Conducting Microbiome study, a How to guide

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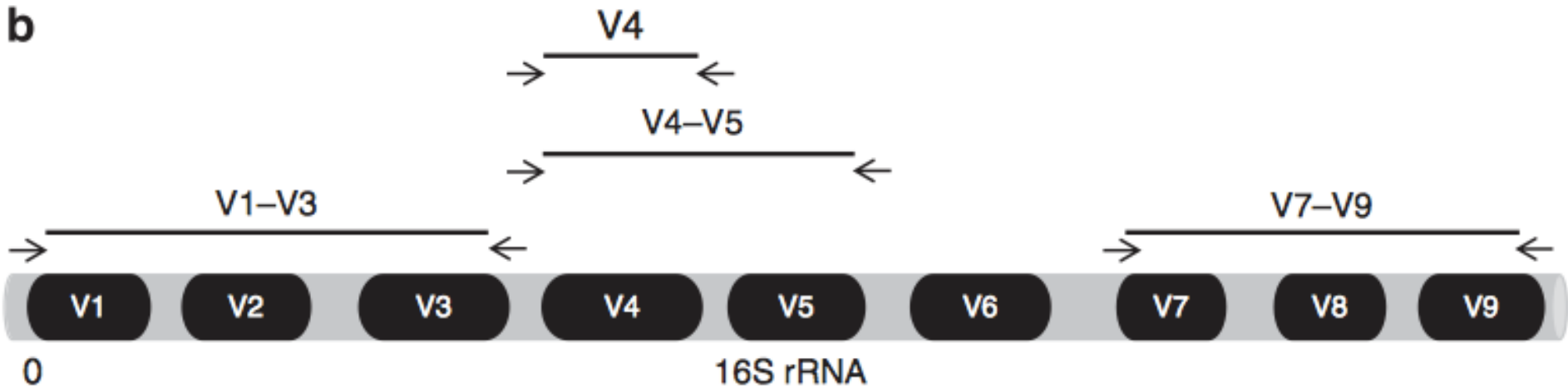
Why study Microbiome?

- ✓ Essential component, e.g. Gut microbes
- ✓ Disbiosis (microbial imbalance) & disease
  - ➢ *Obesity, diabetes, inflammatory bowl disease*
- ✓ Limitation of traditional culture methods.

16S rRNA gene

- Metagenomic Study methods based on:
  - ***16S rRNA gene*** *or Whole Genome Sequencing*
- 16S rRNA gene is widely accepted, because:
  - ✓ *9 hypervariable regions (Identification, phylogenetic)*
    + highly conserved regions (universal primers binding);
  - ✓ *Well-developed processing procedure;*
  - ✓ *Database is mature: e.g. Ribosomal Database Project (RDP)…*

16S rRNA



Black: hypervariable region

Grey: conserved region

Tyler A D, The American journal of gastroenterology, 2014, 109(7): 983-993.

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Ongoing Microbiome Project

NIH HUMAN MICROBIOME PROJECT

Workflow of Microbiome Study

1. Study Design

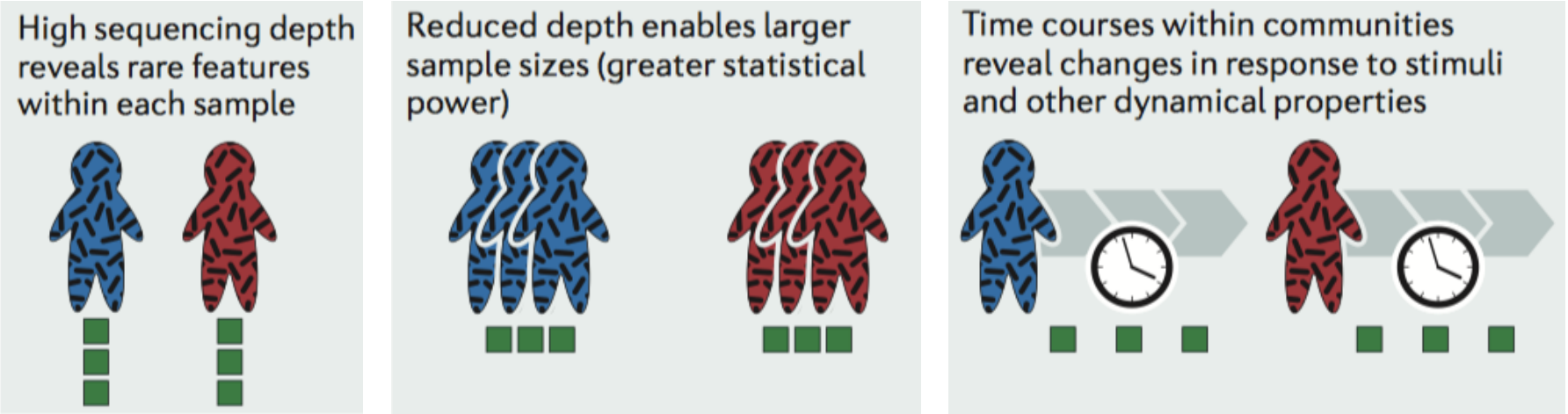
2. Sample Preparation

3. Library Preparation & Sequencing

4. Sequence Analysis

5. Statistical Analysis

Study Design



- Exclusion Criteria?
- Control group?
- Sampling methods?

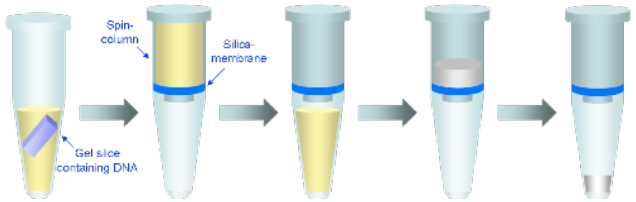
Franzosa E A, Nature Reviews Microbiology, 2015.

2. Sample preparation

1) Storing: immediate freezing, storage at -80℃.

   ➢ *Minimize freeze-thaw cycles.*

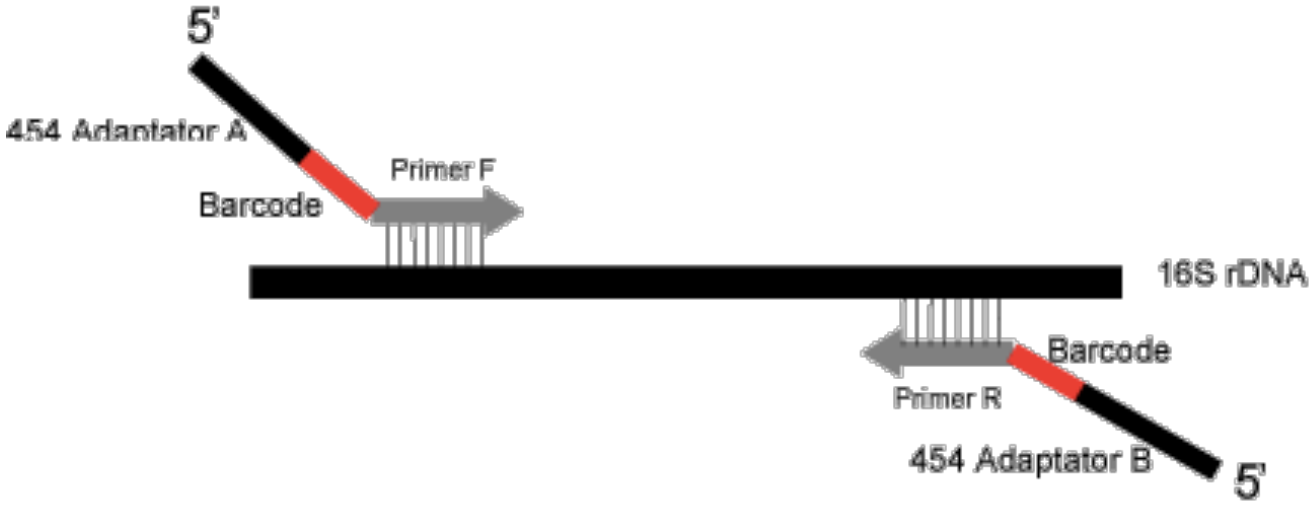
2) DNA extraction: commercially available kits specific to the sample type. (Caution: Run a blank extraction control!)



http://biotechcrunch.blogspot.hk/2011/11/9-factors-affecting-dna-extraction-from.html

3. Library preparation (PCR)

16S Primers & **Barcode & adaptor**



Fabrice A, J Comput Sci Syst Biol, 2009, 2(1): 074-92.

3. Sequencing

- **Platform:** Illumina MiSeq & Ion torrent

Platform	Cost per run	Read Length	Run time	Throughput
Ion torrent (318 chip)	$625	100 bases	3h	1000Mb
MiSeq	$750	150*2 bases	27h	1500Mb

Ion torrent

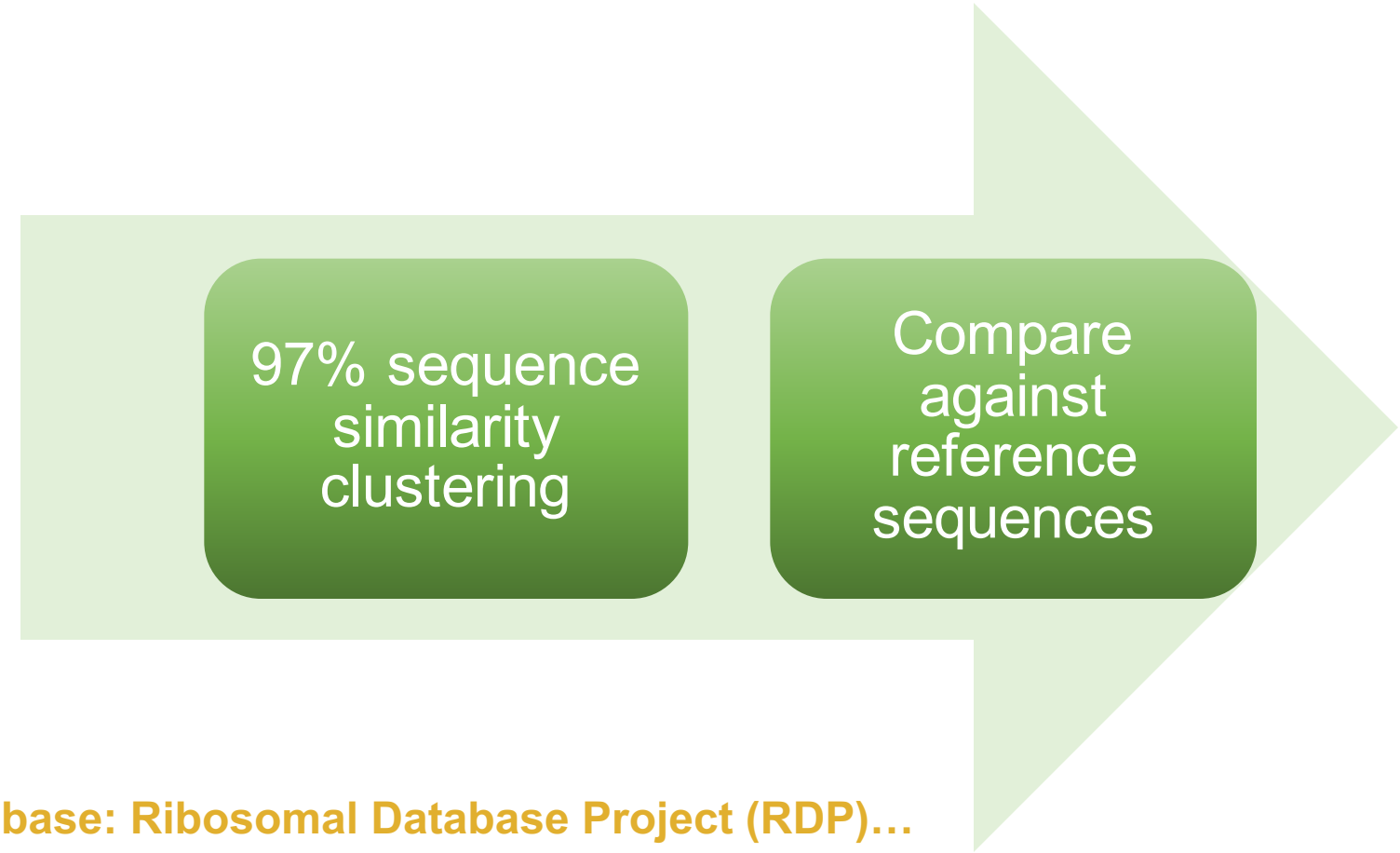
MiSeq

Loman N. Nature biotechnology, 2012, 30(5): 434-439.

4. Sequence Analysis

- **Software packages:** QIIME, mothur
- **Steps:**
    1. Sequence Quality Control
        - ✓ *Trimming adaptors*
        - ✓ *Filtering low quality sequence*
        - ✓ *Filtering Chimeras (5%~45%)*
    2. Taxonomic identification
        - ➢ Operational Taxonomic Units (OTUs) Analysis

Operational Taxonomic Units (OTUs) Analysis



*** Database: Ribosomal Database Project (RDP)…**

5. Statistical Analysis: Measure of Diversity

Relative abundance:

- Quantitative measure
- Frequency of a specific taxon

Alpha diversity:

- Measure of diversity within a sample
- Calculated by algorithms

Beta diversity:

- Comparison of diversity between samples
- Displayed as Principle Coordinate Analysis (**PCoA**) plot

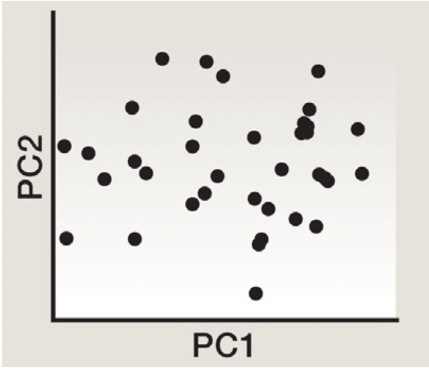
Principle Coordinate Analysis (PCoA) plot

- Microbiome data:

  Multi-dimension, each species = one dimension

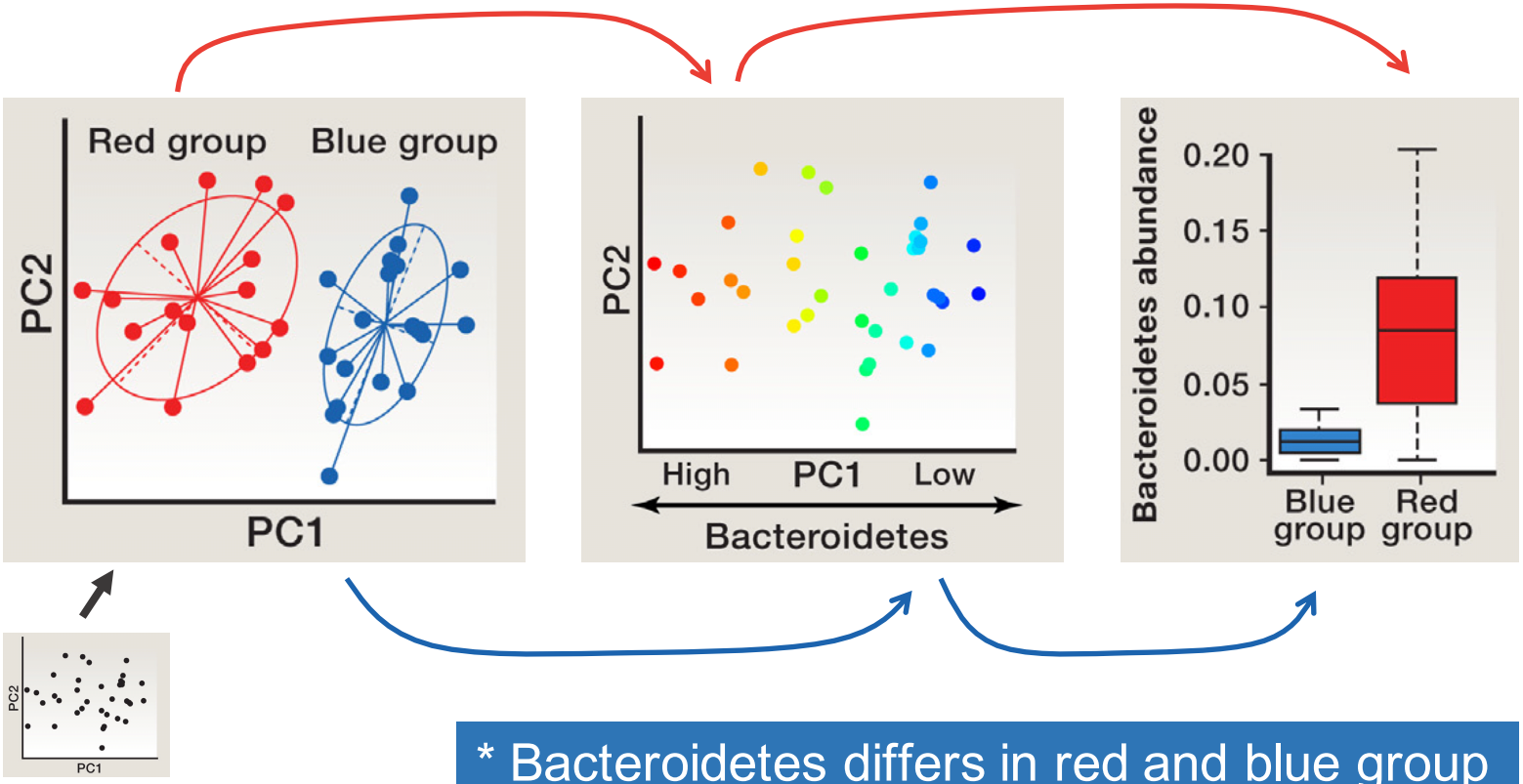
- Mathematically reduce them to two/three dimensions:

  Principle Coordinates (PC)



Data visualization: PCoA plot
*each dot= a sample

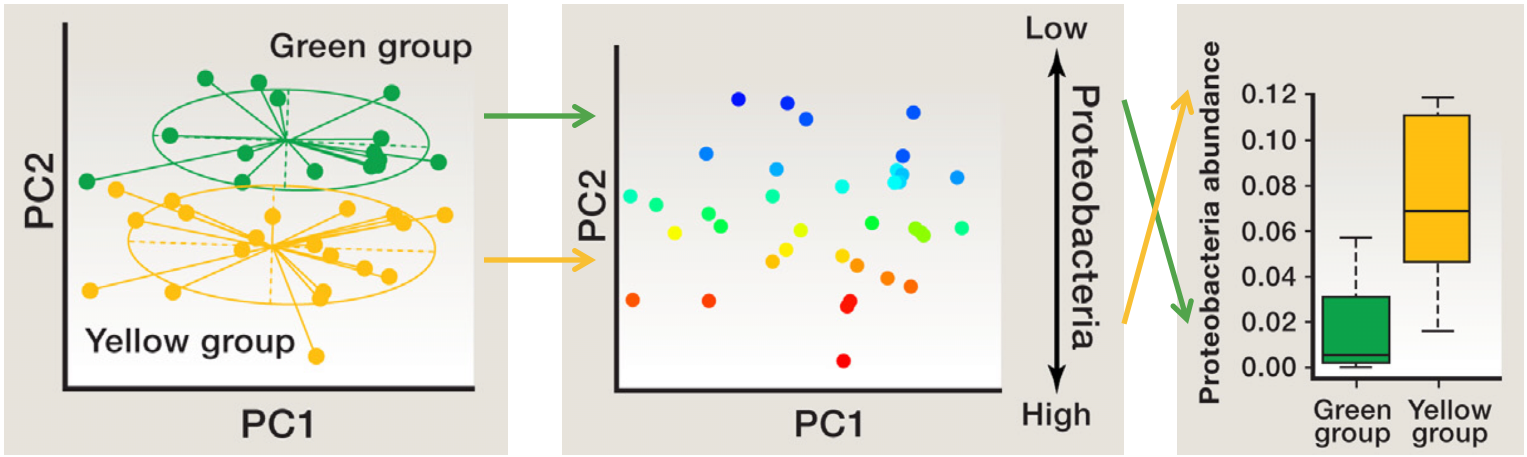
Principle Coordinate Analysis (PCoA) plot

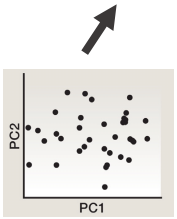


* Bacteroidetes differs in red and blue group

Goodrich J K, Cell, 2014, 158(2): 250-262.

Principle Coordinate Analysis (PCoA) plot





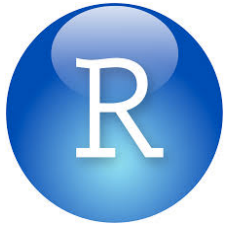
* Proteobacteria differs in green and yellow group

Goodrich J K, Cell, 2014, 158(2): 250-262.

5. Statistical analysis: Regression Analysis

- **Response variables**: alpha diversity, PCs, the abundances of OTUs
- **Statistical models**: Multiple Logistic Regression…
- **Control cofounding factors:**

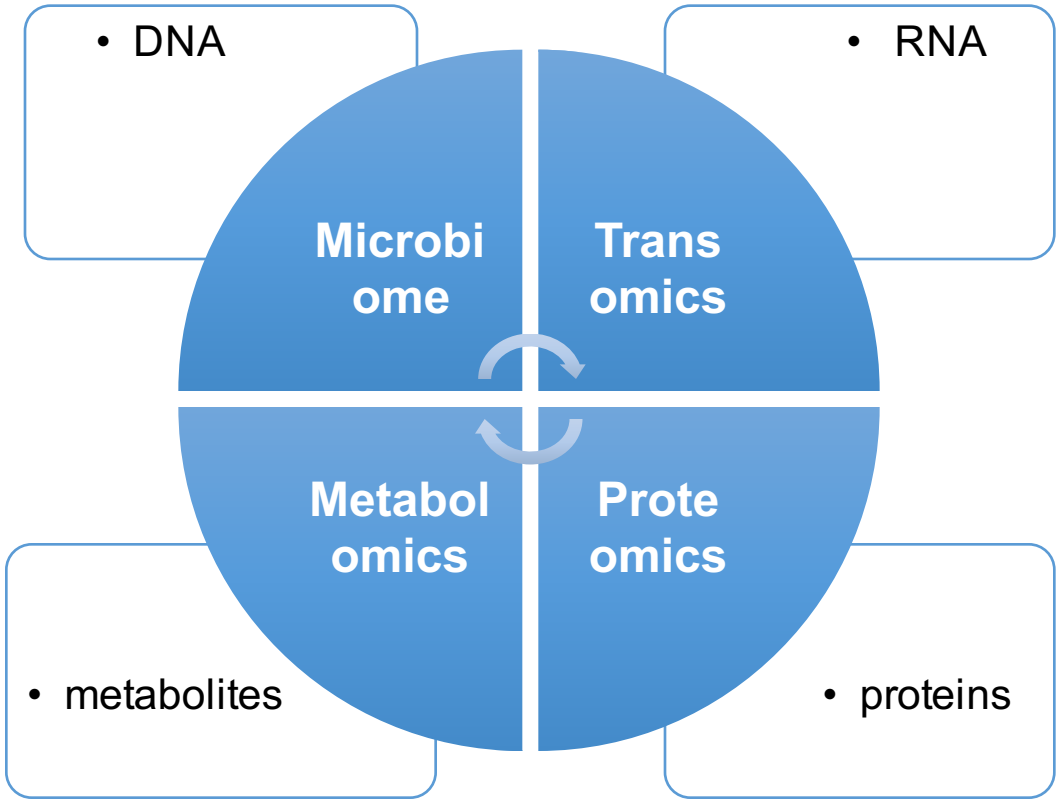
  maternal effects, gender, age, sex, technical sources of variation, season…...



Future

- **Limitations of 16s rRNA gene based methods:**
  - Species level
  - Can’t observe dynamic changes of different conditions
- **Future:**
  - **Multi-omic data** to measure functional activity under given condition

Multi-Omic data



Reference

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Thank you!
Q&A