DTU

DTU Health Technology
Bioinformatics

Metagenomics and Microbiome Analysis:
What is metagenomics ?

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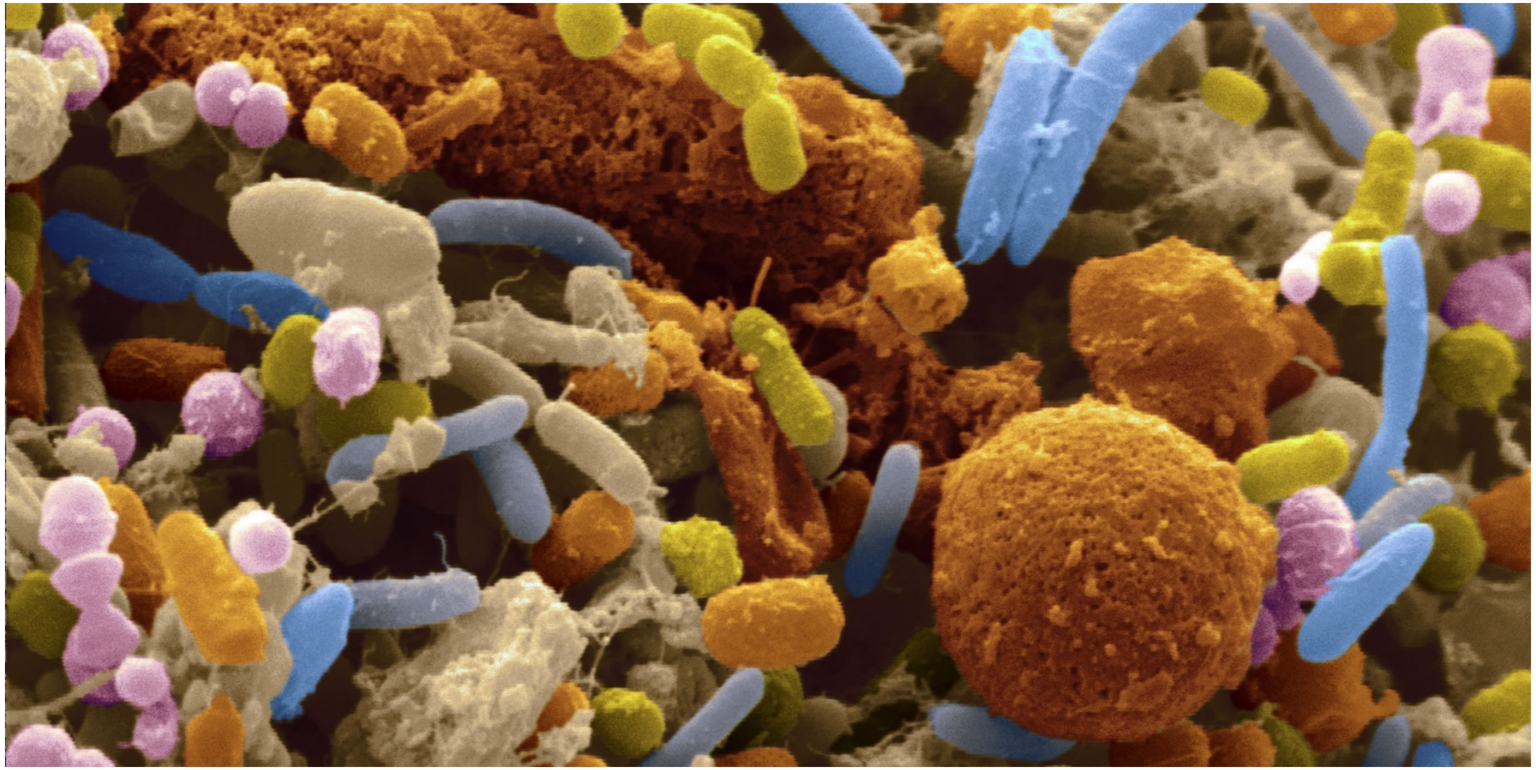
Menu

- What is a metagenome?
- Methods of studying metagenomes
- 16s rRNA amplicon sequencing
- Shotgun metagenomics
- Horses for courses

What is a metagenome

- Metagenome describes the genetic material of an entire microbial community or microbiome



Where are these microbiomes?

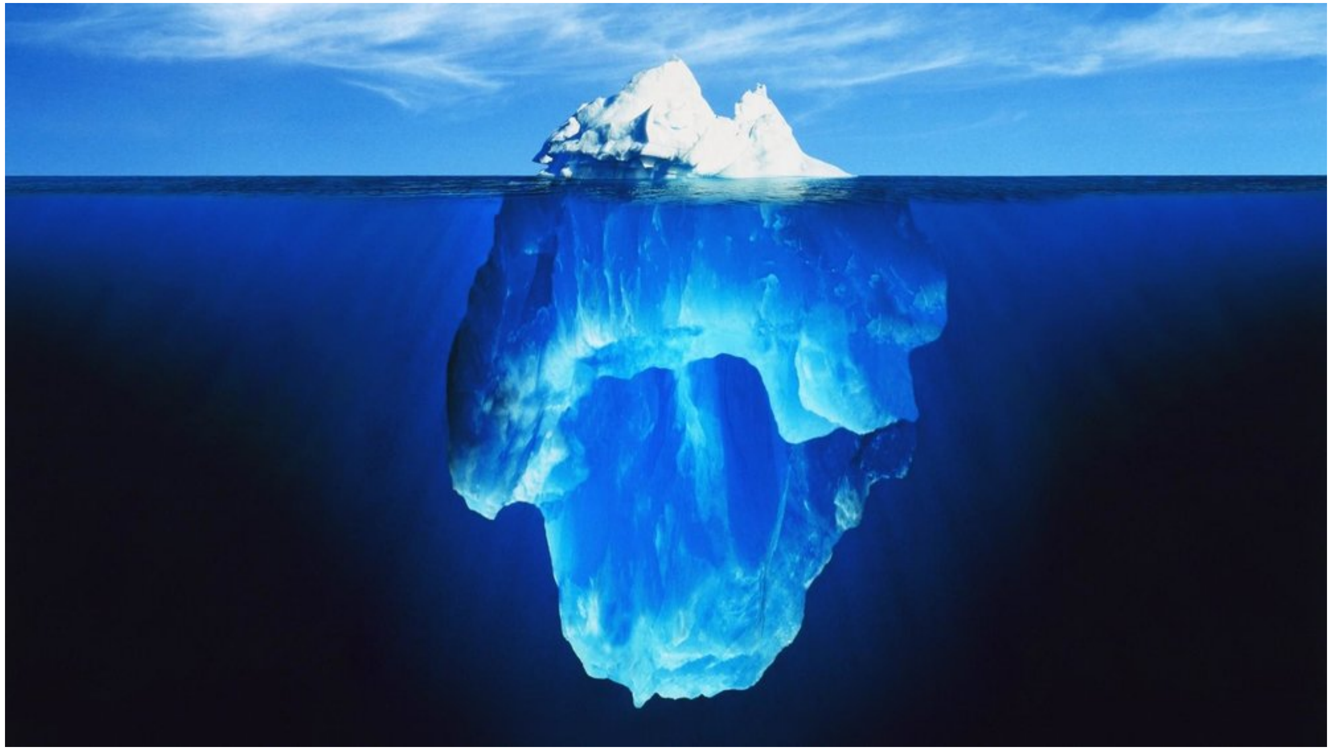
- Everywhere and we can study them!

Pre-sequencing days

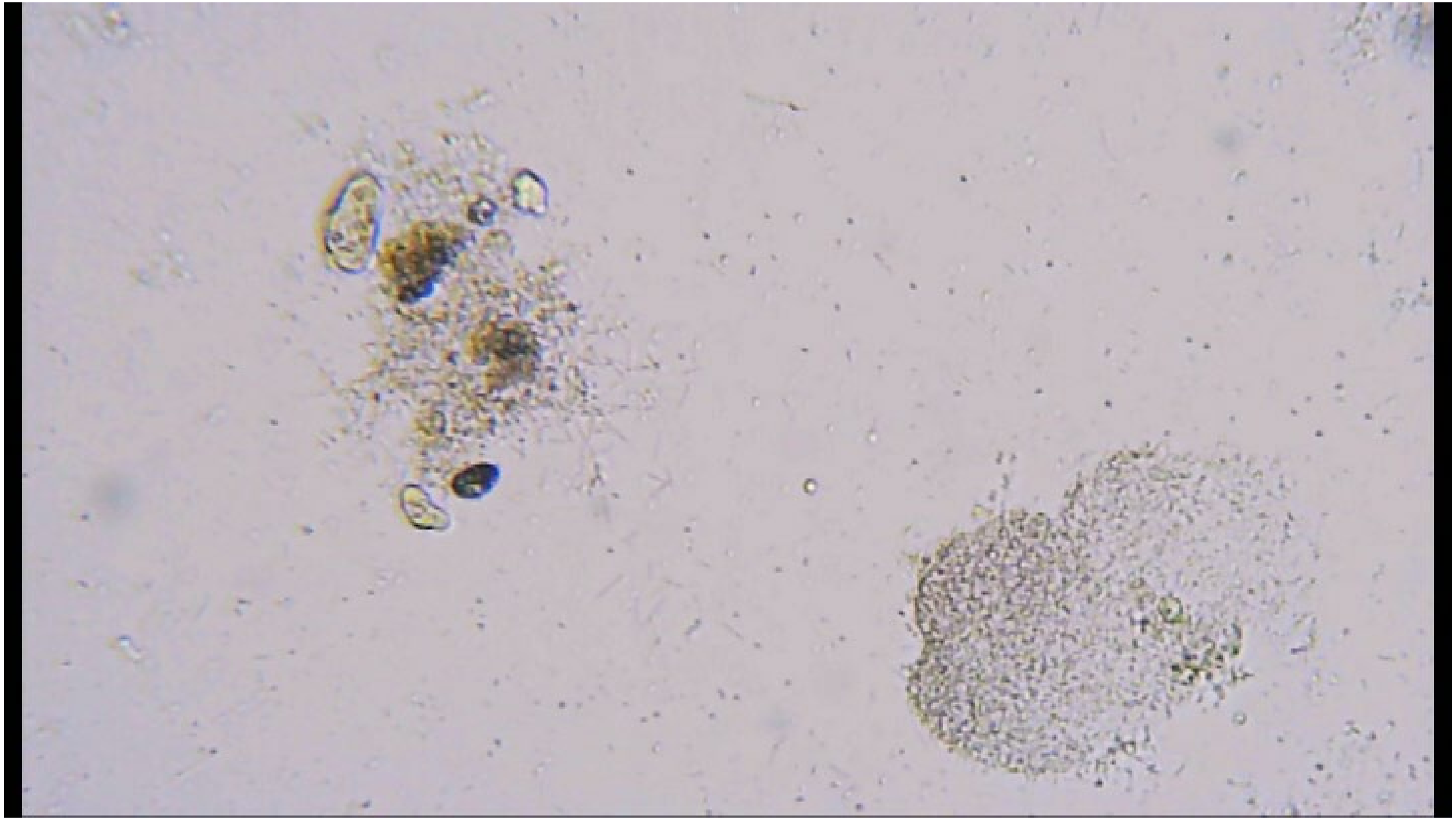
- Culturable organism chosen as models
- Might not be representative even for close relatives

From culturing to sequencing

- Microbiome research previously limited to culturable organisms
- +99% of prokaryotes in the environment cannot not be cultured

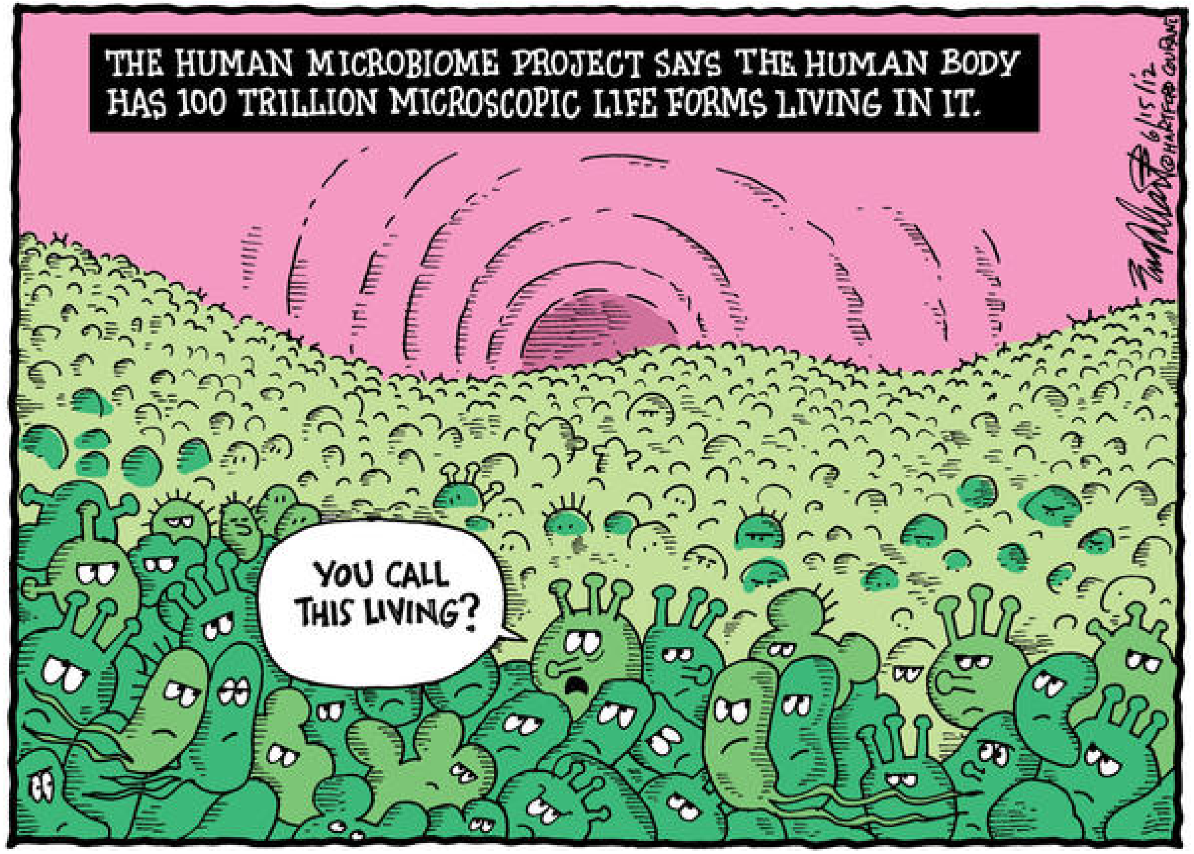


How do microbes live?



More bacteria in you than the human population

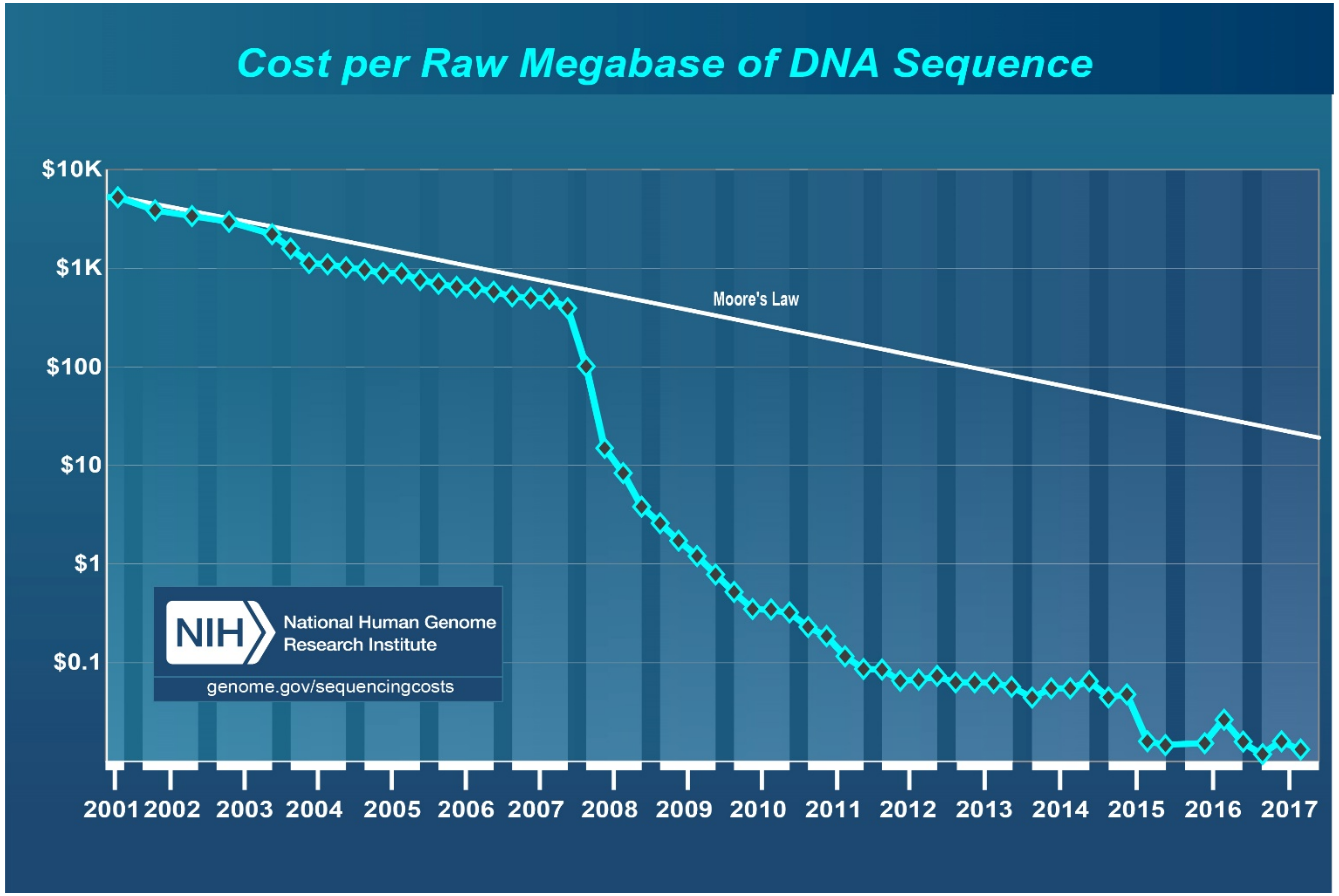
- Estimated 39 trillion microbial cells in you



Development in metagenomics is linked to technological advancements

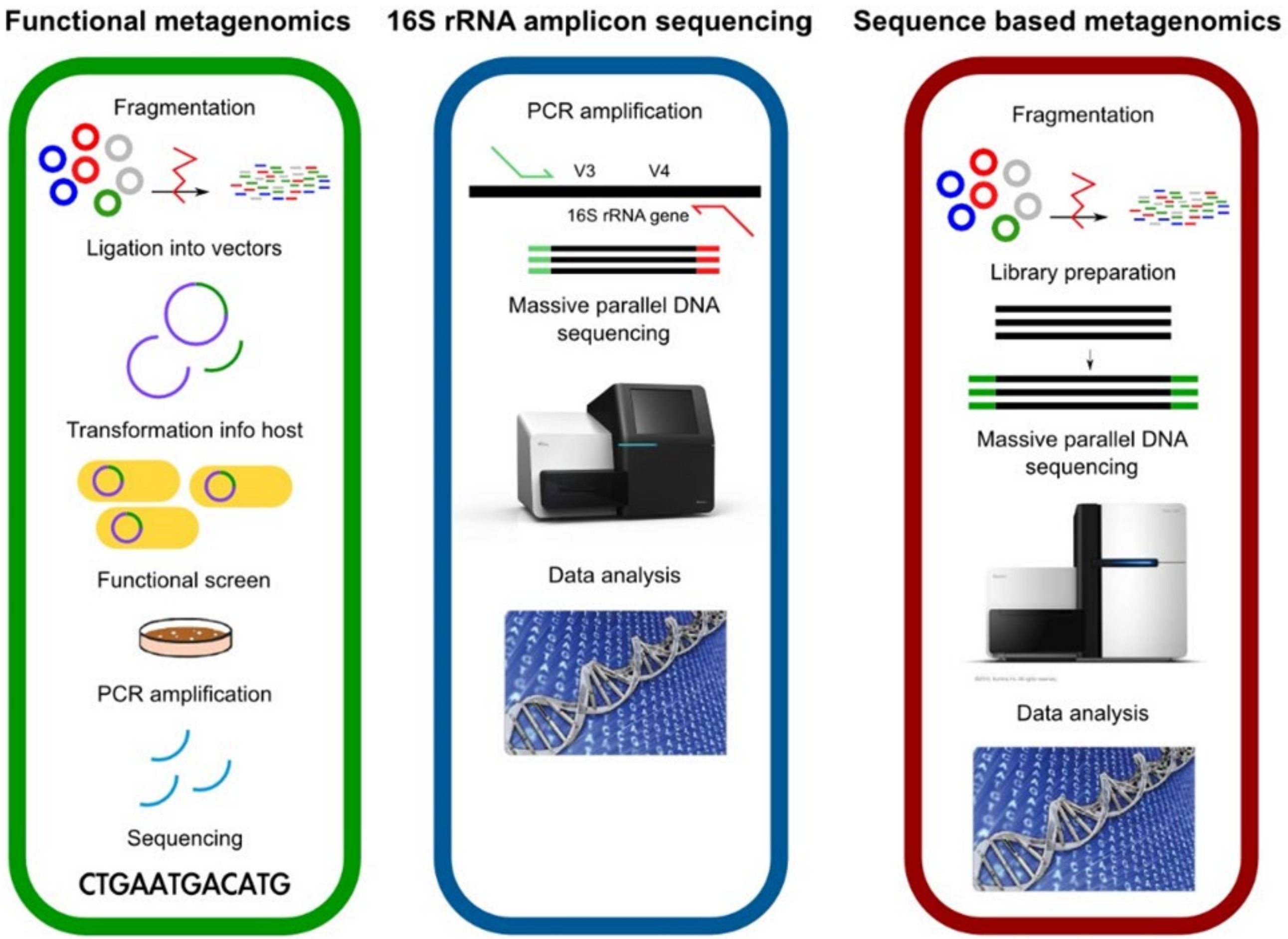
- Better, cheaper, faster

1 human Genome
3.000.000.000 USD
13 years



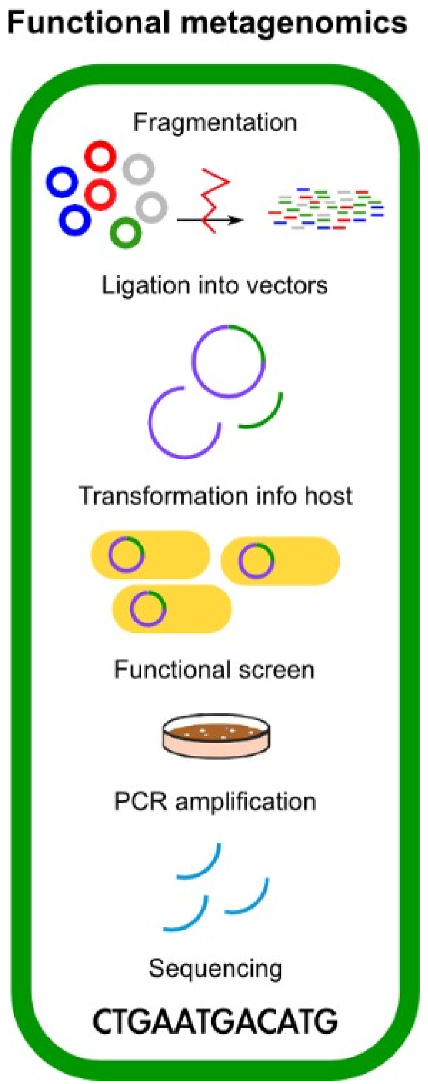
1 human Genome
< 1000 USD
1 day

Methods


Functional metagenomics
16S rRNA amplicon sequencing
Sequence based metagenomics
Fragmentation
Ligation into vectors
Transformation info host
Functional screen
PCR amplification
Sequencing
CTGAATGACATG
PCR amplification
V3
V4
16S rRNA gene
Massive parallel DNA sequencing
Data analysis
Fragmentation
Library preparation
Massive parallel DNA sequencing
Data analysis

Functional metagenomics

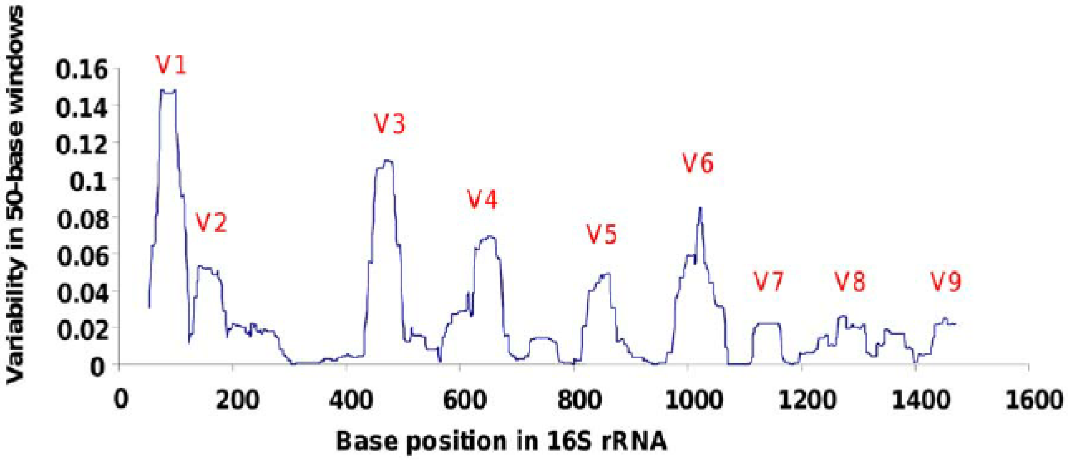
- Industrial large scale screening
- Time consuming due to functional screens
- Setup determines screening specificity



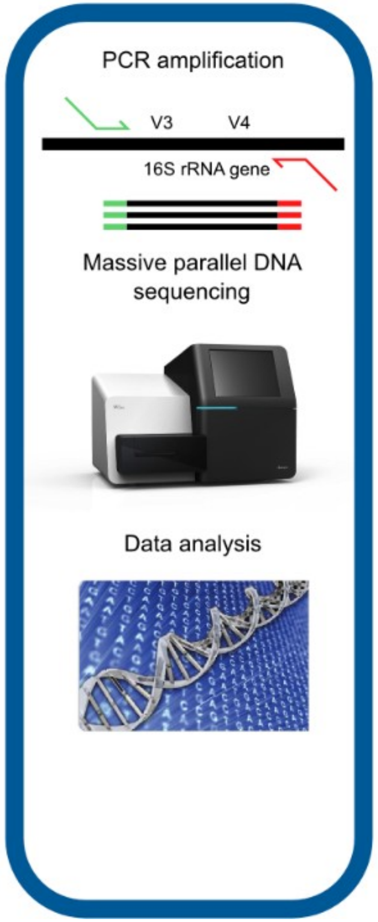
Not part of this course

16S rRNA

- Ribosomal RNA gene conserved in Bacteria and Archaea
- Conserved areas interspaced with hypervariable regions
- Allows the determination of the phylogenetic composition of a microbiome
- Bias by primers selected

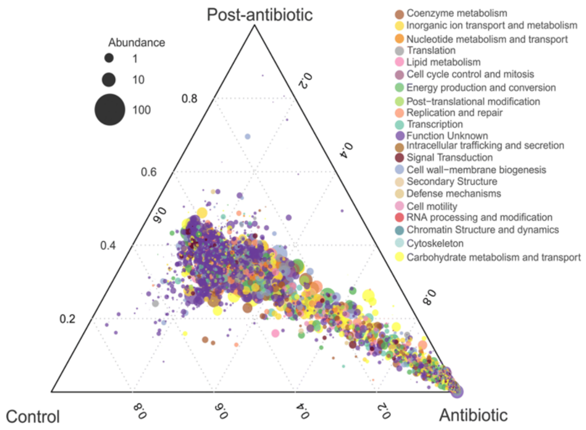


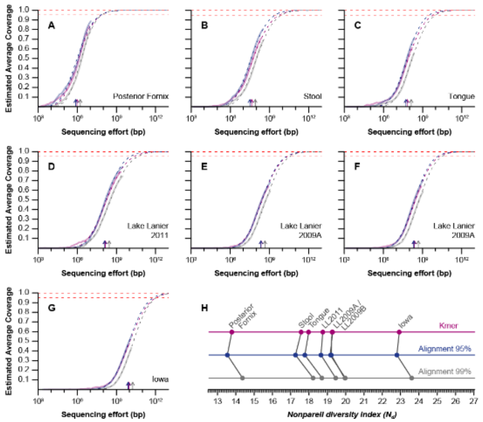
16S rRNA amplicon sequencing



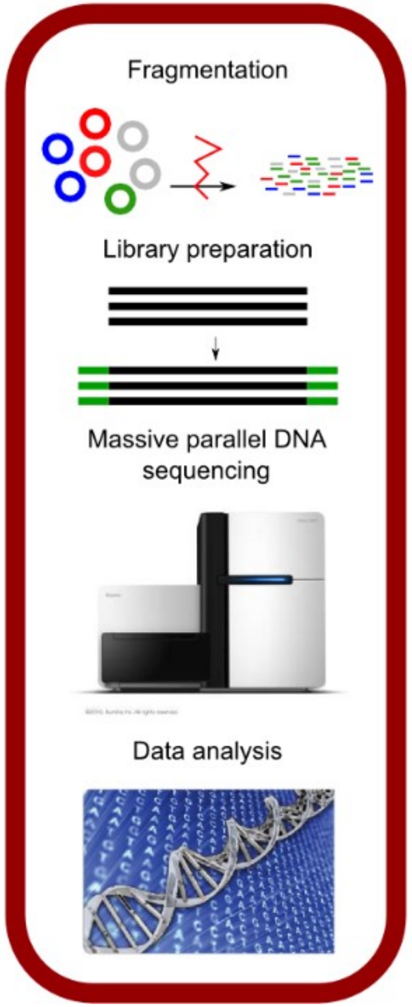
Shotgun metagenomics

- Extract all DNA
- Fragment
- Sequence all
- Analyse all
- Capture all the diversity IF sequencing depth is high enough
- Requires enough biomass for DNA extraction
- Functional analysis possible
- Metagenome diversity analysis is possible



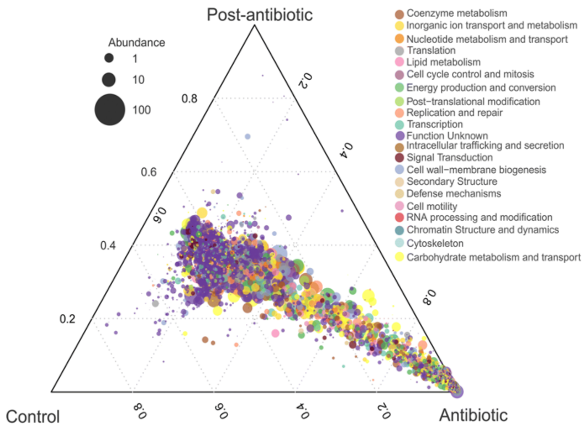


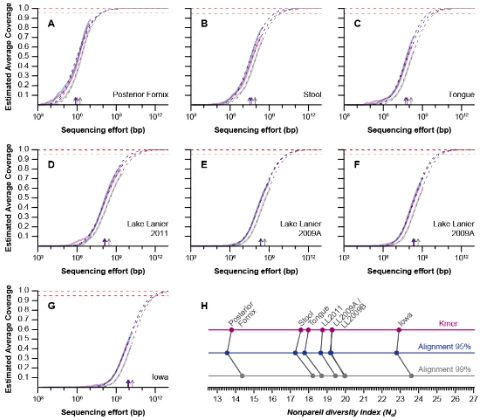
Sequence based metagenomics



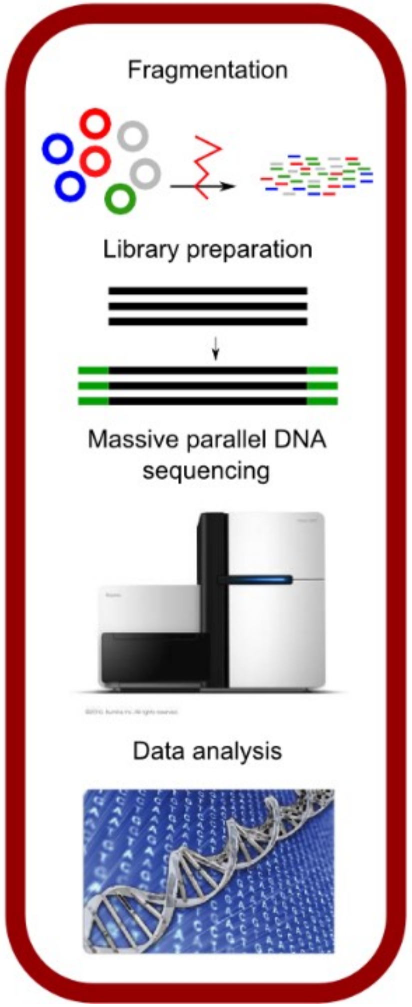
Shotgun metagenomics – the downside

- Large datasets
- Varying abundance, detection problems due to low depth or bias
- Expensive
- Lack of references
- Shared and/or similar regions hinders assembly





Sequence based metagenomics


Fragmentation

Library preparation

Massive parallel DNA sequencing

Data analysis

What kind of questions can we ask?

- Examples of questions we can ask with amplicons?
- Examples of questions we can ask with metagenomics?

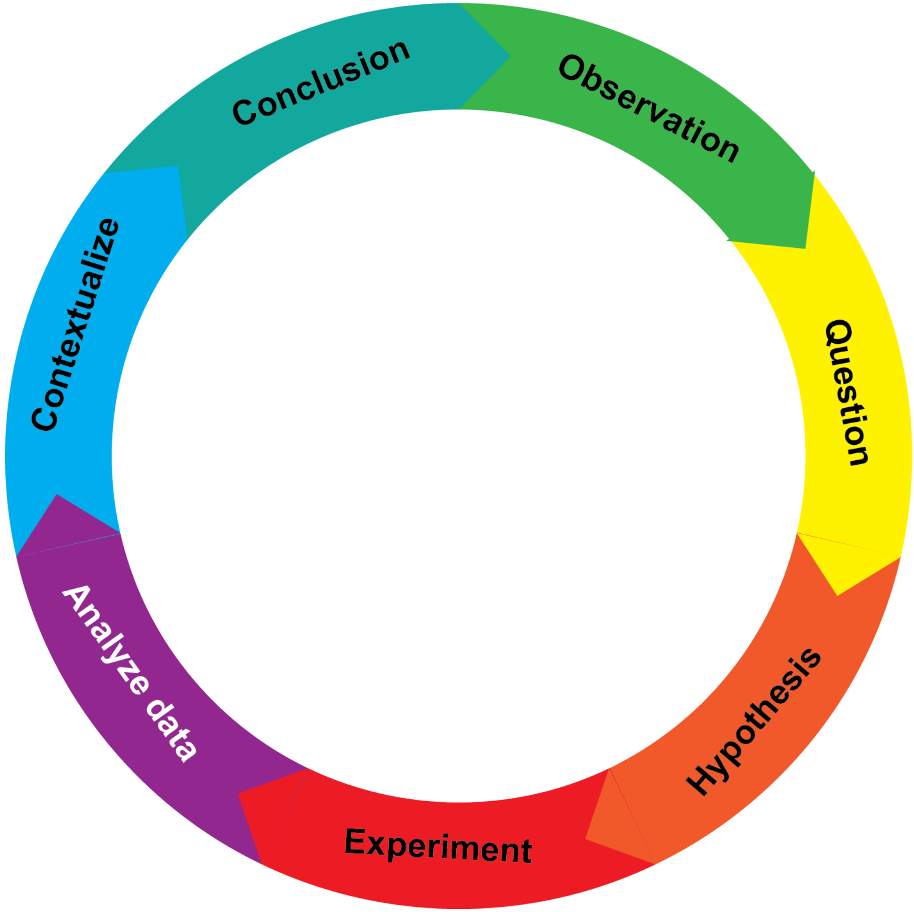
Primers define the analysis of amplicon studies

Metagenomics coming from amplicon studies

- Many projects end up being data-driven and exploratory
- So many options…

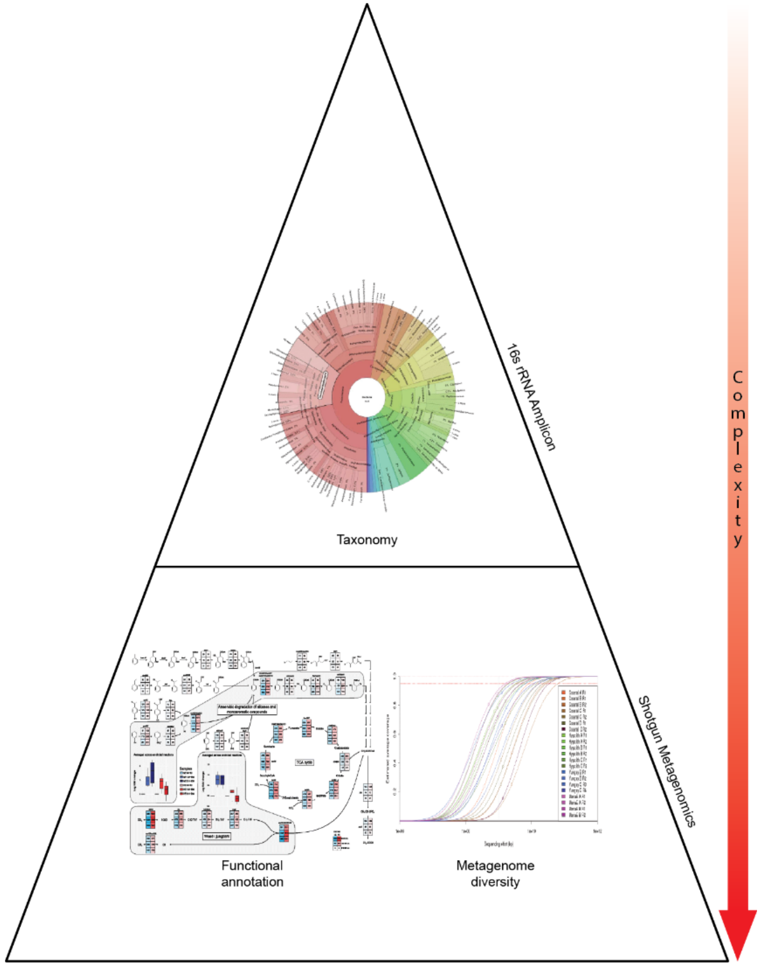
The Scientific method is critical to metagenomics

- Hypothesis helps to dictate the analysis



Horses for courses

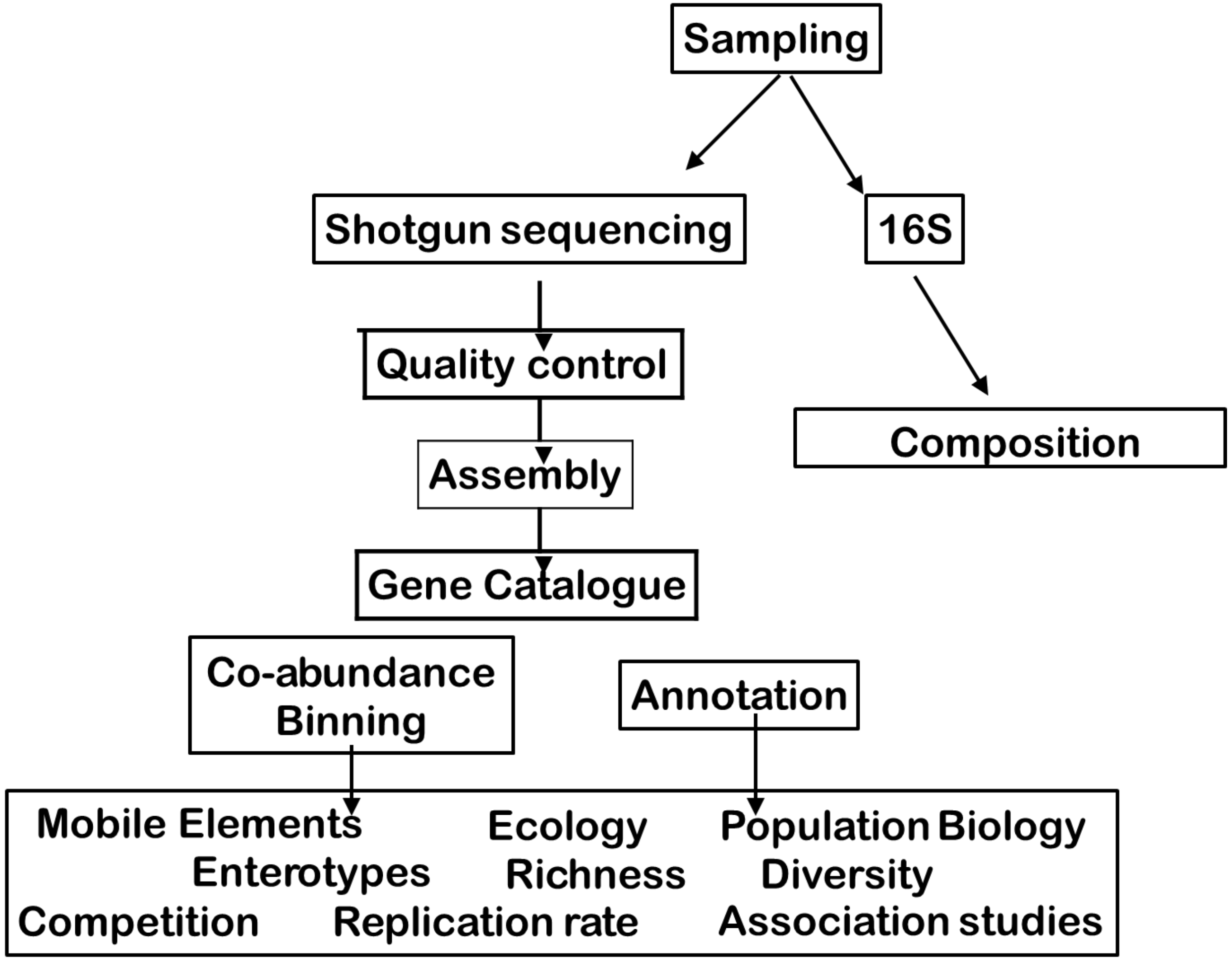
- Question dictates the appropriate tool
- Amplicon sequencing is easy & cheap
- Metagenomics allows more questions
  - – Discovery of novel proteins, antibiotics etc.
- Same microbiome composition does not mean same metagenome!



This course

- Fundamental metagenomics & microbiome knowledge
- Sampling properly IS important
- How to extract DNA and prepare sequencing libraries: do it yourself
- How to analyse shotgun metagenomics data (NGS): do it yourself
- Formulating a study with a focus on intervention studies

What you will learn



Sampling

Shotgun sequencing

16S

Quality control

Assembly

Gene Catalogue

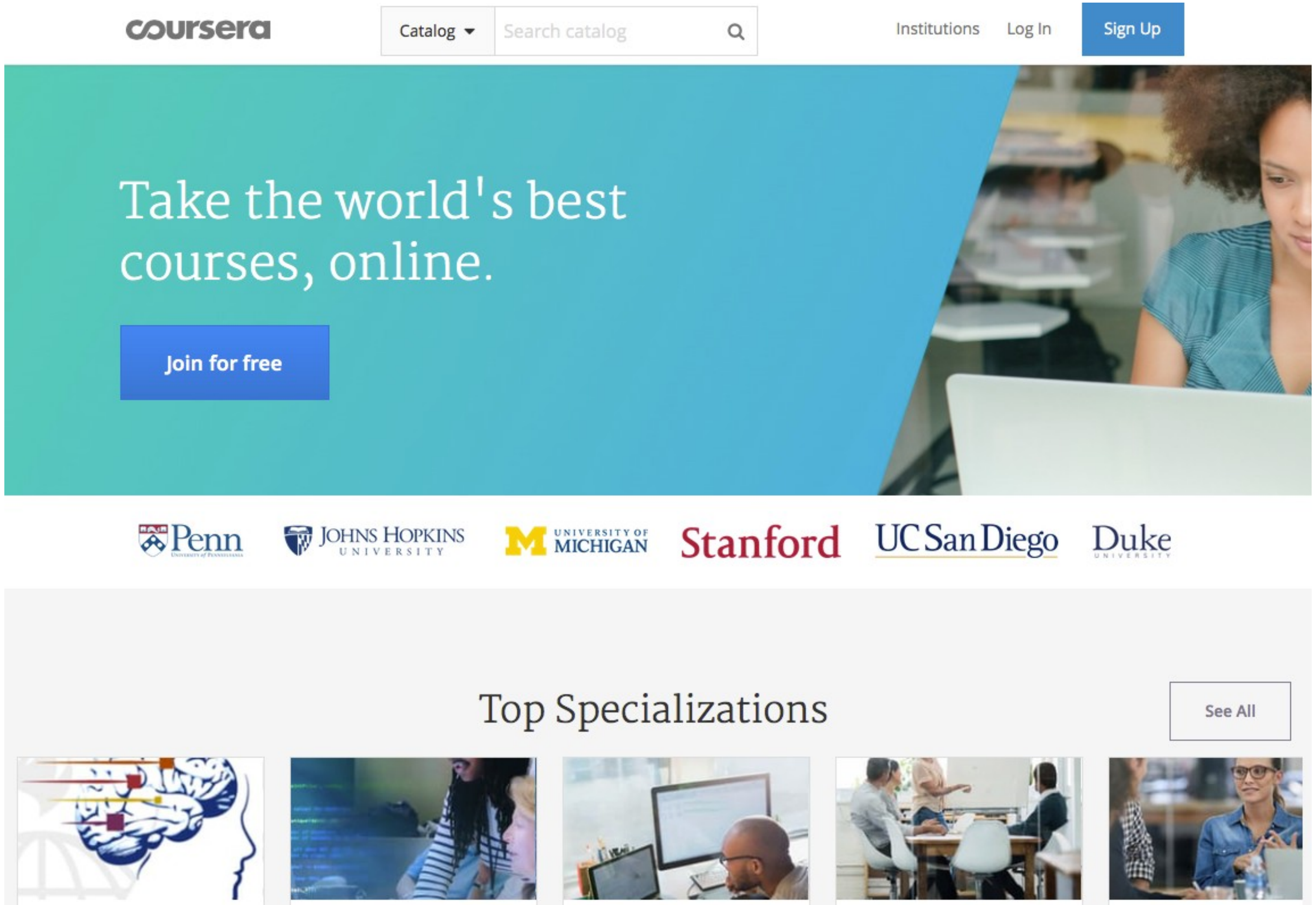
Composition

Co-abundance Binning

Annotation

Mobile Elements
Enterotypes
Competition
Replication rate
Ecology
Richness
Population Biology
Diversity
Association studies

Coursera



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Summary

- Metagenome describes the genetic material of an entire microbial community or microbiome
- Can be analyzed via Functional metagenomics, 16s rRNA amplicon studies or ”proper” shotgun metagenomics
- Horses for courses. Sometimes you do not need more than 16s rRNA amplicons
- What is the question should be your first question