

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34668
MMRRC Strain Name	C3NcrI.FRA(Cg)- <i>Smpd3</i> ^{fro} /Mmnc
Gene Name(s)	sphingomyelin phosphodiesterase 3, neutral; fragilitas ossium (<i>Smpd3</i> ^{fro})
Breeding Protocol(s)	Sib-mating
Protocol Date	1/14/13

MMRRC# 34668 PCR Reaction

NOTE: 1, 3 primer 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 5X Buffer**

	<u>1X</u>
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 Rev	1
Taq	0.5
DNA	2

Bands: MUTANT: 358bp

WT: 316bp

Primer sequences 5' to 3':

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

Mut-F(34668): 5'-CTCCCCTTCTTCTCCCTCTTACGTTATG-3'

Rev(34668): 5'-GCTTCAGGGCACCAACATTTTAGAGG-3'

Run on 3.0% agarose gel in TAE.