

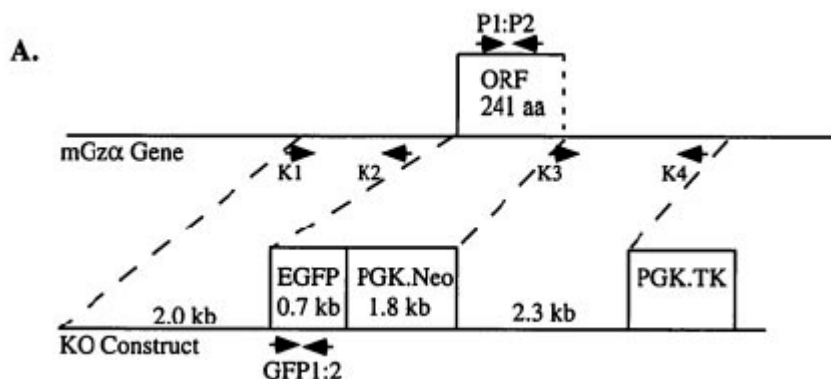
10.12.09 MS
 07.30.10 HB updated
 07.29.13 MLS

Genotyping Protocol: **MMRRC 29580**

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

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). 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.

Strain Description: In this strain, the coding region of the guanine nucleotide binding protein, alpha z subunit gene (*Gnaz*) was replaced by an enhanced green fluorescent protein (EGFP) promoter trap cassette. Details can be found in Yang et al (2000) Proc. Nat. Acad. Sci. 97:9984-89.



Primer Information:

- | | |
|-------------------------|---|
| 1) Name: M29580 mGzP28f | Sequence: 5'-CTG GGC ACC AGC AAC TCG G-3' |
| 2) Name: M29580 mGzP29 | Sequence: 5'-GTT CTG ACC TCT GCC CGC C-3' |
| 3) Name: M29580 GFP1 | Sequence: 5'-GAT CGT CGA CCA TGG TGA GCA AGG GCG-3' |
| 4) Name: M29580 GFP2 | Sequence: 5'-TCT AGA CTC TAC TTG TAC AGC TCG TCC-3' |

Primer location: M29580 mGz28f is located at the beginning of exon 3 and M20580 mGzP29 is located at the end of exon 3, both in the *Gnaz* gene on Chromosome 10. M29580 GFP1 and GFP2 are located on the inserted cassette.

Assay name: **Gnaz tm1Lfb PCR**

Mut PCR:

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M29580 GFP1	Sigma	25µM	0.3
M29580 GFP2	Sigma	25µM	0.3
FastStart Taq	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:

Final Reaction: 19µl master mix & 1µl 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.

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Cycle Parameters:

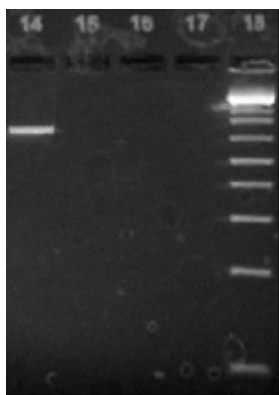
- 1) 95°C 3 minutes
- 2) 94°C 30 seconds
- 3) 68°C 30 seconds
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

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

Expected product: 700bp

Example gel:



Lane 14 displays a sample positive for the mutant allele (700bp band).
Lanes 15 and 16 are blanks.
Lane 17 is a WT control – no band.
Lane 18 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).

WT PCR:

PCR Master Mix Components:

component	manufacturer	concentration	μl/rxn
buffer	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M29580 mGzP28f	Sigma	25μM	0.3
M29580 mGzP29	Sigma	25μM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/μl	0.2
sterile water			13

PCR Setup:

Final Reaction: 19μl master mix & 1μl 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.

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Cycle Parameters:

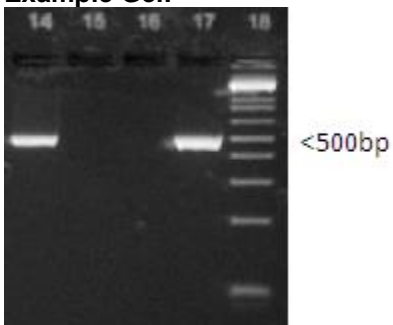
- | | | |
|----|--|--------------------------------|
| 1) | 95°C | 3 minutes |
| 2) | 94°C | 30 seconds |
| 3) | 70°C | 30 seconds |
| 4) | 72°C | 30 seconds |
| 5) | Repeat steps 2-4 34 times for a total of 35 cycles | |
| 6) | 72°C | 10 minutes |
| 7) | 4°C | hold until refrigerate product |

Product Analysis:

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

Expected products: 500bp

Example Gel:



Lane 14 is a sample positive for the WT allele (500bp band).
Lanes 15 and 16 are blanks.
Lane 17 is a WT control (500bp band)P.
Lane 18 is 1 Kb+ Ladder (Invitrogen Cat# 10787-018).

Genotype Analysis:

Homozygous: 700bp band with the Mut PCR, no band with the WT PCR

Heterozygous: 700bp band on the Mut PCR and 500bp band on the WT PCR

Wild type: 500bp band with the WT PCR, no band with the Mut PCR