

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	33038
MMRRC Strain Name	B6;129- <i>Hprt1</i> ^{tm5(Myh7-GFP,Myh6-CFP)Unc} /Mmnc
Gene Name(s)	hypoxanthine guanine phosphoribosyl transferase (Hprt)
Breeding Protocol(s)	Sib-Mated
Protocol Date	4/8/11

MMRRC# 33038 PCR Reaction

NOTE: This is a 4 Primer Reaction.

Thermal Cycler:

Step 1: 94°C 5 min
Step 2: 94°C 30 sec
Step 3: 57°C 30 sec
Step 4: 72°C 1 min
35 cycles step 2-4
Step 5: 72°C 7 min

	<u>1X</u>
DNA	2.0
5X Buffer	5.0
10mM dNTPs	0.5
10 uM Primer	1/1/1/1
Taq	0.5
ddH ₂ O	13.0
25mM MgCl ₂	2.0

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

Bands: MUTANT: 406bp
WT: 504bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

GFP FOR2(33038): 5'- GCCTGCAGGAGATCATGAGCTT

GFP REV2(33038): 5'- GCCGGTGATGGTCTTCTTCA

Hprt-3F(33038): 5'- TCCAATTTGTAGACCAGACTGGCCTT

Hprt-3R(33038): 5'-TACCGCTCCGGAAAGCAGTGAG

Run on 2.0% agarose gel in TAE.