

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	419
MMRRC Strain Name	C3;B6-Tg(SOD1*)125Dbo/Mmnc
Gene Name(s)	Superoxide dismutase 1, soluble(amyotrophic lateral sclerosis 1) (SOD 1 Human)
Breeding Protocol(s)	Tg males x female C3/B6J FI
Protocol Date	1/27/14

MMRRC #419 PCR Reaction

	1X
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

Note: This strain genotyping requires *Fat1* restriction digestion to distinguish between the strain #416 and the strain #419.

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 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.

hSOD1-F (416): GAGGATTAAGGGTAGCGTGTGGTGGT

hSOD1-R (416): GCCTCAGACTACATCCAAGGGAATGTTTA

Bands expected:

Tg+: 423 bp

WT: no band

***Fat1* restriction digestion mix for each PCR sample**

9.6 μ l H₂O

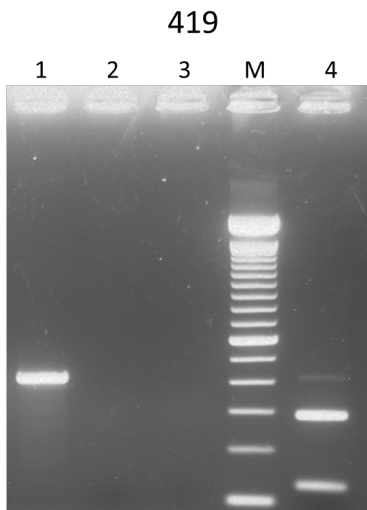
4.0 μ l NEB Buffer 2, 10X

0.4 μ l BSA 100X

1.0 μ l *Fat1*

Add 15 μ l Mix to each 25ul PCR product: incubate at 55 °C for 3 hours - overnight. Then 80°C for 20 minutes

Expected Results: Tg+: 296 bp and 127 bp



Lane 1: PCR product of Tg+; Lane 2: WT; Lane 3: H₂O; Lane 4: Tg+ after *Fat1* digestion; M: 100 bp DNA ladder (Invitrogen)