ILLUMINA 16S AMPLICON LIBRARIES

Objective

- Use PCR to create Illumina 16S sequencing libraries

Principle: The 16S (aka 'small subunit' or SSU) ribosomal RNA gene is present in the genomes of all bacteria and archaea. It is possible to design 'universal' PCR primers that anneal to highly conserved sequences within 16S rRNA genes that will amplify a product that includes more variable intervening sequences. By amplifying 16S gene sequences from a community of microorganisms, it is possible to identify which organisms are present and in what relative abundances. We will use this strategy to survey the microbial diversity of our soil samples by amplifying the 'v4' variable region of the 16S rRNA gene.

The bacterial ribosome. The small subunit rRNA is in green.

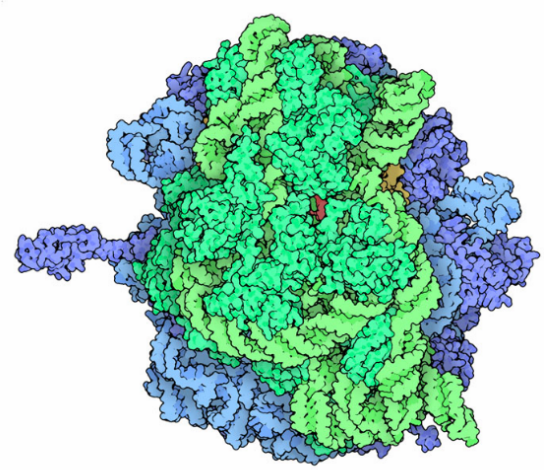
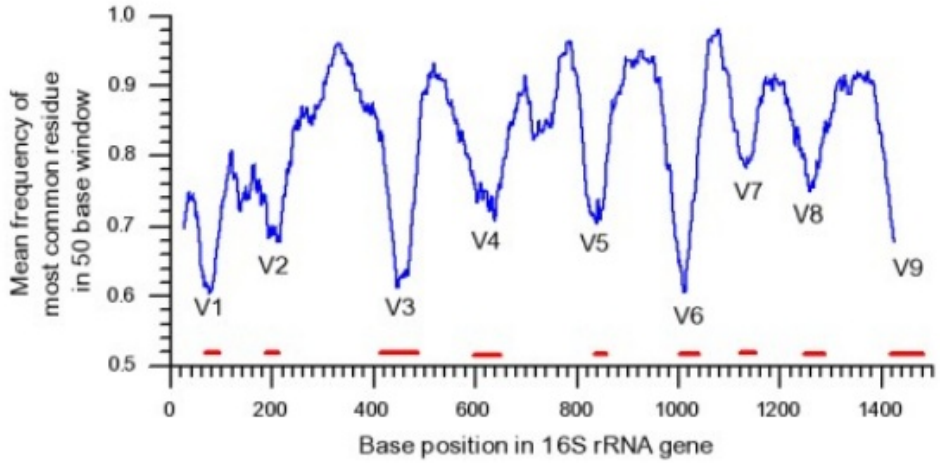


Image credit: http://pdb101.rcsb.org/motm/121

Levels of sequence conservation across bacterial rRNA genes.



Materials and Equipment

- Soil DNA sample
- 2x Crystal Taq Master Mix**
- 16S primers (see below)
- PCR tubes
- Thermal cycler
- Pipets (10 µl; 20 µl; 200 µl) and tips

**Note that for true sequencing library construction, it is important to use a high-fidelity polymerase such as KAPA HiFi or NEB Q5. For the purposes of the demonstration, we will use a regular Taq polymerase, which would introduce a much larger number of errors into individual library molecules and the corresponding reads if these libraries were actually run on a sequencer.

Primer Sequences

We will use the following two primers. The bolded sequence is the indexing barcode that would differ for each library in a multiplexed pool. The 3´ end of each sequence match conserved regions of the 16S rRNA (with ambiguities to account for common variants that distinguish bacterial species). The 5´ end includes the P5 and P7 elements of Illumina adapters.

v4.SA501
5´-AATGATACGGCGACCACCGAGATCTACAC**ATCGTACG**TATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3´

v4.SA701
5´-CAAGCAGAAGACGGCATACGAGAT**AACTCTCG**AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3´

Protocol

We will essentially be following the Schloss Lab's (http://www.schlosslab.org/) 16S v4 region amplification protocol, described here:

https://github.com/SchlossLab/MiSeq_WetLab_SOP/blob/master/MiSeq_WetLab_SOP.md

1. Setup a PCR as follows. Be careful pipetting 1 µl DNA: make sure that your pipet tip contains liquid and that it gets transferred into the tube.

Reagent	**Stock concentration**	**Final concentration**	**Volume for 1 reaction**
Soil DNA			1 µl
2x Crystal Taq Master Mix	2x	1x	12.5 µl
16S index primers (mix of 2 primers)*	5 µM each	0.5 µM each	2.5 µl
dH2O			9 µl
			25 µl total

2. Run the PCR as follows:

98C	30 sec	
98C 55C 72C	10 sec 20 sec 60 sec**	35 cycles
10C	hold	

**Note that the Schloss lab protocol uses a 5-minute extension time, which they have found reduces chimeric products. Here, we are using a 60 second extension in the interest of time.