# Overview of Current Genetic Sequencing Technologies

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## Introduction

- Next-generation sequencing technologies have evolved since the days of Sanger sequencing.
- These technologies are categorized based on read length.
- Two paradigms of sequencing are short-read or "next generation" technologies and long-read or "third-generation" technologies. [5]
- Short-read sequencing is thought to provide high accuracy but with limited read-length. [5]
- Long-read technologies afford much longer read-lengths at the expense of accuracy. [5]
- Emerging developments for third-generation technologies hold promise for the next wave of sequencing evolution, with the co-existence of longer read lengths and high accuracy. [5]

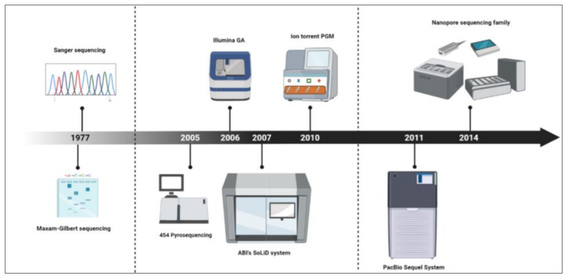


Figure 1. The milestones of genetic sequencing technology [1]

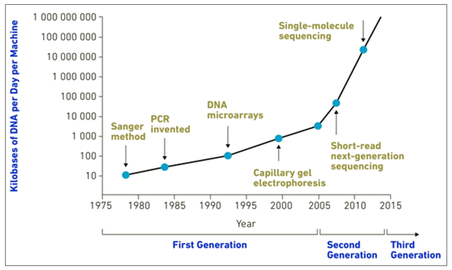


Figure 2: Throughput of sequencing methodologies over time

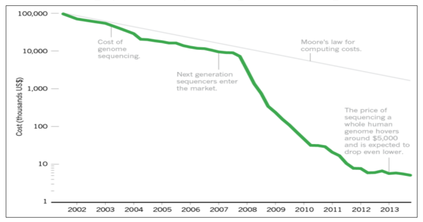


Figure 3: Cost to sequence a human genome over time

## Works Cited

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## First Generation Sanger Sequencing

- The chain-termination method is also called Sanger sequencing.
- It uses a DNA sequence as a template for PCR. [6]
- ddNTPs, are added during the extension step of PCR. [6]
- Incorporation of ddNTPs leads to the termination of the extension. [6]
- Generates numerous copies of the DNA sequence of all lengths spanning the amplified fragment. [6]
- Chain-terminated oligonucleotides are separated by gel electrophoresis or automated capillary sequencers. [6]
- DNA sequence is determined based on the size separation of chain-terminated oligonucleotides. [6]

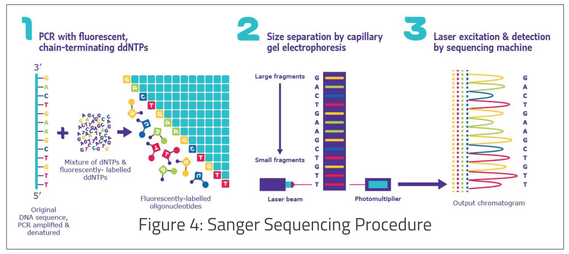


Figure 4: Sanger Sequencing Procedure

## Next Generation Sequencing

- Next generation sequencing (NGS) has replaced conventional sequencing methods. [4]
- NGS enables sequencing of multiple DNA strands at the same time.
- NGS fragments the genetic material and attaches known sequence oligonucleotides through adapter ligation. [4]
- Bases of each fragment are identified by emitted signals. [4]
- NGS allows processing of millions of reactions in parallel resulting in high-throughput, sensitivity, speed, and reduced cost. [6]
- Projects that took years with Sanger sequencing can now be completed within hours using NGS. [6]

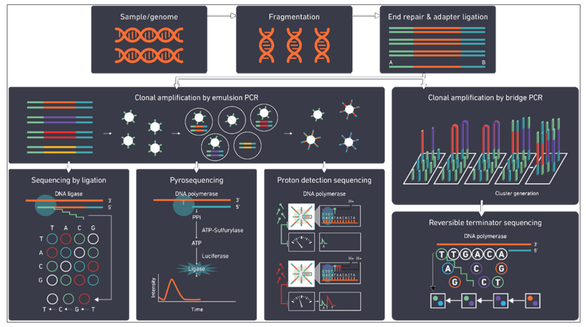


Figure 5: Overview of Next Generation Sequencing Technologies [6]

- Proton detection sequencing counts hydrogen ions released during DNA polymerization. pH changes are detected by semiconductor sensor chips and converted to digital information. [6]
- Pyrosequencing detects pyrophosphate generation and light release to determine the incorporation of specific bases in a DNA chain. [6]
- Reversible terminator sequencing uses bridge-amplification where fragments bind to oligonucleotides on the flow cell creating a bridge that is amplified and detected using fluorescently-labeled nucleotides. [6]
- Sequencing by ligation uses DNA ligase and fluorescence to determine the target sequence. Digital images taken after each reaction are used for analysis. [6]
- DNA nanoball sequencing uses rolling circle replication to compact DNA copies into nanoballs that are bound to sequencing slides for ligation-based sequencing reactions. [6]

## Pacific Biosciences: SMRT

- The PacBio sequencing method is based on DNA sequencing by synthesis. [3]
- SMRT sequencing is performed in SMRT cells that contain ultra-microwells at a zeptoliter scale. [3]
- One molecule of DNA polymerase is immobilized at the bottom of each well using the biotin-streptavidin system in nanostructures known as zero-mode waveguides (ZMWs). [3]
- Fluorescently labeled dNTP analogs are added and detected when the nucleotide is incorporated into the growing strand. [3]
- Lasers and CCD cameras continuously monitor the ZMWs and enable the simultaneous and parallel detection of thousands of single-molecule sequencing reactions. [3]
- The signal is translated into nucleotide sequence through a process termed basecalling. [2]

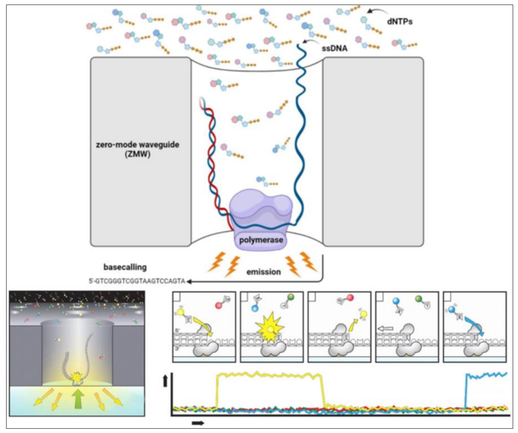


Figure 6: PacBio SMRT Sequencing ZMW Cell [2]

- A circular double-stranded DNA adapter called "SMRTbell" is attached to the DNA target, DNA polymerase will elongate large templates multiple times. [2]
- The amplified elongation produces longer reads and improved accuracy to >99.999%. [3]
- All four nucleotides are added simultaneously and measured in real time. [3]
- The time of base incorporation is longer for modified bases, called "interpulse duration", and indicates DNA modification events. [1]

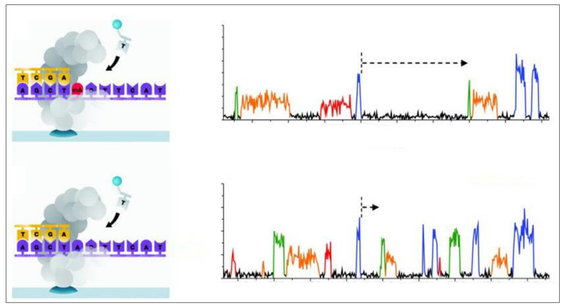


Figure 7: A methylated base sequenced by the PacBio; Interpulse duration is dotted arrow [1]

- SMRT technology enables direct RNA sequencing. [2]
- Sequel IIe System has 8 million ZMWs and generates up to 4,000,000 reads in a run. [2]
- Average length of the reads produced by Sequel IIe is 15 kb. [2]
- Sequel IIe System can produce up to 500 Gb total sequence output. [2]

## Oxford Nanopore Technologies: Nanopore

- Nanopore sequencing uses pores formed from proteins to detect changes in electric current during DNA or RNA strand sequencing in real-time. [3]
- The Nanopore system consists of nano-sensors and special "channels" that denature and direct single-stranded DNA through the pore. [2]
- Alterations of the ionic current are unique for each nucleotide and generate a signature for each base. [2]
- ONT systems detect short nucleotide sequences called k-mers, generating more than 1000 different signals that can be detected simultaneously. [2]
- Each flow cell accommodates 512 different channels (nanopores) that perform sequencing simultaneously. [2]

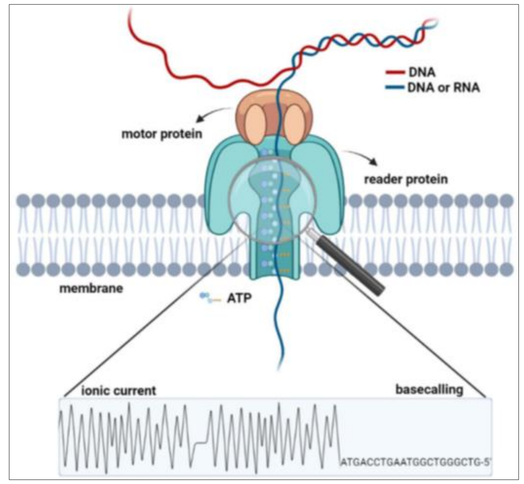


Figure 8: Oxford Nanopore Technologies Nanopore Cell [2]

- R9 chemistry used in flow cells enables >98.3% accuracy per molecule [2]
- R10 chemistry, which has >99% single-molecule accuracy. [2]
- A hairpin structure is designed in DNA library preparation to ligate double DNA strands for continuous reading. [1]
- Nanopore sequencing includes methylation detection and direct RNA sequencing. [3]

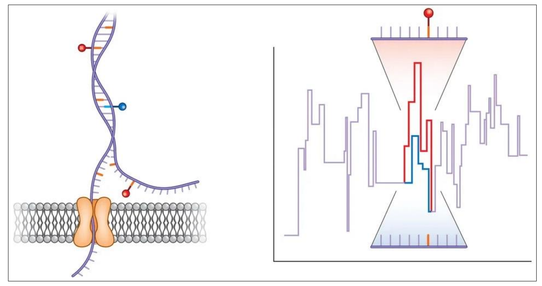

Figure 9: A methylated base (red) sequenced by the ONT [1]

- PacBio and ONT technologies have high error rates in sequencing runs for first generation machines. [2]
- The error rate (~15%) limits the accurate detection of SNPs or point mutations using these technologies. [2]
- NGS remains the best technology for mutational analysis due to its accuracy. [2]
- Improvements in TGS sequencing chemistry are expected to reduce error rates and improve accuracy. [2]