McCoy and Macpherson Lab
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Protocol 902_16S PCR

Material needed:

1. Bacterial genomic DNA (it is highly recommended to use the Qiagen Stool Kit according to our modified protocol and using the Retsch bead beater to prepare genomic DNA from complex mixtures of bacteria, such as intestinal content, or the quick and dirty method for bacterial isolates as described in protocol 901)

2. Primers:
   - fD1: AGA GTT TGA TC**C** TGG CTC AG
   - fD2: AGA GTT TGA TC**A** TGG CTC AG
   - rP1: ACG GTT ACC TTG TTA CGA CTT

   - working concentration is 10µM (stock concentration is 100µM)

Procedure:

5µl	10X Taq polymerase buffer
1.5µl	$MgCl_2$
1µl	10mM dNTP stock
1µl	primer 1 (of 10µM stock)
1µl	primer 2 (of 10µM stock)
1µl	primer 3 (of 10µM stock)
34.1µL	water
0.4µL	Qiagen Taq

Pipette 5µL of DNA sample into PCR tubes

Program:

First step:	94ºC	5 min
35× repeat of:		
	94ºC	1 min
	43ºC	1 min
	72ºC	2 min
last step:		
	72ºC	7 min
	10ºC	forever

Reference:

1. Weisburg, W.G., S.M. Barns, D.A. Pelletier, and D.J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173:697-703.