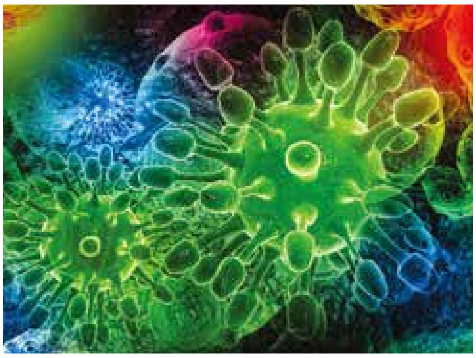
Shotgun Metagenomics



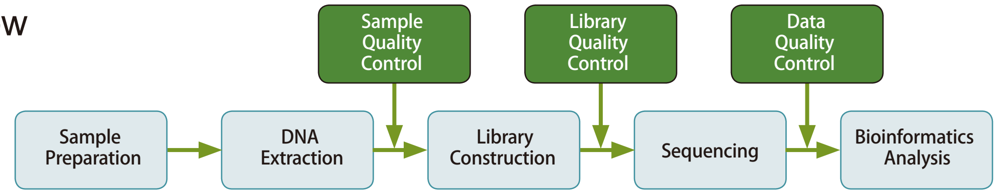
Shotgun metagenomics is a powerful technique for studying microbial communities in their natural habitat, with a broad range of applications. In shotgun metagenomics, genomes from environmental samples are analyzed without the prior isolation and cultivation of individual species.

Novogene helps you with expertise in NGS sequencing and bioinformatics analysis to explore the abundant genetic repertoire of microbial communities and to identify the species, genes, and pathways present in their samples. With shotgun metagenomics sequencing on Illumina platforms, assembly-first strategy and bioinformatics analyses, we could provide you with high-quality and publication-ready data including gene predictions, function annotations, and taxonomic annotations.

Novogene's Advantages

- Highly experienced: We have completed thousands of shotgun metagenomic sequencing and have published dozens of metagenomic studies together with our clients.
- Outstanding service: We provide high-quality sequencing, an efficient standard workflow, and bioinformatics analyses at a cost-effective price.
- Effective methodology: Our techniques enhance the generation of data from low-abundance species.
- Comprehensive analysis: Expert bioinformatics analysis with three databases (KEGG, eggNOG, CAZy) provides comprehensive data on annotated genes and metabolic pathways.

Project Workflow



Sample Quality Control

Library Quality Control

Data Quality Control

Sample Preparation → DNA Extraction → Library Construction → Sequencing → Bioinformatics Analysis

SEQUENCING STRATEGY

- 350-bp insert DNA library
- Illumina platform, paired-end 150 bp.

DATA QUALITY GUARANTEE

- Q30 ⩾ 80%.

TURNAROUND TIME

- Within 15 working days from verification of sample quality (without data analysis)
- Additional 10 working days for data analysis.

RECOMMENDED SEQUENCING DEPTH

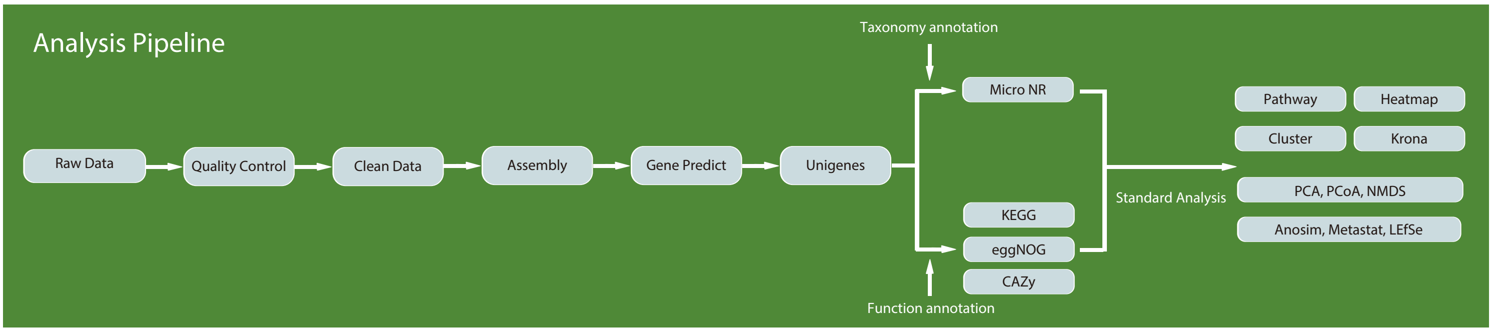
- Environmental samples: ⩾6G/sample, 3-5 biological replicates

  Biological samples: ⩾12G/sample, 3-10 biological replicates

SAMPLE REQUIREMENTS

- DNA amount: ⩾ 1.6 μg
- DNA concentration: ⩾ 30 ng/μl
- Purity: OD260/280 = 1.8 - 2.0 without degradation or RNA contamination.

Analysis Pipeline



Raw Data → Quality Control → Clean Data → Assembly → Gene Predict → Unigenes

Taxonomy annotation: Micro NR

Function annotation: KEGG, eggNOG, CAZy

Standard Analysis: Pathway, Heatmap, Cluster, Krona, PCA, PCoA, NMDS, Anosim, Metastat, LEfSe

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List of Analyses

- Our standard analysis package includes gene prediction, function annotation, and taxonomic annotation, mPATH, heatmaps, PCA, Krona, cluster analysis, MetaStats, and OG-Taxa.
- Our advanced analysis package includes MRPP, ANOSIM, NMDS (Non-metric Multidimensional Scaling), CCA/RAD, and LEFSe (LDA Effect Size).
- For other analysis, please contact your local Novogene representative or europe@novogene.com.

Demo Data

The following table shows sample data from sequencing projects conducted by Novogene. The effective rate, comparing clean data to raw data, was very high, with an average of over 94%, indicating that the base calling was highly accurate.

Sample	Insert Size (bp)	Raw Data	Clean Data	Clean Q20	Clean Q30	GC (%)	Effective Rate (%)
Test 1	300	5,491.78	5,273.46	94.06	88.54	52.17	96.03
Test 2	300	5,263.63	5,004.54	94.20	88.82	51.13	95.08
Test 3	300	5,471.88	5,090.86	93.74	87.90	54.05	93.04
Test 4	300	5,337.27	5,142.07	93.81	88.21	50.45	96.34
Test 5	300	5,781.12	5,700.68	95.97	91.90	42.47	98.61
Test 6	300	4,325.69	4,259.49	94.20	88.62	50.23	98.47

Project Example

The following study utilized Novogene's expert metagenomics services.

Impacts of the Three Gorges Dam on Microbial Structure and Potential Function.

Scientific Reports, 5:8605 (2015).

The Three Gorges Dam has significantly altered ecological and environmental conditions within the reservoir region, but how these changes affect bacterioplankton structure and function is unknown. Here, three widely accepted shotgun metagenomic tools were employed to study the impact of damming on the bacterioplankton community in the Xiangxi River. Our results indicated that bacterioplankton communities were both taxonomically and functionally different between backwater and riverine sites, which represent communities with and without direct dam effects, respectively. There were many more nitrogen cycling Betaproteobacteria (e.g., Limnohabitans), and a higher abundance of functional genes and KEGG orthology (KO) groups involved in nitrogen cycling in the riverine sites, suggesting a higher level of bacterial activity involved in generating more nitrogenous nutrients for the growth of phytoplankton. Additionally, the KO categories involved in carbon and sulfur metabolism, as well as most of the detected functional genes, also showed clear backwater and riverine patterns. As expected, these diversity patterns all significantly correlated with environmental characteristics, confirming that the bacterioplankton communities in the Xiangxi River were markedly affected by environmental changes caused by the Three Gorges Dam. This study provides the first comparative shotgun metagenomic insight into evaluating the impact of the large dam on microbial function.

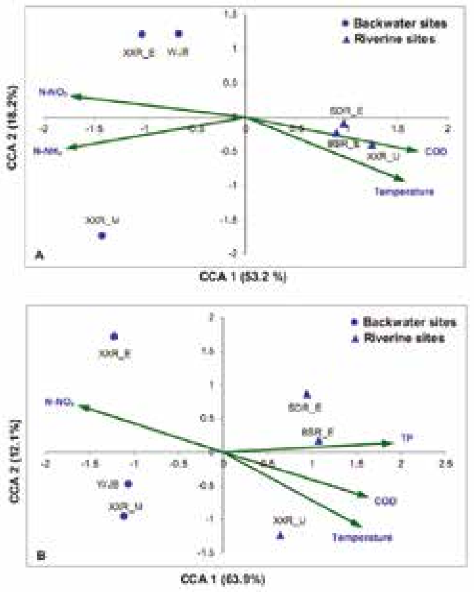

Figure 1. Canonical correspondence analysis (CCA) shows the relationships between environmental variables variables and the bacterial OTUs (A) and functional genes (B). Only variables that were significantly correlated with the community (forward selection with Monte Carlo test, P = 0.05) are shown. Abbreviations: TP, total phosphorus; N-NH4, ammonium nitrogen; N-NO3, nitrate nitrogen; COD, chemical oxygen demand.

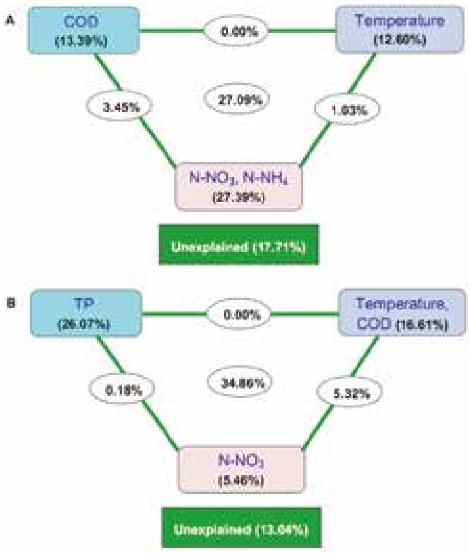

Figure 2. Variance partitioning canonical correspondence analysis (CCA) shows the relative effects of multiple variables on the composition of bacterial taxa (A) and functional genes (B). The squares represent the effect of individual variables by partitioning out the effects of the other variables.The ellipses between the squares represent the combined effects from the variables on either side of the ellipse. The combined effects of all variables are shown by the ellipse in the center. The square at the bottom of each figure represents the effect that could not be explained by any of the variables tested. Abbreviations: TP, total phosphorus; N-NH4, ammonium nitrogen; N-NO3, nitrate nitrogen; COD, chemical oxygen demand.

EXAMPLES OF PUBLICATIONS USING NOVOGENE'S EXPERTISE

Year	Journal	Article
2018	Nature Communications	Hypoxia induces senescence of bone marrow mesenchymal stem cells via altered gut microbiota
2017	Microbiome	Gut microbiota dysbiosis contributes to the development of hypertension
2016	Water Research	Changes of resistome, mobilome and potential hosts of antibiotic resistance genes during the transformation of anaerobic digestion from mesophilic to thermophilic
2015	Water Research	Metagenomic insights into Cr(VI) effect on microbial communities and functional genes of an expanded granular sludge bed reactor treating high-nitrate wastewater
2014	Environment Science and Technology	Prevalence of antibiotic resistance genes and bacterial pathogens in long-term manured greenhouse soils as revealed by metagenomic survey