{tm2Enl} /Mmnc
Gene Name(s)	DNA methyltransferase 3A (Dnmt3a)
Breeding Protocol(s)	Intra-strain mating
Protocol Date	7/16/13

MMRRC #29884 PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

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
 Step 5: 35x from step 2 to step 4
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

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

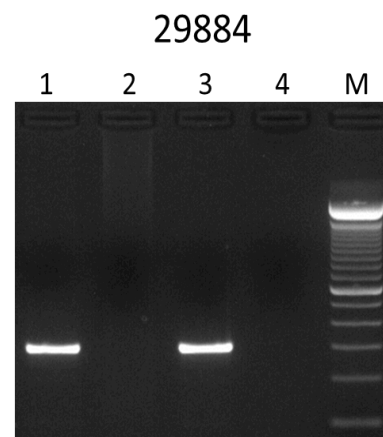
3a Comm: TCC CAG GGT TCC ATG TTA GGT CTG T

IRES-MUT: ACA CTC CAA CCT CCG CAA ACT CCT A

Bands expected:

MUTANT: 259 bp

Run on 1% agarose gel in TAE.



Lane 1, 3: Het; Lane 2: WT; Lane 4: H₂O; M: 100 bp DNA ladder (Invitrogen)