

SEQUENCING 101



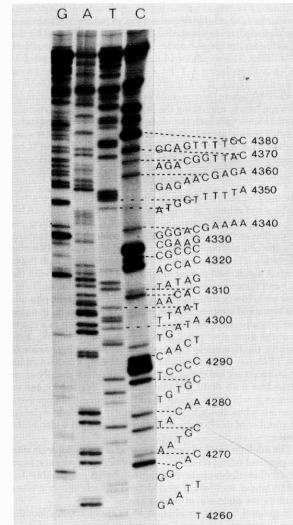
(THE INCOMPLETE) HISTORY OF SEQUENCING

Chain termination:
Sanger sequencing



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Sanger sequencing



Proc. Natl. Acad. Sci. USA
Vol. 74, No. 12, pp. 5463–5467, December 1977
Biochemistry

DNA sequencing with chain-terminating inhibitors

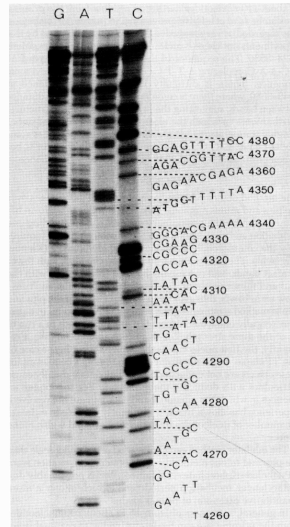
(DNA polymerase/nucleotide sequences/bacteriophage ϕ X174)

F. SANGER, S. NICKLEN, AND A. R. COULSON



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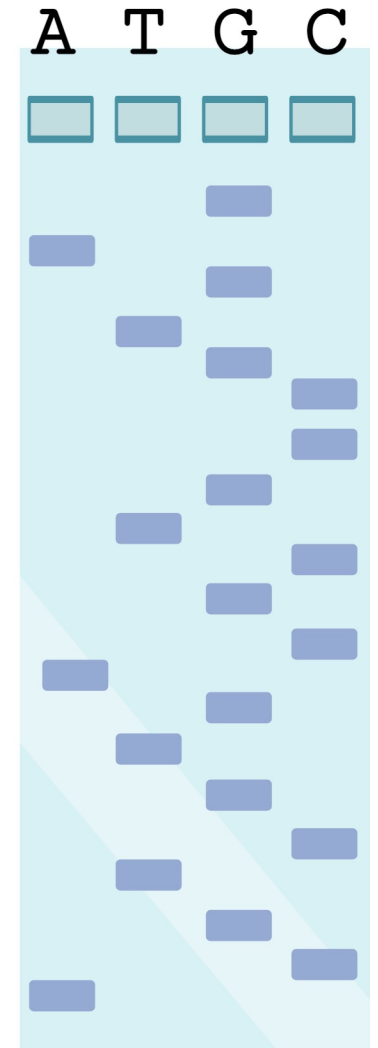


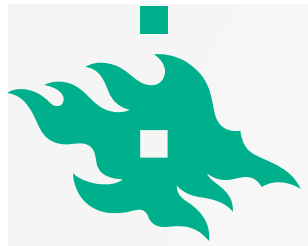
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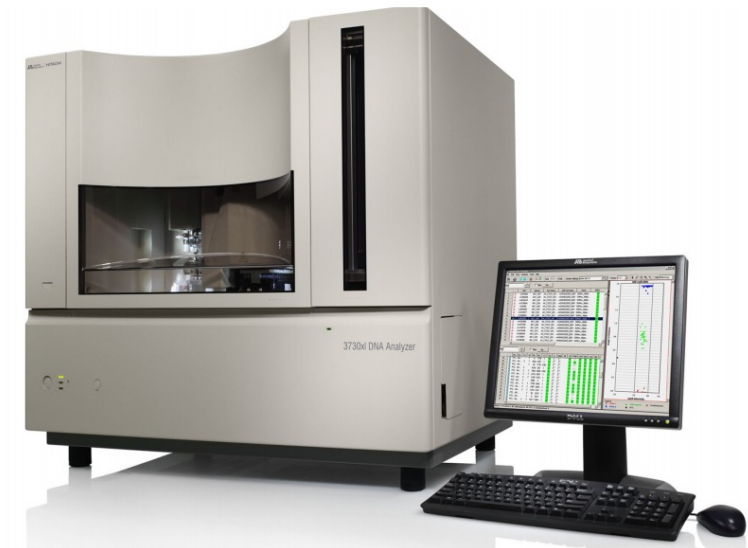
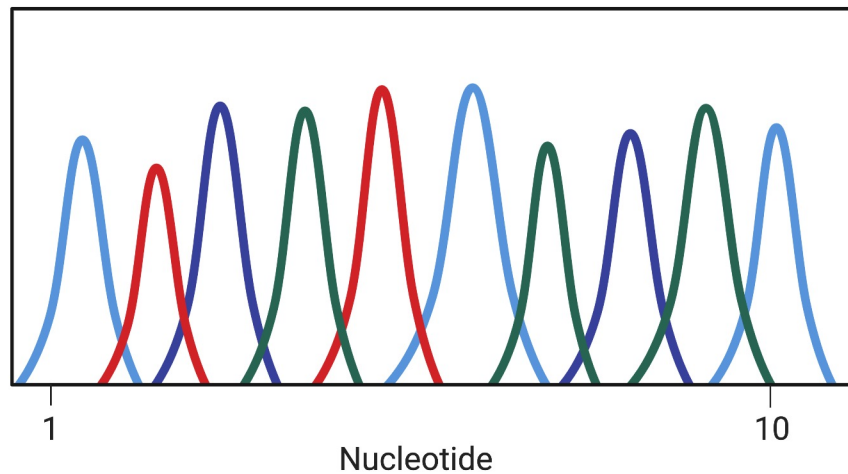
High-throughput Sanger sequencing

Dye-terminators and capillary electrophoresis



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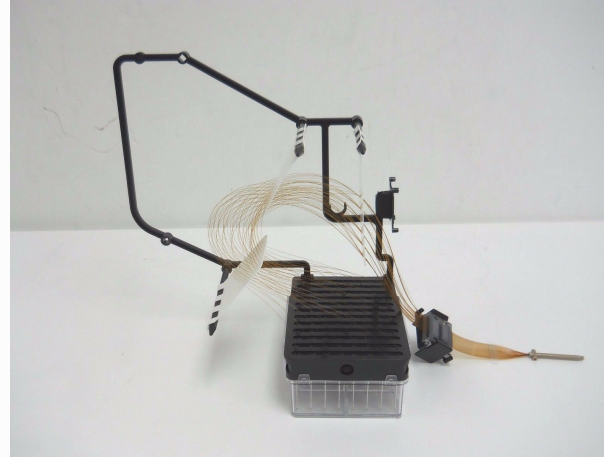
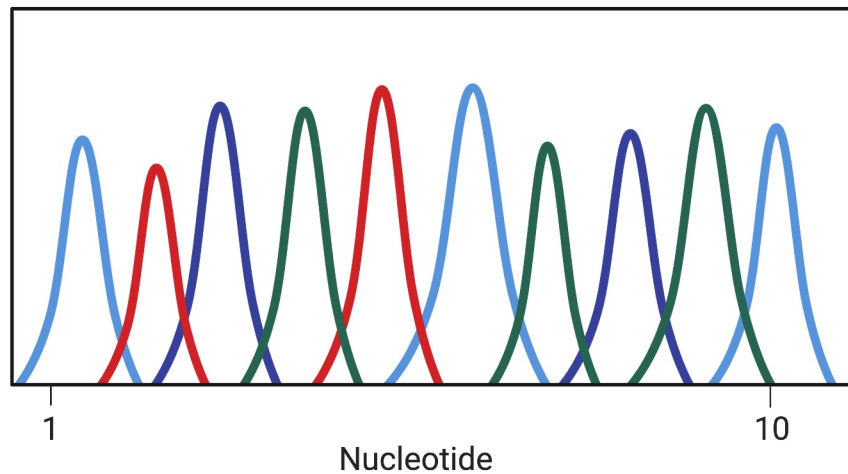
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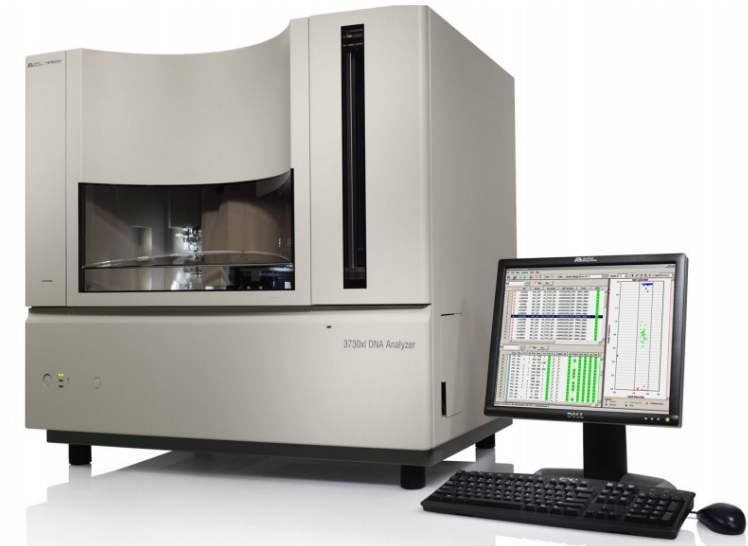


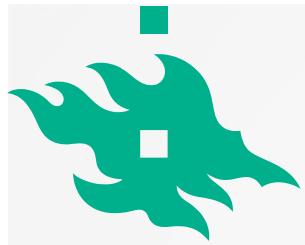
(THE INCOMPLETE) HISTORY OF SEQUENCING

High-throughput Sanger sequencing
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48/96 –capillars
Read length < **900 bp**
< **90 Kbp / run**





(THE INCOMPLETE) HISTORY OF SEQUENCING

Sequencing by synthesis:

Pyrosequencing

Genome sequencing in microfabricated high-density picolitre reactors

Marcel Margulies, Michael Egholm, [...] Jonathan M. Rothberg 

Nature **437**, 376–380(2005) | [Cite this article](#)



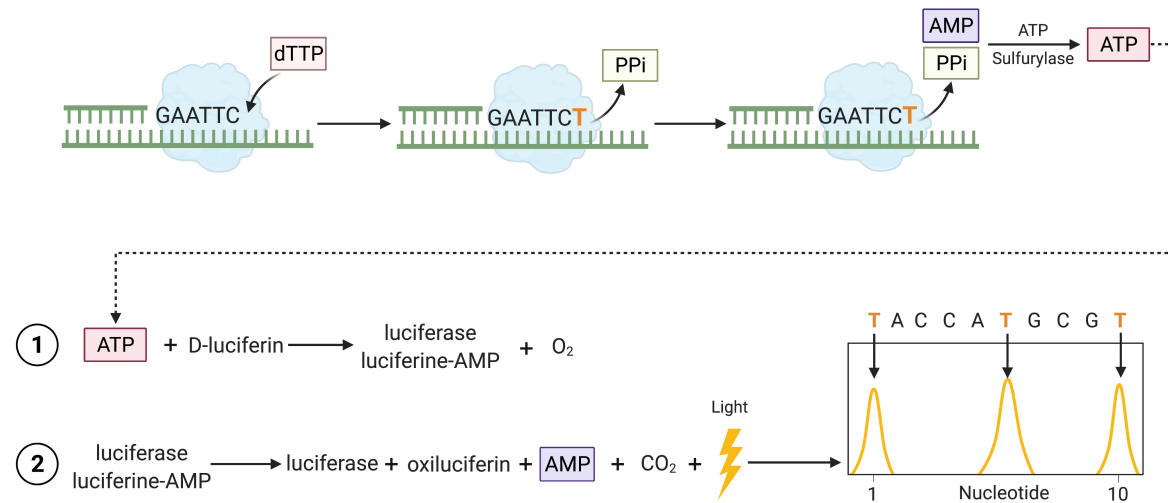
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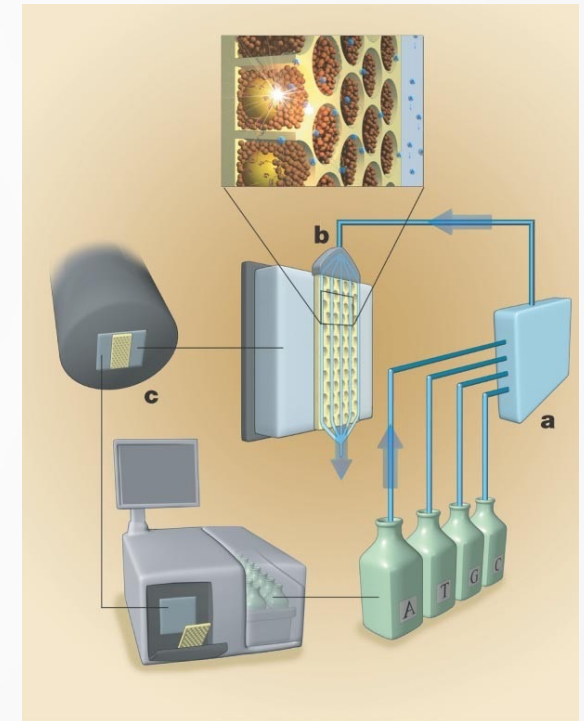
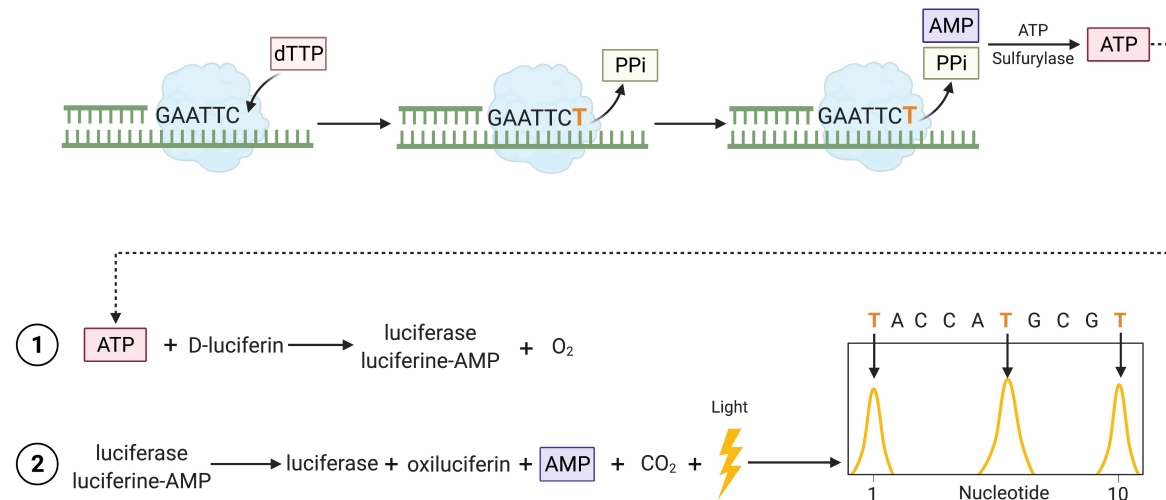
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Margulies et al., 2005. Nature

**Read length ~ 400–1000 bp
20 – 600 Mbp / run**

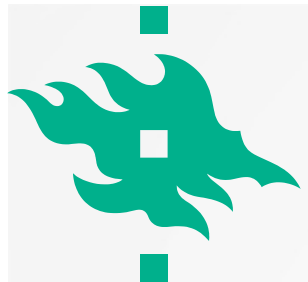


(THE INCOMPLETE) HISTORY OF SEQUENCING

Sequencing by synthesis:
Short-read sequencing

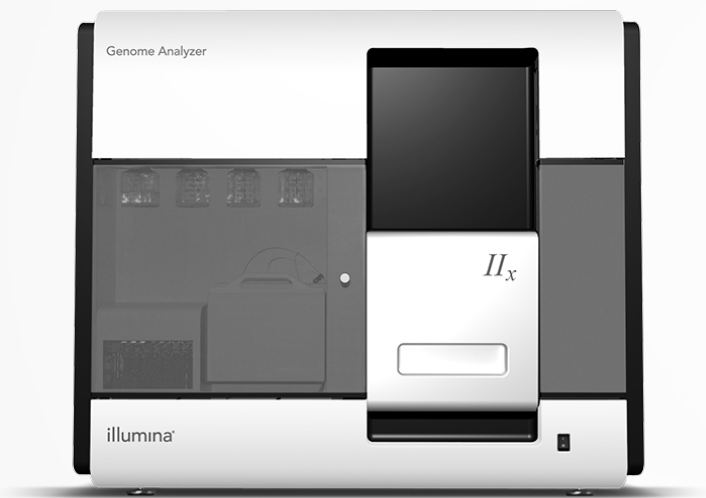


Illumina (Solexa)
Genome Analyzer, 2006
1 Gbp / run

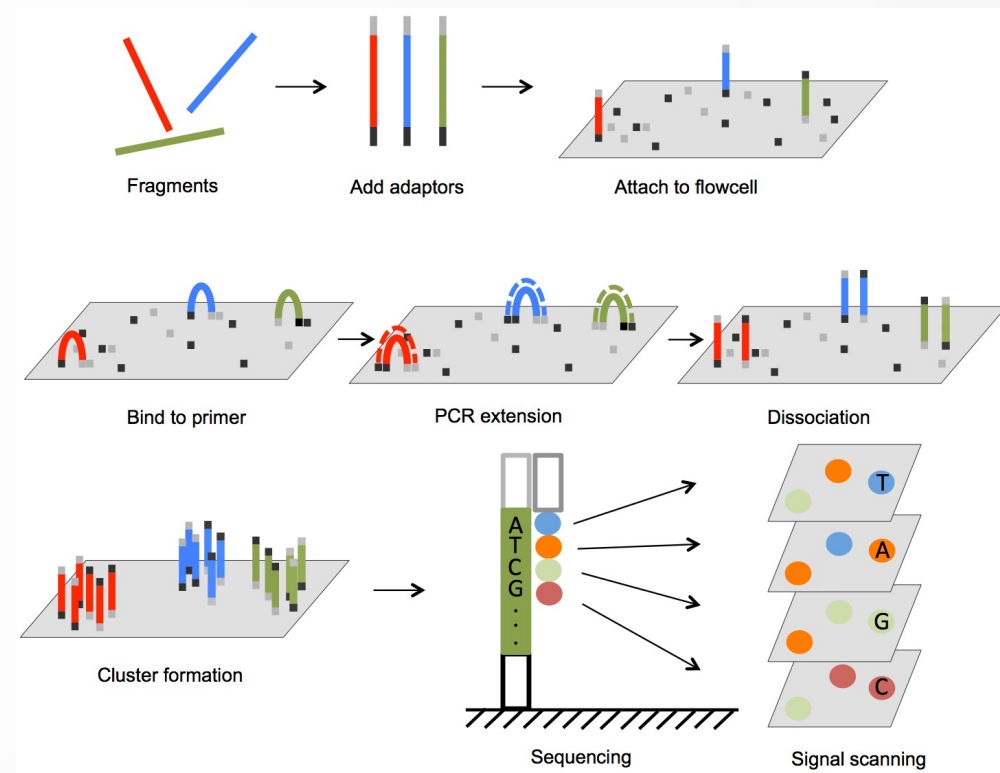


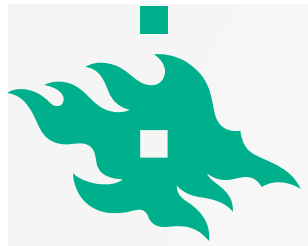
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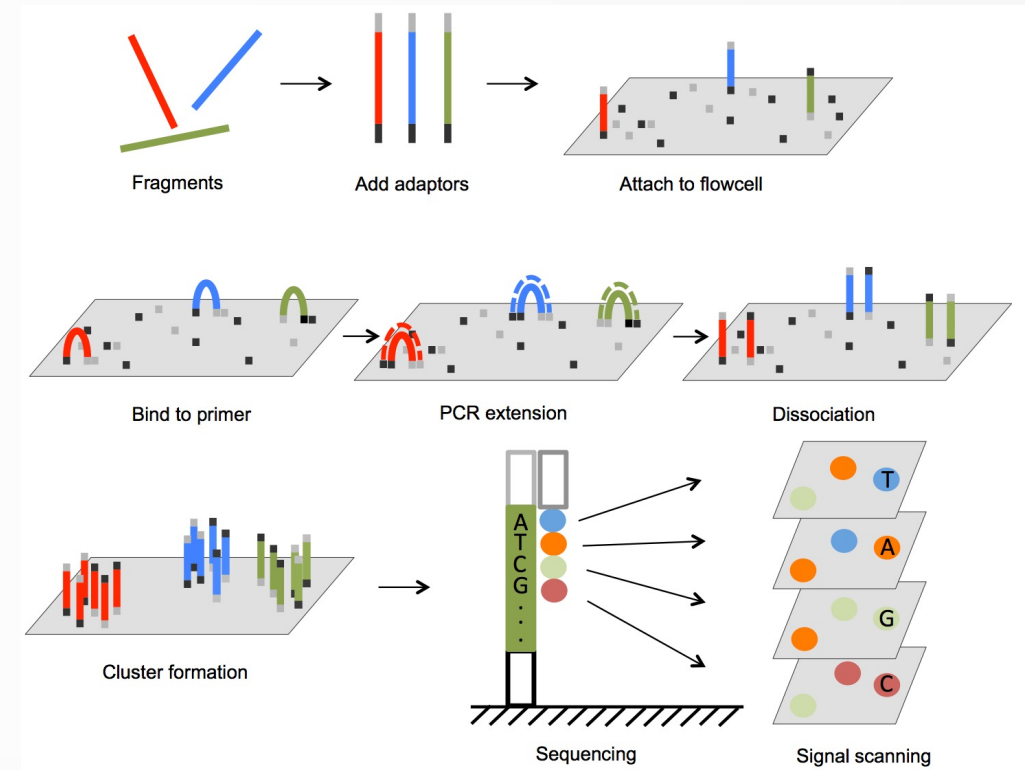
Illumina
Genome Analyzer IIx, 2008
20 Gbp / run (9.5 days)





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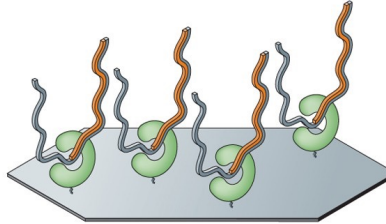




(THE INCOMPLETE) HISTORY OF SEQUENCING

Third revolution:
Long-read sequencing

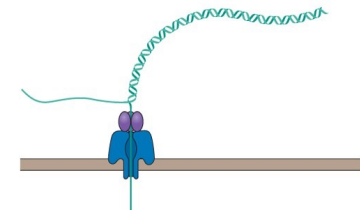
The Third Revolution
Single-molecule sequencing

A diagram illustrating PacBio SMRT sequencing. It shows four green circular templates on a grey surface. Each template has a single-stranded DNA molecule (orange) attached to it. The DNA molecules are shown in various stages of synthesis, with some having longer strands than others.

Pac Bio SMRT sequencing

- Sequencing by synthesis
- Single-molecule templates
- Low accuracy but long read lengths

For example, PacBio RS
(Pacific Biosciences)

A diagram illustrating Oxford Nanopore sequencing. It shows a blue protein complex (pore) embedded in a brown membrane. A single-stranded DNA molecule (green) is shown passing through the pore, with a long, wavy strand extending upwards.

Oxford Nanopore sequencing

- Nanopore sequencing
- Single-molecule templates
- Low accuracy but long read lengths

For example, MinION
(Oxford Nanopore)

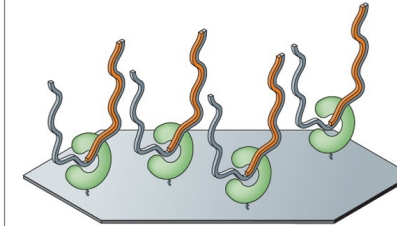


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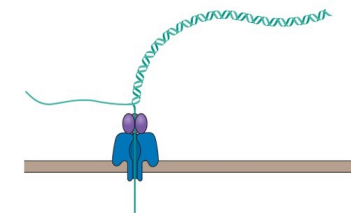
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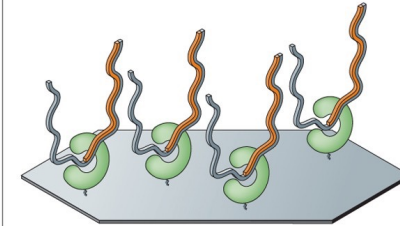


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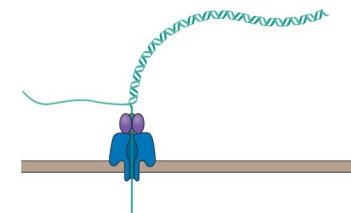
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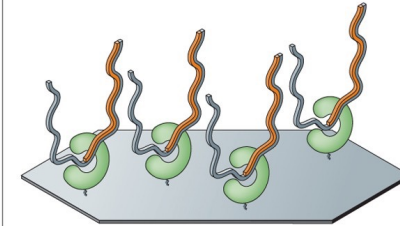


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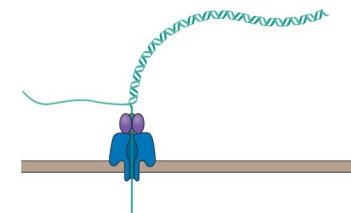
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(Oxford Nanopore)

Sequel II
HiFi reads:
Read length:
20 kb
30 Gbp /run
(99.92 % acc.)

Minion:
Read length:
> 4 Mb
1–50 Gbp
/run
(~ 97 % acc.)
Real-time!