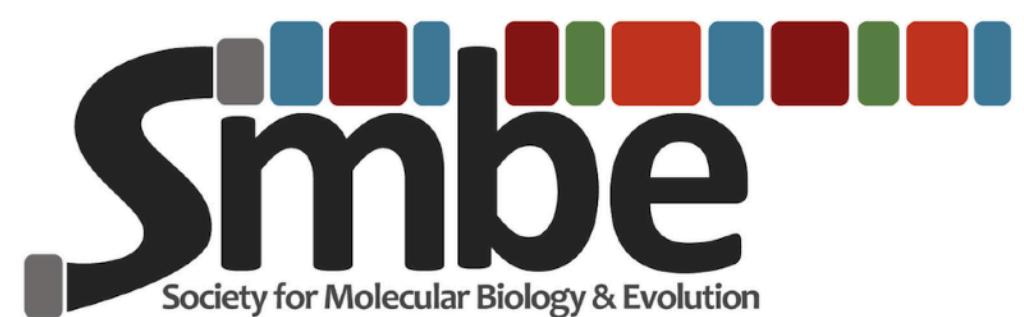


Metagenomics

Mark Stenglein



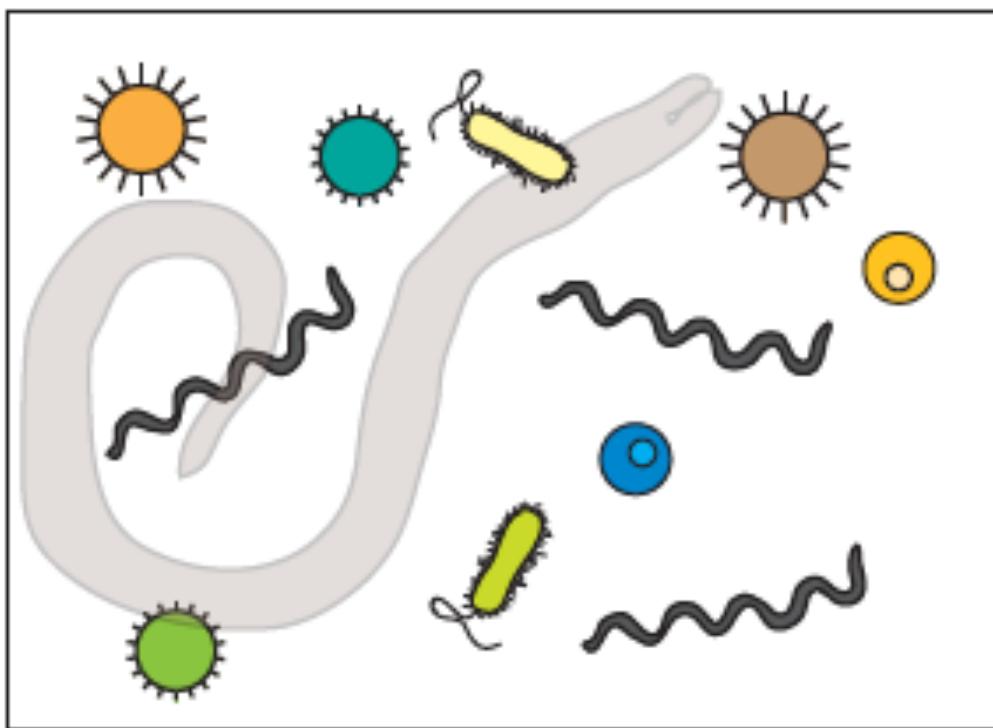
SMBE Regional Workshop
on Computational Biology
in Todos Santos, Mexico



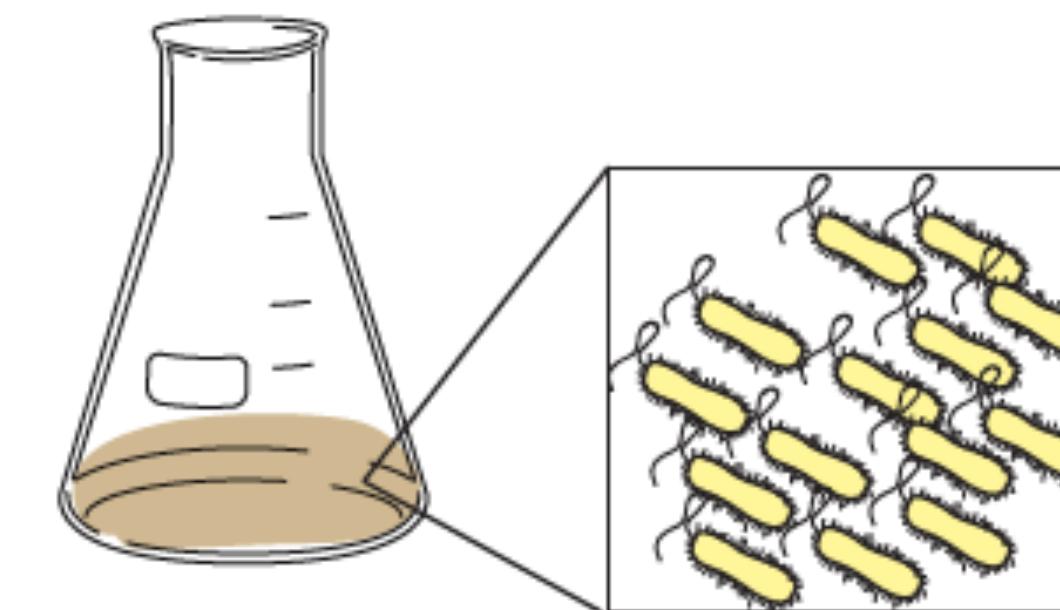
Colorado State University Todos Santos Center
April 8-12, 2019

Metagenomics is the study of >1 genome

soil community



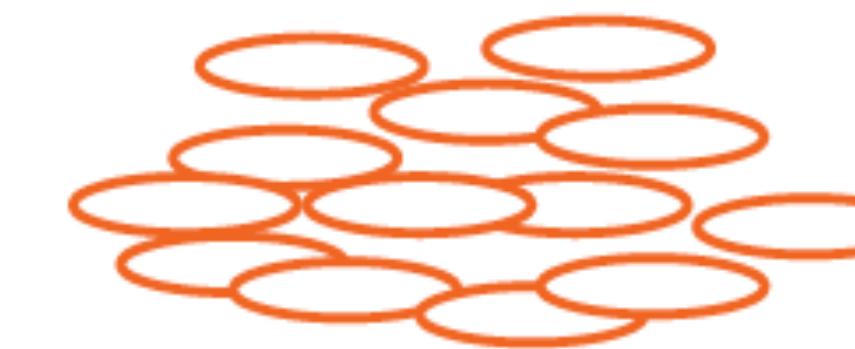
bacterial isolate



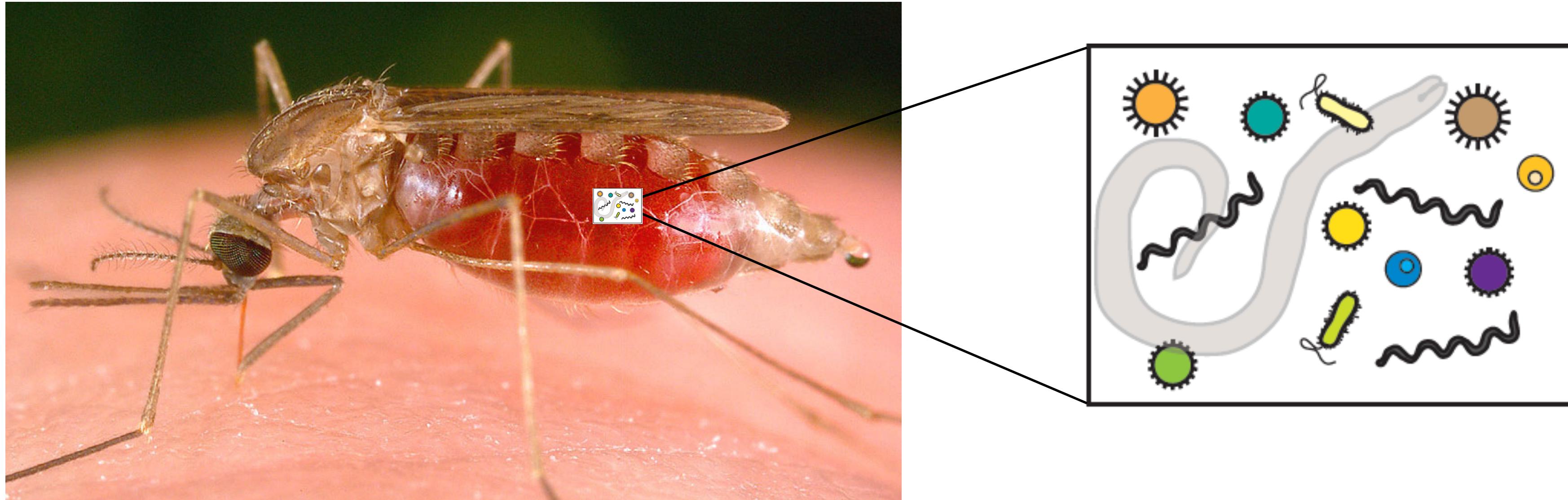
soil 'metagenome'



bacterial genome



If you sequence an intact multicellular organism,
you are doing metagenomics



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

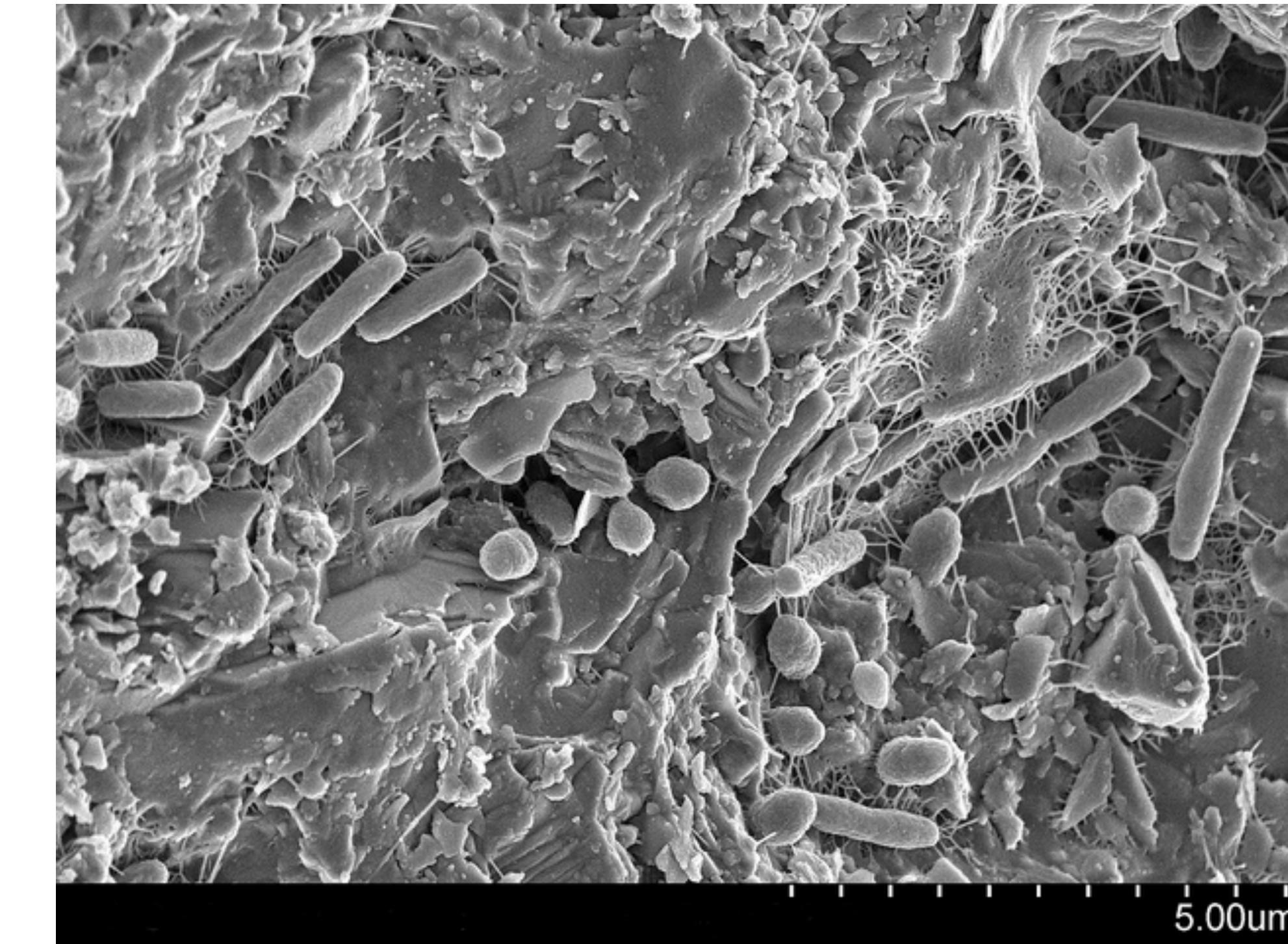


Chemistry & Biology

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

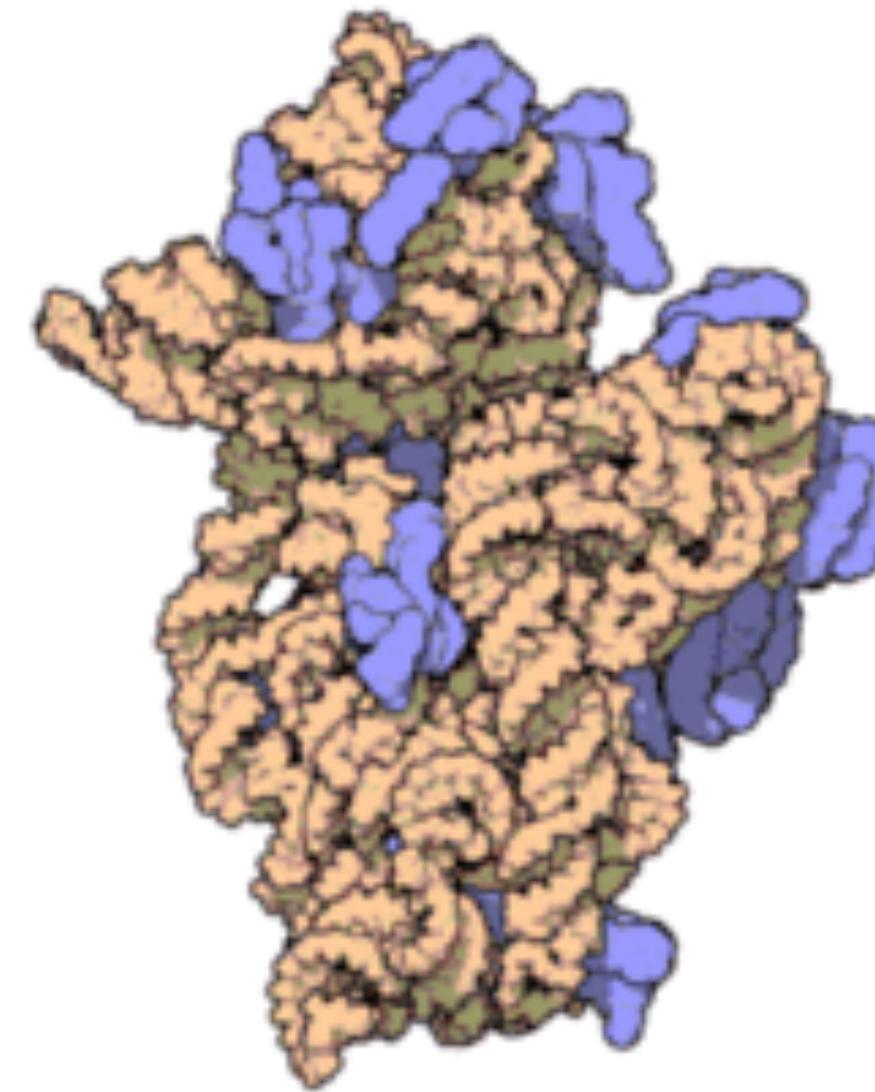
Handelsman et al (1998) Chem & Biol

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



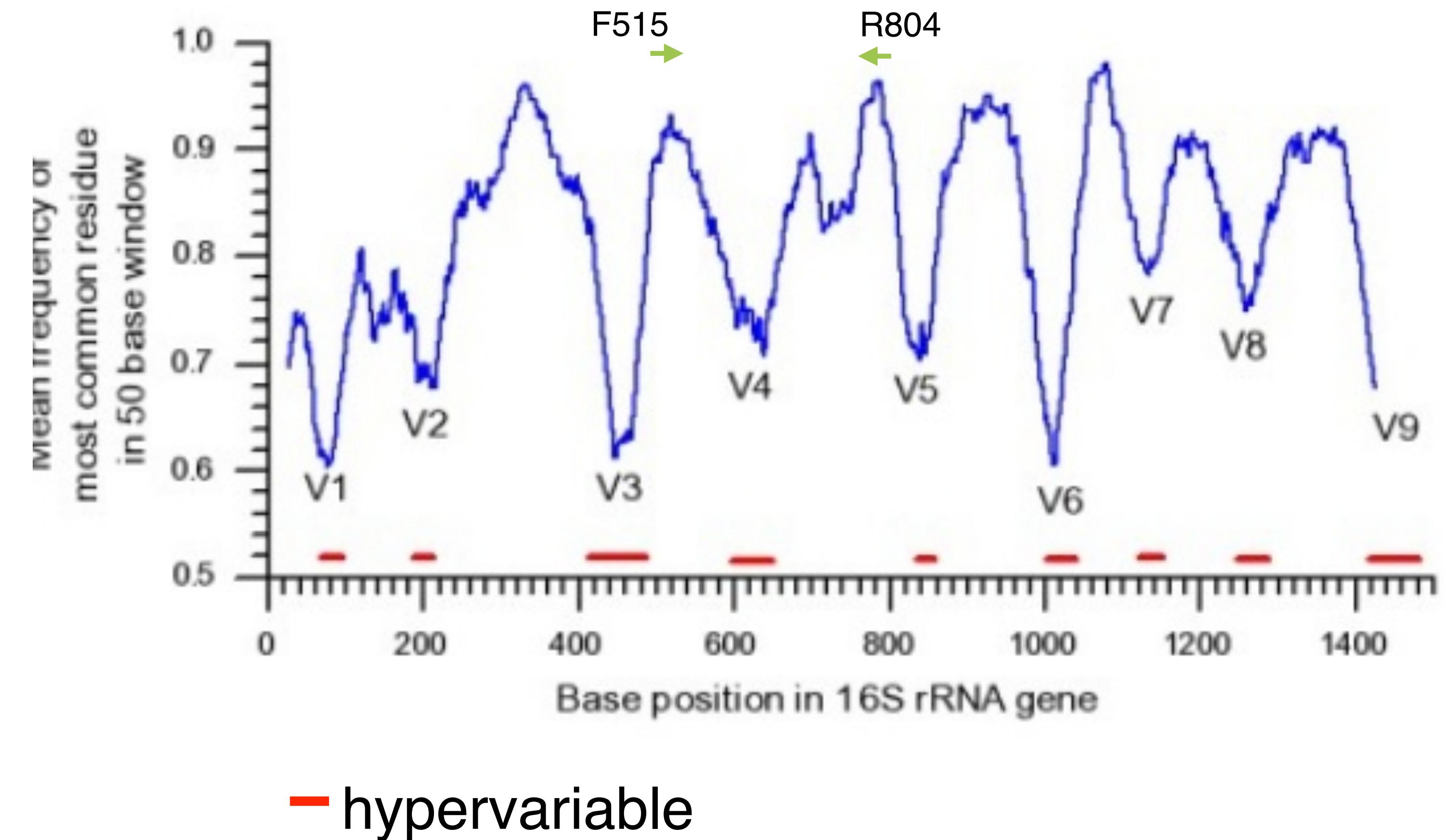
EM: Kim Lewis, Northeastern Univ.

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

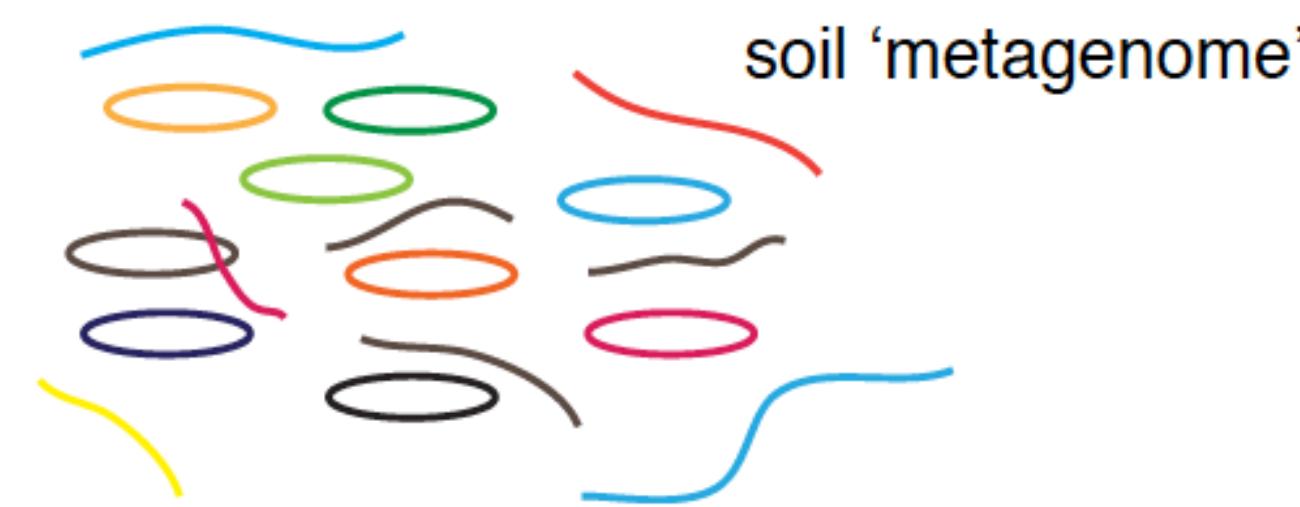
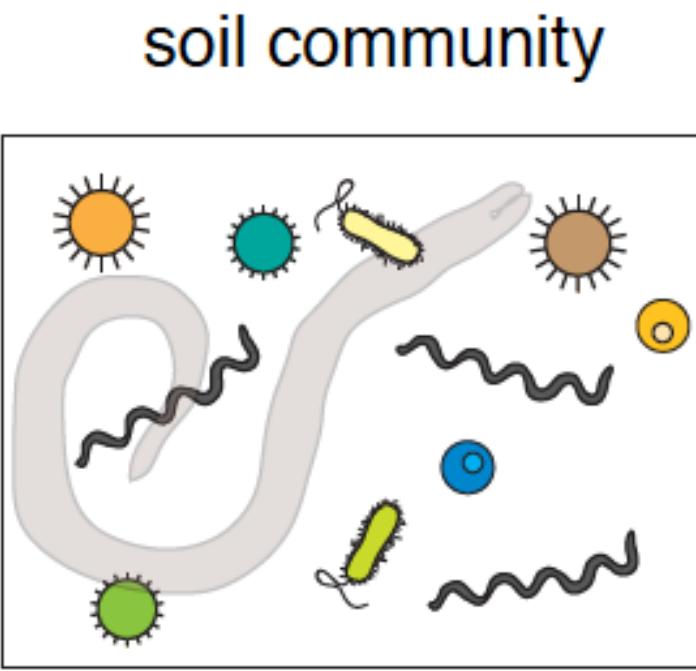
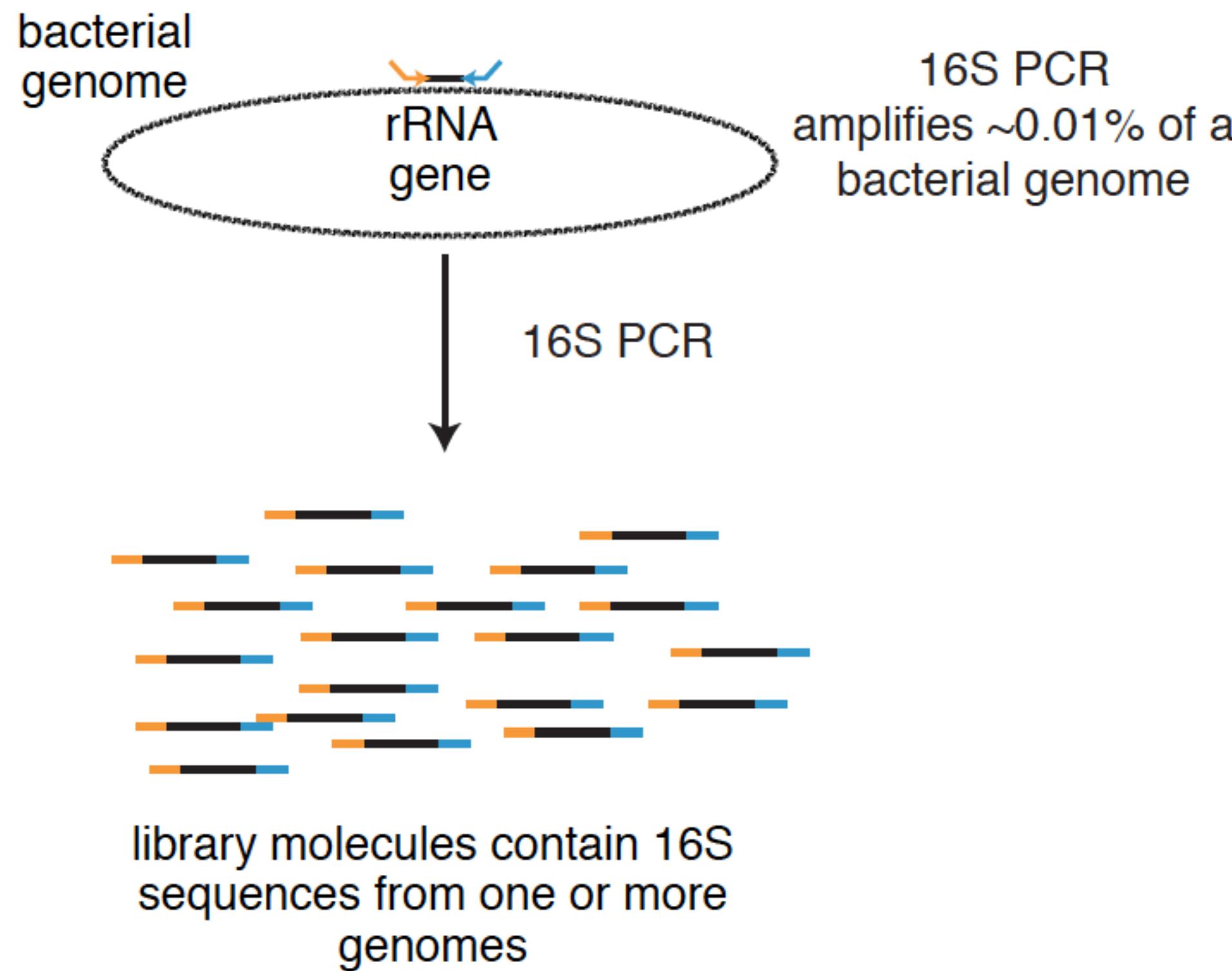


bacterial 30S ribosomal subunit
16S rRNA is in orange
(purple: ribosomal proteins)

image: wikipedia



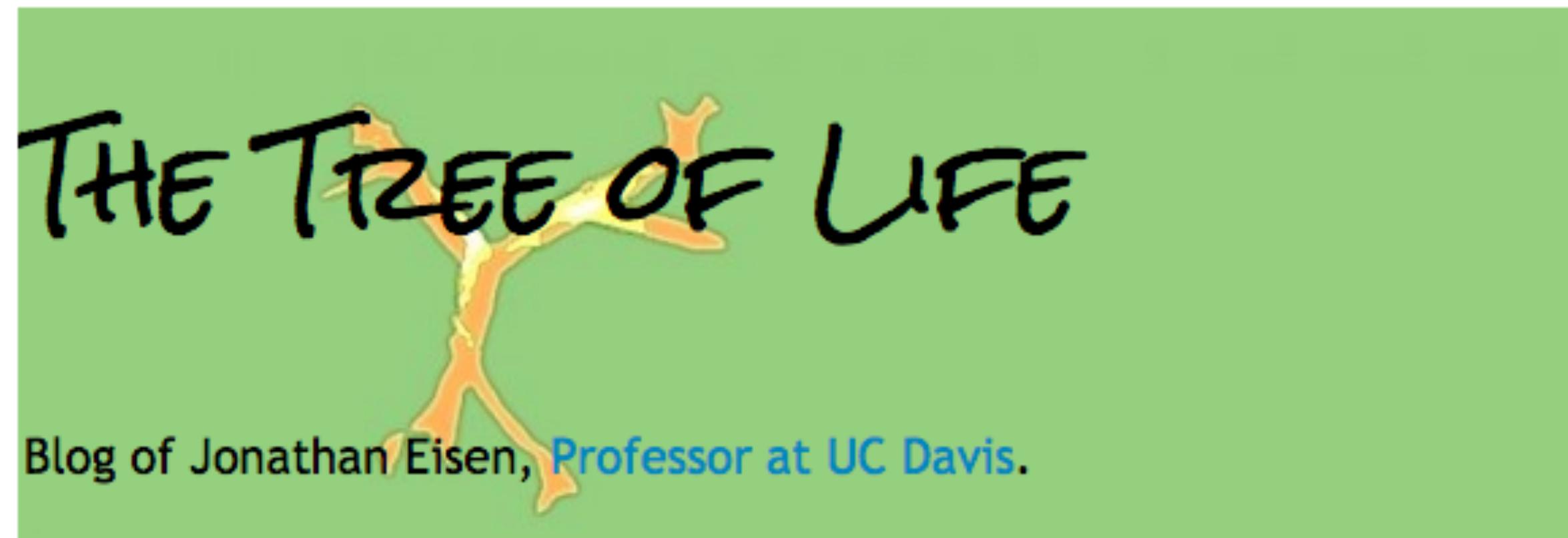
16S sequencing vs. shotgun metagenomics



- 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

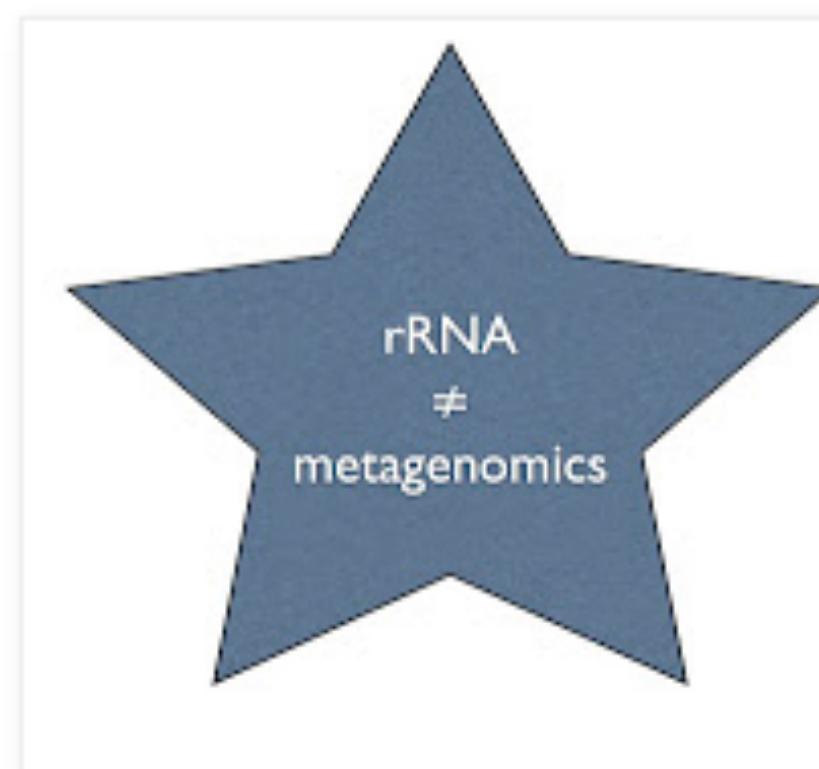
- All organisms studied*
- Decreased sampling depth per \$
- Enables analysis of other genomic features of organisms, e.g. antimicrobial resistant genes
- Analysis is significantly more difficult

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

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[▽]

Linlin Li,^{1,2} Joseph G. Victoria,^{1,2} Chunlin Wang,³ Morris Jones,⁴ Gary M. Fellers,⁵
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

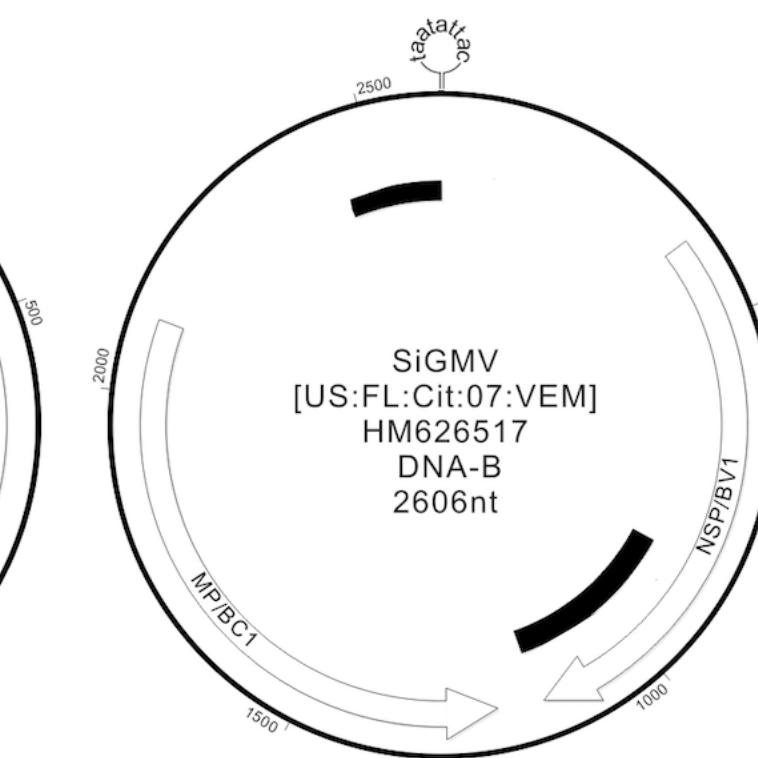
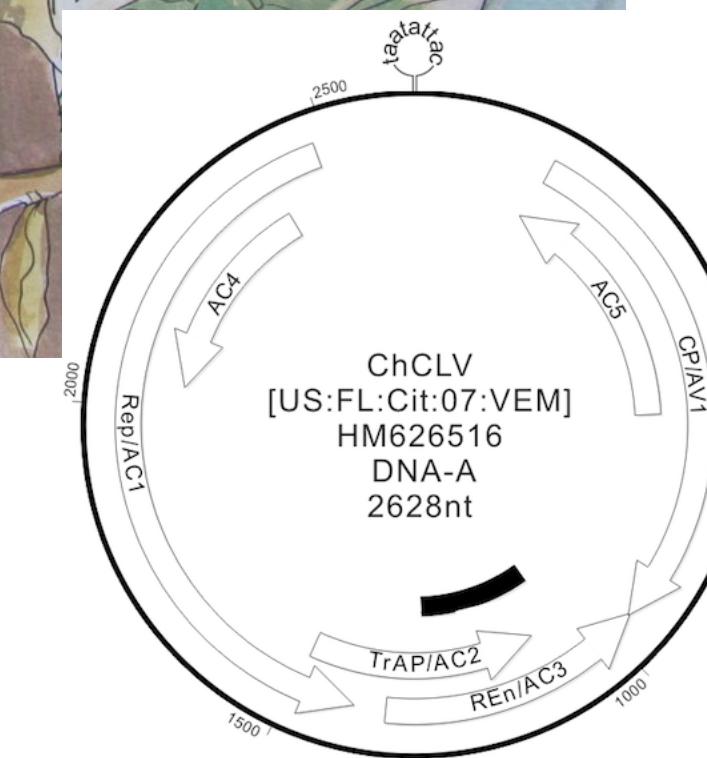
They found:

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

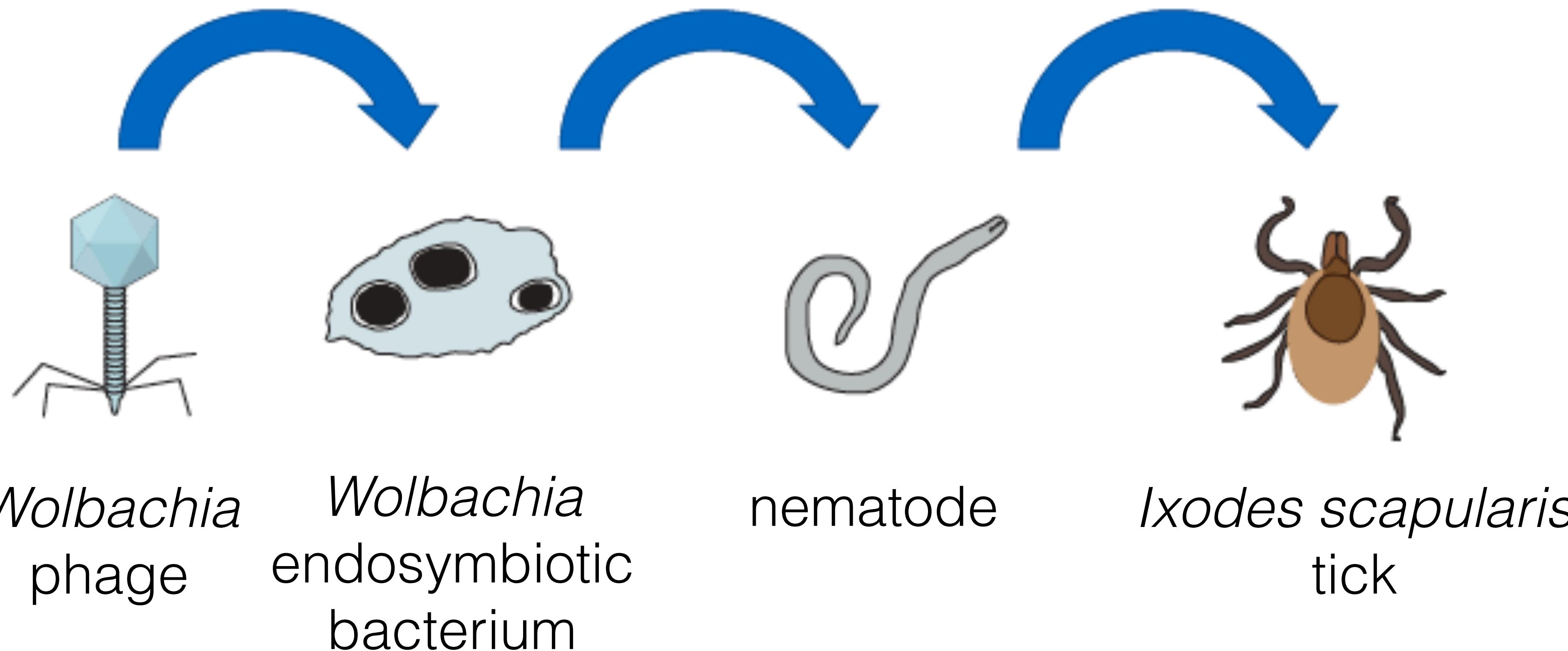
'Vector-enabled metagenomics'



Mya Breitbart lab, Univ of South Florida



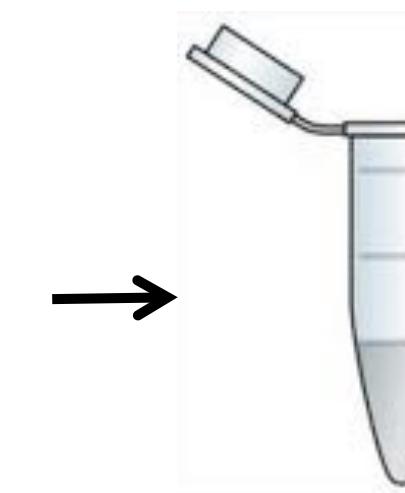
We've had similar results sequencing the metagenome of ticks



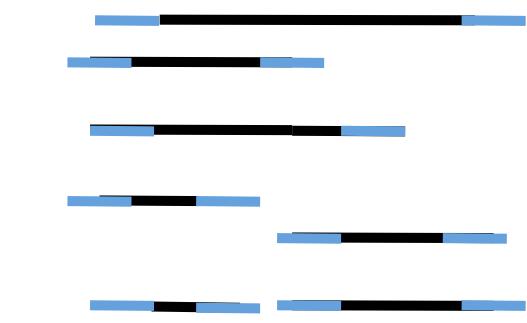
Pathogen discovery using metagenomics sequencing



case and control
tissues



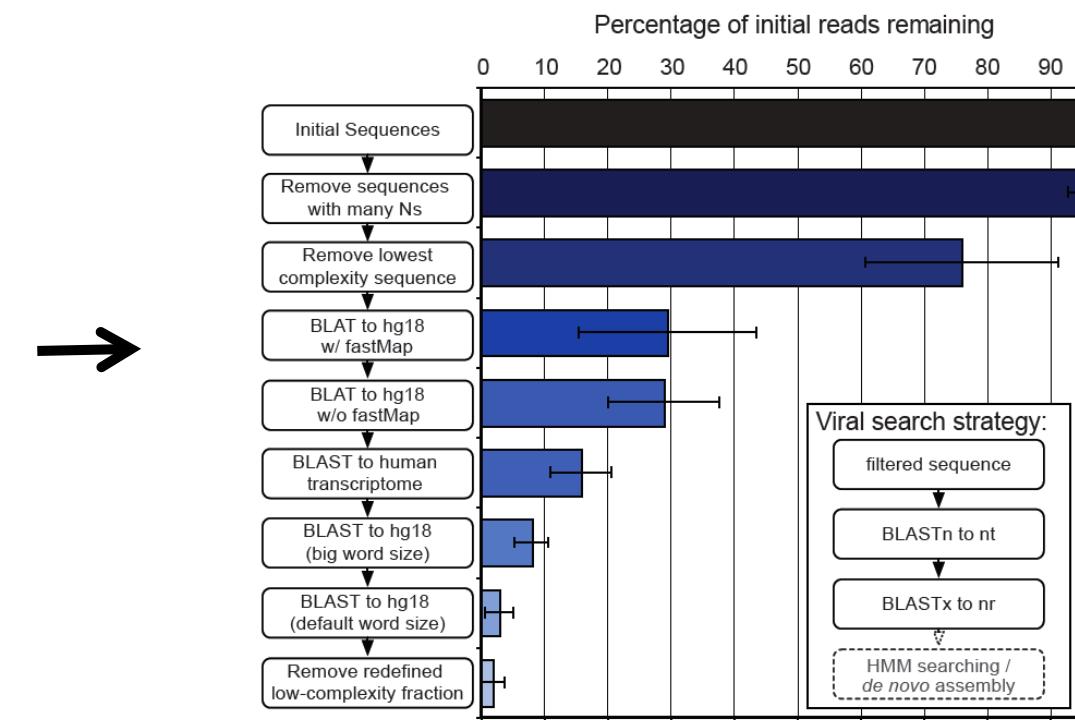
Nucleic acid



Library prep
/ barcode



Illumina
sequencing



Computational
Analysis



Follow-up

Sequencing can only give you sequences

A rabbit facility in TN experienced an outbreak of fatal gastroenteritis

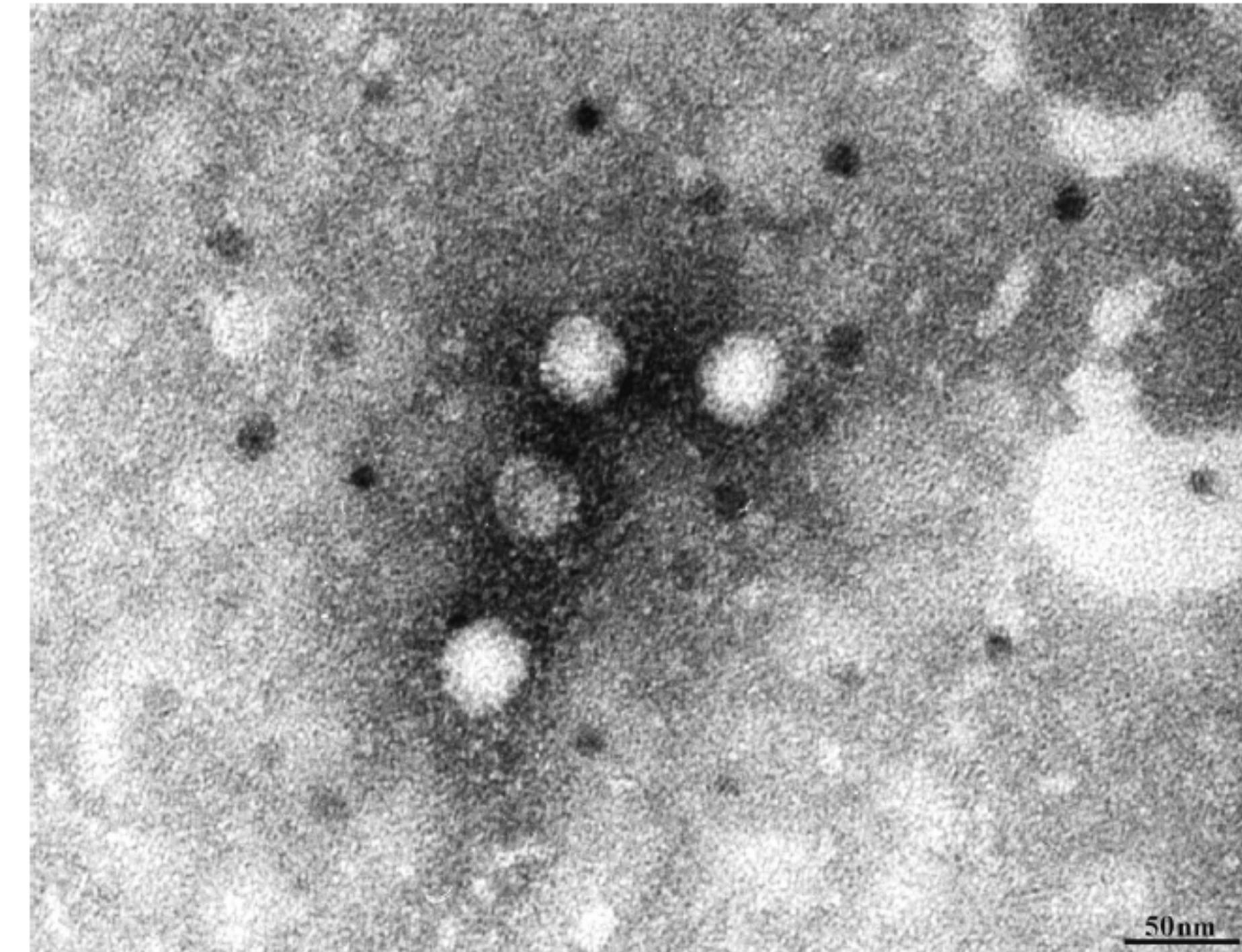


Figure 1 Electron micrograph of virus like particles in the stool of one animal (Table 1). Scale bar indicates 50 nm.

An astrovirus is the likely cause of gastroenteritis in these rabbits



Stenglein et al. Virology Journal 2012, 9:216
<http://www.virologyj.com/content/9/1/216>



RESEARCH

Open Access



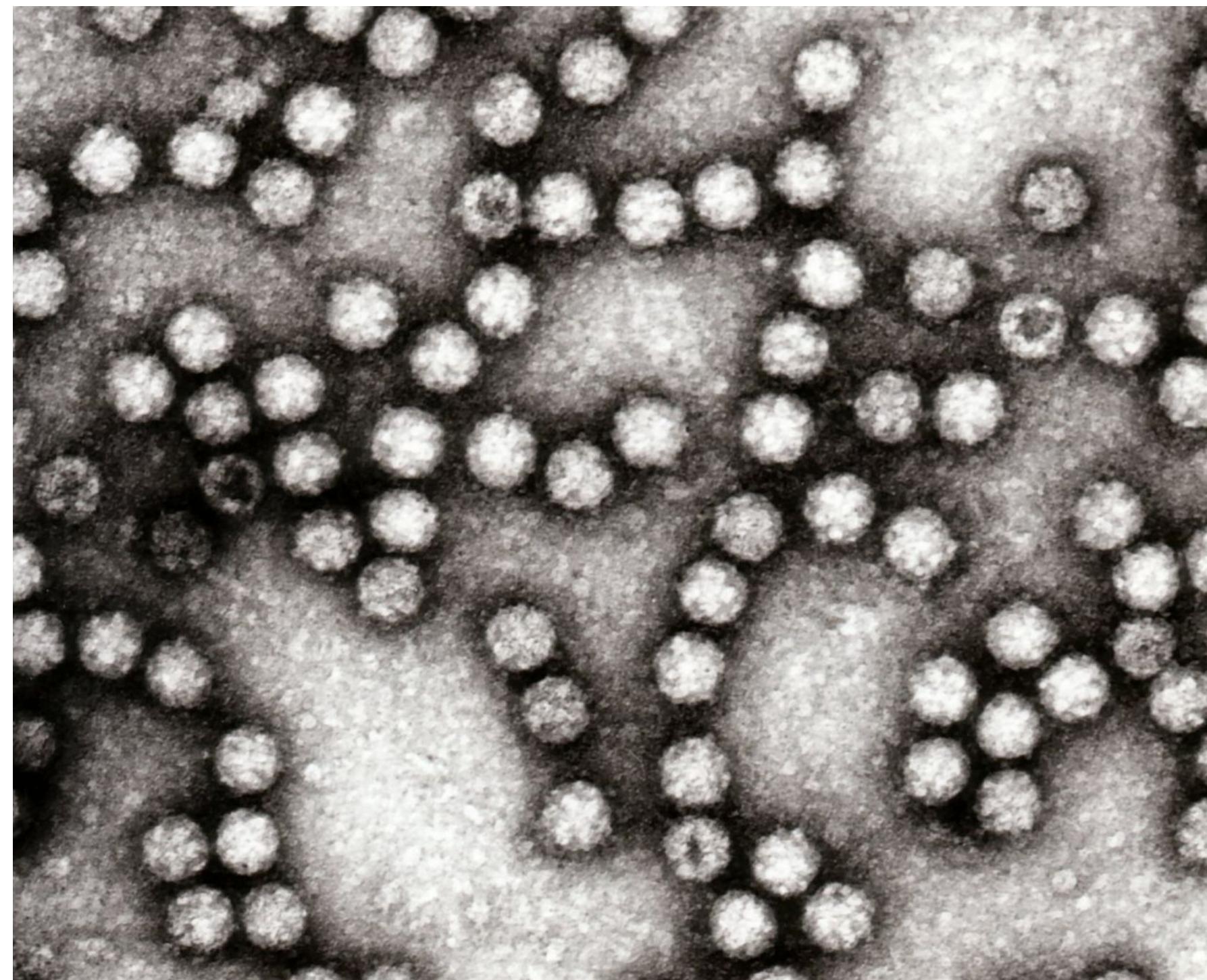
Complete genome sequence of an astrovirus identified in a domestic rabbit (*Oryctolagus cuniculus*) with gastroenteritis

Also lots of plant sequences, presumably from the rabbit's diet



Astroviruses cause diarrhea in a variety of animals, including humans.

Astrovirus particles



JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1993, p. 955-962
0095-1137/93/040955-08\$02.00/0
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Vol. 31, No. 4

Characterization and Seroepidemiology of a Type 5 Astrovirus Associated with an Outbreak of Gastroenteritis in Marin County, California

KAREN MIDTHUN,^{1†*} HARRY B. GREENBERG,^{1‡} JOHN B. KURTZ,² G. WILLIAM GARY,³
FENG-YING C. LIN,⁴ AND ALBERT Z. KAPIKIAN¹

RESULTS

Volunteer study. Nineteen adult volunteers were orally administered a filtrate prepared from a 0.1% suspension of stool from one of the ill individuals in the original Marin County outbreak. None of 17 volunteers who received a 1-ml inoculum became ill. Because of this, the amount of inoculum was increased to 20 ml. Of two volunteers who received the larger inoculum, one developed a gastrointestinal illness characterized by nausea, vomiting, diarrhea, and malaise.

idseq.net is a new online portal that performs metagenomic analysis
it's free, and pretty fast and easy to use.

The screenshot shows the idseq.net homepage. At the top, there is a navigation bar with links to various resources like Apps, Read Later, GenBank, blastn, blastx, blastp, tblastn, NCBI Tax, PubMed, SRA, github, ViralZone, Primer3, FoCo W, Kuali, BDSC, CSU Dir, Google Scholar, and Canvas. Below the navigation bar is a black header with the IDseq logo and a "Join our team" button. The main content area features a large text block about IDseq's purpose, followed by three sections: Discover, Detect, and Decipher, each with an icon and a brief description. To the right, there is a "Learn more about IDseq" box with fields for First Name, Last Name, Email, Affiliated Institution or Company, and a text area for "How would you use IDseq? Optional". A "Submit" button is at the bottom of this box.

← → C https://idseq.net

Apps Read Later GenBank blastn blastx blastp tblastn NCBI Tax PubMed SRA github ViralZone Primer3 FoCo W Kuali BDSC CSU Dir Google Scholar Canvas

IDSEQ Join our team

IDseq is an unbiased global software platform that helps scientists identify pathogens in metagenomic sequencing data.

Discover
Identify the pathogen landscape

Detect
Monitor and review potential outbreaks

Decipher
Find potential infecting organisms in large datasets

Learn more about IDseq
Already have an account? [Sign in.](#)

First Name Last Name

Email

Affiliated Institution or Company

How would you use IDseq? Optional

Submit