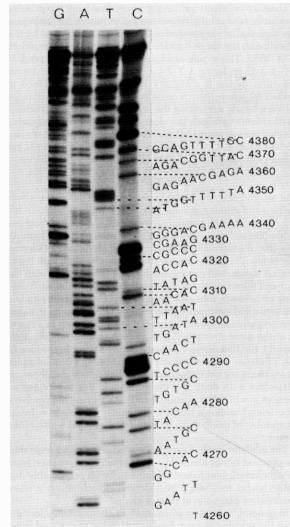# SEQUENCING 101

(THE INCOMPLETE) HISTORY OF SEQUENCING

Chain termination:
Sanger sequencing

(THE INCOMPLETE) HISTORY OF SEQUENCING

Chain termination:
Sanger sequencing

DNA sequencing with chain-terminating inhibitors

(DNA polymerase/nucleotide sequences/bacteriophage φX174)

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

(THE INCOMPLETE) HISTORY OF SEQUENCING

Chain termination:

Sanger sequencing

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

DNA sequencing with chain-terminating inhibitors

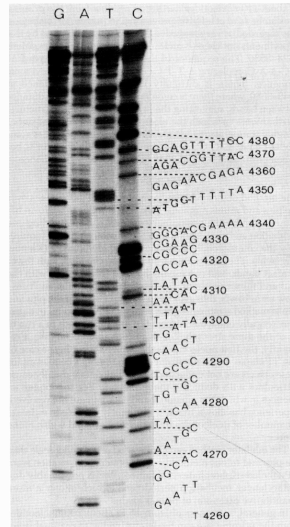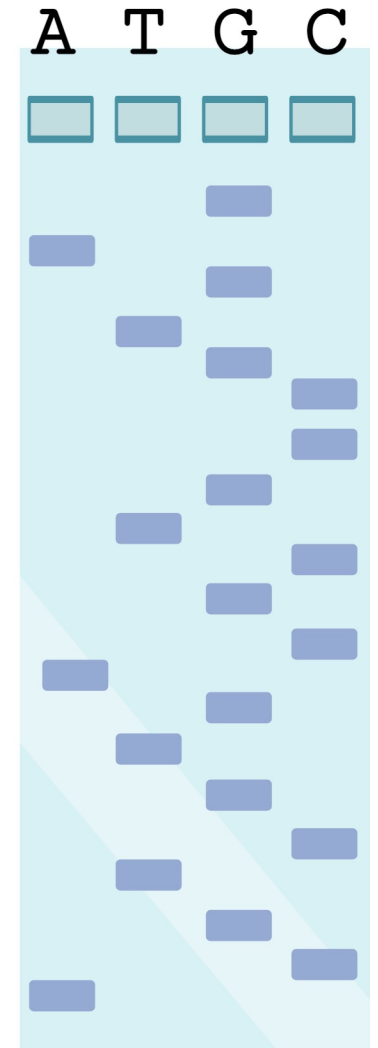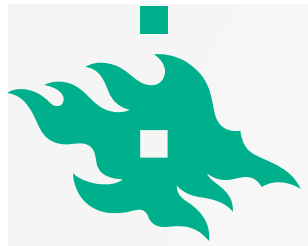(DNA polymerase/nucleotide sequences/bacteriophage φX174)

F. SANGER, S. NICKLEN, AND A. R. COULSON
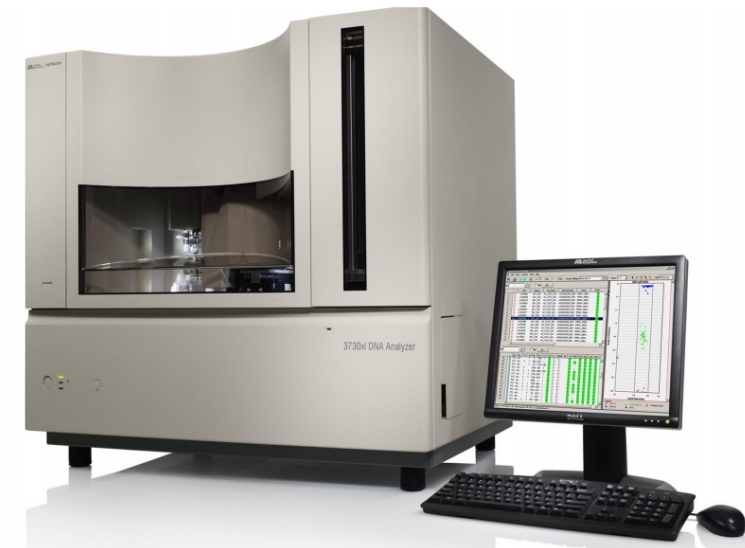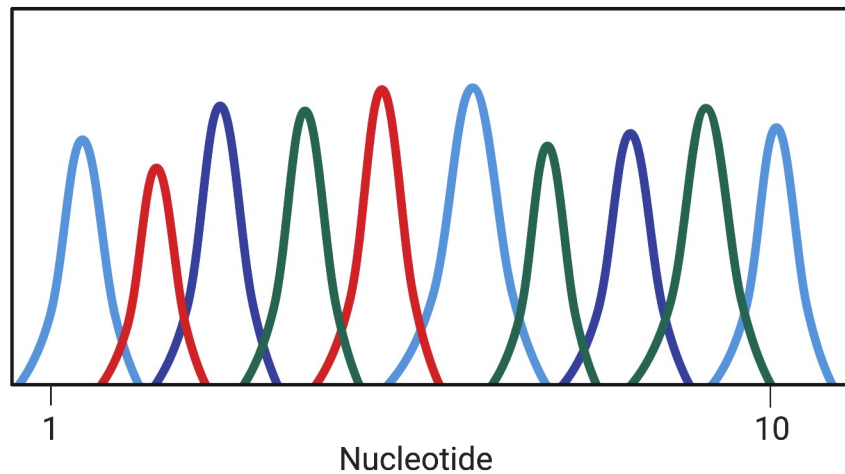
(THE INCOMPLETE) HISTORY OF SEQUENCING

High-throughput Sanger sequencing
Dye-terminators and capillary electrophoresis
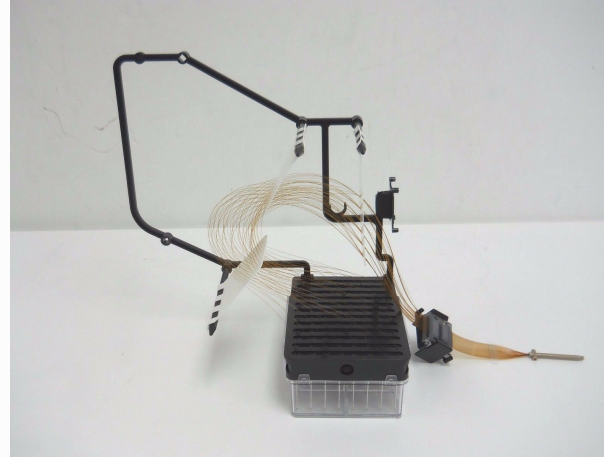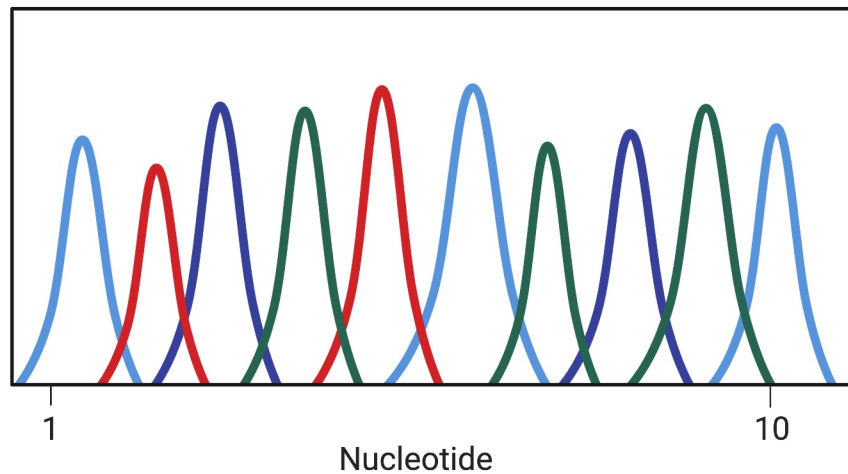
(THE INCOMPLETE) HISTORY OF SEQUENCING

High-throughput Sanger sequencing
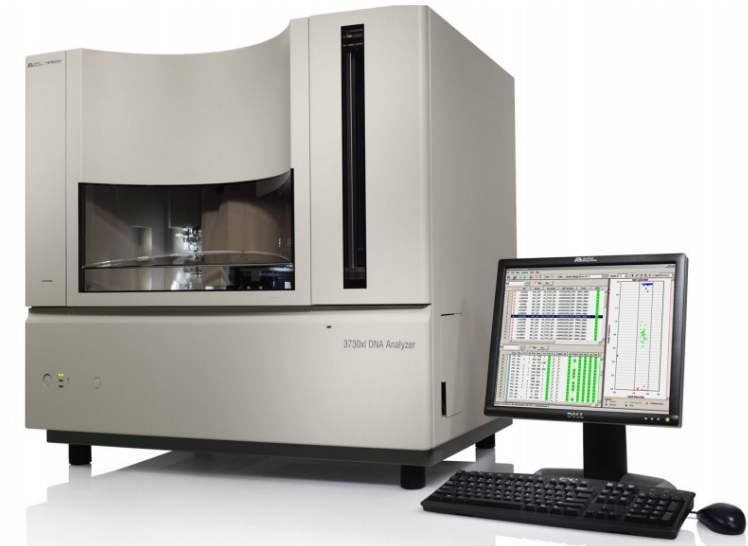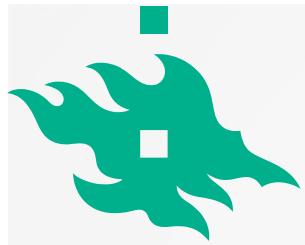Dye-terminators and capillary electrophoresis

(THE INCOMPLETE) HISTORY OF SEQUENCING

High-throughput Sanger sequencing
Dye-terminators and capillary electrophoresis

48/96 –capillars
Read length < **900 bp**
< **90 Kbp / run**

Nucleotide

(THE INCOMPLETE) HISTORY OF SEQUENCING

Sequencing by synthesis:
Pyrosequencing

Genome sequencing in microfabricated high-density picolitre reactors

Marcel Margulies, Michael Egholm, [...] Jonathan M. Rothberg ✉
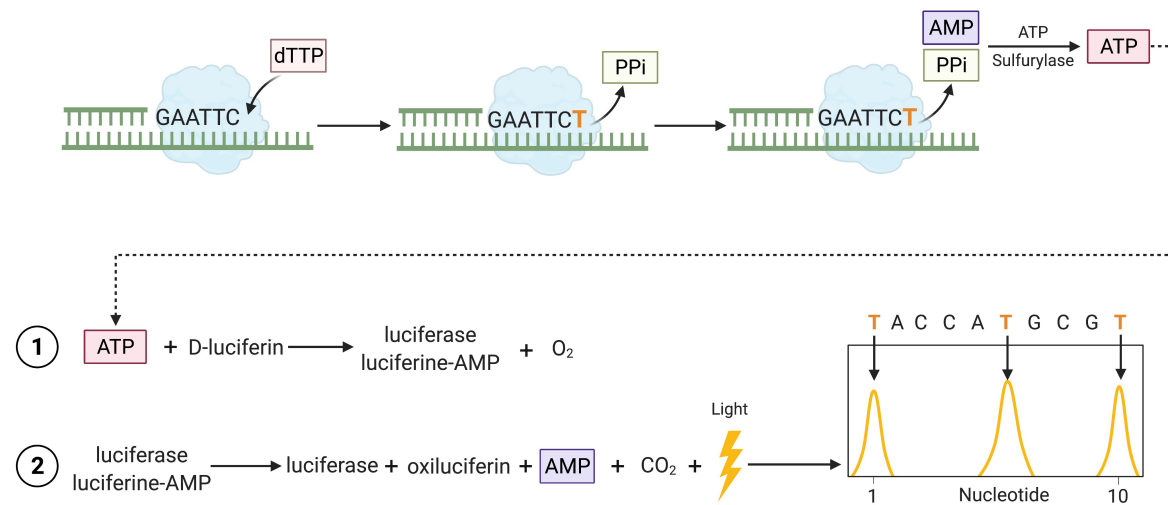
Nature **437**, 376–380(2005) | Cite this article

Sequencing by synthesis:
Pyrosequencing

Genome sequencing in microfabricated high-density picolitre reactors

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

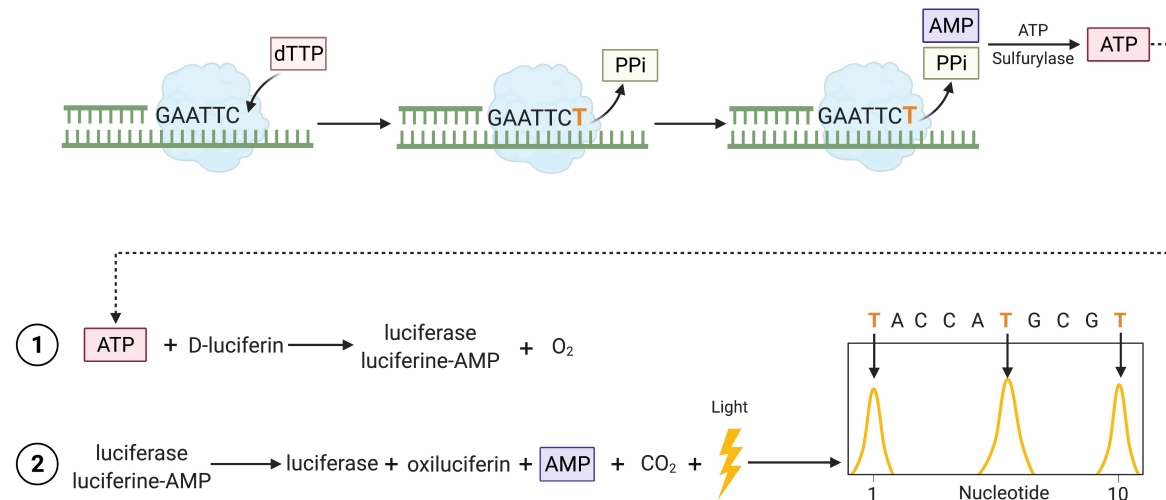*Nature* **437**, 376–380(2005) | Cite this article

(THE INCOMPLETE) HISTORY OF SEQUENCING

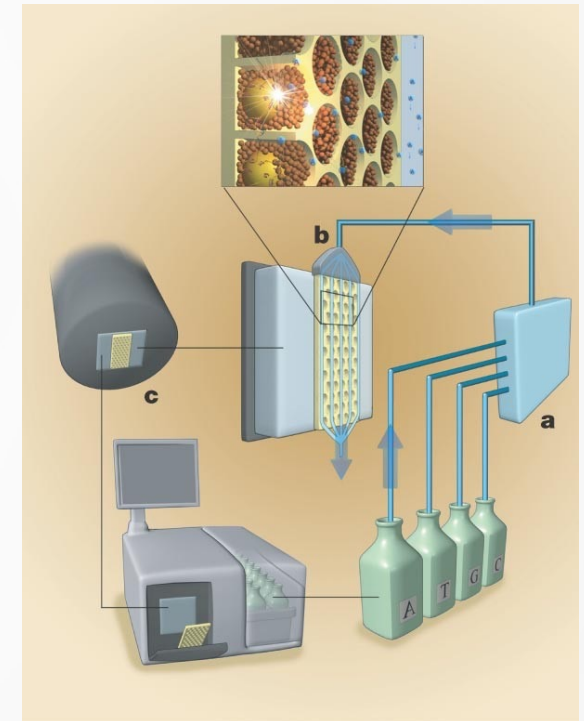**Sequencing by synthesis:**

Pyrosequencing

Genome sequencing in microfabricated high-density picolitre reactors

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

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

Marguelis et al., 2005. Nature

Read length ~ 400–1000 bp

20 – 600 Mbp / run

(THE INCOMPLETE) HISTORY OF SEQUENCING

Sequencing by synthesis:

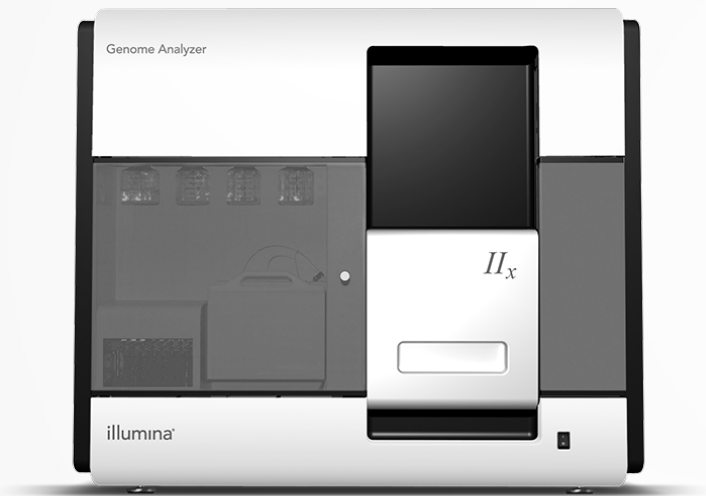Short-read sequencing

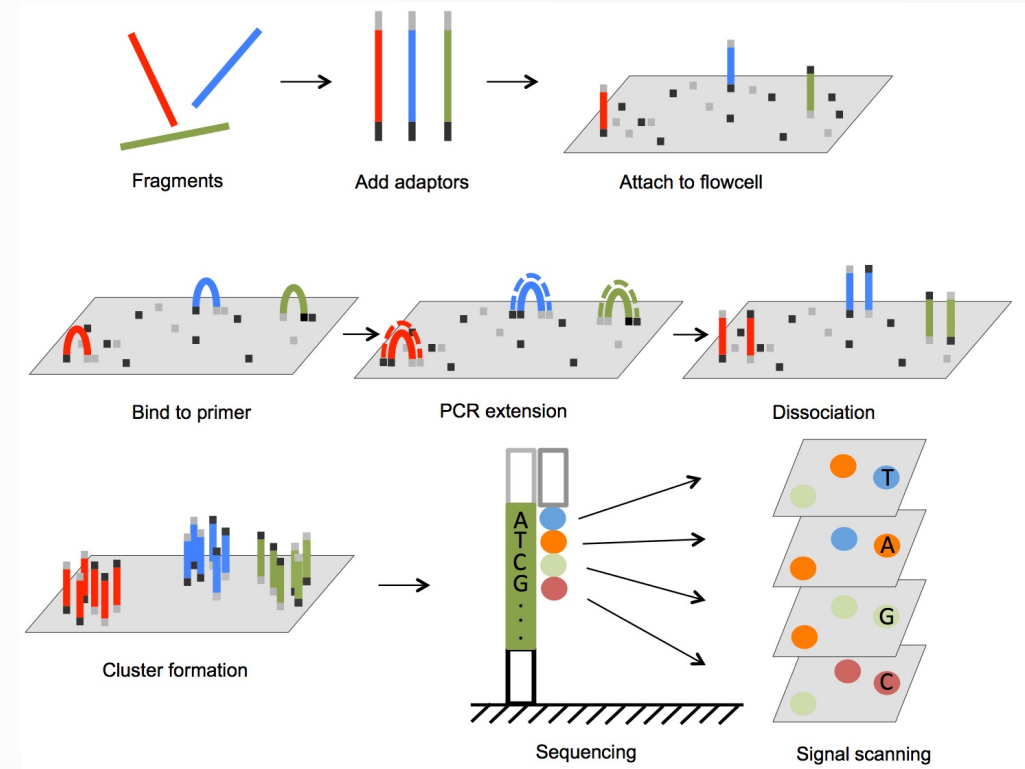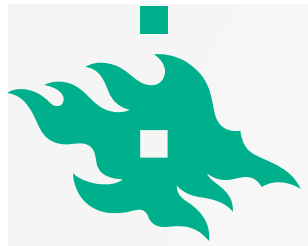Illumina (Solexa)
Genome Analyzer, 2006
1 Gbp / run

(THE INCOMPLETE) HISTORY OF SEQUENCING

Sequencing by synthesis:

Short-read sequencing

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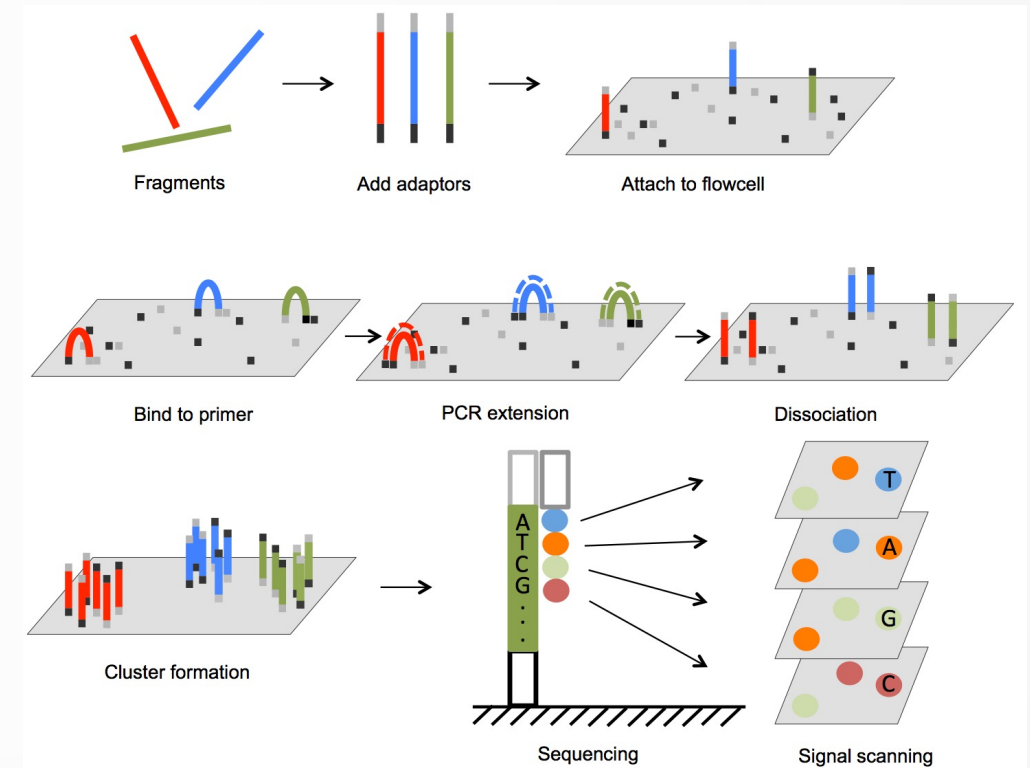Genome Analyzer IIx, 2008
20 Gbp / run (9.5 days)

(THE INCOMPLETE) HISTORY OF SEQUENCING

Sequencing by synthesis:
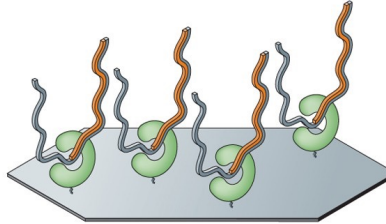
Short-read sequencing

1 Gbp – >1 Tbp / run (~1–2 d)

(THE INCOMPLETE) HISTORY OF SEQUENCING
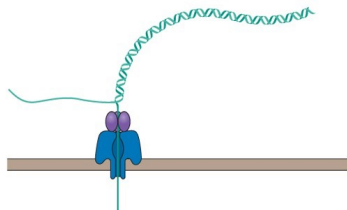
Third revolution:
Long-read sequencing

(THE INCOMPLETE) HISTORY OF SEQUENCING

Third revolution:
Long-read sequencing

(THE INCOMPLETE) HISTORY OF SEQUENCING

Third revolution:
Long-read sequencing

The Third Revolution
Single-molecule sequencing

Pac Bio SMRT sequencing
• Sequencing by synthesis
• Single-molecule templates
• Low accuracy but long read lengths

For example, PacBio *RS*
(Pacific Biosciences)

Oxford Nanopore sequencing
• Nanopore sequencing
• Single-molecule templates
• Low accuracy but long read lengths
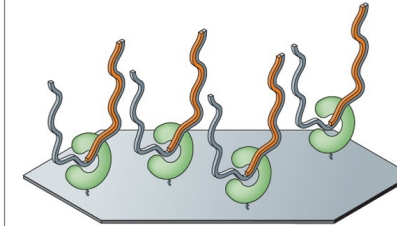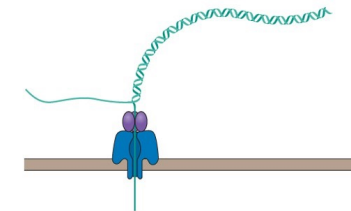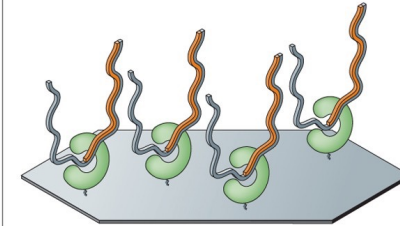
For example, MinION
(Oxford Nanopore)

Nature Reviews | Microbiology

(THE INCOMPLETE) HISTORY OF SEQUENCING
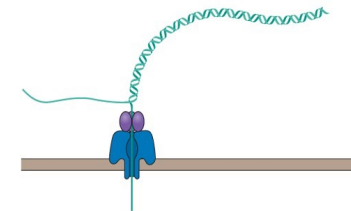
Third revolution:
Long-read sequencing

The Third Revolution
Single-molecule sequencing

Pac Bio SMRT sequencing
- Sequencing by synthesis
- Single-molecule templates
- Low accuracy but long read lengths

For example, PacBio *RS*
(Pacific Biosciences)

Oxford Nanopore sequencing
- Nanopore sequencing
- Single-molecule templates
- Low accuracy but long read lengths
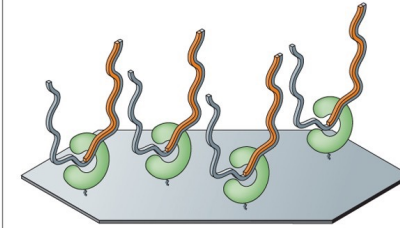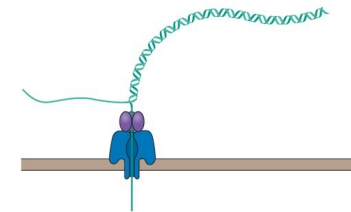
For example, MinION
(Oxford Nanopore)

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!

Nature Reviews | Microbiology