

Genotyping Protocol: **Rosa26 GFP mouse**

Assay Type: PCR - can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy kit. Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Primer Information:

1) Name: Rosa26 s1 F Sequence: TCT CAG ACT AGC CAG AGC TAT ACA
2) Name: Rosa26 s1 R Sequence: CTG GCT TTC TTC CCT CAT TTC CCT
2) Name: Rosa26 3F-3 Sequence: CAC TGC ATT CTA GTT GTG GTT TGT CC

Primer location: Rosa26 s1 F & R on either side of insertion on mouse chromosome 3-F2 @ 91.31 Mb

Primer location: Rosa26 3F-3 in Rosa26/GFP transgene

PCR Master Mix Components:

| component | manufacturer | concentration | µl/rxn |
|------------------|---------------------|----------------------|---------------|
| buffer | Roche | 10X | 2 |
| dNTP | Roche | 1.25mM | 3.2 |
| Rosa26 s1 F | IDT | 25µM | 0.3 |
| Rosa26 s1 R | IDT | 25µM | 0.3 |
| Rosa26 3F-3 | IDT | 25µM | 0.3 |
| FastStart taq | Roche | 5 U/µl | 0.2 |
| sterile water | | | 12.7 |

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template

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 5 minutes
- 2) 94°C 30 seconds
- 3) 61°C 30 seconds
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 7minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

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

Wildtype = 357 bp

Transgene = 268 bp & 203 bp