1st BASE
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NGS Handbook

NGS and TGS
approaches to Microbial Genomics & Transcriptomics

SINGAPORE ☎ +65-67757318 ✉ custcare@axilscientific.com www.axilscientific.com
MALAYSIA ☎ +603-89433252 ✉ custcare@apicalscientific.com www.apicalscientific.com
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#1
Amplicon Sequencing In-House Service Provider

16s/ITS Amplicon Metagenomic Sequencing Services

20,000+ Samples Sequenced

Available in Illumina Short-Read and Oxford Nanopore Full-Length.

NGS-grade DNA extraction service available for a wide range of sample types
(coffee, water, stool, sludge, gut content, and many more).

Conducted with ISO Certified Protocols
Standard protocol recommended by Illumina, verified, and validated by ISO Accreditation body (ISO 17025), maintaining the consistency and accuracy of the results.

200k Raw Reads for Each Sample
Ensures more accurate results, reduce biasness, and maximise discovery of ASV available in the samples.

Fast Turnaround Time
As fast as 6 weeks after QC has been passed.

Prompt Technical Support
Post-sequencing technical support from our Bioinformatics team.

Applications offered by 1st BASE

16s rDNA Amplicon Metagenomic Sequencing

The 16S rRNA amplicon metagenomic sequencing technique is a microbiome analysis to uncover and study the diversity of Bacteria within a given sample. Choose between the 16s region of V1 – V9 for better resolution in your data, or simply choose region V3-V4 for uncovering general diversity in your samples.

Custom Metagenomics Sequencing

Custom Metagenomic amplicon libraries are targeting diverse population of organisms (Such as aquatic animal, plant or many more) using targeted specific genes. Just provide us your primer sequence of interest to get started.

Nanopore Full Length Amplicon Sequencing

Nanopore Full length amplicon sequencing is a targeted long-read sequencing method on Oxford nanopore platform. This approach is particularly useful for studying genetic diversity, structural variants, and regions of interest without limitation of size of Amplicon. Comparing to Illumina approach, this method would give better taxanomy resolution

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Amplicon Seq and Amplicon Seq Lite Comparison Chart

	Amplicon Seq	Amplicon Seq Lite
Read Coverage	**High -200k raw reads**	**Low -100k raw reads**
Turnaround Time	**Faster (4-6 weeks after QC passed)**	**Slower (6-8 weeks after QC passed)**
Sample Type	**Soil samples, Water sample or sample type that is high biodiversity .**	**Saliva sample, Gut content, fermentated food, that expected to have low biodiversity**
Bioinformatics Analysis	**More comprehensive analysis including Sample Pairwise Comparison, Picrust (16s Only), Krona Chart**	**Less Comprehensive with essential analysis (ASV table, Alpha &Beta Diversity, Phylogenetic Tree)**
Post-Analysis	**AmpliCloud by 1st BASE and Nova Lifetech** **(FOC 3 months from Date of result receival)** **Worth up to RM 1,500!** **Amplicon Metagenomic Post-Analysis Online tools.** • **Exclusive and Comprehensive tools developed by Apical Scientific and BI partner** • **Read Count Analysis** • **Taxonomy (Bar Plot, Taxanomy Table, Bubble Plot, Heatmap plot, Box Plot, Ternary Plot and Table, Radar Plot, Sankey Plot)** • **Alpha-Diversity (Alpha Plot, Alpha-Diversity Table, Statistical Tests, Rarefaction Plot, Rarefaction Table)** • **Ordination (Plot & Table)** • **Venn Diagram** • **Differential Abundance (Plot & Table)** **** Allow regenerate graphical presentation for publication on own click.**	**Not applicable.**
Why Choose This?	Good to **high biodiversity samples,** which allow close to or **achievement of Pleataue** to avoid Result Biases With **AmpliCloud** by 1st BASE and Novalifetech complimentary Online Bioinformatics tools to help research and navigate changes to tailor-fit Thesis and Multiple paper Publication **Fast TAT and ISO 17025:2017 Accredited.** 1 Price to cater all from gDNA QC, PCR QC, Library Preparation and Sequencing, Data Deliver in **Standard/Comprehensive** Bioinformatics Analysis and can include Metadata such as pH, Temperature for better comparison data. **Save cost, Human resources and Energy for Complex Library Preparation** by our In-house Laboraroty Experts- 1 Price for all	Good with Project with very tight budget constraint Do not require high sequencing depth due to low sample biodiversity **1 Price to cater all** from gDNA QC, PCR QC, Library Preparation and Sequencing, Data Deliver in Basic/Essential Bioinformatics Analysis which are sufficient for Publication and Comparison. **Save cost, Human resources and Energy for Complex Library Preparation** - 1 Price for all

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Illumina / Oxford Nanopore Amplicon Seq Comparison Chart

	Short-read Amplicon Metagenomic	Full-Length Amplicon Metagenomic
Sequencing Platform	Illumina	Oxford Nanopore
Read Coverage	100 -200k raw reads	10 -30k raw reads
Turnaround Time	4-8 weeks after QC passed	4-6 weeks after QC passed
Suggested Sample Type	All type of samples	All type of samples: Medical Gut Microbiome, Bac ID metagenome for contaminated Cultures or Products.
Similarities	To Discover microbiome available in the samples, comparison of treatment or changes that could affect the microbiomes and study on possible causes host's health	
Differences	• Use Short-length primer (16s V3-V4 or ITS 1 or ITS 2) that limited below 550bp. • Lower Taxanomy resolution. • Higher sequencing reads to provide overview of Taxanomy availables.	• Uses Full length Primer Such as 16s V1-V9, ITS 1 to ITS 2, Can be extended further as request. • Higher Resolution in Taxanomy. • Useful Especially study on Targeted Species Microbes that caused Diseases or pathogenic strain discovery. • More Applicable to less diverse samples with higher taxanomy resolution required.
Why Choose This?	Overview of Available Microbial Diversity Suitable for High biodiversity samples.	1st BASE NGS: The only Oxford Nanopore Certified Service Provider in Malaysia and Top 5 in South East Asia. In-House Sequencing Run Contantly upgrading technology and extensive R&D to upkeep standard Objective to study Pathogen Strain that responsible for certain disease or related Host's health.

eDNA NGShield

Next-Gen eDNA for Conservation, Sustainability and Environmental Research

Unlock

New Frontiers in Environmental Research with Apical Scientific's NGS Custom Amplicon Sequencing

Explore Gene Regions

trnL Understand the dietary habits of herbivores.

COI Discover land, marine and freshwater animal biodiversity through the COI gene.

12S MiFish Unveil the fish community composition in water bodies.

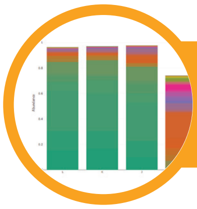
Custom Region

Be the pioneer Principal Investigator to explore other gene region of interest.

Bioinformatics
Deliverables

1. Data QC and trimming
2. ASV table generation and Taxonomic characterisation
3. ASV Analysis and Species Annotation, including KRONA results, Heatmap, Taxonomic Abundance and up to 5 Venn diagrams*
4. Alpha-diversity Analysis, including Alpha Indices table with ANOVA, Rarefaction curve, Rank abundance curve
5. Beta-diversity Analysis, including Ordination (CCA, RDA, DPCoA, NMDS, MDS, PCoA), Unifrac distance analysis, UPGMA (Unweighted Pair-group Method with Arithmetic Means)
6. Phylogenetic Trees, including ASV co-occurrence networks & Metacoder
7. Statistics Between Groups: DESeq2, ANOSIM, LEfSE
8. Metagenome Functional Prediction using Picrust2 (only applicable for 16s)

Key Diagrams



Taxa Barplot

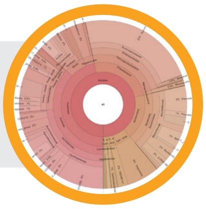
This graphs depicts the Taxonomy overview across

Top 10 Taxa (Boxplot)

A box plot of the top **10 OTUs/ ASV** by group. For each sample type, the median and range of abundances of the most abundant OTUs/ASV are plotted.

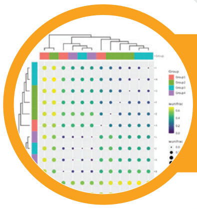
Heatmap

Heatmap exhibits the relative abundances of taxa (phyla) using shades of colour. The higher a value is, the darker the represented colour



Krona Chart

Krona allows visualization of hierarchical data through a zoomable pie chart. It presents the abundances of taxa across multiple levels of the phylogenitic hierarchy simultaneously



Distant Matrix

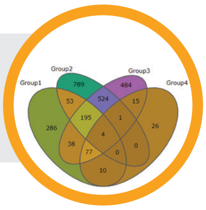
UniFrac measures the phylogenetic distance between sets of taxa in a phylogenetic tree as the fraction of the branch length of the tree that leads to descendants from either one environment or the other, but not both.

UPGMA

UniFrac measures the phylogenetic distance between sets of taxa in a phylogenetic tree as the fraction of the branch length of the tree that leads to descendants from either one environment or the other, but not both.

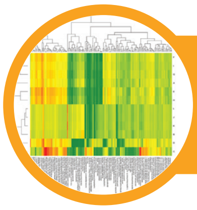
Alpha Diversity

In ecology, alpha diversity is the mean species diversity in sites at a local scale, whereas beta diversity refers to the ratio between regional and local species diversity.



Venn Diagram

A Venn diagram is an illustration of the relationships between and among sets, groups of samples with their own unique OTU/ ASVs and those that share OTU /ASVs in common.



Picrust

Picrust2 predict2 metagenome functional content based on the microbiome composition. Complete Picrust2 prediction results based on KO, Metacyc and EC and be found in the Picruts2 folder (only applicable for 16s Amplicon sequencing)

What Our Customers Are Saying

“ Great service, molecular work can't happen without it ”

The samples are sent very easily and the results are always given on time. The service has been integral part of my research during my doctorate.

Muhammad Moman Khan, Institute for Biotechnology,
BTU Cottbus-Senftenberg (June 2022)

“ Bioinformatic support has been very helpful! ”

Apical Scientific has provided excellent support from sample preparation to amplicon data analysis. The analysis report was comprehensive and the figures provided were attractive and neatly organised. More importantly, the local bioinformatic support has been very helpful where we have had interactive discussion and received professional advice to ensure our research is properly designed and correctly analysed. Thank you Apical Scientific for such a great assistance and help throughout our research journey at UPM!

Dr. Amalia Mohd. Hashim
Biotech Faculty, UPM (September 2022)

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Shotgun Metagenomic Sequencing

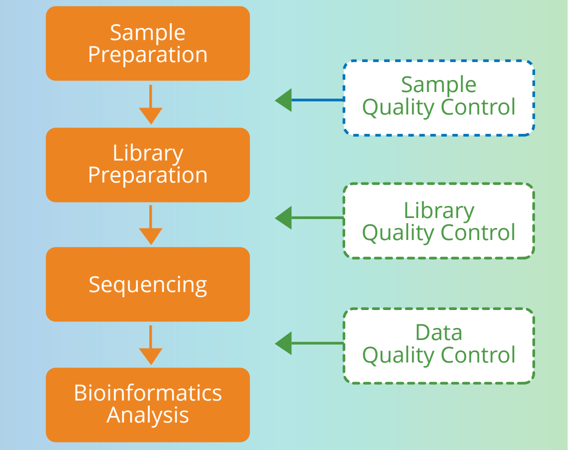
Shotgun Metagenomic Sequencing offers an approach to identify and characterize the entire microbiome in a specific environment without the need for microbial isolation or culturing. It can elucidate the community structure, species classification and provide insights into systemic evolution, gene function, metabolic network, and antibiotic resistance gene information for multidisciplinary research.

Our Key Features & Advantages

- **Demo Report Available on request.**
- **Post-analysis support by over 10-years experiences Data Scientist.**
- **6-8 weeks turnaround time after QC passed.**
- **Customized Bioinformatics Analysis on request. Please inquire for more details.**

Dry Ice Reverse pick-up available outside Klang Valley area with additional charge, please inquiry for more information.

Project Workflow



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Sample Requirements

Library Type	Sample Type	Amount	Volume	Concentration	Purity
Shotgun Metagenomic Library	Total DNA	≥ 200 ng	≥ 20 μL	≥ 10 ng/μL	OD260/280= 1.8-2.0; No degradation, No contamination, No color

Analysis Content

Standard Analysis

1. Data quality control (host contamination removal if required)

2. Metagenome Assembly

3. Gene Prediction

4. Taxonomy Annotation

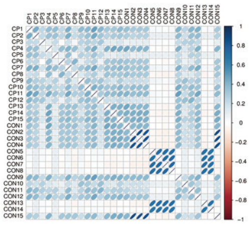
5. Function Annotation (KEGG, eggNOG, CAZY...etc)

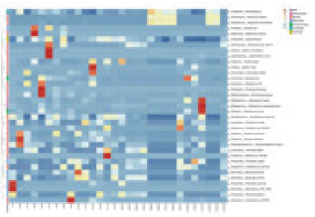
6. Alpha and Beta Diversity Analyses

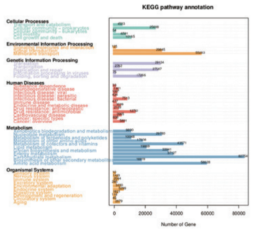
7. Antibiotic Resistance Gene and Mobile Genetic Elements Annotation and Analyses

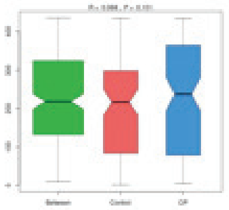
8. Differential analysis between groups of species and function

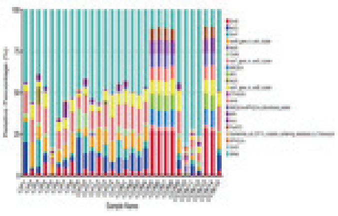
Demo Analysis Results

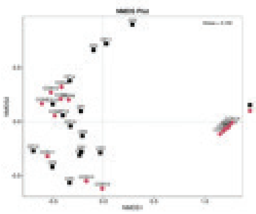

Correlation Analysis between Samples

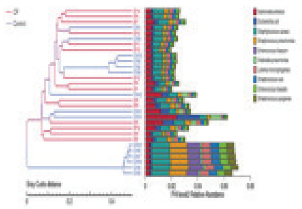

Taxonomic Abundance Heatmap at Species Level

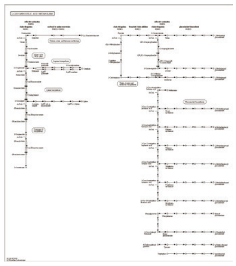

KEGG Pathway Annotation Cartogram


Anosim of CAZy


Relative Abundance of Antibiotic Resistance Gene


NMDS of Plasmid Annotation


Pathogen-Host Interaction Clustering Tree based on Bray-Curtis Distance


Metabolic Pathway Analysis

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NEXT GENERATION SEQUENCING (NGS)

WHOLE GENOME SEQUENCING (WGS)

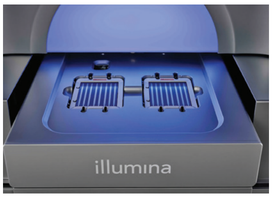
For BACTERIA / FUNGUS Sample

- **Premium Local QC with DNA Barcoding* to Confirm Pure Isolation**
- **Demo Report Available**
- **Data Support by Local Data Scientist**
- **6-8 Weeks After QC Passed**

*Certified with ISO 17025:2017

Option 1

ILLUMINA PE 150 SHORT READ WGS



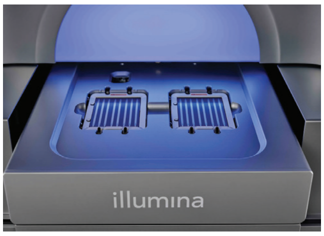
- Suitable for SNP and INDEL with Reference Genome (Resequencing)

NANOPORE LONG READ WGS

- For longer contigs requirement
- Suitable for both *de novo* and Resequencing.

Option 2

ILLUMINA SHORT READ + NANOPORE LONG READ HYBRID WGS



- Suitable for *de novo* Bacteria and Fungus Genome.

Terms & Conditions apply:

1. All Inclusive gDNA QC, DNA Barcoding, Library Prep, Sequencing and Standard Bioinformatics Analysis.
2. For Outstation, Please Enquire Us for Cold Shipment Pick-Up.

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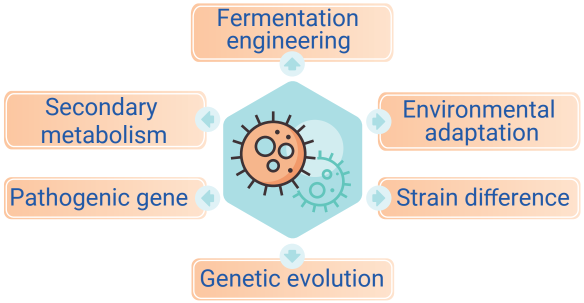
Application



Fermentation engineering

Secondary metabolism

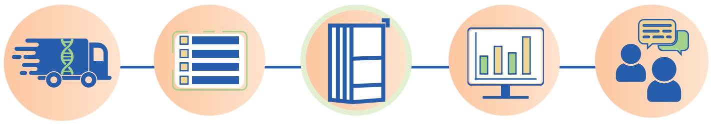
Environmental adaptation

Pathogenic gene

Strain difference

Genetic evolution

Service Workflow



Sample delivery

Library construction

Sequencing

Data analysis

After-sale services

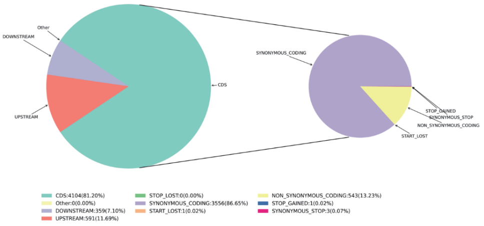
Service Specifications

Library	Read Length	Recommended Strategy	Assembly
Illumina	PE150	100X NGS	Draft map
PacBio	HiFi-10 Kb	30X HiFi	Complete map 0-Gap
Nanopore	20 Kb	100X ONT + 100X NGS	Complete map 0-Gap

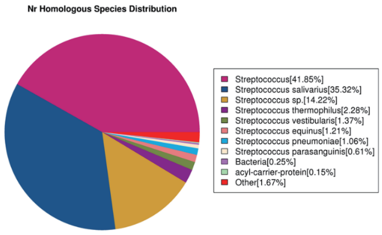
Sample Requirements

Platform	Conc. (ng/μL)	Amount (μg)	Volume (μL)
Illumina	1	0.06	20
PacBio	20	1.2	20
Nanopore	40	2	20

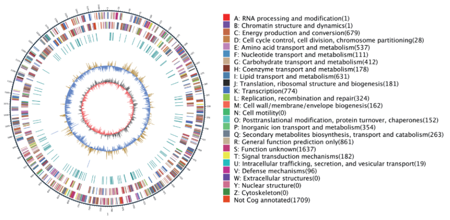
Demo Results



1.SNP classification and annotation



2. Coding gene annotation



3. Genome visualization

Featured Publications

Year	Journal	Article	Applications	DOI
2024	Nature	Inducing novel endosymbioses by implanting bacteria in fungi	Endosymbioses	10.1038/s41586-024-08010-x
2024	Nature Microbiology	Non-canonical start codons confer context-dependent advantages in carbohydrate utilization for commensal E. coli in the murine gut	Metabolic competition	10.1038/s41564-024-01775-x
2024	Journal of Infection	Pandrug-resistant Klebsiella pneumoniae isolated from Ukrainian war victims are hypervirulent	Pathogenic bacteria	10.1016/j.jinf.2024.106312

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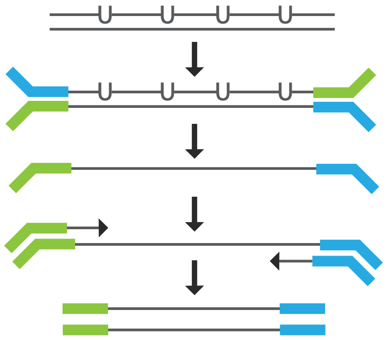
1st BASE Prokaryotic mRNA Sequencing (NGS)

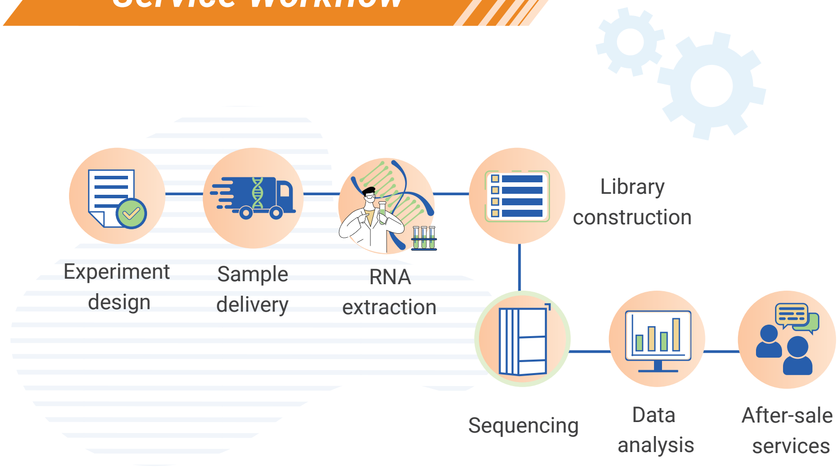
Prokaryotic RNA sequencing harnesses the power of next-generation sequencing (NGS) to unlock dynamic cellular transcriptomes. 1st BASE's cutting-edge technology is tailored for prokaryotes with a reference genome, providing comprehensive transcriptome analysis, gene structure analysis, and more. Widely applied in basic scientific research, drug development, and other fields, it helps unravel the mysteries of prokaryotic gene expression.

Technical Features

rRNA depletion and stranded library construction



Service Workflow



Bioinformatics

- Gene expression analysis;
- Differential expression analysis;
- Function annotation and enrichment analysis;
- sRNA prediction and annotation;
- Transcript structure analysis.

Service Advantages

- Unlock comprehensive and high-resolution insights into gene expression and regulation in prokaryotes.
- The reference genome based prokaryotic transcriptome can be more accurate.
- Dig deep into gene structure and SNP/InDel information to discover new genes or gene expression elements.
- Nine interactive analysis modules, including advanced analysis, to realize the comprehensive analysis of transcriptome data.
- After-sale services: After-sale services are valid for 3 months upon project completion, including project follow-up, trouble-shooting, results Q&A, etc.

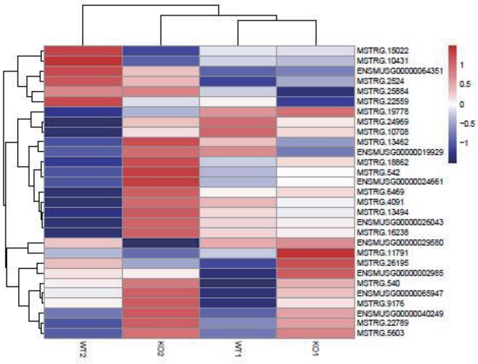
Service Specifications

Library	Read Length	Recommended Data	Data Quality
rRNA depletion	PE150	2 Gb	Q30 ≥ 85%

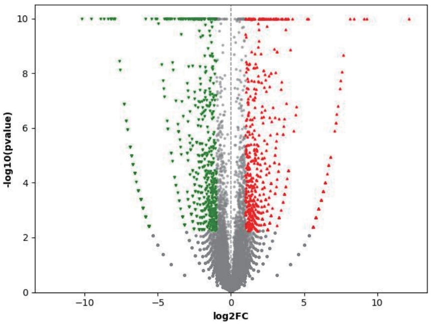
Sample Requirements

Amount	Purity	Integrity
Conc. ≥ 50 ng/μL; Volume ≥ 20 μL; Total ≥ 1 μg	OD260/280=1.8-2.0 OD260/230=1.0-2.5 Limited or no protein or DNA contamination shown on gel.	RIN ≥ 6.5

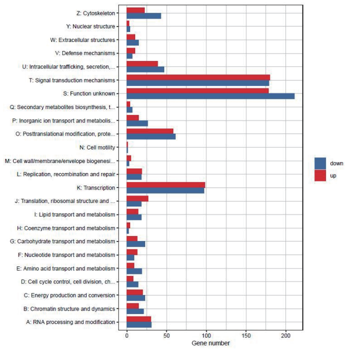
Demo Results



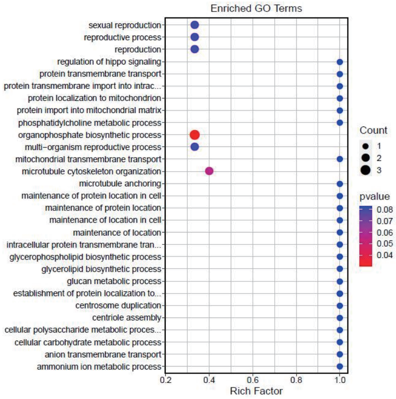
1. Sample expression heatmap



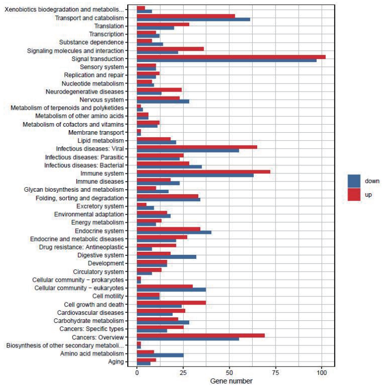
2. Differential expression analysis



3. eggNOG annotation on DEGs



4.GO annotation on DEGs



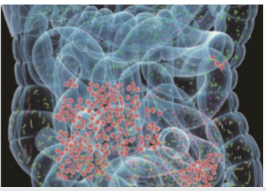
5.KEGG annotation on DEGs

1st BASE
Metatranscriptomics Sequencing (NGS)

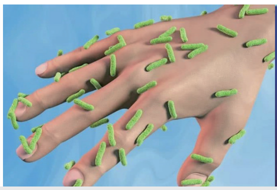
Through high-throughput sequencing technology, 1st BASE metatranscriptomics delves into the gene expression of diverse microbes, from eukaryotes to prokaryotes, thriving in natural environments like soil, water, sea, feces, and the gut. With our comprehensive service, gain insights into the whole gene expression profiles of complex microbial communities, conduct taxonomic analysis of species, explore functional enrichment of differentially expressed genes, and beyond.

Application


Feces


Gut

Mouth


Skin


Alveolar lavage fluid


Vaginal samples

Service Workflow

Sample delivery
Library construction
Sequencing
Data analysis
After-sale services

Bioinformatics

- Transcript assembly;
- Taxonomy annotation:
  Alpha diversity and beta diversity analysis;
- Functional annotation:
  GO, KEGG, eggNOG, CAZy, CARD, PHI,etc.;
- Statistics and differential analysis.

Service Advantages

- Study the changes of complex microbial communities at the transcriptional level and explore potential new genes.
- Explaining microbial community interactions with the host or environment.

Full suite of metatranscriptome analysis software to obtain maximum transcriptome information of microbial communities.

The latest gene function databases for more accurate gene expression information of microbial communities.

After-sale services: After-sale services are valid for 3 months upon project completion, including project follow-up, trouble-shooting, results Q&A, etc.

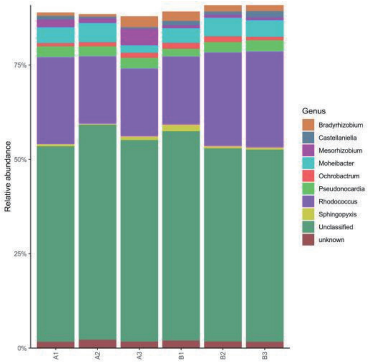
Service Specifications

Platform	Read Length	Recommended Data
Illumina	PE150	12 G

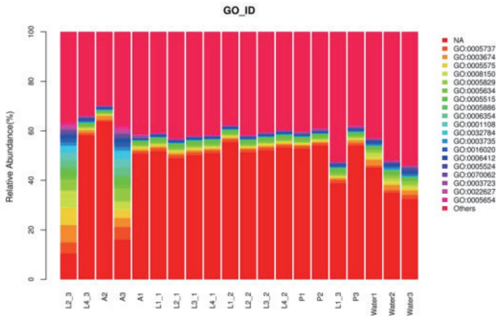
Sample Requirements

Conc. (ng/μL)	Amount (μg)	Volume (μL)	OD260/280	OD260/230	RIN
50	1.0	20	1.8-2.0	1.0-2.5	≥ 6.5

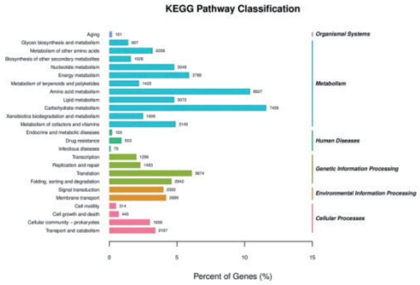
Demo Results



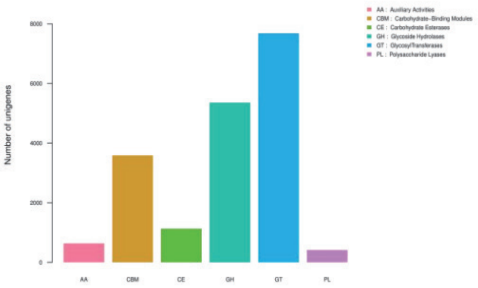
1. Relative abundance histogram



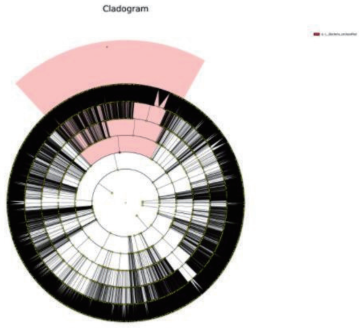
2. GO annotation



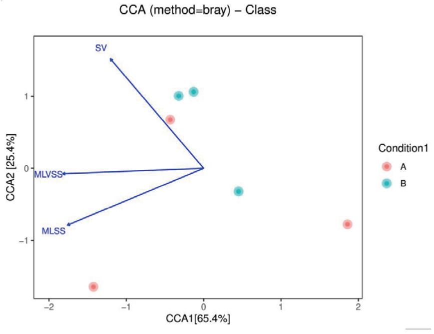
3. KEGG annotation



4. CAZy classification



5. LEfSe analysis



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