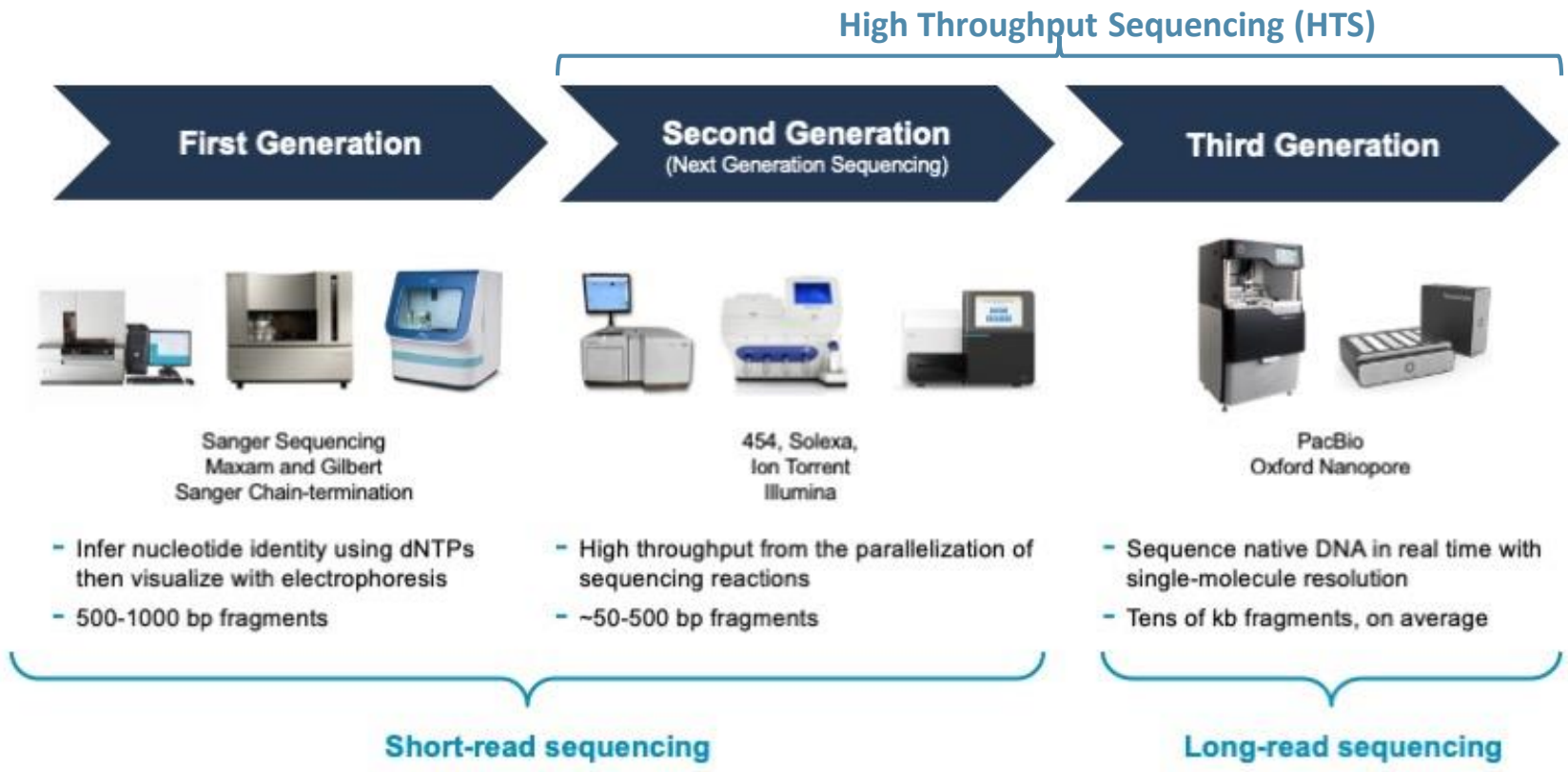
Introduction to NGS data analysis

Dr Gustavo A. Silva-Arias

Technische Universität München

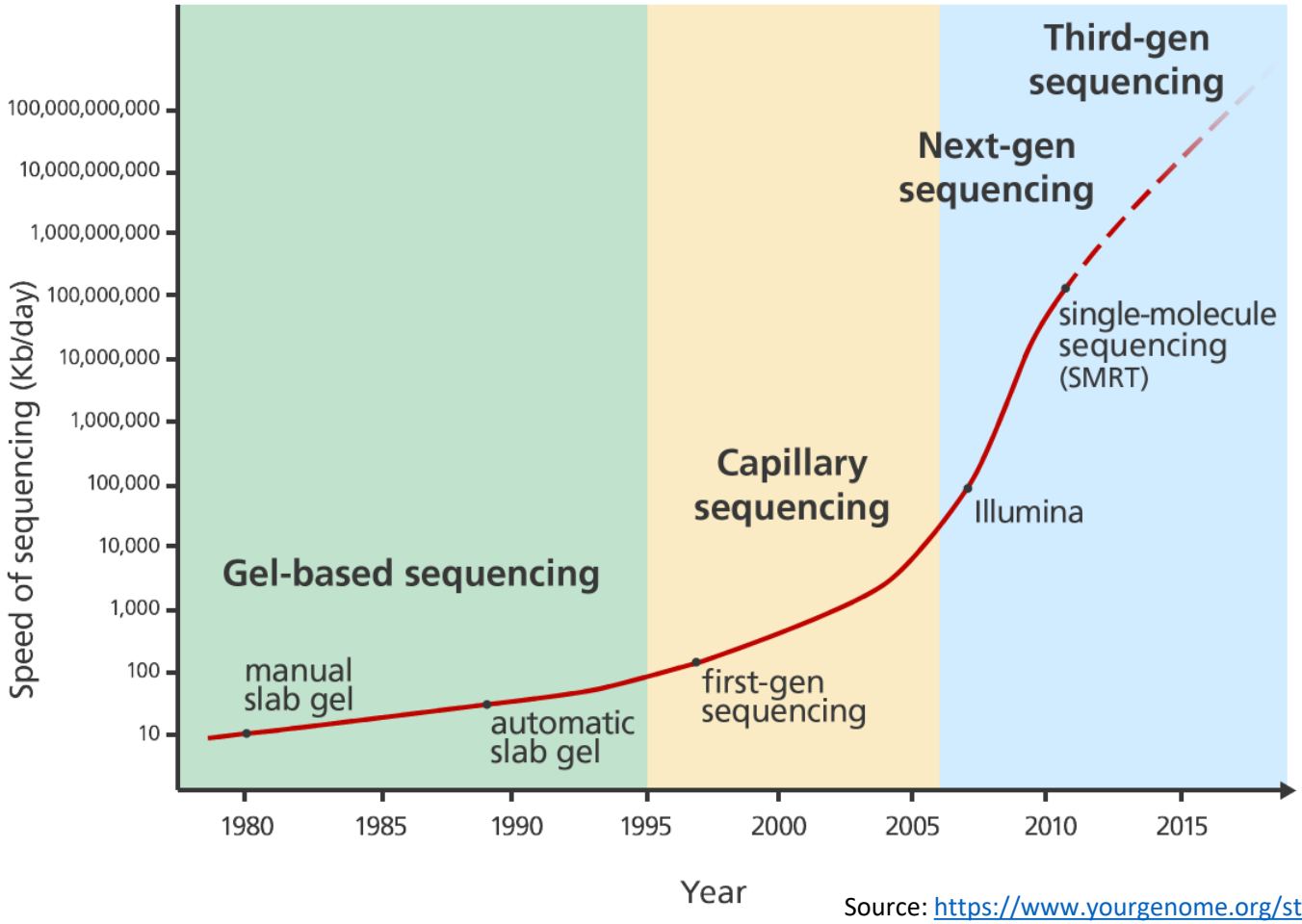
Bogotá, 23 de Agosto 2021

The evolution of sequencing technology



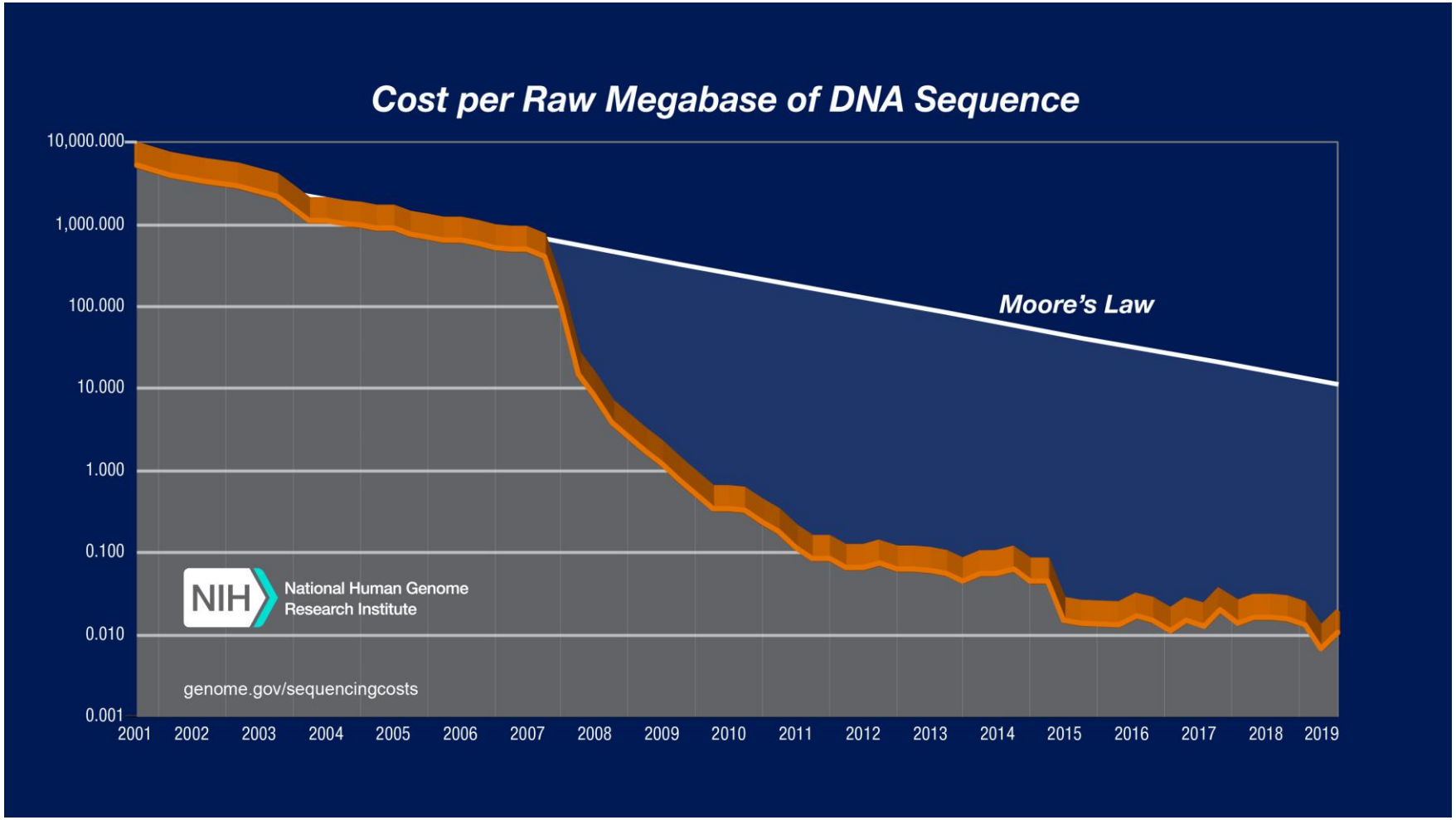
Source: https://www.pacb.com/blog/the-evolution-of-dna-sequencing-tools/

The evolution of sequencing technology - efficiency



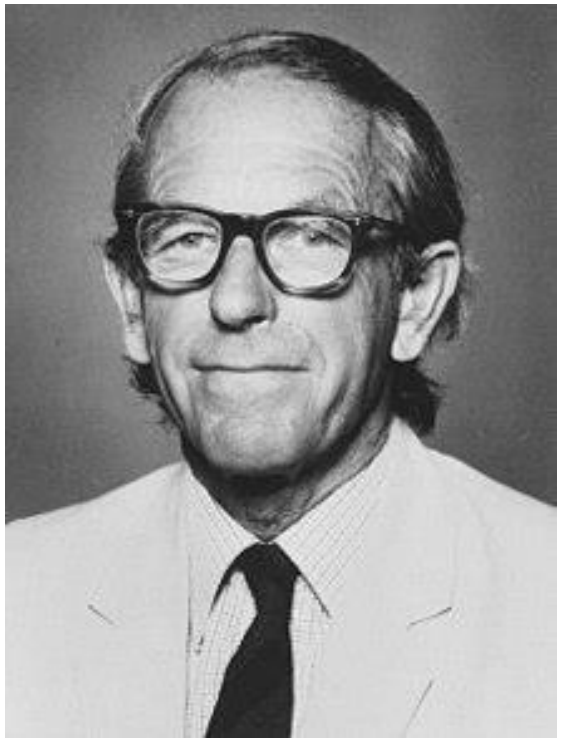
Source: https://www.yourgenome.org/stories/third-generation-sequencing

The evolution of sequencing technology - cost



Source: https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data

First Generation: Sanger sequencing



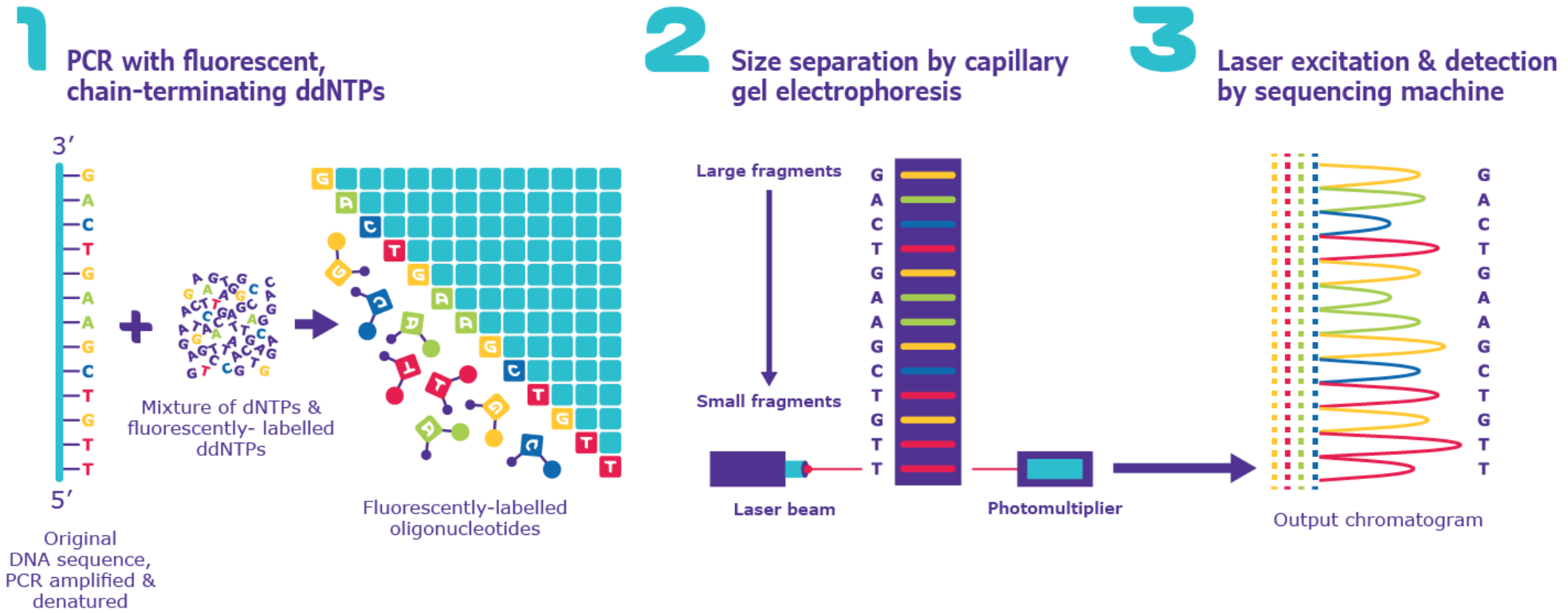
- Frederick Sanger (1918-2013)
- British biochemist

- **Nobel Prize in Chemistry 1958:**
  - *"for his work on the structure of proteins, especially that of insulin.“*
  - He determined the complete aminoacid sequence of bovine insuline using electrophoresis and chromatography

- **Nobel Prize in Chemistry 1980:**
  - *"for their contributions concerning the determination of base sequences in nucleic acids.“*
  - Developed the "dideoxy" chain-termination method for sequencing DNA molecules, now known as the “Sanger method“.

Source: https://www.nobelprize.org/prizes/chemistry/

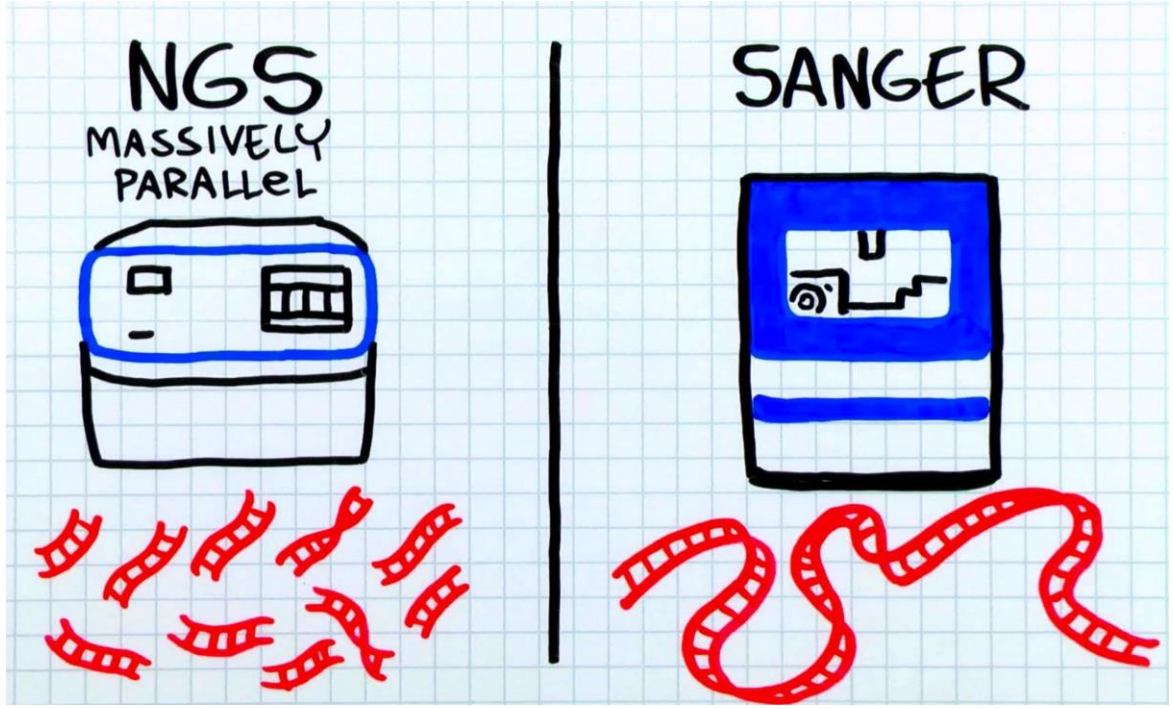
First Generation: Sanger sequencing



- Old gel „slab“ method: https://www.youtube.com/watch?v=3M0PyxFPwkQ
- Capillary gel method: https://www.youtube.com/watch?v=x7PUqNA0eOA

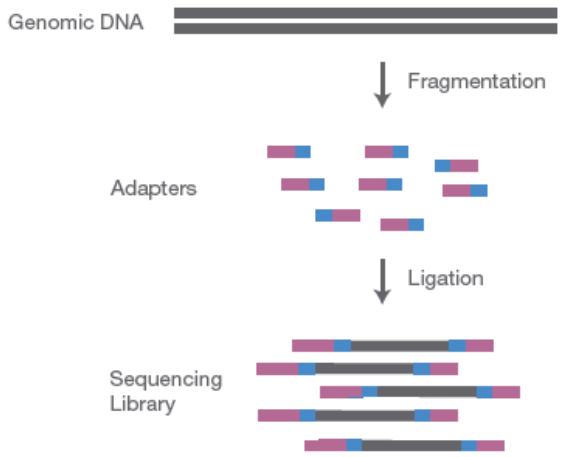
Source: https://www.sigmaaldrich.com/technical-documents/articles/biology/sanger-sequencing.html

Second Generation:
Massive Parallel Sequencing of Short Reads

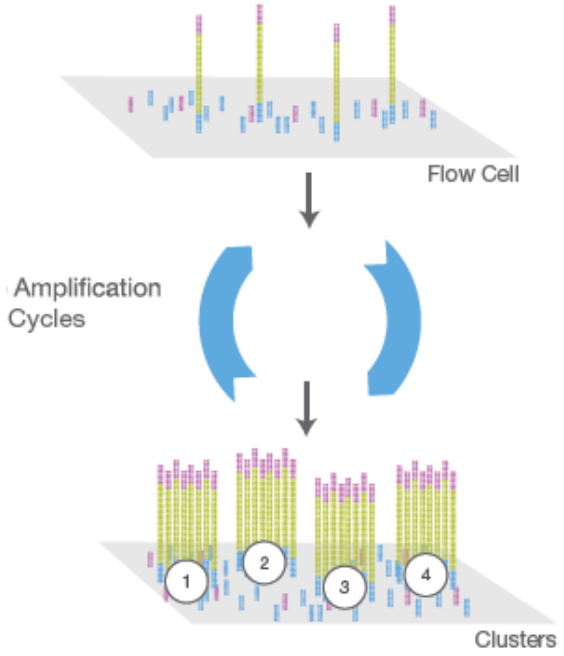


Second Generation:
Massive Parallel Sequencing of Short Reads

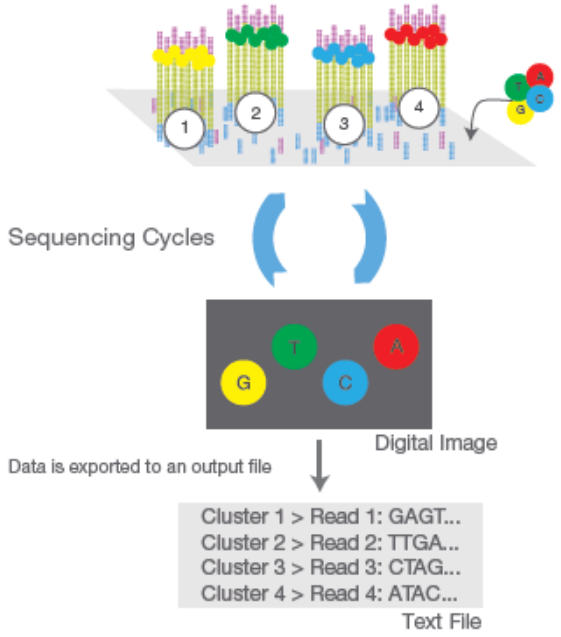
- 1. Library preparation



2. Template Amplification



- 3. Sequencing



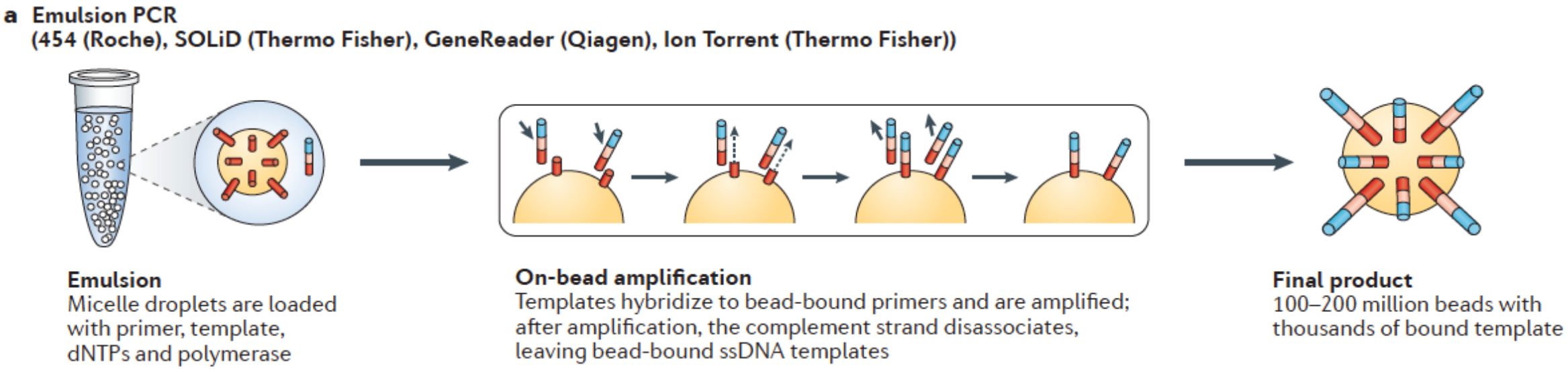
Source: https://emea.illumina.com/content/dam/illumina-marketing/documents/products/other/ivf-reproductive-genetic-health-ngs-primer-1570-2015-012.pdf

Second Generation:
Massive Parallel Sequencing of Short Reads

Template amplification methods

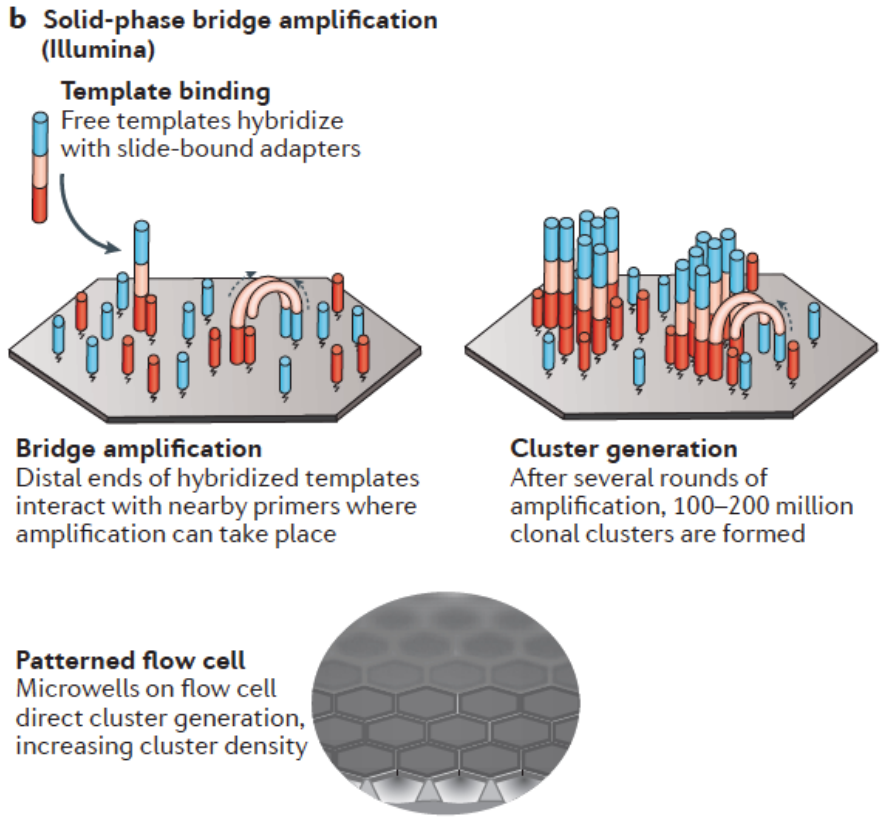
- Emulsion PCR
  - 454 (Roche)
  - SOLiD (Thermo Fisher)
  - GeneReader (Quiagen)
  - Ion Torrent (Thermo Fisher)
- Solid-phase bridge amplification
  - Illumina
- Solid-phase template walking
  - SOLiD Wilfire – Thermo Fischer
- In-solution DNA nanoball generation
  - Complete Genomics - BGI

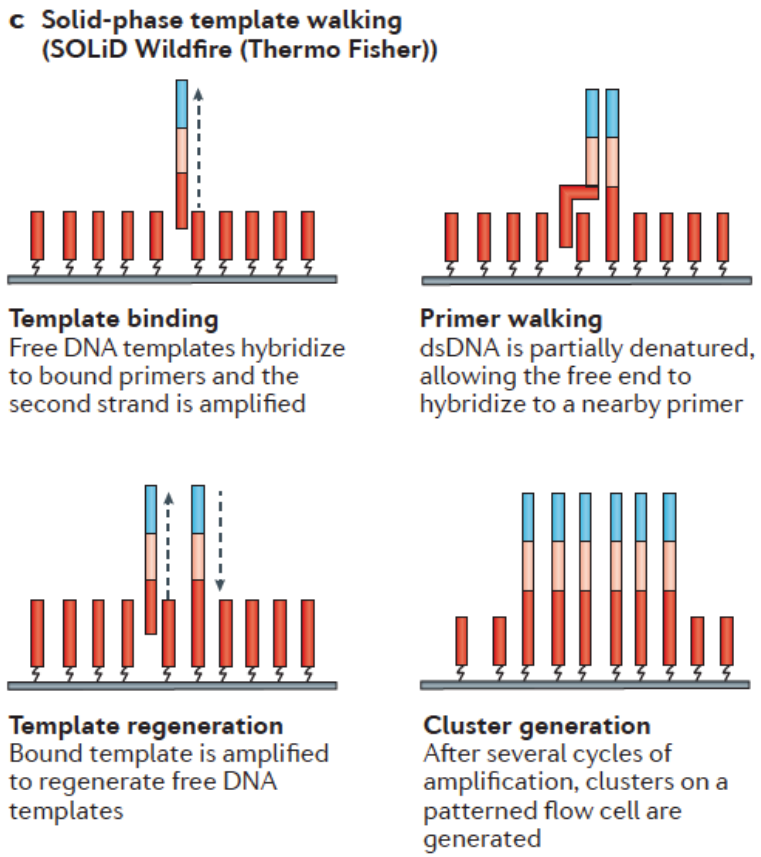
Template amplification methods



Source: Sara Goodwin *et al.* 2016 - *Nature Reviews Genetics* - https://doi.org/10.1038/nrg.2016.49

Template amplification methods





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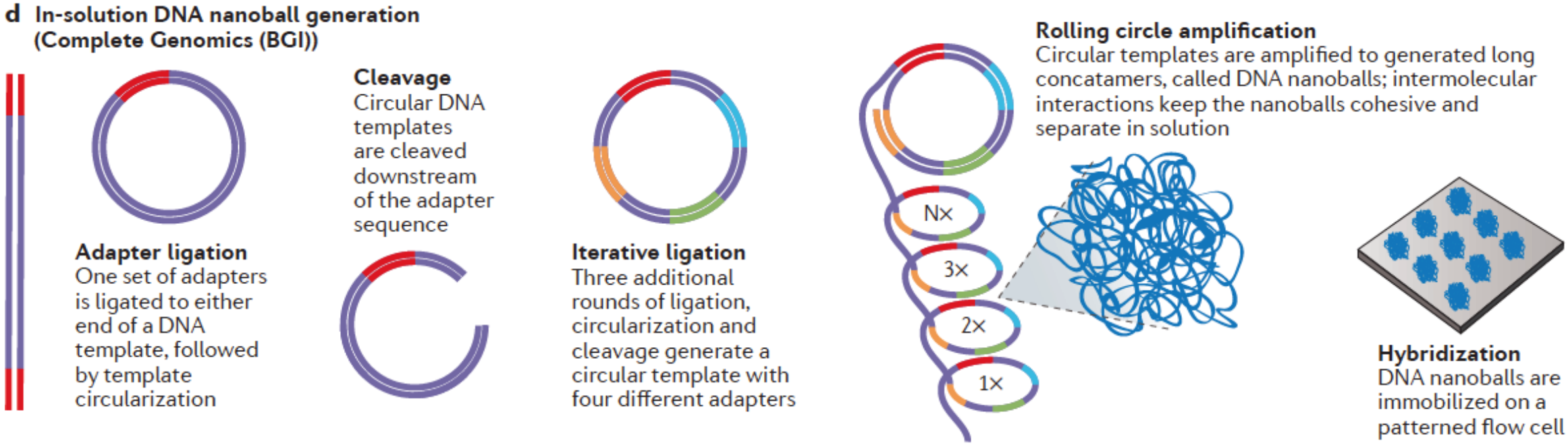
Template amplification methods

DNBSEQ (BGI)



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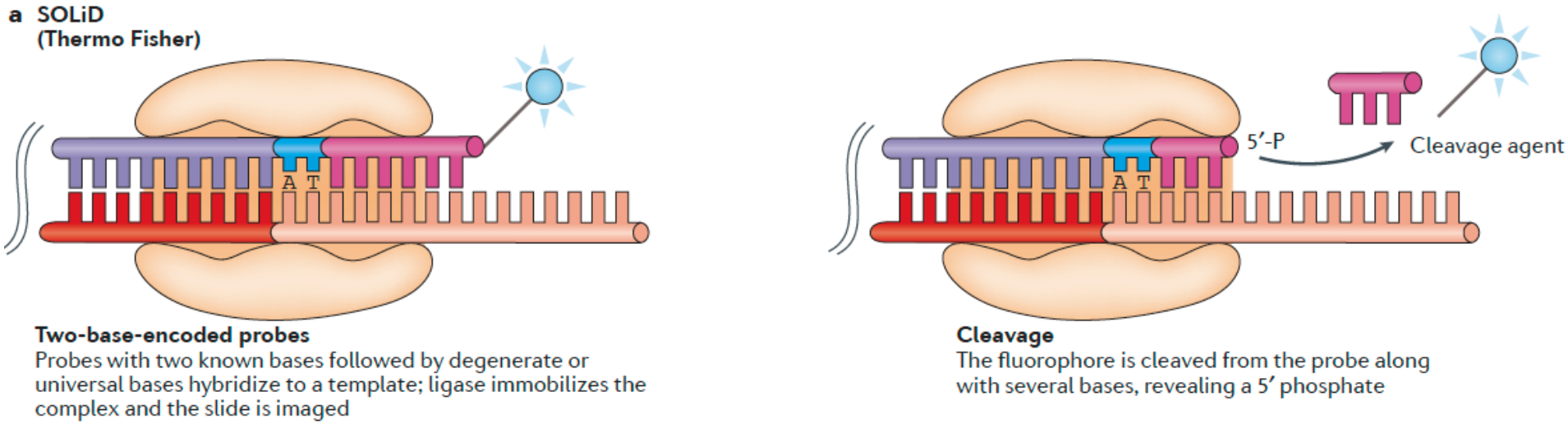
Second Generation:
Massive Parallel Sequencing of Short Reads

<u>Sequencing Methods</u>

- By Ligation (SBL)
  - SOLiD (Thermo Fisher)
- By Synthesis (SBS)
  - Cyclic Reversible Termination (CRT)
    - Illumina
    - GeneReader (QiaGen)
    - DNBSEQ (BGI)
  - SNA: Single Nucleotide Addition
    - 454 (Roche)
    - IonTorrent (ThermoFisher)

Sequencing Methods - By Ligation (SBL)



Two-base-encoded probes
Probes with two known bases followed by degenerate or universal bases hybridize to a template; ligase immobilizes the complex and the slide is imaged

Cleavage
The fluorophore is cleaved from the probe along with several bases, revealing a 5′ phosphate

Full explanation: https://www.youtube.com/watch?v=PPEKybWYOBA

***Note:** DNBSEQ (BGI) also used a ligation technique but has switched to SBS to obtain longer reads

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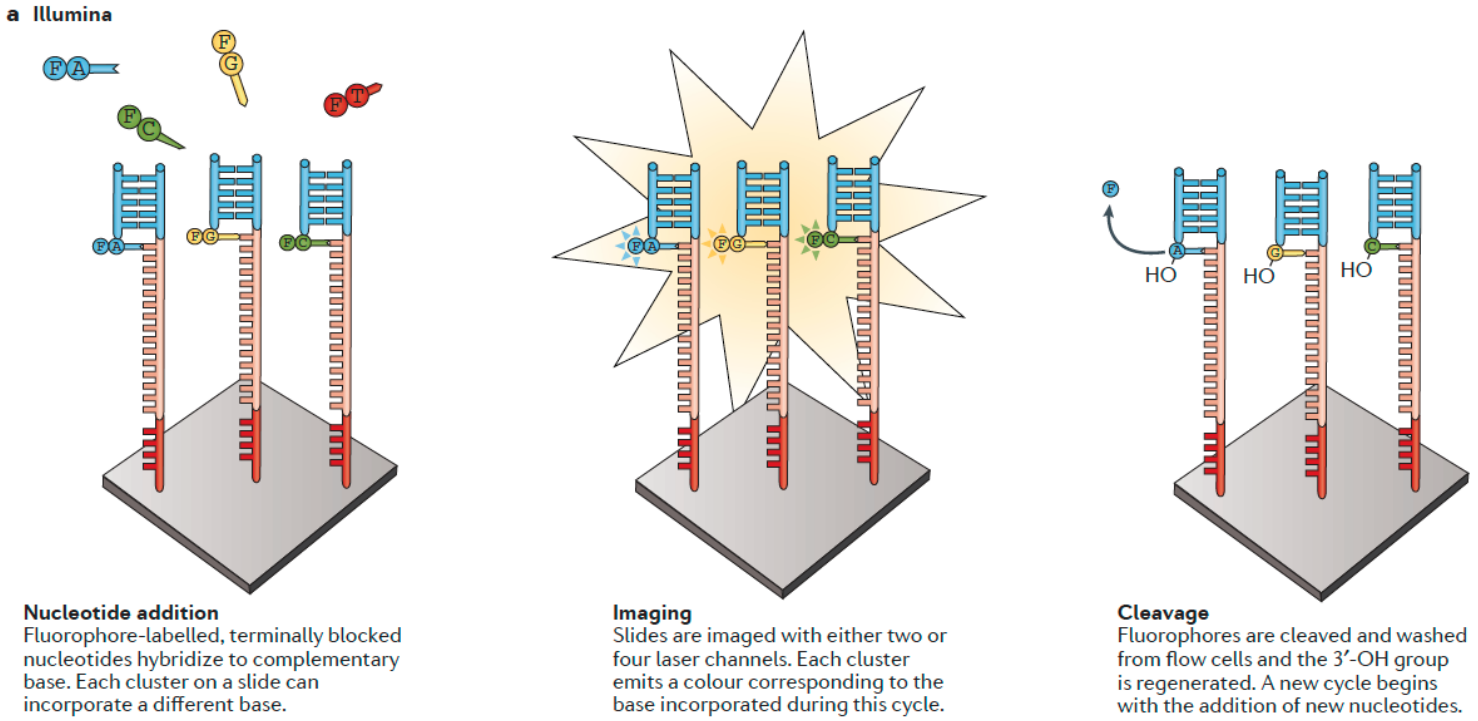
Sequencing Methods - By Synthesis (SBS)

- CRT: Cyclic Reversible Termination, used by Illumina, GeneReader (QiaGen), DNBSEQ (BGI)



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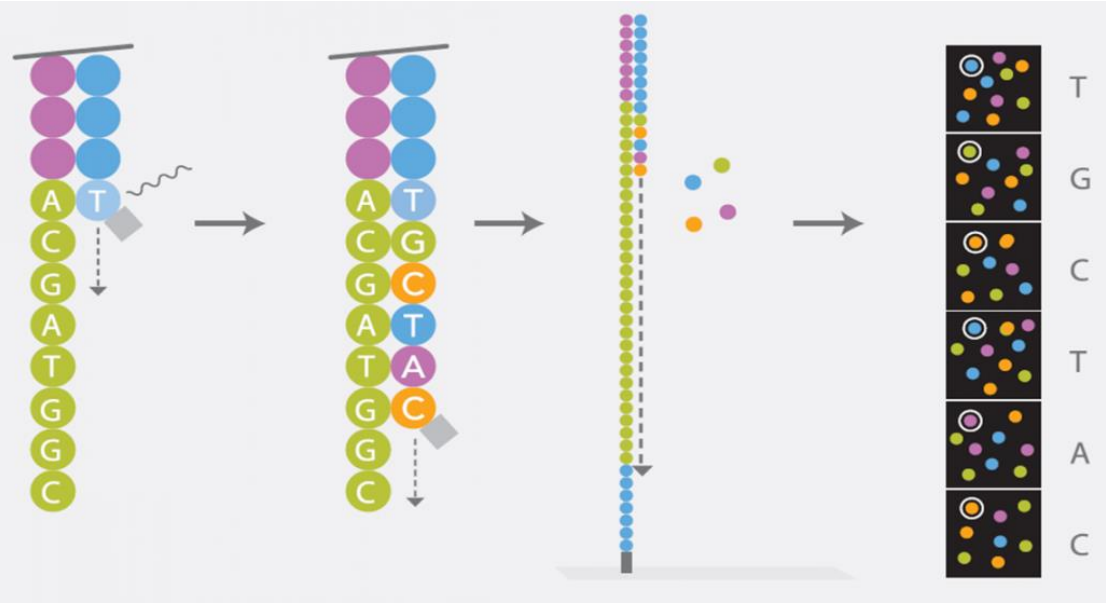
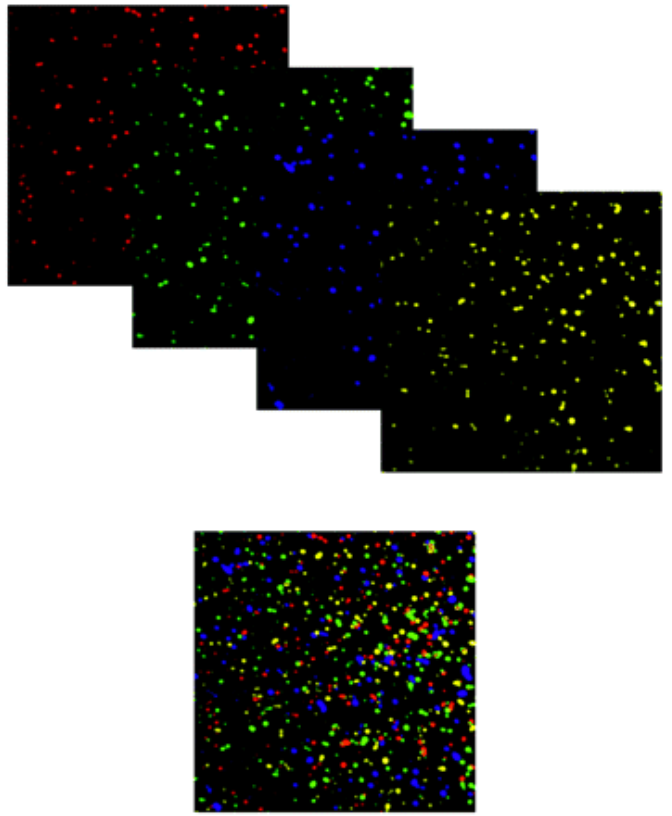


Cycle 1
Cycle 2
Cycle 3
Cycle 4
Cycle 5
Cycle 6
Cycle 150

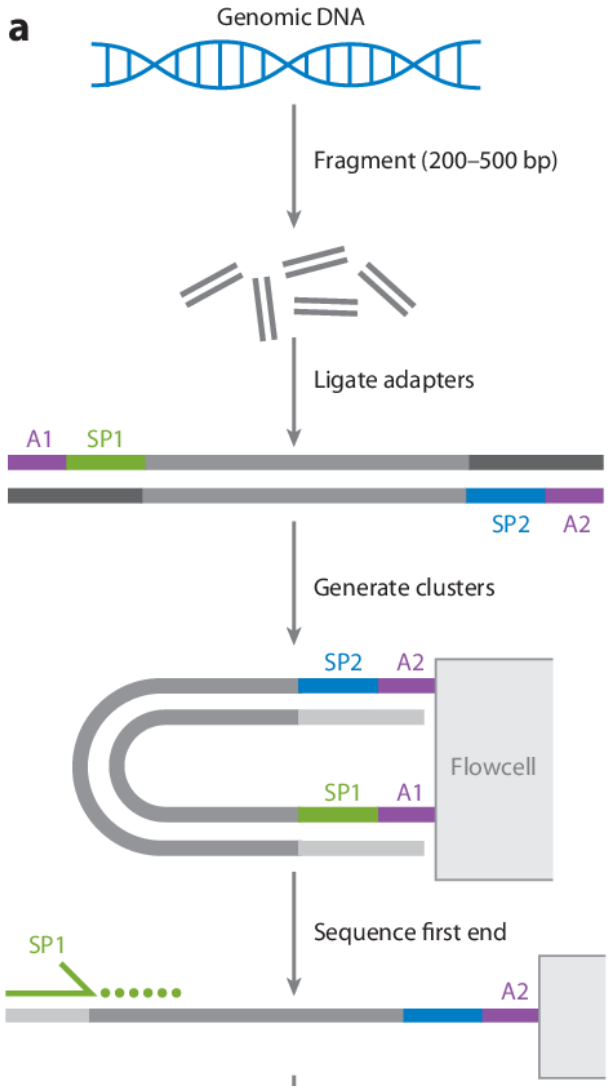
Store an image per cycle to reconstruct the sequence of each cluster

Source: Sara Goodwin *et al.* 2016 - *Nature Reviews Genetics* - https://doi.org/10.1038/nrg.2016.49



Source: https://doi.org/10.1373/clinchem.2008.112789

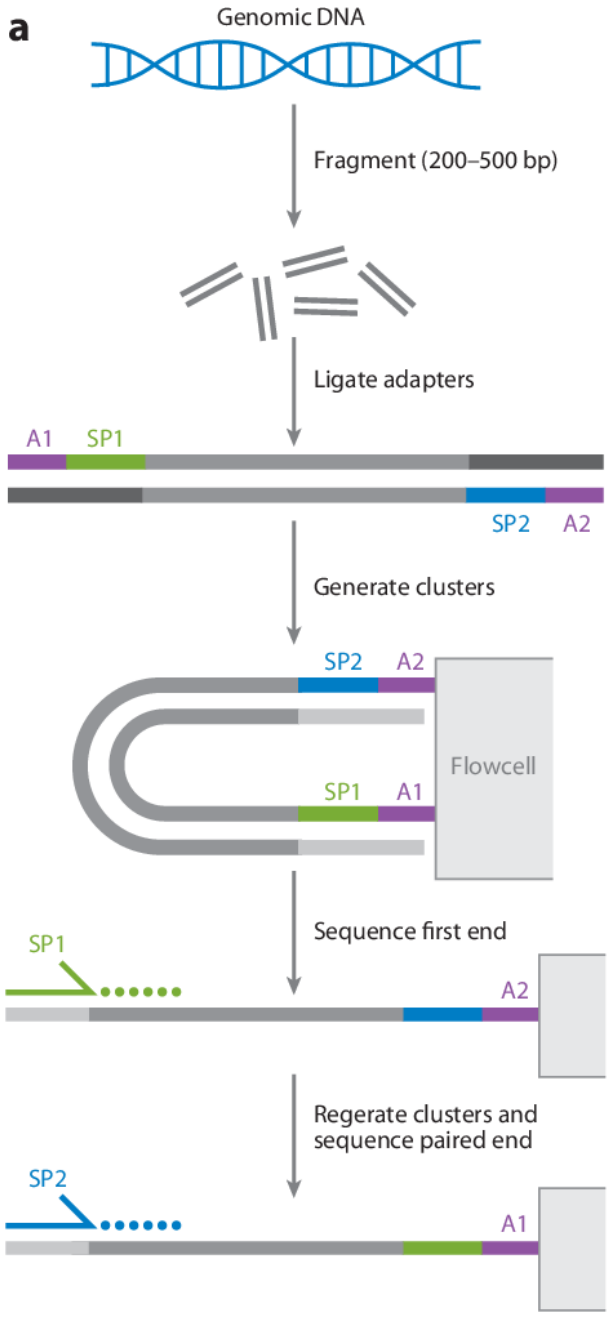
Single-End vs. Paired-End (Illumina, DNBSEQ)



Single-End

Source: Elaine R. Mardis 2013 - *Annual Review of Analytical Chemistry* - https://doi.org/10.1146/annurev-anchem-062012-092628

Single-End vs. Paired-End (Illumina, DNBSEQ)



Paired-End

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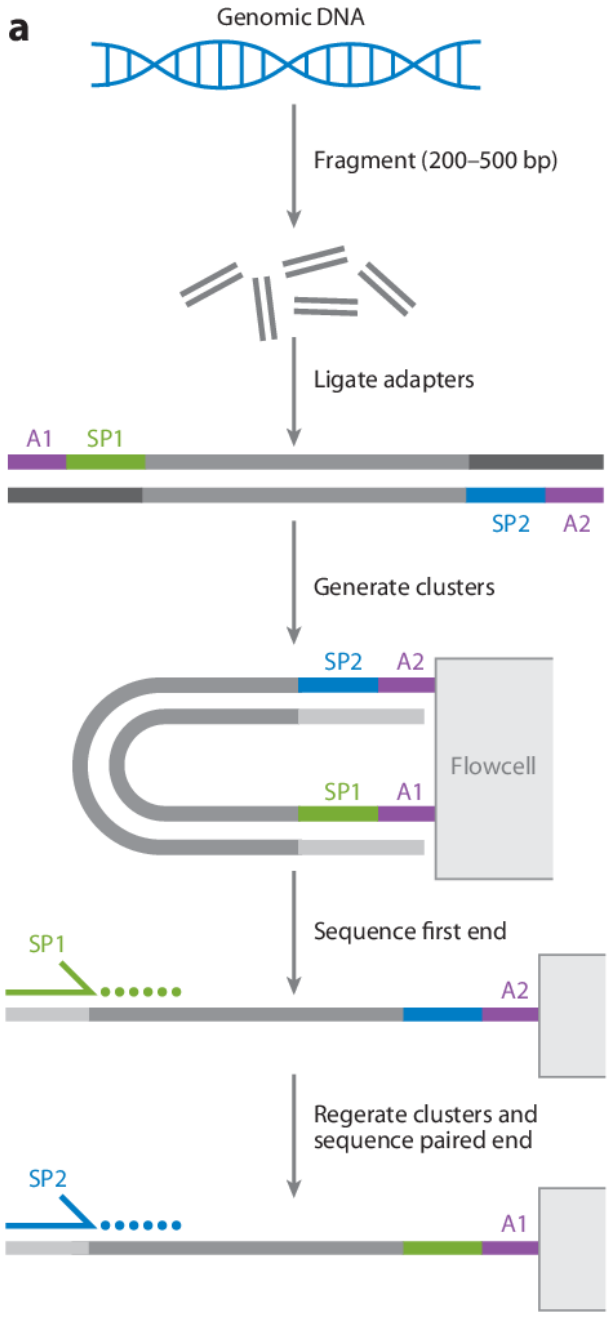
Single-End vs. Paired-End (Illumina, DNBSEQ)



Read length 150 (x2)

Insert length

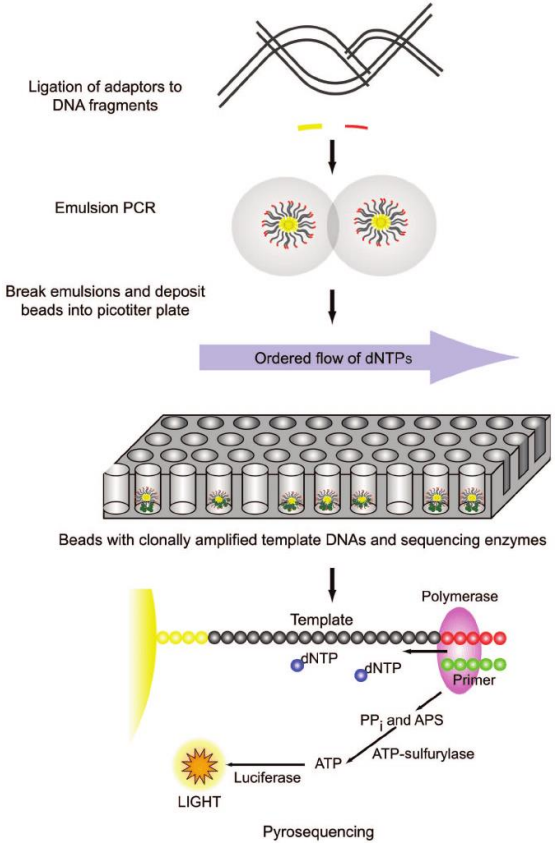
Ideally the insert length (genomic DNA fragment) should be longer than read length x 2

Paired-End

Source: Elaine R. Mardis 2013 - *Annual Review of Analytical Chemistry* - https://doi.org/10.1146/annurev-anchem-062012-092628

Sequencing Methods - By Synthesis (SBS)

- SNA: Single Nucleotide Addition. 454 (Roche), IonTorrent (ThermoFisher)



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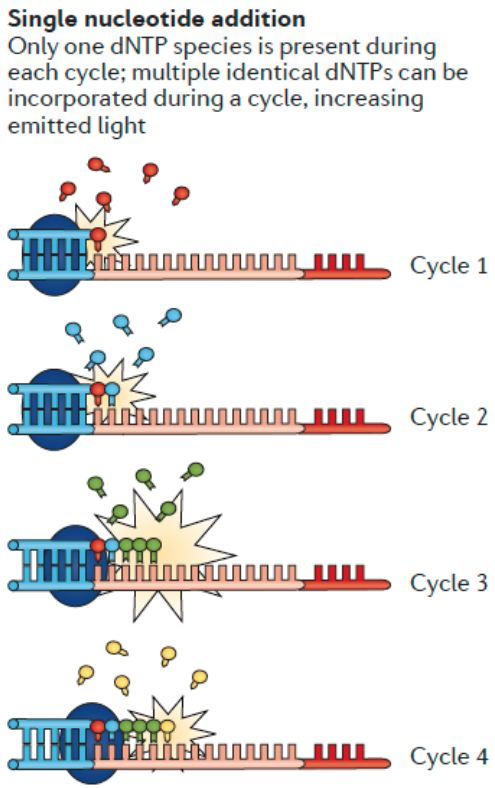

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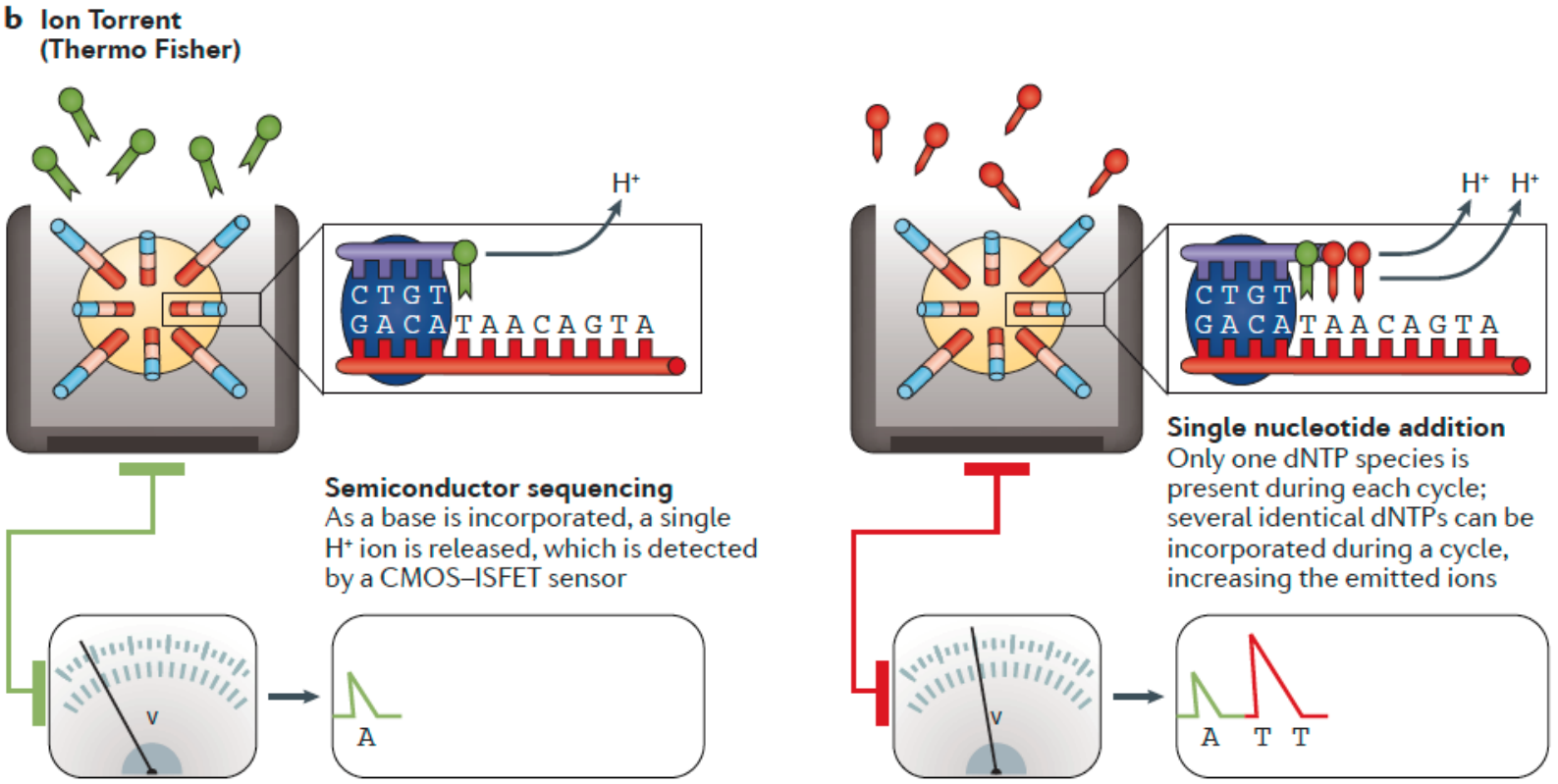
Sequencing Methods - By Synthesis (SBS)

- SNA: Single Nucleotide Addition, used by 454 (Roche), IonTorrent (ThermoFisher)



Source: Sara Goodwin *et al.* 2016 - *Nature Reviews Genetics* - https://doi.org/10.1038/nrg.2016.49

Sequencing Methods - By Synthesis (SBS)

Full explanation videos:

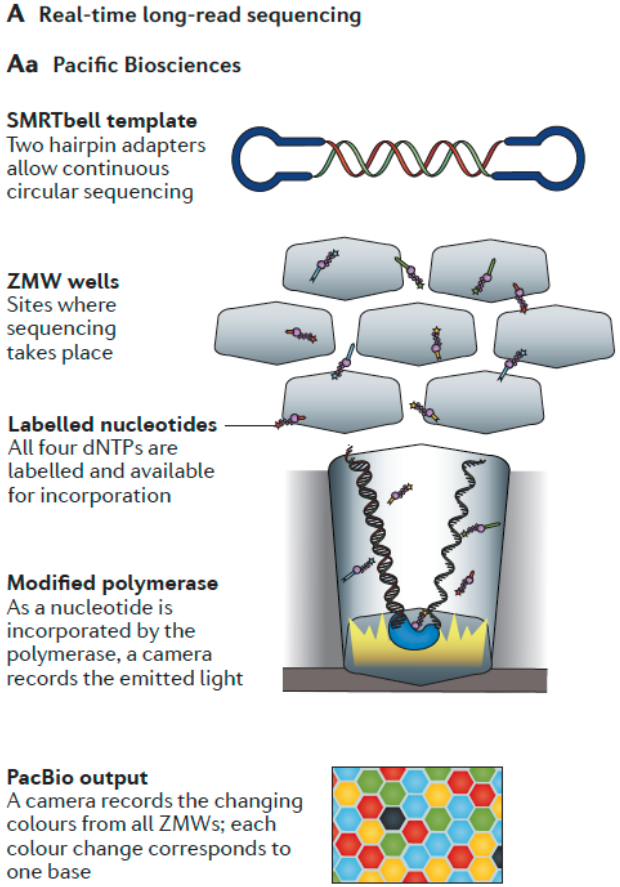
- Illumina [CRT]:
  https://www.youtube.com/watch?v=fCd6B5HRaZ8

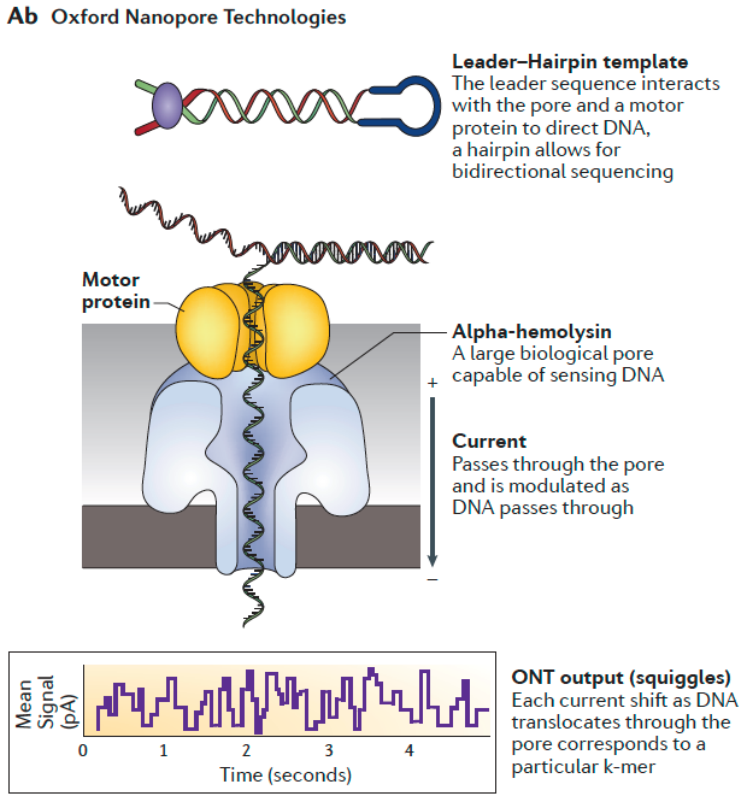
- DNBSEQ (BGI) [CRT]:
  https://www.youtube.com/watch?v=RGcpftDHpng&t

- 454 pyrosequencing [SNA]:
  https://www.youtube.com/watch?v=bNKEhOGvcaI

- IonTorrent [SNA]:
  https://www.youtube.com/watch?v=ZL7DXFPz8rU

Third Generation:
Parallel Sequencing of Long Reads in Real Time





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Third Generation:
Parallel Sequencing of Long Reads in Real Time

Full explanation videos:

- PacBio:

  https://www.youtube.com/watch?v=v8p4ph2MAvI

  https://www.youtube.com/watch?v=NHCJ8PtYCFc

  https://www.youtube.com/watch?v=_lD8JyAbwEo

- Nanopore:

  https://www.youtube.com/watch?v=E9-Rm5AoZGw

  https://www.youtube.com/watch?v=CGWZvHIi3i0