

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34368
MMRRC Strain Name	B6.129S4- <i>Gcdh</i> ^{tm1Dmk} /Mmnc
Gene Name(s)	glutaryl-Coenzyme A dehydrogenase (<i>Gcdh</i>)
Breeding Protocol(s)	Random Intra-strain Mating
Protocol Date	7/22/13

MMRRC #34368 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 72°C for 2 min

Step 4: 35x from step 2 to step 3

Step 5: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3':

Primers are 10 µM with respect to each primer.

KF: TTAGGCCTAGTGTGCTGGTCCCGGA

KR: TCTGGTGCCGGAACAGGCAAAGC

F13: CTCCGTAAGTACTGCCAGGAGCGG

R21: AGCTCTCGGGTCAGGAGCCCATAGG

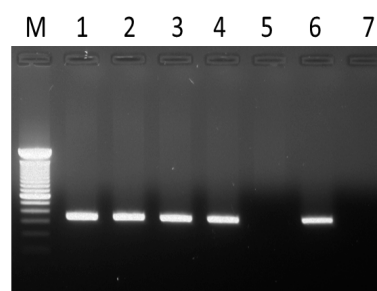
Bands expected: WT: primer F13 + R21: 565 bp

MUTANT: primer KF + KR: ~390 bp

Run on 2% agarose gel in TAE. Combine two Rxn products to run gel

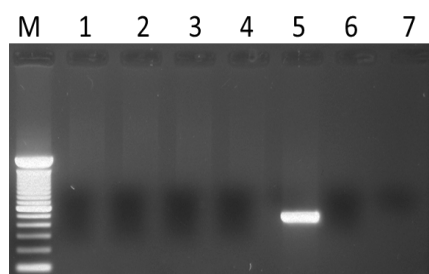
	1X
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10 µM Primer Forward	1
10 µM Primer Reverse	1
Apex Taq	0.5
DNA	2

34368 (Mut Rxn)



Mut primers: KF + KR

34368 (WT Rxn)



WT primers: F13 + R21

Lane 1-4, 6: Hom; Lane 5: WT; Lane 7: H₂O; M: 100 bp DNA ladder (Invitrogen)

Lane 1-4, 6: Hom; Lane 5: WT; Lane 7: H₂O; M: 100 bp DNA ladder (Invitrogen)