

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34667
MMRRC Strain Name	B6NCrI.FRA(Cg)- <i>Smpd3</i> ^{fr0} /Mmnc
Gene Name(s)	sphingomyelin phosphodiesterase 3, neutral; fragilitas ossium (<i>Smpd3</i>)
Breeding Protocol(s)	Sib-mate (heterozygous x heterozygous)
Protocol Date	6/27/13

MMRRC # 34667 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 60°C for 30 sec
 Step 4: 72°C for 30 sec
 Step 2 to 4 Cycles: 35
 Step 6: 72°C for 7 min

Taq: **Apex and chromataq Buffer**

	1X
ddH ₂ O	12
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 uM Primer WT-F	1
10 uM Primer Mut-F	1
10 uM Primer TM R	1
Taq	0.5
DNA	2

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

TM-WT-F(34668): 5'-GCCAACGTGTGACTAGAGCCAGTCC-3'

TM-Mut-F(34668): 5'-CTCCCCTTCTTCTCCCTTTACGTTATG-3'

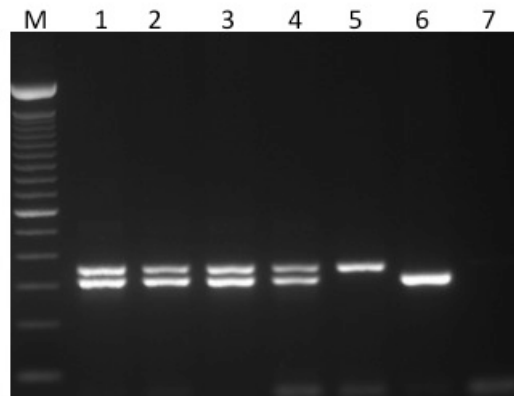
TMR(34668):GCTTCAGGGCACCAACATTTTAGAGG-3'

Bands: WT-F (34668) + TM R (34668): WT 316 bp

Mut-F (34668) + TMR (34668): MUTANT: 358 bp

34667

Run on 3 % agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen); Lane 1-4: Het; Lane 5: Hom; Lane 6: WT; Lane 7: ddH₂O