## A Real-time long-read sequencing

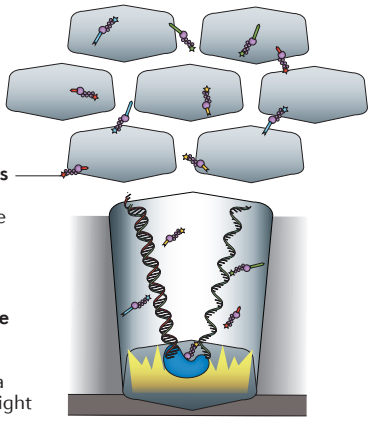
### Aa Pacific Biosciences

**SMRTbell template**
Two hairpin adapters allow continuous circular sequencing

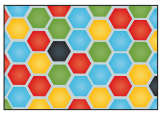
**ZMW wells**
Sites where sequencing takes place

**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

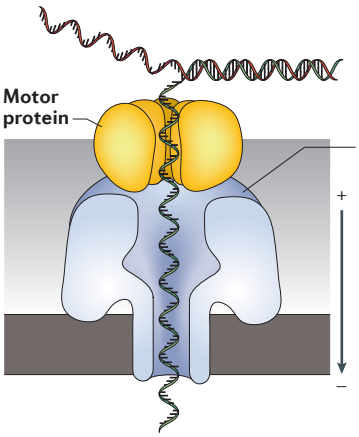


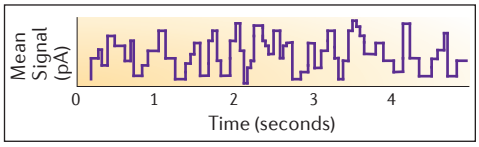
**PacBio output**
A camera records the changing colours from all ZMWs; each colour change corresponds to one base



### 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




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

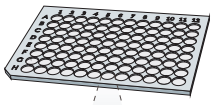
## B Synthetic long-read sequencing

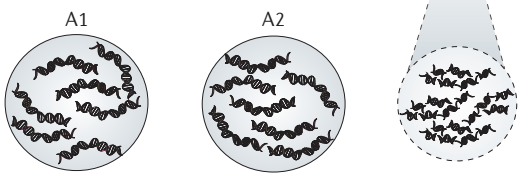
### Ba Illumina

**DNA fragment**
DNA is fragmented and selected to ~10 kb

~3,000 molecules per well

**Enzymatic cleavage**
DNA is barcoded and fragmented to ~350 bp





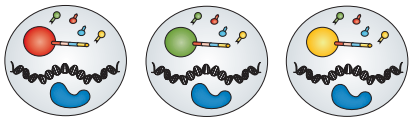

**Barcodes**
DNA from the same well shares the same barcode

**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

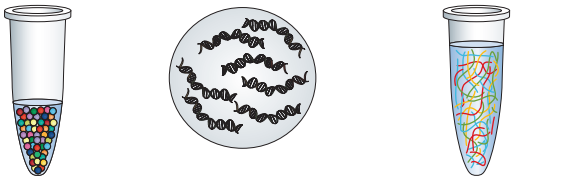
**Emulsion PCR**
Arbitrarily long DNA is mixed with beads loaded with barcoded primers, enzyme and dNTPs



**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



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