

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34256
MMRRC Strain Name	B6.129(C)- <i>Mthfd2</i> ^{tm1Rmk} / <i>Mmnc</i>
Gene Name(s)	methylenetetrahydrofolate dehydrogenase (NAD ⁺ dependent), methenyltetrahydrofolate cyclohydrolase (<i>Mthfd2</i>)
Breeding Protocol(s)	Backcross to C57BL/6NCrl Intra-Strain (-/+ x -/+), line is -/- lethal
Protocol Date	3/1/11

MMRRC# 34256 PCR Reaction

NOTE: Run 2 reactions for each sample (NMDMC 1+ NMDMC7 for wild-type) and (NMDMC5 + NMDMC7 for mutant)

Thermal Cycler:

94°C for 5 min
 94°C for 30 sec
 64°C for 30 sec
 72°C for 30 sec
 35x
 72°C for 10 min

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

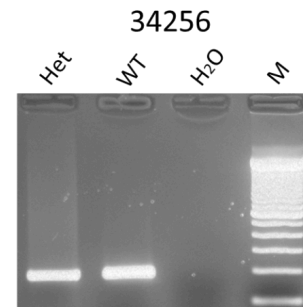
Bands: MUTANT: 141bp
 WT: 194bp

Primer sequences 5' to 3':

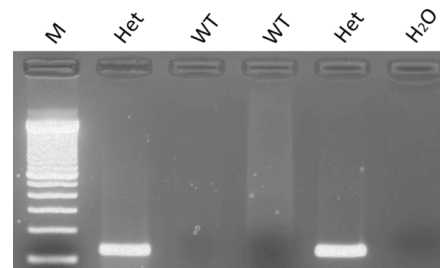
NMDMC-1: 5'-GAA AGT GCT AAG ACC AGA GGA GC-3'
 NMDMC-5: 5'-GCA TCG CCT TCT ATC GCC TTC TT-3'
 NMDMC-7: 5'-GCG CGC ACA CAG CCA CCC GTT-3'

Run on 2.0% agarose gel in TAE.

	<u>1X</u>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	0.5
10 uM Primer F/R	1/1
Taq	0.5
ddH ₂ O	14.0
25mM MgCl ₂	2.0



Primers: NMDMC 1 + NMDMC7
 M: 100 bp DNA ladder (Invitrogen)
 34256



Primers: NMDMC5 + NMDMC7
 M: 100 bp DNA ladder (Invitrogen)