PROTOCOL 2

Degenerate s*igB* PCR using GoTaq Green

Objective: To amplify a 570bp fragment of the *sigB* gene to be used for sequence determination and subsequent phylogenetic analyses.

In PCR room, aseptically prepare the Master Mix using GoTaq polymerase and its accompanying 5X Green buffer and 25 mM $MgCl_2$. GoTaq polymerase should be added last to the Master Mix as shown in the Master Mix set-up below. Do not votex the GoTaq polymerase tube. Mix thoroughly and aliquot 49 ul of master mix to each PCR tube. Keep the tube rack on ice until placed in a thermocycler.

Master Mix:	**1X (uL) (50 uL rxn)**
dH_2O	33.75
5X Green buffer	5.0
25 mM $MgCl_2$	4.0
10mM dNTP's	2.0
12.5uM sigBdegF	2.0
12.5uM sigBdegR	2.0
5 u/µL GoTaq polymerase	0.25
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	49 ul per tube

At a lab bench, add 1 ul of DNA template to corresponding tubes of Master Mix.

PCR Cycling Conditions:

3 min @ 95˚C
30 sec @ 94˚C
30 sec @ 60˚C
1 min @ 72˚C
} *20 cycles (decreases by 0.5˚ C per cycle)
30 sec @ 94˚C
30 sec @ 50˚C
1 min @ 72˚C
} *20 cycles
7 min @ 72˚C
∞ @ 4˚ C

Primer sequences:

sigBdegF (forward) = 5' – ATGAAAAGCAGGTGGAGGAGAATGC – 3'
sigBdegR (reverse) = 5' - CCSGTTTCTTTTTGACTRCGRTTTTC -3'