

MMRRC UNC – Genotyping Protocol

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| MMRRC Strain ID | 10480 |
| MMRRC Strain Name | B6;129- <i>Btrc</i> ^{tm1Paga} /Mmnc |
| Gene Name(s) | Beta-transducin repeat containing protein (Btrc) |
| Breeding Protocol(s) | Intra-Strain Mating (Het x WT) |
| Protocol Date | 12/16/13 |

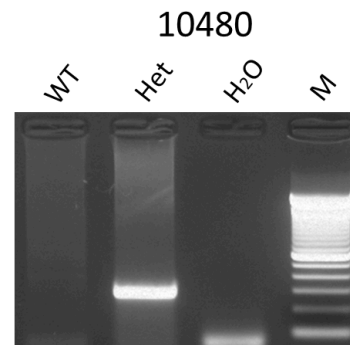
MMRRC# 10480 PCR Reaction

| | <u>1X</u> |
|------------------------|-----------|
| DNA | 2.0 |
| 5X Buffer | 5.0 |
| 10mM dNTPs | 0.5 |
| 20 uM Primer F/R | 1.0 |
| Taq | 0.5 |
| ddH ₂ O | 14.0 |
| 25mM MgCl ₂ | 2.0 |

NOTE: 2 PCR Reactions: (Beta-CMD3+BetaWTD1) and (BetaCMD3 + Betaneo)

Thermal Cycler:

94°C for 30 sec
 56°C for 30 sec
 72°C for 60 sec
 36x



Primers: Beta-CM-D3 + Betaneo
 M: 100 bp DNA ladder (Invitrogen)

Taq: Denville and Chromataq 5X Buffer

Bands: MUTANT: 261bp
 WT: 372bp

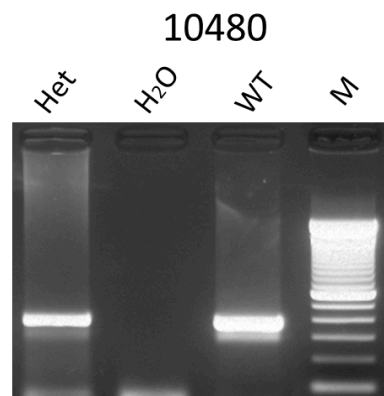
Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

Beta-CM-D3 5'- CTTCTTATCTAACAGAAGATGGA

Beta Wt-D1 5'- TCCTGACCATCCTCTCGATG AGC

Betaneo 5'- TCTAATTCCATCAGAAGCTG ACT

Run on 2.0% agarose gel in TAE.



Primers: Beta- CM-D3 + BetaWT-D1
 M: 100 bp DNA ladder (Invitrogen)