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The bionivid Science Blog

A BEGINNER'S GUIDE TO SEQUENCING PLATFORMS

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– Team Molecular Biology Lab, Bionivid

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In the world of genomics, sequencing technology has transformed science, medicine, and even agriculture. But with so many platforms available, it can be overwhelming to understand which technology suits your needs. Let's break down the most popular DNA sequencing platforms: Illumina, Pacific Biosciences (PacBio), and Oxford Nanopore Technologies (ONT).

Technological Divergence

The reason behind the variety of sequencing platforms lies in the trade-offs between speed, accuracy, cost, and read length. Different research goals require different capabilities as some demand ultra-high accuracy, while others benefit from longer reads or rapid, portable sequencing. As a result, companies have developed specialised technologies to meet these diverse needs, driving innovation and diversity in the sequencing landscape.

Illumina: The Giant of Short-Read Sequencing

When people think about modern DNA sequencing, Illumina is usually the first name that comes to mind. Dominating the field for over a decade, Illumina's Next-Generation Sequencing (NGS) platforms have made high-throughput and high-accuracy sequencing widely accessible.

How it works

Illumina uses a method called sequencing by synthesis. DNA fragments are attached to a flow cell, amplified to form clusters, and sequenced one base at a time as fluorescent signals are detected.

Popular Illumina platforms

- **MiSeq:** Great for small projects (e.g., targeted panels, small genomes).
- **NextSeq:** Mid-throughput, ideal for exomes or transcriptomes.
- **NovaSeq:** Ultra-high throughput, perfect for population-scale genome projects.

MiSeq

NextSeq

NovaSeq

PacBio: Long Reads with High Accuracy

Pacific Biosciences (PacBio) is best known for its Single Molecule, Real-Time (SMRT) sequencing technology. Unlike Illumina, PacBio specializes in long reads, which can span thousands to tens of thousands of base pairs.

How it works

PacBio uses SMRT (Single Molecule Real-Time) sequencing to track DNA synthesis in real time, capturing fluorescent signals from nucleotides within a zero-mode waveguide (ZMW).

Recent innovation

PacBio's HiFi sequencing combines long reads with high accuracy (>99.9%), solving one of the traditional limitations of long-read technologies.

Popular PacBio platforms

- **Revio:** Scalable, next-gen platform delivering cost-efficient HiFi reads for large-scale genomic studies.
- **Sequel IIe:** Produces highly accurate long reads, ideal for genome assembly and transcriptomics.
- **RS II:** Early SMRT platform, still used in niche or legacy long-read sequencing applications..

Revio

Sequel IIe

RS II

ONT: Ultra-Long Reads and Portability

Oxford Nanopore Technologies (ONT) offers a radically different approach to DNA sequencing — and it's changing the game with its unique portable devices and ultra-long reads.

How it works

DNA strands are passed through a nanopore (a tiny hole), and the changes in ionic current are measured to determine the sequence.

Recent innovation

Oxford Nanopore's Q20+ chemistry significantly improves raw read accuracy (~98–99%), addressing earlier concerns and enabling more reliable ultra-long read sequencing.

Popular Illumina platforms

- **GridION:** Higher throughput for labs.
- **MinION:** A pocket-sized sequencer you can plug into a laptop.
- **PromethION:** Industrial-scale sequencing.

GridION

MinION

PromethION

Comparative Overview

	Why Choose?	Limitations
Illumina	• Extremely accurate (error rates ~0.1%). • Cost-effective for large numbers of short reads. • Versatile: works for everything from RNA-seq to whole genome sequencing.	• Short read lengths (usually 150–300 bp). • Struggles with repetitive regions or large structural variants.
PacBio	• Long reads (average ~15–20 kb, can reach >50 kb). • Exceptional accuracy with HiFi reads. • Best for genome assembly, structural variant analysis, and full-length RNA sequencing.	• Higher cost per Gb compared to Illumina. • Lower throughput compared to Illumina for large-scale projects.
ONT	• Ultra-long reads (can reach over 1 million base pairs!). • Portability: Sequence anywhere — even in the jungle or on a space station. • Real-time sequencing: Start analyzing data immediately as it's generated. • Direct RNA sequencing: Capture native RNA without needing reverse transcription.	• Historically, lower raw accuracy (although now improved to ~98–99% with newer chemistries like Q20+). • More susceptible to biases in homopolymer regions (e.g., runs of 'AAAA').

Final Thoughts:

Which Sequencing Platform is Right for You?

- For precision and scalability in short-read data, Illumina remains the gold standard. It's ideal for high-throughput projects like population genetics, transcriptomics, and exome sequencing where accuracy and cost-efficiency matter.

- For high-accuracy long reads, PacBio excels. It's the platform of choice for assembling complex genomes, detecting structural variants, and analysing full-length transcripts.

- For flexibility, real-time analysis, and ultra-long reads, Oxford Nanopore Technology offers a uniquely portable and adaptable solution, suited to in-field sequencing, metagenomics, and regions inaccessible to short-read technologies.

Ultimately, the choice of platform depends on your research question, budget, and desired read characteristics. In fact, many cutting-edge projects now combine Illumina and PacBio or ONT data to get the best of both worlds: short-read accuracy with long-read structural insights.

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