

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34322
MMRRC Strain Name	B6;129S4-Myh9 ^{tm3(GFP/MYH9/MYH10)Rsad} /Mmnc
Gene Name(s)	myosin, heavy polypeptide 9, non-muscle (Myh9)
Breeding Protocol(s)	Sib-mating
Protocol Date	7/22/13

MMRRC #34322 PCR Reactions

Mutant Reaction

Thermal Cycler:

- Step 1: 94°C for 5 min
- Step 2: 94°C for 30 sec
- Step 3: 57°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

Taq: Apex and Chromataq 5X Buffer

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

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

TM3-FOR (34322): CCGACGTCATCATAGGGTTC

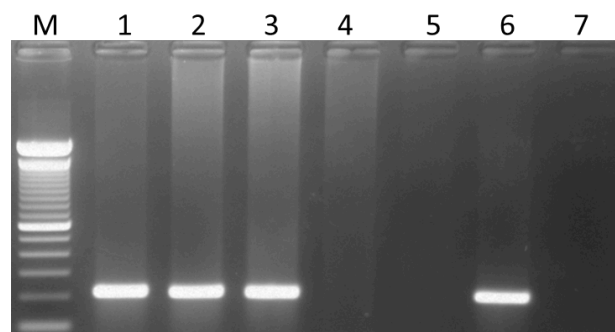
TM3-REV (34322): GGCCTGAAGTCTCTCCTCT

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Bands expected: MUTANT: ~200bp

WT: no band

Run on 2.0% agarose gel in TAE.



Mut Primers: TM3-FOR (34322) + TM3-REV (34322)

Lane 1-3, 6: Het; Lane 4, 5: WT; Lane 7: H₂O; M: 100 bp DNA ladder

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>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	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

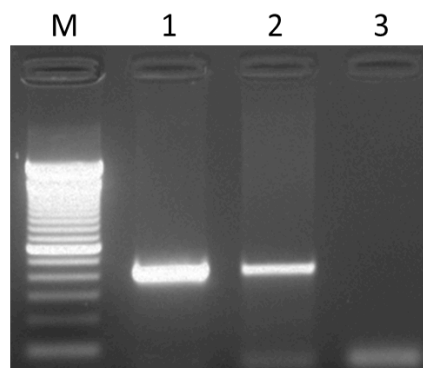
Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

P1 (34321): TCATGTTCTGTCGTTGTCC
 P2 (34321): CAGTGGGATAAAGAGACTCC

Bands expected: 421 bp

Run on 2% agarose gel in TAE.

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

Lane 1, 2: WT; Lane 3: H₂O; M: 100 bp DNA ladder