

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	16822
MMRRC Strain Name	129S6.129X1(B6)- <i>Cyp1b1</i> ^{tm1Gonz} /Mmnc
Gene Name(s)	Cytochrome P450, family 1, subfamily b, polypeptide 1 (<i>Cyp1b1</i>)
Breeding Protocol(s)	Sib-mating
Protocol Date	1/6/14

MMRRC #16822 PCR Reaction

NOTE: Run 2 reactions for each sample (*Cyp1b1* F/R for WT) and (Neo F/R for Mutant).

Thermal Cycler:

For WT Reaction

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 52°C for 30 sec
 Step 4: 72°C for 30 sec
 Step 5: Repeat 35x
 Step 6: 72°C for 10 min

For Mutant Reaction

Step 1: 94°C for 5 min
 Step 2: 94°C for 45 sec
 Step 3: 60°C for 45 sec
 Step 4: 72°C for 60 sec
 Step 5: Repeat 36x
 Step 6: 72°C for 10 min

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

Taq: Denville (5U/ul) for WT and Apex Taq for Mutant and Chromataq 5X Buffer

Bands: MUTANT: 424bp
 WT: 187bp

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

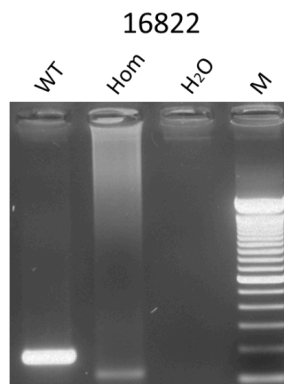
Cyp1b1 FOR1: 5'-TTCTCCAGCTTTTGCCTGT

Cyp1b1 REV1: 5'-TAATGAAGCCGTCCTTGTC

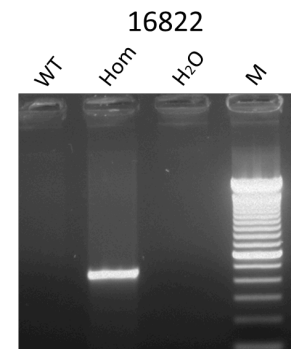
Neo FOR: 5'- ATTGAACAAGATGGATTGCAC

Neo REV: 5'- CGTCCAGATCATCCTGATC

Run on 2.0% agarose gel in TAE.



Primers: *Cyp1b1* FOR 1+*Cyp1b1* REV1
 M: 100 bp DNA ladder (Invitrogen)



Primers: Neo FOR + Neo REV
 M: 100 bp DNA ladder (Invitrogen)