MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36805
MMRRC Strain Name	B6.Cg-Tg(Rgs21-TagRFP)6Dski/Mmnc
Gene Name(s)	regulator of G-protein signalling 21 (Rgs21), Tag-RFPT variant of Red Fluorescent Protein (TagRFP(Sea anemone, Entacmaea quadricolor))
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	7/29/13

MMRRC #36805 PCR Reaction

Note: Genomic DNA prepared by "HotSHOT" — NaOH digestion buffer.
See reference: Biotechniques. 2000. 29(1): 52

Reagents

Alkaline Lysis Reagent	
Reagent	Final concentration
NaOH	25 mM
Disodium EDTA	0.2 mM
pH will be 12	

Neutralizing Reagent	
Reagent	Final concentration
Tris-HCl	40 mM
pH will be 5.	

Protocol

1. Obtain tissue
   a. 0.2 cm tail snip or 25-mg pieces of spleen
   b. 2 mm ear punch biopsy
2. Place tissue in a 96 well thermal cycler plate or thermal cycler strip tubes.
3. Add 75 µL of Alkaline Lysis Reagent
4. Heat to 95°C for 10 min to 1 h (30 min is optimal)
5. Cool to 4°C
6. Add 75 µL Neutralization Buffer
7. Use 1 to 5 µL per PCR reaction

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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

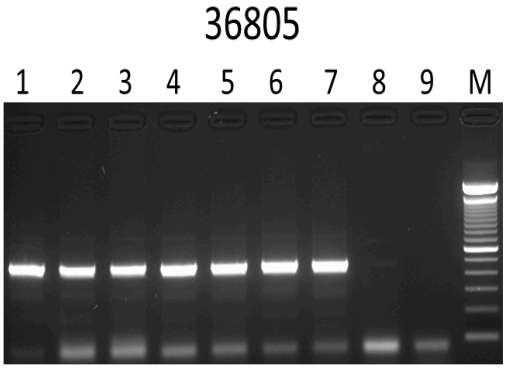
Taq: **Apex and Chromataq 5X Buffer**

	1X
ddH_2O	13
5X Buffer	5
25mM $MgCl_2$	2
10mM dNTPs	0.5
10 µM Primer Forward	1
10 µM Primer Reverse	1
Apex Taq	0.5
DNA	2

Primer sequences 5' to 3': Primers are 10 µM with respect to each primer
polya-F: GGAAGACAATAGCAGGCATGCTGGGGATGC
polya-R: GCTGCTTCCTTACCCAGCACTGTCTAGCCAT

Bands expected: Tg+, 480bp

Run on 1% agarose gel in TAE.



Lane 1-7: Tg+; Lane 8: WT; Lane 9: H_2O; M: 100 bp DNA ladder (Invitrogen)

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