DTU

DTU Health Technology
Bioinformatics

Metagenomics and Microbiome Analysis: What is metagenomics ?

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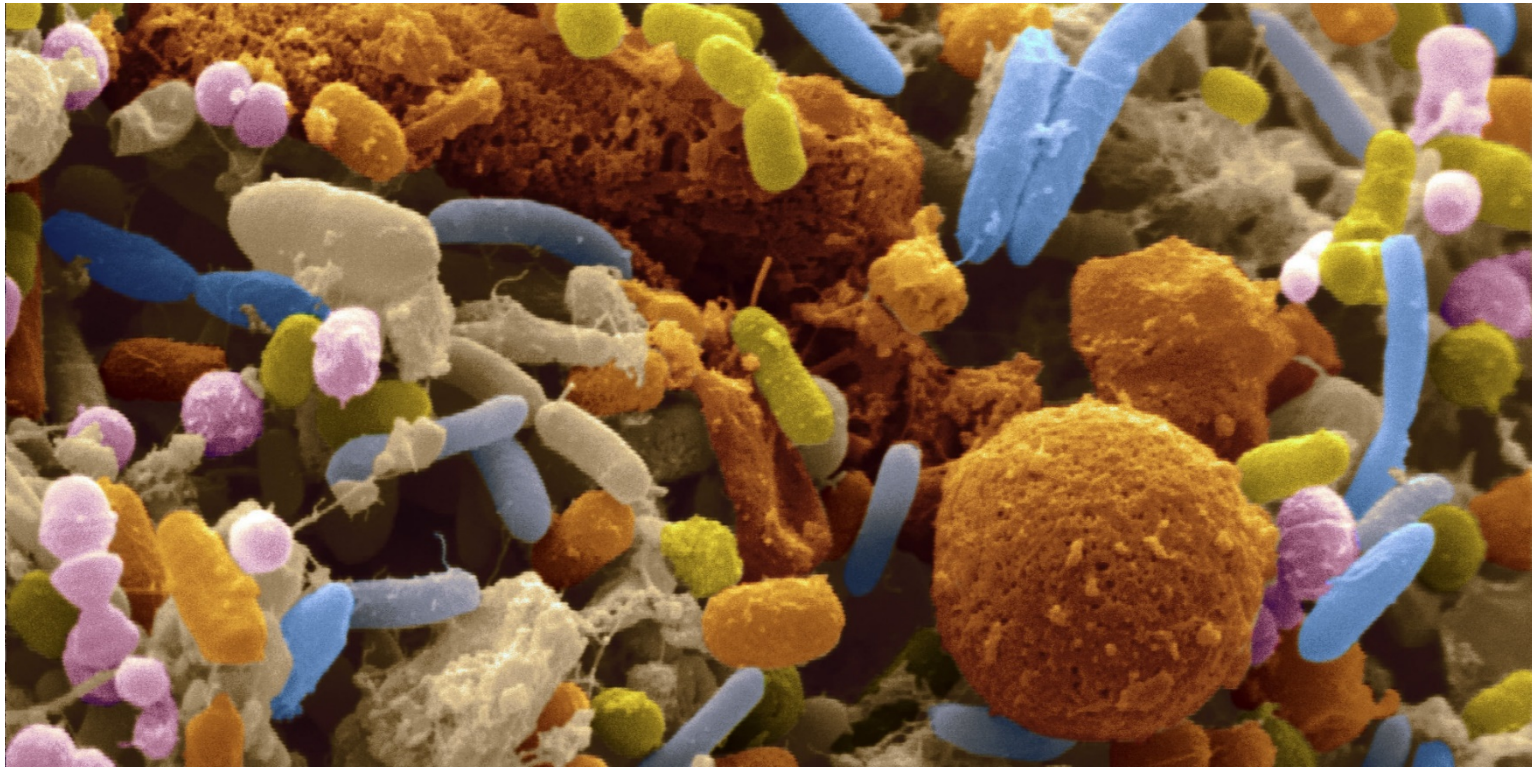
Menu

- 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?

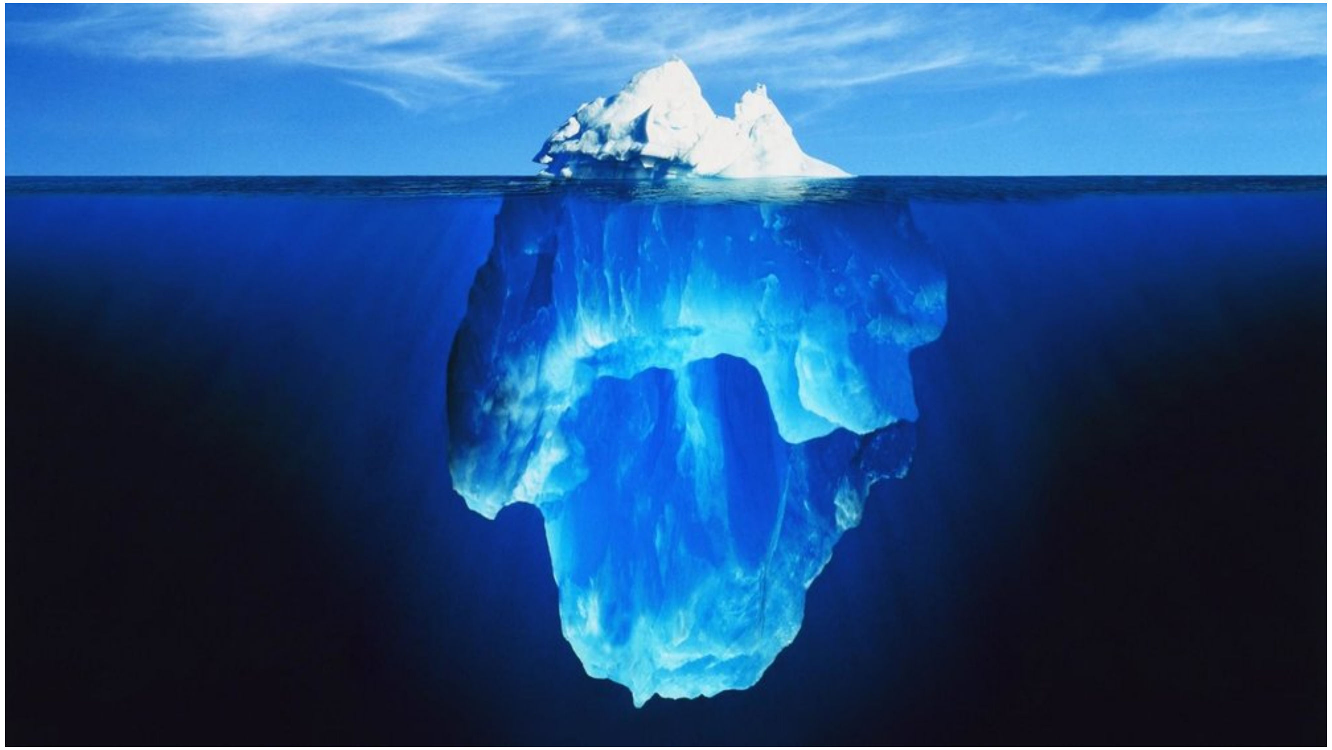
- Everywhere and we can study them!

Pre-sequencing days

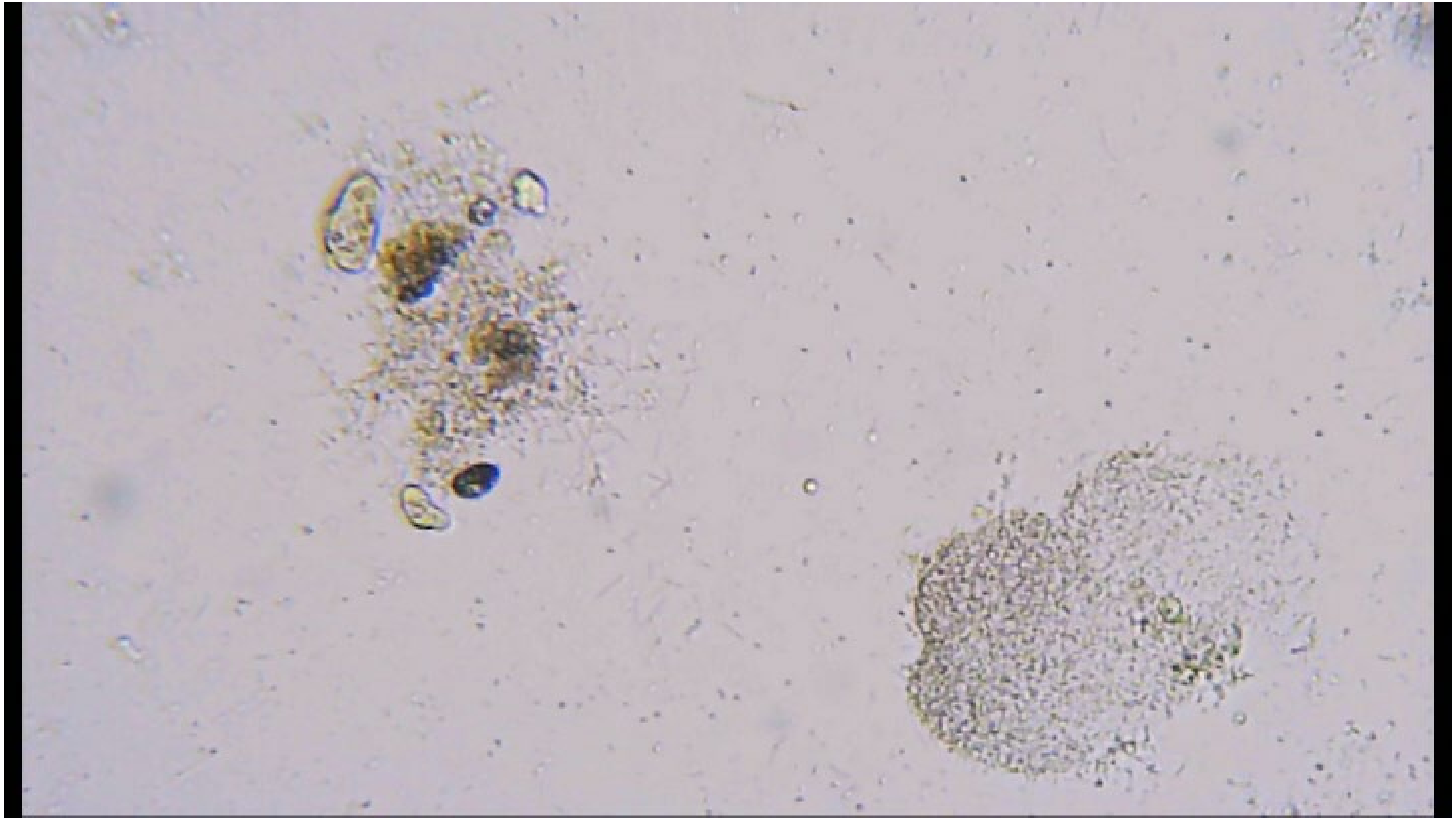
- Culturable organism chosen as models
- Might not be representative even for close relatives

From culturing to sequencing

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

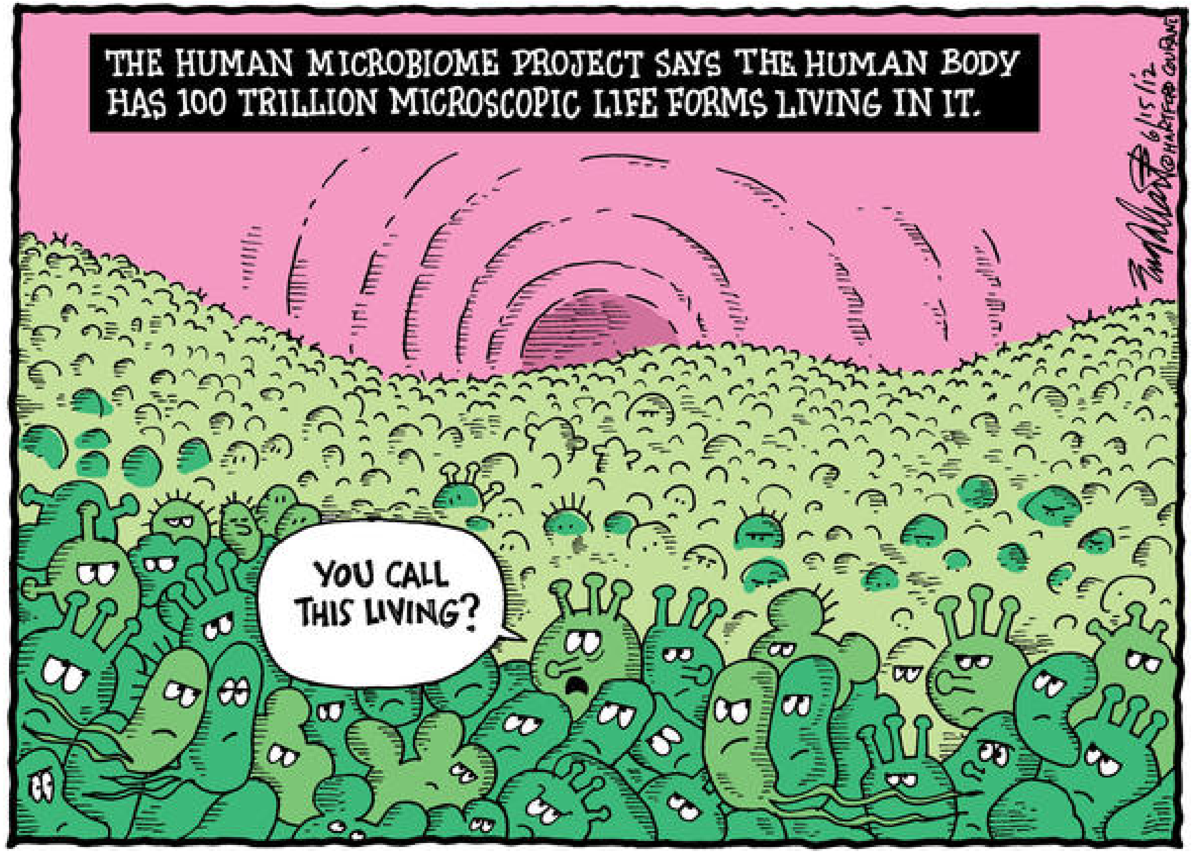


How do microbes live?



More bacteria in you than the human population

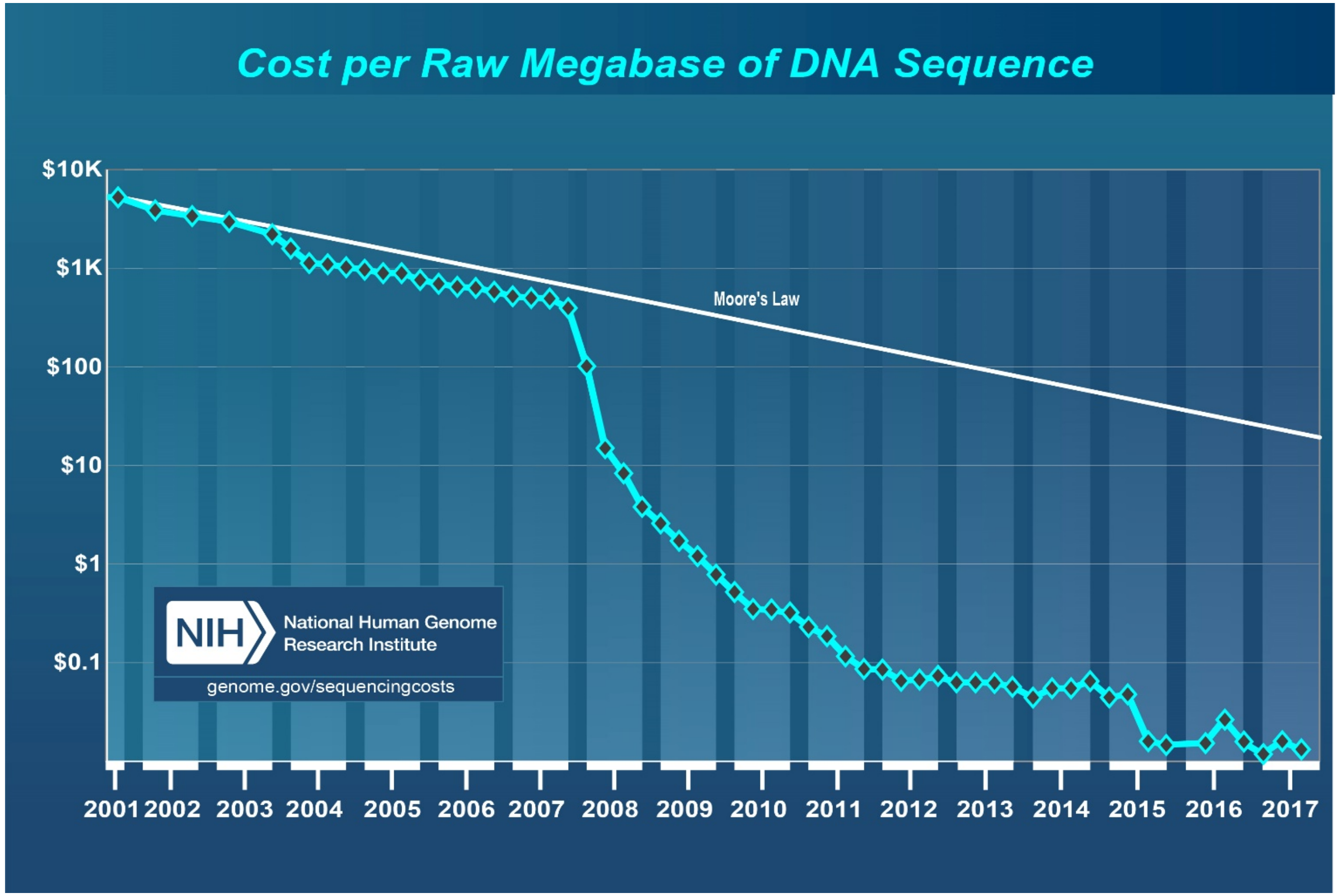
- Estimated 39 trillion microbial cells in you



Development in metagenomics is linked to technological advancements

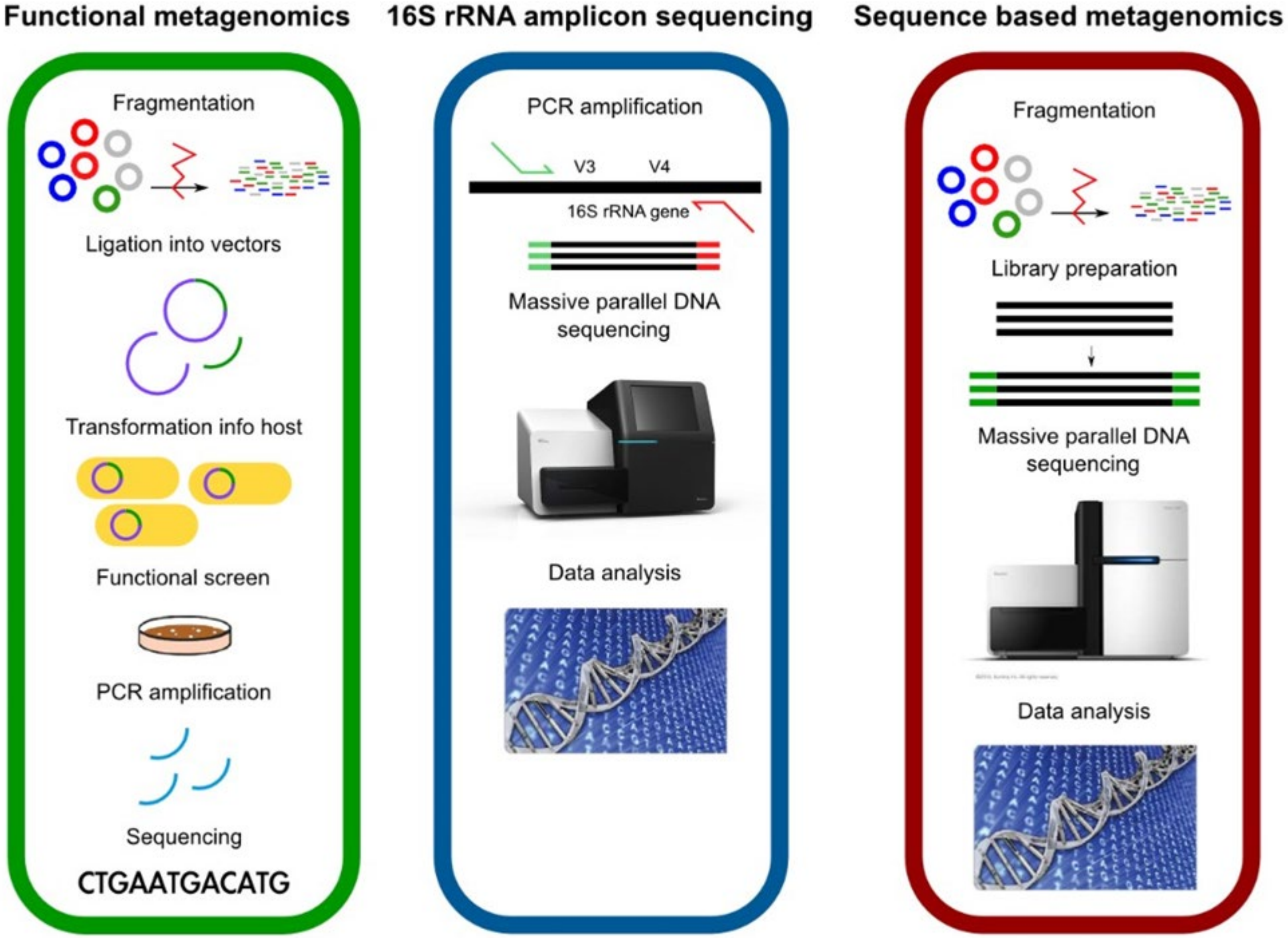
- Better, cheaper, faster

1 human Genome
3.000.000.000 USD
13 years



1 human Genome
< 1000 USD
1 day

Methods



Functional metagenomics

Fragmentation

Ligation into vectors

Transformation info host

Functional screen

PCR amplification

Sequencing

CTGAATGACATG

16S rRNA amplicon sequencing

PCR amplification

Massive parallel DNA sequencing

Data analysis

Sequence based metagenomics

Fragmentation

Library preparation

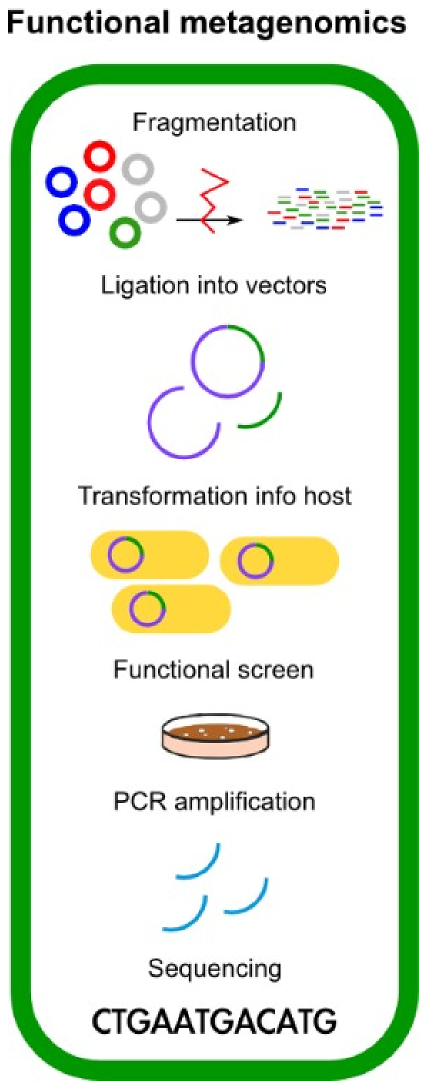
Massive parallel DNA sequencing

Data analysis

Functional metagenomics

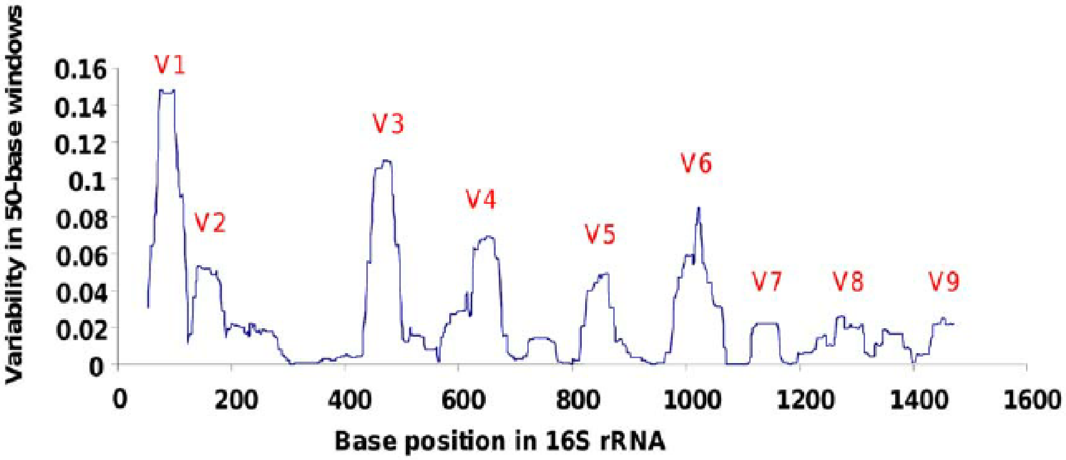
- Industrial large scale screening
- Time consuming due to functional screens
- Setup determines screening specificity

Not part of this course

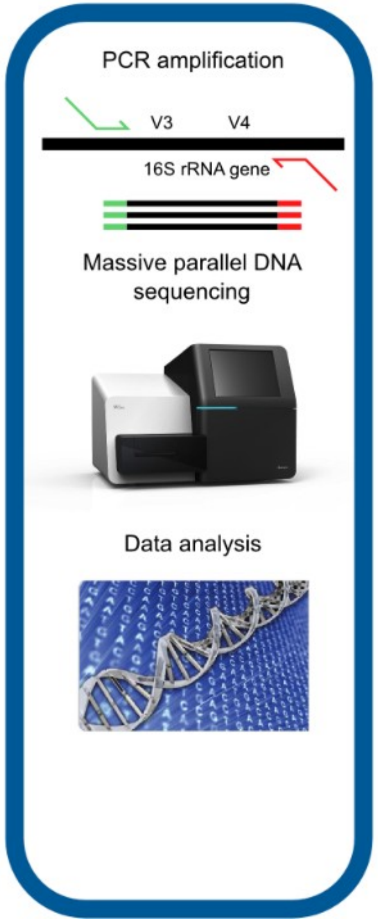


16S rRNA

- 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

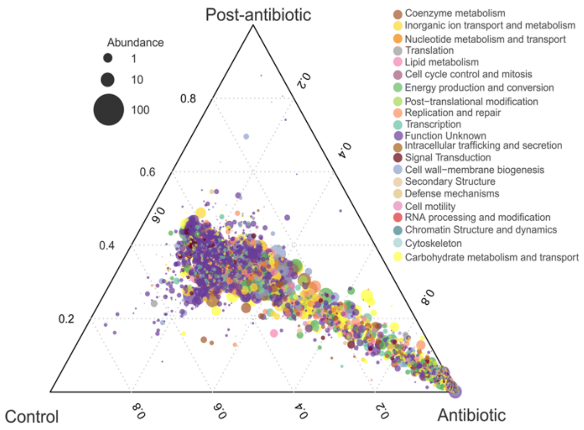


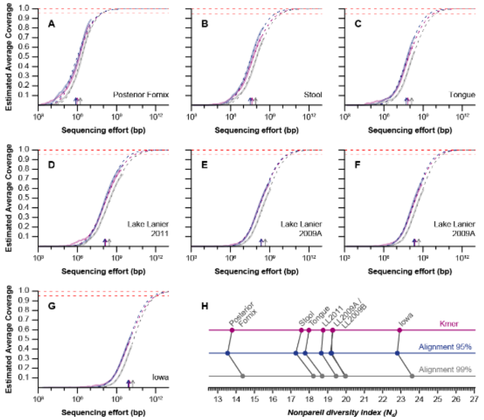
16S rRNA amplicon sequencing



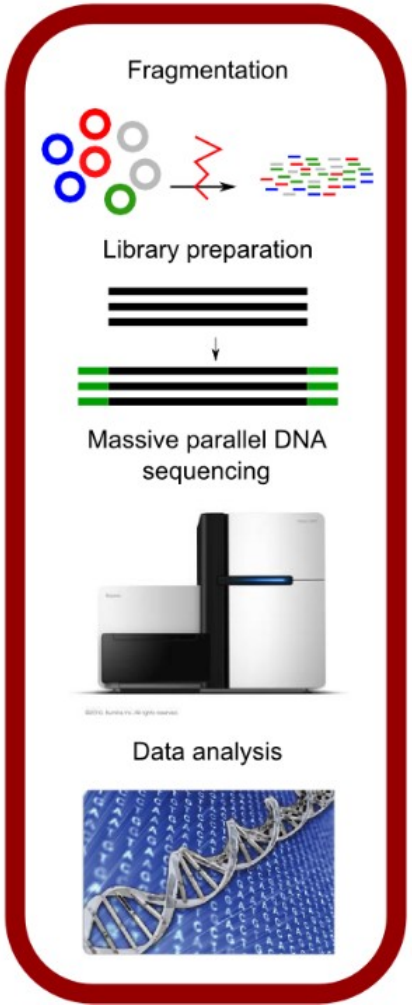
Shotgun metagenomics

- 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



Fragmentation

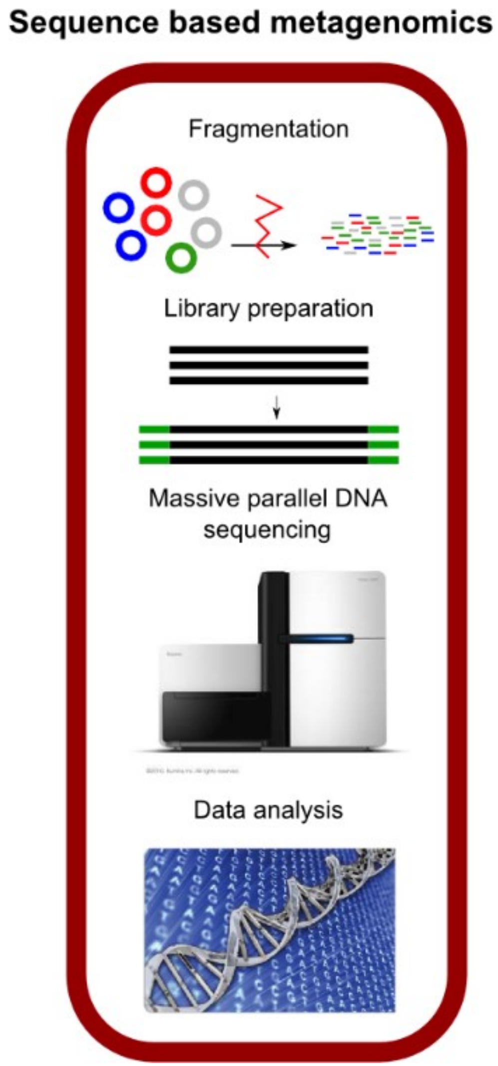
Library preparation

Massive parallel DNA sequencing

Data analysis

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

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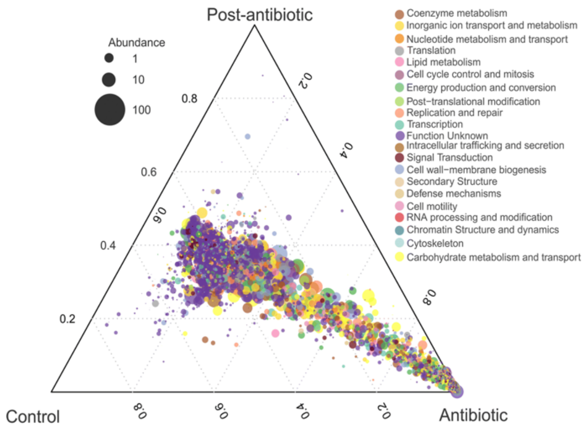
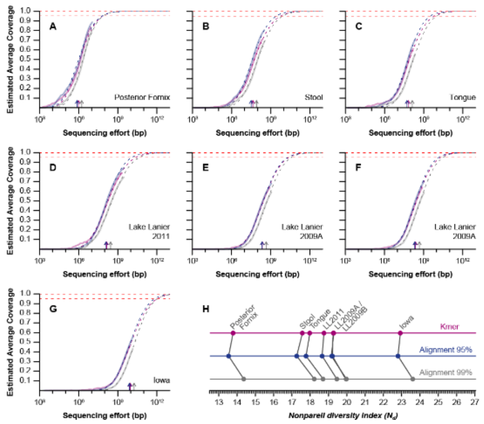
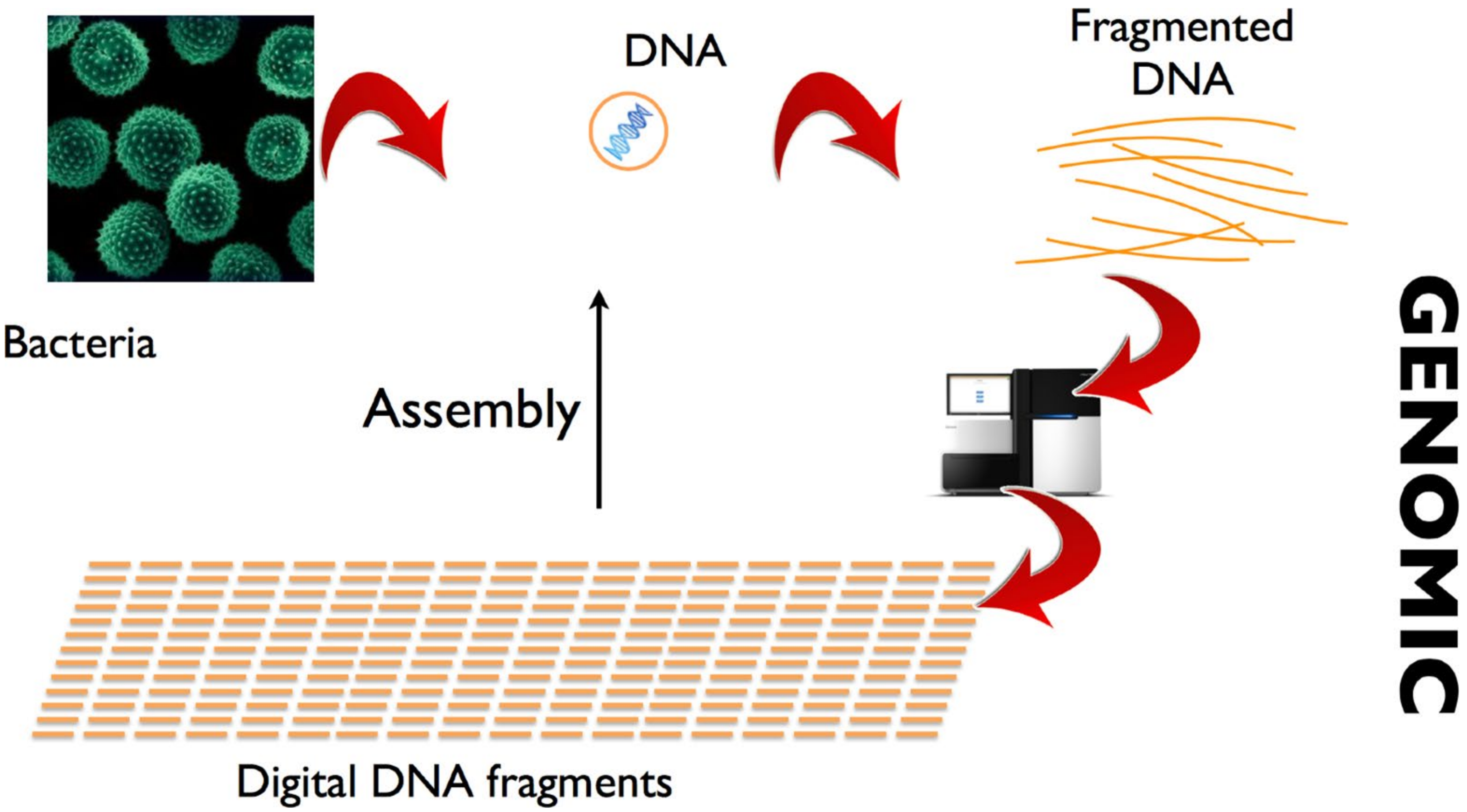




A needle in a haystack…and the haystack is on fire…and you have no clue what a needle is

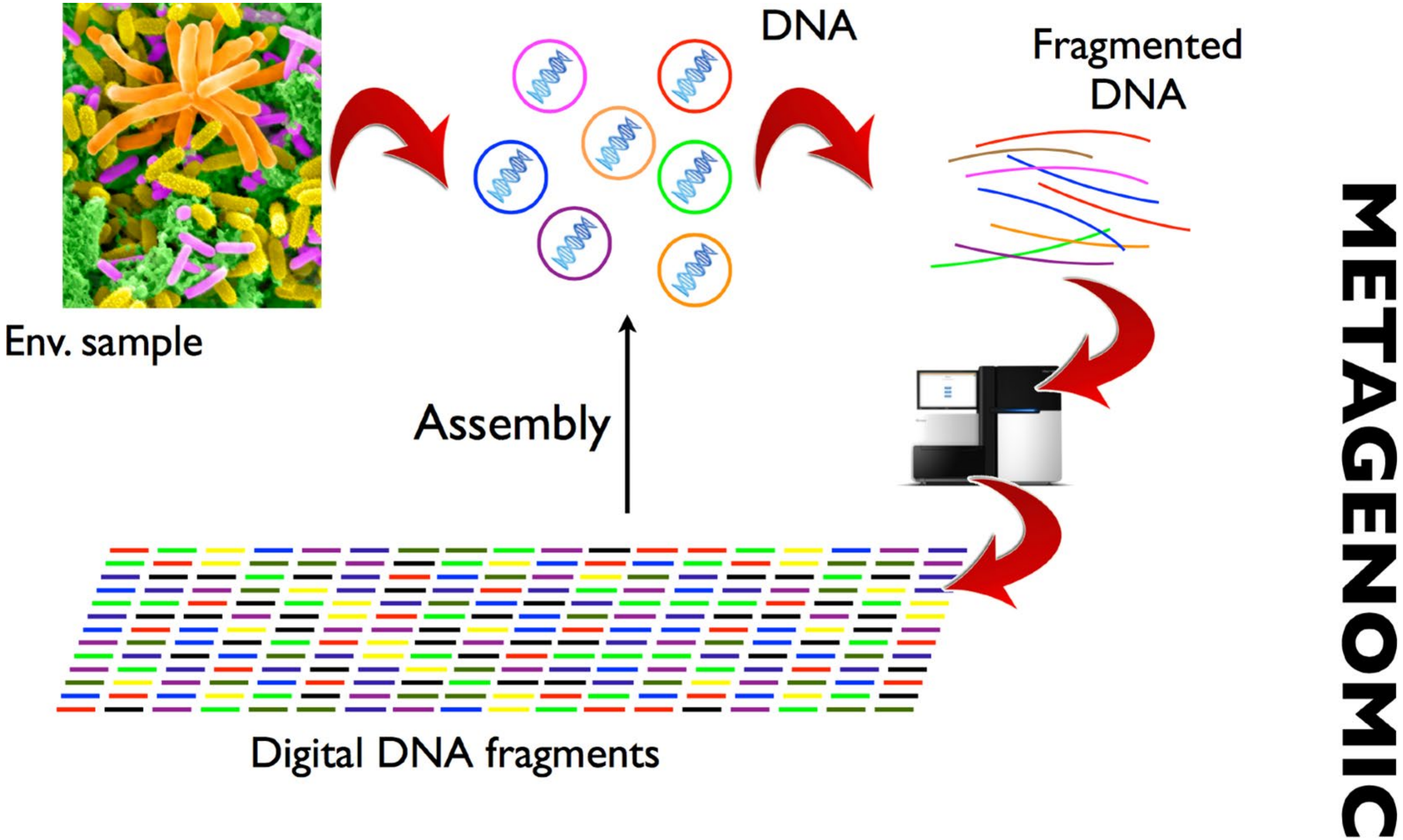
Genomics vs Metagenomics

- Genomics is often like assembling a puzzle



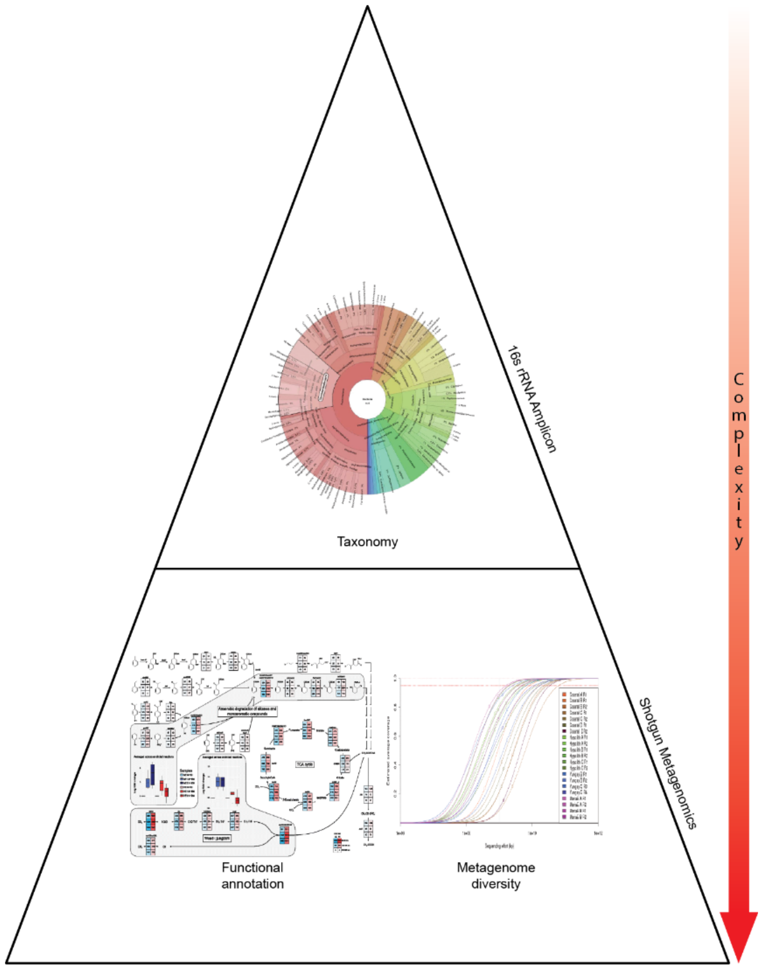
Genomics vs Metagenomics

- Metagenomics is often like assembling a million puzzles all scrambled together



Horses for courses

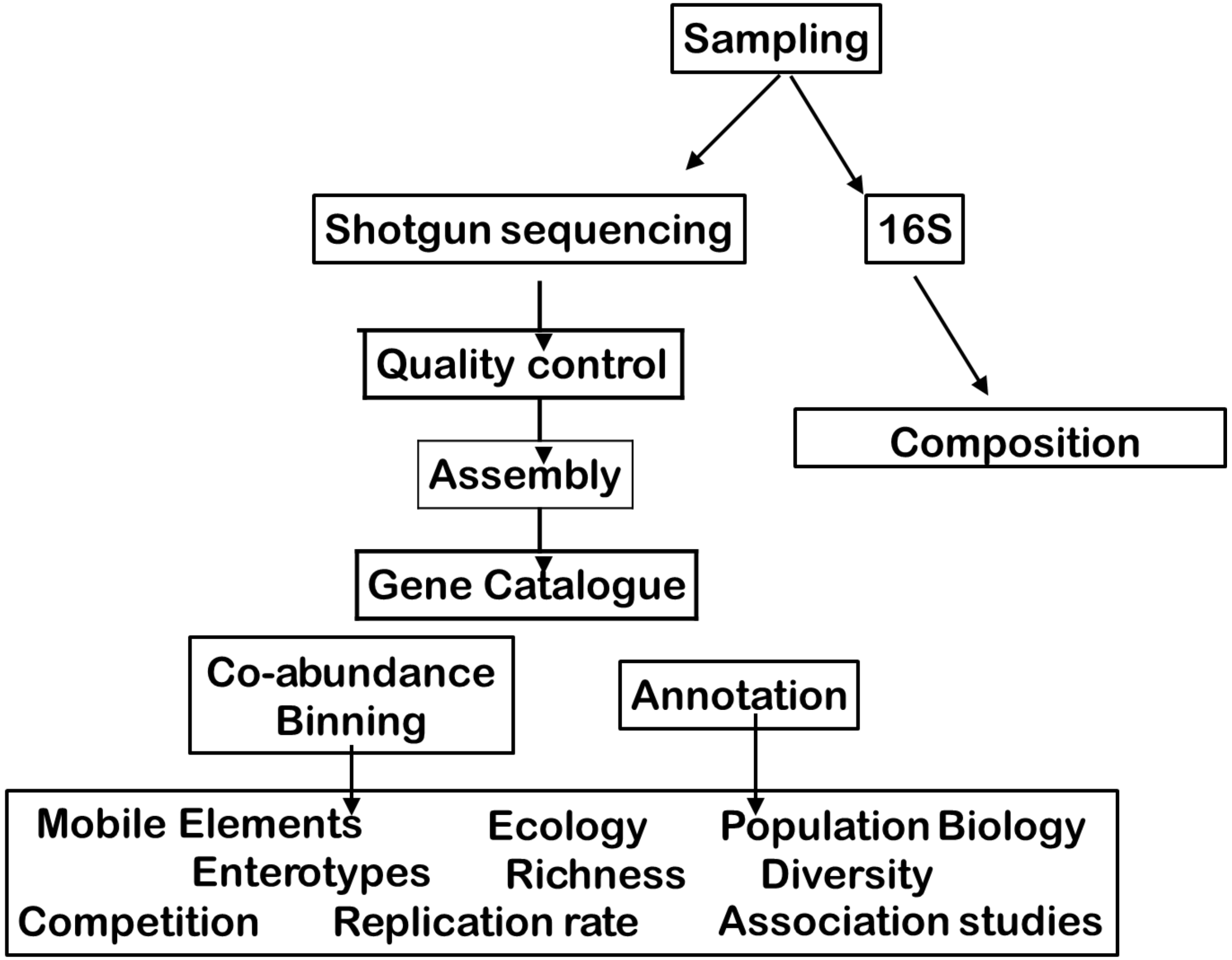
- Question dictates the apropriate tool
- Amplicon sequencing is easy & cheap
- Metagenomics allows more questions
  - Discovery of novel proteins, antibiotics etc.
- Same microbiome composition does not mean same metagenome!



This course

- First time NOT focussing on the human gut microbiome
- 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

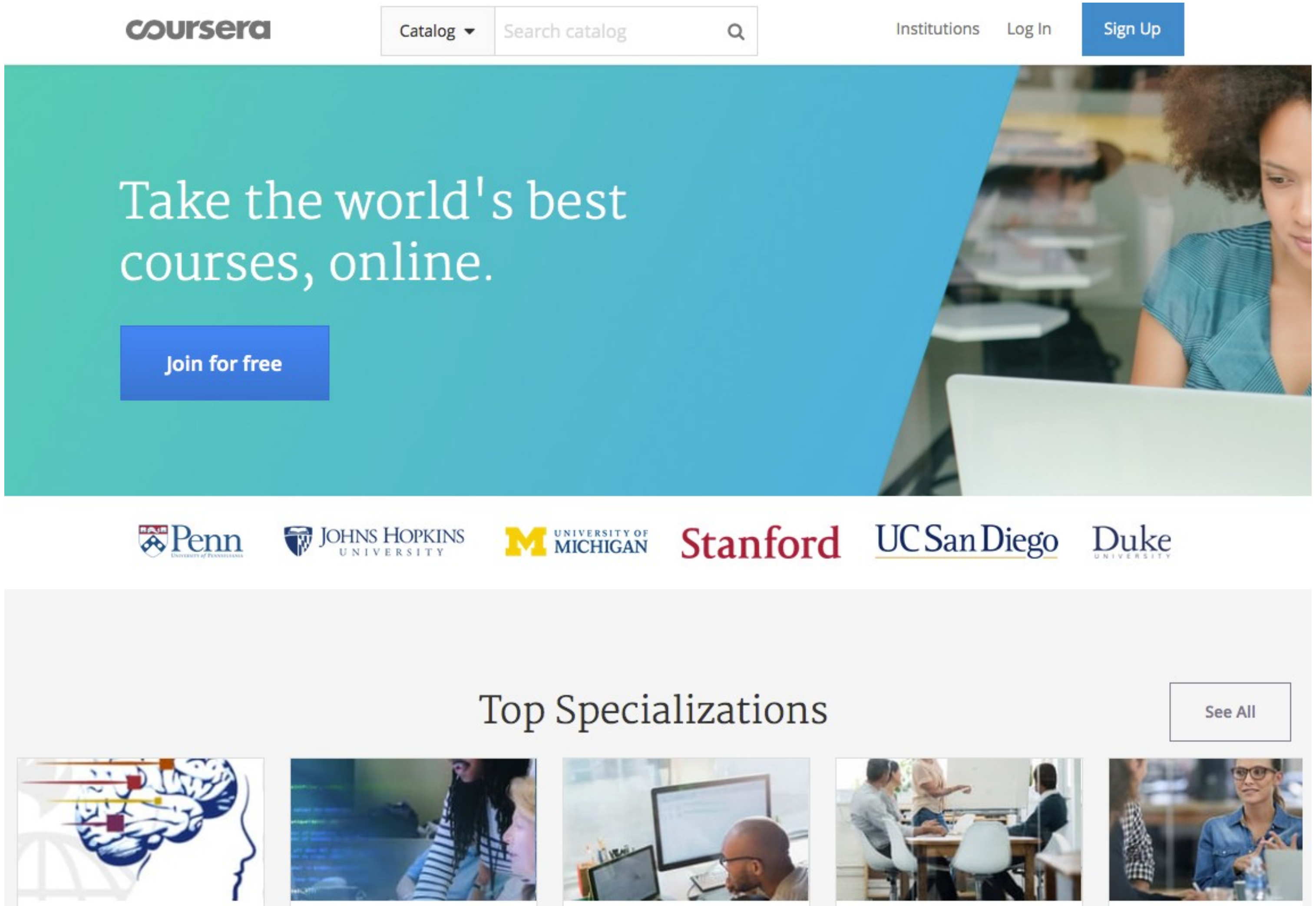
Co-abundance Binning

Annotation

Composition

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

Coursera



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Top Specializations

Gut check: Exploring your microbiome

- Voluntary, will point to certain parts of the course
- www.coursera.org/learn/microbiome
- Sign up and join course (No verified certificate)
- Complete lectures for week 1 & 2
- Complete quizzes for week 1 & 2

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