Novogene

# Full-length 16S Amplicon Metagenomic Sequencing

Full-length 16S Amplicon Metagenomic Sequencing is frequently used to identify and differentiate microbial species. Pacbio Sequel Systems are powered by Single Molecule Real-Time (SMRT) sequencing technology and deliver highly accurate long reads. Using the PacBio Sequel, full-length 16S amplicon sequencing resolves the limitations of short read lengths (e.g. shattered gene distribution and minor hypervariable region coverage) in next generation sequencing (NGS), and improves the resolution of the strain.

## Advantages and Highlights

- **Long Read Lengths**

  No fragmentation enables easy reading of full-length 16S rDNA, with PacBio SMRT sequencing technology to avoid GC bias compared to short read sequencing

- **High Resolution**

  Guaranteed >99.9% single-molecule sequencing accuracy enables more accurate species classification and more low-abundant species discovery

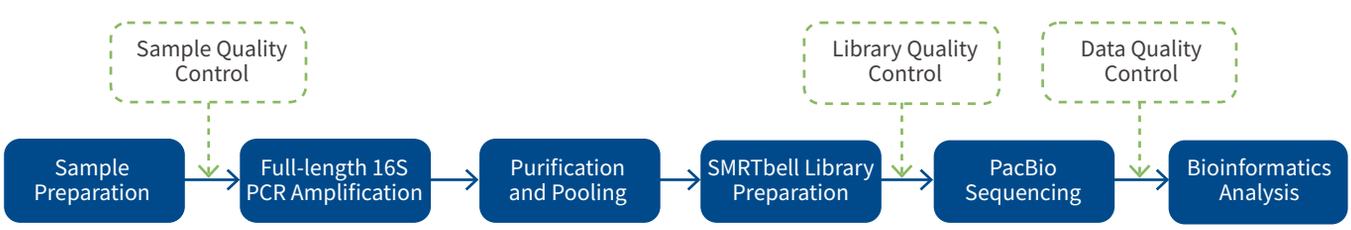
- **Clean Reads Delivery In WBI Option***

  Our advanced filters help you get rid of primers and chimeras in raw reads. 5,000 or 10,000 clean CCS reads per sample enables more efficient data analysis (*Clean reads are delivered only for WBI projects )

- **Updated Analysis Software**

  Amplicon Sequence Variants (ASV) generated from QIIME 2 software infer the biological sequences in the sample prior to the introduction of amplification, can distinguish as little as one nucleotide

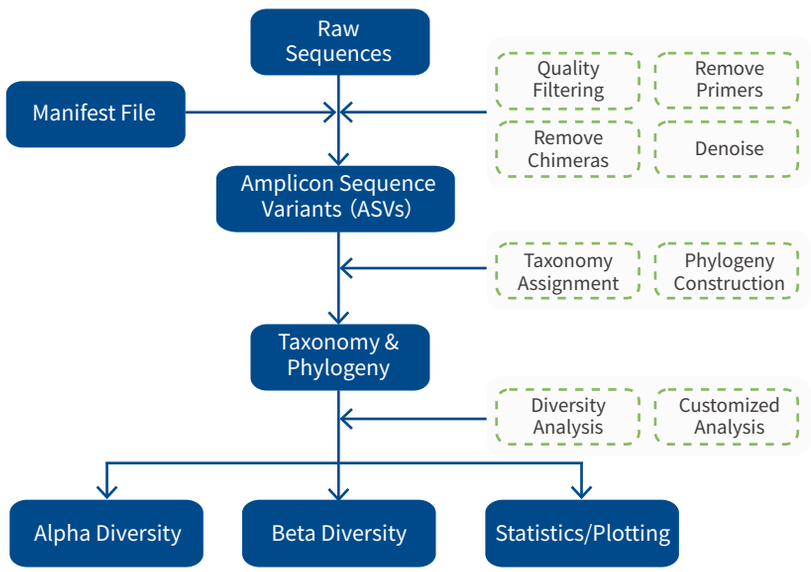
## Project Workflow




## Sample Requirements

| SAMPLE TYPE | AMOUNT | CONCENTRATION | VOLUME | PURITY |
|---|---|---|---|---|
| Genomic DNA | ⩾ 150 ng | ⩾10 ng/μL | ⩾ 15 μl | OD260/280=1.8-2.0, no degradation, no RNA or protein contamination |

## Standard Analysis Pipeline




## Novogene Client Publication

### Species-level bacterial community profiling of the healthy sinonasal microbiome using Pacific Biosciences sequencing of full- length 16S rRNA genes (Earl *et al,* 2018)

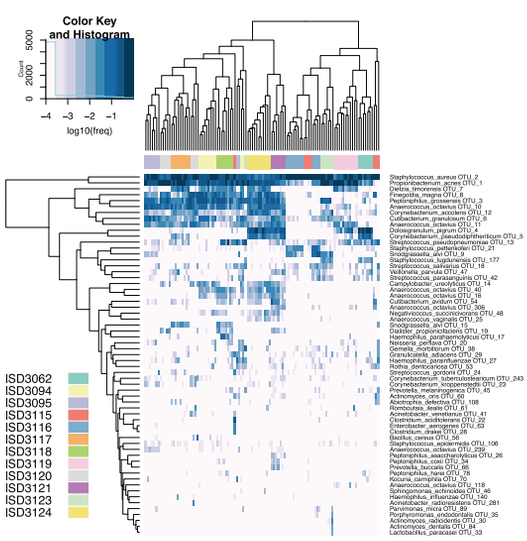



Fig.1 Heatmap of human sinonasal microbiome from 12 subjects. Columns are subjects; rows are species. OTU counts were summed by species-level centroid classification, samples with < 500 reads were excluded, and species with < 0.2% relative abundance in all samples were dropped.

#### Research Methods

- DNA isolated from sinonasal samples of patients
- 2Kb SMRTbell libray preparation
- PacBio Sequel platform sequencing
- Circular Consensus Sequences (CCS) polishing
- ASV/OTU clustering
- Taxonomic classification
- Abundance heatmap
- Ecological analysis

#### Conclusions

The microbial composition analysis pipeline for single-molecule real-time 16S rRNA gene sequencing, by using clustered OTU sequences generated from CCS reads, increased taxonomic classification resolution, made abundance of species-level analysis easier to achieve (shown in Fig.1), as well as strengthened the specificity of associations between microbial unities and ecology.

Reference: Earl J.P, Adappa N.D, Krol J *et al.* Species-level bacterial community profiling of the healthy sinonasal microbiome using Pacific Biosciences sequencing of full-length 16S rRNA genes. *Microbiome*, 2018, 6:190.



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