

## PROTOCOL S1

### *sigB* PCR using AmpliTaq Gold

**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<sub>2</sub>. Amplitaq gold should be added last to the Master Mix as shown in the Master Mix set-up below. Do not vortex 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.

<u>Master Mix:</u>	<u>1X (50 ul rxn)</u>
dH <sub>2</sub> O	33.75 ul
Amplitaq 10X PCR buffer	5.0 ul
Amplitaq MgCl <sub>2</sub> 25mM	4.0 ul
dNTP's 10mM	2.0 ul
SigB15 10uM	2.0 ul
SigB16 10uM	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 Decreases by 0.5° C through first 20 cycles; Final 20 cycles @ 44° C  
1 min @ 72° C  
7 min @ 72° C  
∞ @ 4° C

#### Primer sequences:

LM sigB15 (forward) = AATATATTAATGAAAAGCAGGTGGAG

LM sigB16 (reverse) = ATAAATTATTTGATTCAACTGCCTT