

<https://www.sanger.ac.uk/tool/wormbase/>

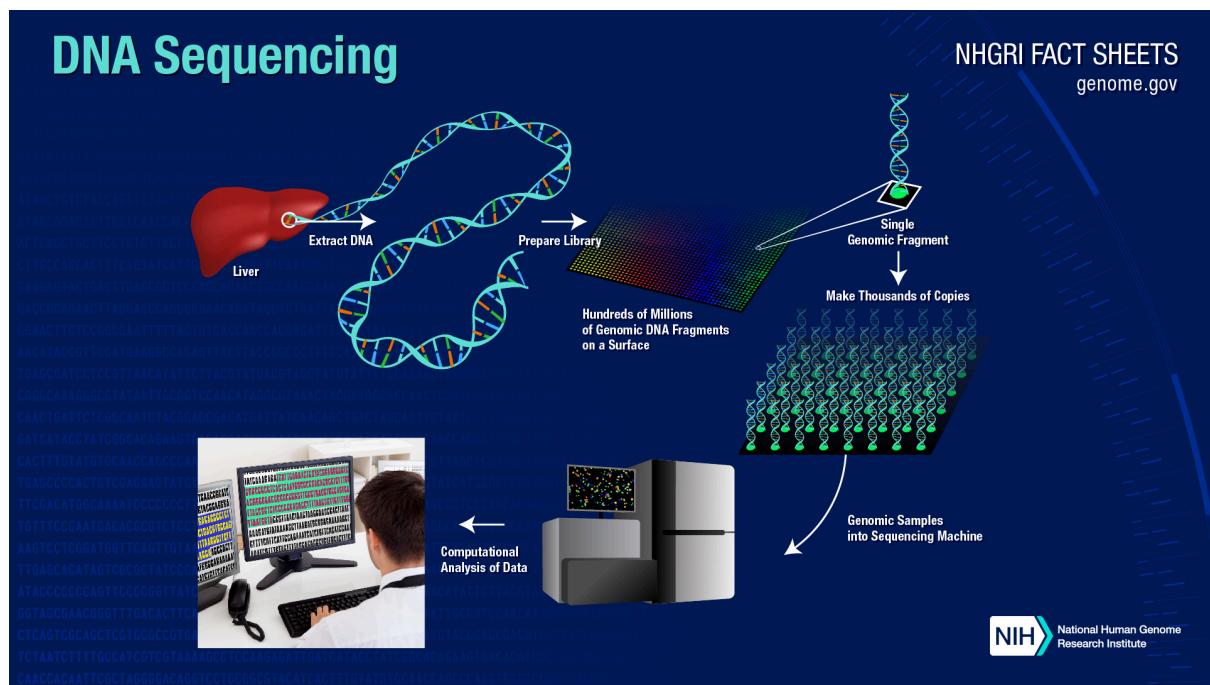
DNA sequencing is the process of determining the order of the four chemical bases (A, T, C, G) that make up DNA. This sequence reveals genetic information, helping scientists identify genes, regulatory regions, and disease-causing mutations.

In the DNA double helix, bases always pair in a specific way: adenine (A) with thymine (T) and cytosine (C) with guanine (G). This pairing allows DNA to be copied during cell division and forms the basis of sequencing techniques. The human genome has about 3 billion base pairs that guide human development and maintenance.

Since the Human Genome Project, advancements in technology have made DNA sequencing faster and cheaper. It's now common to sequence individual genes, and labs can sequence large amounts of DNA each year at a lower cost. The goal is to eventually sequence a human genome for under \$1,000.

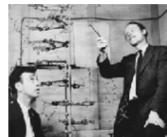
New sequencing technologies are being developed, including methods that track DNA polymerase with a fast camera and dye colors, and nanopore sequencing, where DNA strands pass through tiny pores, identifying bases based on their electrical effects.

Improved sequencing methods are advancing human health by enabling rapid comparisons of DNA, revealing links between genetics, diseases, and environmental factors. For example, in cancer care, sequencing helps doctors choose the best treatments based on a patient's specific genetic information. DNA sequencing is also being used to identify genetic causes of rare diseases, screen newborns, and study common diseases like heart disease and diabetes. Comparing DNA across species helps scientists understand development and evolution.



A quick history of sequencing

1869 – Discovery of DNA
1909 – Chemical characterisation
1953 – Structure of DNA solved
1977 – First genome ($\Phi X 174$) Sequencing by synthesis (Sanger) - Sequencing by degradation (Maxam- Gilbert)
1986 – First automated sequencing machine 1990
– Human Genome Project started
1992 – First “sequencing factory” at TIGR
1995 – First bacterial genome – *H. influenzae* (1.8 Mb) 1998 – First animal genome – *C. elegans* (97 Mb)
2003 – Completion of Human Genome Project (3 Gb)
– 13 years, \$2.7 bn
2005 – First “next-generation” sequencing instrument
2013 – 10,000 genome sequences in NCBI database



This article explains seven key methods used for DNA sequencing:

Sanger's Method
Maxam and Gilbert Method
Hybridization Method
Pal Nyren's Method
Automatic DNA Sequencer
Slab Gel Sequencing Systems
Capillary Gel Electrophoresis

DNA sequencing determines the exact order of nucleotides in DNA. Before direct sequencing methods, the process was indirect, requiring DNA to be converted to RNA. This method was slow and could only sequence short DNA strands. In the 1970s, researchers like Walter Gilbert and Alan Maxam developed ways to sequence DNA more directly, with the Lac operator being the first long sequence determined.

With advances in sequencing technology, from traditional methods to newer techniques like pyro-sequencing, sequencing has become faster and cheaper, allowing for detailed genetic studies. Pyrosequencing, a key method, is based on DNA synthesis, generating light signals to determine sequences in real time.

Key Methods:

1. **Sanger's Method (Dideoxy Method)**: This method uses modified nucleotides (dideoxynucleotides) that stop DNA strand elongation, allowing scientists to determine the DNA sequence based on the length of resulting fragments. It is a popular and widely used sequencing method.

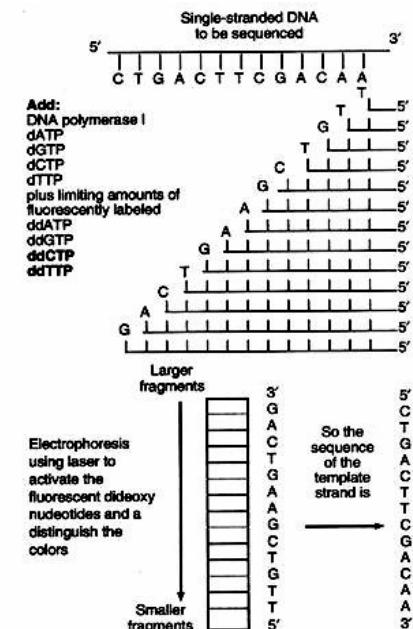
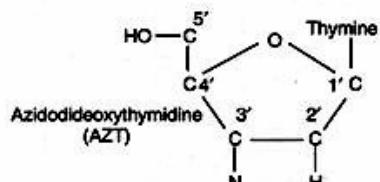
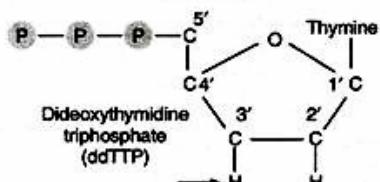
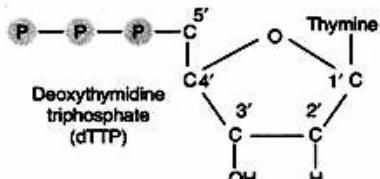


Fig. 23.7. Structure of dideoxynucleotides.

Fig. 23.8. Sanger's method of DNA sequencing.

- Maxam and Gilbert Method:** This technique uses chemical reactions to cleave DNA at specific bases. The fragments are then separated by size and analyzed. However, it's less popular due to its use of hazardous chemicals and slower processing time.
- Hybridization Method:** This method involves hybridizing DNA to arrays of short sequences (oligonucleotides), then using a computer to analyze the pattern. It's used for detecting specific DNA sequences but can have ambiguities.
- Pal Nyren's Method (Pyrosequencing):** Pyrosequencing uses an enzymatic reaction to generate light signals that correspond to nucleotide incorporation during DNA synthesis, offering real-time results. It's efficient and has been improved for high-throughput sequencing.
- Automatic DNA Sequencer:** This automated version of the Sanger method uses fluorescent dyes to label nucleotides, allowing for easier detection and faster sequencing. It uses capillary or slab gel electrophoresis to separate DNA fragments.

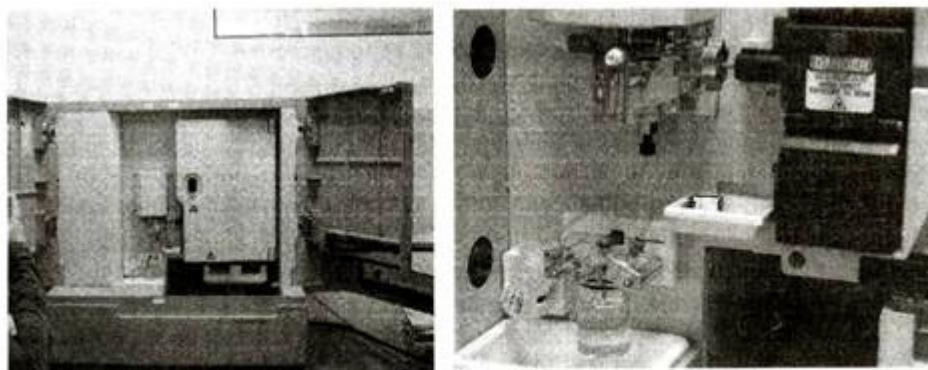


Fig. 23.9. Automated DNA sequencer (left) and details of sample loop (right).

6. **Slab Gel Sequencing Systems:** These systems use thin gel slabs and automated sample loading to sequence DNA faster and more efficiently, allowing multiple samples to be processed at once.
7. **Capillary Gel Electrophoresis:** This technique uses capillary tubes instead of gels, allowing for continuous sample processing and more efficient sequencing. It requires less manual intervention and provides a steady flow of data.

Additionally, DNA sequencing has been instrumental in sequencing the genomes of various organisms, including the human genome, model organisms, and plants. As technology advances, sequencing continues to progress, enabling more in-depth studies of genetics.

Genomic DNA Sequencing is a method used to examine the full genetic material of an organism. It helps scientists understand the genetic information, how genes relate to diseases and traits, and the biological processes behind evolution. This technique is vital for biological research.

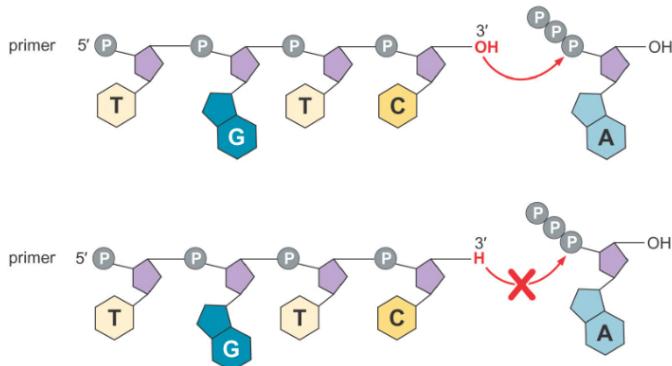
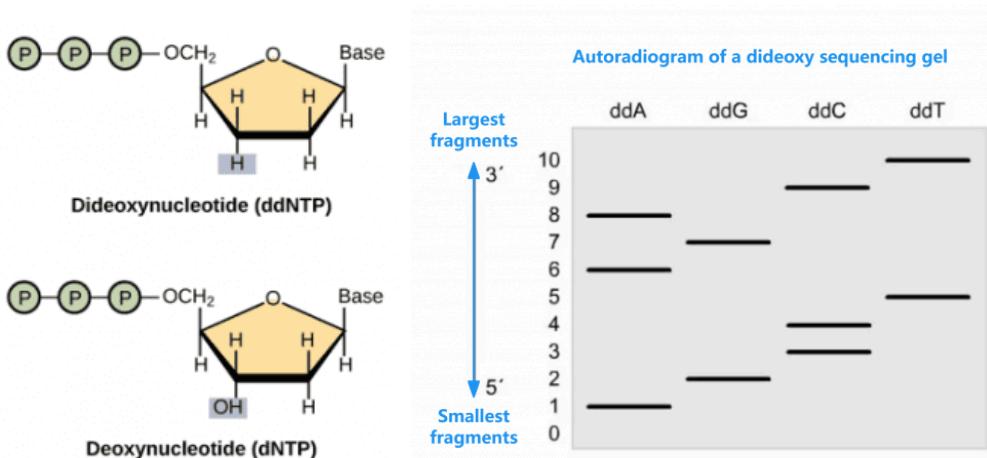
Methods of Genomic DNA Sequencing:

1. **Sanger Sequencing:** This traditional method is widely used in clinical diagnostics. It works by adding specific nucleotides to a template DNA strand, stopping at a terminator to end the process.

What is Sanger Sequencing?

Sanger sequencing, developed by Frederick Sanger in 1977, is a method for determining the order of nucleotide bases in a DNA strand, typically for smaller DNA fragments (less than 1,000 base pairs). It's highly accurate, with 99.99% base accuracy, making it the "gold standard" for validating DNA sequences, even those obtained through newer methods like next-generation sequencing (NGS). It was used in the Human Genome Project to sequence small DNA fragments.

In the presence of the four deoxynucleotide triphosphates (dNTPs: A, G, C, and T), the polymerase extends the primer by adding the complementary dNTP to the template DNA strand. To determine which nucleotide is incorporated into the chain of nucleotides, four dideoxynucleotide triphosphates (ddNTPs: ddATP, ddGTP, ddCTP, and ddTTP) labeled with a distinct fluorescent dye are used to terminate the synthesis reaction. Compared to dNTPs, ddNTPs have an oxygen atom removed from the ribonucleotide, hence cannot form a link with the next nucleotide. Following synthesis, the reaction products are loaded into four lanes of a single gel depending on the diverse chain-terminating nucleotide and subjected to gel electrophoresis. According to their sizes, the sequence of the DNA is thus determined.

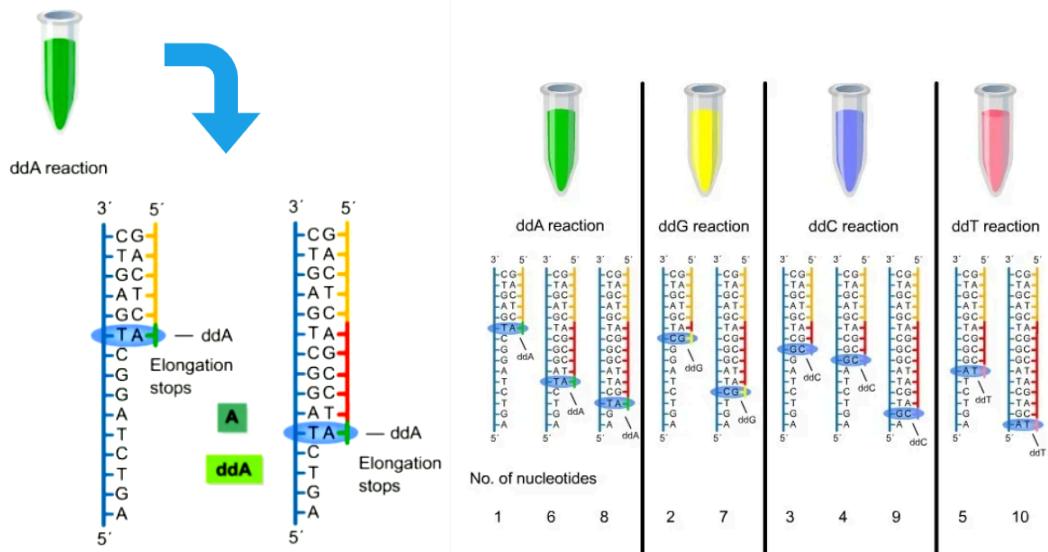


If the template DNA sequence is 5'-AGTCTAGGCTGAGTC-3' and the primer is 5'-GACTC-3', the sequencing process could generate sequences like 5'-GACTCAGCCT-3' or 5'-GACTCAGCCTAGACT-3', depending on where the synthesis stops at the T bases of the template strand (where ddT is used).

It's important to use the correct amount of ddT, because too much or too little can affect where the DNA strands stop. If too much ddT is used, most strands will stop early, leading to an incorrect pattern. This can cause issues in identifying the correct positions of T bases in the DNA sequence.

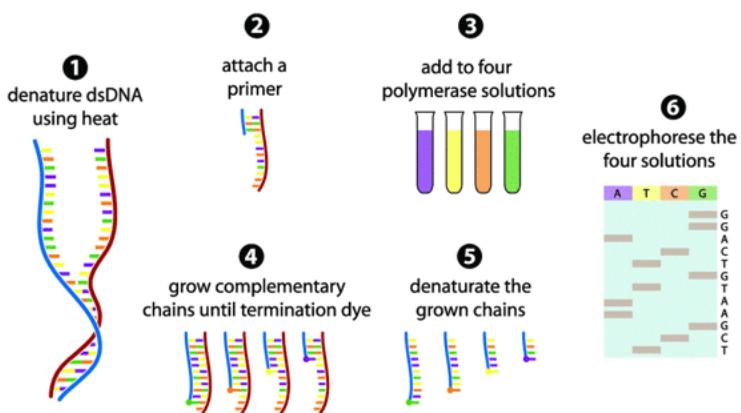
Once the DNA strands are created, they are analyzed using gel electrophoresis to determine the sequence. The lengths of the fragments help pinpoint where the adenine (A) bases match the thymine (T) bases in the template strand.

In the Sanger method, four types of dideoxynucleotide triphosphates (ddNTPs) are added, which stop the DNA synthesis at specific points, allowing the sequence to be determined based on where these stops occur.



How Sanger Sequencing Works:

- DNA is split into two single strands.
- A primer is added to one strand to start DNA synthesis.
- Four types of normal nucleotides (dNTPs) and four special terminator nucleotides (ddNTPs) are added. The ddNTPs stop DNA synthesis.
- The DNA is synthesized, and the process randomly stops when a ddNTP is added.
- The fragments are separated by size using gel electrophoresis.
- The sequence is determined by reading the order of these fragments.



Sanger sequencing is still widely used for specific tasks like gene cloning, SNP identification, and validating other sequencing methods.

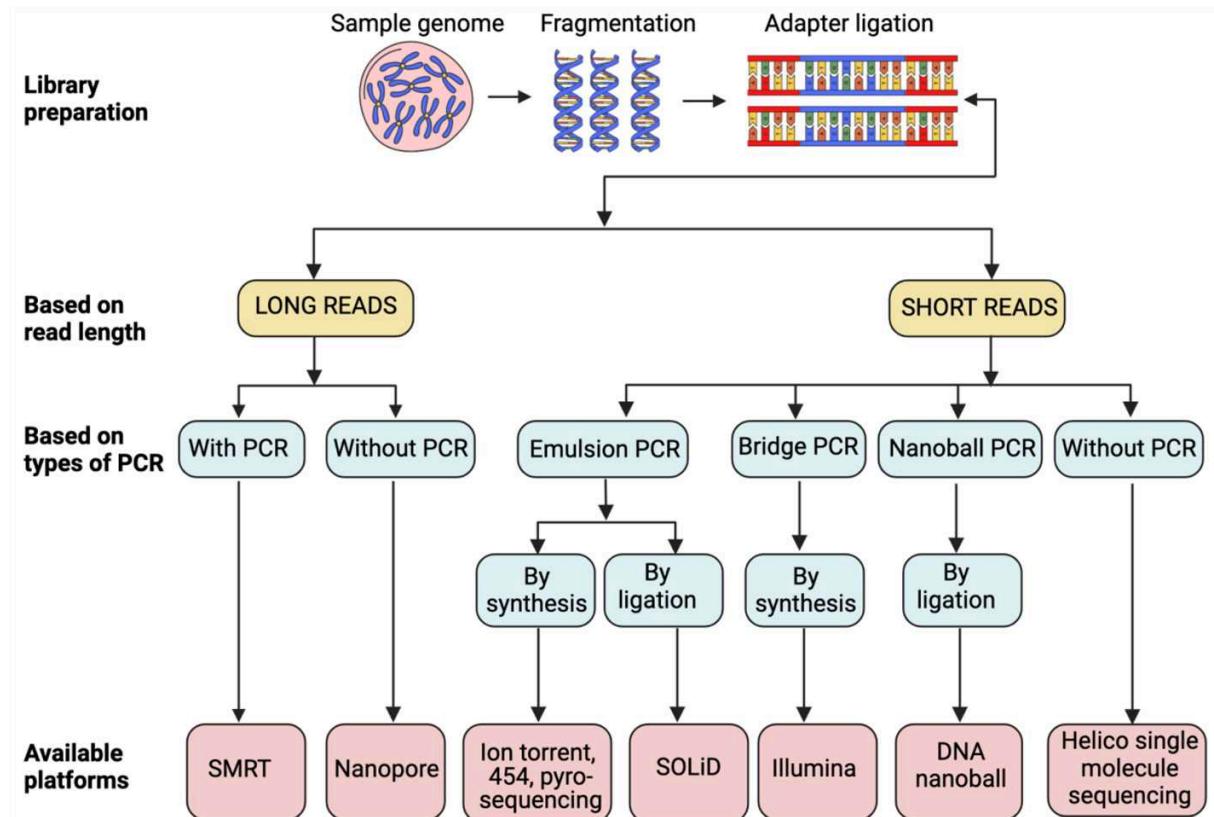
Sanger Sequencing vs. NGS:

Sanger Sequencing: Best for small DNA fragments or single gene sequencing. It's more accurate but slower and more expensive for large-scale projects.

NGS: Allows sequencing of whole genomes or many genes at once, much faster and more

cost-effective for large projects but less accurate per read.

2. **Next-Generation Sequencing (NGS):** NGS technologies, like Illumina sequencing, are more efficient, faster, and cost-effective than Sanger sequencing. They can process millions of DNA sequences simultaneously, making them popular in research and diagnostics. NGS has expanded the use of DNA sequencing in fields like medicine and forensics, though it requires strong computational tools due to short sequence lengths.



Transcriptome Sequencing Overview

Transcriptome sequencing is the study of all RNA molecules in a cell, including mRNA, rRNA, tRNA, and non-coding RNA. It helps in understanding gene expression, mutations, and alternative splicing events by cataloging RNA transcripts. This method is more advanced than older techniques like microarrays, offering higher accuracy and resolution, especially for studying early development, cellular changes, gene variations, and biomarker discovery.

Transcriptome Sequencing Process:

1. **RNA Isolation:** Total RNA is extracted from the sample.
2. **Library Preparation:** RNA is enriched (e.g., ribosomal RNA depletion or poly-A selection), fragmented, and converted to cDNA for sequencing.

3. **Sequencing:** High-throughput sequencing is done using platforms like Illumina or PacBio, with long-read strategies providing full-length isoforms without assembly.
4. **Bioinformatics Analysis:** Data is processed, including quality control, alignment to reference genomes, and gene expression quantification.

Types of Transcriptomics Services:

1. **RNA-Seq:** Identifies RNA presence, expression levels, gene fusions, SNPs, and alternative splicing.
2. **Small RNA Sequencing:** Analyzes miRNA expression and function, identifies novel miRNAs, and predicts mRNA targets.
3. **LncRNA Sequencing:** Analyzes both long non-coding RNAs and mRNAs in one sequencing run.
4. **CircRNA Sequencing:** Focuses on circular RNAs with single-base resolution.
5. **Degradome Sequencing:** Studies RNA degradation and miRNA cleavage sites.
6. **Bacterial RNA Sequencing:** Analyzes bacterial gene expression.
7. **Ribosome Profiling:** Investigates translation by sequencing ribosome-protected mRNA fragments.
8. **Total RNA-Seq:** Analyzes both coding and non-coding RNAs for a complete transcriptome view.
9. **Targeted RNA-seq:** Measures differential expression, allele-specific expression, and gene fusions.
10. **Exosomal RNA Sequencing:** Investigates RNA in exosomes.
11. **Ultra-Low Input RNA Sequencing:** Works with very small RNA samples, ideal for limited-cell samples.
12. **Dual RNA-seq:** Studies interactions between host and pathogen RNA.

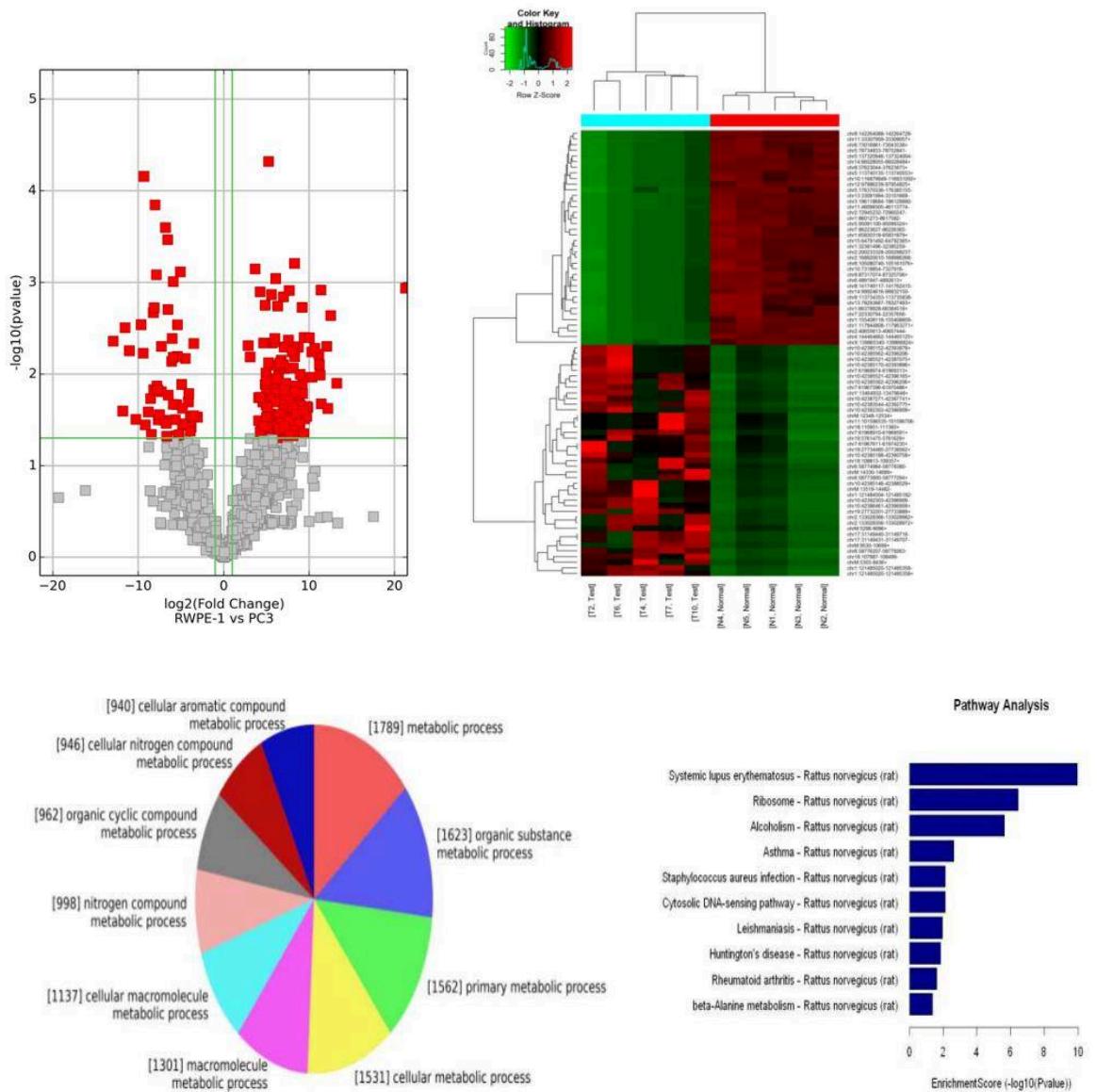
Service Advantages:

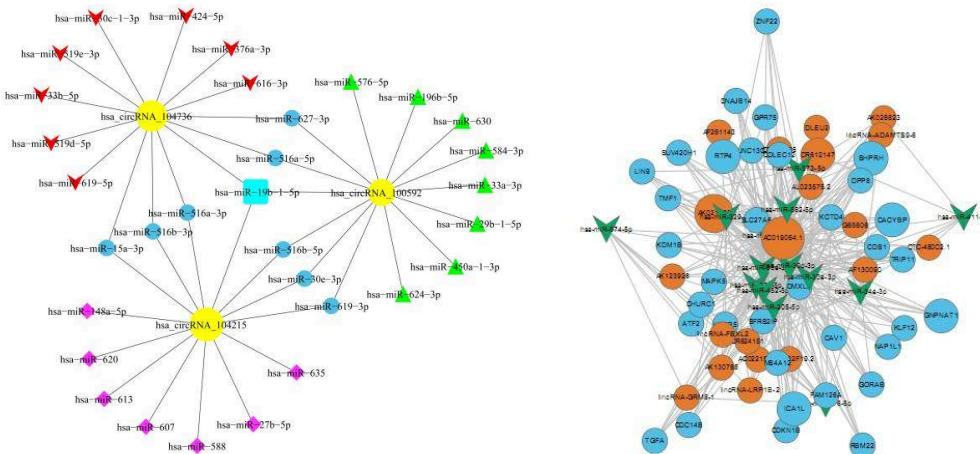
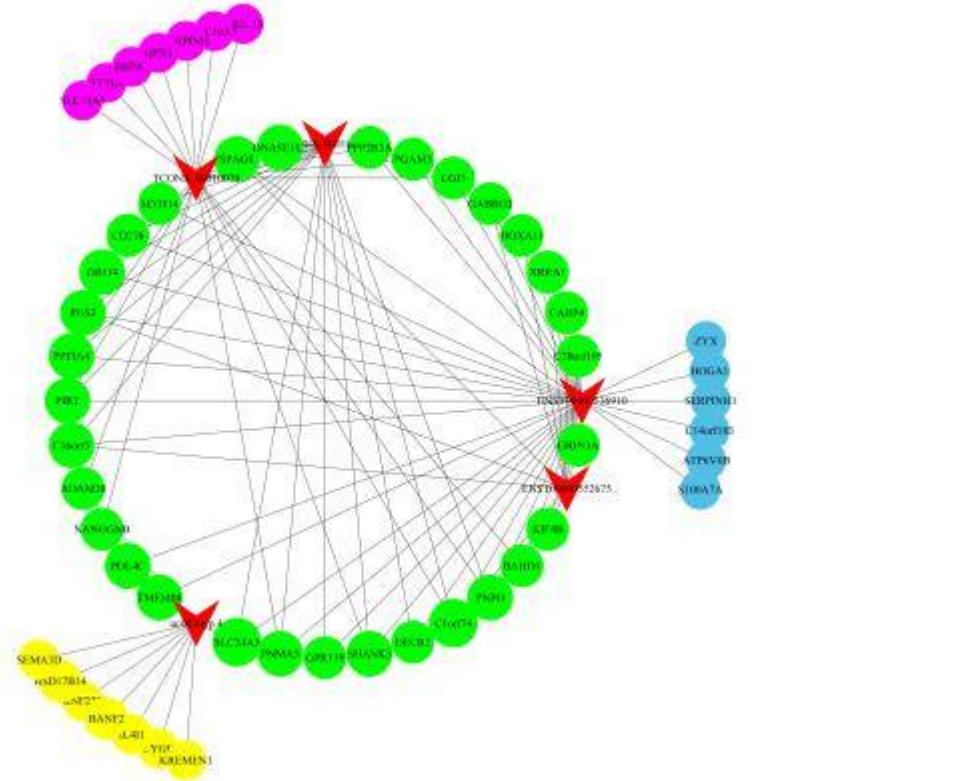
- **Comprehensive RNA Detection:** Uses rRNA depletion kits to retain all RNA types, including non-poly(A) RNA.
- **Suitable for Degraded Samples:** Works with degraded samples using specialized kits.

- **Strand-Specific Detection:** Detects antisense RNAs that traditional methods miss.
- **Specialized Bioinformatics:** Offers in-depth data analysis by a dedicated bioinformatics team.
- **High Resolution:** Identifies subtle gene differences and single-nucleotide variations.
- **Broad Detection Range:** Accurately quantifies both abundant and rare RNA transcripts.

Data Analysis:

- **Differential Expression:** Identifies significant changes in mRNA, circular RNA, and long non-coding RNA with statistical thresholds (Fold Change ≥ 2 , P-value ≤ 0.05).

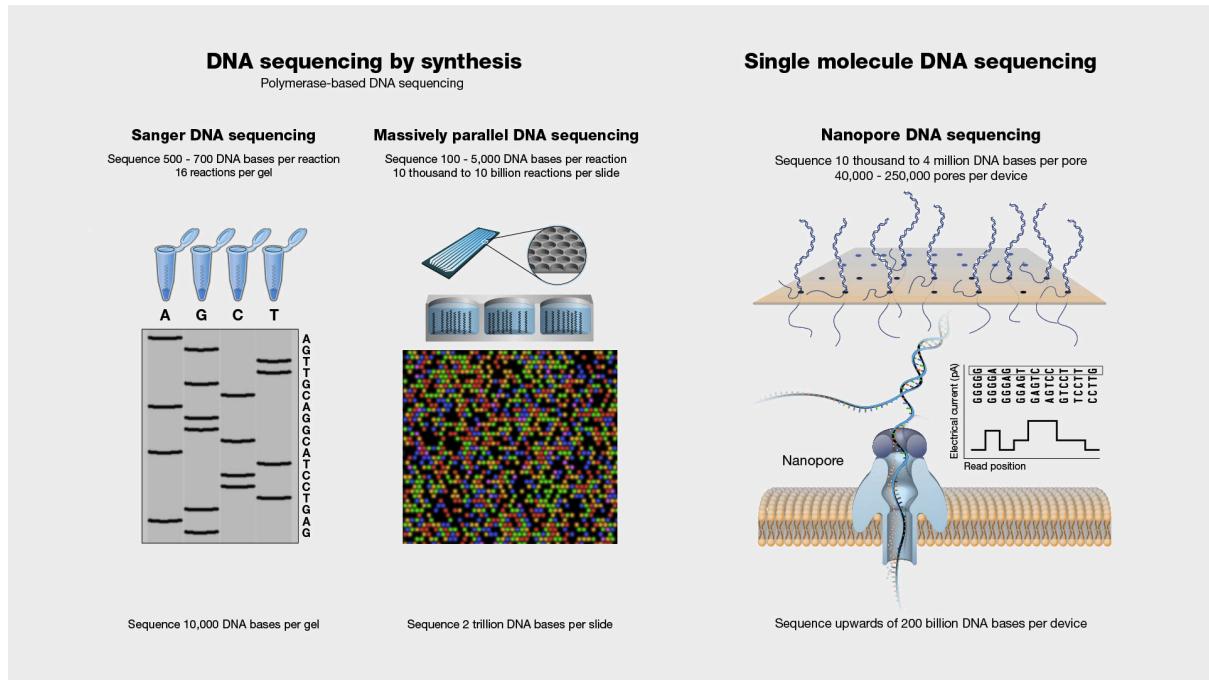




Certain circRNA/LncRNA molecules can regulate the expression of miRNA target genes by binding to miRNAs. Through the analysis of circRNA/LncRNA-miRNA-mRNA associations, we can assist in deducing the circRNA/LncRNA molecules acting as miRNA sponges and their mechanisms of action.

3. **Single-Molecule Sequencing:** This newer method, including Single-Molecule Real-Time (SMRT) and nanopore sequencing, excels at reading longer DNA sequences without needing amplification. It's becoming more popular for its ability to

read long stretches of DNA.



These sequencing technologies help scientists explore genetic links to diseases, traits, and biological evolution.

What is Single-Cell Sequencing?

Single-cell sequencing allows scientists to study individual cells at the genomic, transcriptomic, epigenomic, and proteomic levels, offering insights into how cells function and interact. Unlike traditional bulk sequencing, which averages data from many cells, single-cell sequencing reveals the unique molecular profiles of each cell, highlighting the complexity within tissues or organisms.

Why Perform Single-Cell Sequencing?

Traditional RNA sequencing averages gene expression across many cells, which can miss important variations within a population, such as in tumors. Single-cell RNA sequencing (scRNA-seq) examines these differences at the individual cell level, allowing researchers to track cancer cell evolution, metastasis, drug resistance, and other factors that influence disease progression.

Methods for Single-Cell Sequencing

- **Microplate-Based Sequencing:** Individual cells are placed in wells of a microplate, where they release their genetic material to be captured by magnetic beads. These beads are then sequenced.
 - **Microdroplet-Based Sequencing:** Cells are encapsulated in tiny droplets with capture beads, which capture their genetic material for sequencing.

- **10x Genomics:** A leading platform that offers solutions for various types of single-cell sequencing, including RNA sequencing and immune profiling.

Our Single-Cell Sequencing Services

We use advanced technologies, including 10x Genomics Chromium, to provide detailed analysis of individual cells, tumor heterogeneity, and developmental processes. Services include:

- Single-Cell RNA Sequencing
- Single-Cell DNA Sequencing
- Single-Cell DNA Methylation Sequencing

Advantages of Single-Cell Sequencing

- **Comprehensive Workflow:** Complete analysis of the entire transcriptome of individual cells.
- High Throughput:** Process up to 96 cells at once.
- Cost-Effective:** One of the most affordable methods for single-cell sequencing.
- Cutting-Edge Technology:** Use of advanced platforms like 10x Genomics ensures reliable results.
- Bioinformatics.**

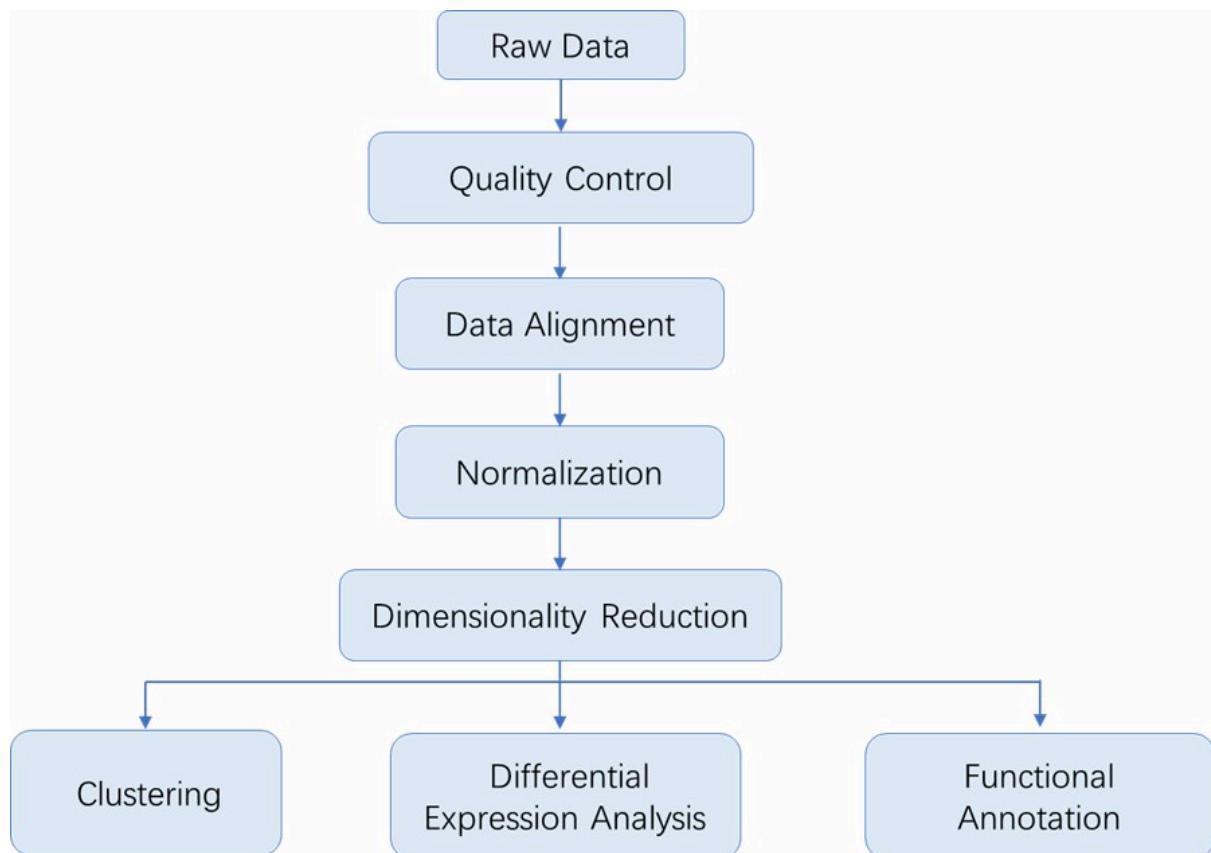
Applications of Single-Cell Sequencing

- Profiling rare clinical samples
- Studying tumor heterogeneity and chemotherapy responses
- Investigating immune cell diversity
- Analyzing developmental biology and neurological disorders
- Exploring microbial ecosystems

Single-Cell Sequencing Workflow

The process involves isolating single cells, amplifying their DNA or RNA, and sequencing. This enables detailed profiling of each cell's genetic material, providing insights into cellular functions.





Sample Requirements

- **ScRNA-seq:** 2×10^6 cells (minimum 1×10^6 cells)
- **10X Visium Spatial Transcriptome:** Tissue samples (6.5mm x 6.5mm)
- **Single-Cell Genome Sequencing:** Requires cells stored in 1xPBS buffer.
- <https://www.cd-genomics.com/single-cell-sequencing.html>

