Of Mudwatts and Microbiomes

Phylogenetic structure of the prokaryotic domain: The primary kingdoms

Carl R. Woese and George E. Fox

PNAS November 1, 1977. 74 (11) 5088-5090; https://doi.org/10.1073/pnas.74.11.5088

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

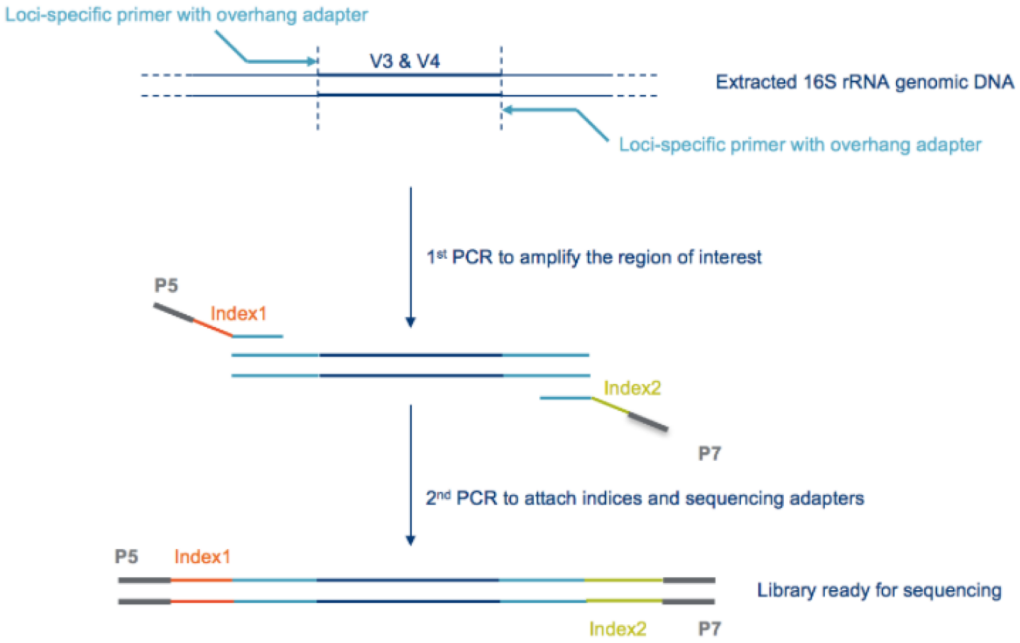
Collection of all the micro-organisms
that exist in an environment of interest

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

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

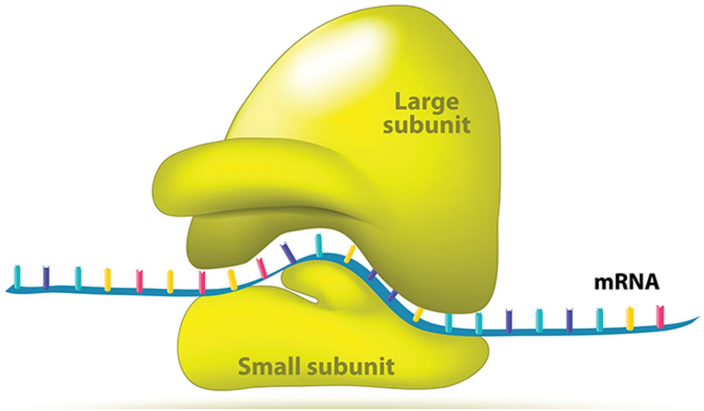


Use DNA primers to well conserved regions
Designed to amplify 16S in all/most bacteria

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

Ribosome

Translates mRNAs to proteins



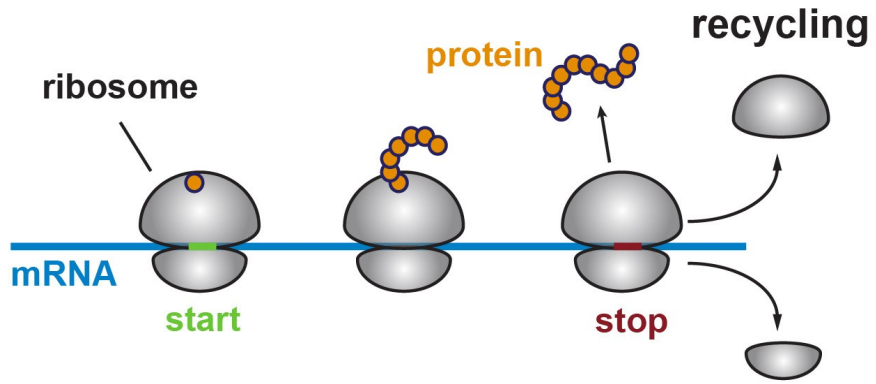
Bacterial Ribosome (70S)
Two subunits – 50S and 30S

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

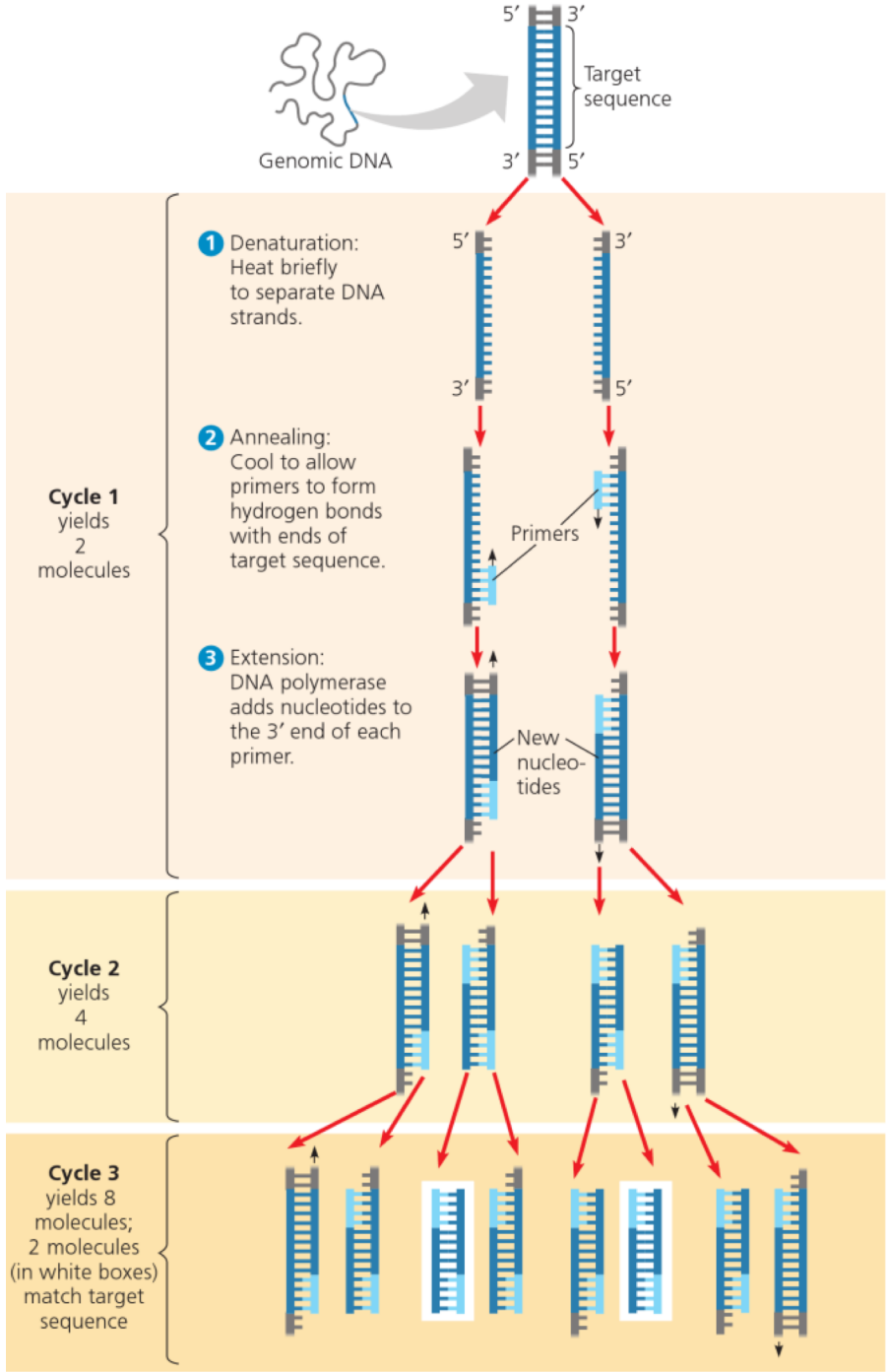
23S (large subunit), 16S (small subunit), and 5S (large subunit) rRNAs



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
Extention 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

RESEARCH ARTICLE

Environmental Genome Shotgun Sequencing of the Sargasso Sea

J. Craig Venter,[1]* Karin Remington,[1] John F. Heidelberg,[3] Aaron L. Halpern,[2] Doug Rusch,[2] Jonathan A. Eisen,[3] Dongying Wu,[3] Ian Paulsen,[3] Karen E. Nelson,[3] William Nelson,[3] Derrick E. Fouts,[3] Samuel Levy,[2] Anthony H. Knap,[6] Michael W. Lomas,[6] Ken Nealson,[5] Owen White,[3] Jeremy Peterson,[3] Jeff Hoffman,[1] Rachel Parsons,[6] Holly Baden-Tillson,[1] Cynthia Pfannkoch,[1] Yu-Hui Rogers,[4] Hamilton O. Smith[1]

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.

Science, 2004

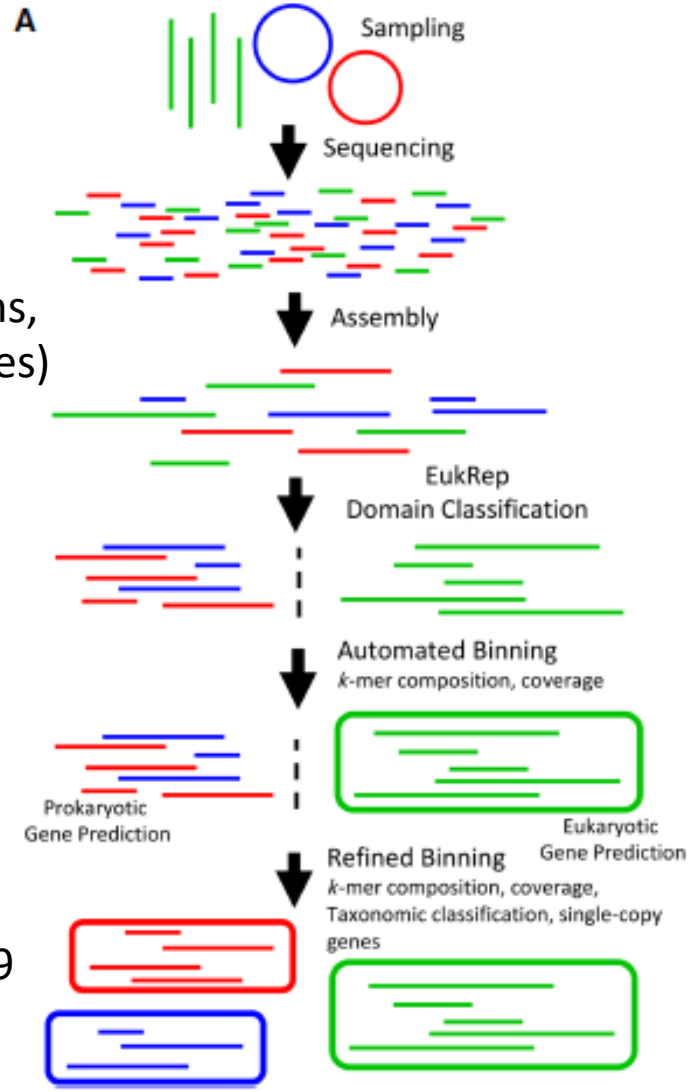


Figure 2
Genome Res 28: 569

Available online at www.sciencedirect.com
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Agriculture and Agricultural Science Procedia

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

Comparison of Total DNA Extraction Methods for Microbial Community form 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 0.5gglass 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).

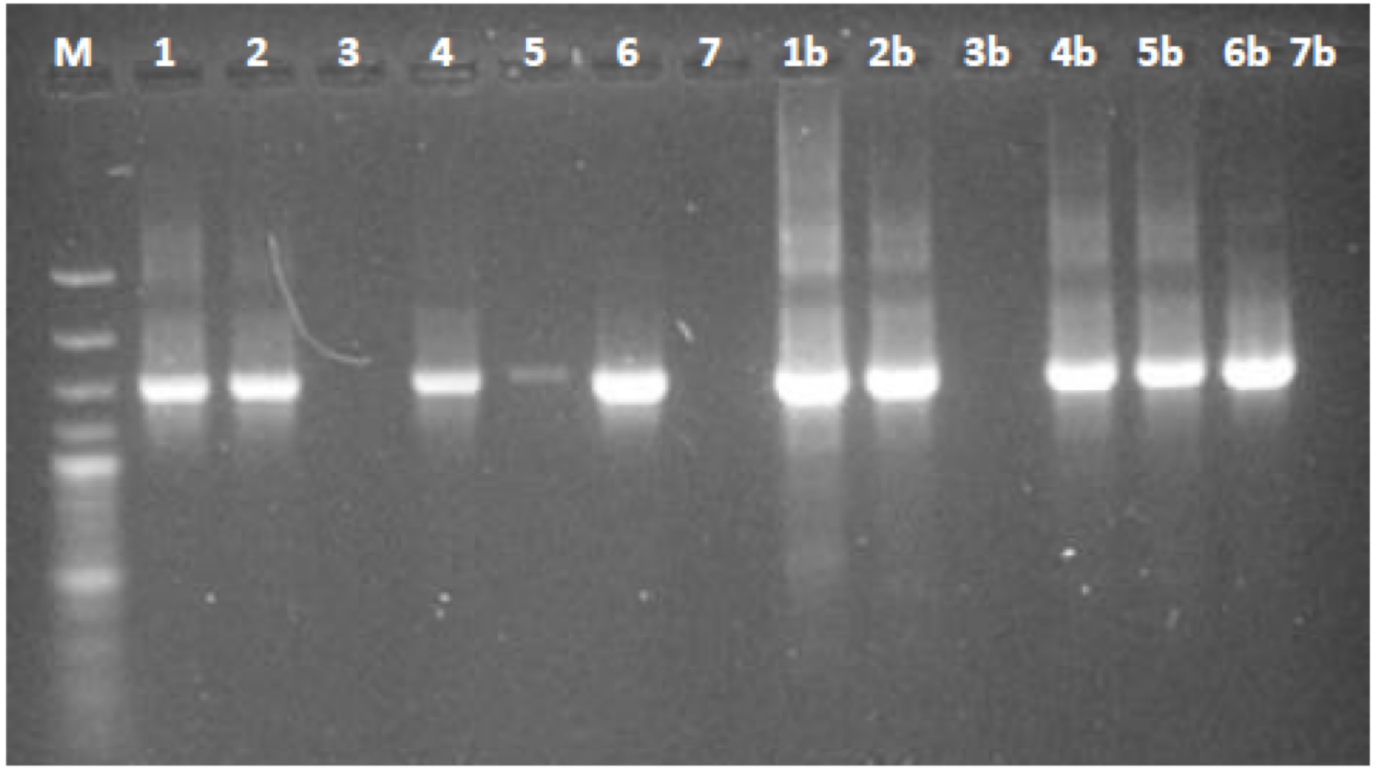


Fig. 3. Agarose gel of 16S rDNA amplicon obtained using various procedures from soil samples (b lanes = +3mg/ml BSA): DNA Ladder λ/ EcoRI and HindIII (M); Soil DNA Isolation Kit NorgenBiotek (1, 1b); S (2, 2b); SP(3, 3b); S-CTAB (4, 4b); GnS-GII (5, 5b); P. aeruginosa PAO1 (6,6b); No DNA control (7, 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 µl of extraction buffer (50 mM Na-phosphate buffer [pH 8], 50 mM NaCl, 500 mM Tris-HCl [pH 8], and 5% SDS) and 300 µl 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 µl proteinase K stock solution, and incubated for 30 min at 37^{o}C. The supernatant was then precipitated with 1 volume of ice-cold isopropanol, and incubated at -20^{o}C for 20 min.

S protocol worked best, and has the simplest components

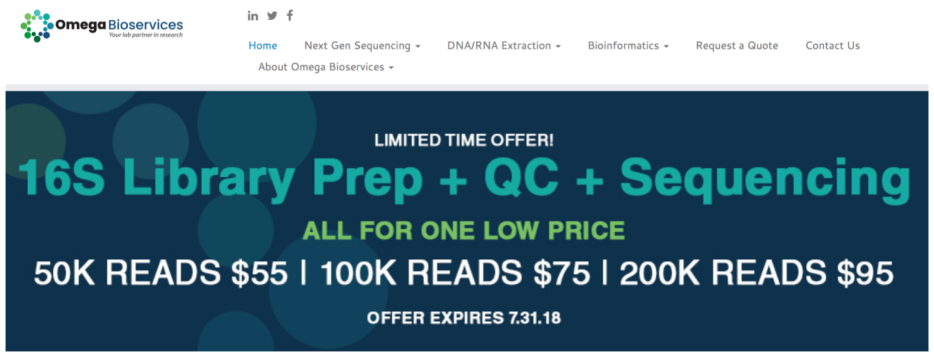
Simple extraction buffer:
50 mM Na-phosphate buffer, pH 8
50 mM NaCl
500 mM Tris-HCl, pH 8
5% SDS

Modifications for us:
Use chloroform-isoamyl (24:1) only

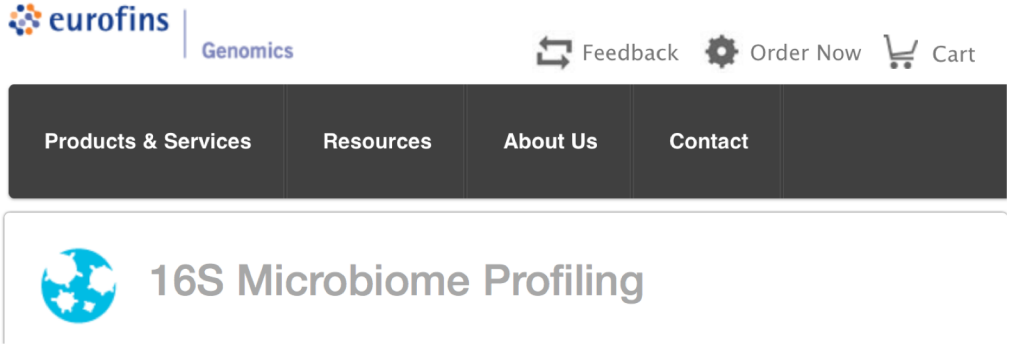
The quality of DNA isolates was estimated by quantifying amplification of 16S rRNA genes using the universal primers specific for Eubacteria: GM3F (5'-AGAGTTTGATCMTGGC-3') and GM4R reverse (5'-TACCTTGTTACGACTT-3') according to Muyzer et al. (1995), using a 50^{o}C annealing temperature on a Mini Cycler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

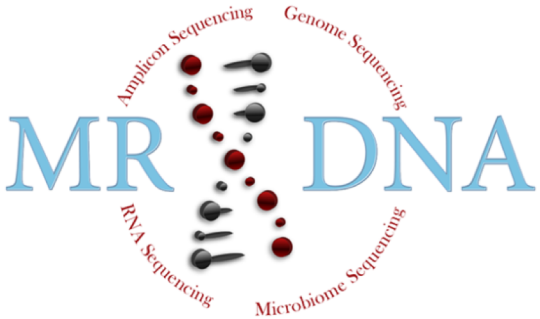
Template DNA concentration was determined and we used 25ng of crude DNA extract for each PCR reaction. Amplification products from 10 reactions for each procedure were checked for correct size (1500 bp) by 1% agarose gel electrophoresis.



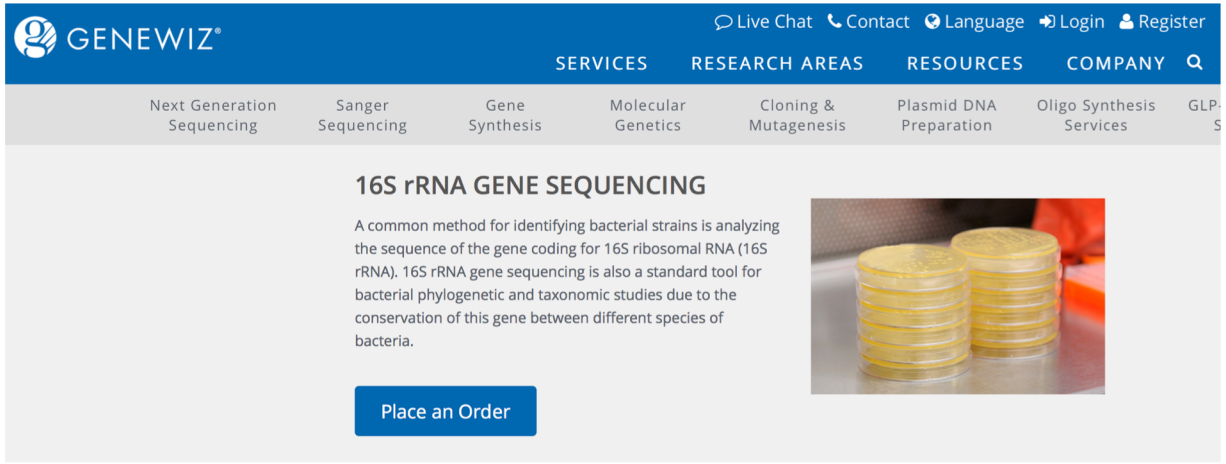
http://omegabioservices.com



https://www.eurofinsgenomics.com/en/products/next-generation-sequencing/16s-microbiome-profiling/

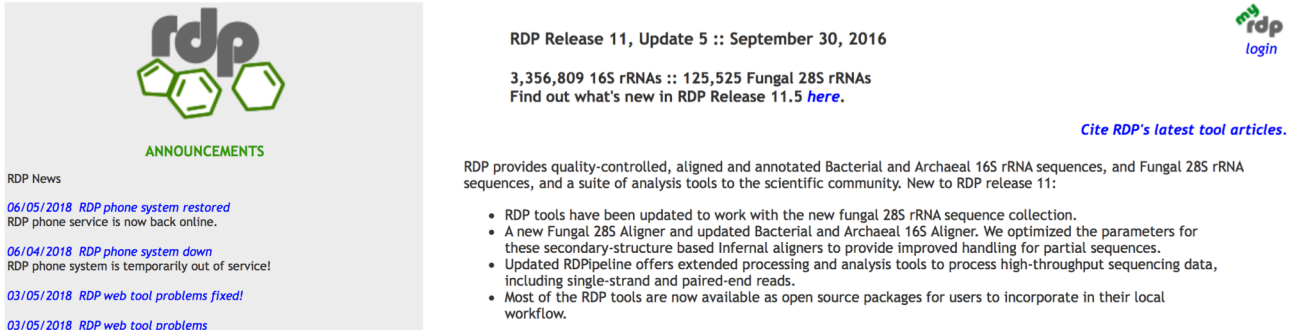


http://www.mrdnalab.com/16s-ribosomal-sequencing.html

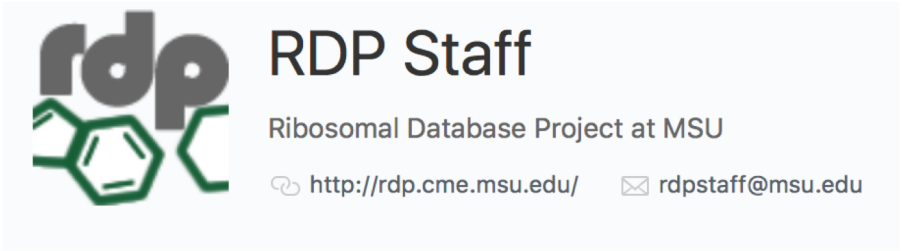


https://www.genewiz.com/en/Public/Services/Molecular-Genetics/16S-rRNA-Sequencing

Ribosomal Database Project



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

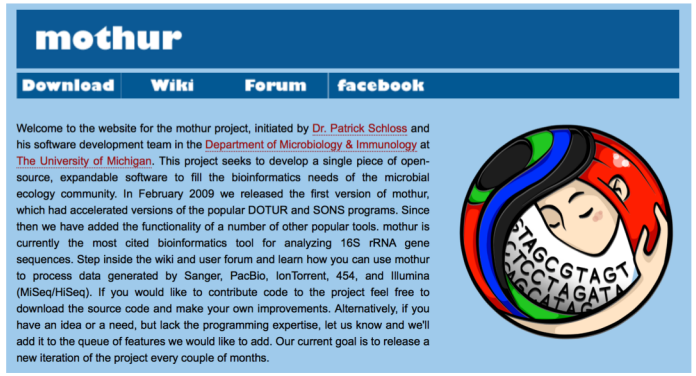


https://github.com/rdpstaff

GREENGENES

The 16S rRNA Gene Database and Tools

http://greengenes.secondgenome.com



https://www.mothur.org

Quantitative Insights Into Microbial Ecology

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^{o}C
Multiple locations
Separate sequencing runs or pool samples

Eubacteria vs archaea primers

Time course
Initial establishment, every three months for a year?

Others???