Standardizing Microbiome Research: From The Lavatory To The Laboratory

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Disclosures

- None

Overview

1. Introduction
2. Sources of variability
3. Methods to minimize variability
4. Standardization in patients with cancer

The human microbiome and cancer

Cancer risk
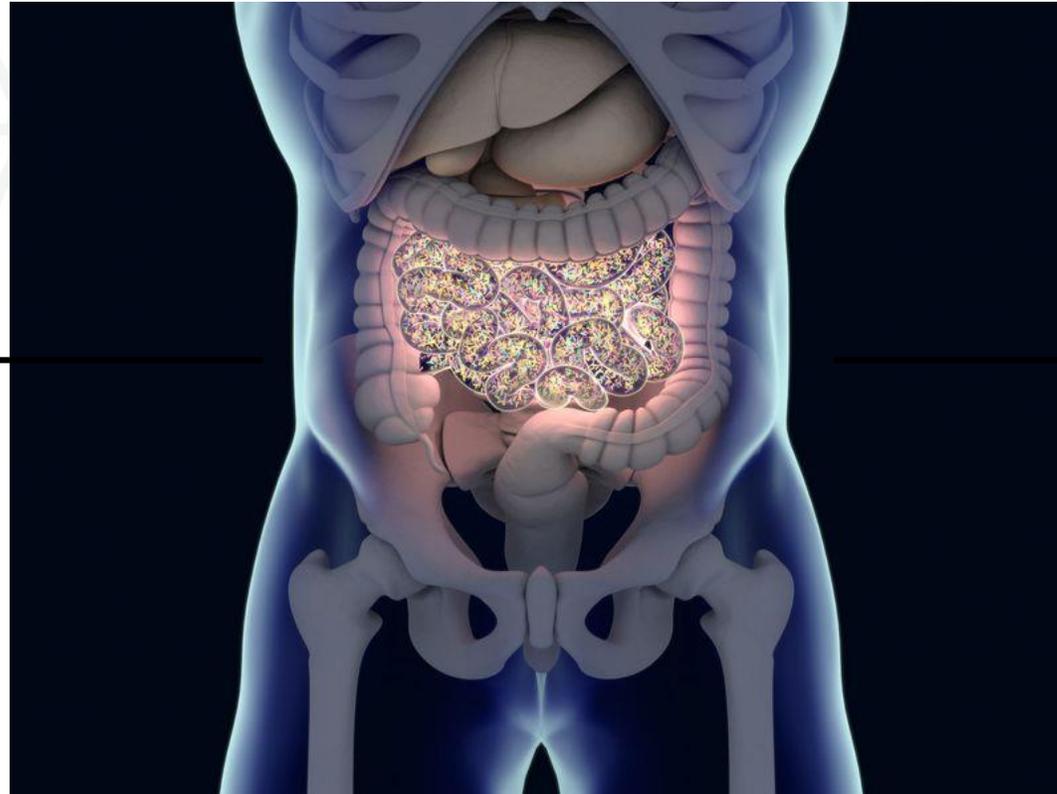
Cancer treatment response and toxicity

https://www.smithsonianmag.com

Stool processing and analysis workflow


1. Collection

2. Processing and storage

3. DNA extraction

4. Library preparation

5. Sequencing

6. Bioinformatic analysis

Samples

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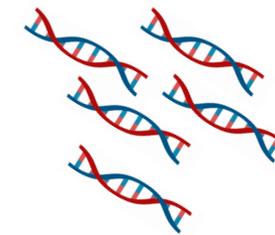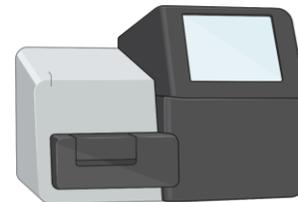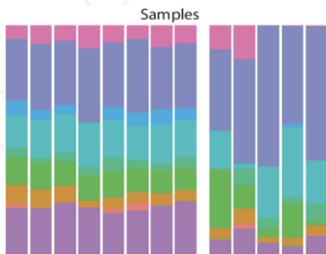
Microbiome sequencing methods

16S ribosomal RNA sequencing

Gross taxonomic classification

Metagenomic sequencing + limited gene analysis

Higher resolution taxonomic classification

Non-bacterial data
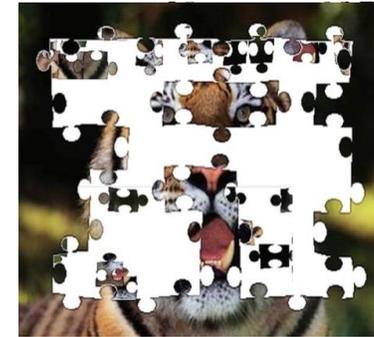
Metagenomic sequencing + whole genome analysis

Species/strain level classification

Non-bacterial data

Metabolic pathways

Reproducibility is a major challenge in microbiome research
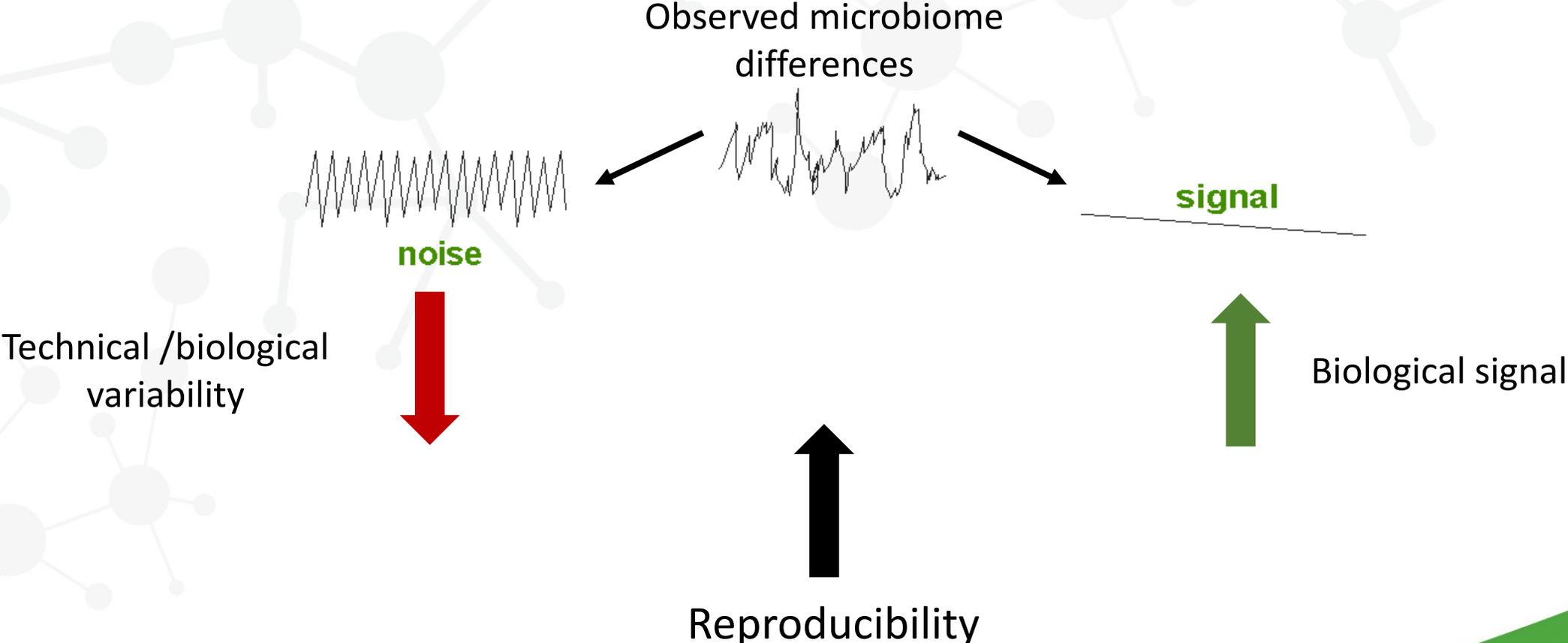
Technical and biological variability can occur at every step

1. Collection
2. Processing and storage
3. DNA extraction
4. Library preparation
5. Sequencing
6. Bioinformatic analysis
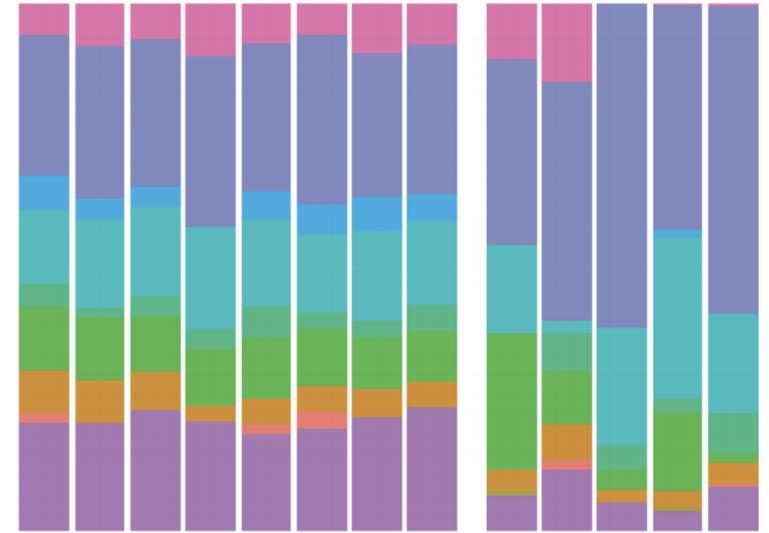
# Sources of technical and biological variability

1. **DNA extraction method**
2. **Bioinformatic processing**

3. Study design
4. Sample collection
5. Storage conditions
6. Batch variation
7. Contamination
8. Different reference databases
9. Many others….

Sources of variability: DNA extraction methods

Knudsen BE et al., mSystems, 2016
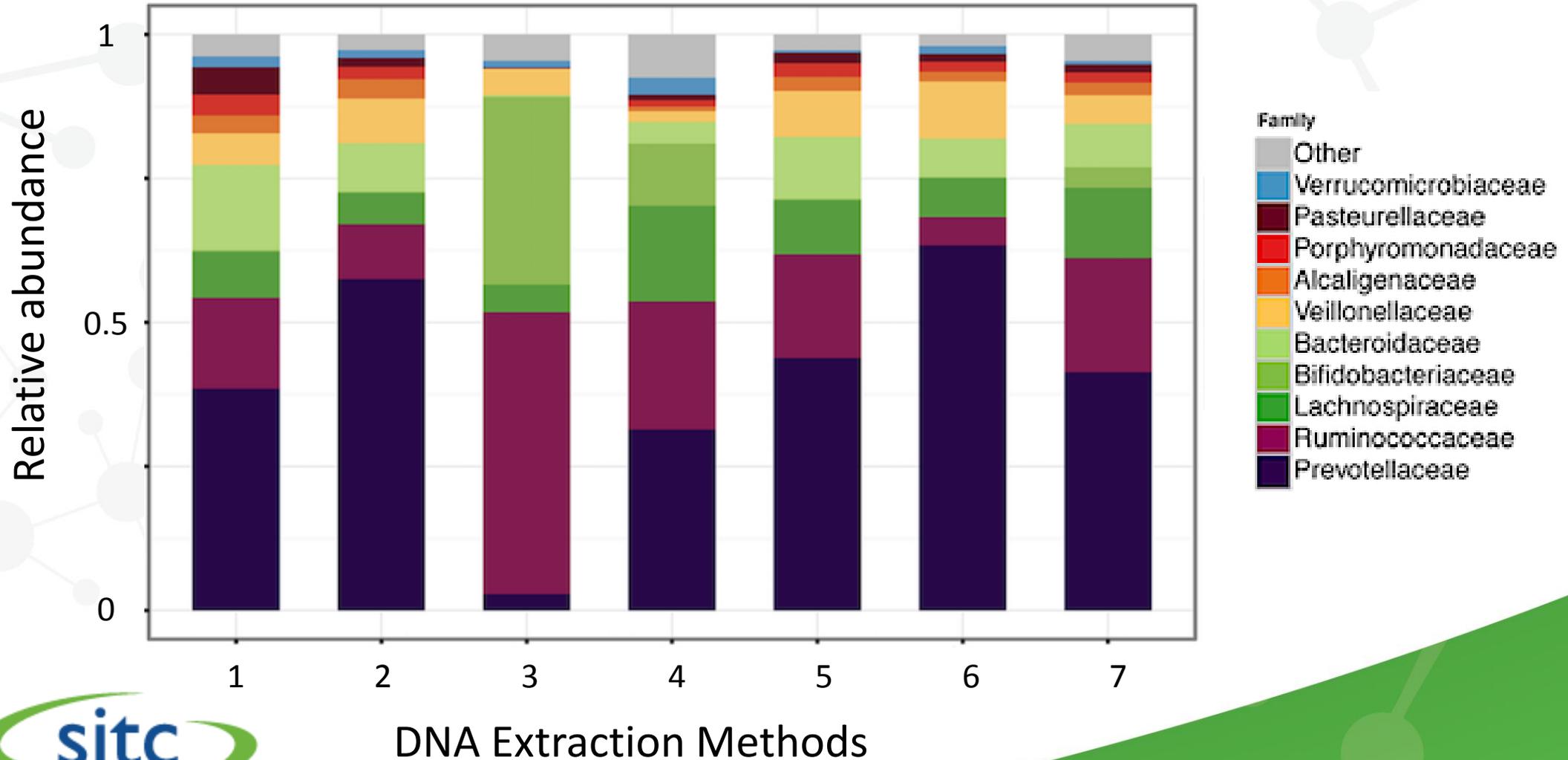
Poor correlation of diversity measurements between different labs processing the same sample
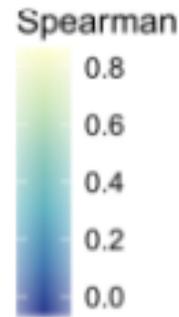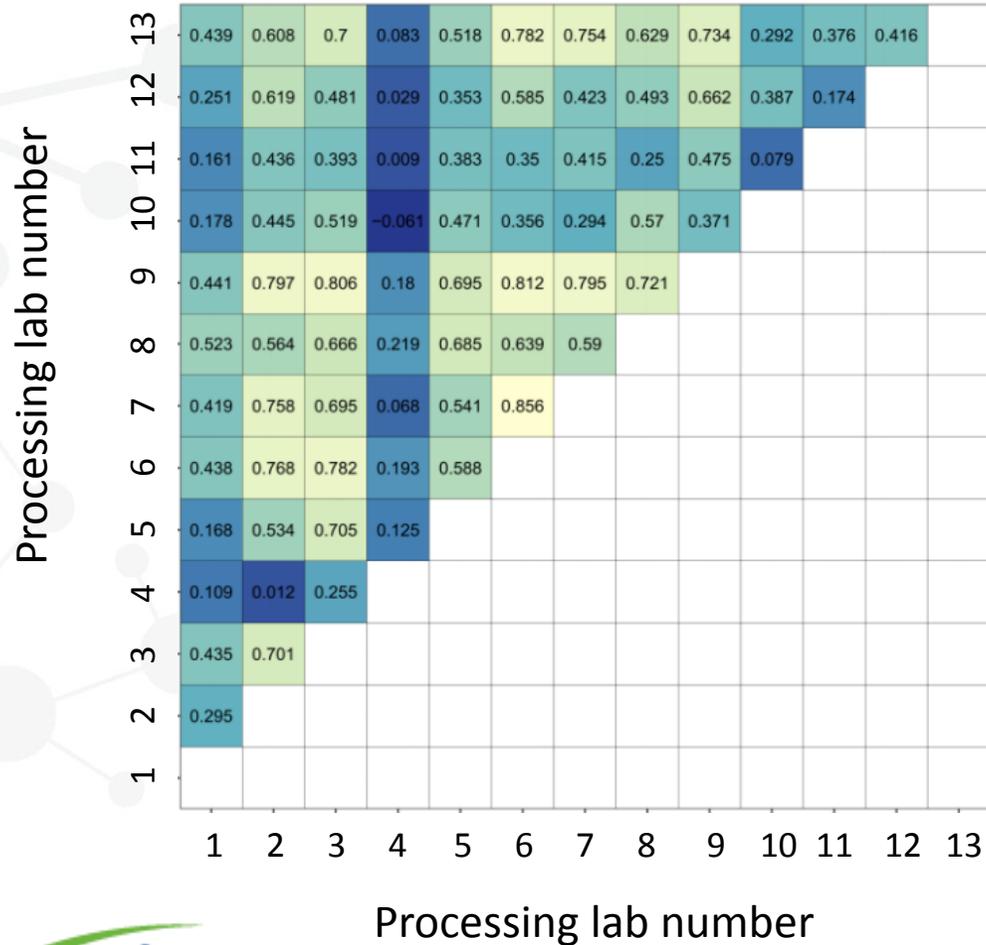
Correlation of diversity measurements:

0.08 (blue)-0.77 (yellow)

Sinha R et al (Microbiome Quality Control, MBQC) Nature Biotech, 2017

# Improved correlation of diversity measurements between different labs <u>analyzing</u> the same sample
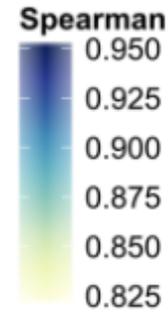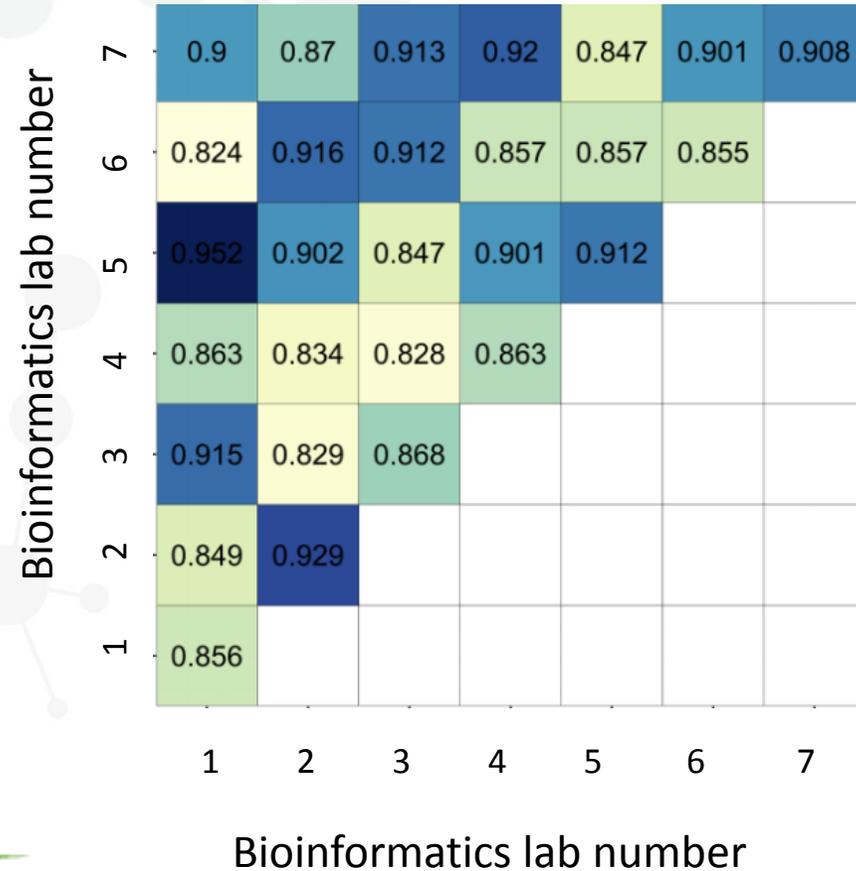
Correlation of diversity measurements:

0.82-0.95

Sinha R et al (Microbiome Quality Control, MBQC) Nature Biotech, 2017

Relative contributions to variability in microbiome research: International Human Microbiome Standards (IHMS)

Sources of variability

Costea PI et al, Nature Biotech, 2017

Relative contributions to variability in microbiome research: International Human Microbiome Standards (IHMS)


Sources of variability

Within protocols (measurement error)
Between library preparations
Between preservation methods
Within stool specimens
Between protocols
Within individuals over time
Between individuals

0   0.25   0.50   0.75   1

Spearman distance

Costea PI et al, Nature Biotech, 2017
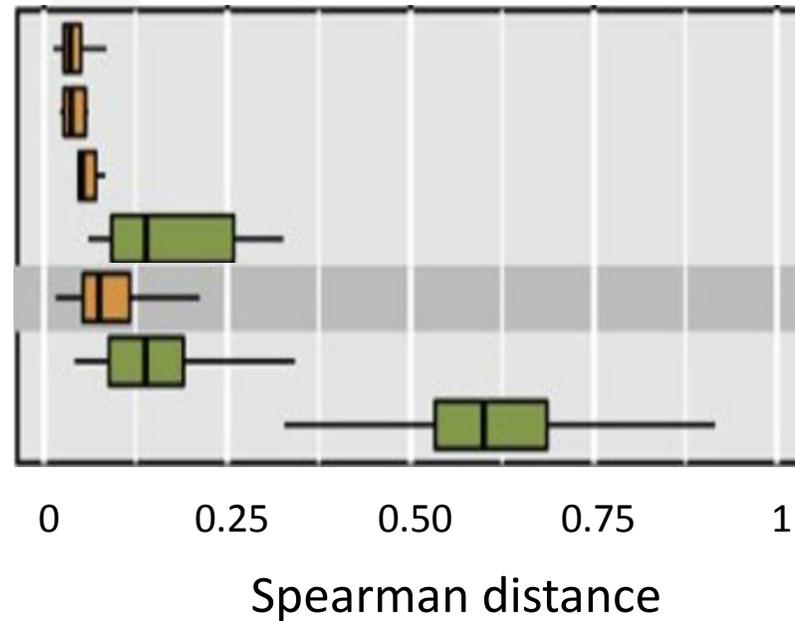
Sources of biological variability
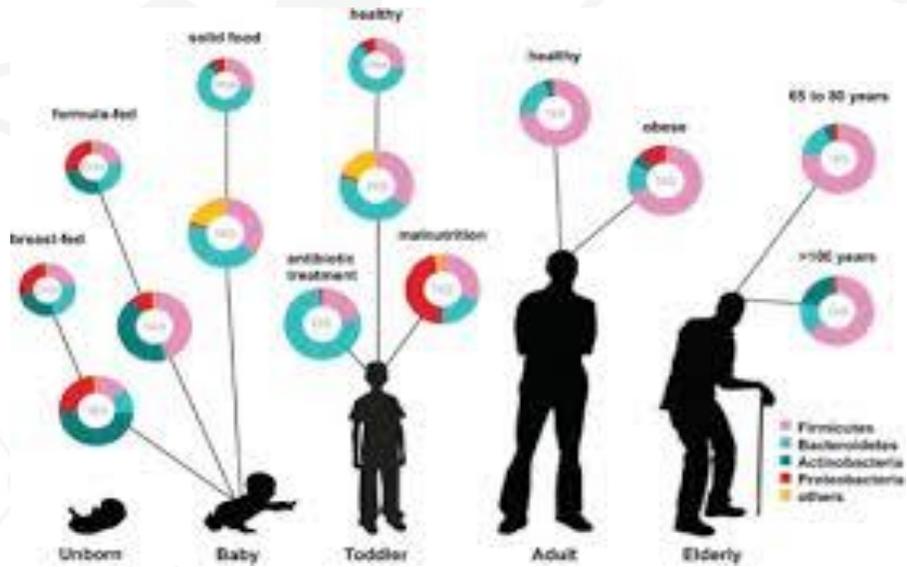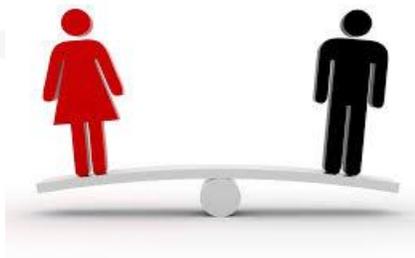
Demographic variables

Medications

Diet

How can we minimize technical and biological variability?

1. **Standardization**

2. **Use of controls (+/-)**

3. Accurate measurement of confounders

Minimizing technical and biological variability: Standardization in healthy participants

- Human Microbiome Project (HMP): https://hmpdacc.org/

- Microbiome Quality Control Project (MBQC): https://www.mbqc.org/

- International Human Microbiome Standards (IHMS): http://www.microbiome-standards.org/

Minimizing technical and biological variability:
+/- Controls

+ controls: Microbiome standards

- controls: Buffer blanks

Microbiome standards
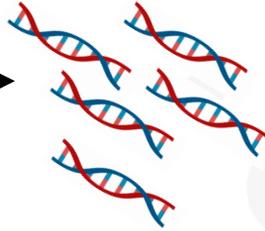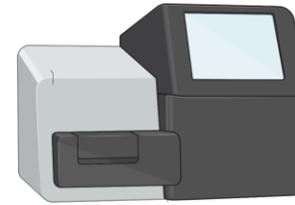
3. DNA extraction
4. Library preparation
5. Sequencing
6. Bioinformatic analysis
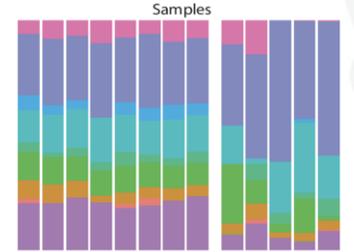
Whole cell standardized microbial community

Microbiome standards

+

Whole cell standardized microbial community

3. DNA extraction

4. Library preparation

5. Sequencing

6. Bioinformatic analysis

Samples
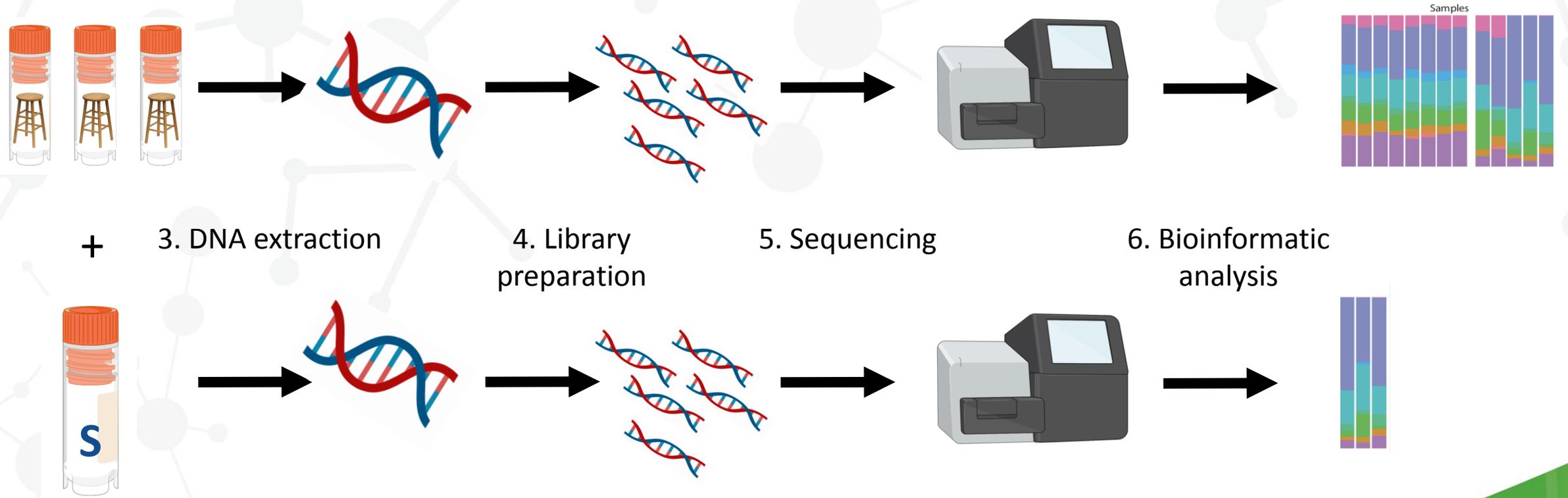
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Microbiome standards
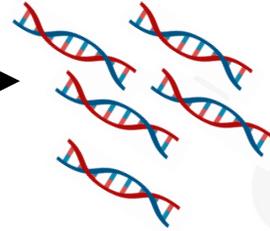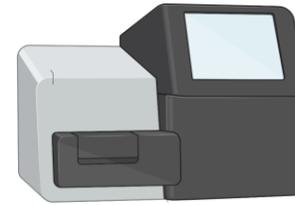
+ 3. DNA extraction   4. Library preparation   5. Sequencing   6. Bioinformatic analysis
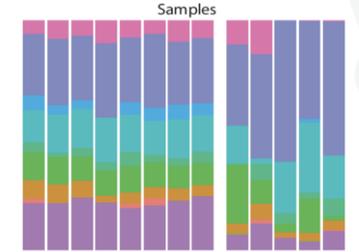
Genomic DNA standard
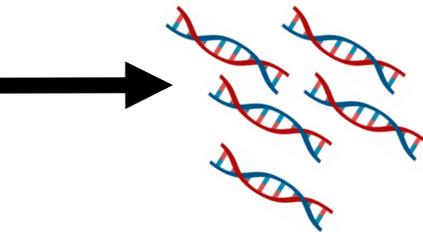
The importance of negative controls: The microbiome of contamination

DNA extraction method A
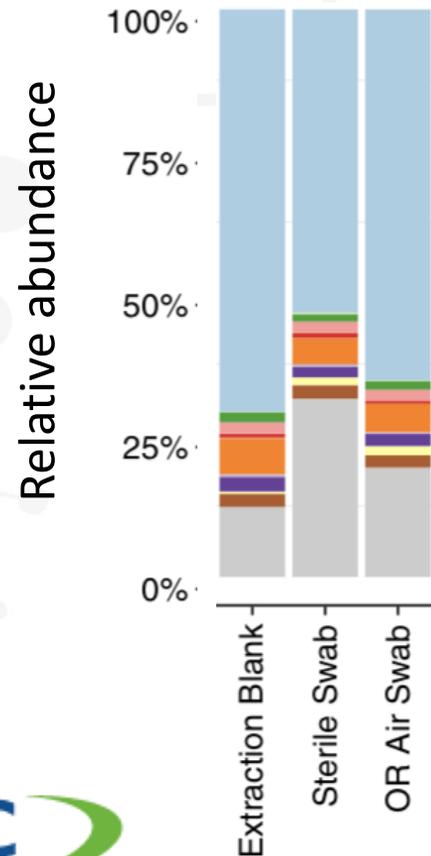
DNA extraction method B

Source

Kim D et al., Microbiome, 2017
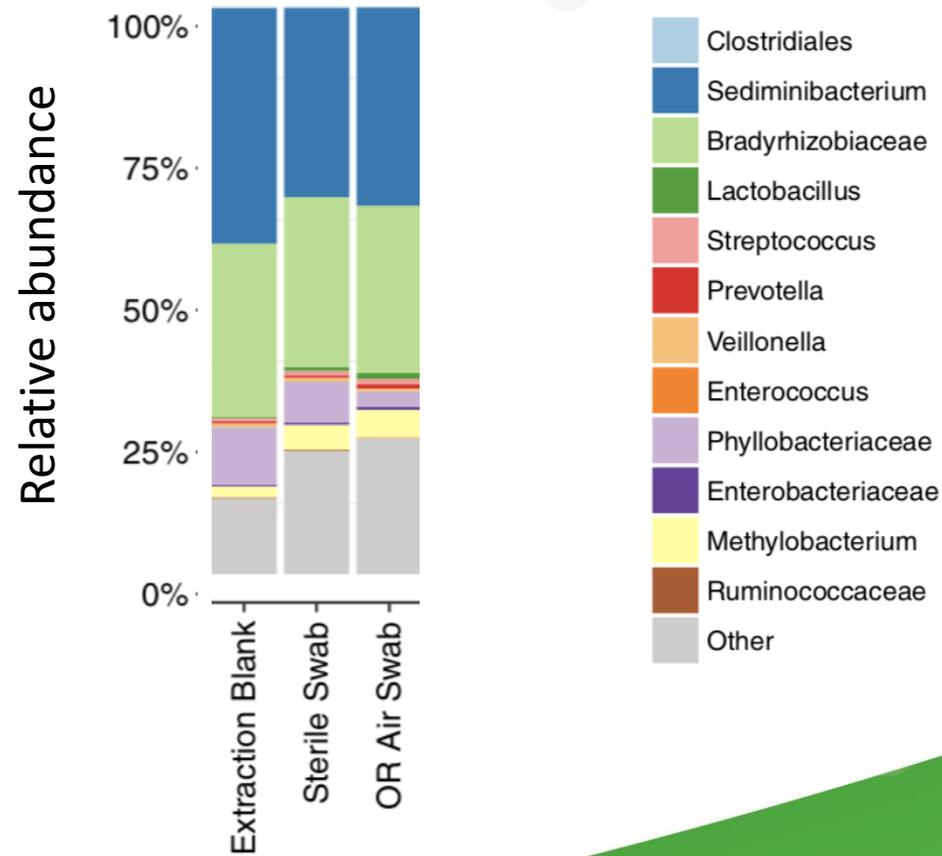
What about standardized protocols in patients with cancer?

- Hospital and clinic based collections

- More variability in microbiome taxonomic composition between patients

- More variability over time within patients

- Low DNA biomass in patients with diarrhea

BMT-CTN 1801: Mi Immune Study

- Microbiome and Immune Reconstitution in Cellular Therapies and Hematopoietic Stem Cell Transplantation (Mi-Immune)

  - **Leslie S. Kean** MD, PhD, **Miguel-Angel Perales**, MD, and **Ami Bhatt** MD, PhD

- Companion study to <u>Progress III</u> (BMT-CTN 1703)
  - Multi-center phase III RCT
  - Post-transplant cyclophosphamide in reduced-intensity conditioning (RIC) allogeneic hematopoietic stem cell transplant (HCT) recipients

Mi Immune Schema
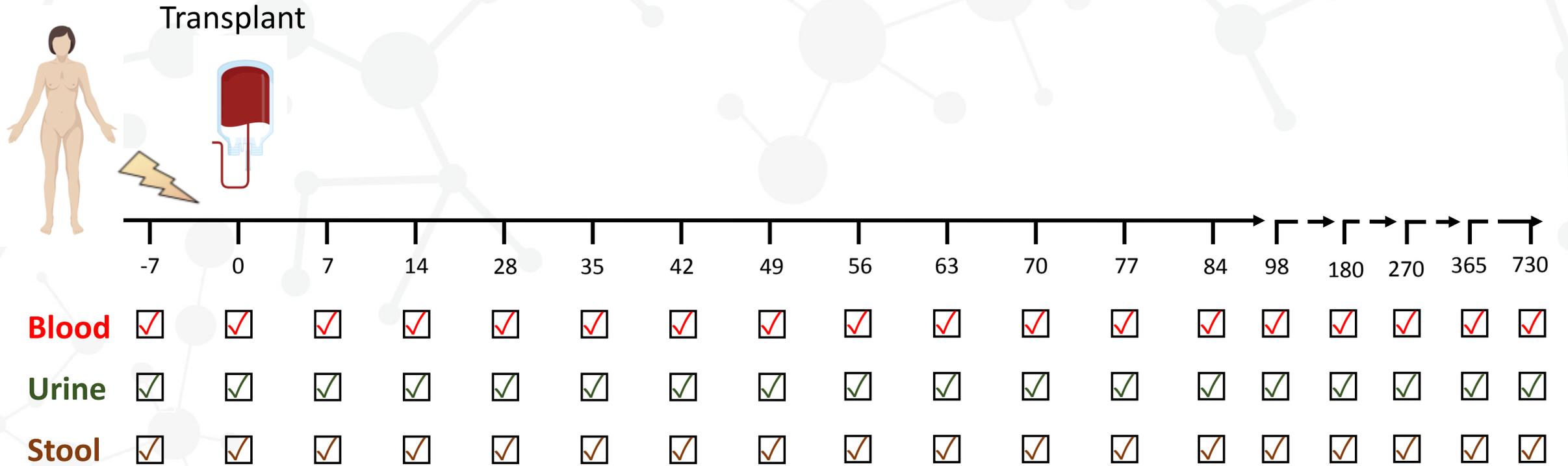
Days relative to transplant

Goals of Mi Immune Biobanking

- Standardized protocols in patients undergoing HCT

- Multi-center collection of banked blood, urine, and stool

- Resource linking clinical metadata to biobanked samples

Summary

- Reproducibility is a major challenge
  - Standardization
  - Controls
  - Measurement of confounders

- Standards exist for microbiome research in the general population

- BMT-CTN 1801 standardization and biobanking in HCT

Thank you!

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