

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34369
MMRRC Strain Name	C57BL/6J- <i>Cars2</i> ^{m1B^{tlr}} /Mmnc
Gene Name(s)	cysteinyl-tRNA synthetase 2 (mitochondrial)(putative)
Breeding Protocol(s)	Intra-Strain Mating
Protocol Date	1/13/14

MMRRC# 34369 PCR Reaction

NOTE: Run 2 reactions for each sample (WT-For +WT-Rev) and (WT-Rev + Mut-For).

Thermal Cycler:

94°C for 30 sec

58°C for 30 sec

72°C for 30 sec

35x

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

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

Bands: MUTANT: 168bp

WT: 168bp

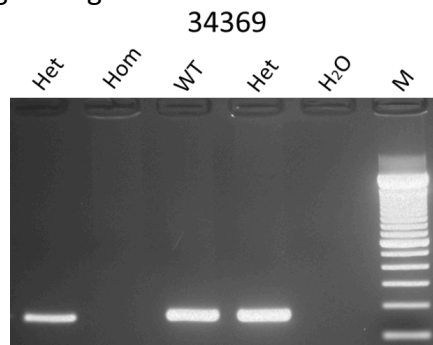
Primer sequences 5' to 3':

Cars2-WT-For: 5'- GCATGTGAAAGGCACAGAAGAAAAGATCT-3,'

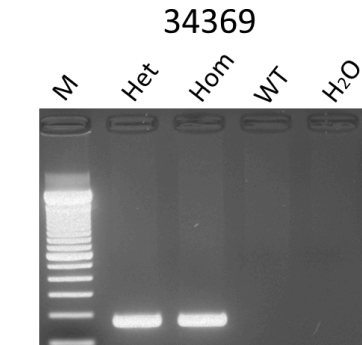
Cars2-WT-Rev: 5'- ATGCAGGGCTAGGTCAGACTACATGTCTA-3'

Cars2-MUT-For: 5'- GCATGTGAAAGGCACAGAAGAAAAGATCA-3',

Run on 2.0% agarose gel in TAE.



Primers: WT- For +WT-Rev
M: 100 bp DNA ladder (Invitrogen)



Primers: WT-Rev + Mut-For
M: 100 bp DNA ladder (Invitrogen)