

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	34323
<b>MMRRC Strain Name</b>	B6;129S4-Myh9 <sup>tm4(GFP/MYH10/MYH9)Rsad</sup> /Mmnc
<b>Gene Name(s)</b>	myosin, heavy polypeptide 9, non-muscle (Myh9)
<b>Breeding Protocol(s)</b>	Sib-mating
<b>Protocol Date</b>	7/22/13

### MMRRC #34323 PCR Reactions

#### Mutant Reaction

**Thermal Cycler:**

Step 1: 94°C for 5 min  
 Step 2: 94°C for 30 sec  
 Step 3: 53°C for 30 sec  
 Step 4: 72°C for 60 sec  
 Step 5: 35x from step 2 to step 4  
 Step 6: 72°C for 7 min

	<u>1X</u>
ddH <sub>2</sub> O	13.0
5X Buffer	5.0
25mM MgCl <sub>2</sub>	2.0
10mM dNTPs	0.5
10 μM Primer Forward	1.0
10 μM Primer Reverse	1.0
Taq	0.5
DNA	2.0

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

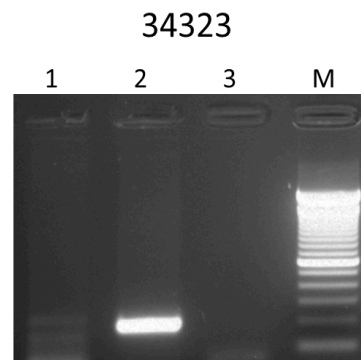
**Primer sequences 5' to 3':** Primers are 10 μM with respect to each primer.

TM4-FOR (34323): ACCTGAAATTACGGCACTGG

TM4-REV (34323): CTGCAATTTCTCTGCCATGA

**Bands expected:** MUTANT: ~200bp  
 WT: no band

Run on 2.0% agarose gel in TAE.



Mut Primers: TM4-FOR (34323) + TM4-REV (34323)

Lane 1: WT; Lane 2: Het; WT; Lane 3: H<sub>2</sub>O; M: 100 bp DNA ladder

Page 1 of 2

## Wild Type Reaction

**Thermal Cycler:**

- Step 1: 94°C for 5 min
- Step 2: 94°C for 30 sec
- Step 3: 51°C for 30 sec
- Step 4: 72°C for 60 sec
- Step 5: 35x from step 2 to step 4
- Step 6: 72°C for 7 min

	<u><b>1X</b></u>
ddH <sub>2</sub> O	13
5X Buffer	5.0
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10 μM Primer Forward	1.0
10 μM Primer Reverse	1.0
Taq	0.5
DNA	2

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

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

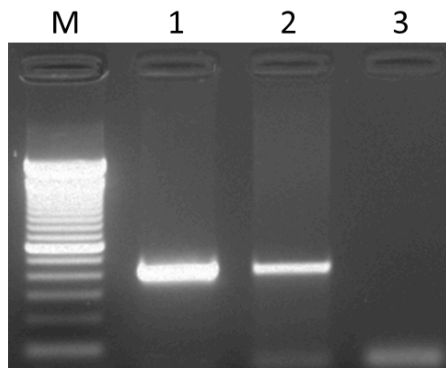
P1 (34321): TCATGTTCTGTCGTTGTCC

P2 (34321): CAGTGGGATAAAGAGACTCC

**Bands expected:** 421 bp

Run on 2% agarose gel in TAE.

**34323**



WT Primers: P1 (34321) + P2 (34321)

Lane 1, 2: WT; Lane 3: H<sub>2</sub>O; M: 100 bp DNA ladder