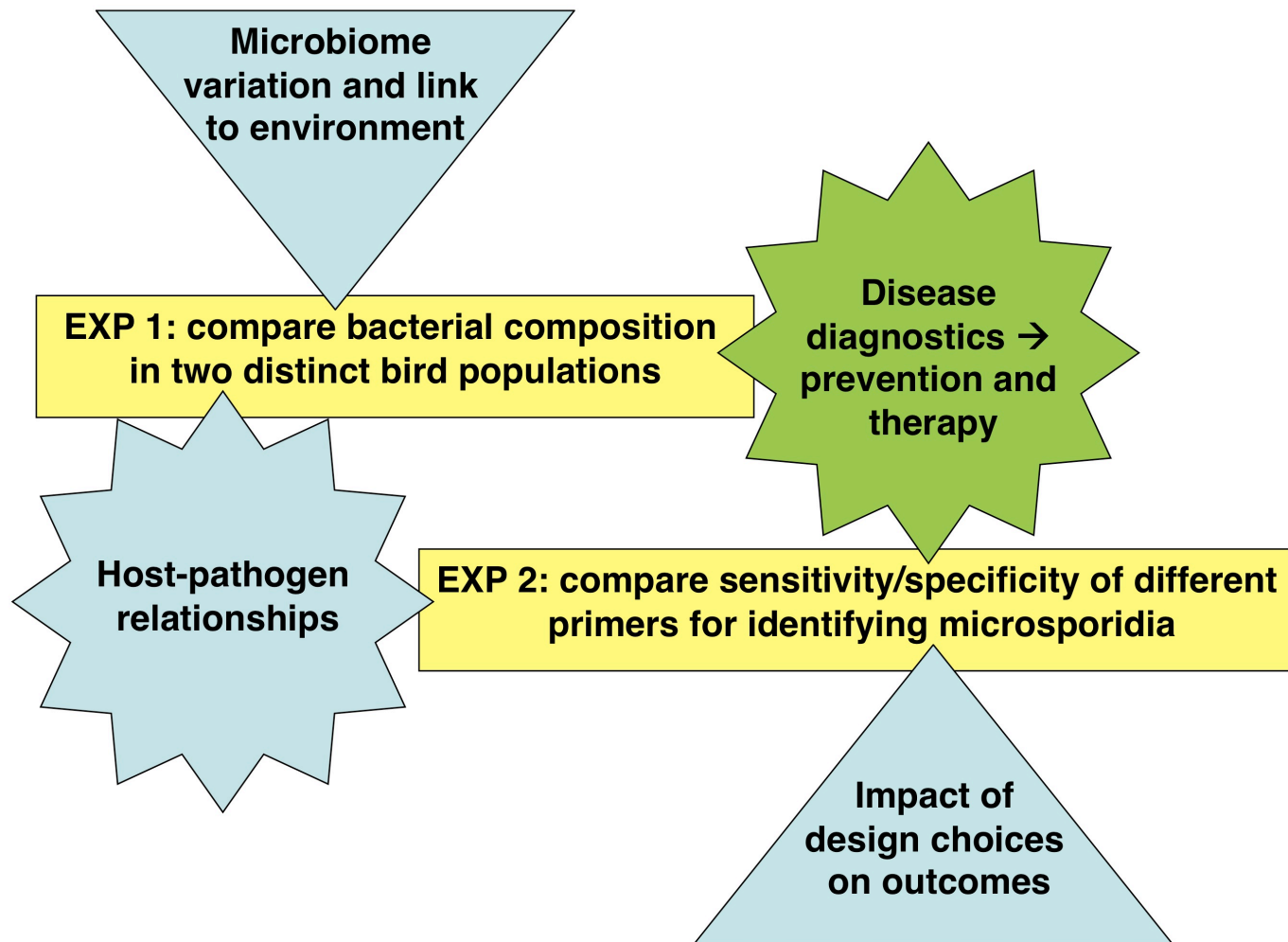# 20.109
Laboratory Fundamentals in Biological Engineering
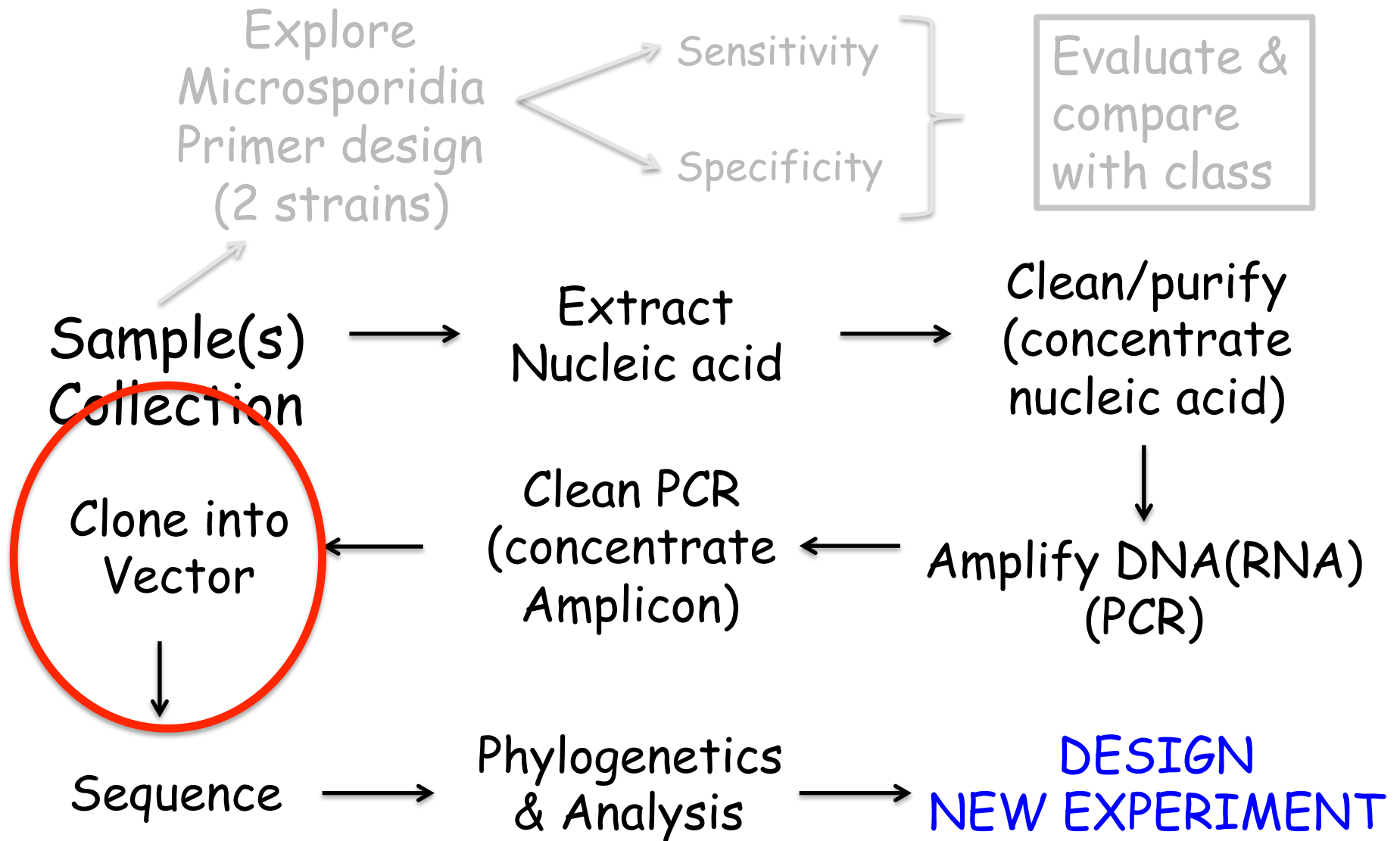
Module 1
Nucleic Acid Engineering
Lecture 4

Office hours: by appt.

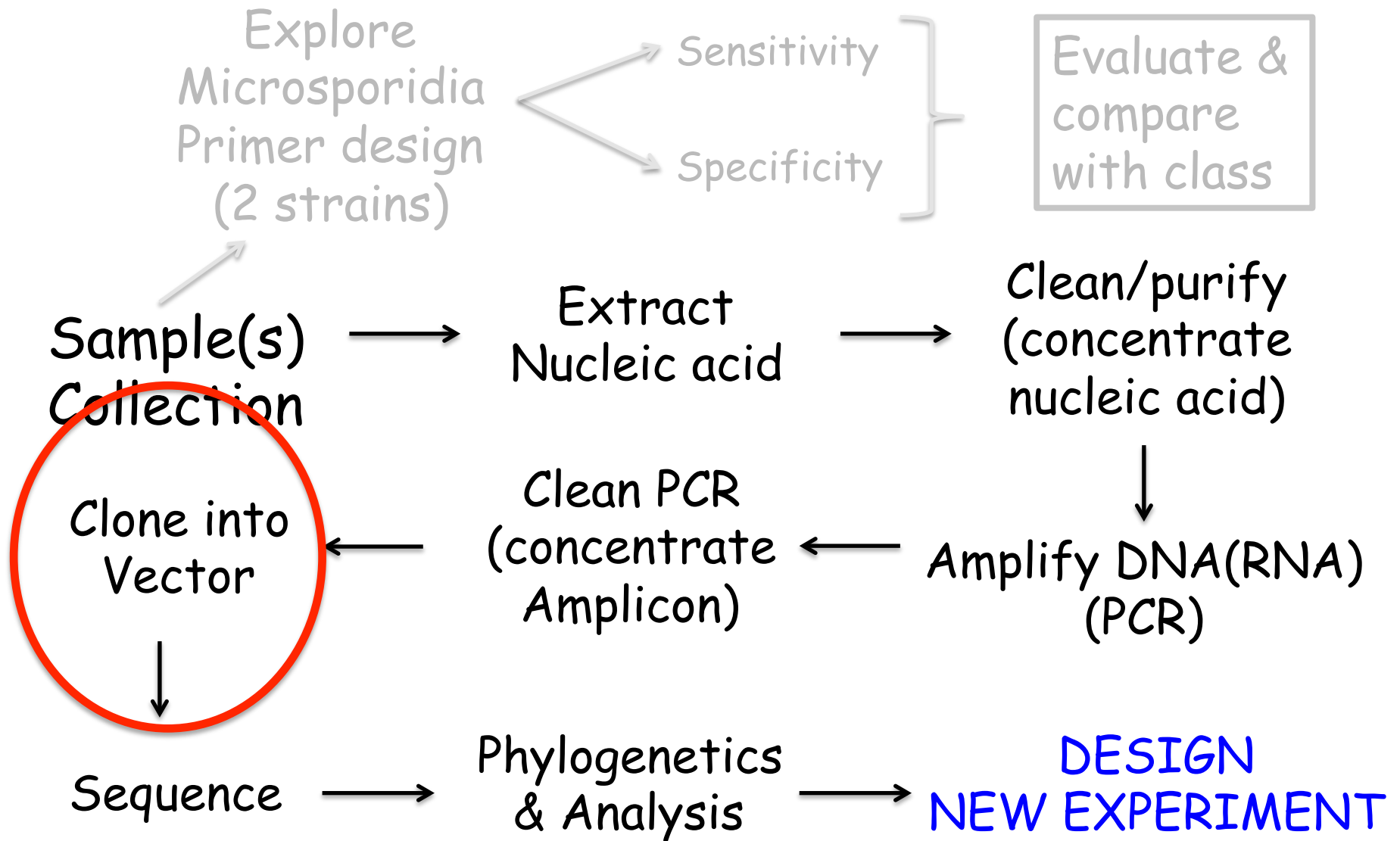# DNA engineering: investigating host/microbe relationships

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

Explore
Microsporidia
Primer design
(2 strains)

Sensitivity

Specificity

Evaluate &
compare
with class

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

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

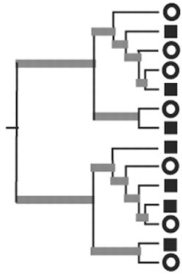Sequence → Phylogenetics
& Analysis → DESIGN
NEW EXPERIMENT

What is cloning

- A brief history
- Choice of vector
- Creation of recombinant DNA
- Creation of transgenic bacteria
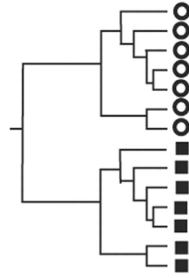- Selection for the clones you're looking for

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)

Clone into
Vector

Clean PCR
(concentrate
Amplicon)

Amplify DNA(RNA)
(PCR)

Sequence

Phylogenetics
& Analysis

DESIGN
NEW EXPERIMENT

Calculation of the UniFrac distance metric

Sequence analysis

3 major axes:
1) Alpha and beta diversity

2) Qualitative or quantitative

3) Phylogeny or taxon based

Characteristics of microbes in the microbiome

- Genome size
  - Bacteria   0.5-10 Mb
  - Viruses   1-1000 Kb
  - Eukaryotic   10-50 Mb

Characteristics of microbes in the microbiome

- Number of taxa
  - Bacteria: Thousands
  - Viruses: unknown
  - Eukaryotes: unknown – likely fewer

Characteristics of microbes in the microbiome

- Relative abundance
  - Bacteria: Variable
  - Viruses: Variable
  - Eukaryotes: unknown, likely variable

Characteristics of microbes in the microbiome

- Ways to target
  - Bacteria: 5S and 16S rRNA
  - Viruses: Specific PCR
  - Eukaryotes: 18S or ITS rRNA region

Characteristics of microbes in the microbiome

- Sequence variation
  - Bacteria: Modest + HGT
  - Viruses: High
  - Eukaryotes: unknown

Back to the core questions

- What do we know?

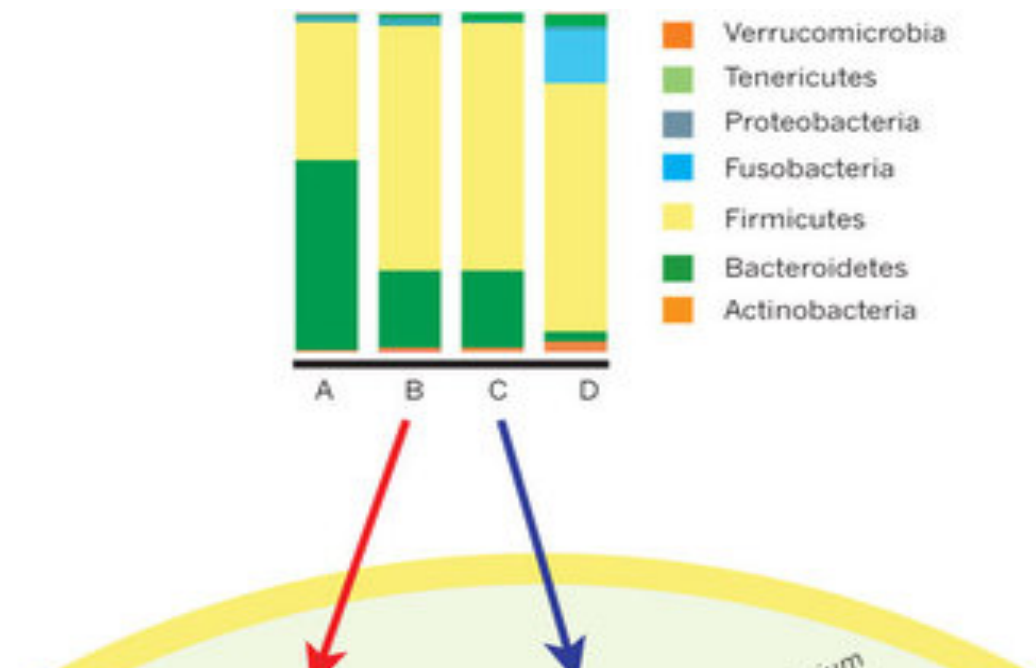- How does it go awry?

- How might we possibly fix/engineer it?

Models of a core microbiome
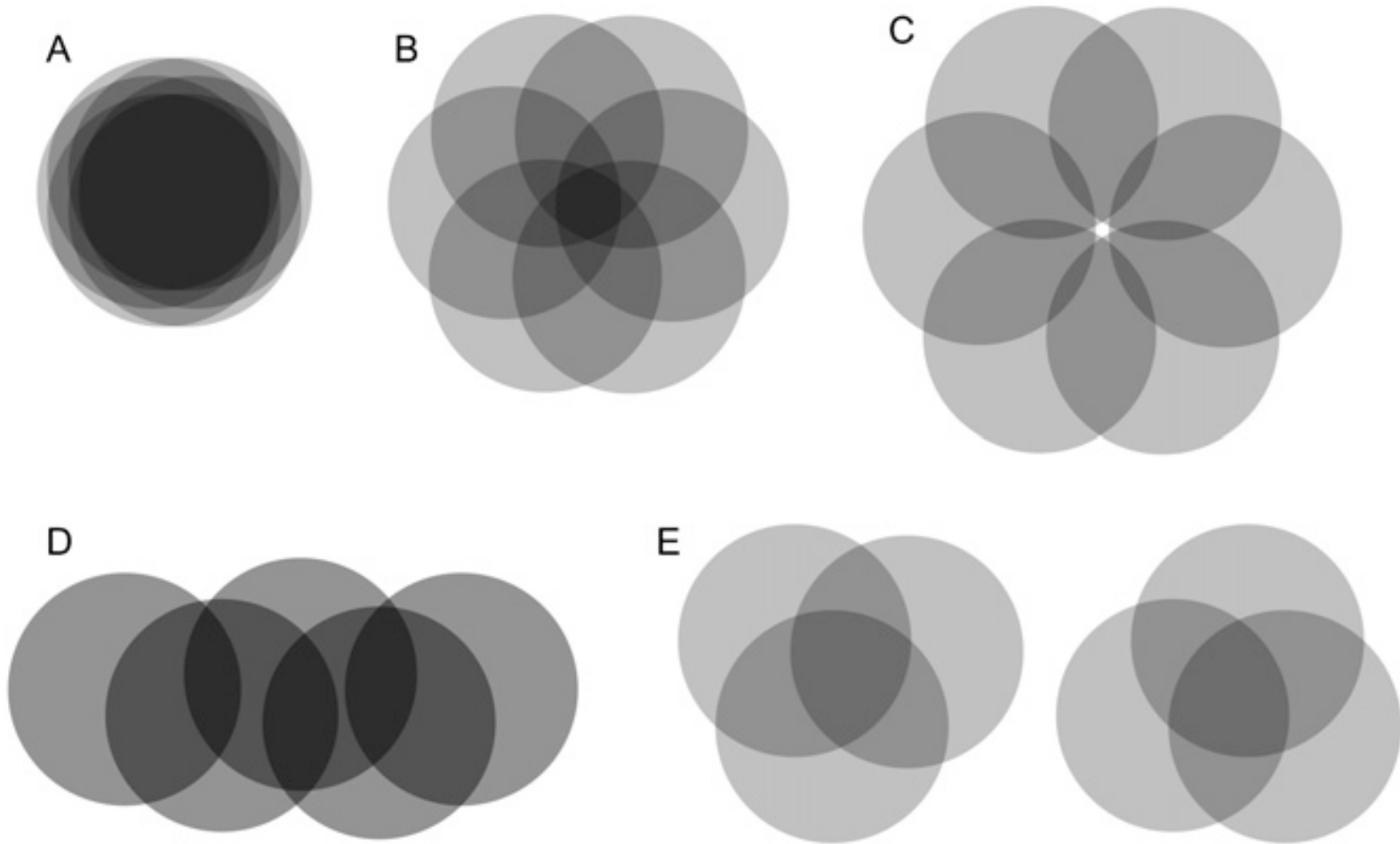
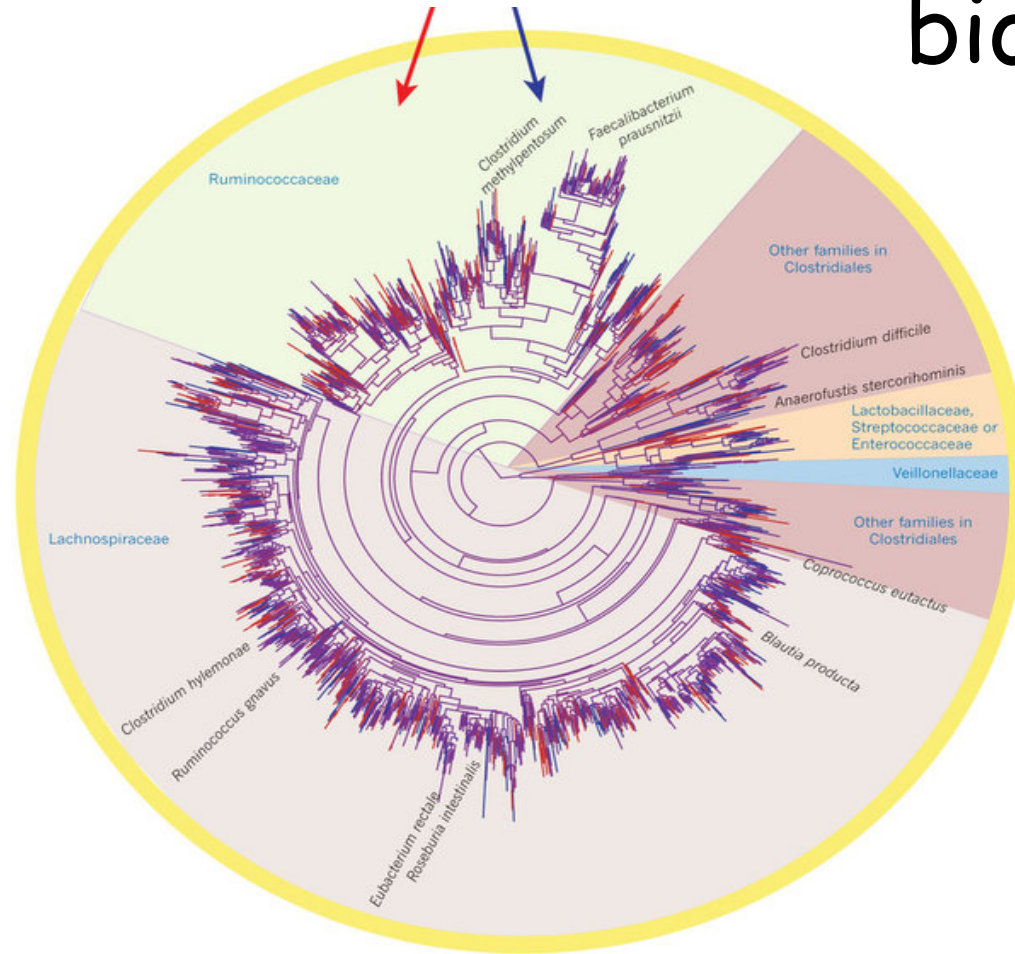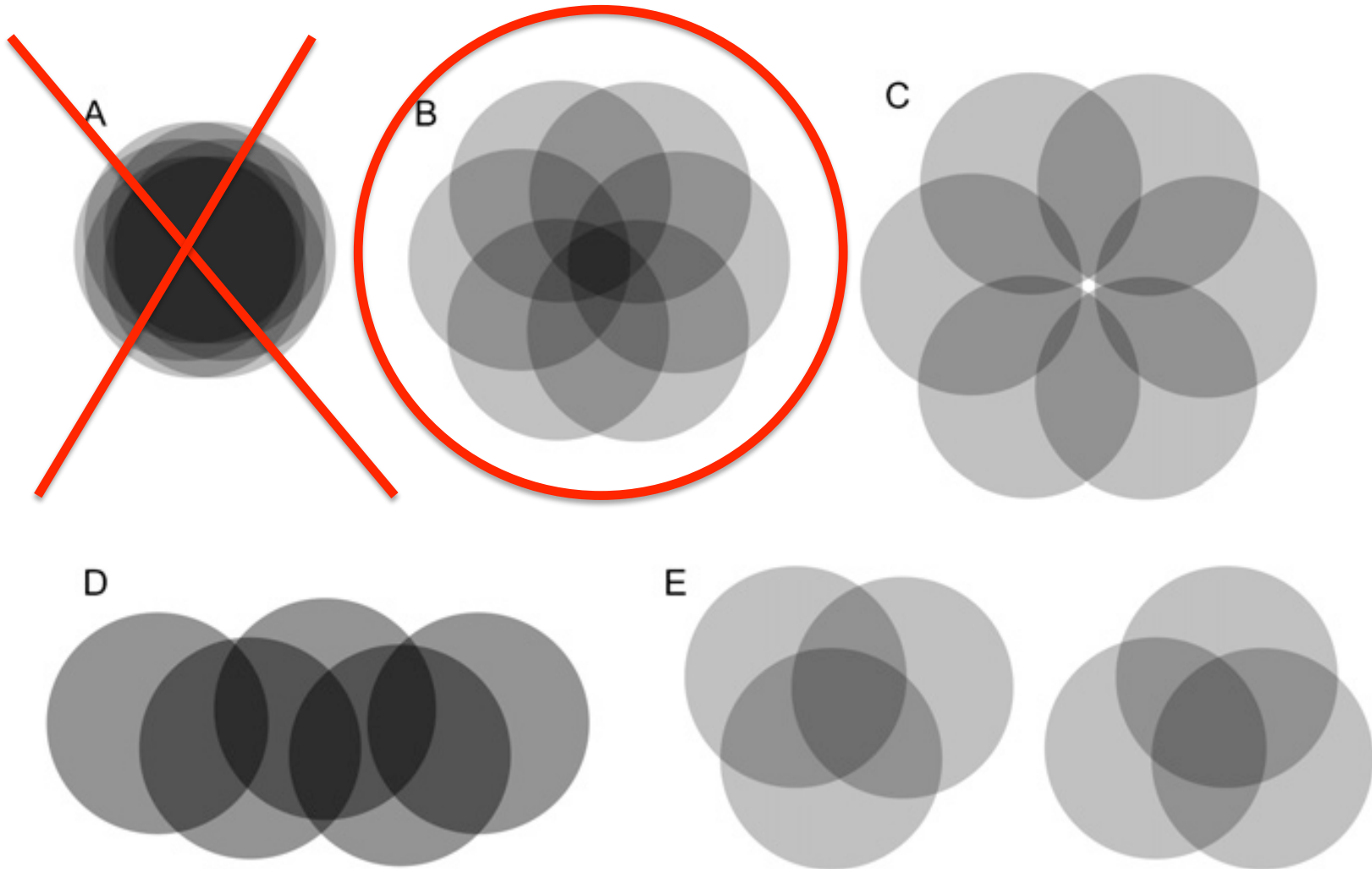# The microbiome is diverse and variable

Yatsunenko et al, Nature 2012

The microbiome is diverse and variable

Yatsunenko et al, Nature 2012
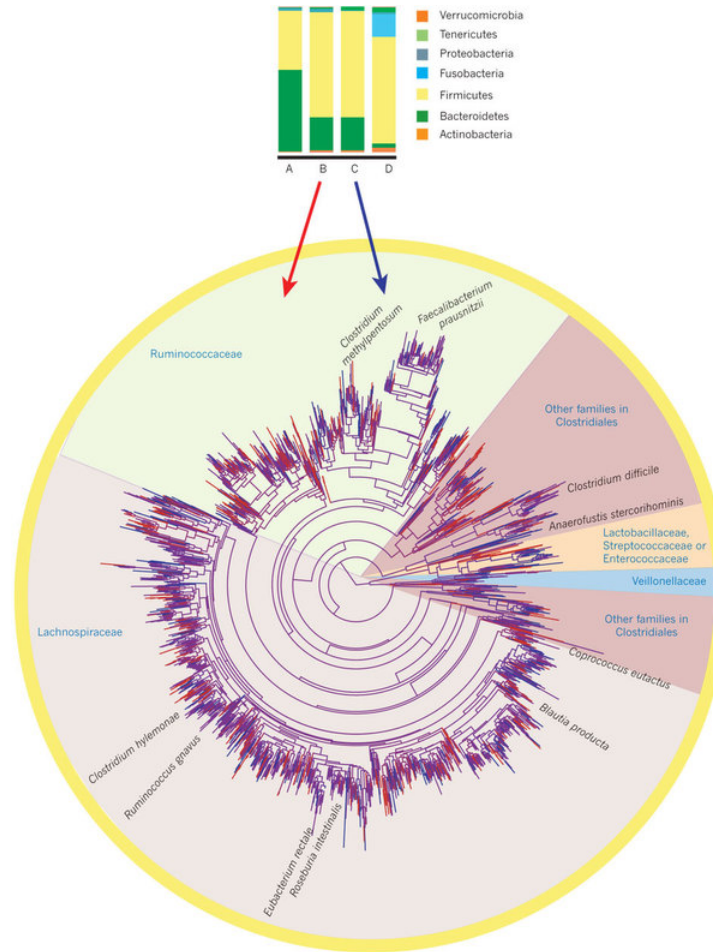
The microbiome is diverse and variable = small core biome?
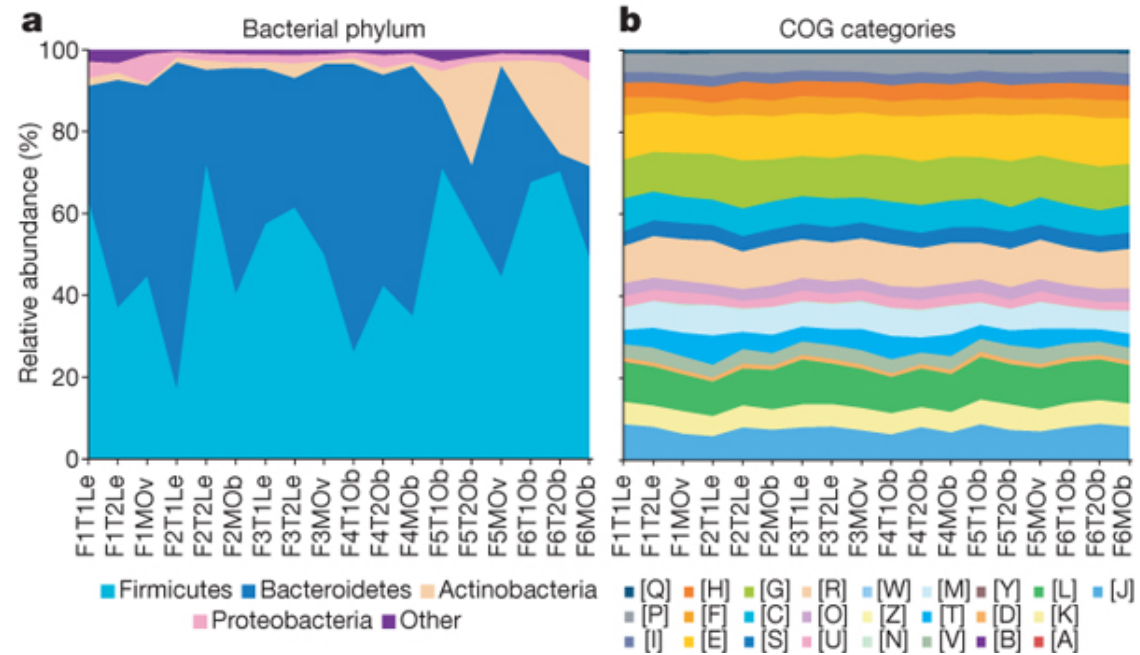

Yatsunenko et al, Nature 2012

Models of a core microbiome

If taxonomy is not conserved, what does that mean for function?
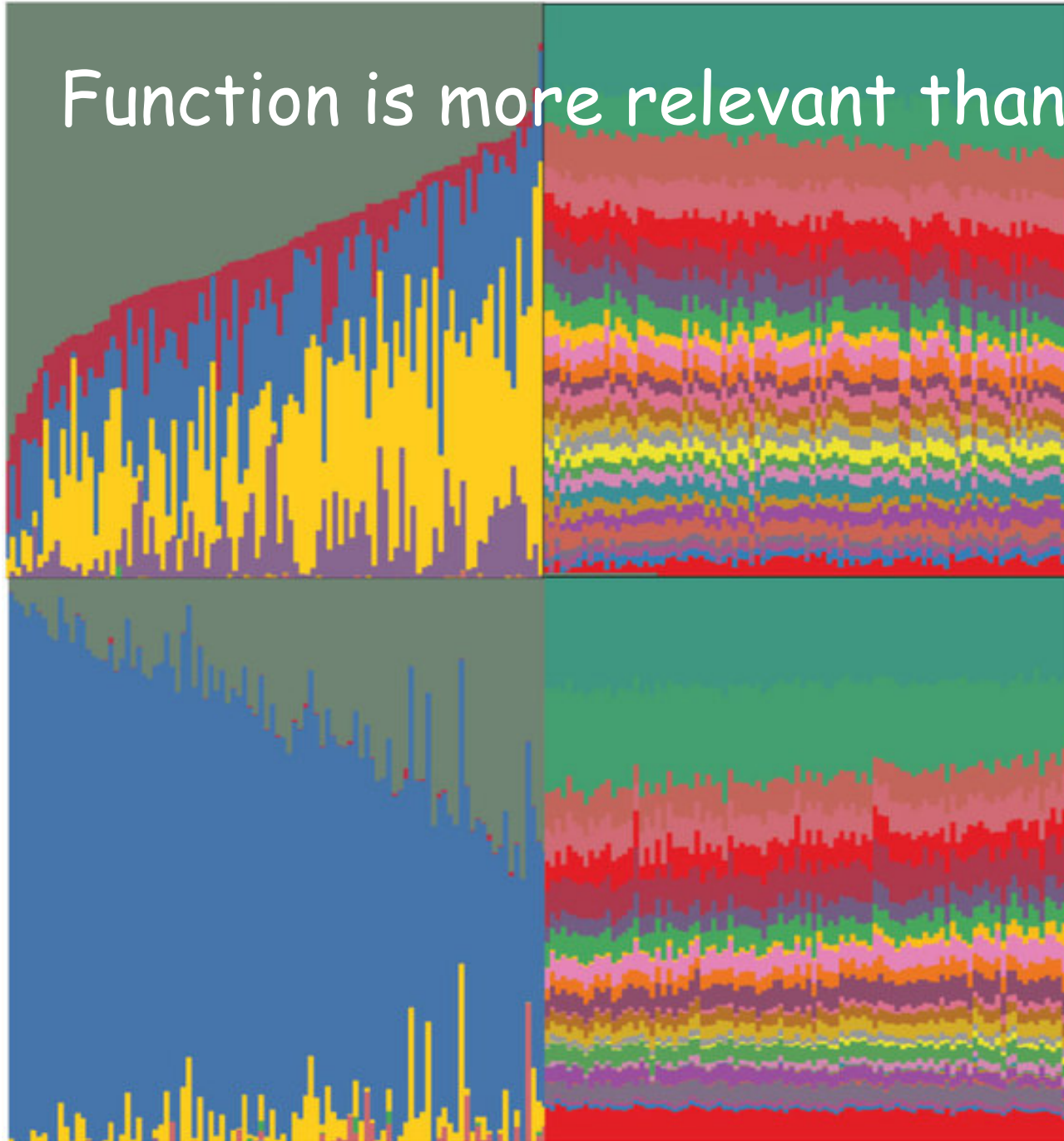
- Functional core?
- Interchangeable parts?

Comparison of taxonomic and functional variations

Function is more relevant than taxonomy

Phylum

Function

Nature 486 (2012)