

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	10859
MMRRC Strain Name	B6.129- <i>Asna1</i> ^{tm1Hbha} /Mmnc
Gene Name(s)	arsA(bacterial) arsenite transporter, ATP-binding, homolog 1 (Asna1)
Breeding Protocol(s)	Sib-mating
Protocol Date	1/27/14

MMRRC #10859 PCR Reaction

	1X
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 55°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 32x from step 2 to step 4
 Step 6: 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.

P85 (10859): CAG AGG GTC AGA TAA TGT GTC

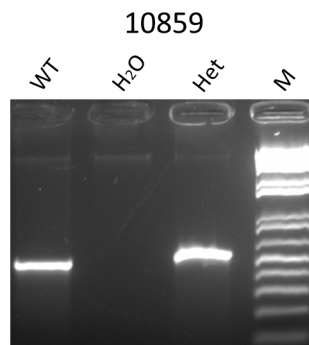
P90 (10859): CGG GCC TCA CAC ATC TTG

P142 (10859): CGC CTT CTA TCG CCT TCT TGA CGA GTT CTT

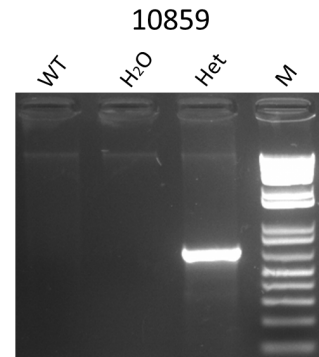
Bands expected: WT, P85 (10859) + P90 (10859): 552 bp

MUTANT, P142 (10859) + P90 (10859): 660 bp

Run on 2 % agarose gel in TAE.



Primers: P85(10859) + P90 (10859)
 M: 1Kb plus DNA ladder (Invitrogen)



Primers: P142 (10859) + P90 (10859)
 M: 1Kb plus DNA ladder (Invitrogen)