

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36196
MMRRC Strain Name	STOCK Myh9 ^{tm6} (EGFP/MYH9*) ^{Rsad} /Mmnc
Gene Name(s)	myosin, heavy polypeptide 9, non-muscle (Myh9); Myh9 ^{tm6.1(EGFP/MYH9*)^{Rsad}} (Mouse/Human)
Breeding Protocol(s)	Sib-mate
Protocol Date	6/27/13

MMRRC #36196 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 90°C for 30 sec
 Step 3: 58°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

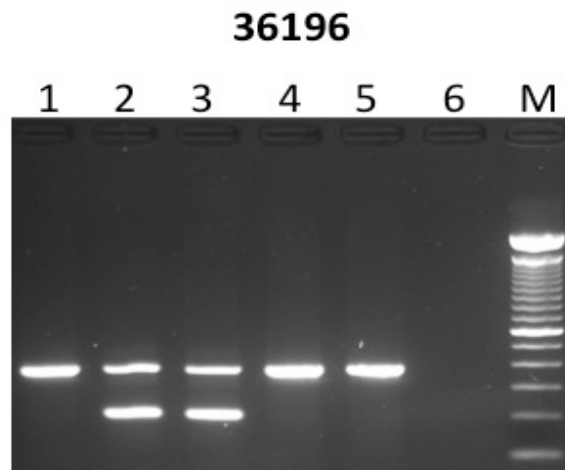
Primers are 10uM with respect to each primer

Primer sequences 5' to 3'

R1: CTGTCACATGGCTCATGTTC
 R2: GCCGGACACGCTGAACTTGT
 R3: GCCCTGAGTAGTATCGCTCC

Bands: R1+R3, WT: 394bp
 R1+R2, MUTANT: 200bp

Run on 2% agarose gel in TAE.



Lane 1, 5 - 6: WT; Lane 2 – 3: Het; Lane 6: ddH₂O;
 M: 100 bp DNA ladder Invitrogen