

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	264
<b>MMRRC Strain Name</b>	STOCK Tg(Mc3r-EGFP)BX153Gsat/Mmnc
<b>Gene Name(s)</b>	Melanocortin 3 receptor (Mc3r)
<b>Breeding Protocol(s)</b>	Outcross to Swiss Webster (Taconic)
<b>Protocol Date</b>	10/16/12

### MMRRC# 264 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

**2,2 Primer Reactions (Com-F + WT-R) for Wild-type and (Com-F + Mut-R) for the mutant reaction**

**Thermal Cycler:**

94°C for 30 sec

61°C for 30 sec

72°C for 30 sec

35x

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

**Bands:** MUTANT: 290bp

WT: 325bp

**Primer sequences 5' to 3':**

Com-F (264): AGGAAAGTTCTTTCTATGTCTCCAAGCCC

WT-R (264): GATGAAGACCTGCTCACAGAACCCAC

Mut-R (264) : TGAAGTGTGGCCGTTTACGTCG

Run on 2.0% agarose gel in TAE.