

# SEQUENCING 101



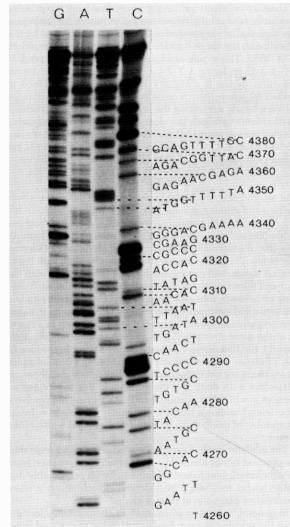
# (THE INCOMPLETE) HISTORY OF SEQUENCING

**Chain termination:**  
Sanger sequencing



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

## **DNA sequencing with chain-terminating inhibitors**

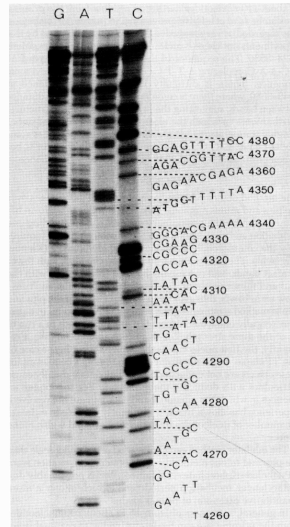
(DNA polymerase/nucleotide sequences/bacteriophage  $\phi$ X174)

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



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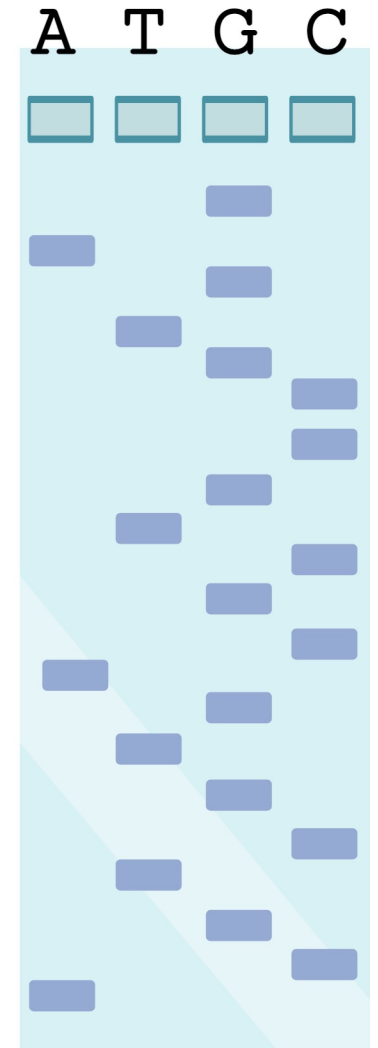


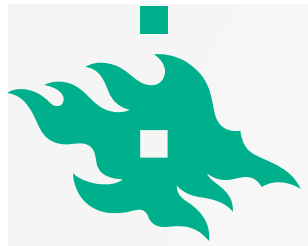
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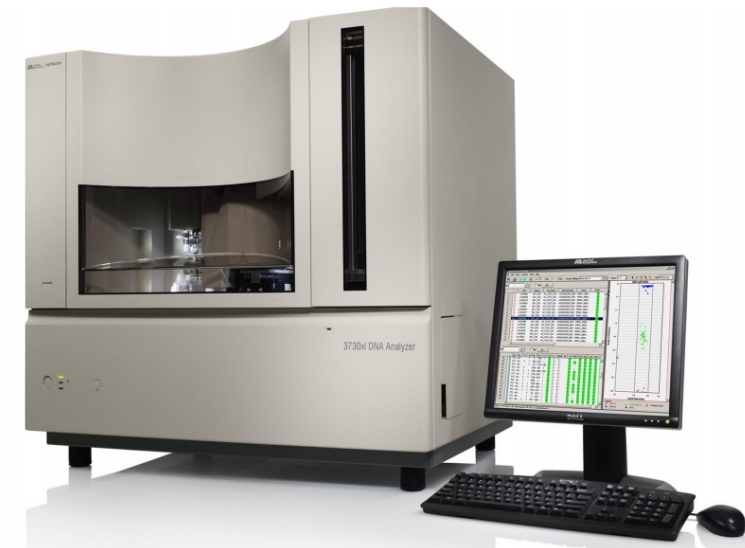
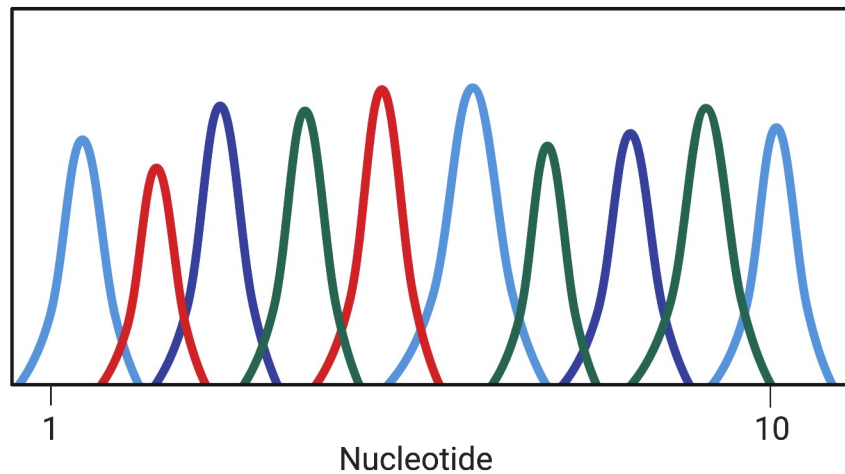
**High-throughput Sanger sequencing**

Dye-terminators and capillary electrophoresis



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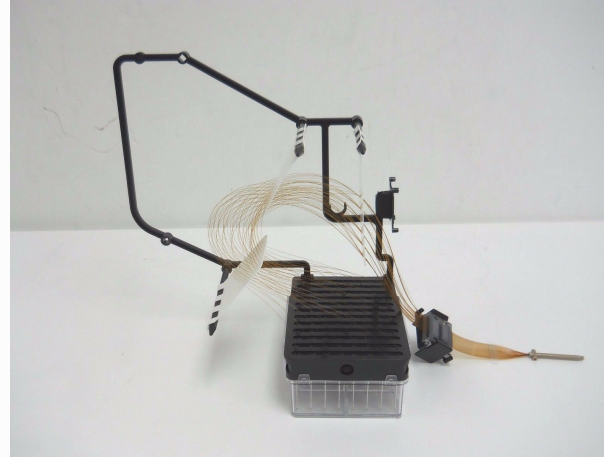
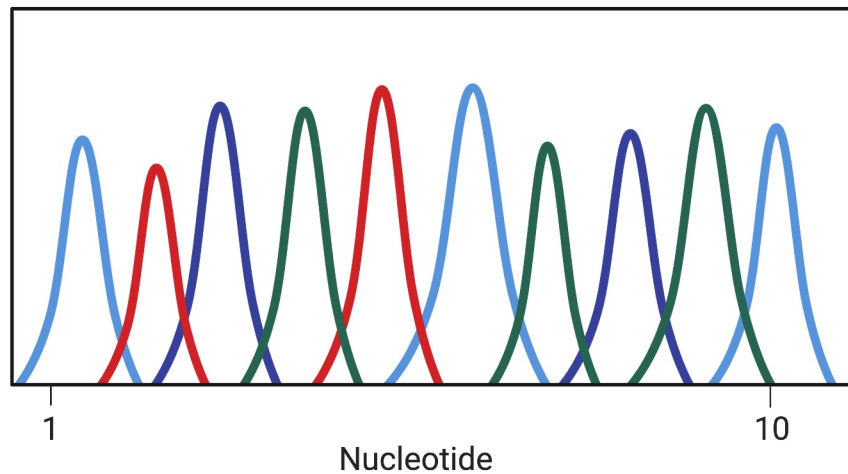
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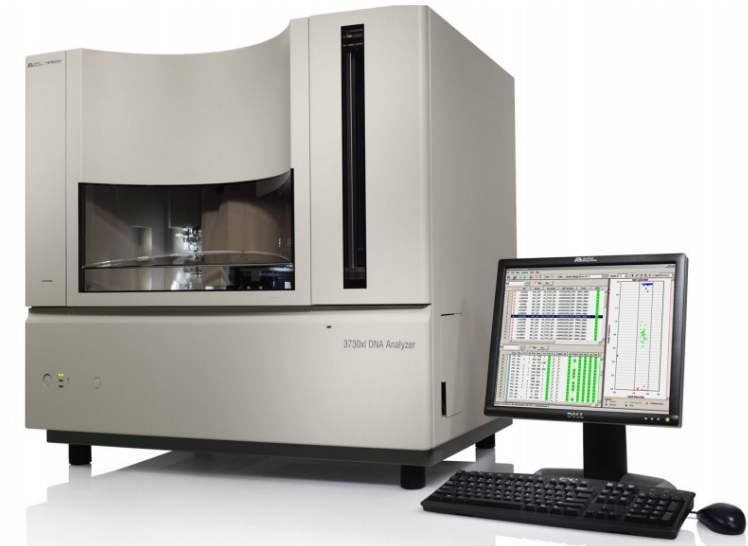


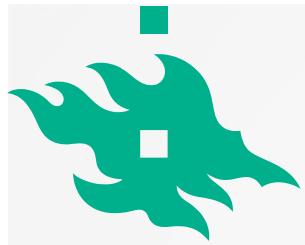
# (THE INCOMPLETE) HISTORY OF SEQUENCING

**High-throughput Sanger sequencing**  
Dye-terminators and capillary electrophoresis



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](#)





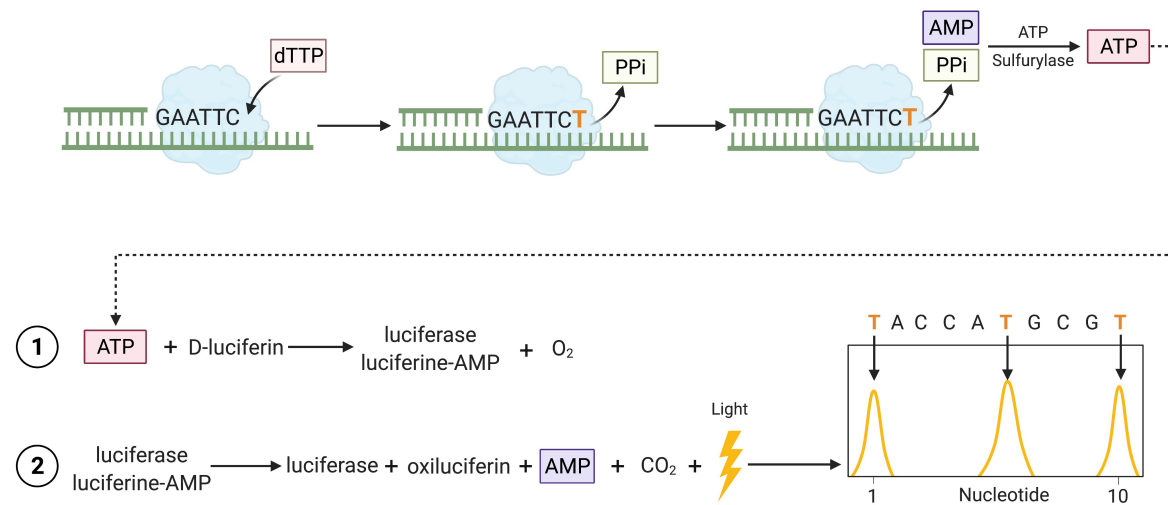
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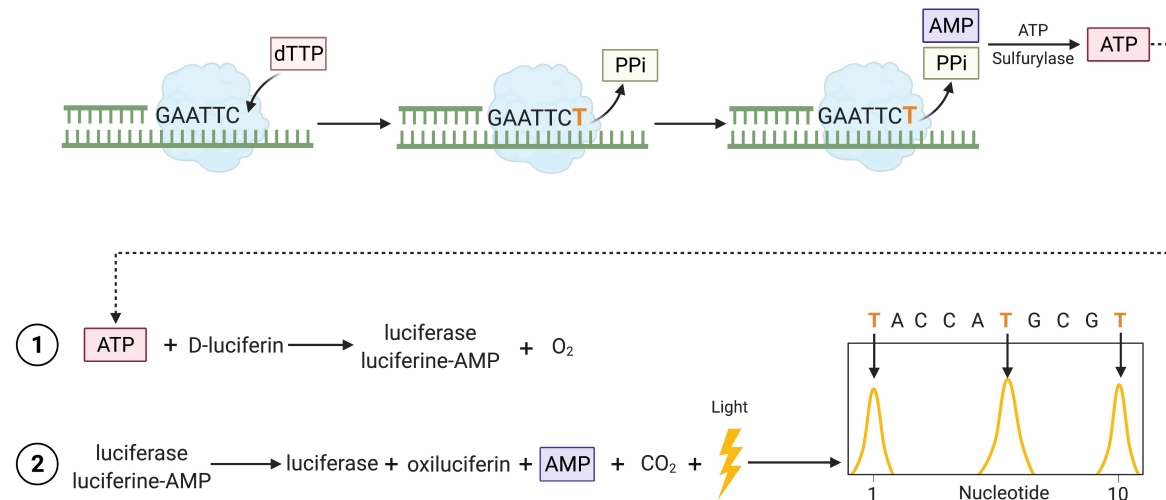
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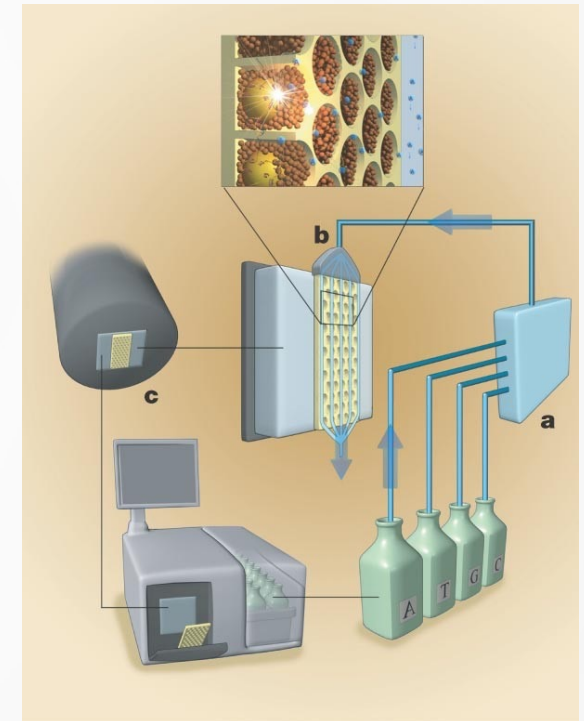
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Margulies et al., 2005. *Nature*

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



# (THE INCOMPLETE) HISTORY OF SEQUENCING

**Sequencing by synthesis:**  
Short-read sequencing

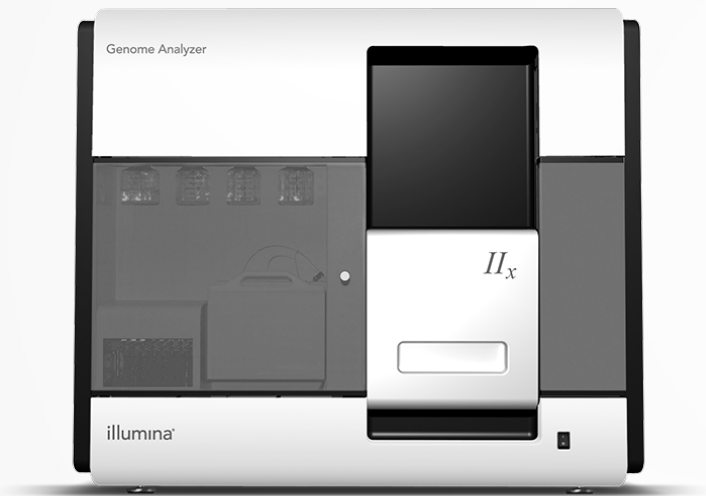


Illumina (Solexa)  
Genome Analyzer, 2006  
**1 Gbp / run**

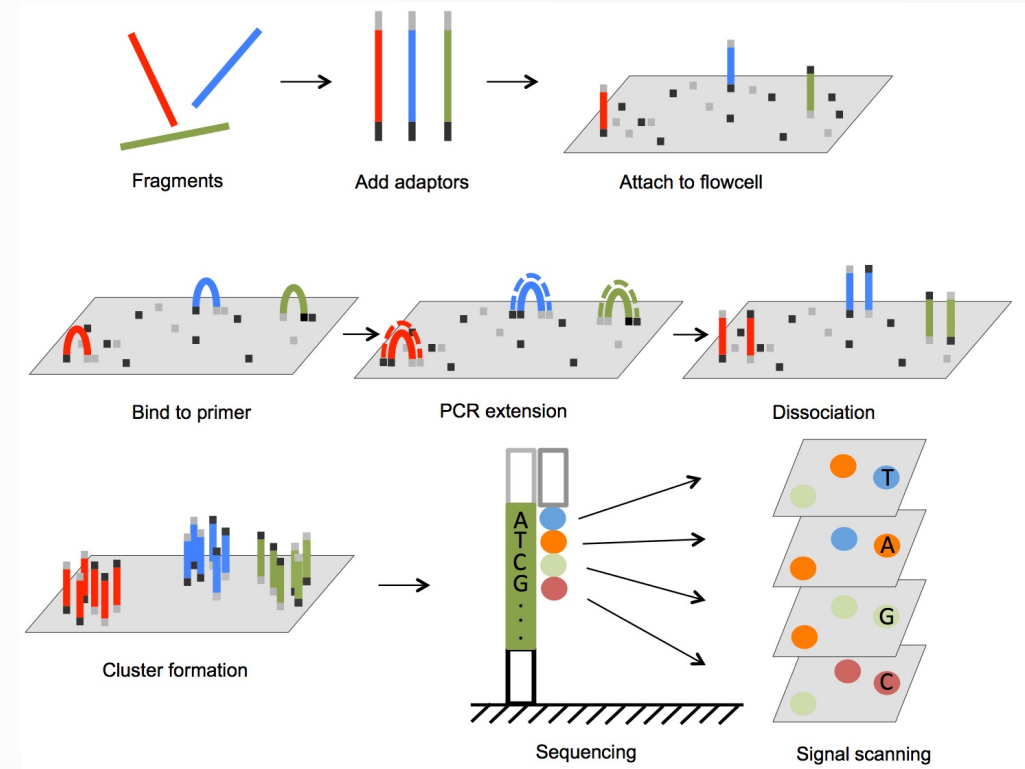


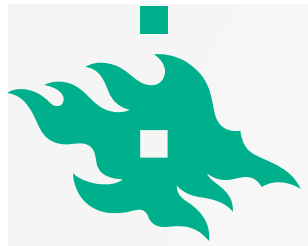
# (THE INCOMPLETE) HISTORY OF SEQUENCING

## Sequencing by synthesis: Short-read sequencing



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



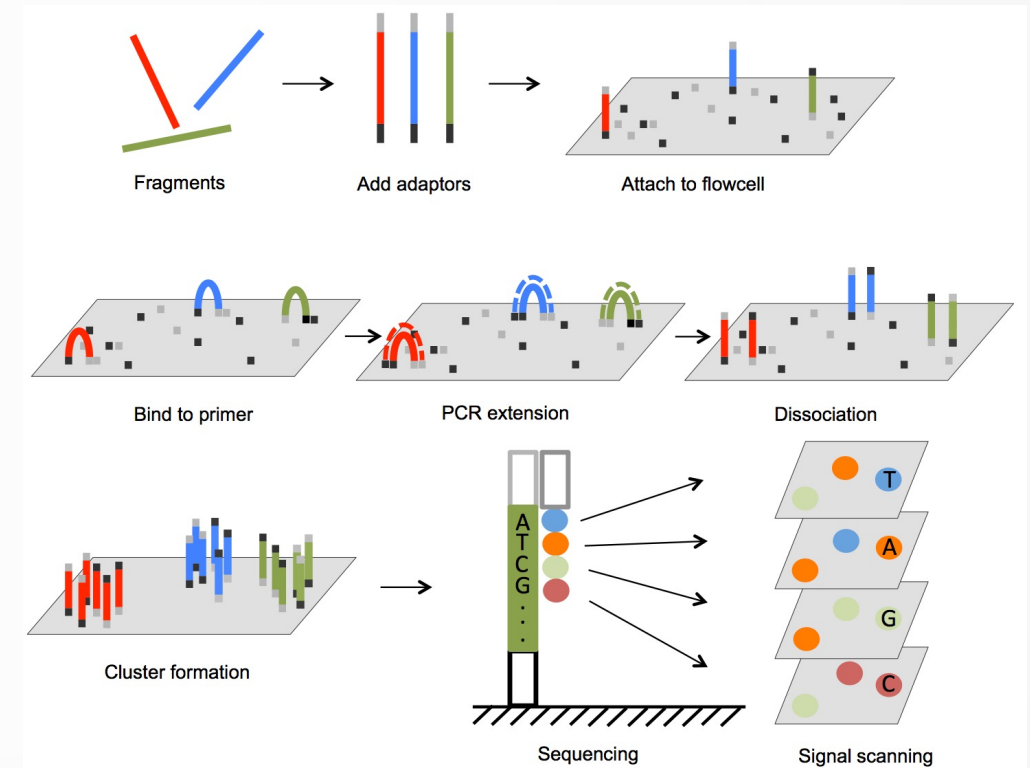


# (THE INCOMPLETE) HISTORY OF SEQUENCING

## Sequencing by synthesis: Short-read sequencing



1 Gbp – >1 Tbp / run (~1–2 d)

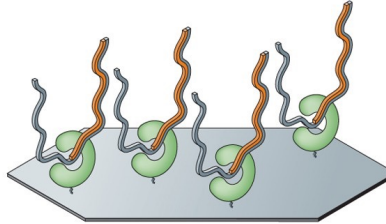




# (THE INCOMPLETE) HISTORY OF SEQUENCING

**Third revolution:**  
Long-read sequencing

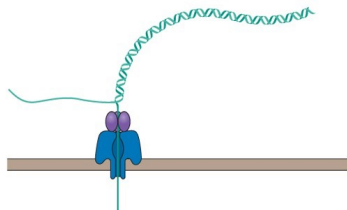
**The Third Revolution**  
Single-molecule sequencing

A diagram illustrating PacBio SMRT sequencing. It shows four green circular templates on a grey surface. Each template has a long, wavy orange strand (DNA) passing through it. The strands are shown in different positions, suggesting the process of sequencing by synthesis.

**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 long, wavy green strand (DNA) is passing through the pore. The strand is shown in a curved position, suggesting the process of sequencing as the strand moves through the pore.

**Oxford Nanopore sequencing**

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

For example, MinION  
(Oxford Nanopore)

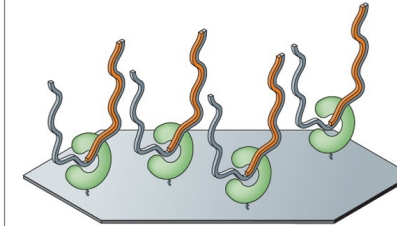


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**Third revolution:**  
Long-read sequencing



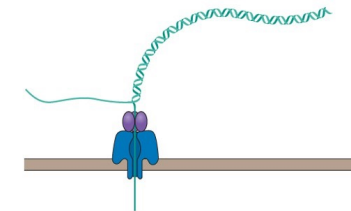
## The Third Revolution Single-molecule sequencing



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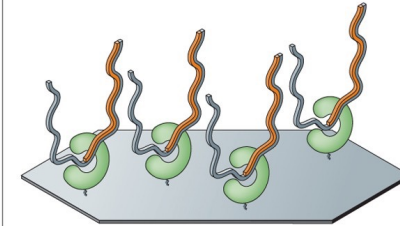


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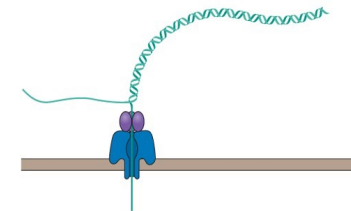
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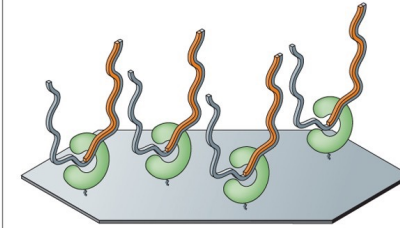


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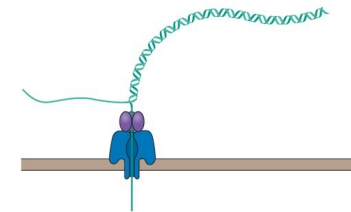
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### Oxford Nanopore sequencing

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For example, MinION  
(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!**