Modified 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.

Procedure: Using a sterile toothpick, remove a single colony from a pure plated culture. Insert wooden toothpick into a 200ul PCR tube (or well in a 96-well plate) containing 100 ul of sterile water and press/swirl colony into bottom of tube. Microwave PCR tubes or 96-well plate for 3 minutes. Let cool at bench

In PCR room, aseptically prepare the Master Mix using Amplitaq and its accompanying 10X PCR buffer & 25 mM $MgCl_2$. Amplitaq gold should be added last to the Master Mix as shown in the Master Mix set-up below. Mix thoroughly and aliquot 49 ul of master mix to each PCR tube. Put tube rack on ice.

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 10uM	2.0 ul
SigB16 10uM	2.0 ul
AmpliTaq gold	0.25
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	49 ul per tube

** At lab bench, add 1 ul of DNA template (or 1.6 ul if using the multichannel pipettor) to corresponding tubes of Master mix. Remember to change tips in between each DNA template to prevent cross contamination!!

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