

Metagenomics

Mark Stenglein

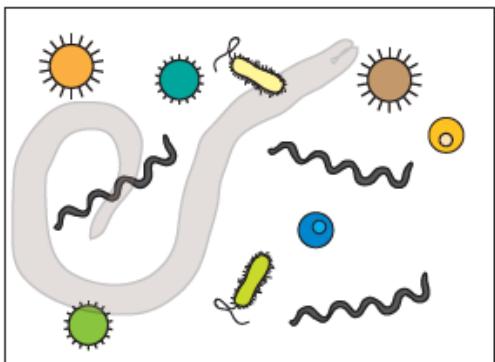


Computational Biology and
Genomics Workshop

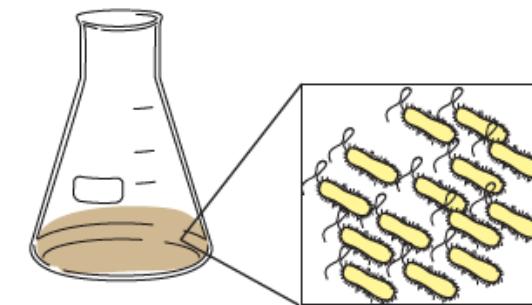
Todos Santos Center
April 9-13, 2018

What is metagenomics?

soil community



bacterial isolate



soil 'metagenome'



bacterial genome



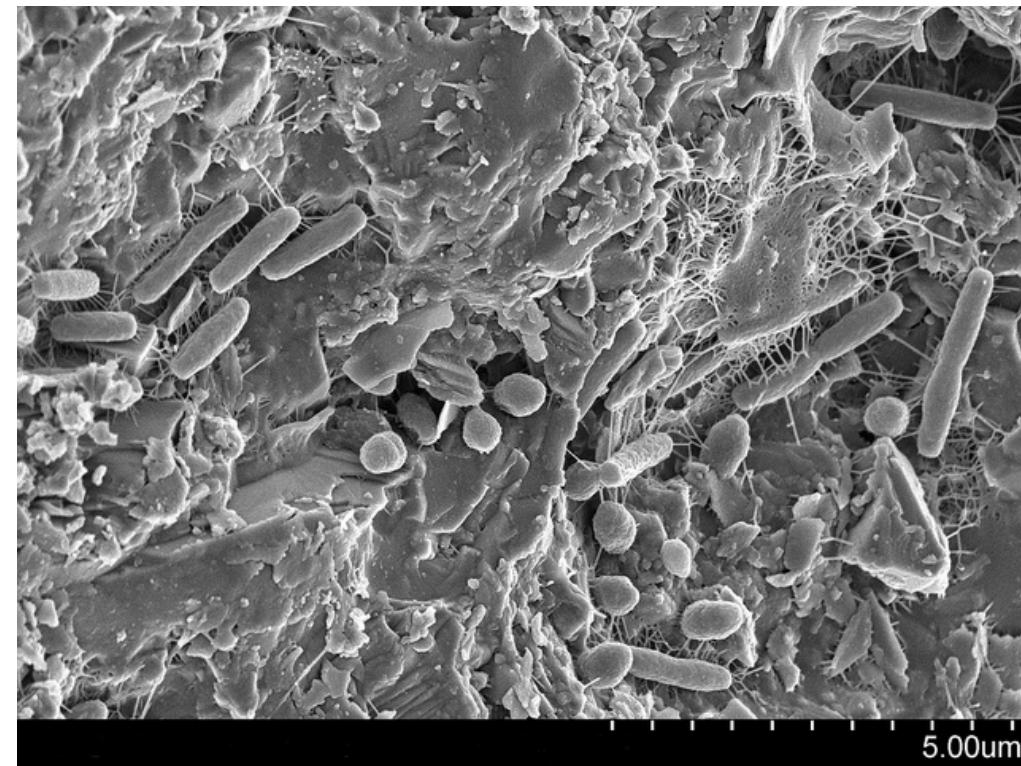
Metagenomics emerged in response to the observation that most micro-organisms can't be cultured



Morphological diversity typical of microorganisms cultured from soil on a broad spectrum medium, tryptic soy agar.

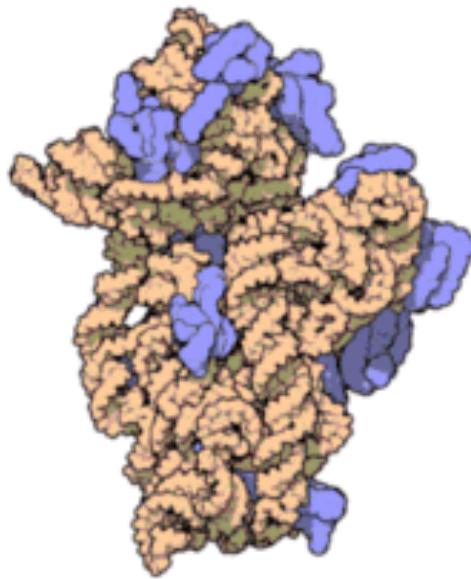
Handelsman et al (1998) Chem & Biol

Estimated: 10^8 bacteria per gram of soil of
6000-8000 different species
Only ~1% culturable (?)

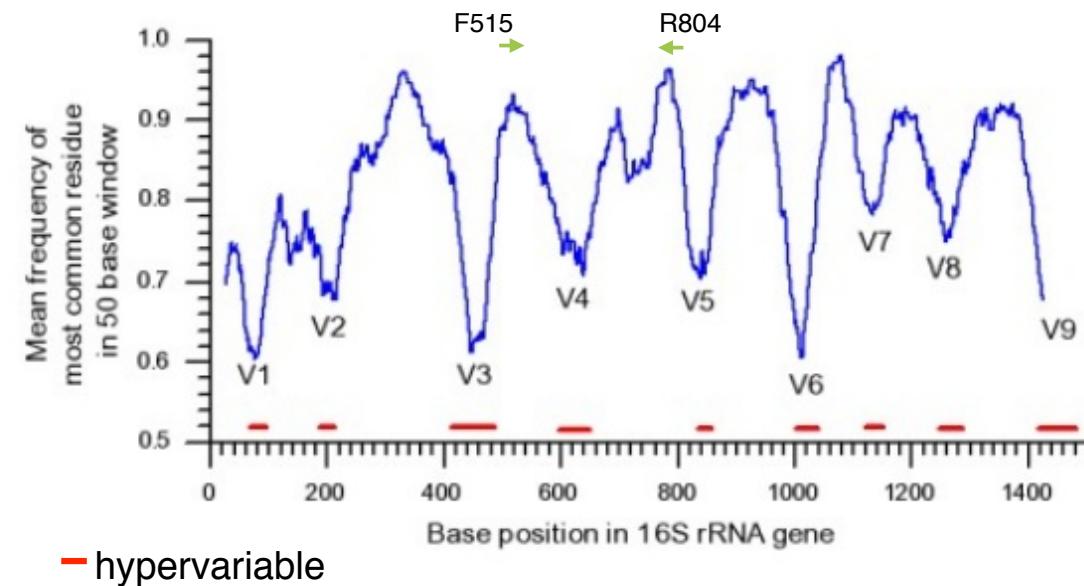


EM: Kim Lewis, Northeastern Univ.

PCR using primers targeting conserved regions of the 16S rRNA gene and sequencing enables genotyping of bacteria and archaea without having to culture them



bacterial 30S ribosomal subunit
16S rRNA is in orange
(purple: ribosomal proteins)
image: wikipedia



One of the earliest “metagenomics” paper, based on 16S sequencing

JOURNAL OF BACTERIOLOGY, July 1991, p. 4371–4378
0021-9193/91/144371-08\$02.00/0
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Analysis of a Marine Picoplankton Community by 16S rRNA Gene Cloning and Sequencing

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Received 7 January 1991/Accepted 13 May 1991

The phylogenetic diversity of an oligotrophic marine picoplankton community was examined by analyzing the sequences of cloned ribosomal genes. This strategy does not rely on cultivation of the resident microorganisms. Bulk genomic DNA was isolated from picoplankton collected in the north central Pacific Ocean by tangential flow filtration. The mixed-population DNA was fragmented, size fractionated, and cloned into bacteriophage lambda. Thirty-eight clones containing 16S rRNA genes were identified in a screen of 3.2×10^4 recombinant phage, and portions of the rRNA gene were amplified by polymerase chain reaction and sequenced. The resulting sequences were used to establish the identities of the picoplankton by comparison with an established data base of rRNA sequences. Fifteen unique eubacterial sequences were obtained, including four from cyanobacteria and eleven from proteobacteria. A single eucaryote related to dinoflagellates was identified; no archaeabacterial sequences were detected. The cyanobacterial sequences are all closely related to sequences from cultivated marine *Synechococcus* strains and with cyanobacterial sequences obtained from the Atlantic Ocean (Sargasso Sea). Several sequences were related to common marine isolates of the γ subdivision of proteobacteria. In addition to sequences closely related to those of described bacteria, sequences were obtained from two phylogenetic groups of organisms that are not closely related to any known rRNA sequences from cultivated organisms. Both of these novel phylogenetic clusters are proteobacteria, one group within the α subdivision and the other distinct from known proteobacterial subdivisions. The rRNA sequences of the α -related group are nearly identical to those of some Sargasso Sea picoplankton, suggesting a global distribution of these organisms.

plankton



image: Smithsonian magazine

Schmidt et al actually made a shotgun library from pico plankton gDNA, identified rDNA clones (38/32000 clones) by colony hybridization, and Sanger sequenced them

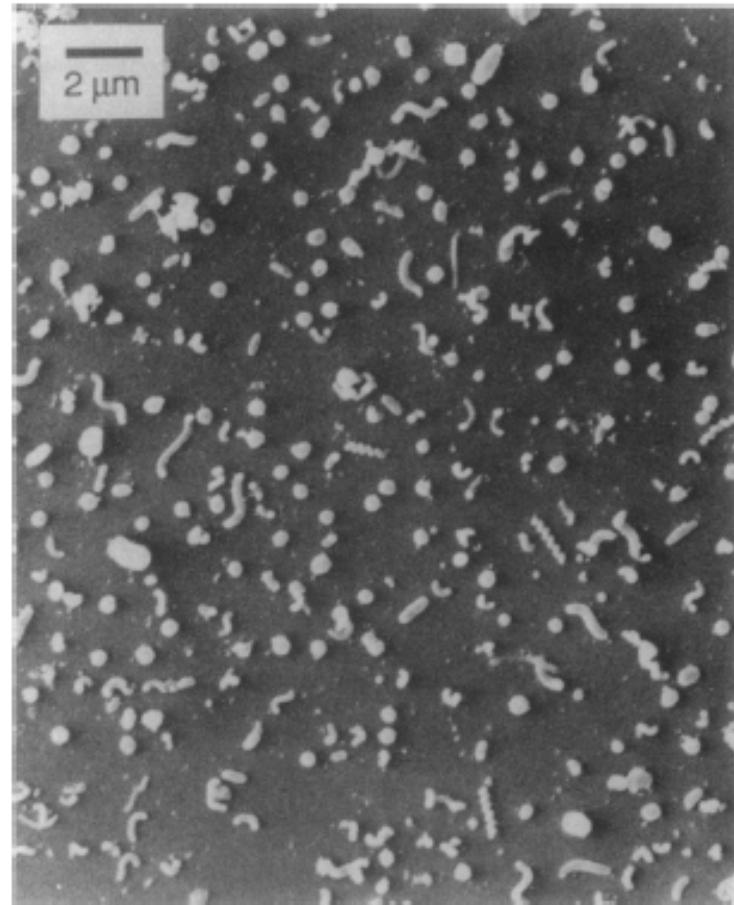


FIG. 2. Scanning electron micrograph of picoplankton represented in the clone library. Picoplankton concentrated from the ALOHA collection site were fixed and prepared for scanning electron microscopy as detailed in Materials and Methods.

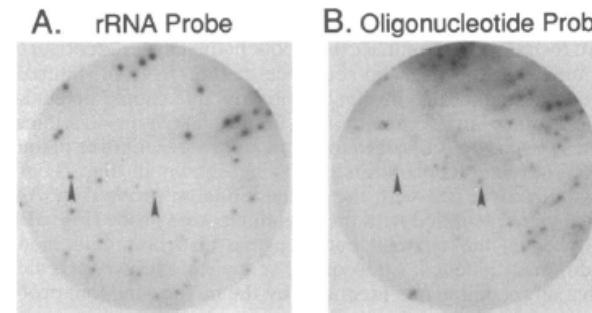


FIG. 3. Identification of rDNA-containing clones. Filter lifts of the recombinant bacteriophage library were probed as detailed in Materials and Methods with a mixed-kingdom rRNA probe consisting of 16S rRNAs from *O. linum* (eubacterium), *S. solfataricus* (archaeabacterium), and *S. cerevisiae* (eucaryote) or an oligonucleotide probe complementary to a universally conserved 16S rRNA sequence.

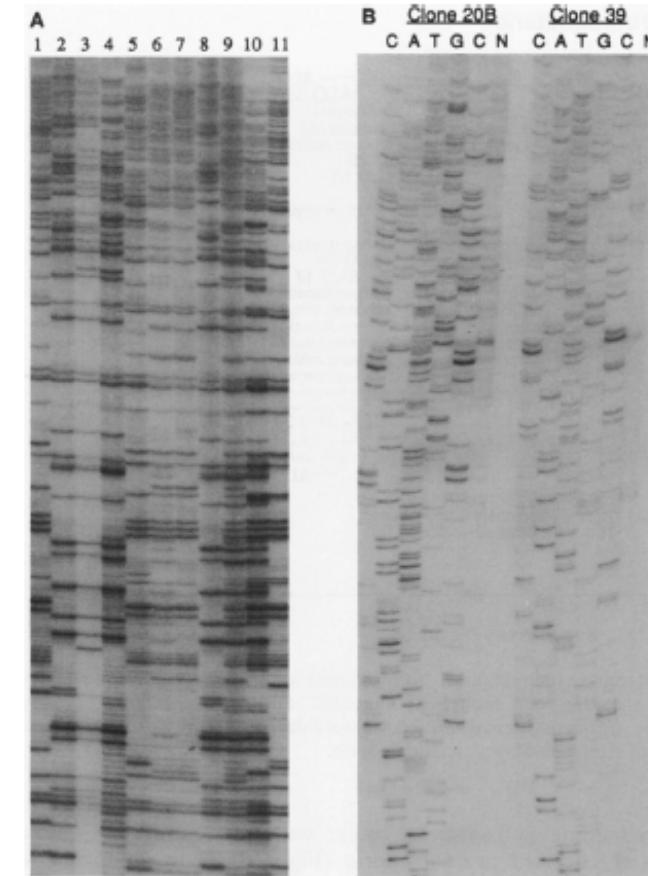
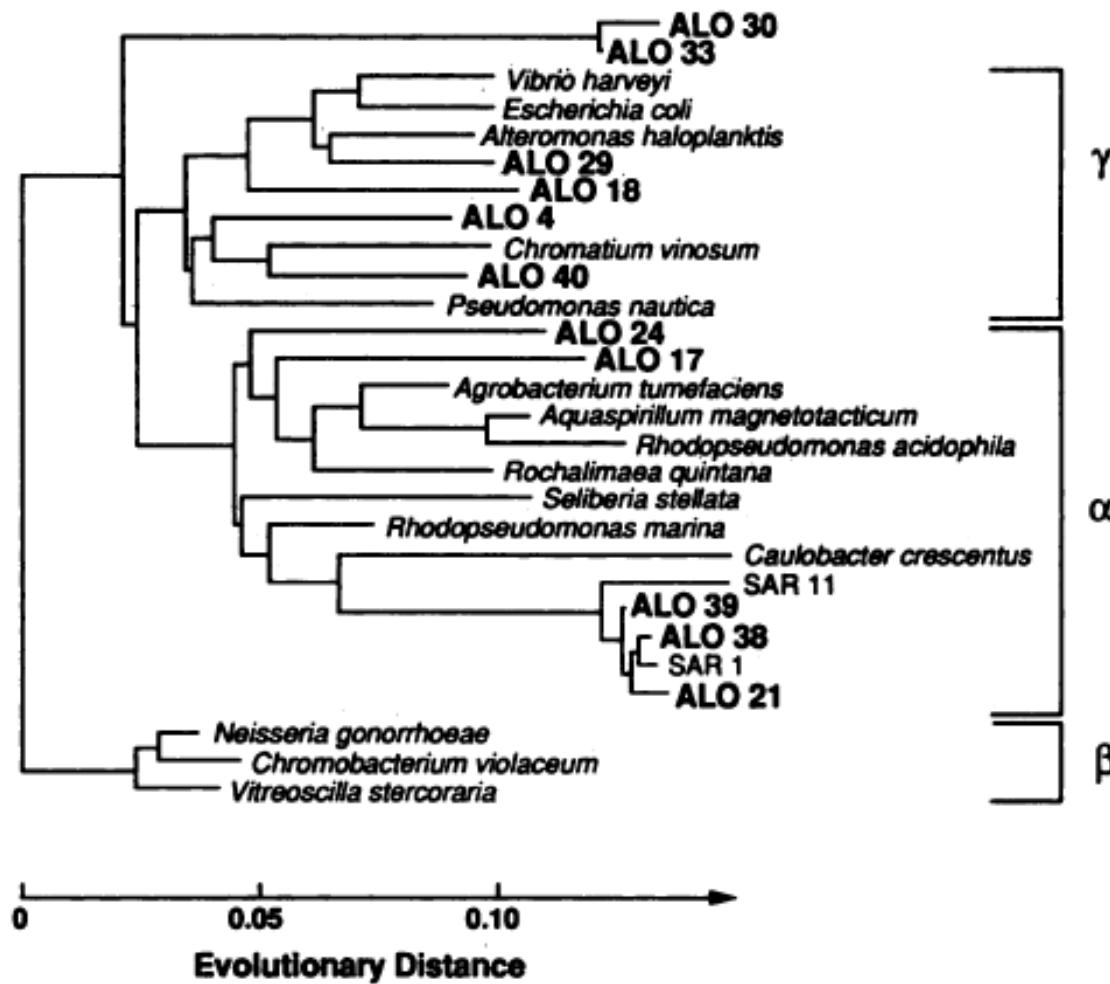


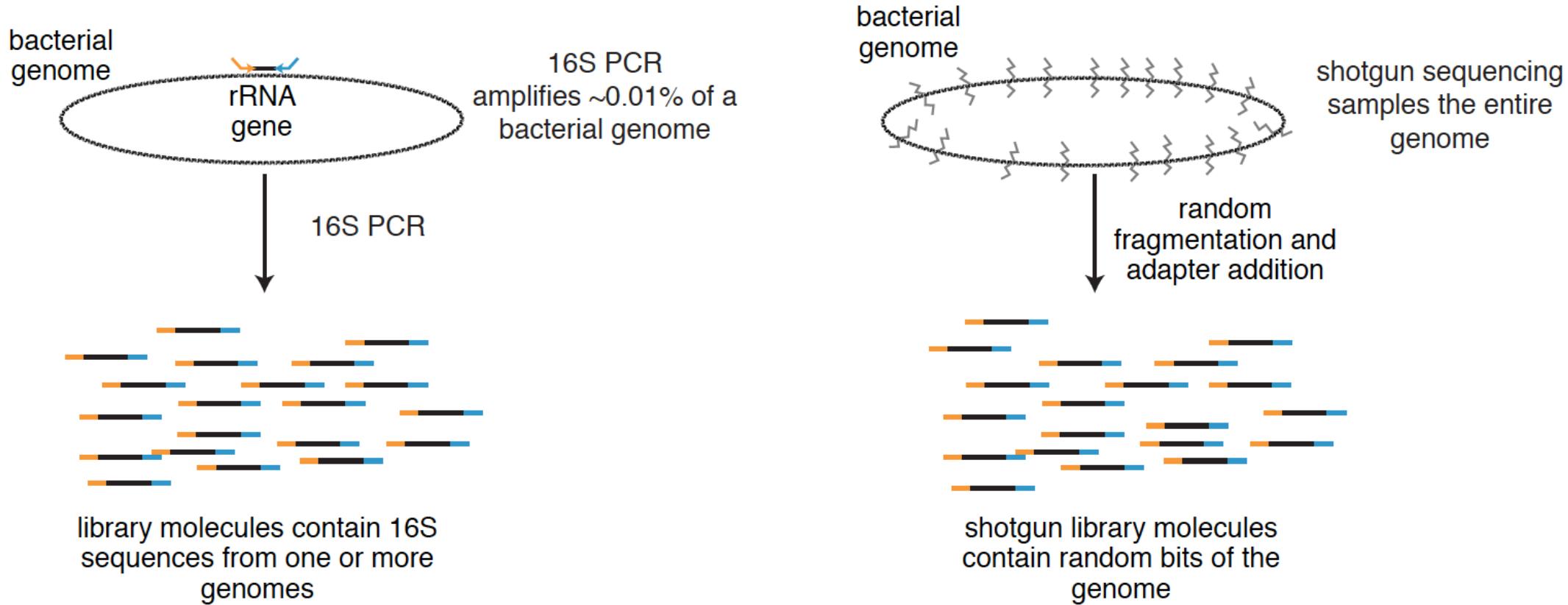
FIG. 5. Sorting and sequence analysis of rDNA-containing clones. (A) Single-nucleotide sequence pattern for 11 picoplankton clones, produced as detailed in Materials and Methods by using a PCR-amplified template, primer 519R, and a single dideoxynucleotide (ddA). (B) Sequence determination by dideoxynucleotide chain termination, using a PCR-generated, single-stranded template from two of the picoplankton clones and primer 519R.

This unbiased survey revealed that the picoplankton contained many previously unknown bacterial species

B. Proteobacteria



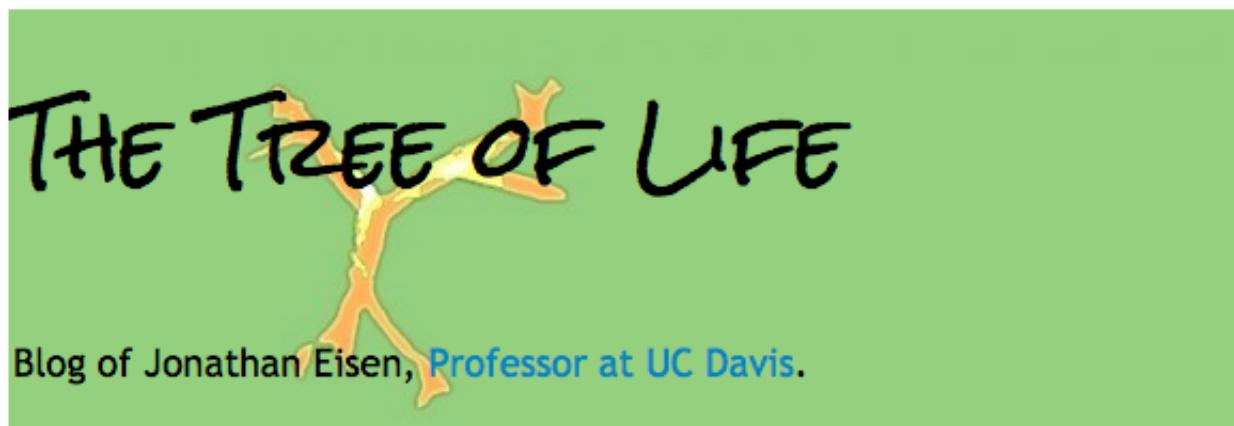
16S sequencing vs. shotgun metagenomics



- Only bacteria and archaea surveyed
- Deeper sampling of bacterial diversity per \$
- Relatively easy to make libraries and interpret results
- Appropriate if all you care about is microbial diversity / ecology

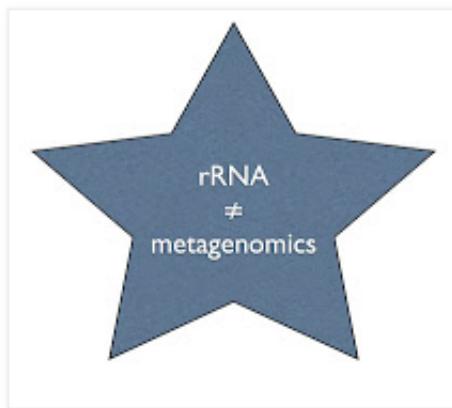
- All organisms studied*
- Decreased sampling depth per \$
- Enables analysis of other genomic features of organisms, e.g. antimicrobial resistant genes
- Analysis is significantly more difficult

Warning! Some will object if you refer to 16S-based studies as “metagenomics”



Wednesday, August 22, 2012

Referring to 16S surveys as "metagenomics" is misleading and annoying #badomics #OmicMimicry



Aargh. I am a big fan if of ribosomal RNA based surveys of microbial diversity. Been doing them for 20+ years and still continue to - even though I have moved on to more genomic/metagenomic based studies. But it drives me crazy to see rRNA surveys now being called "metagenomics".

Here are some examples of cases where rRNA surveys are referred to as metagenomics:

- Deep 16S rRNA metagenomics and quantitative PCR analyses of the premature infant fecal

<http://phylogenomics.blogspot.com/2012/08/referring-to-16s-surveys-as.html>

Viral metagenomics

JOURNAL OF VIROLOGY, July 2010, p. 6955–6965
0022-538X/10/\$12.00 doi:10.1128/JVI.00501-10
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Vol. 84, No. 14

Bat Guano Virome: Predominance of Dietary Viruses from Insects and Plants plus Novel Mammalian Viruses[▼]

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Thomas H. Kunz,⁶ and Eric Delwart^{1,2*}

Blood Systems Research Institute, San Francisco, California¹; Department of Laboratory Medicine, University of California, San Francisco, California²; Stanford Genome Technology Center, Stanford, California³; Clinical Investigation Facility, David Grant USAF Medical Center, Travis Air Force Base, California⁴; U.S. Geological Survey, Western Ecological Research Center, Point Reyes, California⁵; and Center for Ecology and Conservation Biology, Department of Biology, Boston University, Boston, Massachusetts⁶

Received 5 March 2010/Accepted 30 April 2010

Bats are hosts to a variety of viruses capable of zoonotic transmissions. Because of increased contact between bats, humans, and other animal species, the possibility exists for further cross-species transmissions and ensuing disease outbreaks. We describe here full and partial viral genomes identified using metagenomics in the guano of bats from California and Texas. A total of 34% and 58% of 390,000 sequence

Tadarida brasiliensis

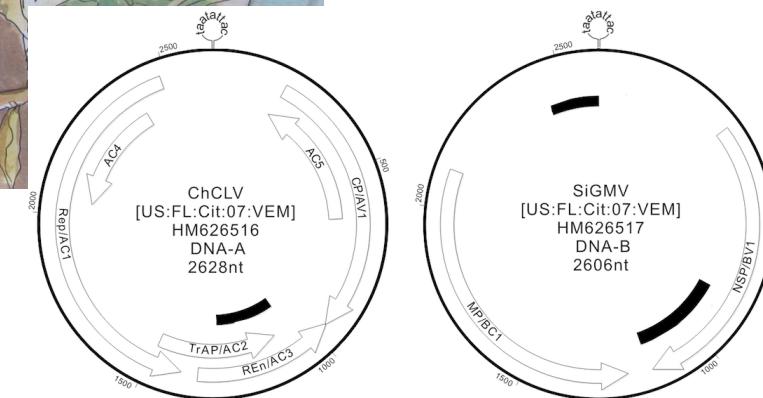


image: Wikipedia/NPS

They found:

- Known bat viruses
- Putative new bat viruses
- Viruses likely infecting the plants and insects that the bats ate

'Vector-enabled metagenomics'

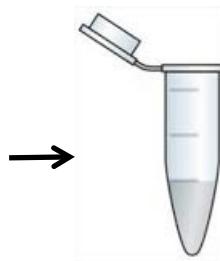


Mya Breitbart lab, Univ of South Florida

Pathogen discovery using metagenomics sequencing



case and control
tissues



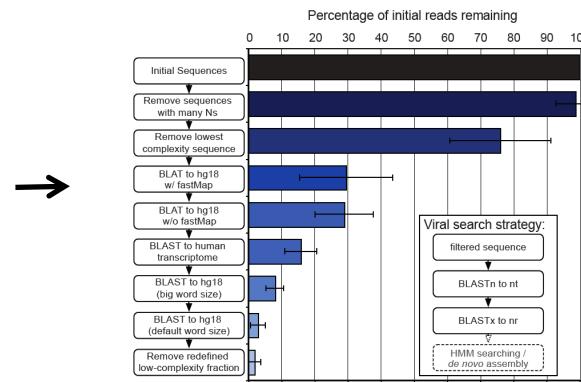
Nucleic acid



Library prep
/ barcode



Illumina
sequencing



Computational
Analysis

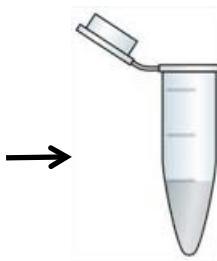


Follow-up

Pathogen discovery using metagenomics sequencing



case and control
tissues



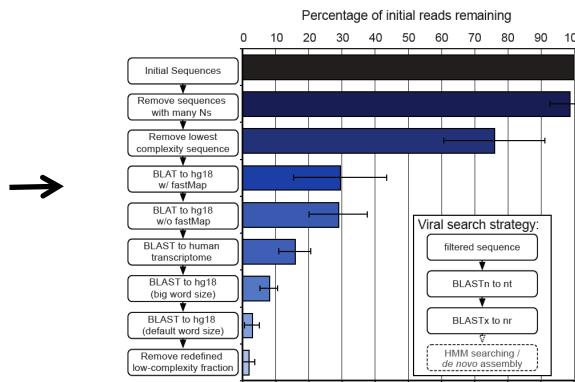
Nucleic acid



Library prep
/ barcode



Illumina
sequencing



Computational
Analysis



Follow-up