

12.23.10 MS
09.03.14 MLS

Genotyping Protocol: **MMRRC 29882**

Assay Type: PCR - can not distinguish heterozygous animals from homozygous animals; Can distinguish transgene positive animals from transgene negative animals.

DNA Extraction: DNA from tail snips was extracted using Sigma's RedExtract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50 µl of Extraction Solution, 12.5 µl Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Strain Information: This is a transgenic line carrying the *MrgA1* gene driven by the tetO promoter. *MrgA1* is a Gq GPCR, a receptor protein involved in calcium signaling. Details can be found in Fiacco et al. (2007) Neuron. 54(4):611-26.

Primer Information:

- 1) Name: M29882 F Sequence: 5'- CAA TGT CTA CAT CAT GGC AGA C -3'
- 2) Name: M29882 R Sequence: 5'- GAT TAT GAT CCC TCA GAG GTC -3'

Primer Location: The forward primer amplifies part of the GFP gene. The reverse primer amplifies part of the mouse transthyretin (*Ttr*) gene.

Assay Name: tetO-MrgA1 PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
M29882 F	Sigma-Genosys	25µM	0.3
M29882 R	Sigma-Genosys	25µM	0.3
sterile water			5.4

PCR Setup:

Final Reaction: 16µl master mix & 4µl DNA template (10-20 ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 3 minutes
- 2) 94°C 1 minute
- 3) 63°C 1 minute
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

All products were analyzed on a 3% agarose gel with ethidium bromide staining

Transgene positive: 350bp product

Transgene negative: no product

Control DNA: transgene positive and wild-type (negative) animals.

Example of Gel:

- Wells 1-3 are transgene positive.
- Wells 4 and 5 are negative controls.
- Well 6 is a wild-type control.
- Well 7 is 1 Kb Plus DNA ladder (Invitrogen Cat.# 10787-018).

