# Next Generation Sequencing, part 2

1. Introduction

After the first three next generation sequencing technologies (Roche-454, Solexa/Illumina, ABI SOLiD) became standard, several newer next-generation sequencing methods were developed and are now commercially available. These offer some advantage over the previous methods in terms of speed, size, cost, or read length.

2. Pacific Biosciences (PacBio)

The first machine that can perform Single Molecule Real Time (SMRT) sequencing. This is a sequencing by synthesis method that uses a DNA polymerase to add nucleotides to a template. In this case, the polymerase enzyme is attached to a surface at the bottom of a nanometer-scale well (known as a ZMW or Zero-Mode Waveguide). A laser is focused very precisely on the polymerase at the bottom of the ZMW. The different nucleotides are labeled with different fluorescent dyes that are released and fluoresce only when the base is incorporated into the growing DNA strand. A "movie" is made of each polymerase and bases are called as the fluorescence changes over time.

**Pro**: fast (base incorporation time is around 1 sec); can give very long reads (up to 25,000 bp or more), which is very useful for *de novo* genome assembly.

**Con:** error rates are high (about 15%); read lengths vary among sequences – some are very long, but others are short. Average read length around 900 bp. Machine is large and relatively expensive.

3. Ion Torrent

This approach is similar to 454 sequencing, but does not rely on light detection. Instead a semi-conductor chip is used to detect very small changes in pH when protons are released during base incorporation.

**Pro:** machine is smaller and cheaper than other next-gen technologies. Fast run-times (7 hours), suitable for individual labs. Uses standard chip production techniques, so should become cheaper and more efficient in the future – just like other microchips.

**Con:** does not have as high sequencing throughput as Illumina or Solid. Read length are a bit shorter than 454-Roche (currently up to 400 bases)

4. Nanopore (MinION)

This method reads the bases of a single-stranded DNA molecule as it passes through a protein channel (or "pore"). The pores can be packed very densely and the sequencing can be very fast – on the order of milliseconds per base. These sequencers are small (hand-held) and can, ideally, sequence up to 1 Gb in 6 hours at low cost. Read lengths can be 10-100's of kb.

In the future, the protein channel may be replaced with a solid-state nano-channel. This may be something like a "DNA transistor". This should be even faster and cheaper than protein nanopores, but technical challenges remain before such a sequencer can be manufactured.

5. Additional reading

Pennisi, E. (2012) Search for Pore-fection. Science, 336: 534–537.

Pennisi, E. (2014) DNA Sequencers Still Waiting For The Nanopore Revolution. Science, 343: 829–830.

Pennisi, E. (2016) Pocket DNA sequencers make real-time diagnostics a reality. Science, 351: 800–801.