

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36210
MMRRC Strain Name	STOCK Myh9 ^{tm7R_{sad}} /Mmnc
Gene Name(s)	myosin, heavy polypeptide 9, non-muscle; targeted mutation 7, Robert S Adelstein (Myh9 ^{tm7(EGFP/MYH9)R_{sa}})
Breeding Protocol(s)	Sib-mating
Protocol Date	6/27/13

MMRRC #36210 PCR Reaction

Thermal Cycler:

Step1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 55°C for 30 sec

Step 4: 72°C for 30 sec

Step 5: Step 2 to 4, Cycles: 35

Step 6: 72°C for 7 min

Taq: **Apex and chromataq Buffer**

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

Primers are 10uM with respect to each primer

Primer sequences 5' to 3':

DN1(36210): CGCTCTTCTAGAGGACAAGTTCCA

DN2 (36210): CAGAGACTCCTCTAGCACTGTA

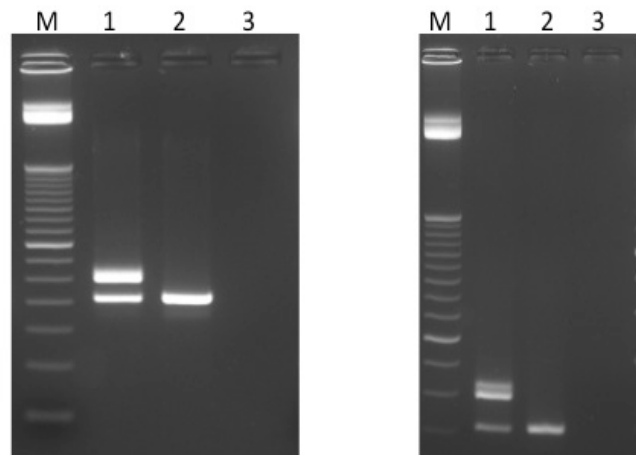
Bands: WT ~200bp

MUTANT ~ 250 bp

* Heterozygous mutant has an unspecific band at ~270bp

Run on 2% agarose gel in TAE.

36210



Lane 1: Het; Lane 2: WT; Lane 3: ddH₂O; M: 50 bp DNA ladder Invitrogen