CD Genomics
The Genomics Services Company

Long-Read Sequencing: PacBio SMRT vs. Oxford Nanopore

Overview

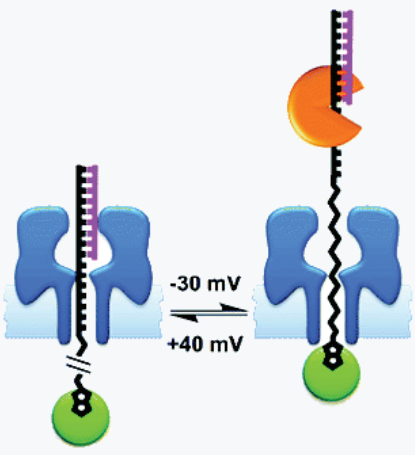
The rapid development of genome sequencing methods over the past few decades has advanced the fields of modern biology and medicine. **Long-read sequencing technologies**, also known as the **third-generation sequencing**, has contributed to this advancement, overcoming the limitations of the inability to fully accomplish complete genome assembly from short reads (100-700 bp in length) by the second-generation sequencing (SGS or next-generation sequencing) technologies in some situations, while maintaining the advantages of the SGS, such as **high throughput**, **cost-efficient** and **accurate**. With the ability to **produce much longer reads**, long-read technologies eliminate the needs for massive computational calculations of genome assembly, transcriptome analysis, and metagenomics. With the ability to **detect epigenetic markers directly**, long-read technologies provide competitive alternations for epigenomic studies. With **excellent portability and speed**, long-read technologies can be applied to even more research scenarios …

Here, we compare two of the most representative and widely used long-read sequencing platforms offered by **Pacific Biosciences (PacBio)** and **Oxford Nanopore Technologies (ONT)**, including the mechanisms, key features, benefits, shortcomings, and applications, to give you the basic idea on how to choose the most suitable long-read sequencing method for your projects.

PacBio SMRT

PacBio Single-Molecule Real-Time (SMRT) Sequencing is a real-time sequencing which captures sequence information during the replication process and adopts a sequencing-by-synthesis strategy that captures a single DNA molecule. **SMRTbell**, the template DNA in SMRT-Seq technology, is a closed single-stranded circular DNA that is prepared by ligating hairpin adaptors to both ends of a dsDNA molecule. SMRTbell is then loaded onto a specialized chip called a SMRT cell that contains numerous sequencing units known as **zero-mode waveguides (ZMWs)**, where the fluorescent light emission provides the detection of single nucleotides being added by the DNA polymerase. SMRT-Seq could generate high-quality single-molecule consensus sequences of cDNA, amplicons, and contiguous sequence reads with an average read length of 10-15 kbp. The long reads and highly contiguous assembly make it well-suited for genomic (e.g. de novo assembly, structural variant discovery), transcriptomic (e.g. gene isoform reconstruction, novel isoform discovery), and epigenetic studies.

Nanopore



Nanopore sequencing is a single-molecule sequencing technology using the basic principle that each nucleotide has a different size and different electrical properties. **PromethION** (and other nanopore sequencers such as **MinION** and **GridION**) directly sequences single-stranded DNA or RNA molecules in massive parallel by measuring characteristic current changes as the bases are guided across a membrane by a molecular motor protein through the nanopores within a flow cell. This technology uses a hairpin adaptor bounding the template and its complement, which is similar to the PacBio circular template. The bases can be measured by characteristic current changes as they are guided across an electrical resistant membrane (which embeds numerous nanopores) by a molecular motor protein through the nanopores. As the template molecules passing through nanopores quickly, the readouts tend to have high error rates. Fortunately, reading two strands through a hole can reduce errors. PromethION contains 48 flow cells that can be run individually or in parallel. Each flow cell contains 3000 channels, which can produce up to 40 Gb of data. Nanopore sequencers provide real-time, long-read, and direct DNA and RNA sequencing at a much larger scale, which is also affordable and field-portable.

Comparison

Platform	PacBio SMRT RS II	PacBio SMRT Sequel	Nanopore PromethION
Average Read Length	10-15 kb	8-12 kb	up to 900 kb
Single Pass Error Rate	13%	13%	2-13%
Sequencing Unit	150k ZMWs	1M ZMWs	144k nanopores
Data Size	500 Mb-1 Gb	5-10 Gb	5 Gb
Number of Reads per Run	50k	500k	up to 1M
Time per Run	0.5-6 h	0.5-6 h	50 h

CD Genomics provides PacBio SMRT sequencing and Oxford Nanopore sequencing to complement our SGS solutions. By taking advantage of the long-read single-molecular sequencing, we offer advanced solutions for genomic, transcriptomic, and epigenetic studies. Our highly experienced expert team can assist you in every procedure to ensure confident and unbiased results. If you have additional requirements or questions, please feel free to contact us.