

Genotyping Protocol: **MMRRC 248**

Assay Type: PCR- cannot distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat# XNAT2R). Kit directions for fresh or frozen tails were performed with a few minor modifications as follows: use 50 µl of Extraction Solution and 12.5 µl of Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Strain Description: This strain carries a transgene in which GFP is inserted directly before the coding region of *Sept4*.

Primer Information:

- 1) Name: GFP F Sequence: 5'-CGC ACC ATC TTC TTC AAG GAC GAC-3'
 2) Name: GFP R Sequence: 5'-AAC TCC AGC AGG ACC ATG TGA TCG-3'

Primer location: GFP F and R are located in the GFP transgene.

Assay name: GFP PCR

PCR Master Mix Components:

Component	Manufacturer	Concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat# XNAT2R)	2X	10
GFP F	IDT	25µM	0.3
GFP R	IDT	25µM	0.3
Sterile Water			5.4

PCR Setup:

Final Reaction: 16µl master mix & 4µl DNA template (10-20ng/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 30 seconds
- 3) 60°C 30 seconds
- 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

Expected products:

Transgene positive: 375 bp
 Transgene negative: no product

Example gel:

Lane 17 is a transgene negative sample (no product).
 Lane 18 is a transgene positive sample (375bp band).
 Lane 19 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).

