A Real-time long-read sequencing

Aa Pacific Biosciences
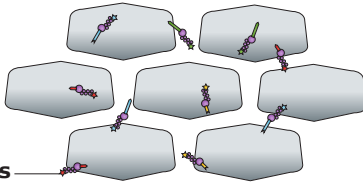
SMRTbell template
Two hairpin adapters allow continuous circular sequencing
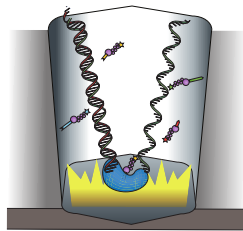
ZMW wells
Sites where sequencing takes place

Labelled nucleotides
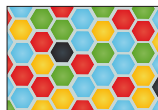All four dNTPs are labelled and available for incorporation

Modified polymerase
As a nucleotide is incorporated by the polymerase, a camera records the emitted light

PacBio output
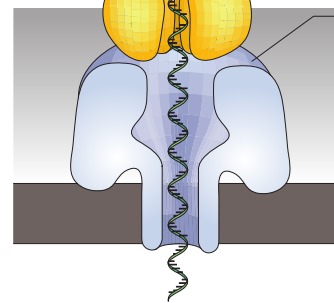A camera records the changing colours from all ZMWs; each colour change corresponds to one base

Ab Oxford Nanopore Technologies

Leader–Hairpin template
The leader sequence interacts with the pore and a motor protein to direct DNA, a hairpin allows for bidirectional sequencing
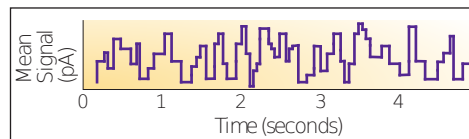
Motor protein

Alpha-hemolysin
A large biological pore capable of sensing DNA

Current
Passes through the pore and is modulated as DNA passes through

ONT output (squiggles)
Each current shift as DNA translocates through the pore corresponds to a particular k-mer

Mean Signal (pA)

Time (seconds)
0   1   2   3   4

B Synthetic long-read sequencing
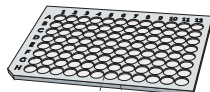
Ba Illumina

DNA fragment
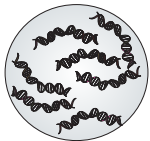DNA is fragmented and selected to ~10 kb

Enzymatic cleavage
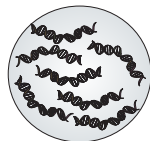DNA is barcoded and fragmented to ~350 bp

~3,000 molecules per well

A1   A2

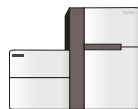**Barcodes**
DNA from the same well shares the same barcode

Pooling
DNA from each well is pooled and undergoes a standard library preparation

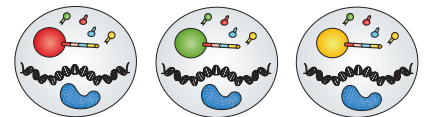**Sequencing**
DNA is sequenced on a standard short-read sequencer

Bb 10X Genomics

Emulsion PCR
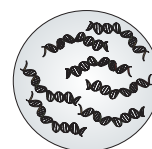Arbitrarily long DNA is mixed with beads loaded with barcoded primers, enzyme and dNTPs

GEMs
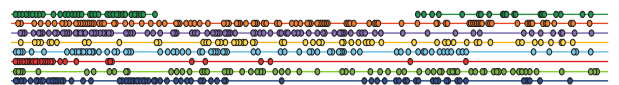Each micelle has 1 barcode out of 750,000

Amplification
Long fragments are amplified such that the product is a barcoded fragment ~350 bp

Pooling
The emulsion is broken and DNA is pooled, then it undergoes a standard library preparation

Linked reads
• All reads from the same GEM derive from the long fragment, thus they are linked
• Reads are dispersed across the long fragment and no GEM achieves full coverage of a fragment
• Stacking of linked reads from the same loci achieves continuous coverage