MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	70
MMRRC Strain Name	B6.Cg-*Myo5aflr Gnb5flr*/Mmnc
Gene Name(s)	Guanine nucleotide binding protein, beta 5/flailer (Gnb5) Mysoin Va/Flailer (Myo5a)
Breeding Protocol(s)	Intra-strain (Mutant x WildType)
Protocol Date	9/30/13

MMRRC# 70 PCR Reaction

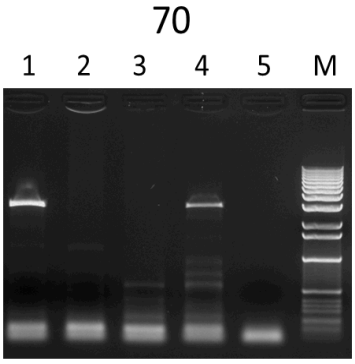
	1X
DNA	1.0
5X Phusion Buffer HF	4.0
10mM dNTPs	1.0
20 uM Primer 1 & 2	1.0
Phusion Taq	0.2
ddH_2O	12.7
DMSO	0.6

Initial – 98°C for 2 min
Denaturing: 98°C for 15 sec
Annealing Temp.: 62°C for 30 sec
Extension: 72°C for 2min
Cycles: 36
Taq: **NEB Phusion Poly. Kit**

Bands: MUTANT 3kb

NOTE: ***Dilute DNA 1:5 in nuclease-free H20 before adding to reaction!***

Set up one 2-primer reaction. Primer pair is: G1F & M5R



Lane 1, 4: Mut; Lane 2, 3 : WT; Lane 5 : H_2O
M: 1Kb DNA ladder (Invitrogen)

Primers are 10uM with respect to each primer (20uM total).

Primer sequences 5' to 3':
G1f (70) TGC AAA GAC AAG CGG AGA ATC GTG
M5R (70) GGA ATA GTG ACA TAT CCA GAG GCA CC

Run on **0.8%** agarose gel in TAE.

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