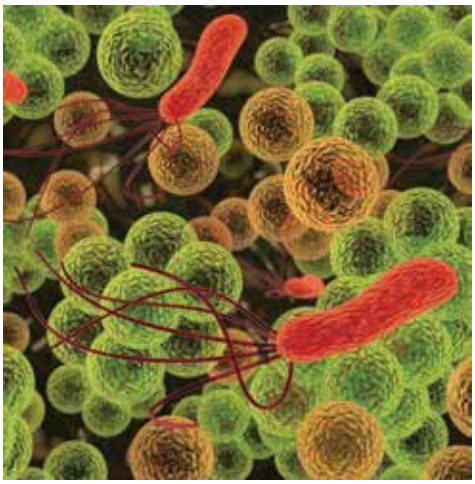
Amplicon-based metagenomic sequencing



Amplicon-based metagenomic sequencing is frequently used to identify and differentiate microbial species. Short (< 500 bp) hypervariable regions of conserved genes or intergenic regions are amplified by PCR, analyzed using NGS technology, and the resulting sequences are compared against microbial databases.

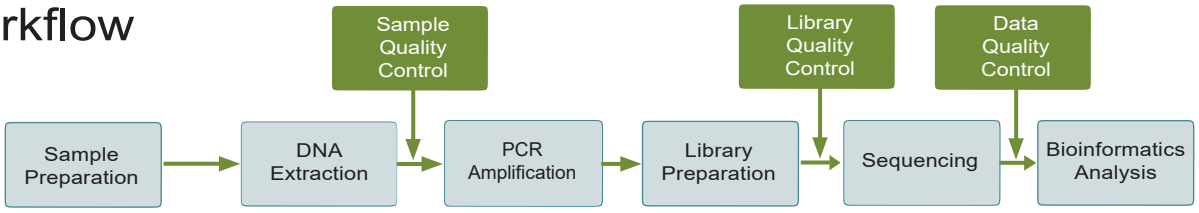
For bacteria and archaea, the 16S rRNA gene is the most common target for Amplicon-based metagenomic sequencing. For fungi, three targets are generally used: the 18S rRNA gene, and two internal transcribed spacers (ITS) located between rRNA genes. These regions are usually sufficiently divergent to separate even highly related species, and can sometimes differentiate subspecies.

At Novogene, we have sequenced over 50,000 microbial samples for our customers. Our standard bioinformatics analyses include alpha-diversity analysis, OTU analysis, species annotation, beta-diversity analysis, and multi-variate statistical analysis. Applications range from identifying a single species in pure culture to characterizing the microbiota of animals or plants to comparing species diversity and population structure in various environmental sources or geographic regions. Our specialists can advise you on the appropriate analyses for your project.

The Novogene Advantage

- **Highly experienced:** We have sequenced over 50,000 samples, and published over 80 research articles.
- **Outstanding service:** We provide high-quality sequencing, an efficient standard workflow, fast turnaround time, and bioinformatics analyses at a cost-effective price.
- **Effective methodology:** Our method features high amplification efficiency of sample DNA (> 95%) and uses PCR free libraries to avoid amplification bias.
- **Comprehensive analysis:** We provide expert bioinformatics analyses using the latest sequence databases and software, generating high-quality, publication-ready data.

Project Workflow



SEQUENCING STRATEGY

- 130-470 bp insert DNA library
- HiSeq platform, paired-end 250 bp

DATA QUALITY GUARANTEE

- The amount of data for each sample is not less than 30,000 tags, 50,000 tags or 100,000 tags.

Target	Region	Fragment Length	Primer	Primer Sequences (5'- 3')
Bacterial 16S rDNA	V4	292 bp	515F	GTGCCAGCMGCCGCGGTAA
			806R	GGACTACHVGGGTWTCTAAT
	V3-V4	466 bp	341F	CCTAYGGGRBGCASCAG
			806R	GGACTACNNGGGTATCTAAT
	V4-V5	393 bp	515F	GTGCCAGCMGCCGCGGTAA
			907R	CCGTCAATTCCTTTGAGTTT
Archaeal 16S rDNA	V4	288 bp	U519F	CAGYMGCCRCGGKAAHACC
			806R	GGACTACNSGGGTMTCTAAT
Eukaryotic 18S rDNA	V4	179 bp	528F	GCGGTAATTCCAGCTCCAA
			706R	AATCCRAGAATTTCACCTCT
	V9	131 bp	1380F	CCCTGCCHTTTGTACACAC
			1510R	CCTTCYGCAGGTTCACCTAC
Fungal ITS*	ITS1	307 bp	ITS5-1737F	GGAAGTAAAAGTCGTAACAAGG
			ITS2-2043R	GCTGCGTTCTTCATCGATGC
	ITS2	386 bp	ITS3	GCATCGATGAAGAACGCAGC
			ITS4	TCCTCCGCTTATTGATATGC

* ITS1 is located between the 18S and 5.8S rRNA genes; ITS2 is located between the 5.8S and 28S rRNA genes.

SAMPLE REQUIREMENTS

- DNA amount: ≥ 200 ng (for one library preparation*)
  * Multiple DNA samples can be mixed together to make one library construction.
- PCR product ≥ 200 ng (for one library preparation)
- DNA concentration: ≥ 20 ng/μl
- Purity: OD260/280 = 1.8 - 2.0 without degradation or contamination.
- Amplified region should be less than 470 bp

TURNAROUND TIME

- Within 15 working days from verification of sample quality (without data analysis)
- Additional 11 working days for data analysis

RECOMMENDED SEQUENCING DEPTH

- 30,000 tags, 50,000 tags, or 100,000 tags

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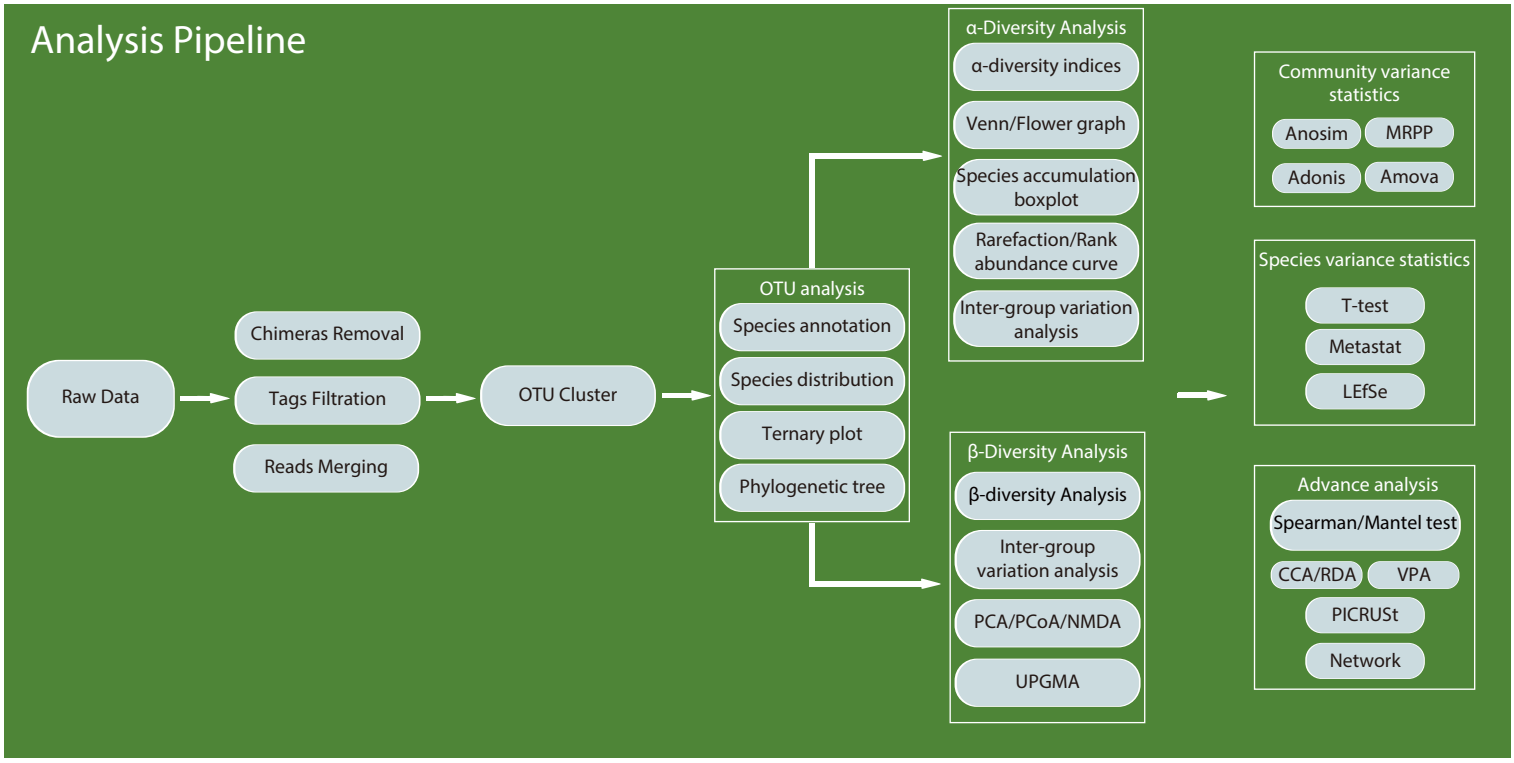
Analysis Pipeline



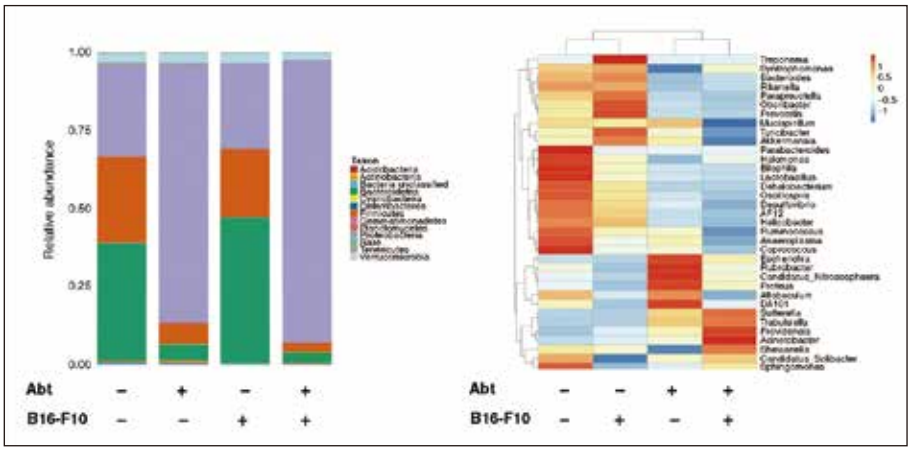
Project Example

The following study utilized Novogene's amplicon-based metagenomic sequencing services.

Microbiota modulate tumoral immune surveillance in lung through a γδT17 immune cell-dependent mechanism

Cancer Research, 74:4030 (2014)

In this study on the effects of commensal bacteria on immune homeostasis, Novogene technology was used to monitor the changes in commensal bacteria following antibiotic treatment, using stool samples from treated and untreated mice. Analysis of 16S rDNA using the Illumina MiSeq platform showed that multiple taxonomic groups were present, and that their relative abundance was altered by antibiotic treatment.



Microbiota variations after different antibiotic treatments

EXAMPLES OF PUBLICATIONS USING NOVOGENE'S SERVICES

Year	Journal	Article
2018	Soil Biology and Biochemistry	Responses of fungal–bacterial community and network to organic inputs vary among different spatial habitats in soil
2017	Frontiers in Microbiology	Effects of copper addition on copper resistance, antibiotic resistance genes, and intl1 during swine manure composting
2017	Environmental Pollution	Effects of biochar on reducing the abundance of oxytetracycline, antibiotic resistance genes, and human pathogenic bacteria in soil and lettuce
2016	Bioresource Technology	High-purity propionate production from glycerol in mixed culture fermentation
2016	Environment International	Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil
2015	Applied Microbiology and Biotechnology	Effects of aeration strategy on the evolution of dissolved organic matter (DOM) and microbial community structure during sludge bio-drying
2014	Biogeosciences	The shift of microbial population composition accompanying the injected water flowing in the water-flooding petroleum reservoirs

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