

Of Mudwatts and Microbiomes

Phylogenetic structure of the prokaryotic domain: The primary kingdoms

Carl R. Woese and George E. Fox

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16S rRNA (1.5 kb)



Part of the ribosomal, a structural scaffold organizing ribosomal proteins of the small subunit of the ribosome.

Ubiquitous – all living organisms have this. Composed of very well conserved (green) sequence and hypervariable regions (blue).

Microbiome

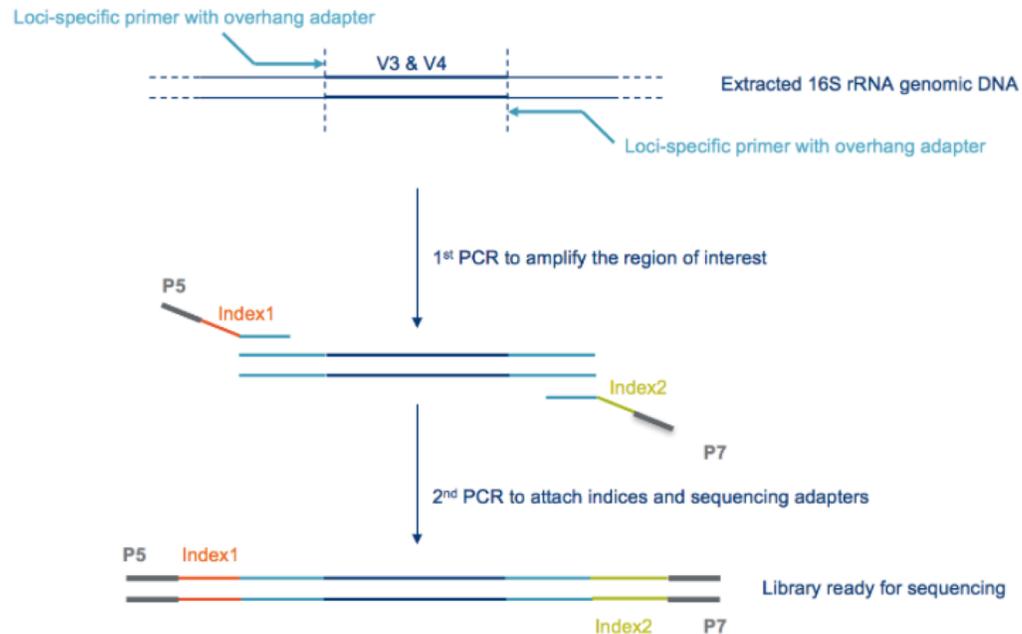
Collection of all the micro-organisms
that exist in an environment of interest

Traditional method to quantify is based on 16S rRNA (bacterial) or 18S rRNA (eukaryotic)

16/18S rRNA is ubiquitous – highly conserved and variable regions
good primers designed; decades of analysis
good reference databases of 16S rRNA sequences (RDP, GreenGenes)
culture independent
established bioinformatics toolkits available

Some bacteria can be cultured, some are hard/impossible to culture or conditions are not known

Need analysis to be culture independent to be comprehensive



Use DNA primers to well conserved regions
Designed to amplify 16S in all/most bacteria

Sequence across variable regions
variable regions are genus or species specific
act as a barcode defining a species

Ribosome

Translates mRNAs to proteins

Bacterial Ribosome (70S)
Two subunits – 50S and 30S

50S subunit – 31 proteins
30S subunit – 21 proteins

S stands for Svedberg coefficient, an old way of measuring size of a protein or complex based on how far it sediments in an ultracentrifuge.
Non-linear, depends on size and conformation.

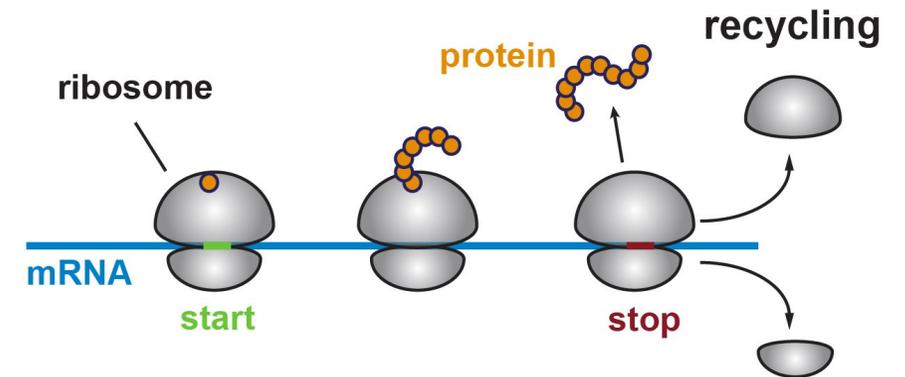
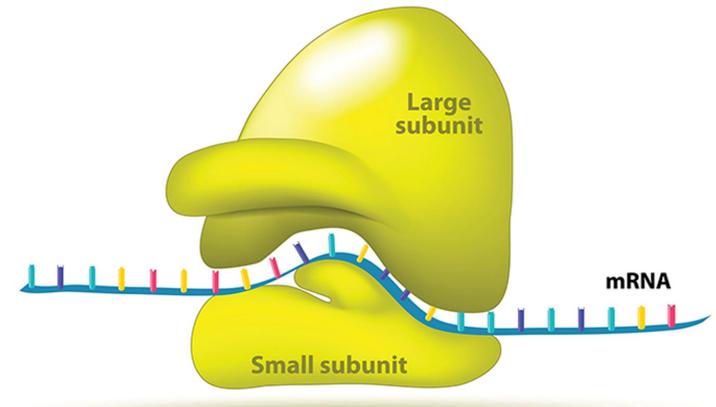
Each are composed of several rRNAs and many proteins

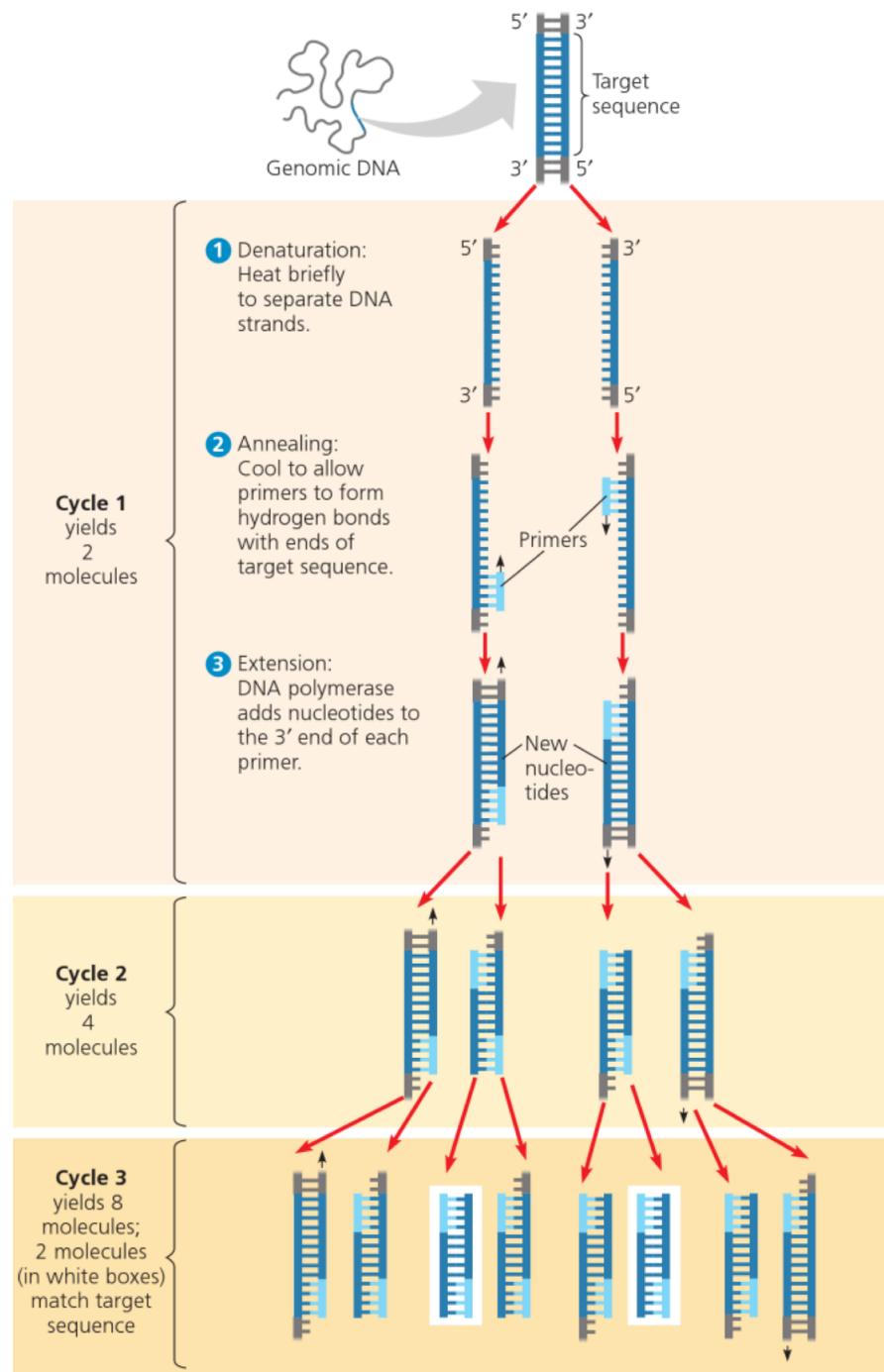
23S (large subunit), 16S (small subunit), and 5S (large subunit) rRNAs

Eukaryotic Ribosome (80S)

60S subunit – 49 proteins
40S subunit – 33 proteins

28S (large subunit), 18S (small subunit), and 5.8S (large subunit) and 5S (large subunit) rRNAs





1 cycle

Denaturing 95C
 Annealing 55-60C
 Extension 72C

Metagenomics based on whole genome sequencing

Metagenome: a collection of assembled genomes of different organisms present in an environment of interest

Advantages

not limited to one specific locus (16S or 18S)

major gaps in eukaryotic reference genomes (skewed to model systems, easily culturable/maintainable organisms and warm and fuzzy creatures)

missing a lot of simpler eukaryotes (protists)

possibility of assembling entirely unknown genomes

not biased towards bacteria

discovery – novel metabolic pathways/natural products

Science, 2004

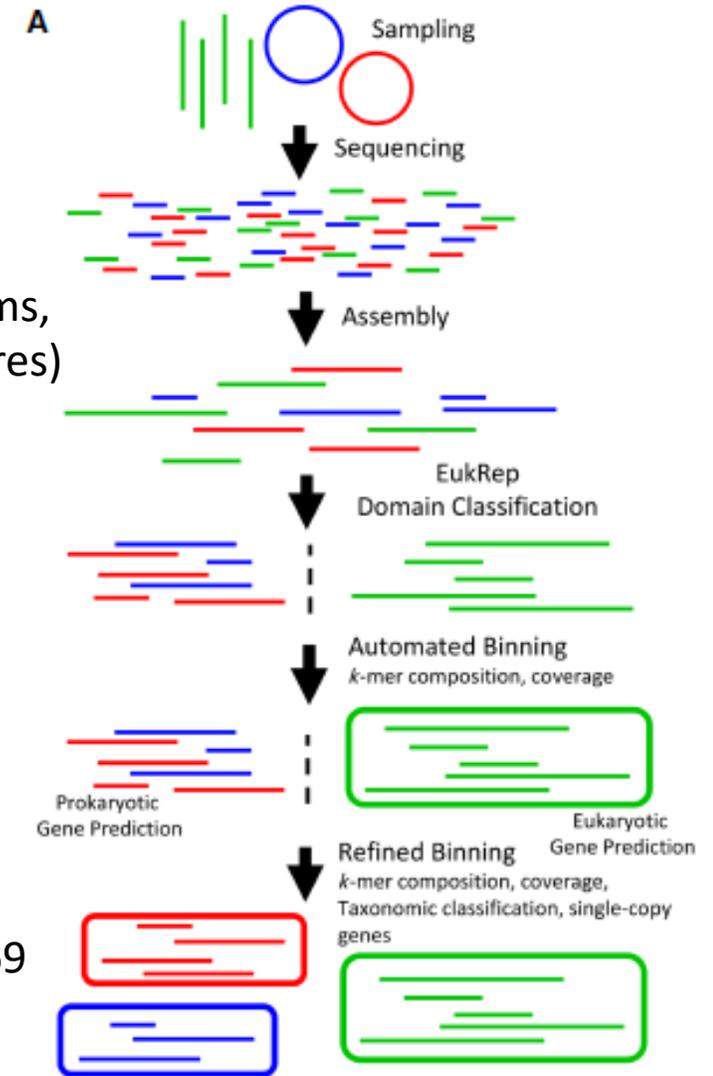


Figure 2
Genome Res 28: 569

RESEARCH ARTICLE

Environmental Genome Shotgun Sequencing of the Sargasso Sea

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 Aaron L. Halpern,² Doug Rusch,² Jonathan A. Eisen,³
 Dongying Wu,³ Ian Paulsen,² Karen E. Nelson,³ William Nelson,³
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 Holly Baden-Tillson,¹ Cynthia Pfannkoch,¹ Yu-Hui Rogers,⁴
 Hamilton O. Smith¹

We have applied "whole-genome shotgun sequencing" to microbial populations collected en masse on tangential flow and impact filters from seawater samples collected from the Sargasso Sea near Bermuda. A total of 1.045 billion base pairs of nonredundant sequence was generated, annotated, and analyzed to elucidate the gene content, diversity, and relative abundance of the organisms within these environmental samples. These data are estimated to derive from at least 1800 genomic species based on sequence relatedness, including 148 previously unknown bacterial phylotypes. We have identified over 1.2 million previously unknown genes represented in these samples, including more than 782 new rhodopsin-like photoreceptors. Variation in species present and stoichiometry suggests substantial oceanic microbial diversity.



“ST26733”, International Conference "Agriculture for Life, Life for Agriculture"

Comparison of Total DNA Extraction Methods for Microbial Community from Polluted Soil

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soil AND microbiome –
2285 results in PubMed

Table 1. Experimental design for comparison of soil DNA extraction methods

Method	Soil pretreatment	Estimated time	DNA extraction
S	0.5 g glass beads 0.5-mm diameter	4h	Modified by Sagova et al. (2008) from Miller et al. (1999)
SP	0.5 g glass beads 0.5-mm diameter	5h	Modified of Sagova et al. (2008)
S-CTAB	0.5 g glass beads 0.5-mm diameter and 0.5g glass beads of 0.1mm diameter	5h	Modified of Sagova et al.(2008)
GnS-GII	0.5 g glass beads 0.5-mm diameter and 0.5g glass beads of 0.1mm diameter	4h	Modified of Plassart et al. (2012)
N	Glass beads provided in the isolation kit	2h	Soil DNA Isolation Kit NorgenBiotech

PCR amplification of the 16S rRNA genes was successful with all DNA extracts, except SP protocol, but resulted in different relative intensity of PCR products (38 successful PCRs out of 50). The intensity of the amplification was higher when 3mg/ml BSA was added to PCR reactions (Figure 3).

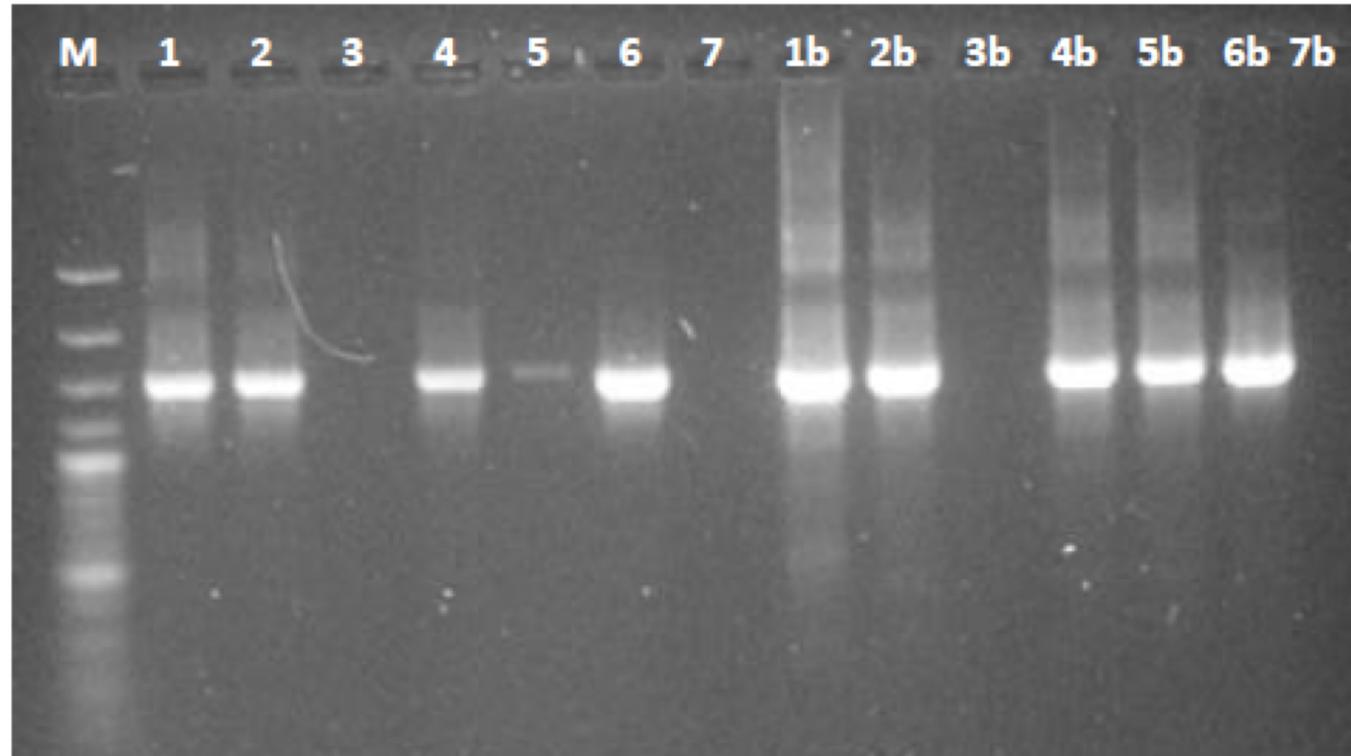


Fig. 3. Agarose gel of 16S rDNA amplicon obtained using various procedures from soil samples (b lanes = +3mg/ml BSA): DNA Ladder λ / EcoRI and HindIII (M); Soil DNA Isolation Kit NorgenBiotek (1, 1b); S (2, 2b); SP(3, 3b); S-CTAB (4, 4b); GnS-GII (5, 5b); *P. aeruginosa* PAO1 (6,6b); No DNA control (7, 7b).

No PCR product of the negative control was detected and the intensity of PCR product for *P. aeruginosa*

DNA purification

Soil (0.5 g) was homogenized on a vortex mixer (S8A Stuart) at 2200 rpm for 5 min with 600 μ l of extraction buffer (50 mM Na-phosphate buffer [pH 8], 50 mM NaCl, 500 mM Tris-HCl [pH 8], and 5% SDS) and 300 μ l of phenol/chloroform-isoamyl alcohol (25:24:1 v/v) and 0.5 g sterile glass beads 0.5-mm diameter, for S and SP protocol.

Resulted lysate was centrifuged at 16000g for 2 minutes. The supernatant was mixed with the same volume of phenol-chloroform-isoamyl alcohol (25:24:1 v/v) and centrifuged at 6,000g for 5 min. The upper layer was transferred and mixed with an equal volume of chloroform-isoamyl alcohol (24:1 v/v) and centrifuged at 16,000g for 5 min for S protocol, and prior to this step at SP protocol, we added a 100 μ l proteinase K stock solution, and incubated for 30 min at 37°C. The supernatant was then precipitated with 1 volume of ice-cold isopropanol, and incubated at -20°C for 20 min.

S protocol worked best, and has the simplest components

Simple extraction buffer:

50 mM Na-phosphate buffer, pH 8

50 mM NaCl

500 mM Tris-HCl, pH 8

5% SDS

Modifications for us:

Use chloroform-isoamyl (24:1) only

The quality of DNA isolates was estimated by quantifying amplification of 16S rRNA genes using the universal primers specific for Eubacteria: GM3F (5'-AGAGTTTGATCMTGGC-3') and GM4R reverse (5'-TACCTTGTTACGACTT-3') according to Muyzer et al. (1995), using a 50°C annealing temperature on a Mini Cyclor MJ Research.

PCR assays were carried out in a 30 µl reaction volume containing PCR Master Mix 2X (ThermoScientific), with and without 3 mg/ml Bovine Serum Albumin (BSA) and 50 µM of each primer, including a no template control and a positive control represented by chromosomal DNA from *P. aeruginosa* PAO1.

Template DNA concentration was determined and we used 25ng of crude DNA extract for each PCR reaction. Amplification products from 10 reactions for each procedure were checked for correct size (1500 bp) by 1% agarose gel electrophoresis.

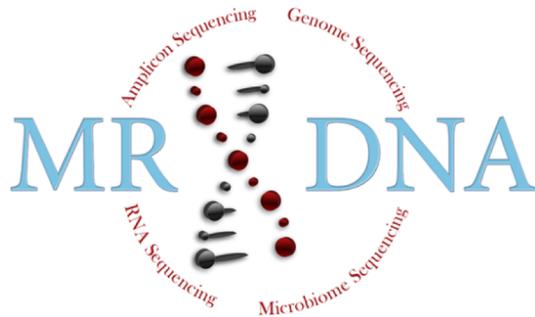


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16S rRNA GENE SEQUENCING

A common method for identifying bacterial strains is analyzing the sequence of the gene coding for 16S ribosomal RNA (16S rRNA). 16S rRNA gene sequencing is also a standard tool for bacterial phylogenetic and taxonomic studies due to the conservation of this gene between different species of bacteria.



Place an Order

<https://www.genewiz.com/en/Public/Services/Molecular-Genetics/16S-rRNA-Sequencing>

Ribosomal Database Project



ANNOUNCEMENTS

RDP News

06/05/2018 RDP phone system restored
RDP phone service is now back online.

06/04/2018 RDP phone system down
RDP phone system is temporarily out of service!

03/05/2018 RDP web tool problems fixed!

03/05/2018 RDP web tool problems

RDP Release 11, Update 5 :: September 30, 2016

3,356,809 16S rRNAs :: 125,525 Fungal 28S rRNAs
Find out what's new in RDP Release 11.5 [here](#).



[Cite RDP's latest tool articles.](#)

RDP provides quality-controlled, aligned and annotated Bacterial and Archaeal 16S rRNA sequences, and Fungal 28S rRNA sequences, and a suite of analysis tools to the scientific community. New to RDP release 11:

- RDP tools have been updated to work with the new fungal 28S rRNA sequence collection.
- A new Fungal 28S Aligner and updated Bacterial and Archaeal 16S Aligner. We optimized the parameters for these secondary-structure based Infernal aligners to provide improved handling for partial sequences.
- Updated RDPipeline offers extended processing and analysis tools to process high-throughput sequencing data, including single-strand and paired-end reads.
- Most of the RDP tools are now available as open source packages for users to incorporate in their local workflow.

<https://rdp.cme.msu.edu/index.jsp>



RDP Staff

Ribosomal Database Project at MSU

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<https://github.com/rdpstaff>



GREEN GENES

The 16S rRNA Gene Database and Tools

<http://greengenes.secondgenome.com>

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. mothur is currently the most cited bioinformatics tool for analyzing 16S rRNA gene sequences. Step inside the wiki and user forum and learn how you can use mothur to process data generated by Sanger, PacBio, IonTorrent, 454, and Illumina (MiSeq/HiSeq). 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 every couple of months.



<https://www.mothur.org>



What is QIIME?

QIIME™ (canonically pronounced *chime*) stands for Quantitative Insights Into Microbial Ecology.

<http://qiime.org>

Considerations for Experimental Design

Where to sample?

Top

Bottom

Single or multiple locations

How, and how much to sample

Need to take a sterile core sample

Replicates and store one at -20°C

Multiple locations

Separate sequencing runs or pool samples

Eubacteria vs archaea primers

Time course

Initial establishment, every three months for a year?

Others???