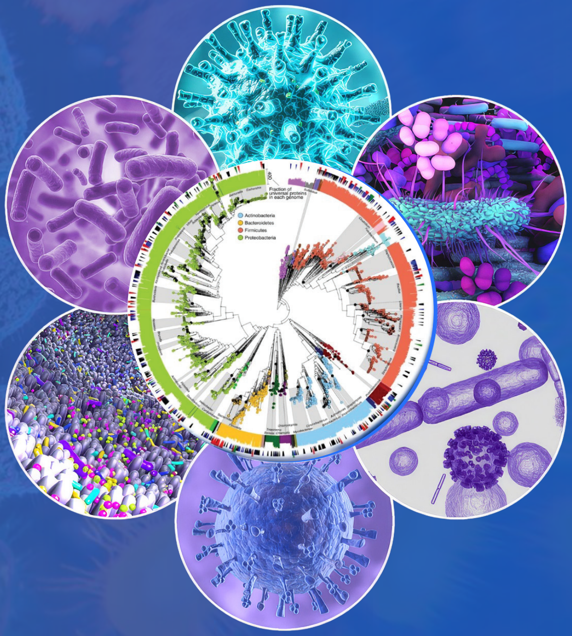
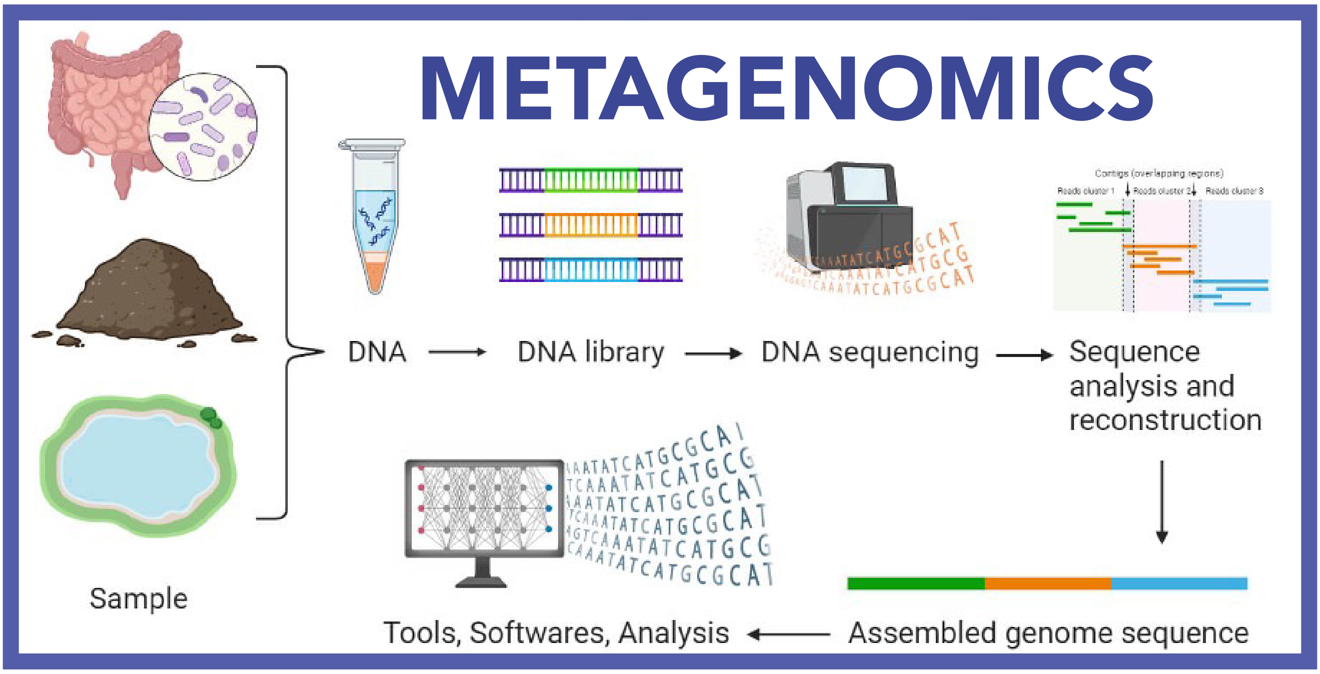
Genetika Science

SHOTGUN METAGENOMIC SEQUENCING



Shotgun Metagenomic Sequencing is the untargeted ("shotgun") sequencing of all ("meta-") genomes of microbial present in a sample. By sequencing the entire DNA content of the samples, this method has emerged as a powerful tool overcoming the constraint of the traditional microbiology analysis techniques. **This makes it possible to study complex microbial ecosystem as closely as their real environment without the need or bias of microbial cultivation.**



METAGENOMICS

Sample

DNA → DNA library → DNA sequencing → Sequence analysis and reconstruction

↓

Assembled genome sequence → Tools, Softwares, Analysis

How Does Shotgun Metagenomic Sequencing Compare to 16S Amplicon Sequencing?

Unlike 16S amplicon sequencing, shotgun metagenomics can be used to **identify not only bacteria but also different domain as well making it suitable for comprehensive analysis of a complex sample.** Moreover, the usage of the whole genetic material within the sample leads to higher taxonomy resolution up to strain level when compared to the 16S amplicon sequencing methods, which mostly only up to species level.

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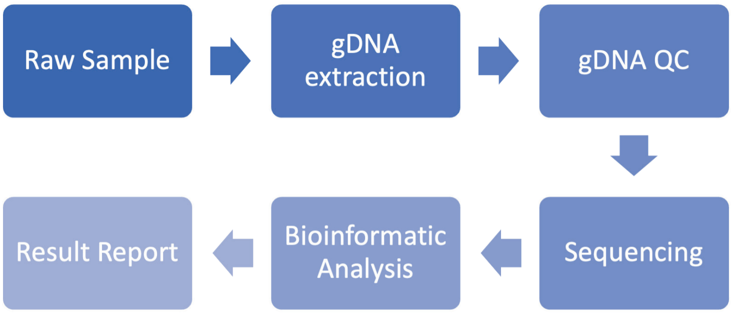
	16S Amplicon Sequencing	Shotgun Sequencing
Bacterial Coverage	High	Limited
Cross-Domain Coverage	No	Yes
Taxonomy Resolution	Genus-Species	Species-Strain
Host DNA Interference	No	Yes
Functional Profiling	No	Yes

Functional profiling analysis which characterizes how a microbial community function within an environment is easily done using shotgun metagenomic sequencing. However, most of the bacterial database caters more toward 16S amplicon data which is easing up the bacterial identification analysis. As well as, the possibility of host DNA contamination is much lower using 16S amplicon sequencing. Either method have its own strengths and weaknesses, no method is suitable for every project.

SAMPLE REQUIREMENT


Raw Sample → gDNA extraction → gDNA QC → Sequencing → Bioinformatic Analysis → Result Report

Platform:

- Illumina NextSeq 2000
- Oxford Nanopore PromethION

Raw sample or Genomic DNA (gDNA)

gDNA Sample Requirements:

- Good DNA purity - A280/260 = 1.8-2.0
- High molecular DNA (>10 kb) must be present.
- DNA concentration (using Nanodrop) ≥ 40 ng/µL in at least 50 µL.

KEY DELIVERABLES:

- **Raw sequencing data in fastq format.**
- **Sequencing data QC**
- **Bioinformatic analysis, includes:**
    - Metagenome assembly
    - Assembly statistic and QC
    - Taxonomy assignment and taxonomical abundance.
    - Functional annotation and abundance based on various databases.
    - Diversity analysis: Alpha diversity indices and ordination analysis (PCA, PCoA, NMDS).
    - Visualization: Bar charts, Bandage, Krona, Venn diagram, Heatmap
    - Assembled genome of top 5 genus in fasta format.

NOTES FOR RAW SAMPLES:

- **Keep the samples in preservation buffer** (Zymo Research DNA/RNA Shield (Cat# R1100 for solid samples), (Cat# R1200 for liquid samples) or **freeze the samples in -20°C or extract the DNA immediately after sampling** when the microbial composition data is time-sensitive (e.g fermentation samples, gut microbiome, fecal samples, and such).
- **When applicable, consider concentrating any sample with low or sparse microbial abundance** (especially water samples) using either filtration or centrifugation to improve microbe detection.

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