



16S-rRNA Amplicon Sequencing

Introduction

Microbes play a fundamental role in the functioning of an ecosystem that encompasses large taxonomic and microbial diversity [1, 2]. The recent high-throughput sequencing technologies facilitate the study and exploration of the microbial world at unrivalled levels [3]. Various microbes present in the ecosystems perform important functions critical for the maintenance of the milieu, but identification and elucidation of their functional roles have remained cryptic primarily due to the inability to isolate and culture them in the laboratory. Metagenomic analysis enables the study of these complex microbial communities by direct sampling of DNA of all the species present obviating the need to isolate it individually. It can help in establishing taxonomic identification and to understand the novel metabolic pathways operating in them that enable them to thrive in that particular niche environment.

The 16S rRNA gene sequences in microbes have a slow rate of evolution and are widely used for the phylogenetic studies and taxonomic identification of the organisms. Amplicon Metagenomics studies involve the sequencing of 16S rRNA gene using specific targeted primers targeted to the hyper-variable regions (HV) that can accommodate variability demonstrating microbial diversity [4]. The studies can be based on short-read sequencing of fragments containing one or more hyper-variable regions, usually the V3 or V4 or V3-V4 regions

The advent of long-read (PacBio-SMRT and Oxford Nanopore Technology) sequencing technologies has enabled us to sequence the complete 16S/18S/ITS rRNA genes. Details of short-read and long-read based 16S rRNA gene metagenomics studies are compared in Figure 1. The distribution of organisms from phylum to species level is estimated through measurement of operational taxonomic unit (OTU) as a scale. The richness and evenness of the species distributed across samples can be studied through calculating several statistical population index methods [5].

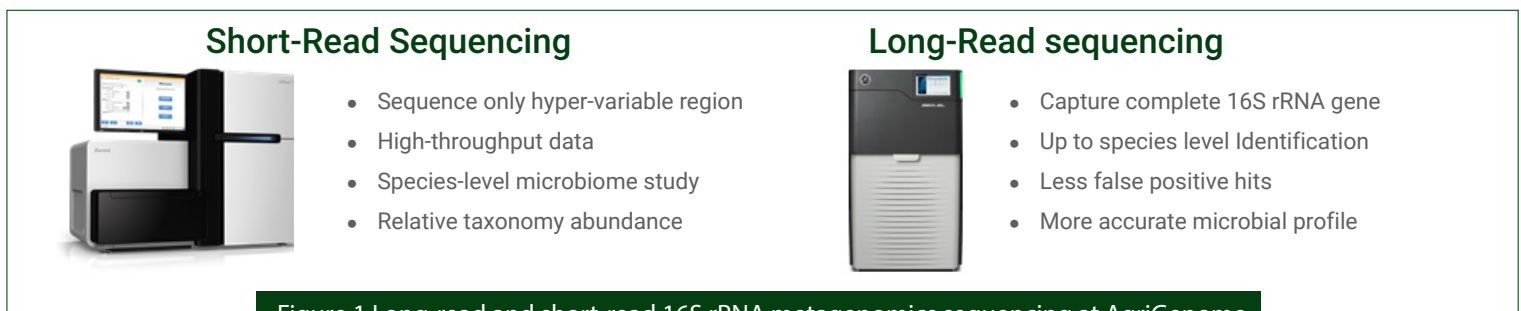


Figure 1 Long-read and short-read 16S rRNA metagenomics sequencing at AgriGenome

Applications

- Identification of agriculturally and industrially important species
- Distribution of microbial community from different environments
- 16S rRNA based meta-barcoding of species
- Core microbiome of host-pathogen interactions
- Antibiotic resistance genes and metabolome studies
- Phylogenetic profile among various environmental conditions

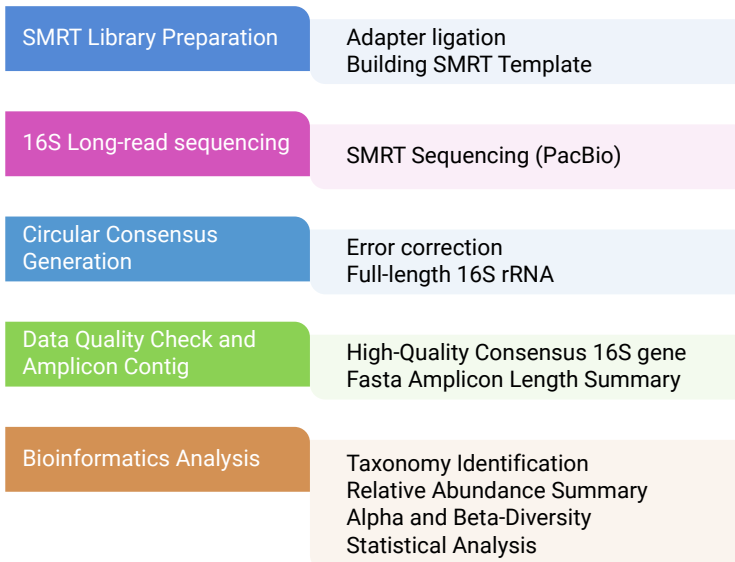


Figure 3. Long-read based 16S-rRNA analysis pipeline at AgriGenome

16S-rRNA gene sequencing at AgriGenome

AgriGenome performs the most accurate and promising protocols and techniques in sequencing to offer customers state-of-the-art methodology and analysis. The advantage of AgriGenome methodology is that specific metagenomic **marker genes (V3, V4, V3-V4 and V5)** can be sequenced with significant depth and this offers excellent value for money. AgriGenome uses the following steps based on the short-read (Figure 2) and long-read (Figure 3) data analysis.



Figure 2. Short-read based 16S-rRNA analysis pipeline at AgriGenome

Deliverables

AgriGenome provides customized bioinformatics analysis as per sample and project requirement:

- Fastq data quality report
- Consensus Fasta of 16S rRNA hyper-variable amplicon from paired end data
- Complete 16S rRNA genes using long-read sequencing and analysis
- Phylogenetic analysis
- Relative taxonomy abundance plot and tables
- Core microbiome studies based on rare and high abundance taxonomy
- Heatmap and Krona plot
- Alpha-diversity with samples and rarefaction curves
- Beta-diversity between samples and beta diversity plots
- Statistical analysis
- Functional prediction analysis

References

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Recent publications from projects executed at AgriGenome

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Hadapad, A.B., Shettigar, S.K.G. & Hire, R.S. Bacterial communities in the gut of wild and mass-reared *Zeugodacus cucurbitae* and *Bactrocera dorsalis* revealed by metagenomic sequencing. *BMC Microbiol* 19, 282 (2019).

<https://doi.org/10.1186/s12866-019-1647-8>

Kalappa DM, Subramani PA, Basavanna SK, Ghosh SK, Sundaramurthy V, Urageyala S, Tiwari S, Anvikar AR, Valecha N. Influence of midgut microbiota in *Anopheles stephensi* on *Plasmodium berghei* infections. *Malar J*. 2018 Oct 25;17(1):385.

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