THE EVOLUTION OF SEQUENCING

There have been numerous sequence modalities developed in the last quarter century, but major advances include Sanger sequencing, sequencing by synthesis, nanopore long-read sequencing from Oxford Nanopore, and, most recently, high-fidelity single-molecule real-time sequencing from PacBio. These differ in the length of reads they generate, their efficiency, and accuracy, with technologies generally evolving to support faster, cheaper, and more-precise sequencing.

### SANGER SEQUENCING

The first sequencing technology invented, and no longer used in modern projects, Sanger sequencing relies on tagging the ends of various sizes of DNA fragments with complementary fluorescent nucleotides. Fragments are then separated by size using gel electrophoresis and the final nucleotides’ fluorescence is read by a laser. The full sequence is inferred by piecing together the end nucleotides of the different-sized fragments.

**YEARS IN USE: 1980–2010**
**READ LENGTH: ~500–1,000 bases**
**CONS:** Low throughput, time intensive

### SEQUENCING BY SYNTHESIS

Sequencing by synthesis (SBS) is the most commonly used type of sequencing today. It relies on synthesizing complementary DNA strands using fluorescently tagged nucleotides and capturing the output signal on a high-resolution camera. Hundreds of thousands of DNA fragments can be read at once, but SBS is limited to short lengths of DNA, making it challenging to assemble whole genomes de novo.

**YEARS IN USE: 2002–today**
**READ LENGTH: ~100–500 bases**
**CONS:** Limited to short reads

### NANOPORE SEQUENCING

Oxford Nanopore devices pull DNA through a bioengineered pore to produce electrical current fluctuations that are then translated into a sequence. This approach generates long reads that can be used for de novo genome assembly or to identify larger structural variations that are difficult to detect with short reads, but it is more accurate than other sequencing technologies.

**YEARS IN USE: 2002–today**
**READ LENGTH: ~10 kb–1 MB**
**CONS:** Limited to short reads

### HIGH-FIDELITY SEQUENCING

Only recently released by PacBio, high-fidelity (HiFi) single-molecule real-time (SMRT) sequencing relies on similar fluorescence strategies as SBS. Like nanopore sequencing, HiFi produces long reads that can be used for de novo genome assembly or to identify structural variants, but it is currently more expensive than other sequencing technologies.

**YEARS IN USE: 2020–today**
**READ LENGTH: ~10 kb**
**CONS:** Currently very expensive