

## Metagenome Human Gut V3-V4

TC-S-MG-HG-16S-V34

## Applications

Method utilizes targeted amplification and next-generation sequencing of the 16S rRNA gene V3-V4 hypervariable regions to profile bacterial communities in human gut samples. This applied genomics approach enables accurate taxonomic characterization and comparative analysis of gut microbiome composition across biological conditions. The V3-V4 metagenome is a cost-effective and scalable method, where the primary objective is to measure changes in microbial diversity and analyze beta-diversity. These methods are ideal for characterized metagenomes with well-documented reference datasets, where the primary objective is understanding treatment differences within a cohort. The method is limited in resolving species- and genus-level taxonomy.

## Pros

- High-resolution profiling of gut bacteria
- Cost-effective microbiome analysis
- Suitable for large cohort studies
- Robust taxonomic classification
- Ideal for comparative gut microbiome studies

## Notes

- Limited to bacterial community profiling
- Species-level resolution may vary on match from V3-V4
- Functional insights are predictive
- Results influenced by sample handling

## Input



DNA ≥ 10 ng recommended



DNA Qubit &gt; 10ng

## Sample Types



Stool samples

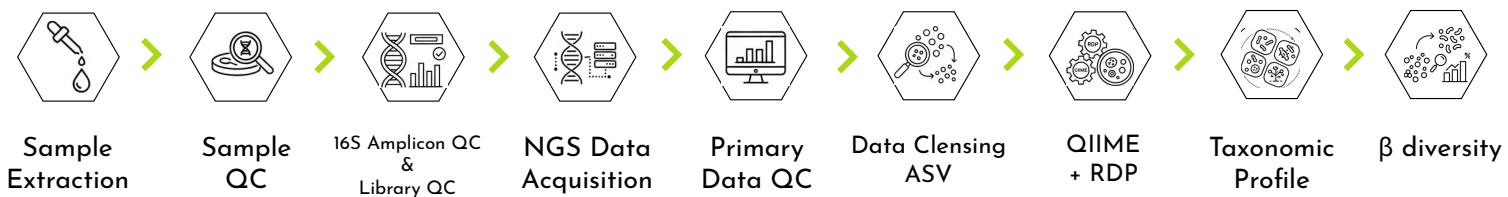


Swabs

\*Stabilized samples | Fresh/Frozen not accepted

Platform Illumina platforms | 0.1 - 0.2M | 300 x 2

## Process Map



## Standard Deliverables

[Download Sample Report](#)

- Sample & Library QC
- Method & Workflow Summary
- Species-level diversity and abundance analysis using SILVA, Greengenes, and 16S-NCBI reference databases
- Read Clustering & Rarefaction Analysis
- Species/Genus Identification with Shannon & Simpson Indices
- PCA /PCoA plots
- KRONA Charts & Predicted Microbial Pathways (optional)

## References

- Estaki M et al. Curr Protoc Bioinformatics. 2020; PMID:32343490.
- Licata AG et al. Microbiol Spectr. 2025; doi:10.1128/spectrum.01673-25.

## Metagenome Human Gut 16s Whole Amplicon

TC-S-MG-HG-16S-WA

## Applications

Method utilizes full-length 16S rRNA gene (full amplicon) sequencing to profile bacterial communities and improve taxonomic resolution. By capturing the complete 16S gene, the approach enables more accurate genus- and species-level classification compared to partial-region sequencing. Compared to the V3-V4 or other fragment-based methods, long amplicon 16S reads are assigned to profile bacterial species and genus. The data provide higher confidence in the discovery of species and inference of microbial pathways, leading to a superior estimate of alpha diversity. This method can be applied to well-referenced metagenomes such as the human gut, as well as environmental samples.

## Pros

- Superior resolution 16S fragments
- Enhanced species-level identification
- Reduced primer-region bias
- Ideal for detailed microbiome profiling
- Suitable for cohort and longitudinal studies where species resolution is desired

## Notes

- Focused on bacterial community analysis
- Functional insights are predictive, not direct
- Species-level resolution depends on reference databases
- Sensitive to DNA quality and extraction/amplification

## Input



Microbial DNA ≥ 10 ng recommended



DNA Qubit &gt; 10ng

## Sample Types



Stool samples



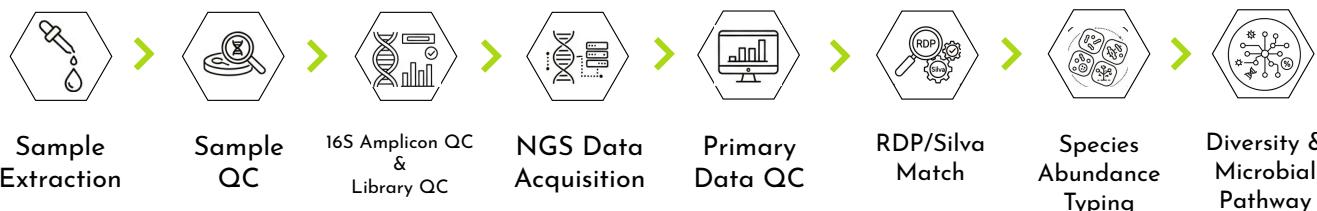
Swabs



Gut-derived clinical samples

**Platform** ONT sequencing platforms | 50K - 100K  
Long reads

## Process Map



## Standard Deliverables

[Download Sample Report](#)

- Sample & Library QC
- Method & Workflow Summary
- Raw Data QC & Processing
- Read Clustering & Rarefaction Analysis
- Species/Genus Identification with Shannon & Simpson Indices
- Differential Taxonomic Abundance with Heatmaps
- KRONA Charts & Predicted Microbial Pathways (optional)

## References

- Estaki M et al. Curr Protoc Bioinformatics. 2020; PMID:32343490.
- Licata AG et al. Microbiol Spectr. 2025; doi:10.1128/spectrum.01673-25.

## Integrated Microbiome Analysis - Fungal &amp; Bacterial

TC-S-MG-INT-MB

## Applications

Method utilizes integrated amplicon sequencing of bacterial and fungal marker genes to comprehensively profile mixed microbial communities within a single study. Bacterial communities are characterized using 16S rRNA gene sequencing, while fungal communities are profiled using ITS (ITS1/ITS2) or 18S rRNA regions. This integrated approach enables simultaneous assessment of bacterial-fungal composition, diversity, and co-occurrence patterns across biological or environmental conditions. The use of long amplicons provides the ability to type microbial as well as fungal/plant samples present in a microbiome. These investigations are well suited to profile species richness, diversity, and abundances in microbiomes to infer changes in time-course or treatment-dependent studies. Inference of alterations in microbial abundances provides clues to shifts in microbial pathways.

These methods are suitable when host DNA is high, sample DNA is limiting, or as a pre-pilot for whole-genome, metagenome, or metatranscriptome investigations.

## Pros

- Simultaneous profiling of bacterial and fungal communities
- Enables cross-kingdom interaction analysis
- Improved ecological and functional interpretation
- Suitable for complex microbiome studies

## Notes

- Based on targeted marker gene sequencing
- Functional insights are predictive
- Taxonomic resolution depends on reference databases
- Sensitive to sample quality and inhibitors

## Input



Microbial / Environmental DNA  
≥ 10 ng recommended



DNA Qubit > 10ng

## Sample Types



Soil



Extreme  
environment  
samples



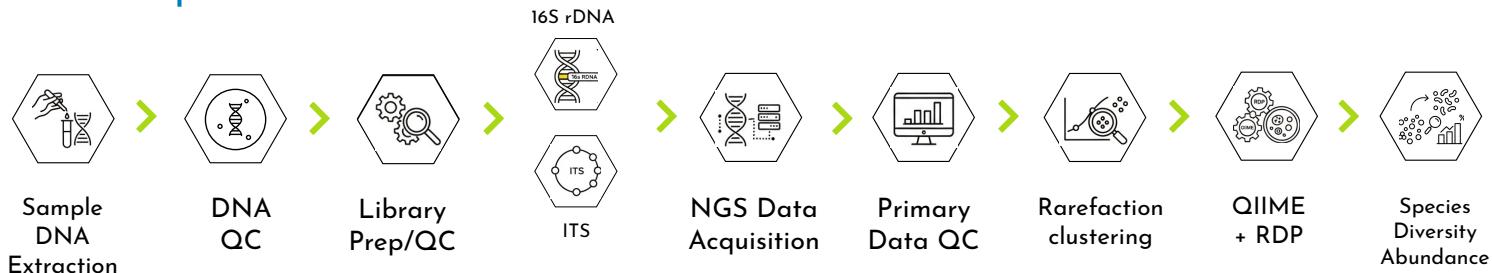
Biofilms and  
sludge



Water

Platform ONT Long-read sequencing | 200K-250K reads 16s | 50K reads ITS

## Process Map



## Standard Deliverables

[Download Sample Report](#)

- Sample QC, Library QC, Summary of methods
- Data QC / Sequence data preprocessing summary
- 16S species match for typing and abundances
- ITS match for fungal and abundances
- Rarefaction Plots
- Species/Genus Identification with Shannon & Simpson Indices
- When multiple samples in time covers / treatment are provided in Beta diversity, differential heatmaps and PCA plots provided

## References

- Estaki M et al. Curr Protoc Bioinformatics. 2020; PMID:32343490.
- Licata AG et al. Microbiol Spectr. 2025; doi:10.1128/spectrum.01673-25.

## Whole Metagenome Sequencing - ONT

TC-S-MG-WMG-ONT

### Applications

Method utilizes shotgun whole metagenome sequencing using Oxford Nanopore Technologies to comprehensively profile the genetic content of complex microbial communities. Long-read sequencing enables improved genome assembly, strain-level resolution, and detection of structural variants, mobile genetic elements, and antimicrobial resistance genes. This approach is suitable for bacteria, archaea, fungi, and viruses within a single experiment.

Whole-genome metagenomics offers the ability to discover novel microbial genomes and new genes/enzymes from complex microbiomes, where some key microbial communities may be uncultivable. The use of long-read data ensures reduced assembly bias and minimizes sequence contamination from closely related microbial genomes. The data are suitable for annotating microbial pathways and inferring mechanisms within microbial communities.

Samples extracted from host tissue may be mixed with significant amounts of host genomic DNA. High host background can skew sequencing results and may require host depletion, enrichment strategies, and/or additional sequencing depth to achieve the required rarefaction.

#### Pros

- Long reads enable improved genome assembly
- Enhanced strain- and species-level resolution
- Detects plasmids, phages, and structural variants
- Suitable for complex and mixed communities
- Enables comprehensive functional profiling

#### Notes

- Requires high-quality, high-molecular-weight DNA
- Bioinformatics analysis is computationally intensive
- Sequencing depth impacts genome recovery

### Input



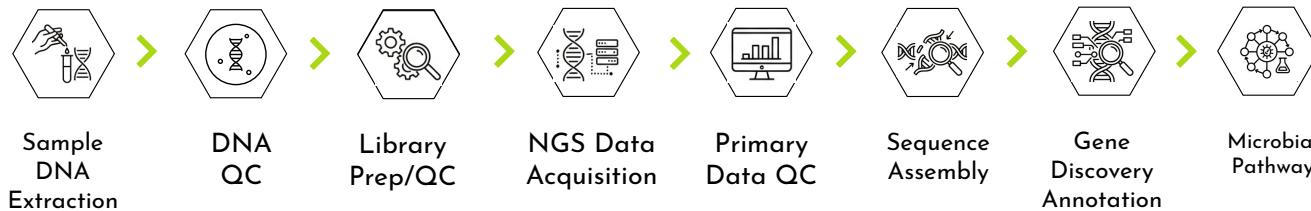
High-molecular-weight Metagenomic DNA  
≥ 1 µg recommended

### Sample Types



**Platform** ONT Long-read sequencing platforms |  
5 - 7 GB data on long re

### Process Map



## Standard Deliverables

[Download Sample Report](#)

- Sample QC, library QC, and method summary
- Raw data QC, adapter trimming, and preprocessing
- Metagenome assembly summary
- Gene annotation and species/genus/strain-level matching against reference databases
- Identification of phages, episomes, and viral DNA with species rarefaction and abundance profiling
- Alpha diversity analysis (Shannon and Simpson indices)
- Beta diversity and inter-sample diversity estimation (for multi-sample studies)
- Host DNA considered as contamination and ideally removed during sample preparation

## References

- Estaki M et al. Curr Protoc Bioinformatics. 2020; PMID:32343490.
- Licata AG et al. Microbiol Spectr. 2025; doi:10.1128/spectrum.OI673-25.

## Whole Metatranscriptome Sequencing

TC-S-MG-WMTS

### Applications

Method utilizes shotgun RNA sequencing of total community RNA to capture the actively expressed genes of complex microbial communities. By sequencing and analyzing community-wide transcripts, this approach reveals functional activity, metabolic pathways, and regulatory responses in real time. The workflow typically includes rRNA depletion followed by cDNA library preparation and NGS. Metatranscriptomics profiles the microbial transcriptome community, providing key insights into the expressed functional genetic complement. The analysis of metatranscriptomes can be applied to discover encoded enzymes and novel pathways that are activated under specific conditions, such as bio-transformation and material degradation. It can also be used to infer metabolic switches when multiple time points are profiled to assess differential gene expression. The use of long-read methods for cDNA sequencing reduces assembly-induced artifacts arising from closely related species in mixed microbial communities.

#### Pros

- Captures active microbial functions
- Reveals real-time metabolic activity
- Complements metagenome sequencing
- Suitable for functional ecology studies
- Enables condition-specific pathway analysis

#### Notes

- RNA is highly sensitive to degradation
- rRNA depletion from samples may be needed
- Sensitive to preparation and inhibitors
- For host + microbial systems, only expressed genomes are profiles unlike whole genomes

### Input



Total Community RNA

≥ 500 ng recommended



DNA Qubit > 1µg



Nanodrop 230/260 | 260/280

### Sample Types



Soil



Extreme environment samples



Biofilms and sludge

Platform ONT Long Read Platform |  
10 - 15 GB data

### Process Map



\*When multiple sample DEG

[Download Sample Report](#)

### Standard Deliverables

- Sample QC, Library QC, Summary of Methods
- Data QC / Sequence Data Pre-processing Summary
- Metatranscriptome Assembly
- Gene Identification, Species/Reference Abundance Estimates
- Identification of Transcripts-Pathway
- For Experiments with Time Course, Differential Expression to Analyze Modulation of Transcript and Species Abundances

### References

- Estaki M et al. Curr Protoc Bioinformatics. 2020; PMID:32343490.
- Licata AG et al. Microbiol Spectr. 2025; doi:10.1128/spectrum.O1673-25.

## Whole Genome Sequencing - Microbe (De novo)

TC-S-AG-WGM-DNV

## Applications

Method utilizes shotgun whole-genome sequencing to generate a de novo assembly of microbial genomes without reliance on a reference sequence. This approach enables complete characterization of bacterial, archaeal, and fungal genomes, including novel species or strains. De novo assembly allows identification of coding and non-coding regions, genomic rearrangements, plasmids, and mobile genetic elements.

## Pros

- Enables discovery of novel genomes and genes
- High-resolution strain characterization
- Suitable for poorly characterized organisms
- Supports functional and evolutionary studies
- Identifies Episomes, Virons etc
- Base modification in DNA

## Notes

- Requires high-quality genomic DNA
- Assembly quality depends on sequencing depth
- Complex genomes may require long-read support
- 16S/18S purity testing suggested

## Input



## High Molecular Genomic DNA

- Short-read sequencing:  $\geq 100$  ng
- Long-read sequencing (if applicable):  $\geq 1$   $\mu$ g
- DIN/Gel analysis suggested

## Sample Types



Bacterial isolates



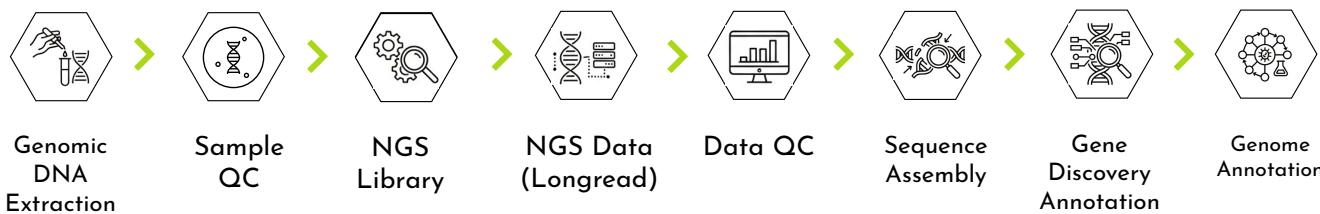
Archaeal isolates



Fungal isolates

**Platform** Illumina platforms / Long-read sequencing platforms | 100X Depth for  $\sim 0.5$  - 2GB data | 30X - 40X depth on long read

## Process Map



## Standard Deliverables

[Download Sample Report](#)

- Sample QC, Library QC, and Summary of Methods
- Data QC, Data Trimming, and Pre-processing
- Genome Assembly Summary Statistics,
- Gene Calling, Gene Annotation
- Identification of Transposable Elements and Gene Clusters
- Antibiotic / Resistance Loci
- GFF3 File, Gene List, Annotation-Pathway Assignment, Gene Ontology Assignment
- CIRCOS Plots, Genome Maps
- \*Where Applicable: Episome Assembly and Annotation

## References

- Estaki M et al. Curr Protoc Bioinformatics. 2020; PMID:32343490.
- Licata AG et al. Microbiol Spectr. 2025; doi:10.1128/spectrum.O1673-25.

## Whole Genome Sequencing - Fungal (De novo)

TC-S-AG-WGF-DNV

## Applications

Method utilizes shotgun whole-genome sequencing to generate a de novo assembly of fungal genomes without reliance on a reference sequence. This enables comprehensive characterization of fungal genomes, including novel species or strains, complex gene families, repetitive regions, and secondary metabolite biosynthetic clusters. De novo assembly supports studies in fungal biology, pathogenicity, industrial strain development, and evolutionary genomics.

## Pros

- Enables discovery of novel fungal genes
- Captures complex and repetitive genomic regions
- Suitable for poorly characterized fungal species
- Supports functional and evolutionary studies
- Structural variation, Synteny and genome organization
- Repeat/Recombination events
- Haplotype phasing

## Notes

- Requires high-quality genomic DNA of high molecular weight
- Assembly quality depends on genome size and complexity
- Long-read sequencing recommended for repeat-rich genomes
- 18S strain purity testing recommended

## Input



## Genomic DNA

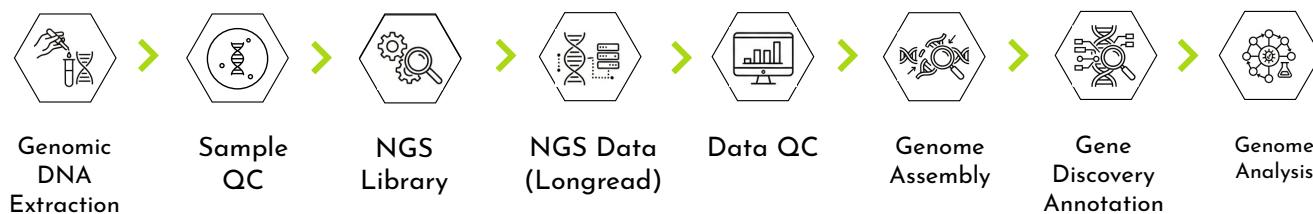
- Short-read sequencing:  $\geq 200$  ng
- Long-read sequencing (recommended):  $\geq 1 \mu\text{g}$
- DIN/Gel analysis for high molecular weight DNA

## Sample Types



**Platform** Long-read sequencing platforms | 50-70X Long read |  
 $\sim 2\text{-}10$  GB | Based on genome/ploidy estimates

## Process Map



## Standard Deliverables

[Download Sample Report](#)

- Sample QC, Library QC, and Summary of Methods
- Data QC, Data Trimming, and Pre-processing
- Genome Assembly Summary, Gene Detection, Genome Annotation
- SNP/Allele identification
- Haplotype information from long read parsing
- GFF3 File, Gene List, Repeat Elements, SINE/LINES, Pathway assignment, Secondary metabolite coding genes
- When multiple Strain/Strain matching scoped - Synteny and comparative genome mapping

## References

- Estaki M et al. Curr Protoc Bioinformatics. 2020; PMID:32343490.
- Licata AG et al. Microbiol Spectr. 2025; doi:10.1128/spectrum.O1673-25.

## Whole Genome Resequencing (Eukaryotic)

TC-S-AG-WGR-EUK

## Applications

Method utilizes high-coverage whole genome sequencing of eukaryotic organisms followed by read alignment to a reference genome to identify genomic variations. This resequencing approach enables accurate detection of single nucleotide variants (SNVs), insertions/deletions (InDels), copy number variations (CNVs), and structural variants across the genome when long reads platform is used. Resequencing methods allow identification of individual mutants/variations and provides a whole genome unbiased discovery approach. The method can be used for generation of data for genetic analysis, discovery of novel/rare SNP's, population structure analysis, discovery of coding and non-coding variations

## Pros

- High-resolution genome-wide variant detection
- Accurate comparison against a reference genome
- Suitable for population and evolutionary studies
- Scalable from small to large genomes
- Supports trait and disease association analysis

## Notes

- Requires a high-quality reference genome
- Structural variant detection benefits from higher coverage is recommended on long read

## Input



High Molecular Genomic DNA

≥ 1 µg recommended

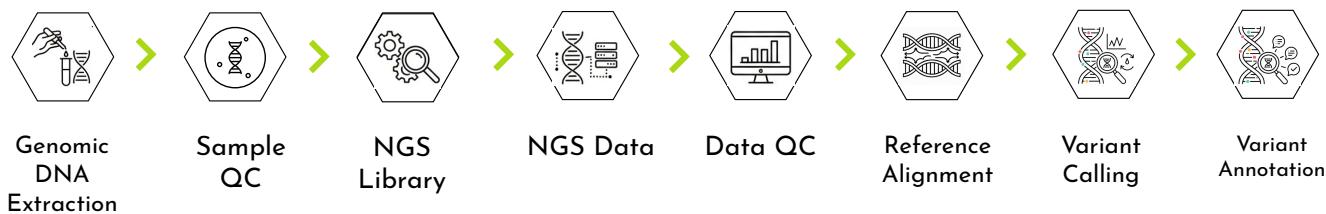
## Sample Types



## Platform

Illumina | ONT Long Read Platforms  
30X - 40X based on genome Illumina;  
Long Reads

## Process Map



## Standard Deliverables

[Download Sample Report](#)

- Sample QC, Library QC, and Summary of Methods
- Data QC, Data Trimming, Pre-processing, and Adapter trimming
- Reference Alignment Summary
- Variant Summary and Variant annotation
- Coding/Non-coding Variants
- Data pre-processing of VCF for genetic analysis based on project requirements

## References

- Estaki M et al. Curr Protoc Bioinformatics. 2020; PMID:32343490.
- Licata AG et al. Microbiol Spectr. 2025; doi:10.1128/spectrum.01673-25.

## Whole Genome Resequencing (Microbial)

TC-S-AG-WGR-MIC

## Applications

Method utilizes high-depth whole genome sequencing of microbial isolates followed by alignment to a reference genome to identify genomic variations at single-nucleotide and structural levels. This resequencing approach enables precise detection of SNPs, InDels, copy number variations, and structural rearrangements, when longread methods are applied.

## Pros

- High-resolution strain-level variant detection
- Ideal for comparative and outbreak studies
- Accurate mutation analysis

## Notes

- Requires a high-quality reference genome
- Closely related reference improves accuracy
- Sequencing depth affects low-frequency variant detection
- Structural variants benefit from long-read
- 16S/18S Strain purity testing recommended

## Input



High Molecular Weight Genomic DNA

≥ 50 ng recommended

## Sample Types



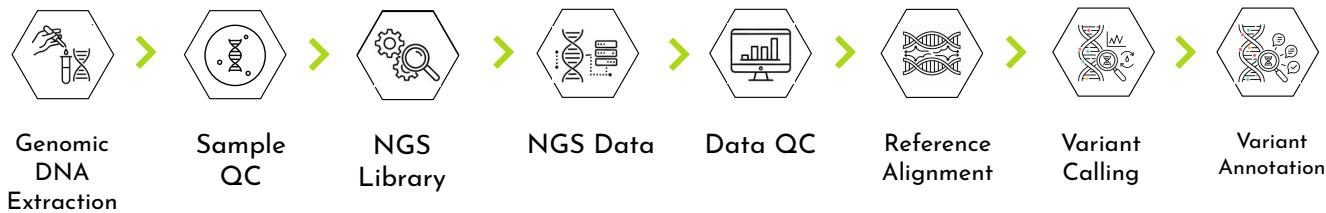
Bacterial isolates

Archaeal isolates

Environmental microbial isolates

**Platform** Illumina | ONT Long Read Platforms  
 30X ~ 0.2 - 0.3 GB | 150 X 2  
 18X - 20X - Long Reads

## Process Map



## Standard Deliverables

[Download Sample Report](#)

- Sample QC, Library QC, and Summary of Methods
- Data QC, Data Trimming, Pre-processing, and Adapter trimming
- Reference Alignment Summary
- Variant Summary and Variant annotation
- Coding/Non-coding Variants
- Data pre-processing of VCF for genetic analysis based on project requirements

## References

- Estaki M et al. Curr Protoc Bioinformatics. 2020; PMID:32343490.
- Licata AG et al. Microbiol Spectr. 2025; doi:10.1128/spectrum.01673-25.

## Methylation Discovery - Applied

TC-S-AG-METH-DIS

## Applications

Method utilizes genome-wide DNA methylation profiling to identify and quantify epigenetic modifications associated with gene regulation and phenotype. The use of long read sequencing allows the analysis of modified bases. The method offers the ability to map individual sequences for multiple base modifications. DNA epimarks can be compared in different tissues/case-control in disease and in normal vs tumor DNA. Methylation analysis can be integrated with gene expression data for matched samples.

## Pros

- Genome-wide discovery of methylation patterns
- High-resolution epigenetic profiling
- Suitable for disease and exposure studies
- Supports integrative multi-omics analysis
- Applicable across diverse sample types

## Notes

- Method requires non-modified genomic DNA
- PCR amplified DNA modifies and removes methylation
- Reference genome provides methylated/modified DNA

## Input



## Genomic DNA

 $\geq 200$  ng recommended

High molecular weight, unmodified DNA recommended for genomic DNA quality analysis

## Sample Types

Blood/  
PBMC

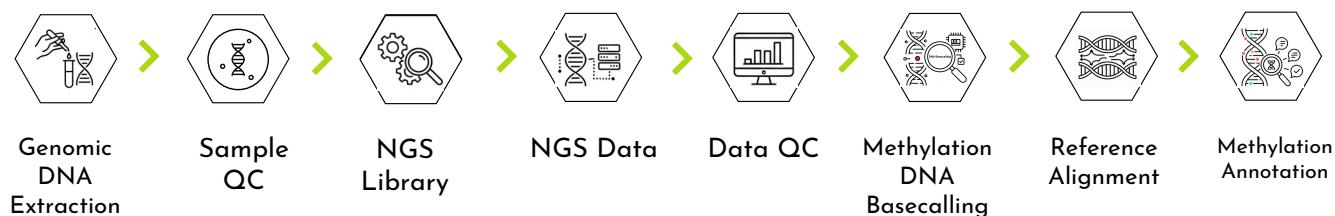
Tissue

Cell lines

Plant/Fungi

**Platform** ONT Long Read platforms |  
30 - 50X | Additional depth for polyploid genomes

## Process Map



## Standard Deliverables

[Download Sample Report](#)

- Sample QC, Library QC, and Summary of Methods
- Data QC, Data Trimming, Pre-processing, and Adapter trimming
- Reference Alignment Summary
- Variant Summary and Variant annotation
- Coding/Non-coding Variants
- Data pre-processing of VCF for genetic analysis based on project requirements

## References

- Estaki M et al. Curr Protoc Bioinformatics. 2020; PMID:32343490.
- Licata AG et al. Microbiol Spectr. 2025; doi:10.1128/spectrum.01673-25.

## Whole Genome De novo Sequencing - Large Genomes (&gt; 500 Mb)

TC-S-AG-WGD-LRG

## Applications

Method utilizes high-depth whole genome sequencing combined with advanced de novo assembly strategies to reconstruct large and complex eukaryotic genomes (> 500 Mb) without reliance on a reference sequence. This approach integrates short-read and long-read sequencing technologies to resolve repetitive regions, structural variations, and complex genome architectures. It is suitable for plants, animals, and other large-genome organisms, including non-model species.

## Pros

- Enables assembly of large and complex genomes
- No reference genome required
- For repetitive and structurally complex regions
- Supports advanced functional genomics

## Notes

- Requires high-quality, high-molecular-weight DNA
- Long-read sequencing strongly recommended
- High sequencing depth and coverage required for genome assembly

## Input



## High-molecular-weight Genomic DNA

- Long-read sequencing:  $\geq$  5-10  $\mu$ g recommended
- Short-read sequencing (polishing):  $\geq$  500 ng

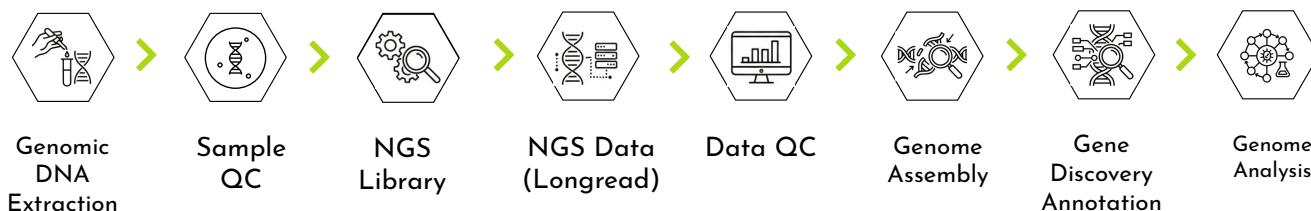
## Sample Types

Plant  
TissueAnimal  
Tissue

## Platform

Long-read sequencing platforms + Illumina platforms |  
 50 - 70X of estimated genome for  $2n$  |  
 Additional depth for polyploid genomes

## Process Map



## Standard Deliverables

[Download Sample Report](#)

- Sample QC, Library QC, and Summary of Methods
- Data QC, Data Trimming, and Pre-processing
- Genome Assembly Summary, Gene Detection, Genome Annotation
- SNP/Allele identification
- Haplotype information from long read parsing
- GFF3 File, Gene List, Repeat Elements, SINE/LINES, Pathway assignment, Secondary metabolite coding genes
- When multiple Strain/Strain matching scoped - Synteny and comparative genome mapping

## References

- Estaki M et al. Curr Protoc Bioinformatics. 2020; PMID:32343490.
- Licata AG et al. Microbiol Spectr. 2025; doi:10.1128/spectrum.01673-25.