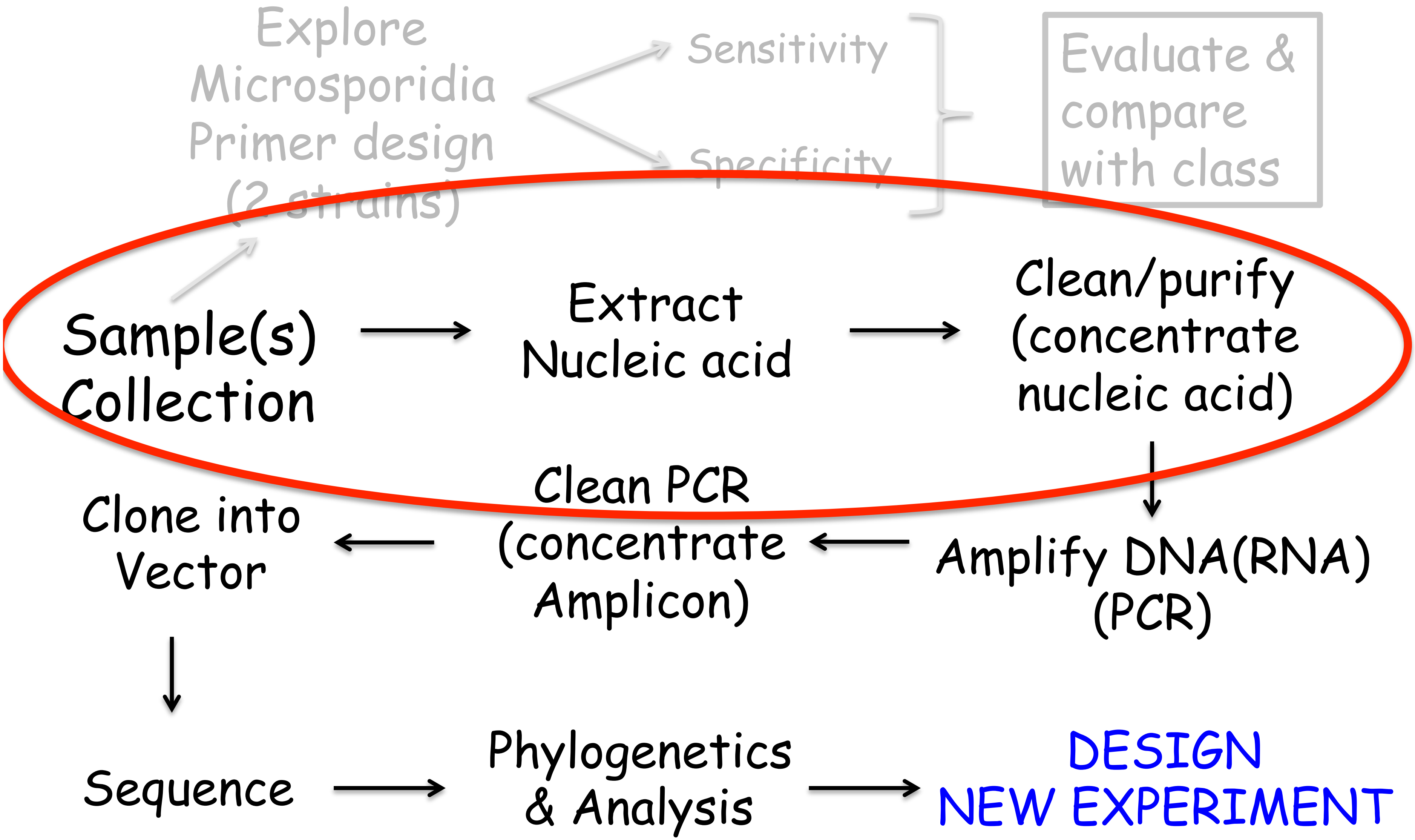
20.109
Laboratory Fundamentals in Biological Engineering

Module 1
Nucleic Acid Engineering
Lecture 3

Module 1



Explore Microsporidia Primer design (2 strains)

Sensitivity

Specificity

Evaluate & compare with class

Sample(s) Collection → Extract Nucleic acid → Clean/purify (concentrate nucleic acid)

→ Amplify DNA(RNA) (PCR) → Clean PCR (concentrate Amplicon) → Clone into Vector

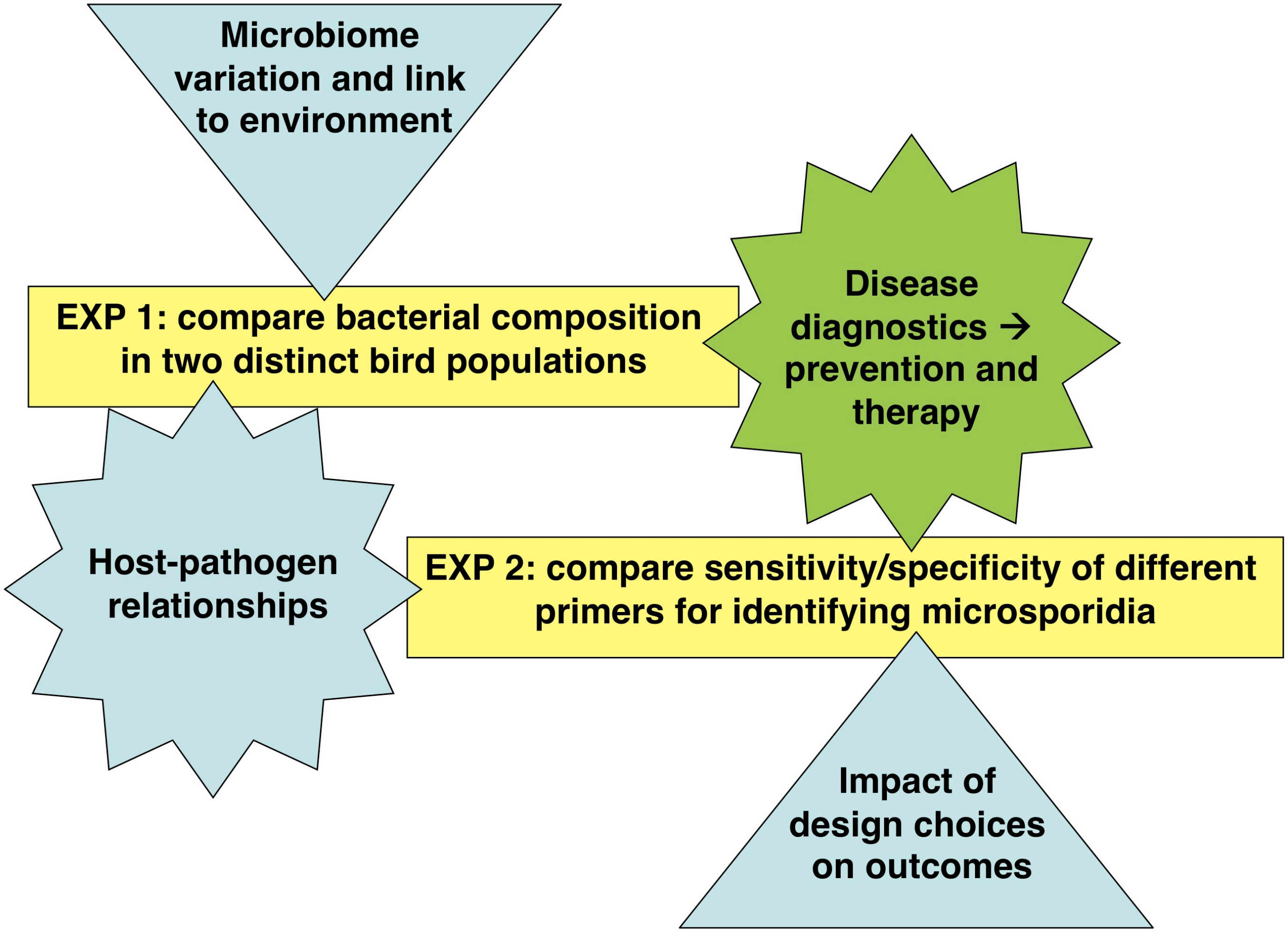
→ Sequence → Phylogenetics & Analysis → DESIGN NEW EXPERIMENT

DNA Extraction

- Break cells open
- Remove lipids
- Remove proteins
- Remove RNA
- Precipitate DNA
- Rehydrate DNA

- Plus special ingredients

DNA engineering: investigating pathogens



Microbiome variation and link to environment

EXP 1: compare bacterial composition in two distinct bird populations

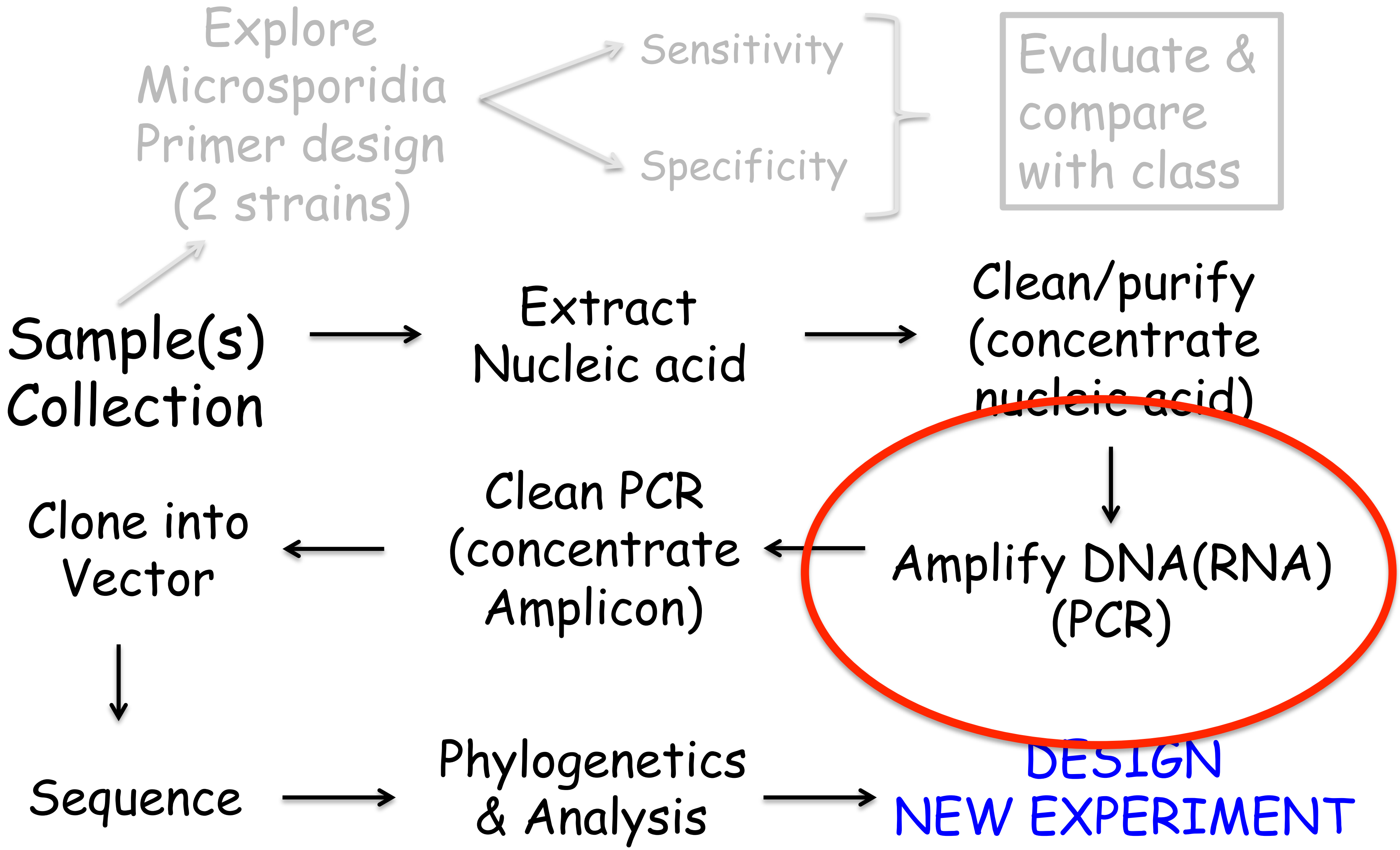
Disease diagnostics → prevention and therapy

Host-pathogen relationships

EXP 2: compare sensitivity/specificity of different primers for identifying microsporidia

Impact of design choices on outcomes

Module 1



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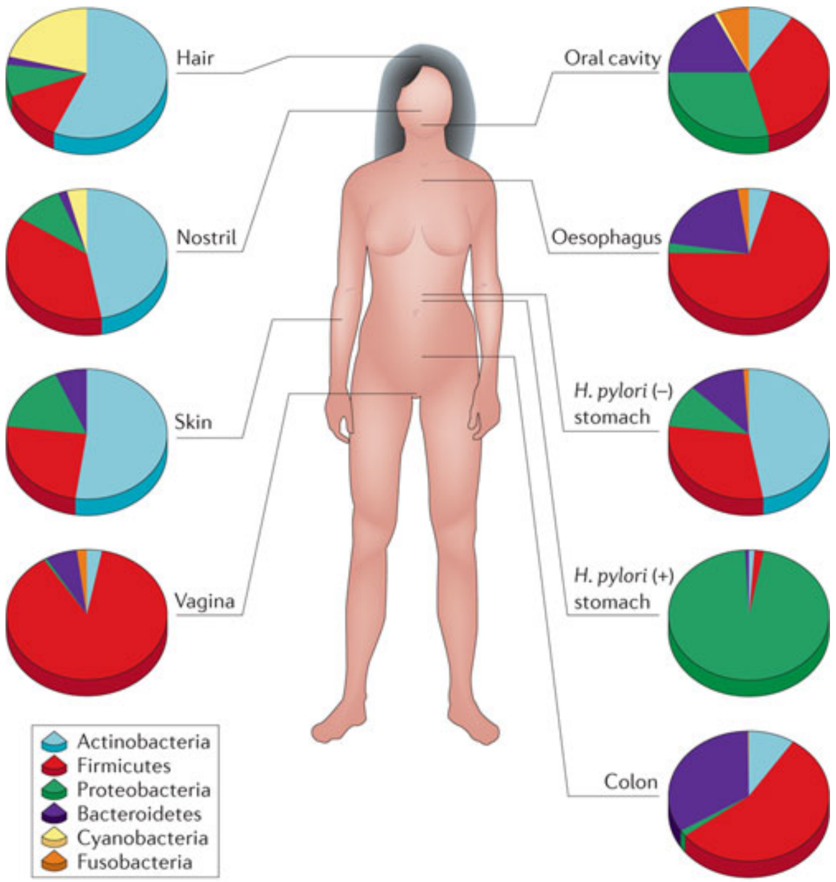
Sequence

Phylogenetics & Analysis

DESIGN NEW EXPERIMENT

A world of environments



Nature Reviews | Genetics

Microbial community profiling

- What criteria make a good molecular target?

1) SSU rRNA
2) Sequencing
3) Something in between

Community profiling

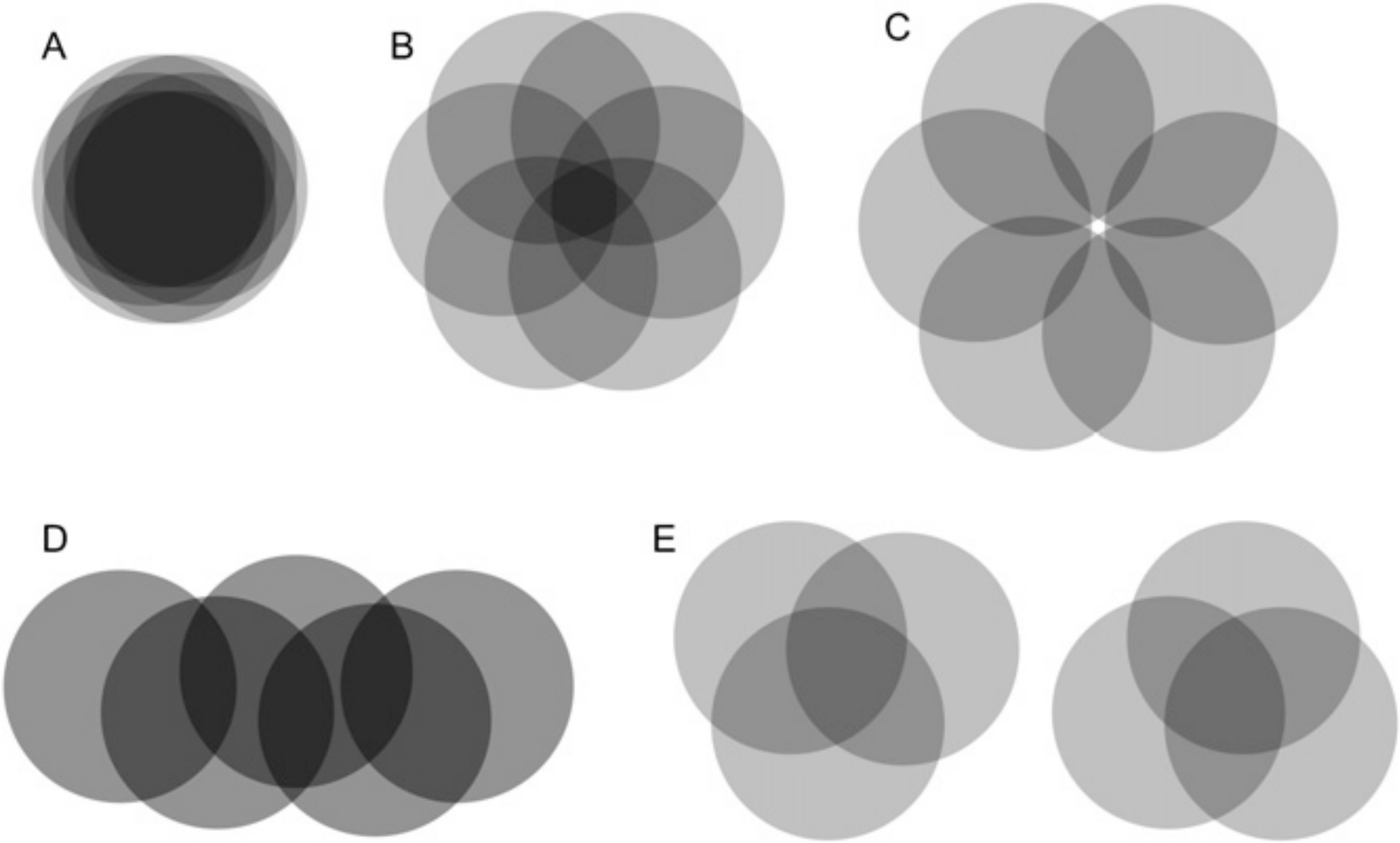
- # unique reads vs # total reads

- # sequences vs taxonomic units (OTUs)

1) Is there a core microbiome?

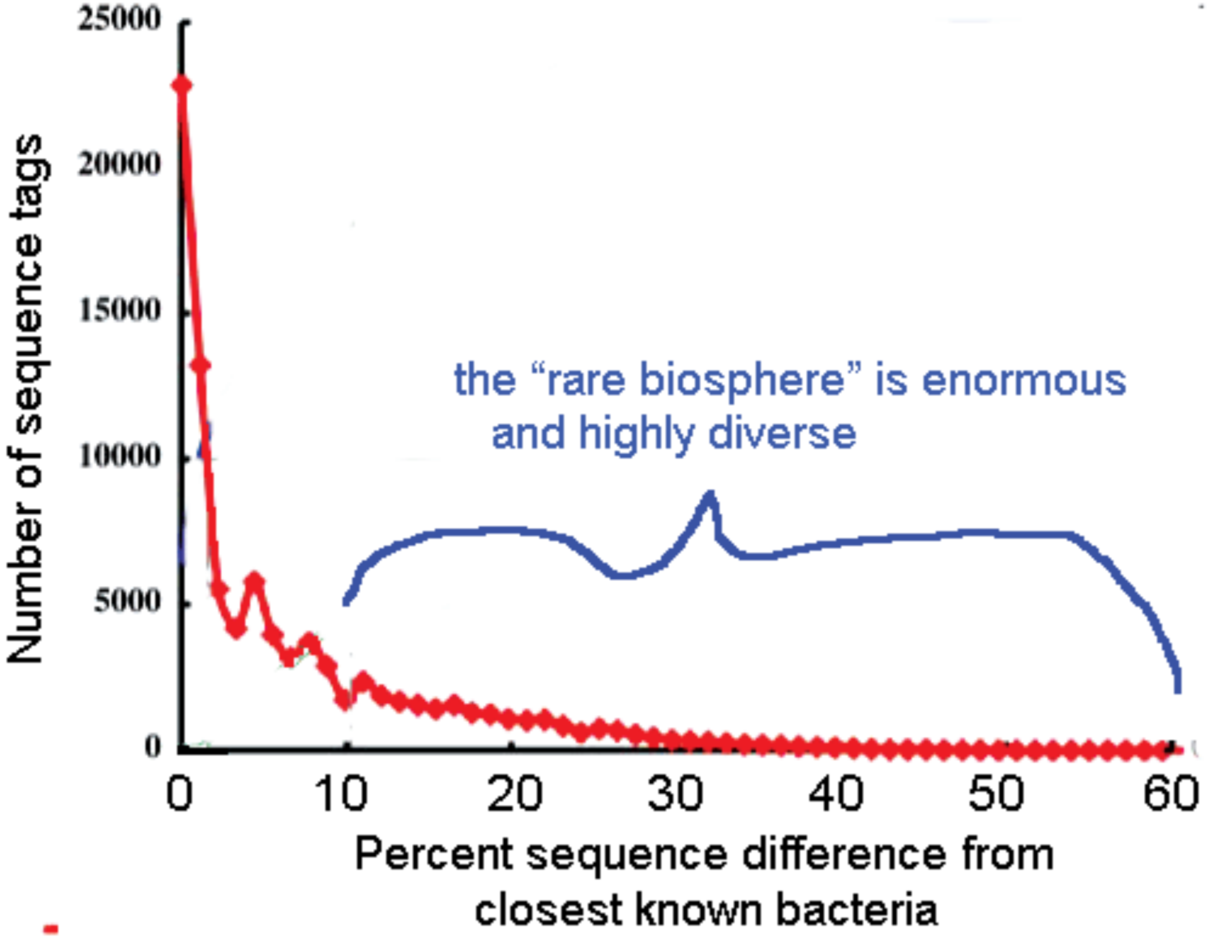
2) Does the rare biosphere matter?

Models of a core microbiome



Hamady and Knight, 2009

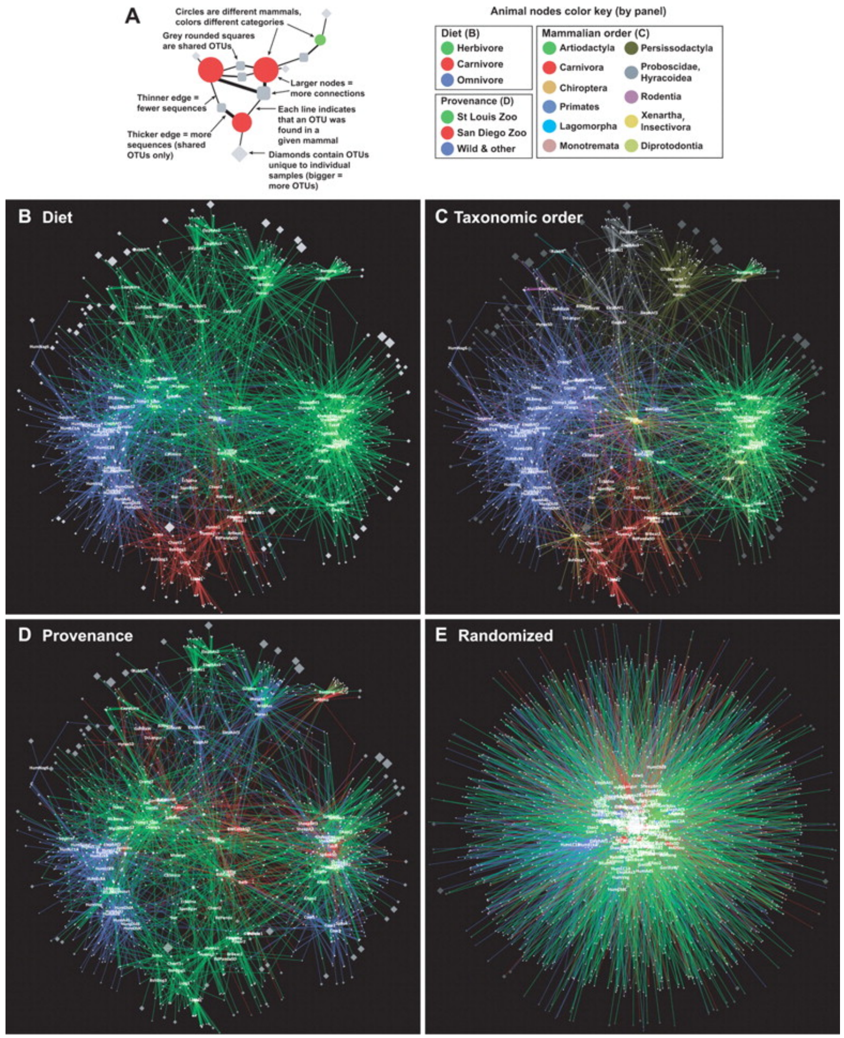
Rare biosphere



Sanger sequencing vs NextGen (3rd gen?)

- Read length
- Cost
- Error
- Bioinformatics
- Sampling depth

Fig. 1. Network-based analyses of fecal bacterial communities in 60 mammalian species.



R E Ley et al. Science 2008;320:1647-1651

16S rRNA

2238 Nucleic Acids Research, Vol. 18, Supplement

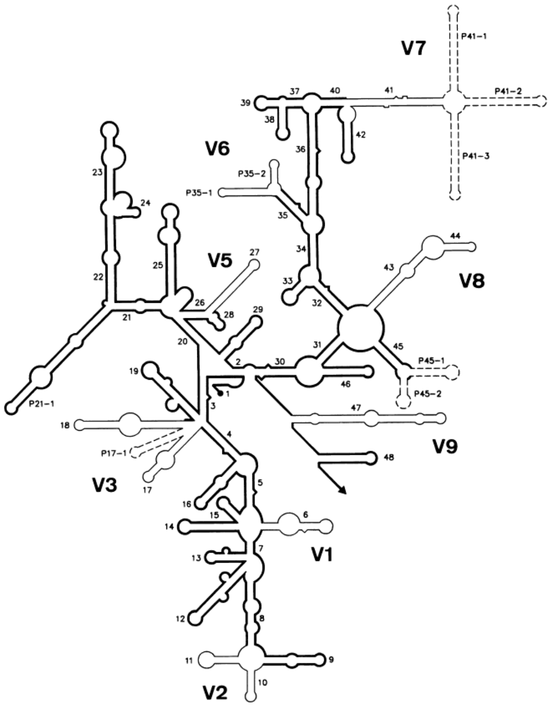
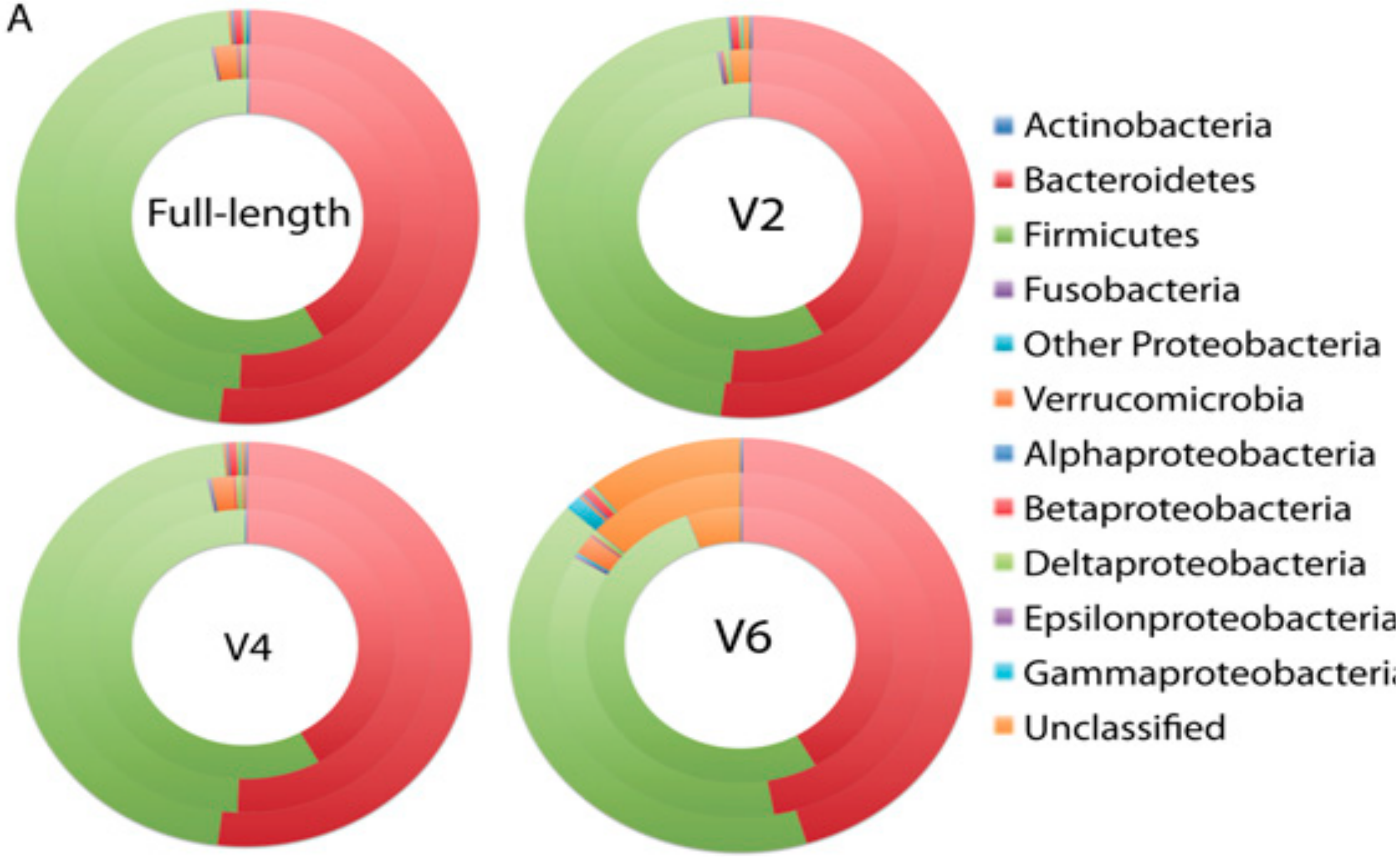


Fig. 1. Secondary structure model for prokaryotic srRNAs.
The 5′-terminus is symbolized by a filled circle and the 3′-terminus by an arrowhead. Helices are numbered in the order of occurrence from 5′- to 3′-terminus. Helices bearing a single number are common to the prokaryotic and eukaryotic (Fig. 2) models. A composite number preceded by P points to a prokaryote-specific helix. Relatively conserved areas are drawn in bold lines, areas of sequence–and length variability in thin lines. Eight variable areas, numbered V1 to V9, are distinguished, V4 being absent in prokaryotic srRNAs. Helices drawn in broken lines are present in a small number of known structures only. Archaebacterial sequences follow the prokaryotic pattern except for helix 35, which is unbranched as in eukaryotes.

Primer bias is a big issue



Hamady and Knight, 2009

Sequence analysis

3 major axes:

1) Alpha and beta diversity

2) Qualitative or quantitative

3) Phylogeny or taxon based