

Probing diversity in a hidden world: applications of *NGS* in microbial ecology



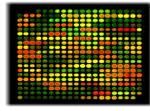
Guus Roeselers

TNO, Microbiology & Systems Biology Group

Symposium on Next Generation Sequencing

October 21, 2013

Royal Museum for Central Africa



Microbial genomics at TNO

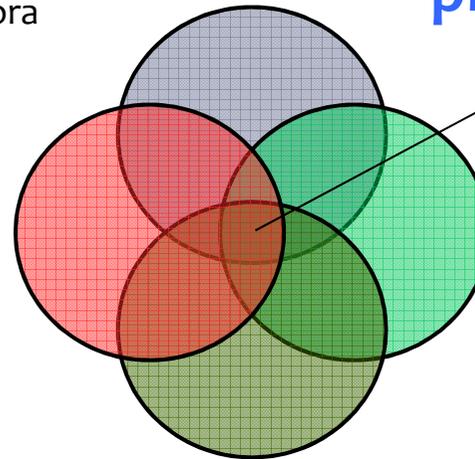
Host associated microbiota (animal & human)

- GastroIntestinal (adult, infant)
- Oral cavity
- Skin flora
- Vaginal flora

platform technology

Clinical microbiology

- MRSA
- ESBL
- VRE, GAS
- Enterobacter
- Pneumococci
- *Candida* sp.
- Novel antibiotics

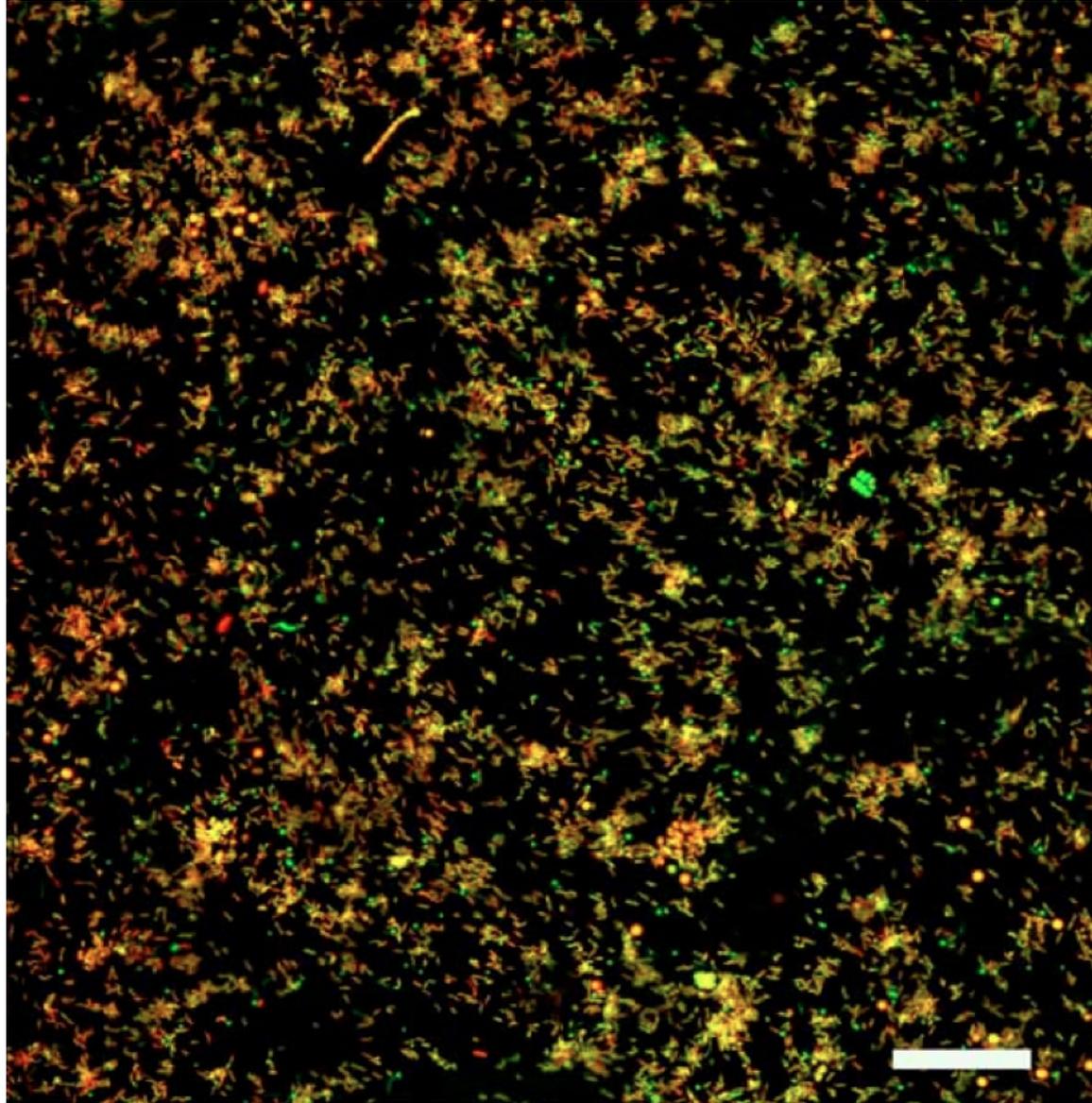
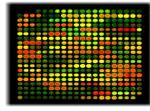


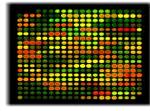
Water

- *Legionella*
- Pathogenic protozoa
- Tox sensor
- Diatoms
- Cyanobacteria

Food Microbiology

- Process optimization
- Food-born pathogen detection & identification
- Hygiene monitoring
- Antibiotics detection in meat
- Design novel preservation strategies

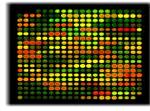




Microbial Ecology

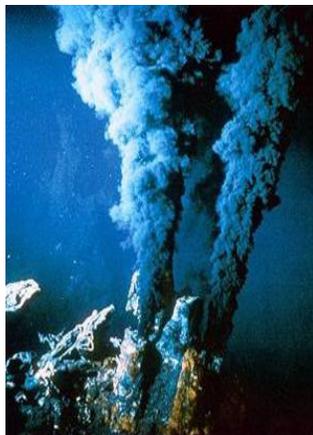
- There are a nonillion bacteria in the world (**10^{30} cells**) give or take a few quadrillion.
(This is more than the number of stars in the known universe)
- Most of these 10^{30} bacterial cells are relatively **small** (0.3 - 3 μm)
- Most of microbes have a **nondescript morphology**
- The vast majority of microbes remains '**unculturable**' using standard methods



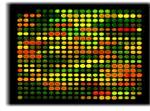


Microbial Ecology & Molecular detection

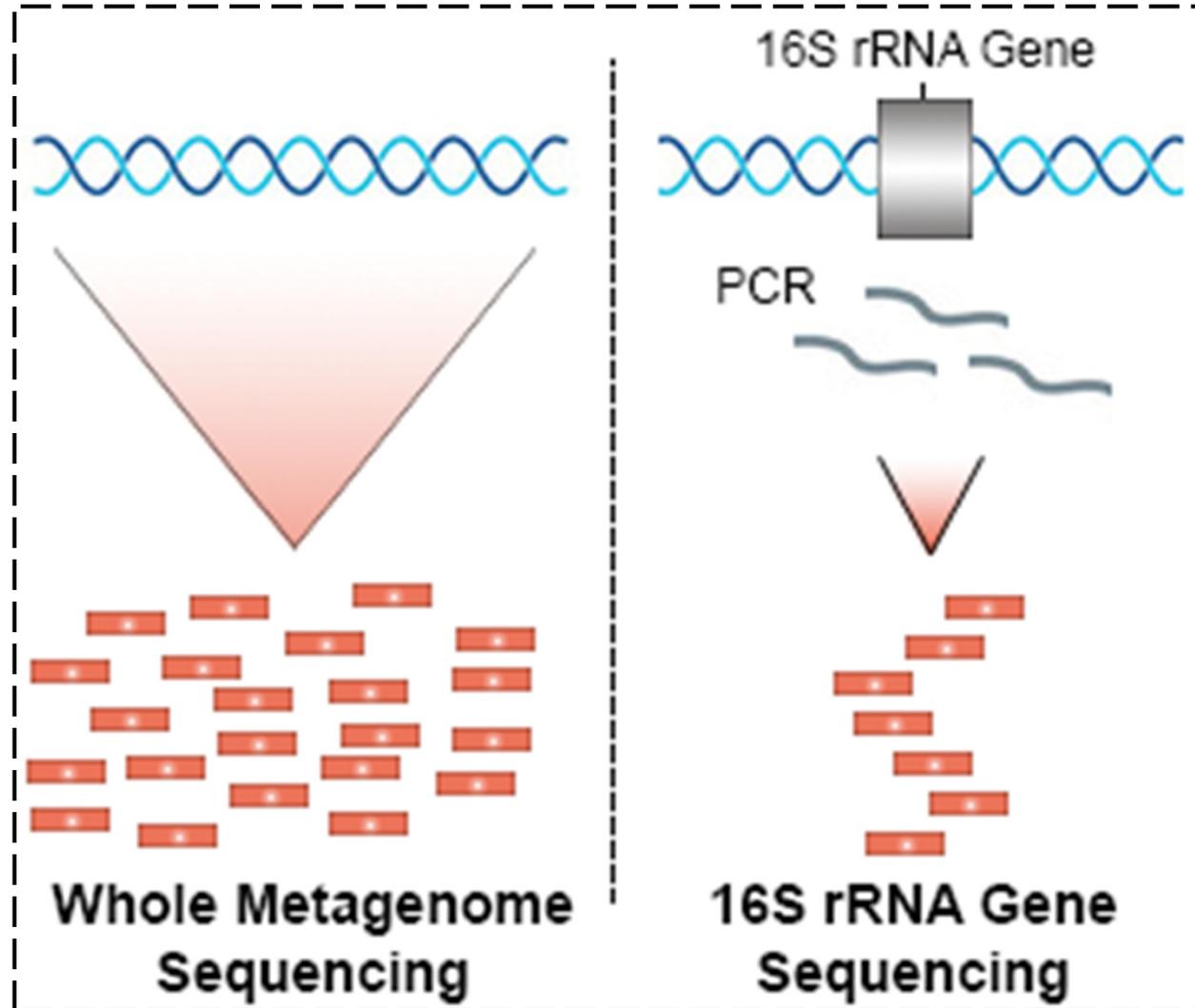
- Sampling from the environment
- Detection and identification of microorganisms in the environment
- Determination of phylogenetic diversity and inferring metabolic diversity
- Microbial activity measurements

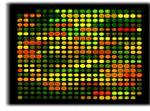


Pace et al., 1985

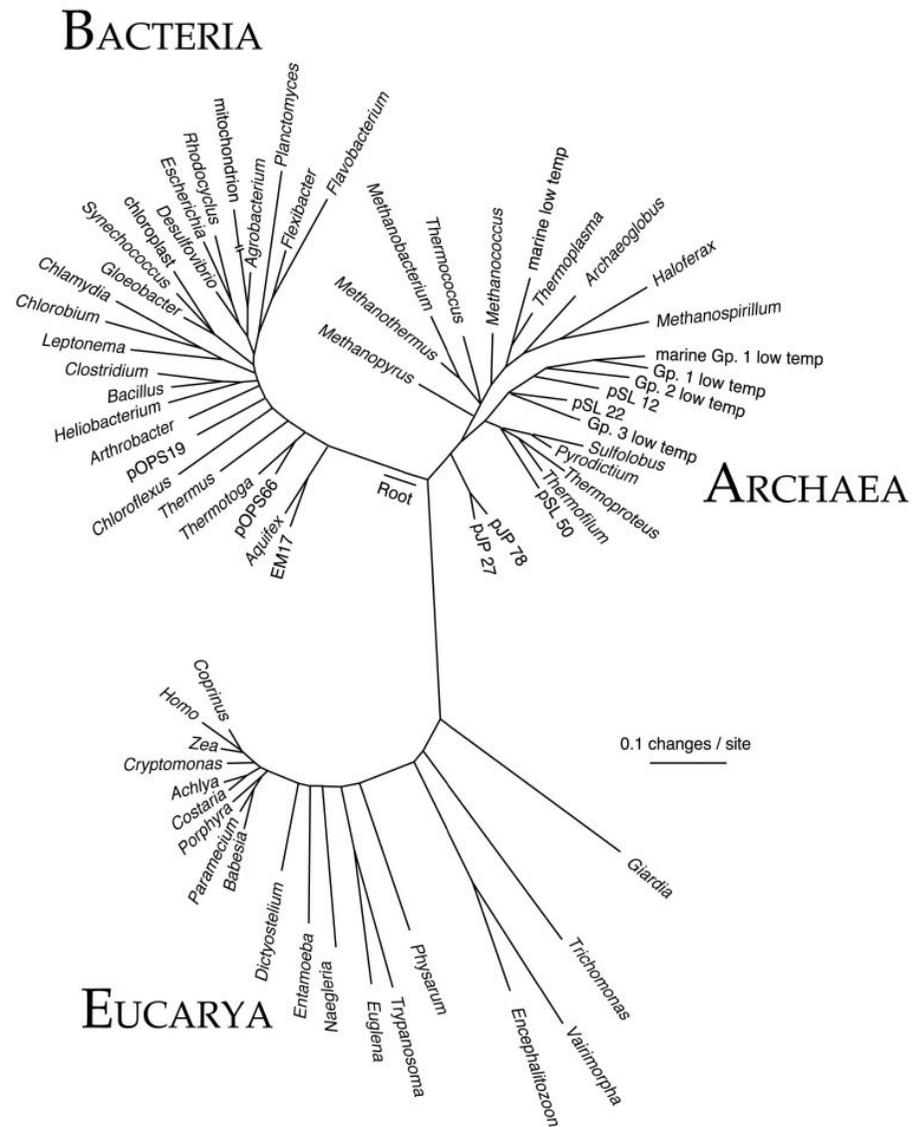


Sequence based surveys of microbiota:

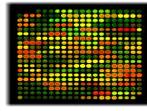




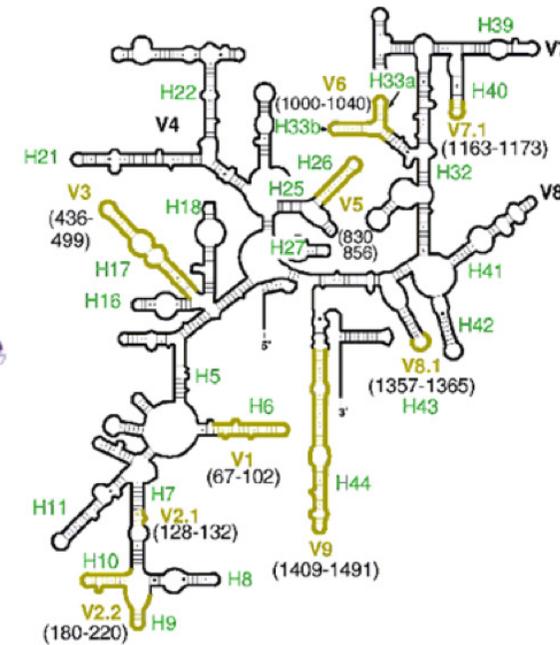
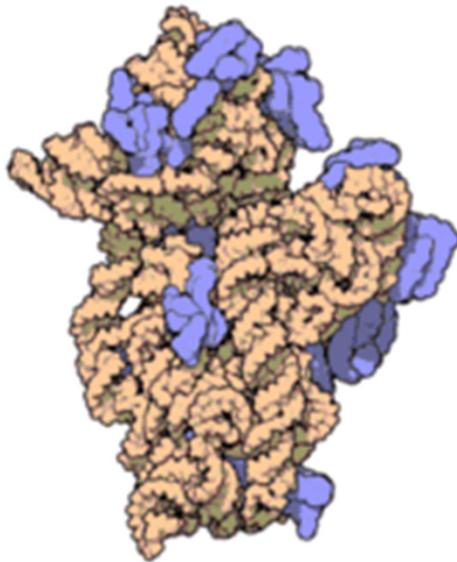
The 16S framework: Three domains of life



Suggested reading: Woese C R (1987) *Microbiol Rev* 51:221–271

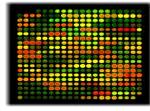


The 16S framework: The Ribosome is a “Ribozyme”



The ribosome is a dynamic molecular machine that manufactures proteins according to the genetic information residing in DNA.

RNA is the catalytic component with proteins being the structural units that support and stabilize it.



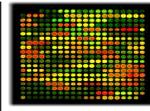
The 16S framework: Ribosomal RNA Sequences as a Tool of Molecular Evolution

Differences in nucleotide or amino acid sequence of functionally similar (homologous) macromolecules are a function of their **evolutionary distance**.

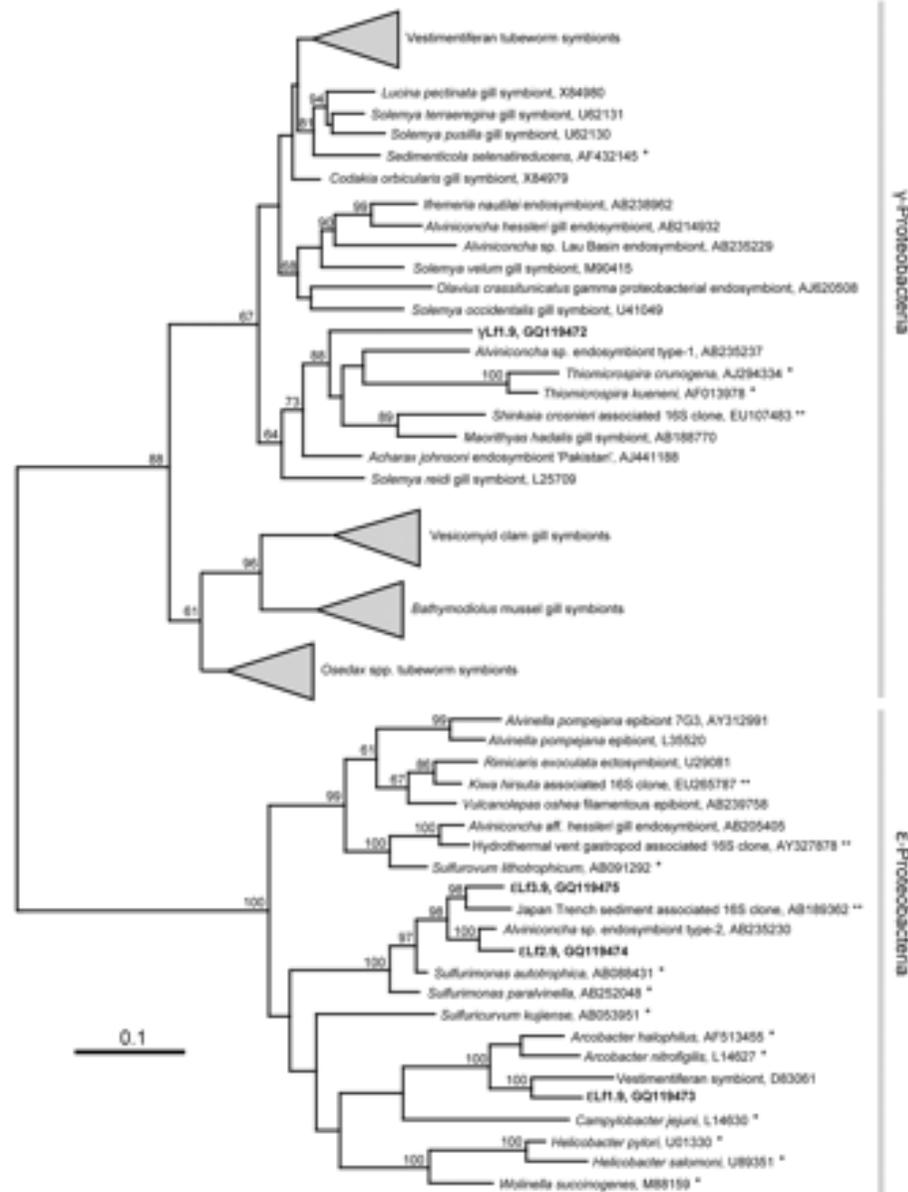
Phylogenetic trees based on ribosomal RNA have now been prepared for all the major prokaryotic and eukaryotic groups.

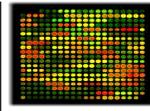
Largedatabases of SSU rRNA sequences exists.

- *Ribosomal Database Project (RDP)*
- *Greengenes*
- *ARB-SILVA*



The 16S framework: Methods for phylogenetic

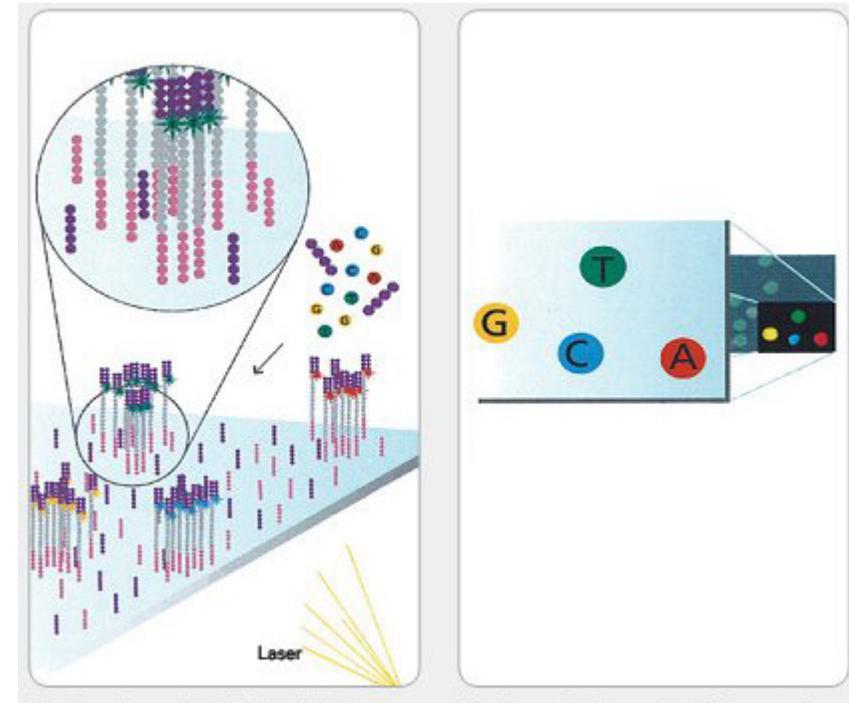
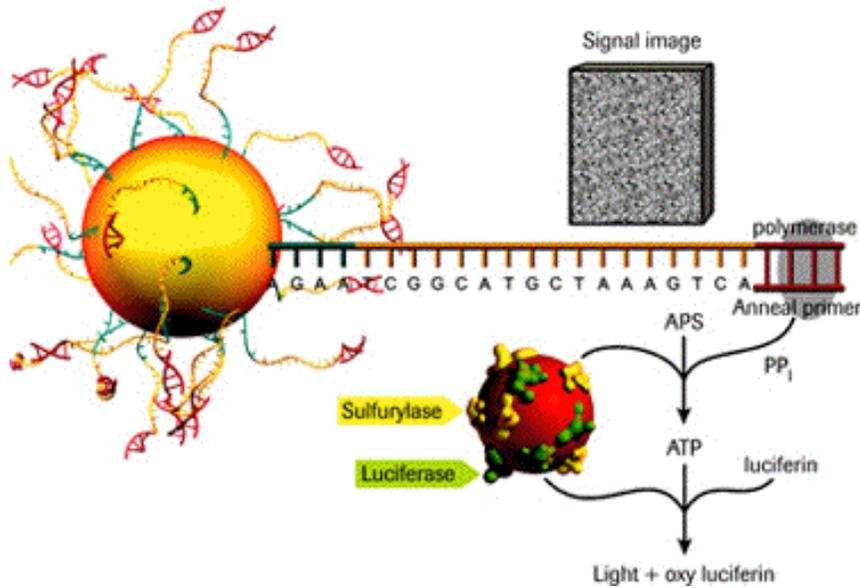




Next generation sequencing (NGS)

Roche/454

Illumina (Miseq)

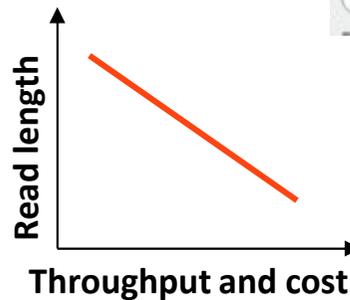


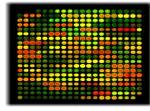
0.5 Milion 450bp reads

\$\$

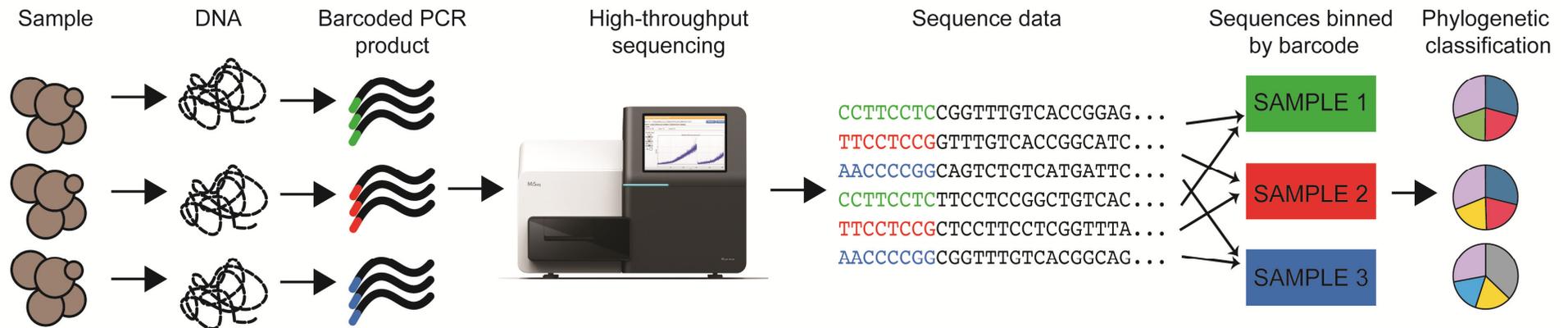
15 Million 250bp reads

\$

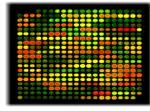




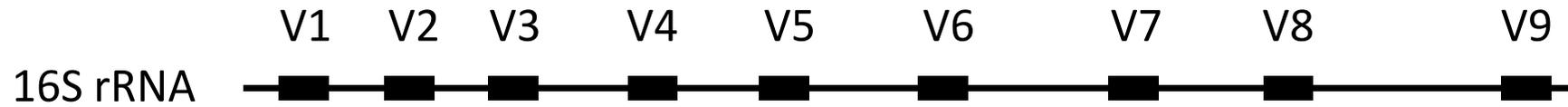
“NextGen Sequencing of barcoded amplicons”



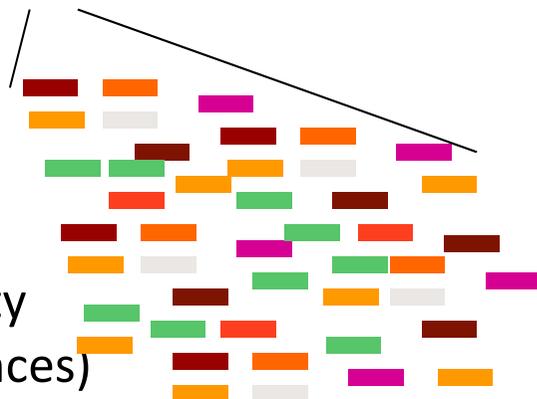
Next generation sequencing technologies have made it possible to obtain **deep sampling coverage** of the 16S rRNA gene



Amplicon tags: Deeper, cheaper, faster



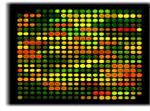
Generate amplicons of a given variable region from bacterial community (many millions of sequences)



Reduce dataset by **dereplication/clustering**

-  X 10
-  X 1
-  X 1,000
-  X 2,000
-  X 200
-  X 1,200
-  X 800
-  X 10,000

Identification

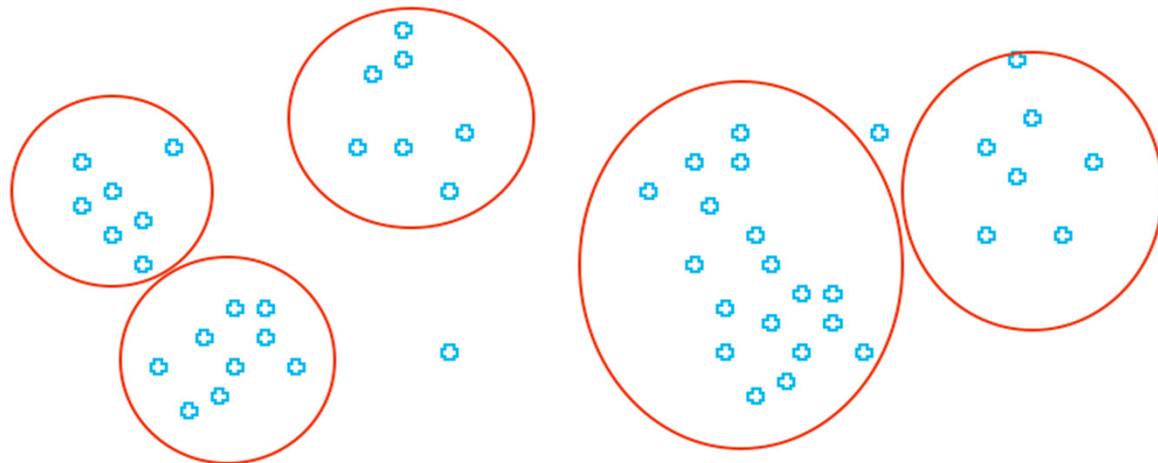


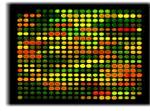
The 16S framework: Clustering

Based on sequence identity sequences can be assigned to **Operational Taxonomic Units (OTUs)** (for different distance levels).

Commonly used Hierarchical Clustering methods:

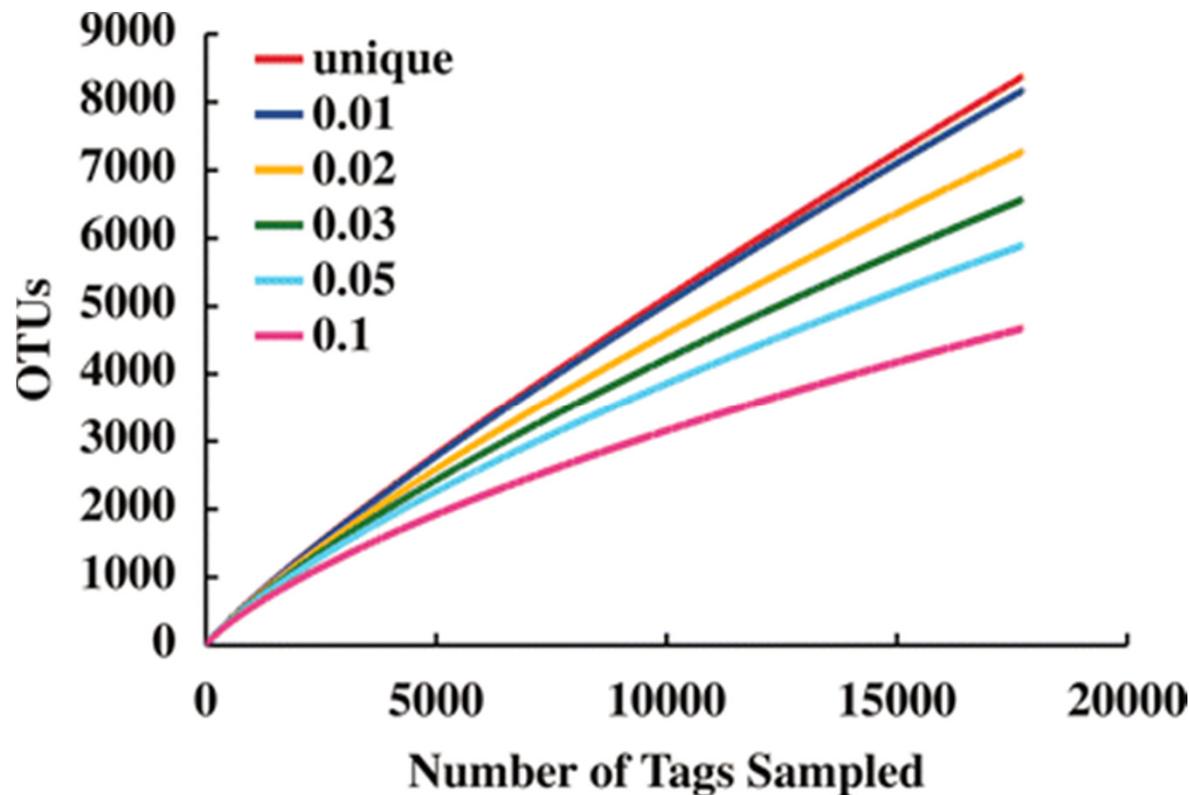
- nearest neighbor
- average neighbor
- furthest neighbor





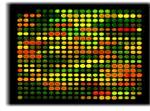
The 16S framework: “Deep sequencing & the Rare Biosphere”

Next generation sequencing technologies have made it possible to obtain **deep sampling coverage** of the 16S rRNA gene



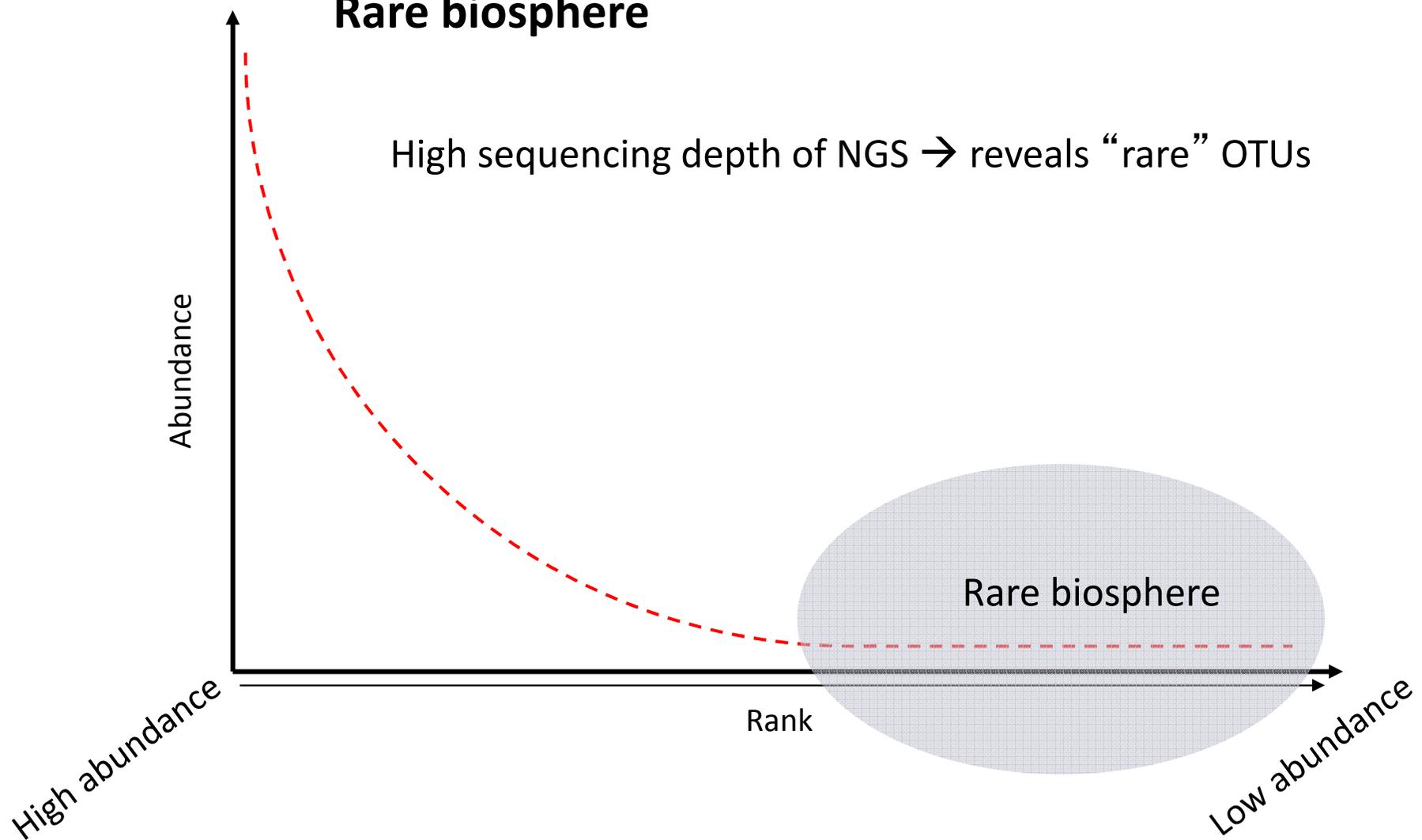
Rarefaction analysis of observed
16S Operational Taxonomic Units
(OTUs)

Sogin et al. PNAS, 2006

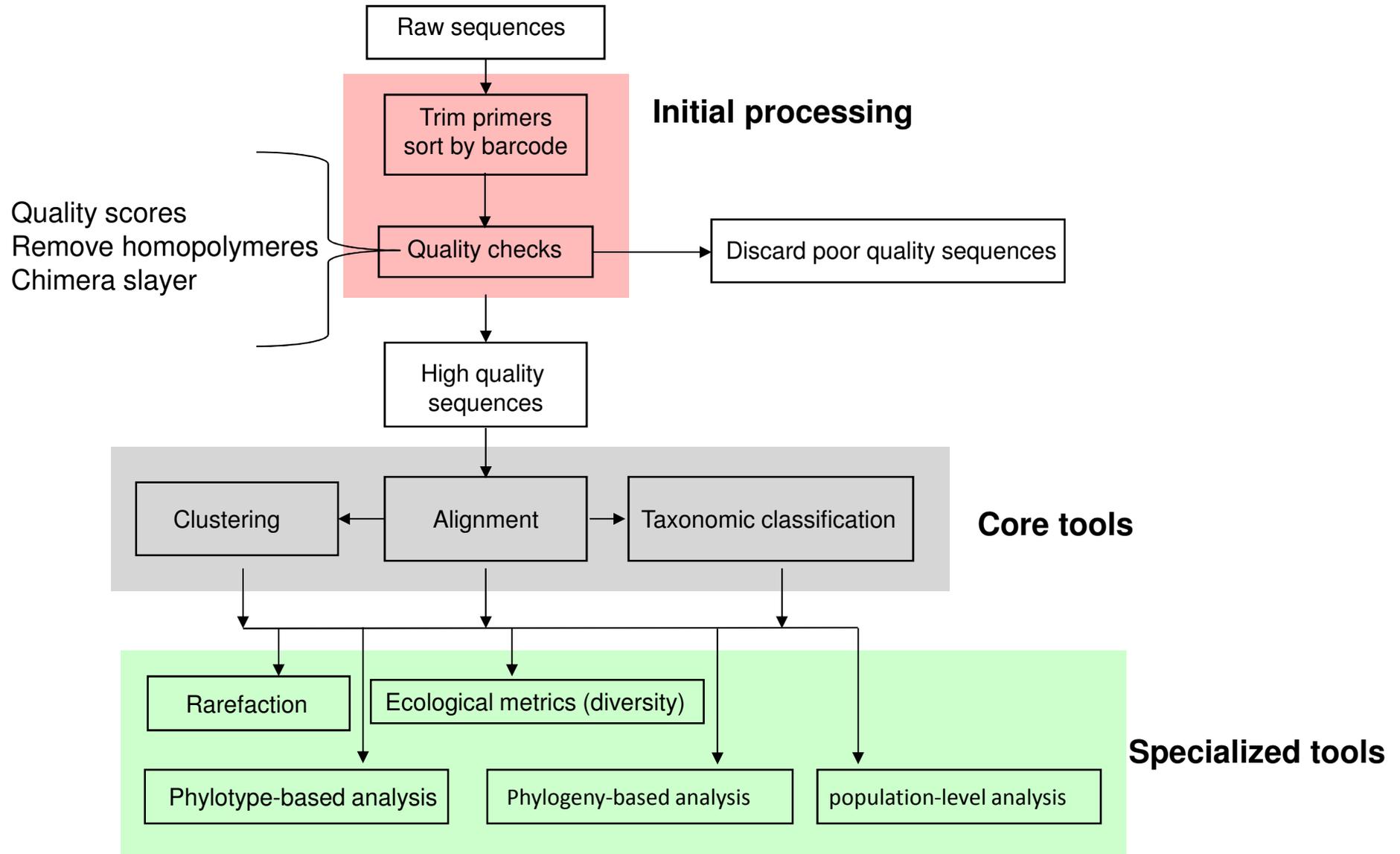
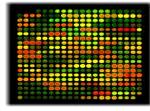


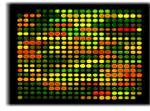
Rare biosphere

High sequencing depth of NGS → reveals “rare” OTUs

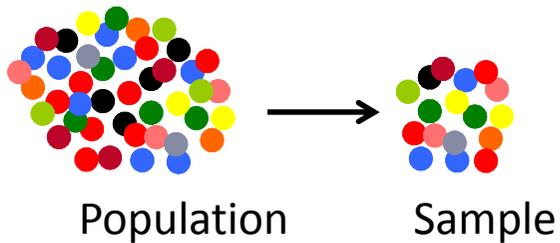


Sequencing error? Chimeras? Background noise?





Sampling a large population: did we sequence deep enough?

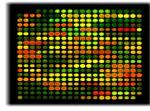


Thus, **coverage** of the population by
your sample would be:

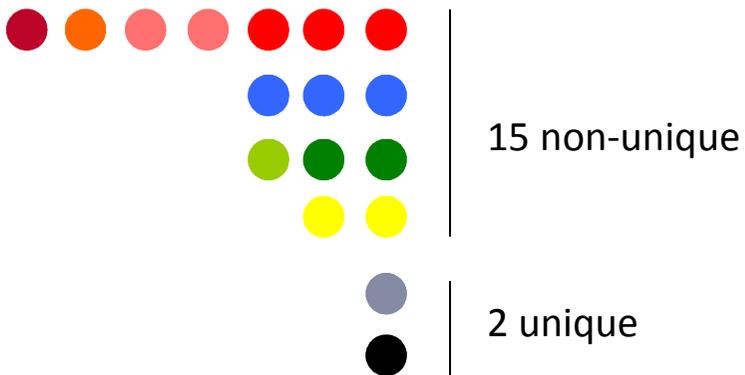
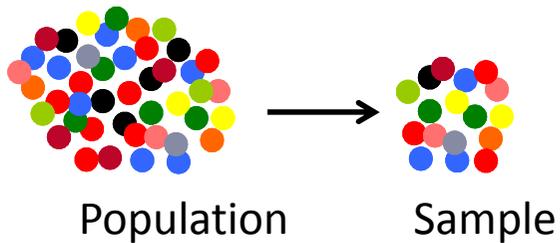
$$C = 1 - (\text{number unique}/\text{total number})$$

$$C = 1 - (5/17)$$

$$C = 70.6\%$$



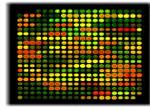
Sampling a large population.



Changing the definition of "unique" such that close colors are considered the same (e.g. red/pink/orange) will change the coverage

$$C = 1 - (2/17)$$

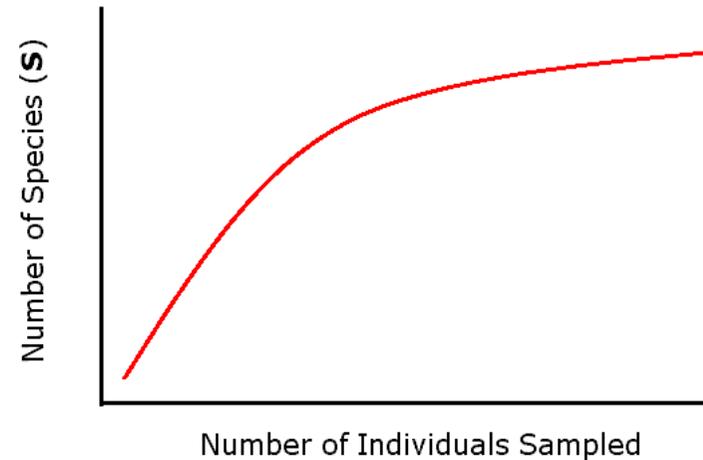
$$C = 88.2\%$$



How to quantify biodiversity

Two aspects of biodiversity:

- Species Richness
(number of species in a community).

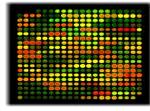


- Relative Abundance or Equitability
(evenness with which the individuals are spread out among the species in a community).

$$H' = -\sum p_i \ln(p_i)$$

H' = The Shannon-Weaver Diversity Index

p_i = the relative abundance of each group of organisms



Phylogenetic Similarity:

UniFrac:

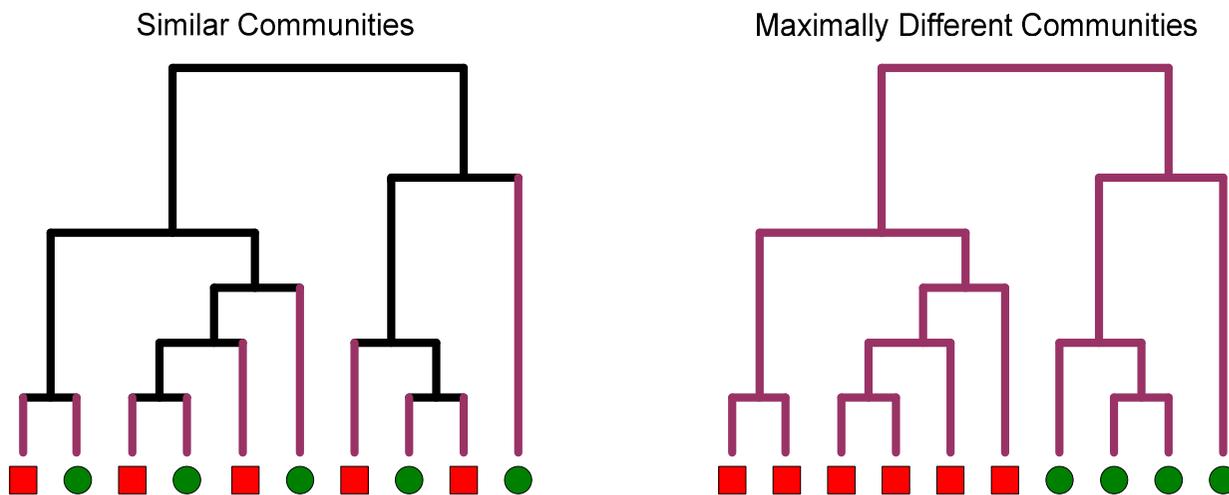
Hypothesis:

There is less evolutionary history shared between a pair of environments than would be expected by chance

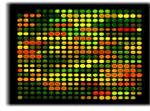
Two communities are compared:

Community 1: ■

Community 2: ●



$$\text{UniFrac Distance Measure} = \frac{\text{purple}}{\text{black} + \text{purple}}$$

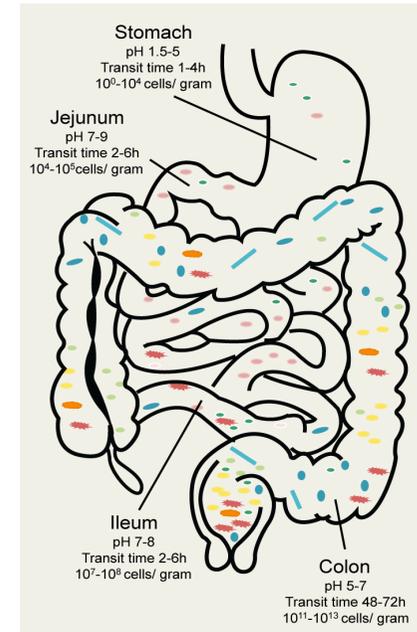


Application: the human Microbiome



Human microbiome
1,000,000+ genes

Human genome
23,000 genes

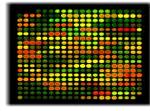


The microbiota of the distal colon contains an estimated 10^{13} individual microbial cells

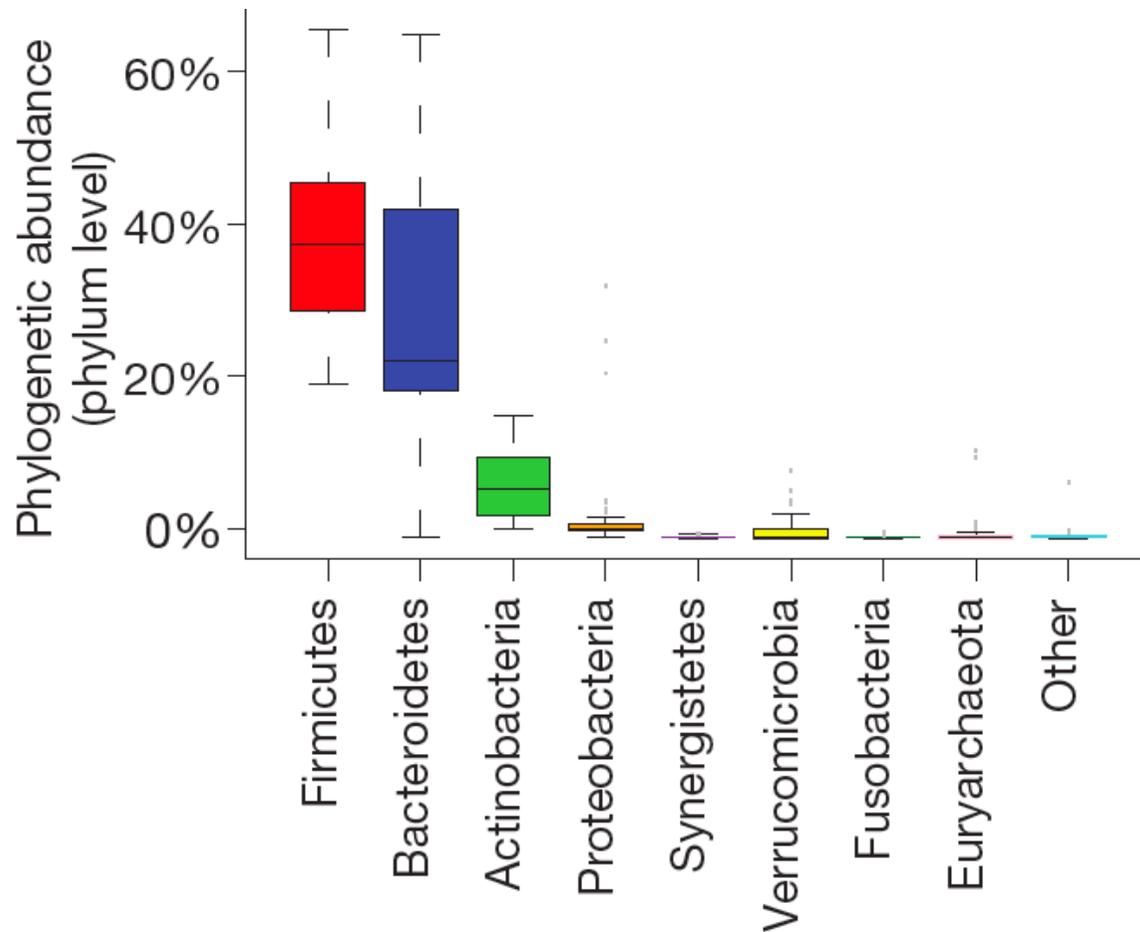
Human metabolic features are combo of human and microbial traits

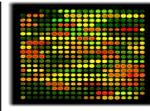
Microbiota: microorganisms that live inside and on humans

Microbiome: the genomes of the microbial symbionts



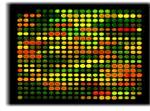
Bacterial phyla in the Human gut



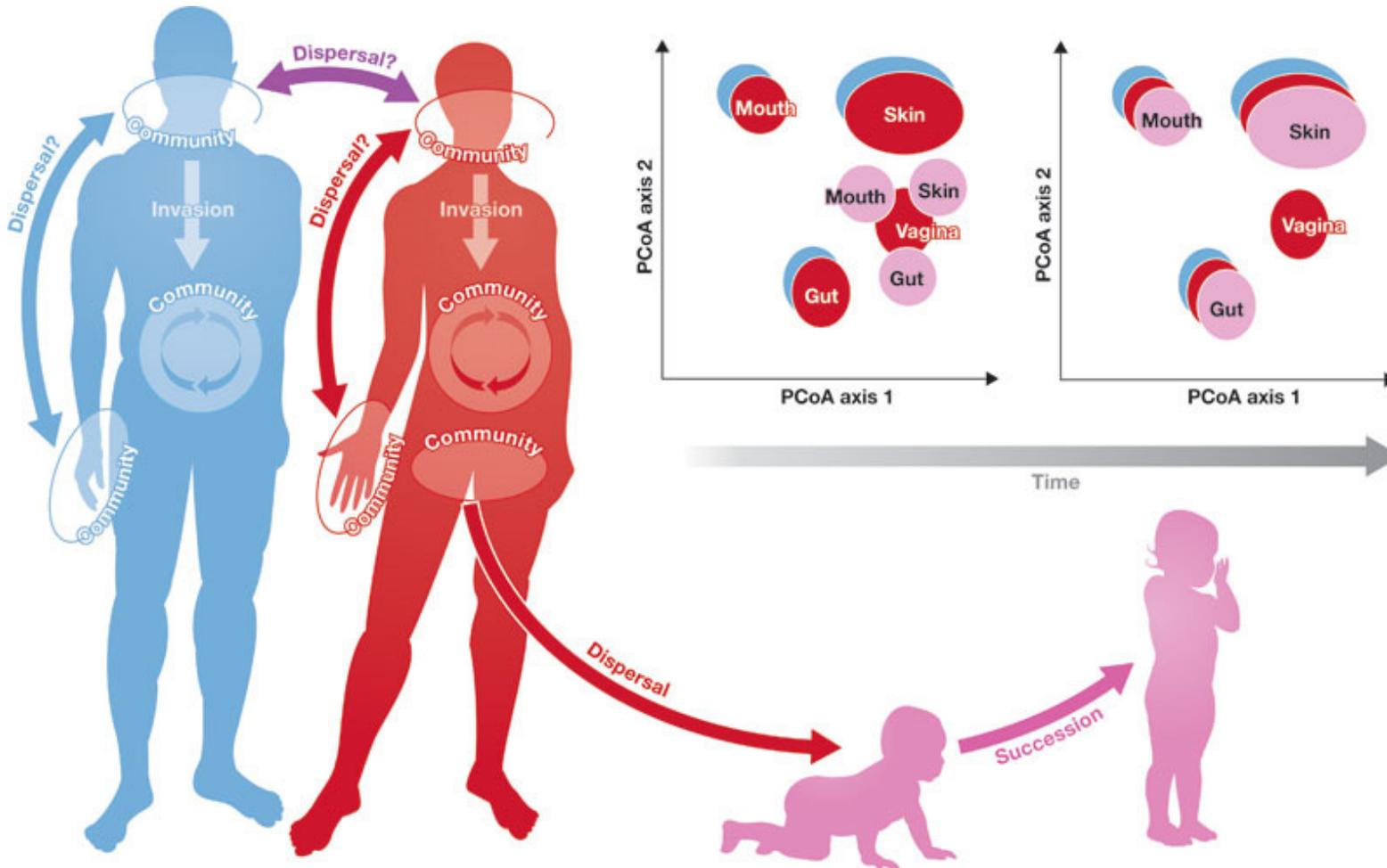


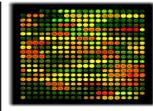
(Gastrointestinal) Health and Microbiota

Disease	Relevant finding
Psoriasis	Increased ratio of Firmicutes to Actinobacteria
Reflux oesophagitis	Oesophageal microbiota dominated by gram-negative anaerobes; gastric microbiota with low or absent <i>Helicobacter pylori</i>
Obesity	Reduced ratio of Bacteroidetes to Firmicutes
Childhood-onset asthma	Absent gastric <i>H. pylori</i> (especially the cytotoxin-associated gene A (<i>cagA</i>) genotype)
Inflammatory bowel disease (colitis)	Larger populations of Enterobacteriaceae
Functional bowel diseases	Larger populations of <i>Veillonella</i> and <i>Lactobacillus</i>
Colorectal carcinoma	Larger populations of <i>Fusobacterium spp.</i>
Cardiovascular disease	Gut-microbiota-dependent metabolism of phosphatidylcholine

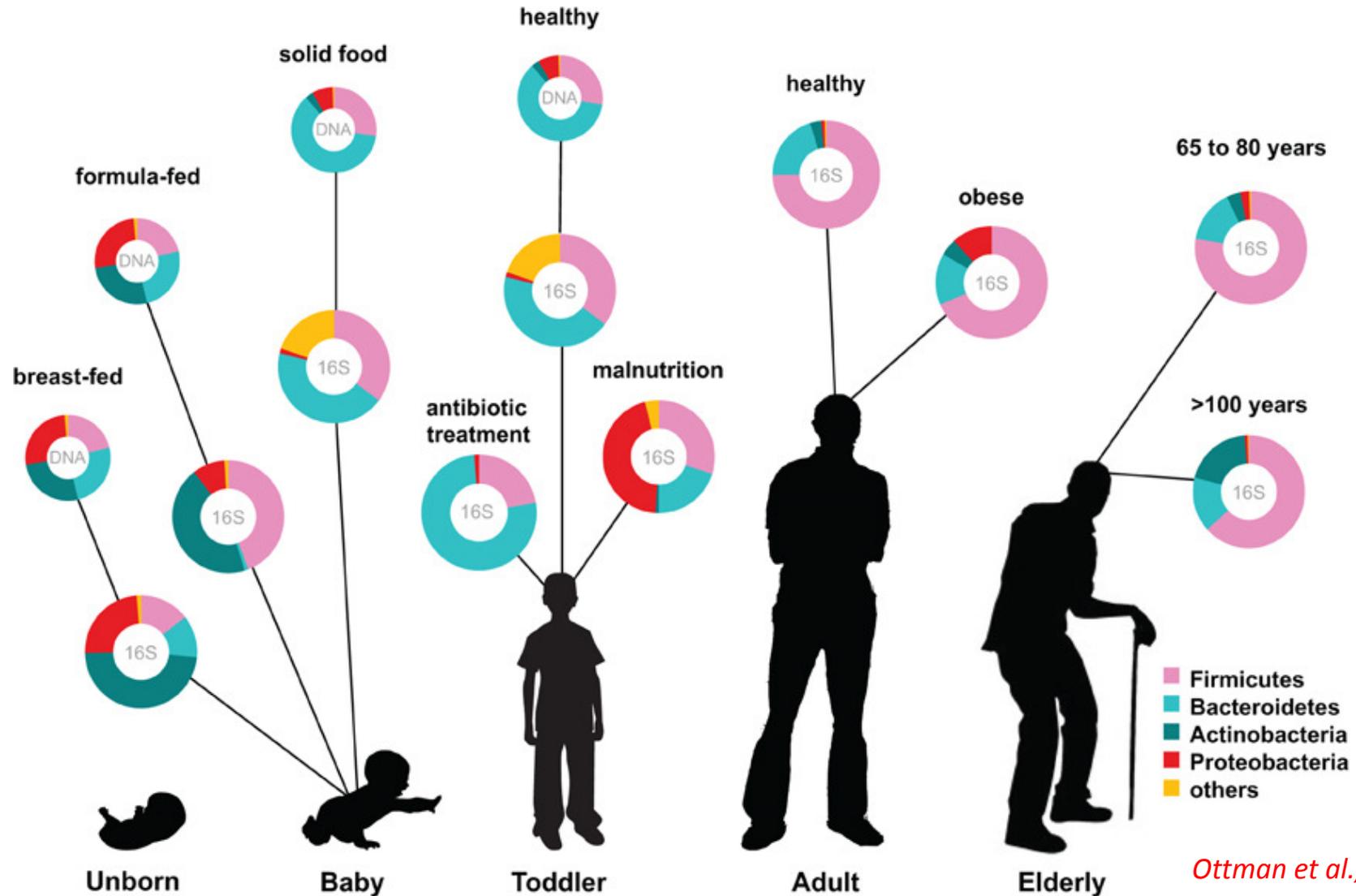


Acquisition of the microbiome: Vertical transmission or Lateral transmission?

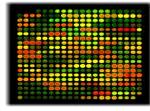




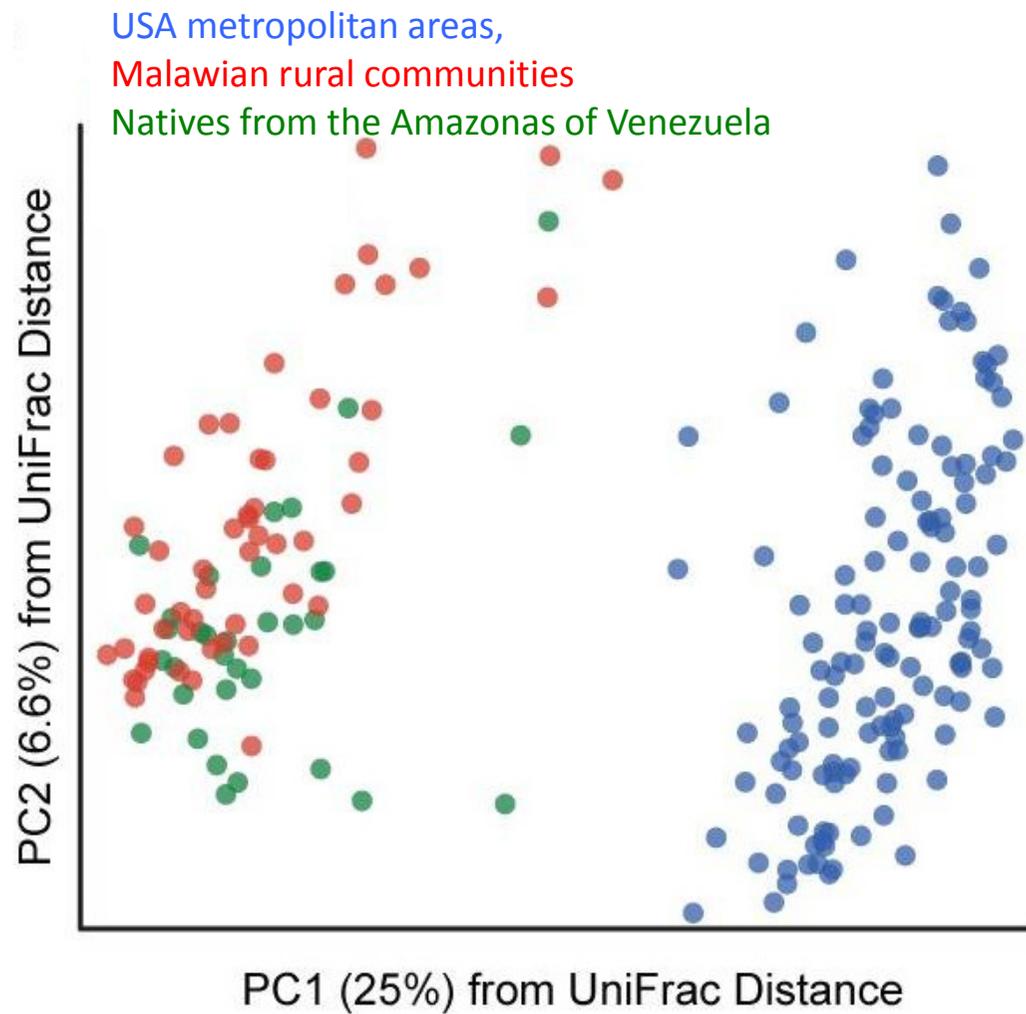
Human Gut Microbiota & Age



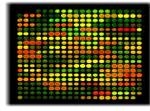
Ottman et al., 2012



Human gut microbiome & geography

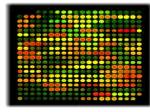


Yatsunenko, Nature, 2012

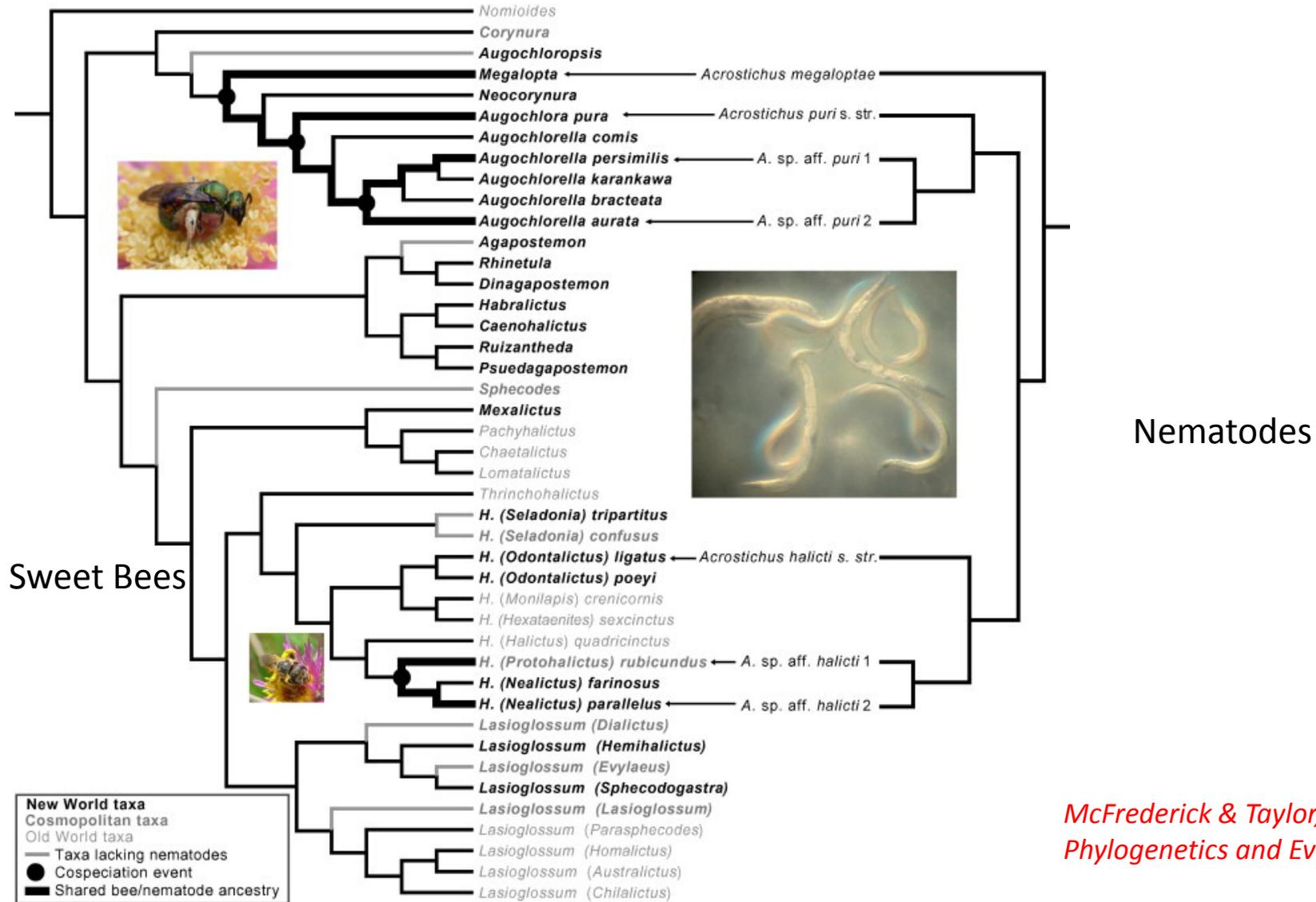


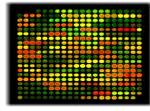
Host-microbiome co-evolution?

- In studying germ-free (GF) mice we can now see that genes are insufficient to produce a “viable” organism.
- If a microorganism can exploit a trait that already exists, then it reduces or even eliminates the selection pressure for such a trait to be manifest in the host genome.
- Consider the role of the microbiota and their influence on the evolution of their our genomes...

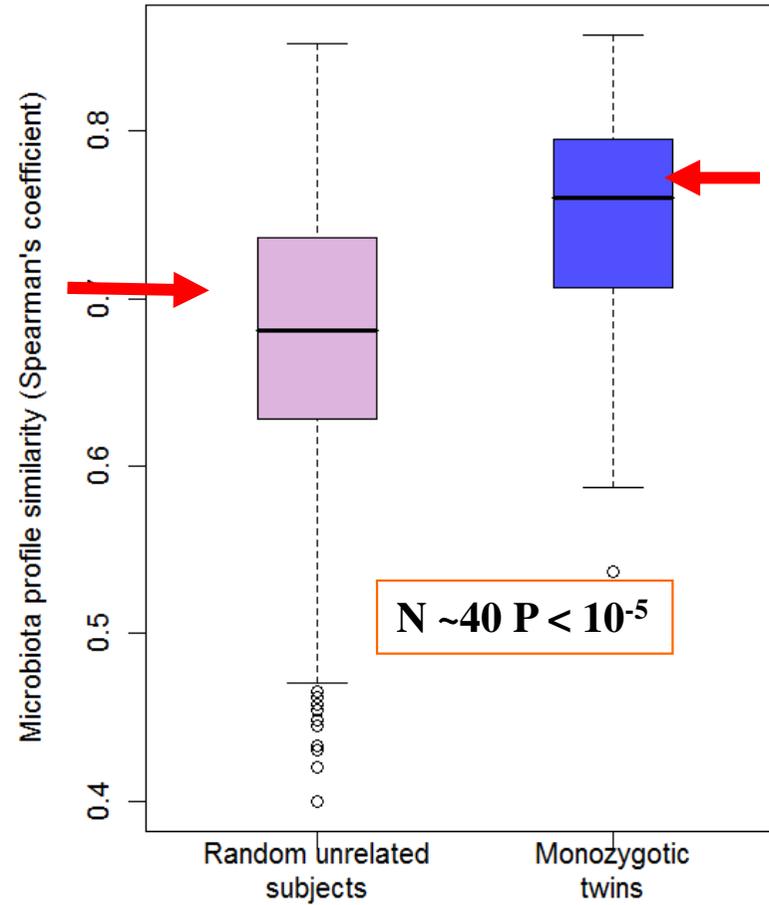


Host parasite Co-speciation



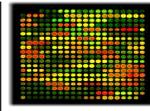


Monozygotic Twins Have Similar Microbiota

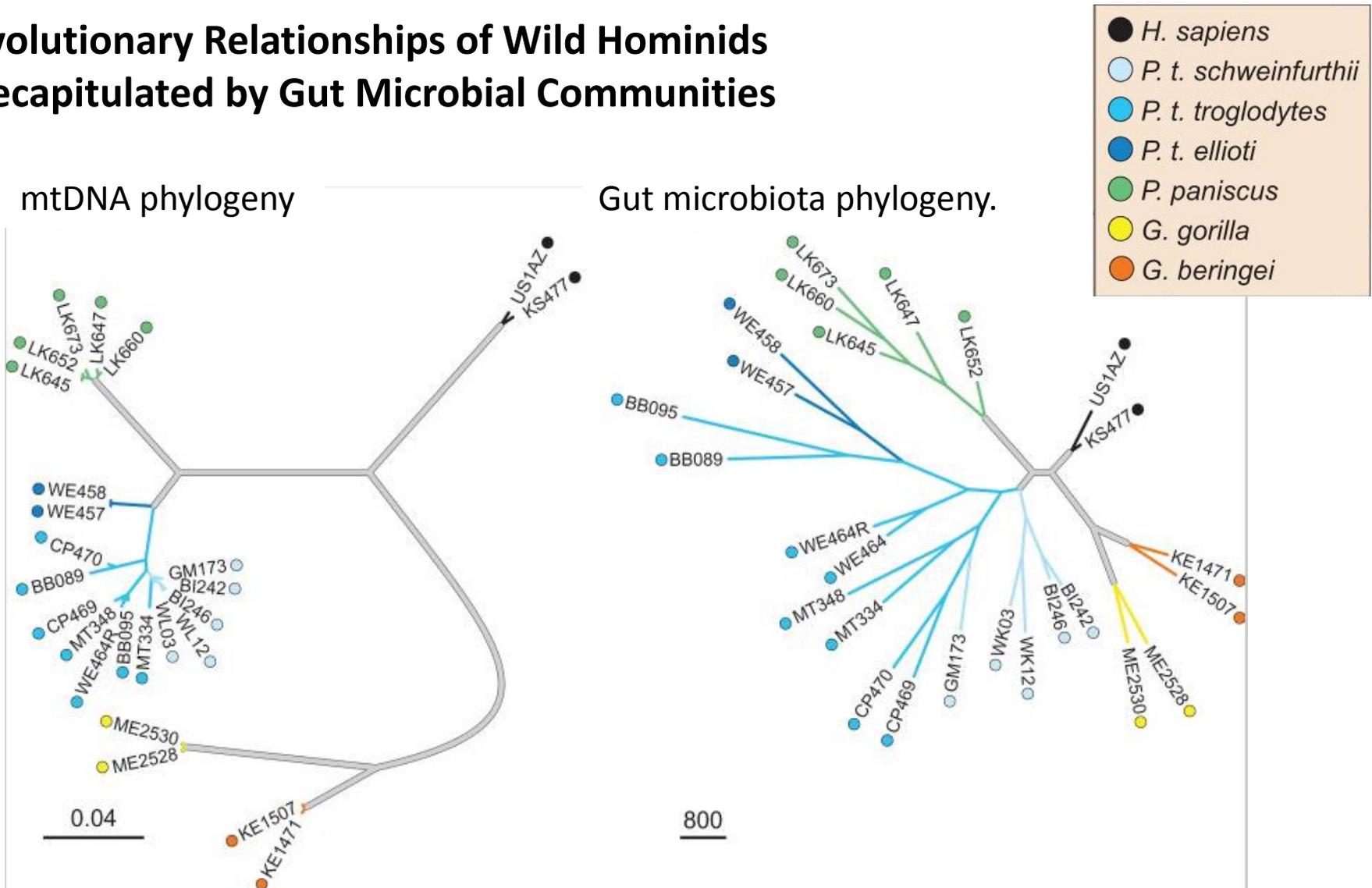


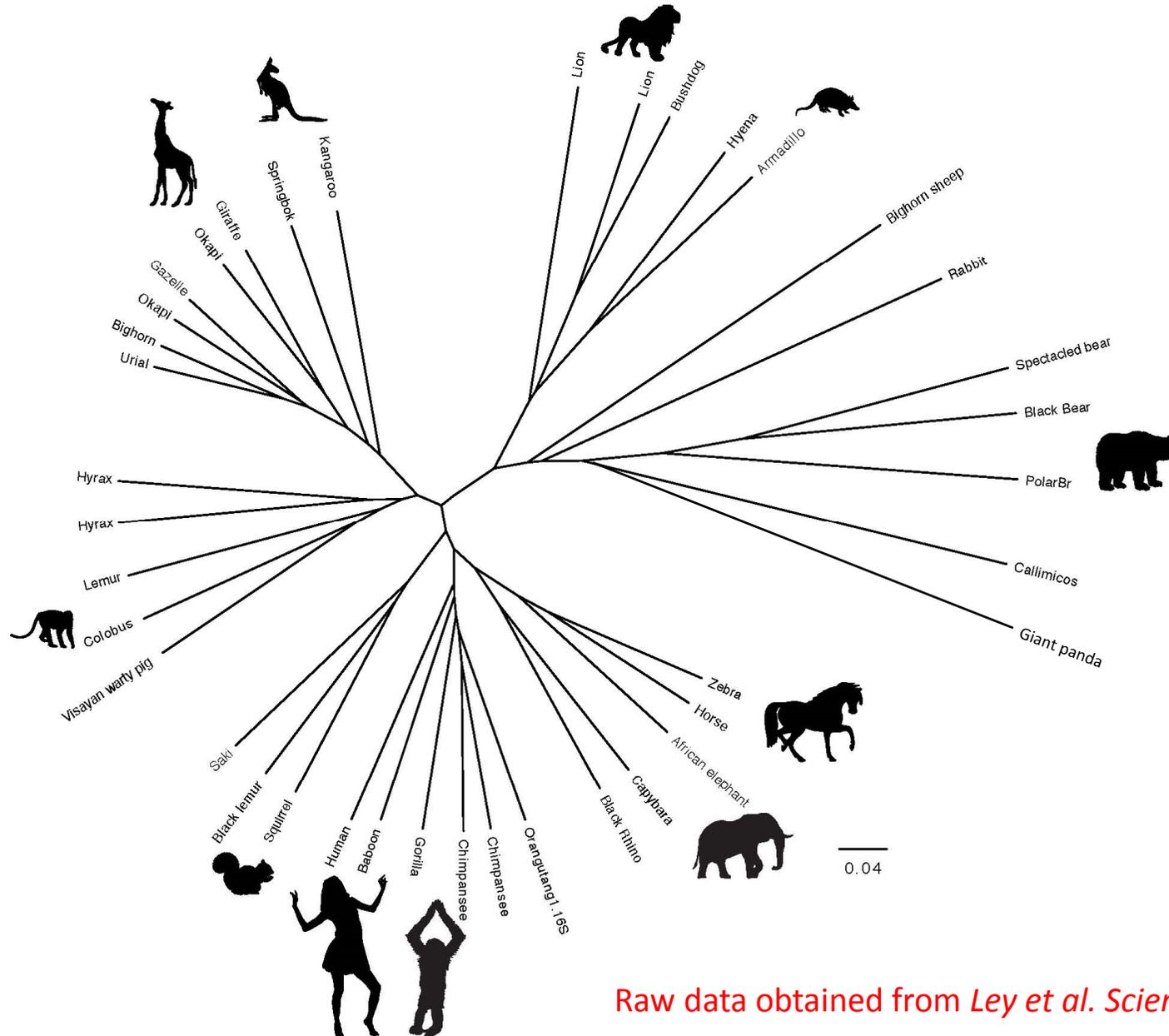
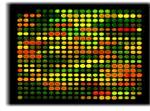
Zoetendal et al., *Microbial Ecology in Health & Disease* 2001

Dicksved et al., *ISME Journal* 2008

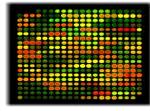


Evolutionary Relationships of Wild Hominids Recapitulated by Gut Microbial Communities





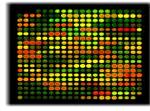
Raw data obtained from *Ley et al. Science 2008*



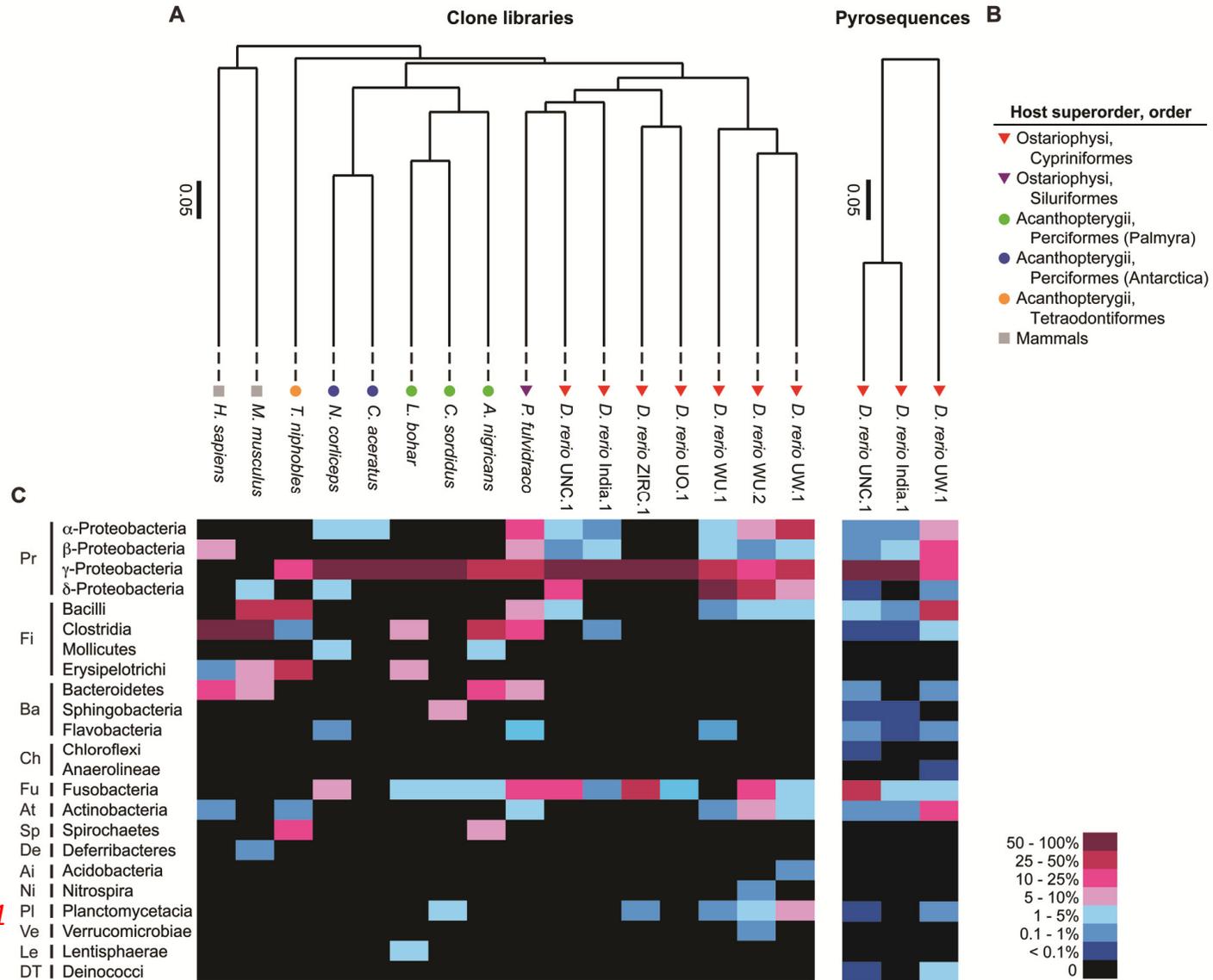
Evolution of Mammals and Their Gut Microbes

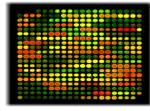
- host diet and phylogeny both influence bacterial diversity, which increases from carnivory to omnivory to herbivory
- the gut microbiota of humans living a modern life-style is typical of omnivorous primates.
- Despite their herbivorous diet, red and giant pandas have simple guts, cluster with other carnivores, and have carnivore-like levels of phylogenetic diversity



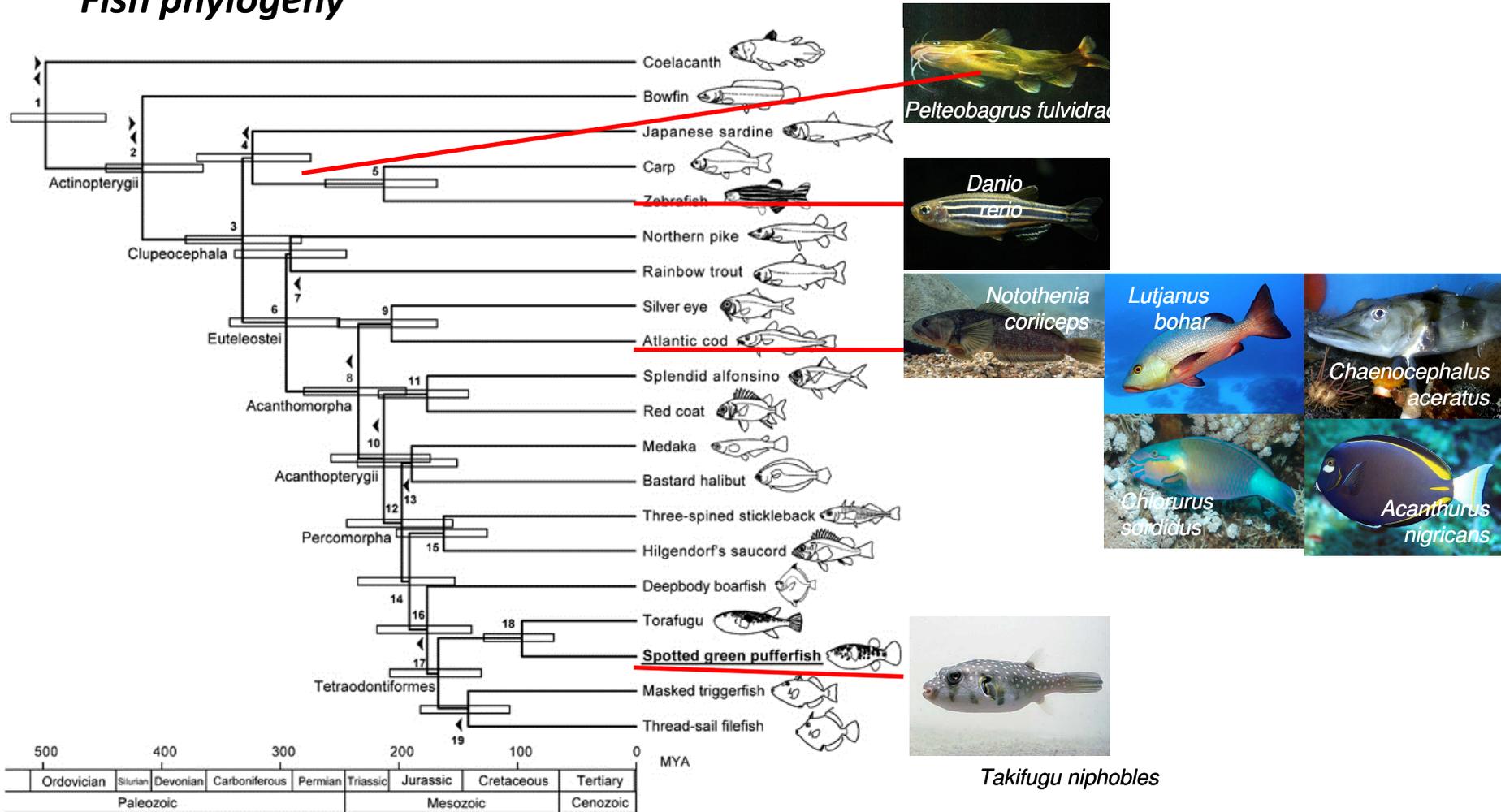


Microbial diversity reflects host phylogeny

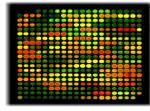




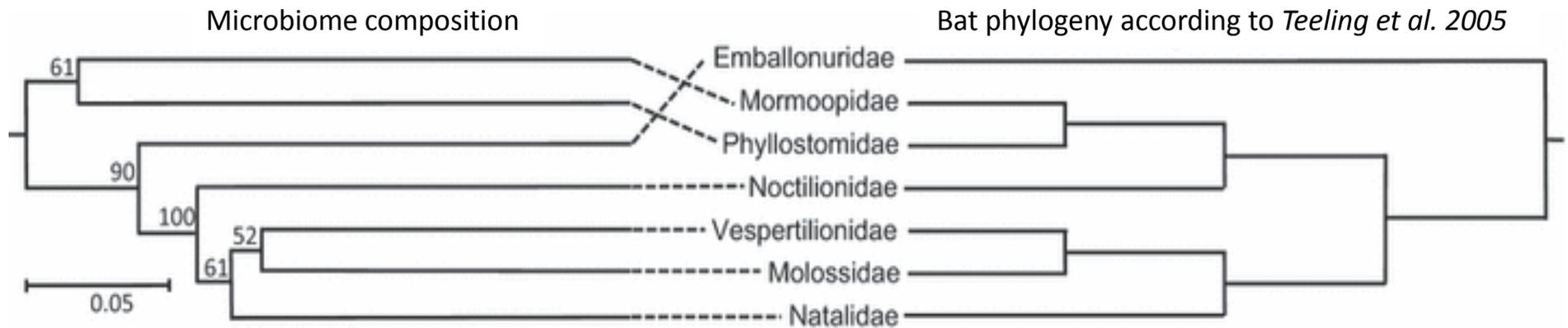
Fish phylogeny

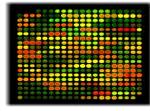


Yamanoue et al. (2006) GGE



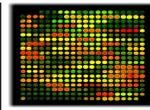
Gut microbiome composition & bat phylogeny





Take home messages

- Microbial ecology is currently experiencing a renaissance spurred by the rapid development of NGS techniques.
- Bioinformatics has become a bottleneck for microbial ecologists as the throughput of NGS instruments increases every quarter.
- Microbial ecology is not only fundamental basis for (bio)geosciences...
... but especially important in understanding the correlation between our microbiome and our health.
- Humans (and other animals) form co-evolving “multi-symbiosis”
- Phenotype is dictated by, the genetically inherited human genome (~25,000 genes) and the environmentally acquired microbiome (> 1 million genes).
- Your genome and microbiome must work in harmonious integration as a *hologenome* to maintain health.



Thank you!

