DNA Extraction and Library Prep

Microbiome Kickstart Workshop
Monday 12th 2024

Veronica Roman-Reyna

Overview

Experimental design

- Sampling
- Sequencing technologies
- Contamination
- DNA extraction
- Library prep

Analysis

- Metabarcoding
- Metagenomics
- Metabolomics

Overview

Experimental design

- Sampling
- Sequencing technologies
- Contamination
- **DNA extraction**
- **Library prep**

Analysis

- Metabarcoding
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DNA extraction

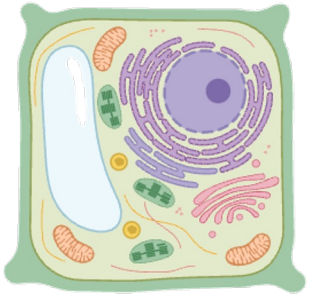
1. Cell lysis
2. DNA Separation
3. DNA Wash
4. DNA Elution/resuspension
5. Quality and Quantity Assessment

DNA extraction

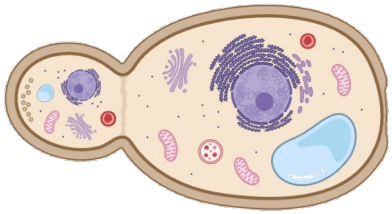
What is DNA extraction?

- Isolate and purify DNA from other cell components.

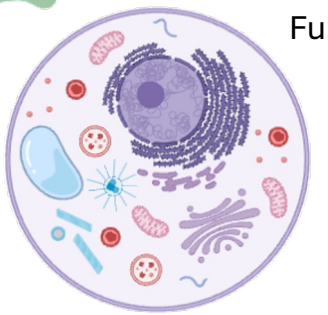
Eukaryotic cells



Plant



Fungi

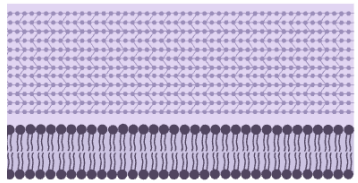


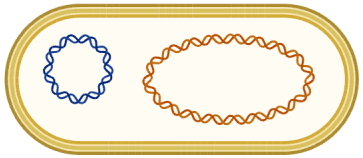
Animal

Prokaryotic cells

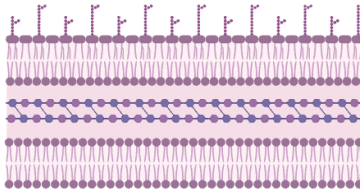
Bacteria, Archaea

Thick wall





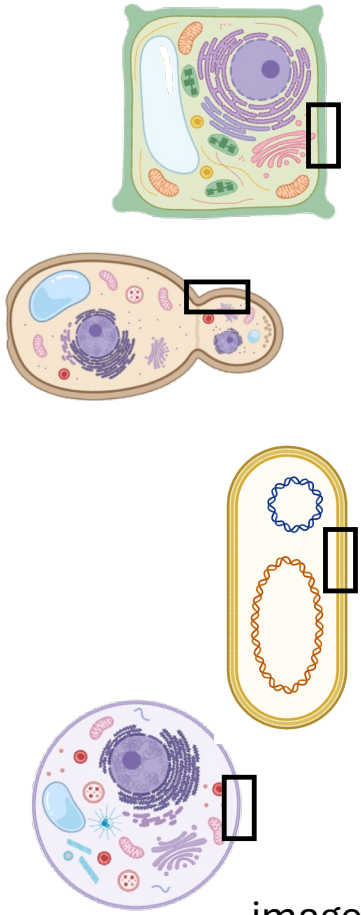
2 membranes



images: BioRender.

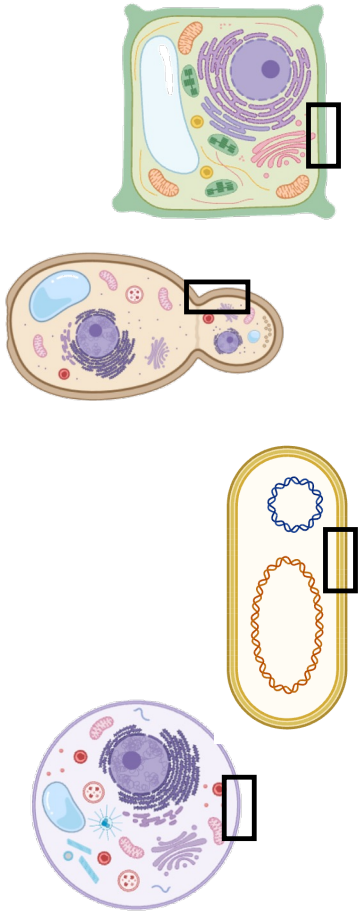
1. Cell lysis

Kit or “manual” extraction



images: BioRender.

1. Cell lysis



Lyse cells
membranes
walls

Mechanical:

- Beads
- Mortar/Pestle

Chemical:

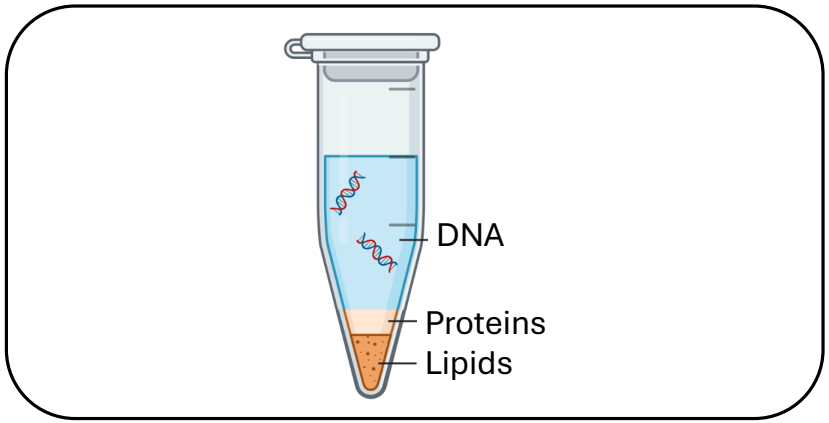
- Surfactants (aka. soaps)
- Enzymes
  - CTAB, SDS, guanidine
  - Lysozymes

images: BioRender, Revvity.

2. DNA separation

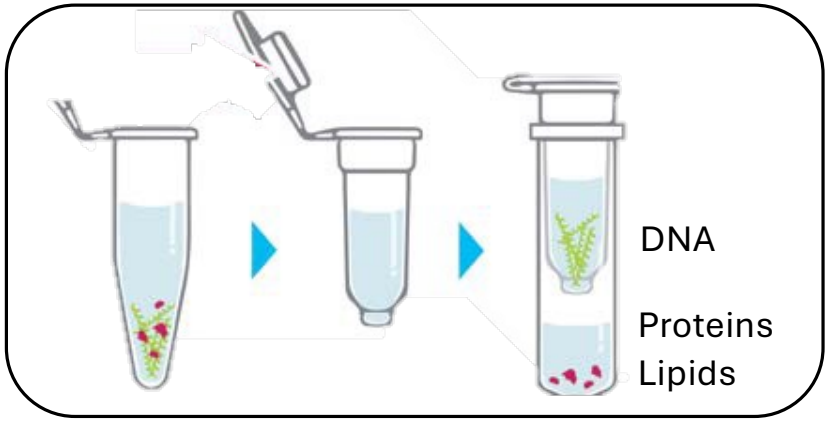
Phase separation

Phenol/chloroform
CTAB



Column binding

DNA binds to silica membrane

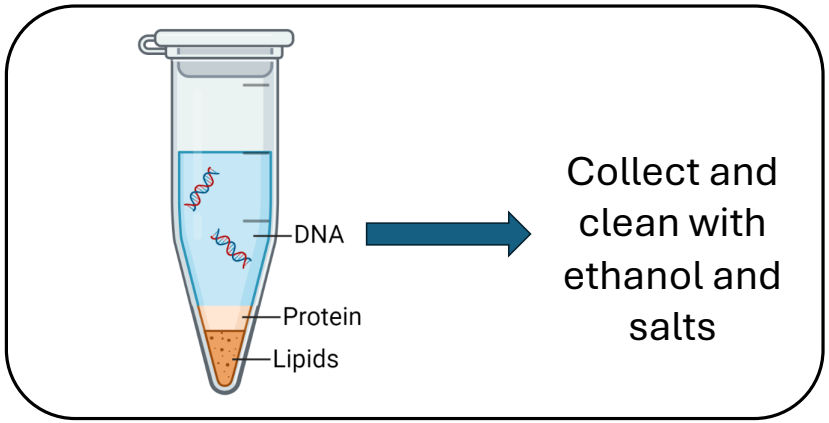


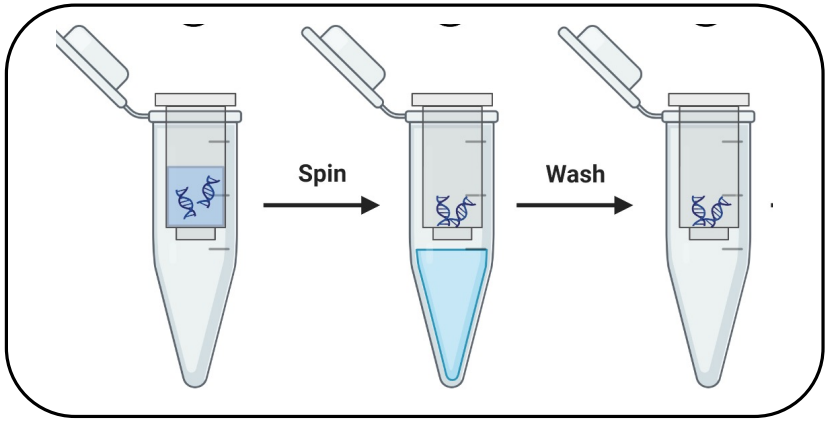
images: BioRender, generi-biotech.

3. DNA wash

Remove any remaining salts

Ethanol
Isopropanol





images: BioRender

4. Elution/resuspension

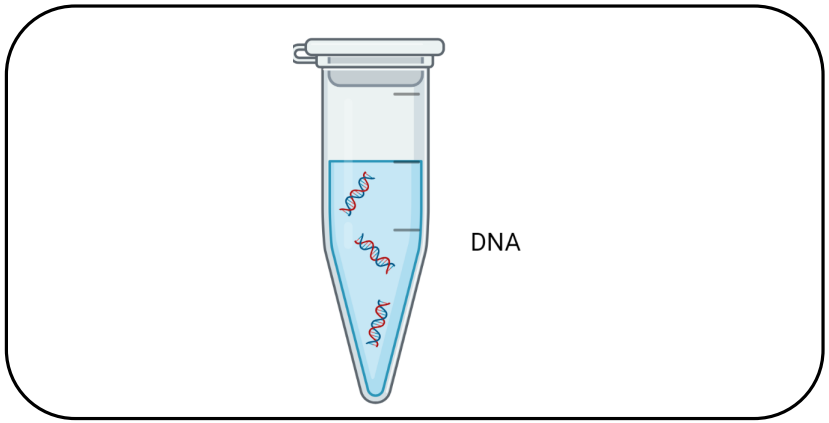
Dissolve DNA

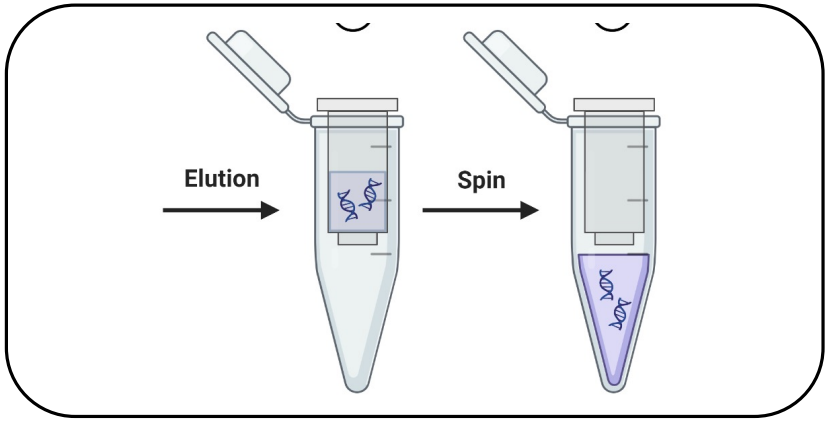
Nuclease-free Water

Buffer

Long term storage

TE buffer





images: BioRender

5. Quality and Quantity Assessment

Spectrophotometry

Nanodrop, UV5nano, Eppendorf

- "Guestimate" of DNA concentration.
- Best for quality assessment.

- A260/A280 < 1.8 = protein contamination
- A260/A230 < 1.8 = organic contaminants

Fluorescence

Qubit or Quantus

- Accurate DNA concentration

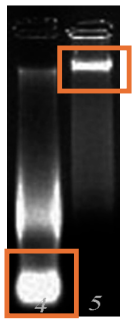
- Dye binds to DNA
- Compares to a standard/curve

Electrophoresis

Agarose gels

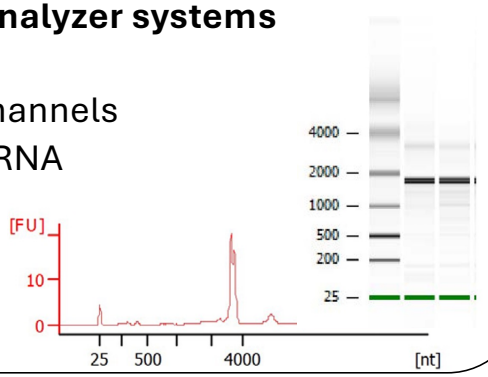
- DNA integrity
  - Degradation (smear)
  - Short/long reads

- Purity
  - RNA "leftovers"



TapeStation and Bioanalyzer systems

- Microfluidic chip/channels
- Accurate DNA and RNA concentration
- DNA integrity



images: Thermo, Promega, Agilent.

DNA extraction

Considerations

- Why **type** of microbe are you extracting from?
  - How harsh/gentle you should lyse cells?
- How much **biomass** are you expecting to collect?
- Are there **inhibitors** from your sample?
- Is this for short or long **read** sequencing?
- What **controls** should you consider?
- Are you **cost-limited**?

Library preparation

1. DNA fragmentation.
2. Adaptor ligation.
3. Library amplification
4. Pooling

Library Preparation

What is library preparation?

- Prepare DNA for sequencing.
- Create a collection of DNA (library) fragments that are compatible with the sequencing platform.
- **Adaptor**: short DNA sequence that enable DNA fragment to attach to the sequencing platform. For example, P5, P7.
- **Barcode** (index): unique short DNA sequence to allow the differentiation of multiple samples.

1. DNA fragmentation

Short-read seq.

Illumina

- Make small fragments (<1000bp)

Long-read seq.

Oxford Nanopore
PacBio

- Direct or DNA fragments.
- Sequence long fragments (>20Kb)

- Mechanical shearing
- Enzymatic digestion
- **Tagmentation**
- **PCR**

2. Fragmentation and Adaptor Ligation

Tagmentation

Is a molecular biology technique used to simultaneously **fragment** DNA and **add** known DNA sequences in a single step.

Transposase Enzyme preloaded with known sequences (“tags”).

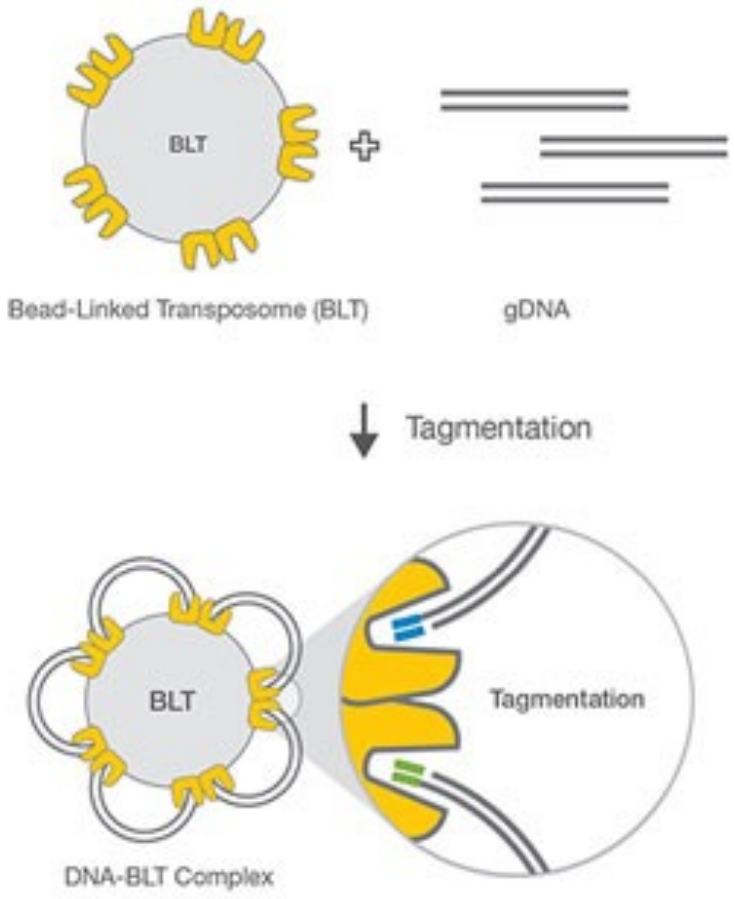
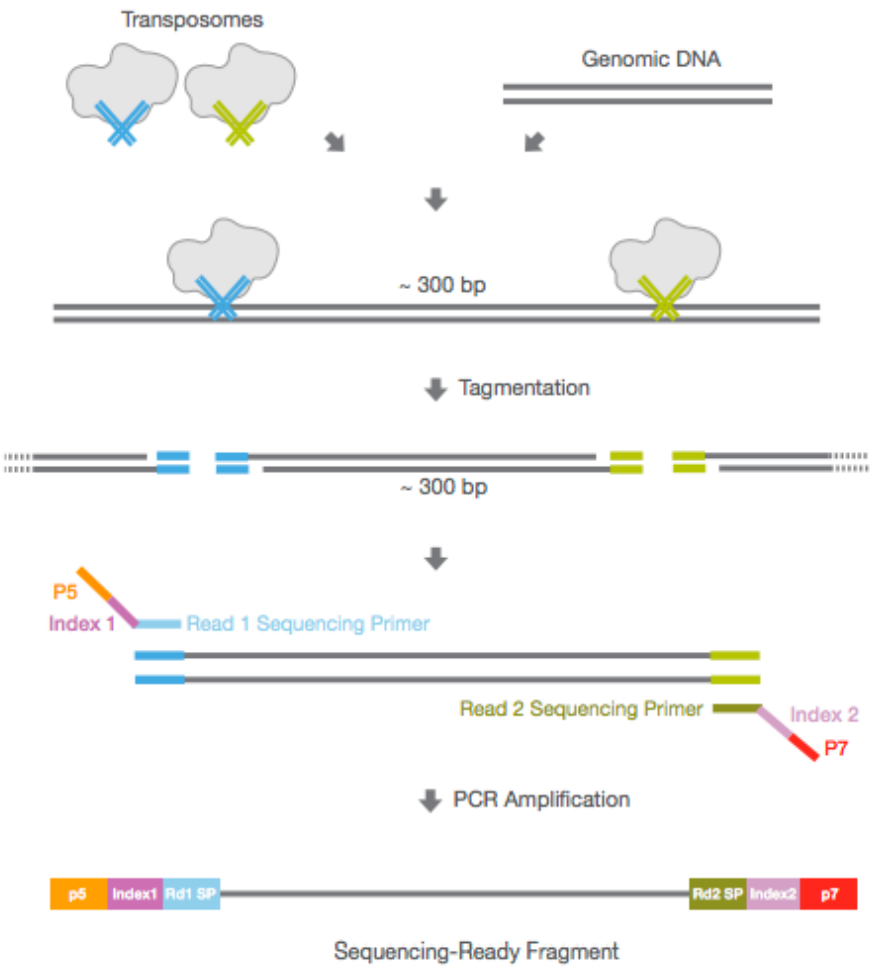


image: Illumina

Metagenomic library (Illumina Nextera)

1. Tagmentation

2. Library amplification.



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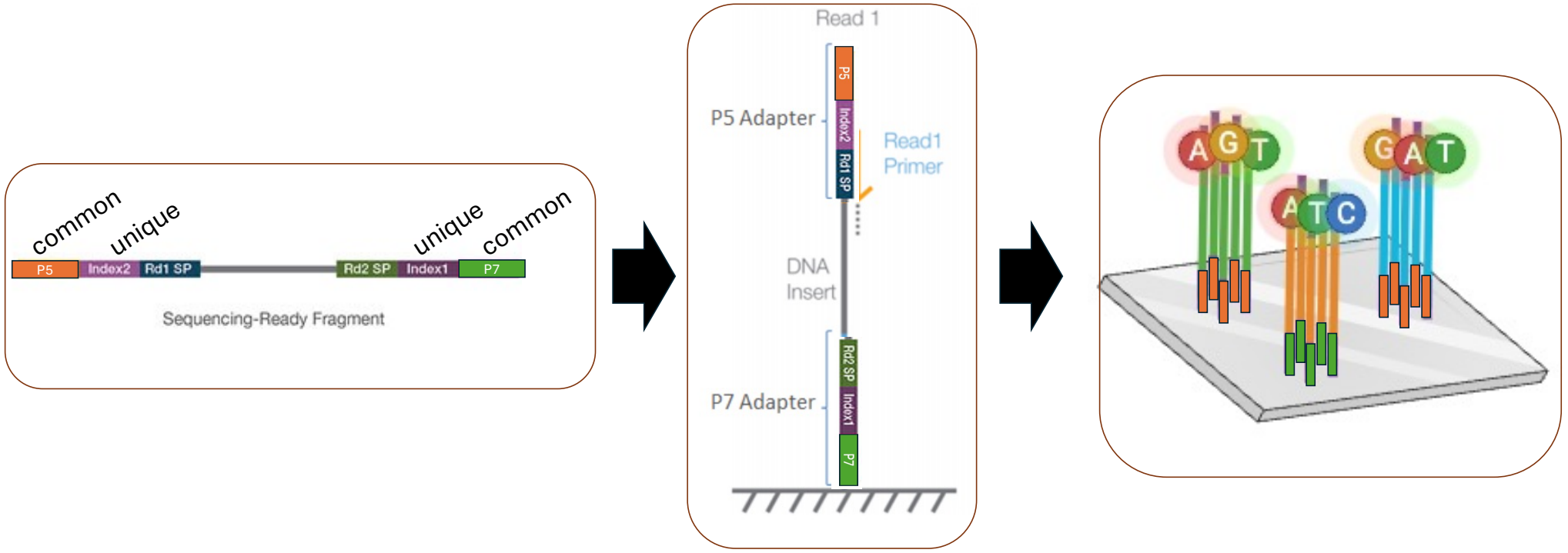


image: Illumina, BioRender

16S rRNA and ITS sequencing libraries

One-step (single PCR)

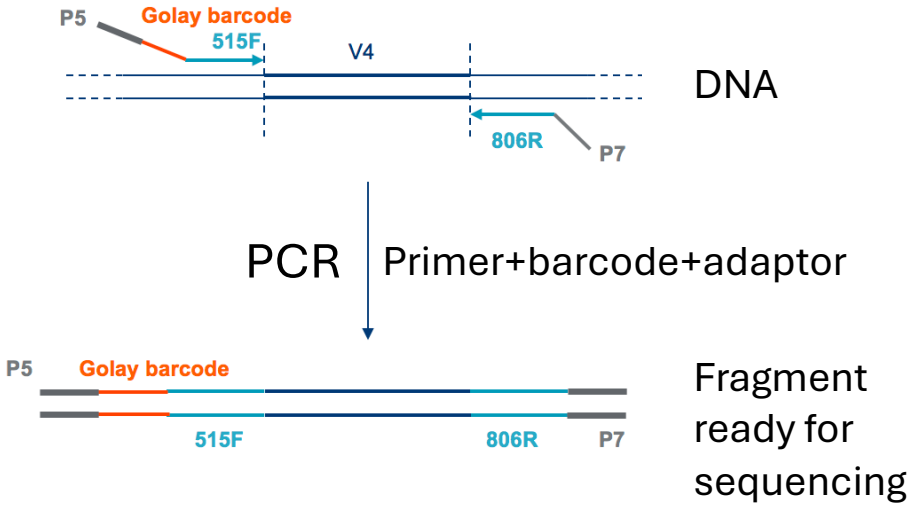


image: IDT.com

Need to order many primers with barcodes in them. (plan barcodes in advance).

Two-step (two PCRs)

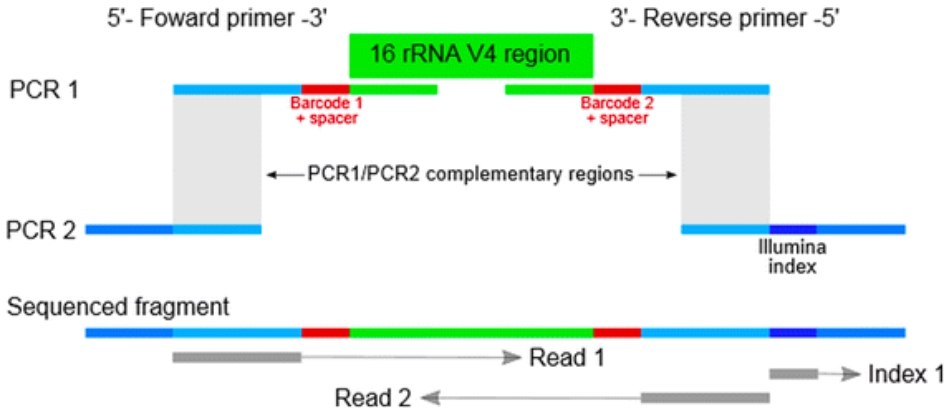
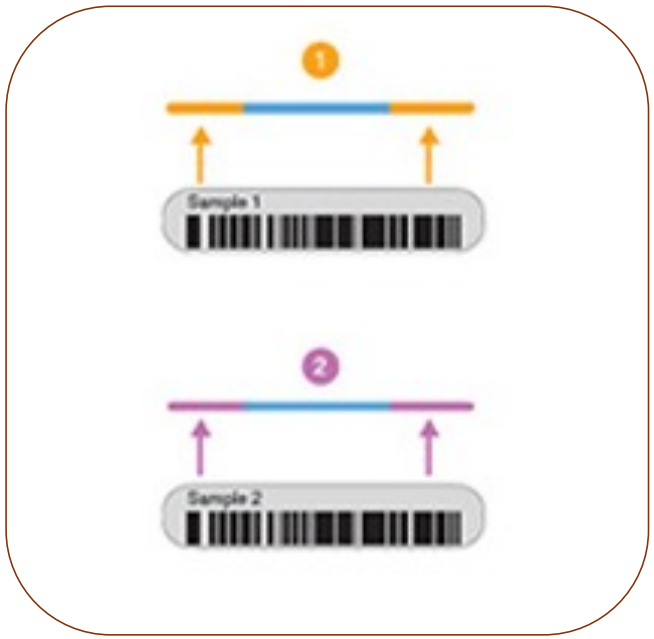


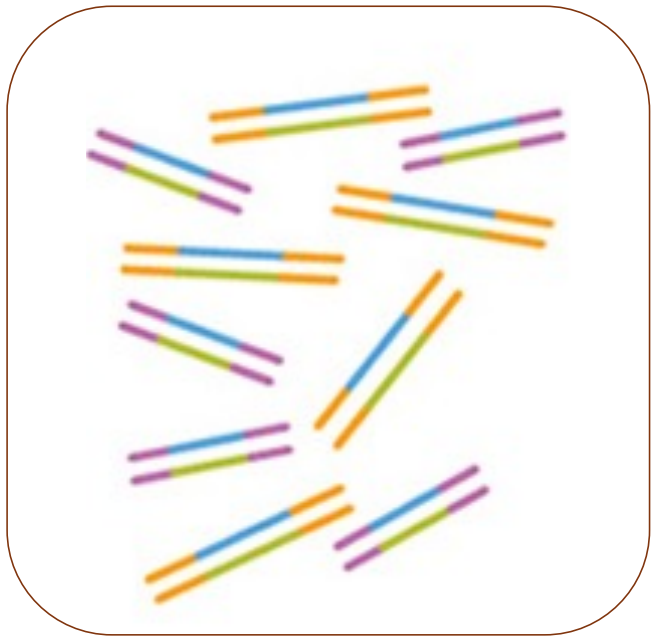
image: de Muinck et al. 2017

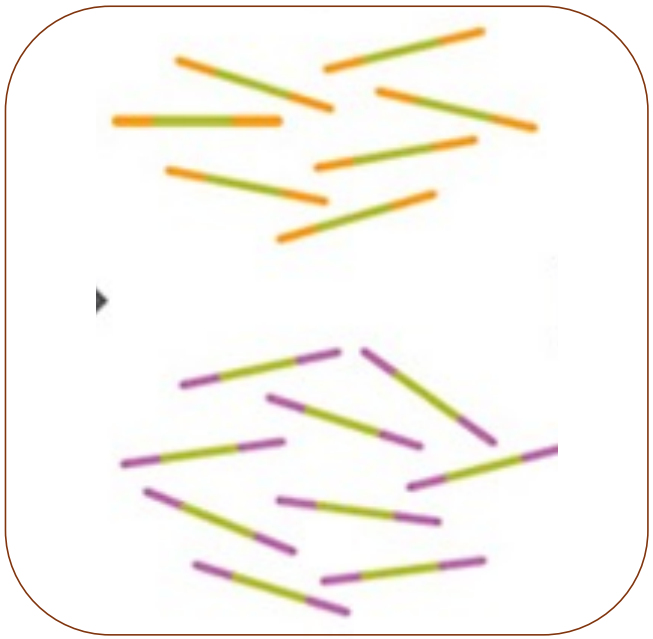
Do not need to plan barcodes in advance.

4. Pooling / multiplexing

Library preparation (unique barcodes)	Pool samples and sequence	De-multiplex



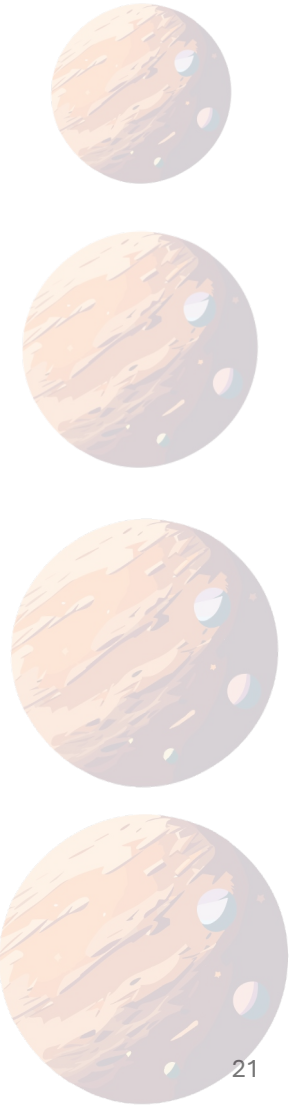




images: Illumina

Breakout rooms!

Running a microbiome study of Mars



1. How would you design DNA extraction and library preparation for your study?
2. What are some considerations of your design during the DNA extraction stage?
3. What are some considerations of your design during the library prep stage?
4. How would you ensure your DNA extractions and library preps are good quality?
5. Do current DNA extraction protocols designed for Earth-based life work effectively with potential Martian biomolecules, which may have different chemical compositions?