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 (containing bacteria) 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. Do not votex the Amplitaq gold tube. Mix thoroughly and aliquot 49 ul of master mix to each PCR tube. Keep tube rack on ice until placed in 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 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 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