PROTOCOL S1

s*igB* PCR using AmpliTaq Gold (updated version)

Objective: To amplify a 780bp fragment of the *sigB* gene to be used for sequence determination and subsequent phylogenetic analyses.

In PCR room, aseptically prepare the Master Mix using Amplitaq and its accompanying 10X PCR buffer and 25 mM $MgCl_2$. Amplitaq gold should be added last to the Master Mix as shown in the Master Mix set-up below. Do not votex the Amplitaq gold 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 (50 ul rxn)**
dH_2O	33.75 ul
Amplitaq 10X PCR buffer	5.0 ul
Amplitaq $MgCl_2$ 25mM	4.0 ul
dNTP's 10mM	2.0 ul
SigB15 12.5uM	2.0 ul
SigB16 12.5uM	2.0 ul
AmpliTaq gold	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:

10 min @ 95˚C
30 sec @ 94˚C
30 sec @ 54-44˚C } *20 cycles (decreases by 0.5˚ C through first 20 cycles)
1 min @ 72˚C
30 sec @ 94˚C
30 sec @ 44˚C } *20 cycles
1 min @ 72˚C
7 min @ 72˚C
∞ @ 4˚ C

Primer sequences:

LM sigB15 (forward) = AATATATTAATGAAAAGCAGGTGGAG
LM sigB16 (reverse) = ATAAATTATTTGATTCAACTGCCTT