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)

1. **Library Preparation** — Fragment DNA and attach adapters
2. **Cluster Generation** — Immobilize fragments and amplify
3. **Sequencing** — Add fluorescent labeled nucleotides
4. **Data Analysis** — Convert fluorescent signals to sequence

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.