

## MMRRC UNC – Genotyping Protocol

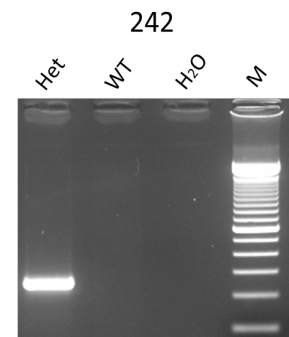
<b>MMRRC Strain ID</b>	242
<b>MMRRC Strain Name</b>	STOCK Tg(Cadps-EGFP)CQ69Gsat/Mmnc
<b>Gene Name(s)</b>	Ca <sup>2+</sup> dependent secretion activator (Cadps)
<b>Breeding Protocol(s)</b>	Backcross to Swiss Webster (Taconic)
<b>Protocol Date</b>	3/10/14

### MMRRC #242 PCR Reaction

	<b><u>1X</u></b>
ddH <sub>2</sub> O	8.5
5X Buffer	5.0
25 mM MgCl <sub>2</sub>	1.5
10 mM dNTPs	0.5
10 μM Primer Forward	1.0
10 μM Primer Reverse	1.0
Denville Taq (5U/μl)	0.5
5 M Betaine	5
DNA	2

#### **Thermal Cycler:**

Step 1: 94°C for 5 min  
 Step 2: 94°C for 30 sec  
 Step 3: 58°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



Primers: TM-Com-F (242) + TM-Mut-R (242)  
M: 100 bp DNA ladder (Invitrogen)

#### **Taq: Denville Taq (5U/μl) and Chromataq 5X Buffer**

**Primer sequences 5' to 3':** Primers are 10 μM with respect to each primer.

TM-Com-F (242): ACACTCGGCATCTTTTCTGGAGGC

TM-WT-R (242): TCCTCTCCAGGATCTCATCTGACTCC

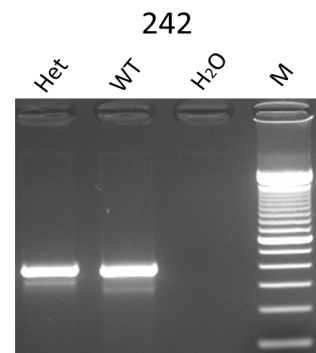
TM-Mut-R (242): TCGCCGGACACGCTGAACTT

#### **Bands expected:**

WT: TM-Com-F (242) + TM-WT-R (242), 394 bp

MUTANT: TM-Com-F (242) + TM-Mut-R (242), ~260 bp

Run on 2% agarose gel in TAE.



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