

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	243
<b>MMRRC Strain Name</b>	STOCK Tg(Chrna3-EGFP)BZ135Gsat/Mmnc
<b>Gene Name(s)</b>	cholinergic receptor, nicotinic, alpha polypeptide 3
<b>Breeding Protocol(s)</b>	Outcross to Swiss Webster
<b>Protocol Date</b>	3/13/20

### PCR Reaction

	<u>1X</u>
ddH <sub>2</sub> O	13
5X Buffer	5.0
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10 uM Primer F	1
10 uM Primer R	1
Taq	0.5
DNA	2

**Note: This protocol has not been utilized by the MMRRC . Primers are given to us by Genesat. Therefore, the MMRRC will have limited ability to aid in troubleshooting**

#### **Thermal Cycler:**

Step 1: 94C, 5min  
 Step 2: 94C, 30sec  
 Step 3: (~T<sub>m</sub>-5C), 30sec  
 Step 4: 72C, 30sec  
 Step 2 to 4 Cycles: 30  
 Step 5: 72C, 7min

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

Bands: MUTANT: ~650bp  
 WT: No Band

Primer sequences 5' to 3':

Chrna(243) 5ABox: GGCGCGCCCAGCATCGTGCTGAAAATAGTT  
 Chrna(243) EGFP-R2: TAGCGGCTGAAGCACTGCA

Run on 2.0% agarose gel in TAE.