

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	16998
MMRRC Strain Name	B6;129S-Myh10 ^{tm6(Myh9/GFP)Rsad} /Mmnc
Gene Name(s)	Myosin, heavy polypeptide 10, non-muscle (Myh10)
Breeding Protocol(s)	Sib-mating (homo lethal)
Protocol Date	8/27/10

MMRRC# 16998 PCR Reaction

Step 1: 94°C for 5 min
 Step 2: 94°C for 45 sec
 Step 3: 60°C for 60 sec
 Step 4: 72°C for 60 sec
 Step 5: Steps 2-4, 34 cycles
 Step 6: 72°C for 10 min
 Step 7: 4°C, indefinite

	1X
DNA	1.0
5X Buffer	5.0
10mM dNTPs	0.5
30 uM Primer F/R	1.5
Taq	0.5
ddH ₂ O	14.5
25mM MgCl ₂	2.0

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

Bands: MUTANT: 1000bp
 WT: 500bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (30uM total).

Primer 1: 5'-GTAGCTATGGCCTGTAGTCAC

Primer 2: 5'-CTCAGATGCTTGGGACATGT

Primer 3: 5'-TTCGCAGCGCATCGCCTTCTA

Run on 1.0% agarose gel 100mV for 2 hours