Strategies and Tools in illumina & Nanopore-integrated metagenomic analysis of microbiome data

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Background: Metagenomic Microbiome Data Analysis

illumina®

Advantages

- **Low price commercial sequencing service** → high community coverage
- **Low requirement on the input DNA,1ng**.
- **High per base accuracy**
  - **High accuracy of assembled contigs**
  - **Various mature bioinformatic frameworks**

Limitations

- **High instrumental cost** → longer turn-around time
- **Unavoidable biases** against high-GC
- SR limitations for analysis
  - **Annotation difficulties**
  - **Fragmented assembly**

Oxford NANOPORE Technologies

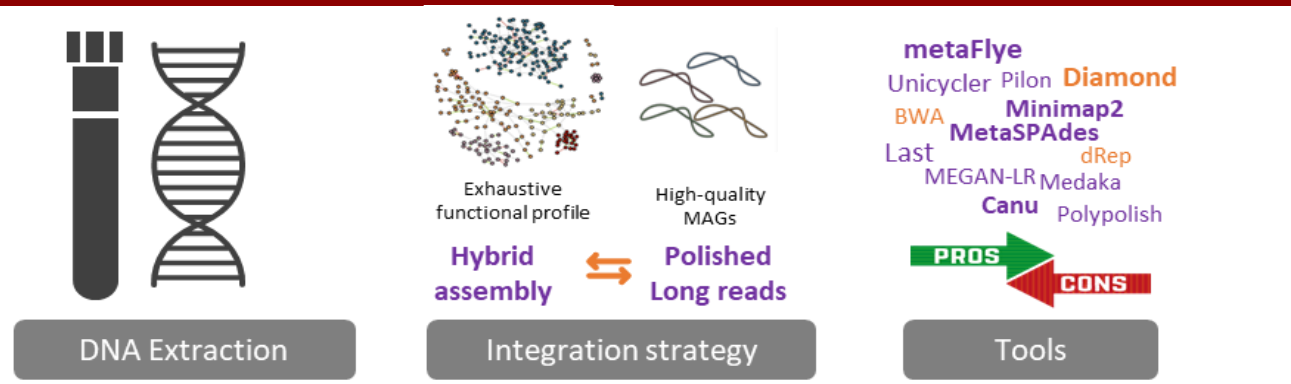
Advantages

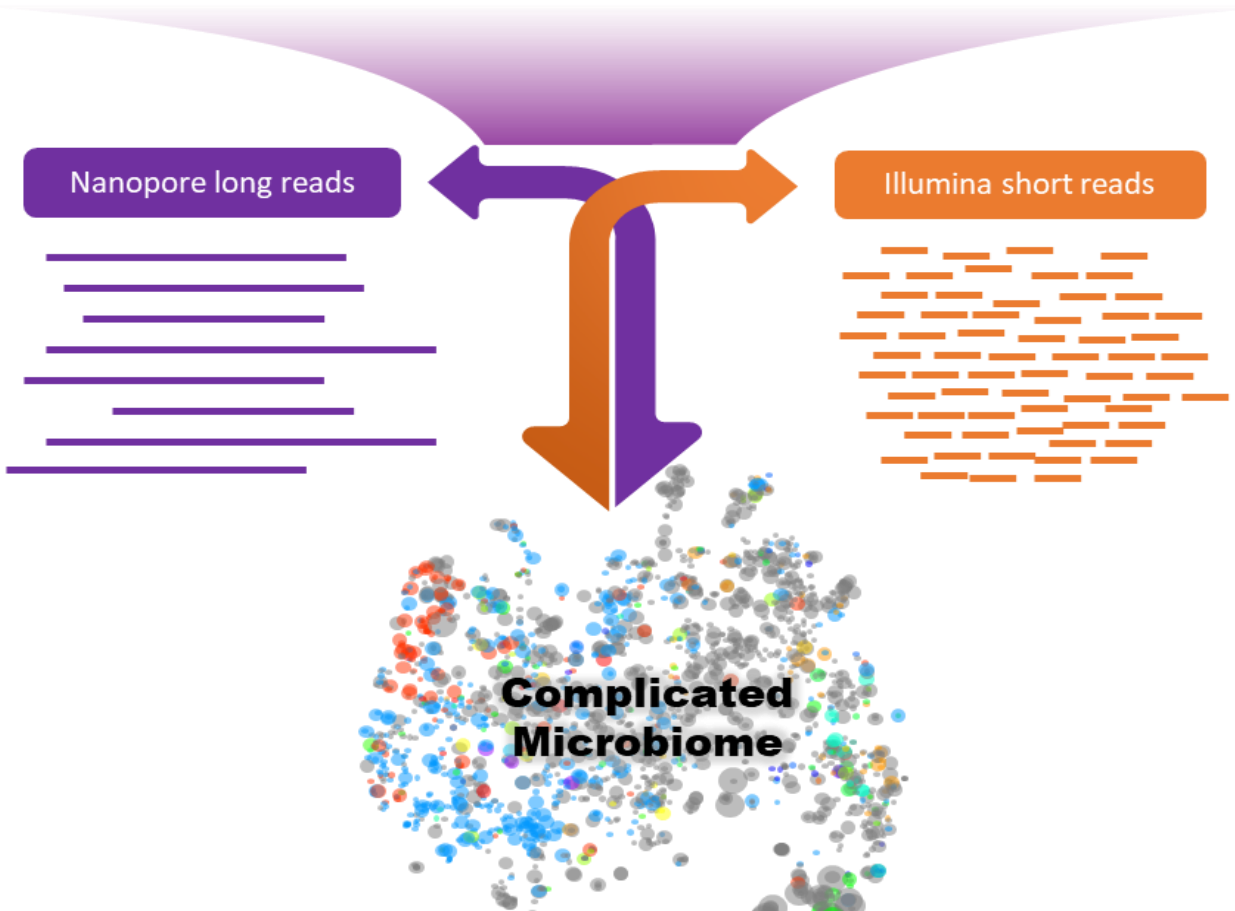
- **Low instrumental cost** → short turn-around time & Higher feasibility
- **New protocols: ReadUntil sequencing**
- **No systematic bias**
- **LRs benefits for analysis**
  - **much longer assembly**
  - **Differentiate closely-related lineage**

Limitations

- **High price for commercial sequencing service** → limited community coverage
- **Strict requirement on DNA purity and quantity**
- **High error rate LRs**
  - **Unmature bioinformatic tools**
  - **Persistence of indel errors on assembled contigs**

Integrating Illumina SRs and Nanopore LRs





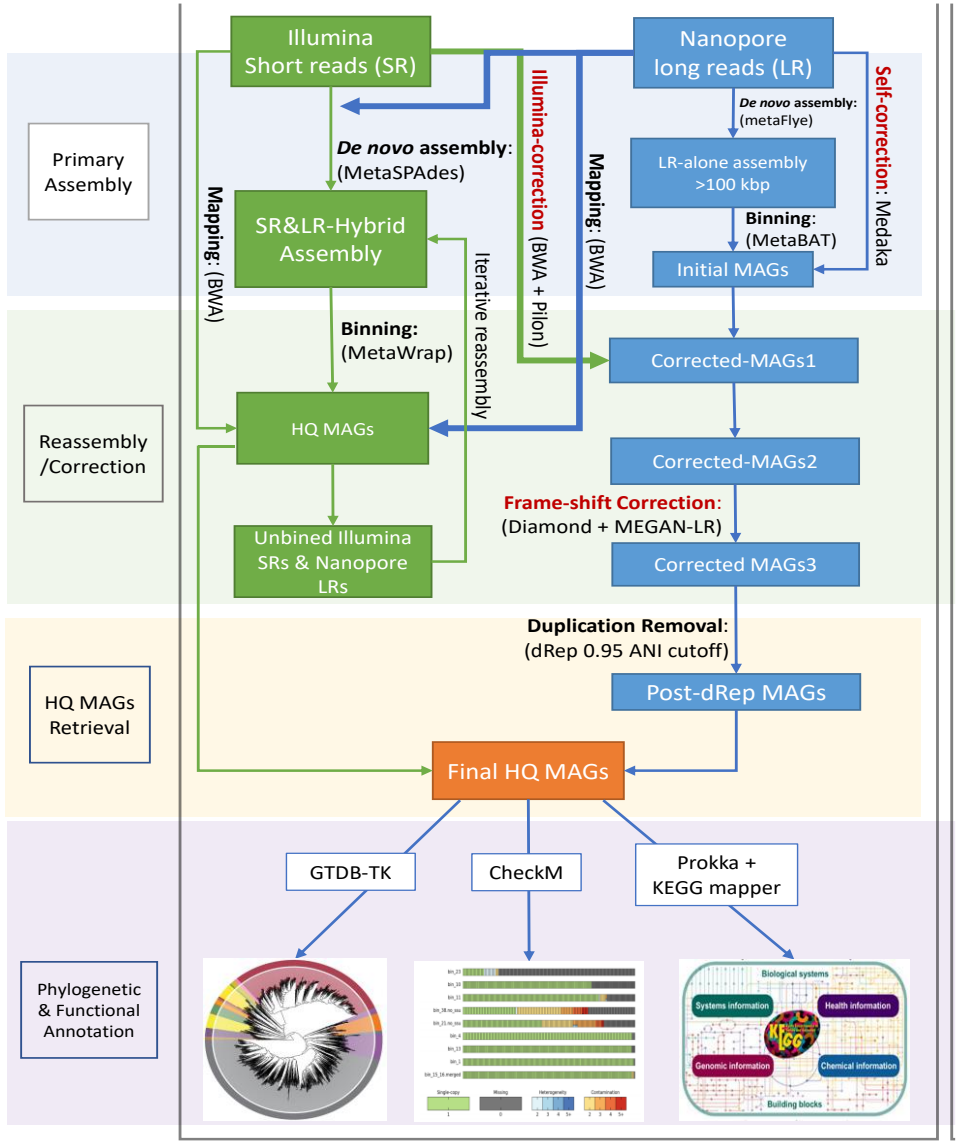
What would you get from this review:

systematic and timely knowledge framework for integrating SRs and LRs in metagenomic microbiome data analysis

- ☐ **The workflow of common analytic strategies for SRs and LRs integration**
- ☐ **Algorithm foundations and application practice of common tools.**
- ☐ **Sample preparation protocols commonly used**

Strategies and Tools

“Genome-centric” strategy: to get high-quality MAGs



☐ Hybrid assembly： **High Computational Cost**

hybrid-assembly of illumine short-reads and Nanopore long-reads

Tool used: MetaSPAdes and Unicycler

Insufficient Community Coverage

☐ Nanopore assembly：

Assembly of nanopore long-reads

Tool used: Miniasm，Canu and Metafyle

☐ Polish1：Self-correction of long-reads

Tool used: Medaka and Racon

☐ Polish2: Illumina-based Indels polish

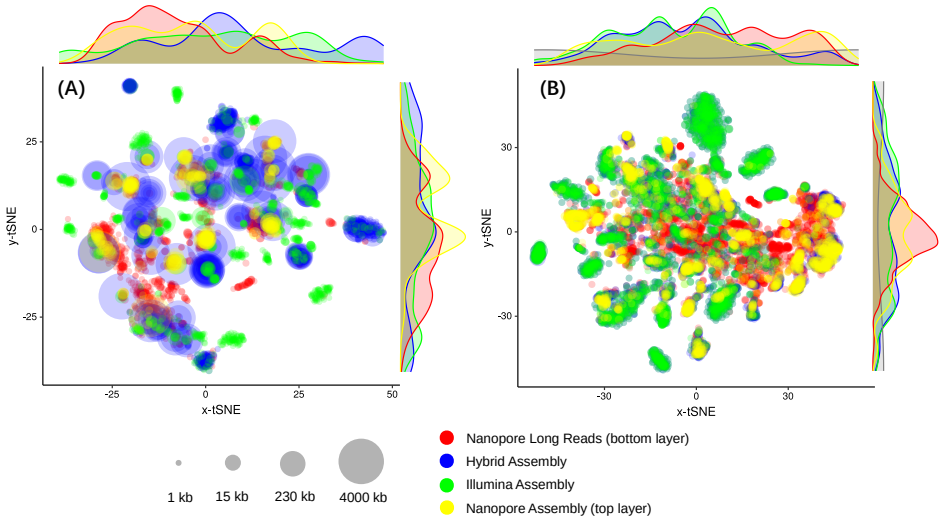
Tool used: Pilon and Polypolish

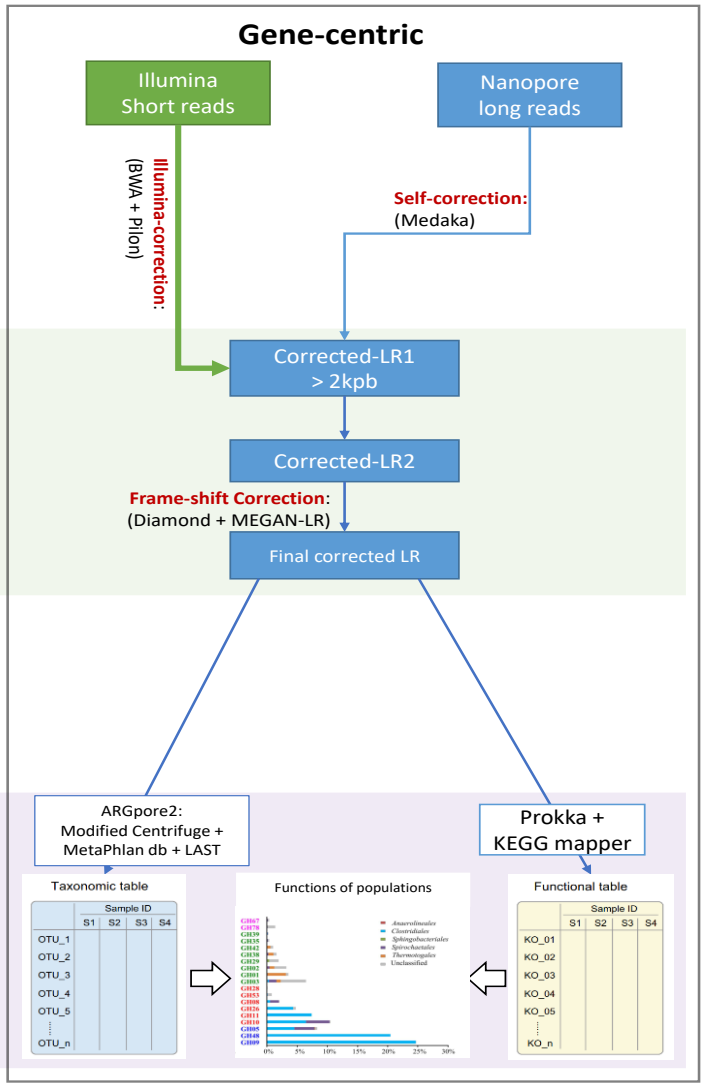
☐ Polish3: frame-shift correction

Tool used: Diamon+MEGAN-LR

Strategies and Tools

Assembly-free “Gene-centric” strategy





- **Core idea:** functional diversity of communities directly Identify and quantify based on corrected LRs rather than assembled MAGs
- **Importance:**
  - LRs are not easy to be assembled due to low coverage LRs is as long thus informative as assembled contigs

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