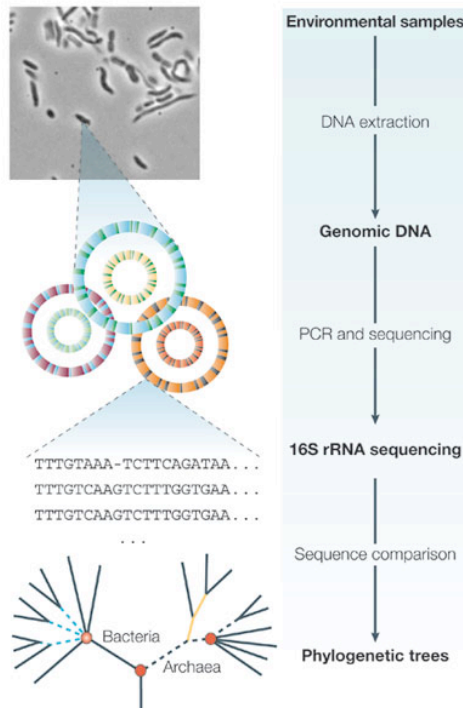


16S rRNA Sequencing

Prokaryotic Community Profiling



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Sequence Analysis

mothur

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Welcome to the website for the mothur project, initiated by [Dr. Patrick Schloss](#) and his software development team in the [Department of Microbiology & Immunology at The University of Michigan](#). This project seeks to develop a single piece of open-source, expandable software to fill the bioinformatics needs of the microbial ecology community. In February 2009 we released the first version of mothur, which had accelerated versions of the popular DOTUR and SONS programs. Since then we have added the functionality of a number of other popular tools including s-libshuff, TreeClimber (i.e. the parsimony test), UniFrac, distance calculation, visualization tools, a NAST-based aligner, and many other features. If you would like to contribute code to the project feel free to download the source code and make your own improvements. Alternatively, if you have an idea or a need, but lack the programming expertise, let us know and we'll add it to the queue of features we would like to add. Our current goal is to release a new iteration of the project monthly.



Department of Microbiology & Immunology
The University of Michigan Medical School
The University of Michigan

This site is maintained by Pat Schloss
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Schloss, P.D., et al., Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol*, 2009, 75(23):7537-41.

The original tools:

Schloss, PD & Handelsman, J. (2006) Introducing SONS, a tool for OTU-based comparisons of membership and structure between microbial communities. *Applied and Environmental Microbiology*, 72:6773-9.

Schloss, PD & Handelsman, J. (2006). Introducing TreeClimber, a test to compare community structures. *Applied and Environmental Microbiology*, 72: 2379-84.

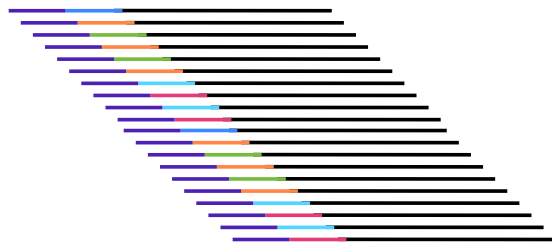
Schloss, PD & Handelsman, J. (2005). Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Applied and Environmental Microbiology*, 71:1501-6.

Schloss, PD, Larget, BR, & Handelsman J. (2004). Integration of microbial ecology and statistics: a test to compare gene libraries. *Applied and Environmental Microbiology*, 70:5485-92.

Sequence Processing

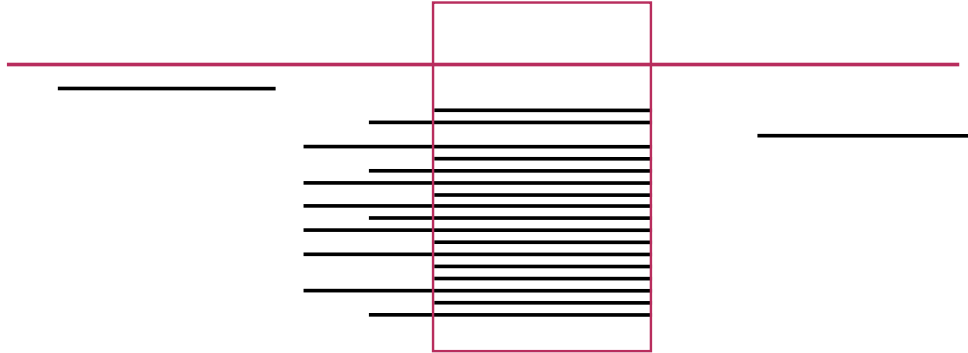
Step I: Denoising, Bin and Trim

- Denoising (using flow grams to reduce errors in sequence)
- Remove Barcodes
- Low Quality Sequences



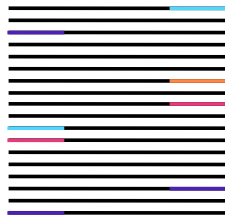
Sequence Processing

Step 2: Align to Reference



Sequence Processing

Step 3: Remove Chimeras



Sequence Processing

Step 4: Classify Sequences

Classification is based on 8-mer frequencies

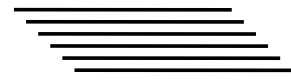
Bacteroidetes



Firmicutes



Actinobacteria

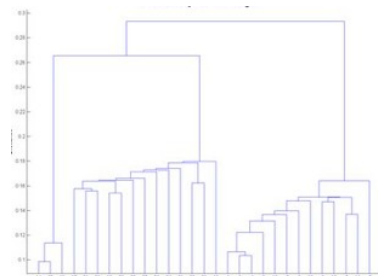
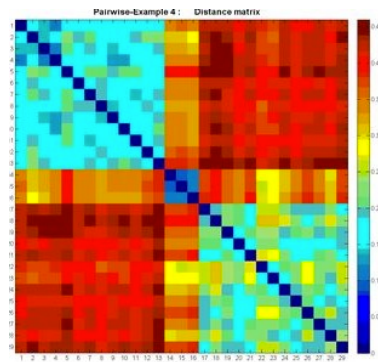


Wang, Q, G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Appl Environ Microbiol.* 73(16):5261-7.

Sequence Processing

Step 5: Determine OTUs

- Calculate distances between sequences using reference based alignment
- Cluster Sequences using a distance cut-off



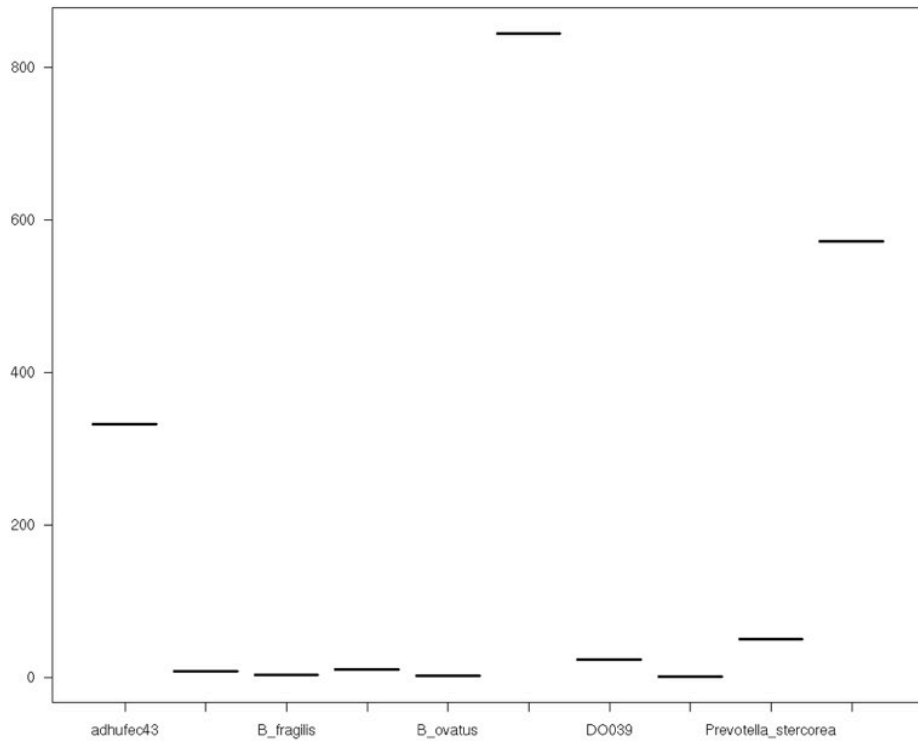
Processed Data

- List of Quality Sequence Reads
- Taxonomic Classification of Reads
- Operational Taxonomic Units of Similar Reads (Species?)

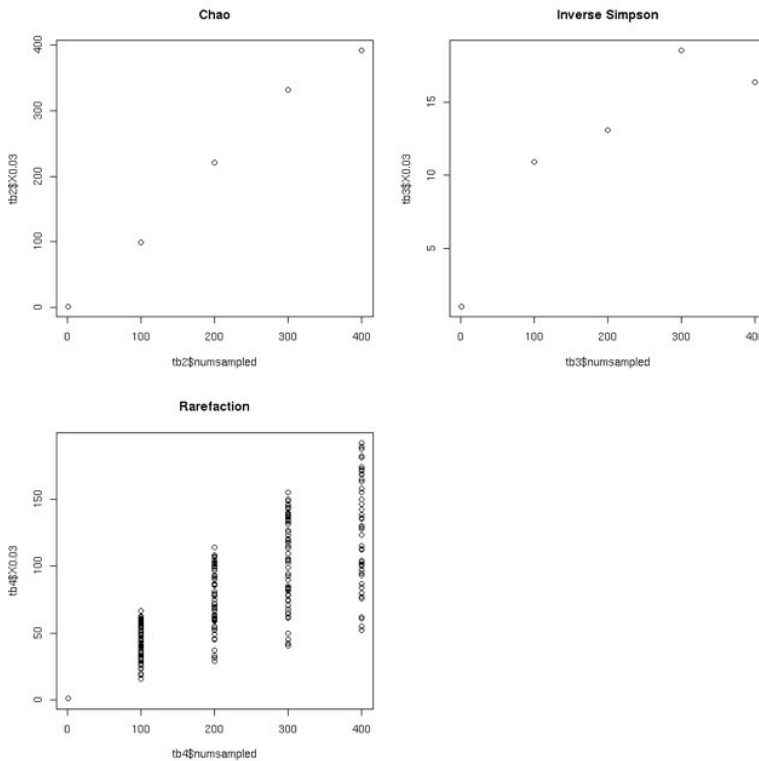
Biological Questions

- What is the taxonomic make-up of the community?
- How rich or diverse is the community?
- How do communities compare?

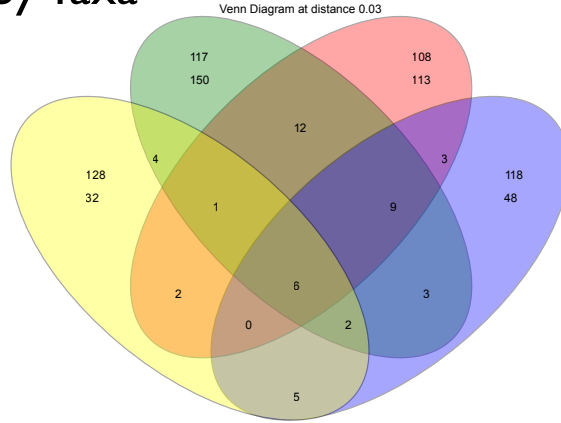
Dominant Bacteroidetes



Reaching Saturation

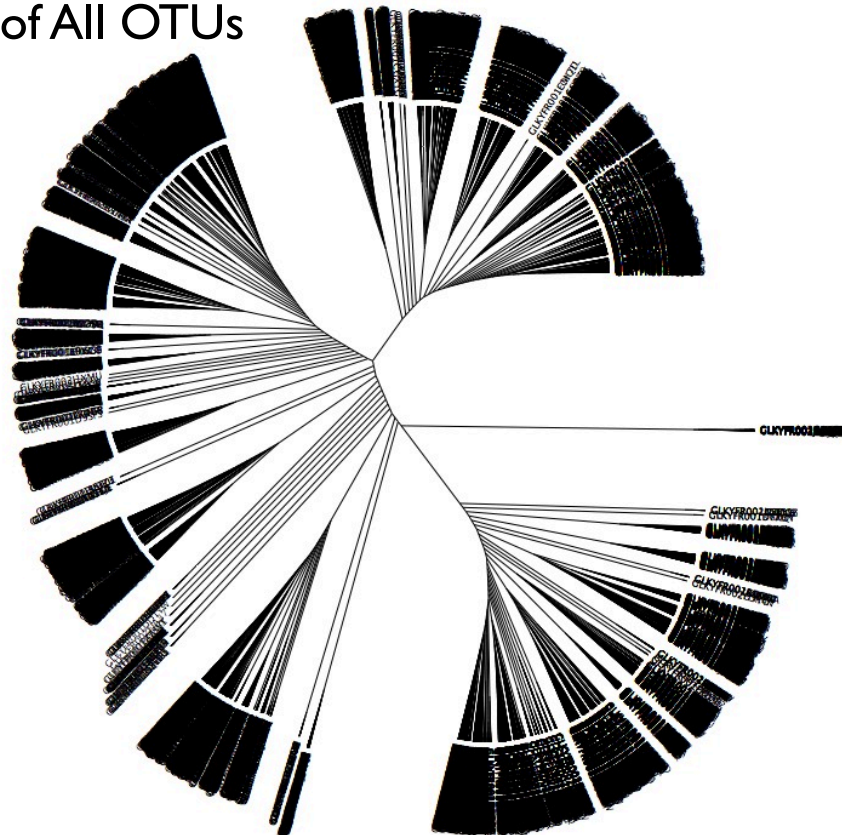


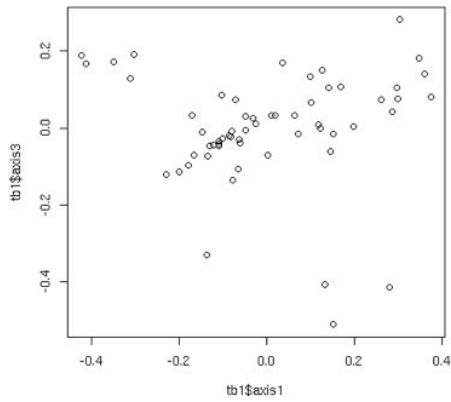
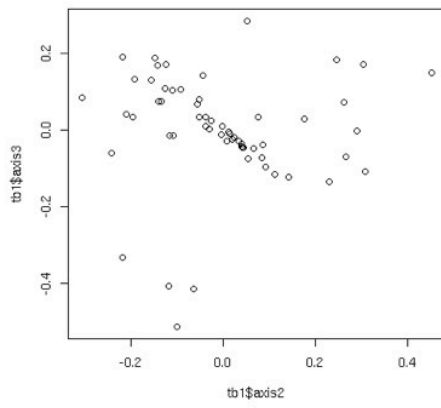
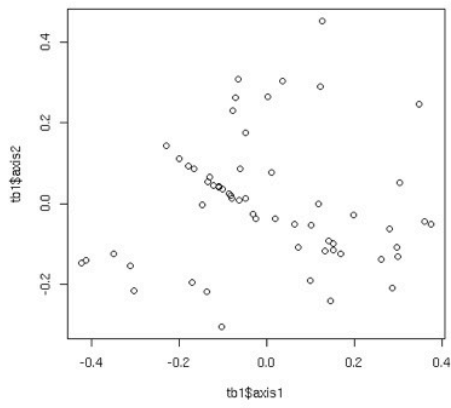
Compare Species Shared by Taxa



The number of species in group 108 is 146
 The number of species in group 117 is 187
 The number of species in group 118 is 76
 The number of species in group 128 is 52
 The number of species shared between groups 108 and 117 is 28
 The number of species shared between groups 108 and 118 is 18
 The number of species shared between groups 108 and 128 is 9
 The number of species shared between groups 117 and 118 is 20
 The number of species shared between groups 117 and 128 is 13
 The number of species shared between groups 118 and 128 is 13
 The number of species shared between groups 108, 117 and 118 is 15
 The number of species shared between groups 108, 117 and 128 is 7
 The number of species shared between groups 108, 118 and 128 is 6
 The number of species shared between groups 117, 118 and 128 is 8
 The total richness of all the groups is 390

Build A Phylogenetic Tree of All OTUs





Separation of Samples?

http://www.mothur.org/wiki/Schloss_SOP