

NGS Revolution Overview

Sanger (Traditional)

Throughput	~1 Kb/day
Cost per Mb	~\$500,000
Parallelization	Single reaction
Time	Hours-Days

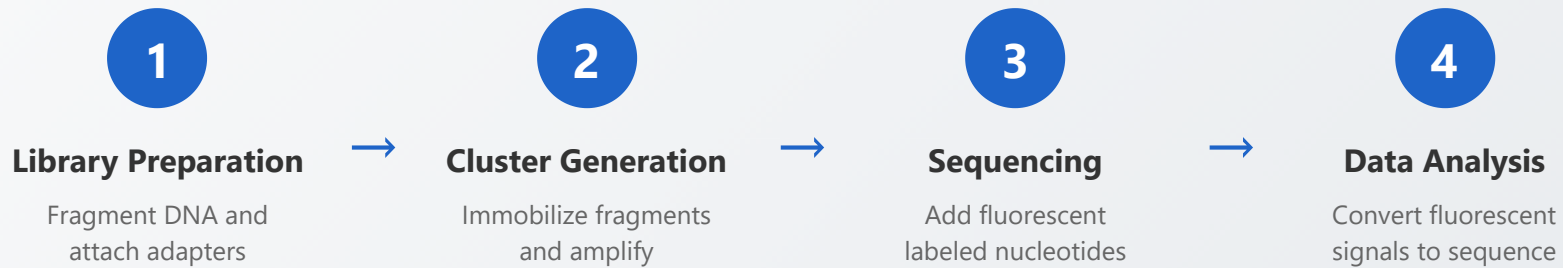
NGS (Next-Gen)

Throughput	~1 Tb/run
Cost per Mb	~\$0.01
Parallelization	Millions of reads
Time	Hours-Days

NGS Key Advantages

- ✓ Massive parallelization - sequence millions of fragments simultaneously
- ✓ Cost-effective - made genome sequencing affordable (\$1000 genome)
- ✓ High throughput - entire human genome in 1-2 days
- ✓ Comprehensive - discover novel variants and structural changes
- ✓ Versatile - DNA, RNA, epigenetic, metagenomic applications

NGS Principles (Sequencing by Synthesis)



Sequencing by Synthesis Process

Cycle 1

Template: 5'-ATCGTAGC-3'

Growing: 3'-T

Red Signal (A)

Cycle 2

Template: 5'-ATCGTAGC-3'

Growing: 3'-TA

Green Signal (T)

Cycle 3

Template: 5'-ATCGTAGC-3'

Growing: 3'-TAG

Cycle 4

Template: 5'-ATCGTAGC-3'

Growing: 3'-TAGC

○ Yellow Signal (G)

● Blue Signal (C)

Detailed Principle Explanation



Library Preparation

DNA Fragmentation: Genomic DNA is fragmented into 200-600bp sizes using physical or enzymatic methods.

Adapter Ligation: Universal primer sequences (adapters) are attached to both ends of fragments by ligation.

Size Selection: Optimal-sized fragments are selected using gel electrophoresis or bead-based methods.



Cluster Generation

Surface Attachment: DNA fragments bind to oligonucleotides immobilized on the flow cell surface.

Bridge PCR: Single DNA molecules are amplified to millions of copies at the same location.

Cluster Formation: Each fragment forms an independent cluster, amplifying the signal for detection.



Sequencing Chemistry

Reversible Terminators: dNTPs labeled with specific fluorophores and 3' blockers are added to each base.

Imaging: Fluorescent signals from each cluster are detected and imaged using lasers.

Cleavage and Repeat: Fluorophores and blockers are chemically removed, then the next cycle repeats.



Data Processing

Base Calling: Fluorescent signal intensity and color from each cycle are analyzed and converted to ACGT sequences.

Quality Score: Accuracy of each base is calculated using Phred scores to assess reliability.

Alignment: Reads are aligned to reference genomes and variants (SNPs, Indels, etc.) are analyzed.

