Illumina sequencing technology

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Illumina sequencing technology

- Introduction to second generation sequencing
- Library preparation and fixation of DNA fragments on a solid support
- Amplification
- Sequencing
- Illumina sequencers and throughput
- Comparison between different generations of sequencing technologies

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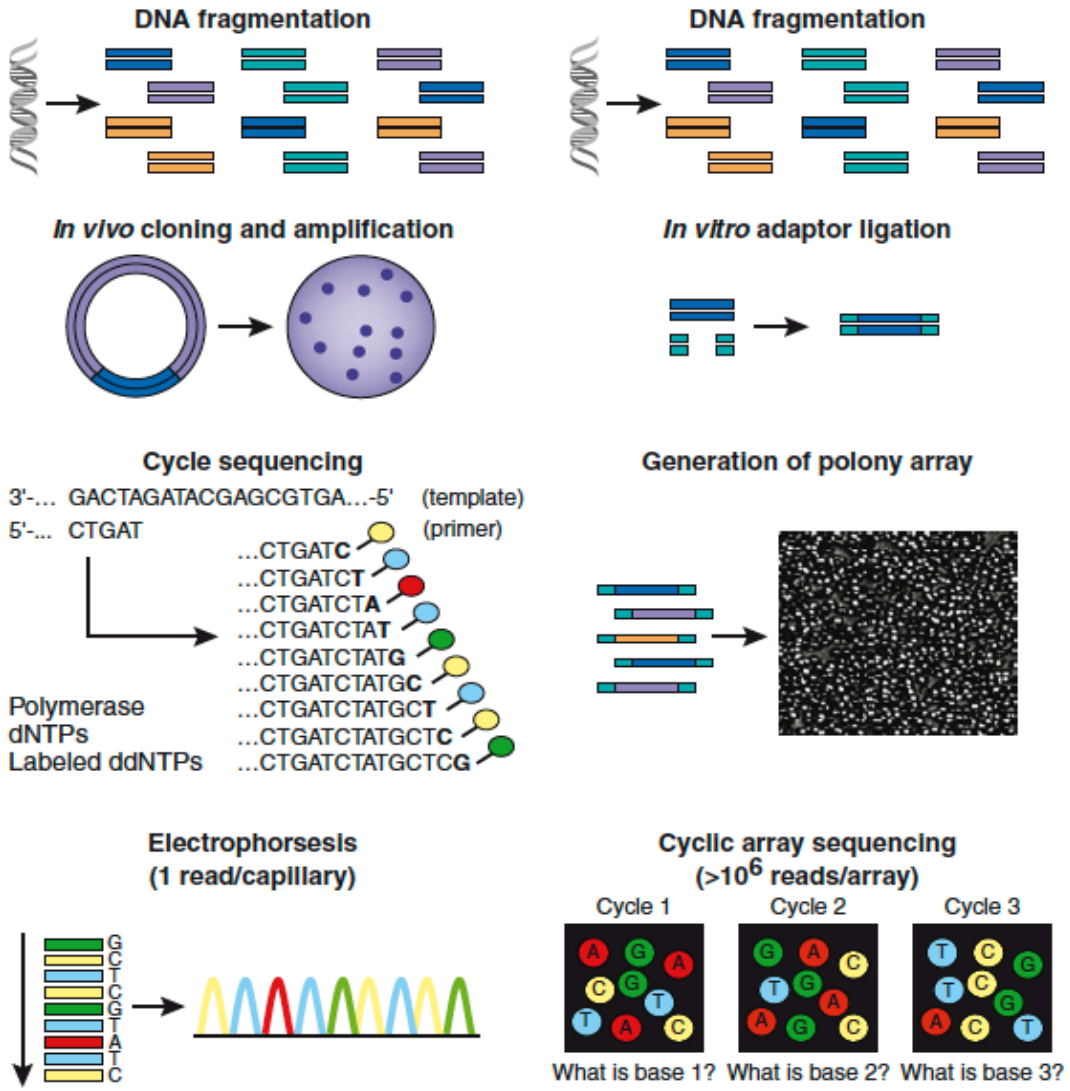
Conventional vs second generation sequencing

Conventional sequencing 2nd generation sequencing



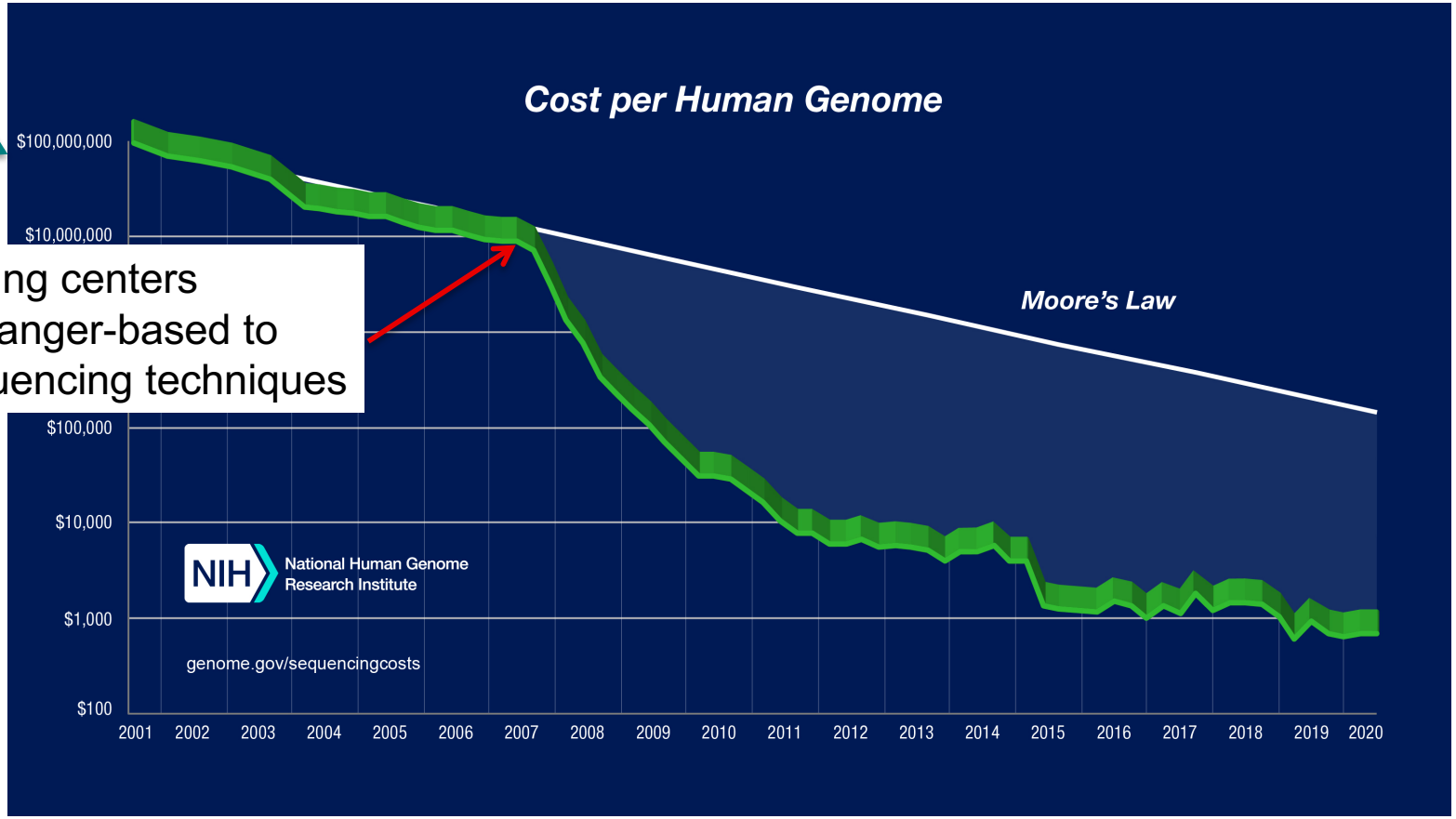
➔ Important decrease of
- Cost per base
- Time needed to obtain sequences

Shendure et al., Nature Biotechnology 2008

Decrease of sequencing costs

Log scale !

When the sequencing centers transitioned from Sanger-based to 2nd generation sequencing techniques

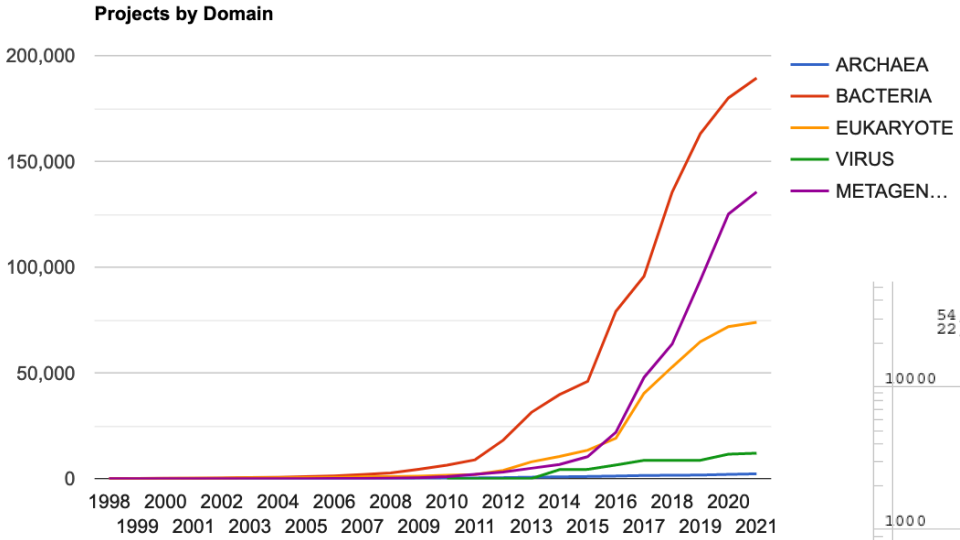


http://www.genome.gov/sequencingcosts/

Human genome with 30X coverage for Illumina sequencing
More information on how these costs are calculated :
https://www.genome.gov/sequencingcostsdata/

Increase of data volume

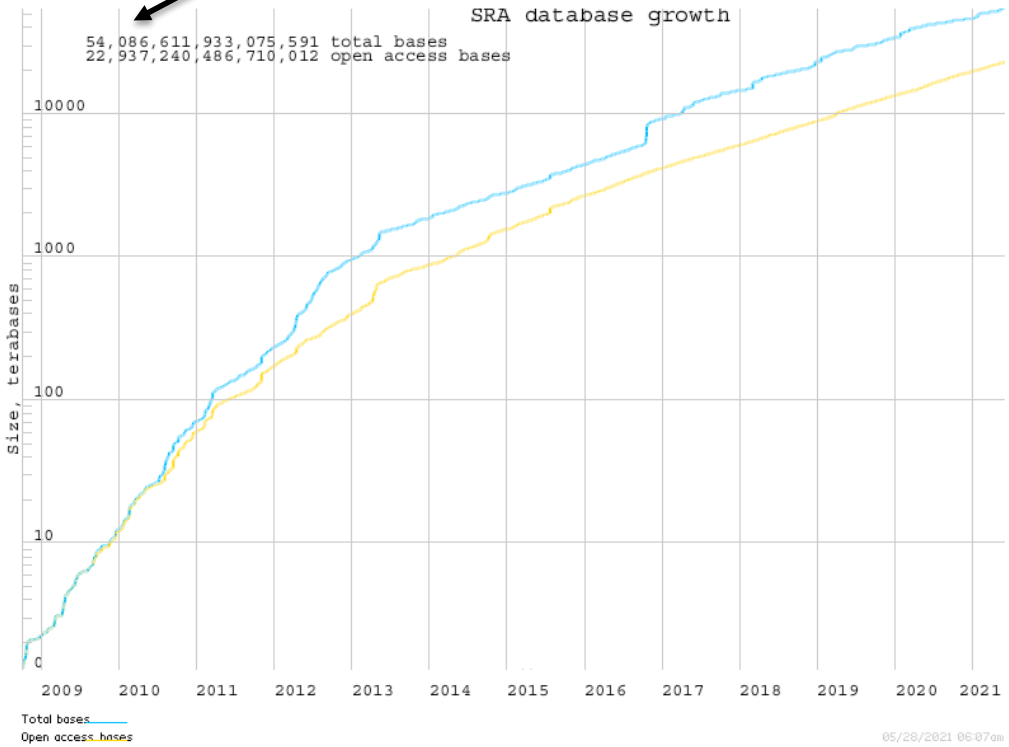
Projects in Genome Online Database



https://gold.jgi.doe.gov/statistics

Bases in Sequence Read Archive

~ 54,000 Tera bases

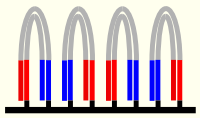
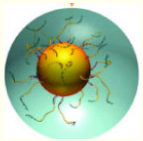
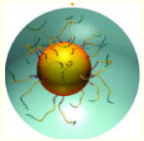
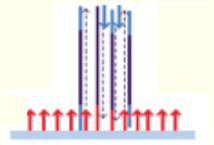
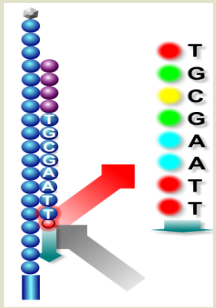
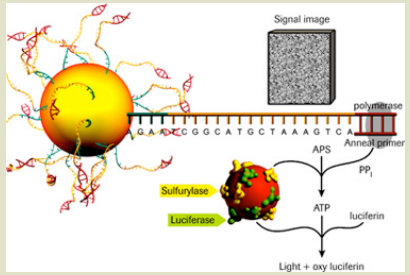
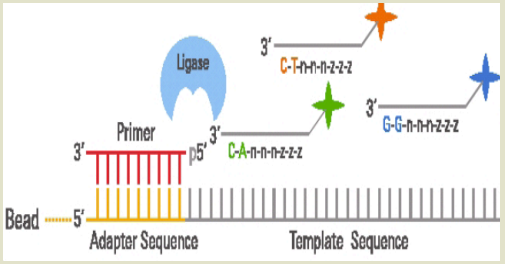
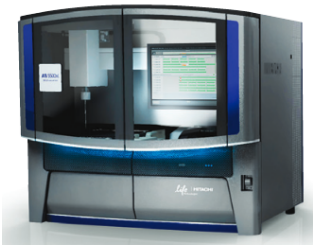


https://www.ncbi.nlm.nih.gov/sra/docs/sragrowth/

Second generation sequencing

- Three main technologies
  - Illumina (formerly Solexa)
    - Fedurco et al., 2006
  - SOLiD DNA Sequencer - Applied Biosystems by Life Technologies (Thermo Fisher Scientific)
    - Shendure et al., 2005
  - Genome Sequencer FLX+ - Roche (formerly 454)
    - Margulies et al., 2005

Second generation sequencing technologies

	Illumina	Roche 454	Applied Biosystems SOLiD	
Amplification	Bridge amplification 	Emulsion PCR 	Emulsion PCR 	Wildfire isothermal amplification 
Sequencing	By synthesis (reversible terminators) 	By synthesis (pyrosequencing) 	By ligation 	
				

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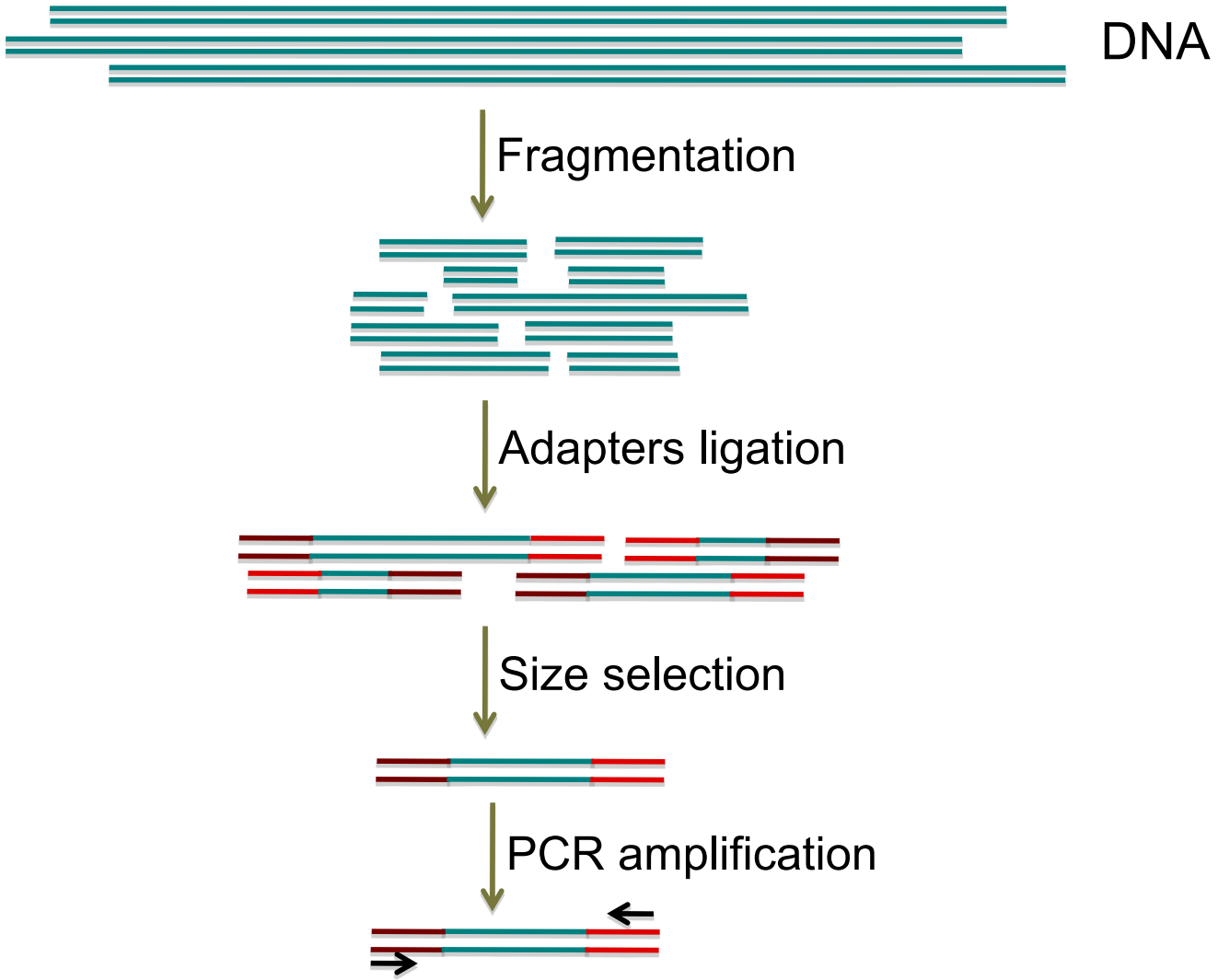
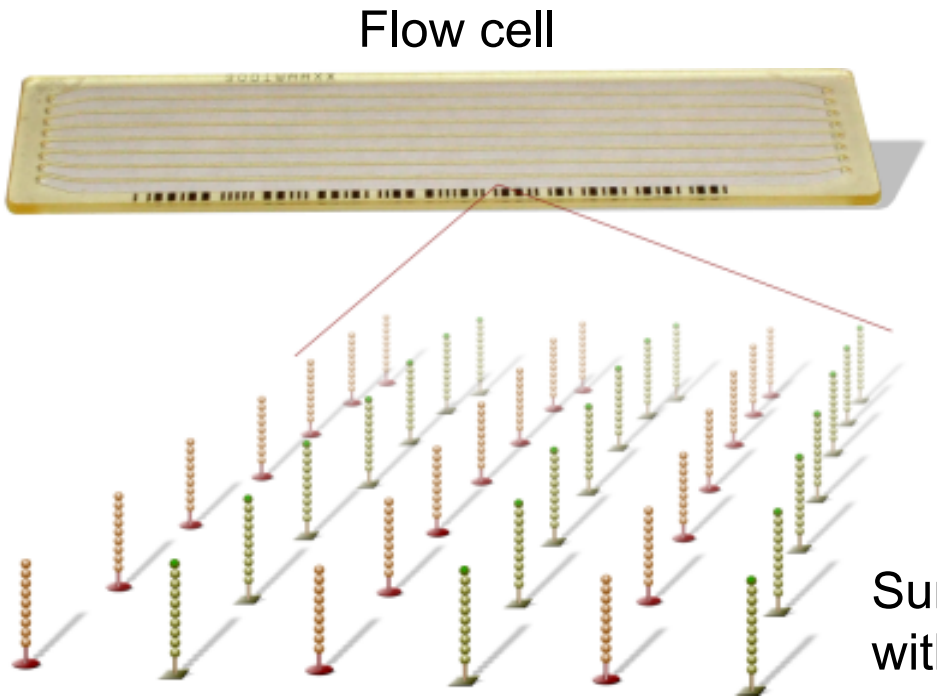
Library preparation



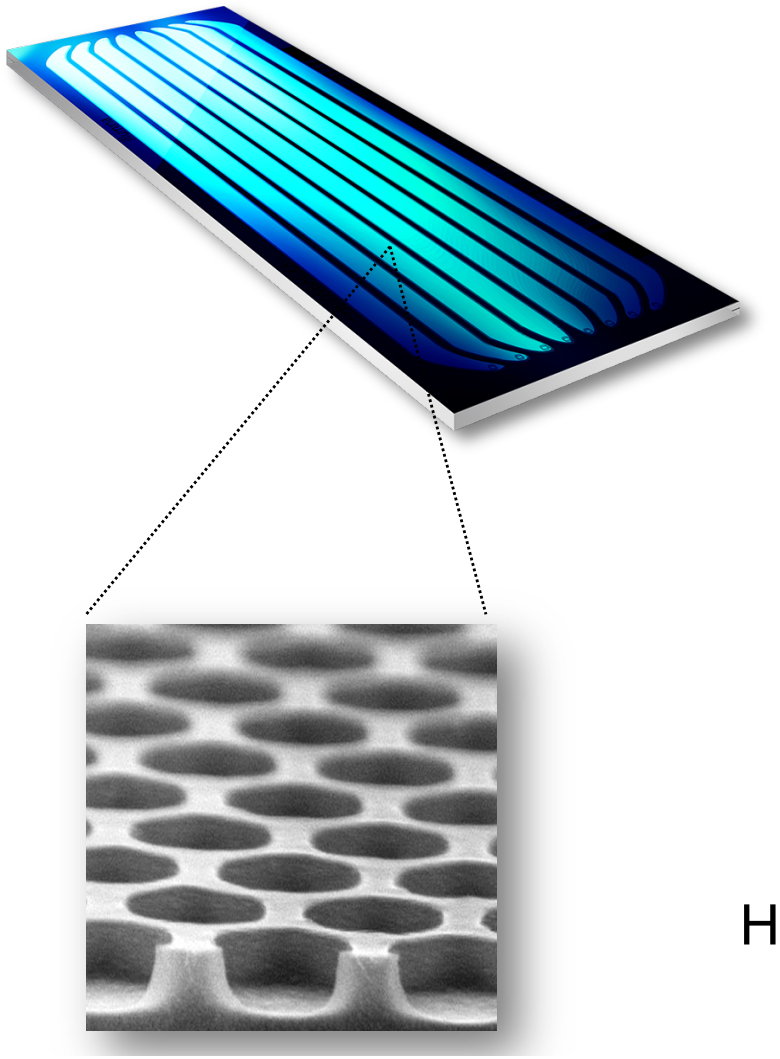
Fixation of DNA fragments on a solid support



https://www.illumina.com

Fixation of DNA fragments on a solid support

Patterned Flow Cell : billions of ordered wells



→ More reads
→ Faster run time

Hiseq3000/4000/X/NovaSeq sequencers

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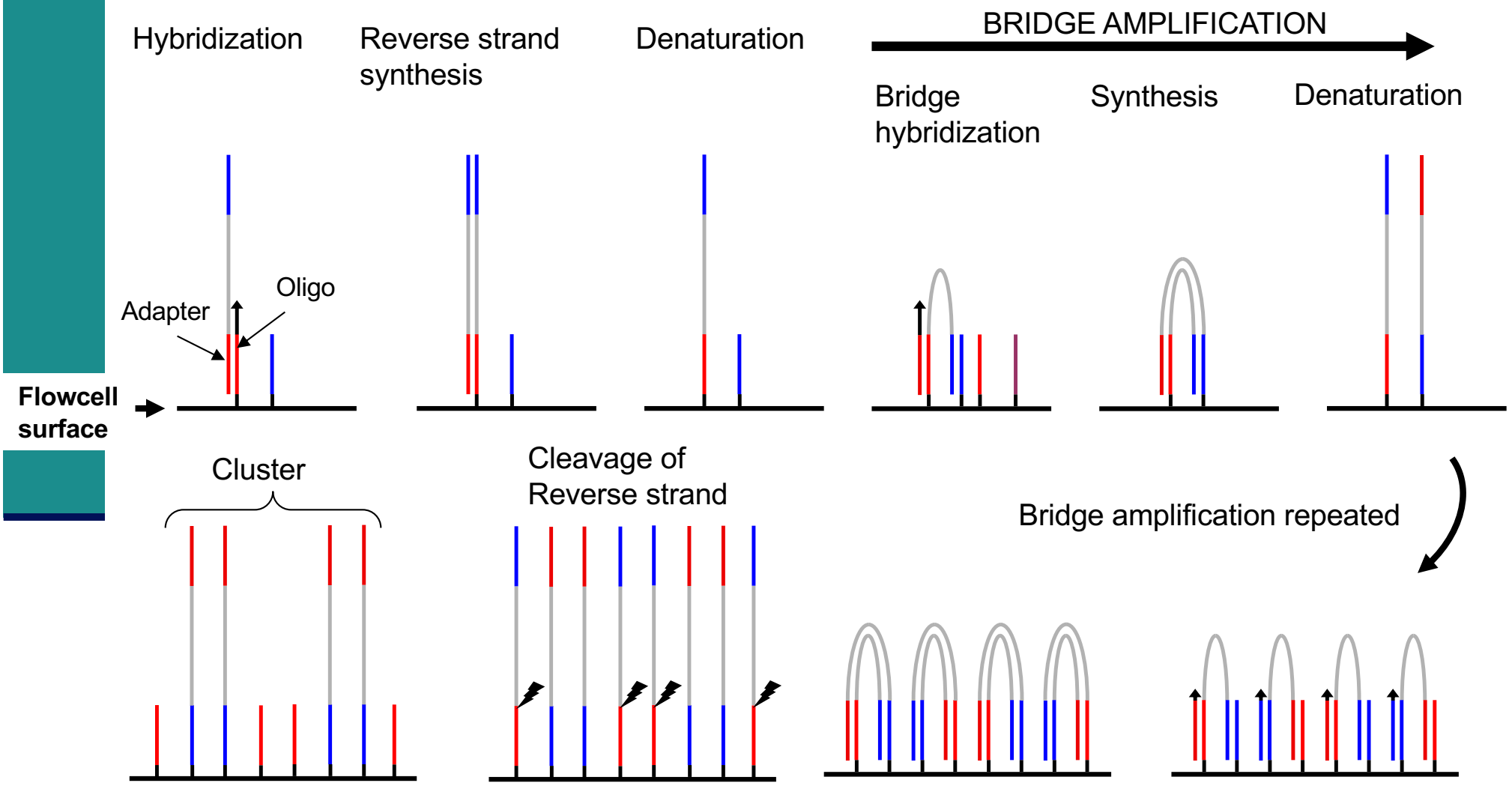
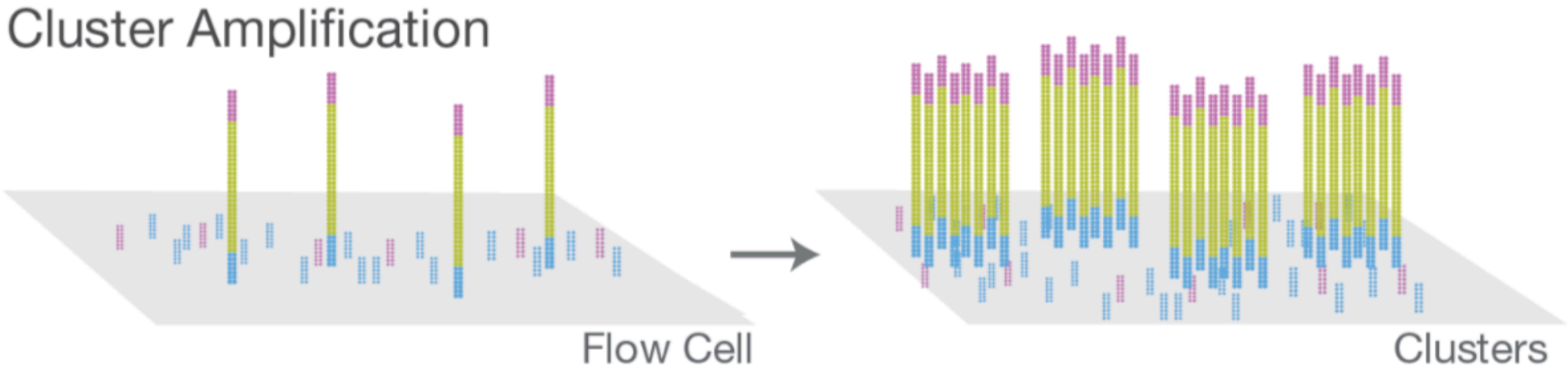
Amplification : method



Amplification : result



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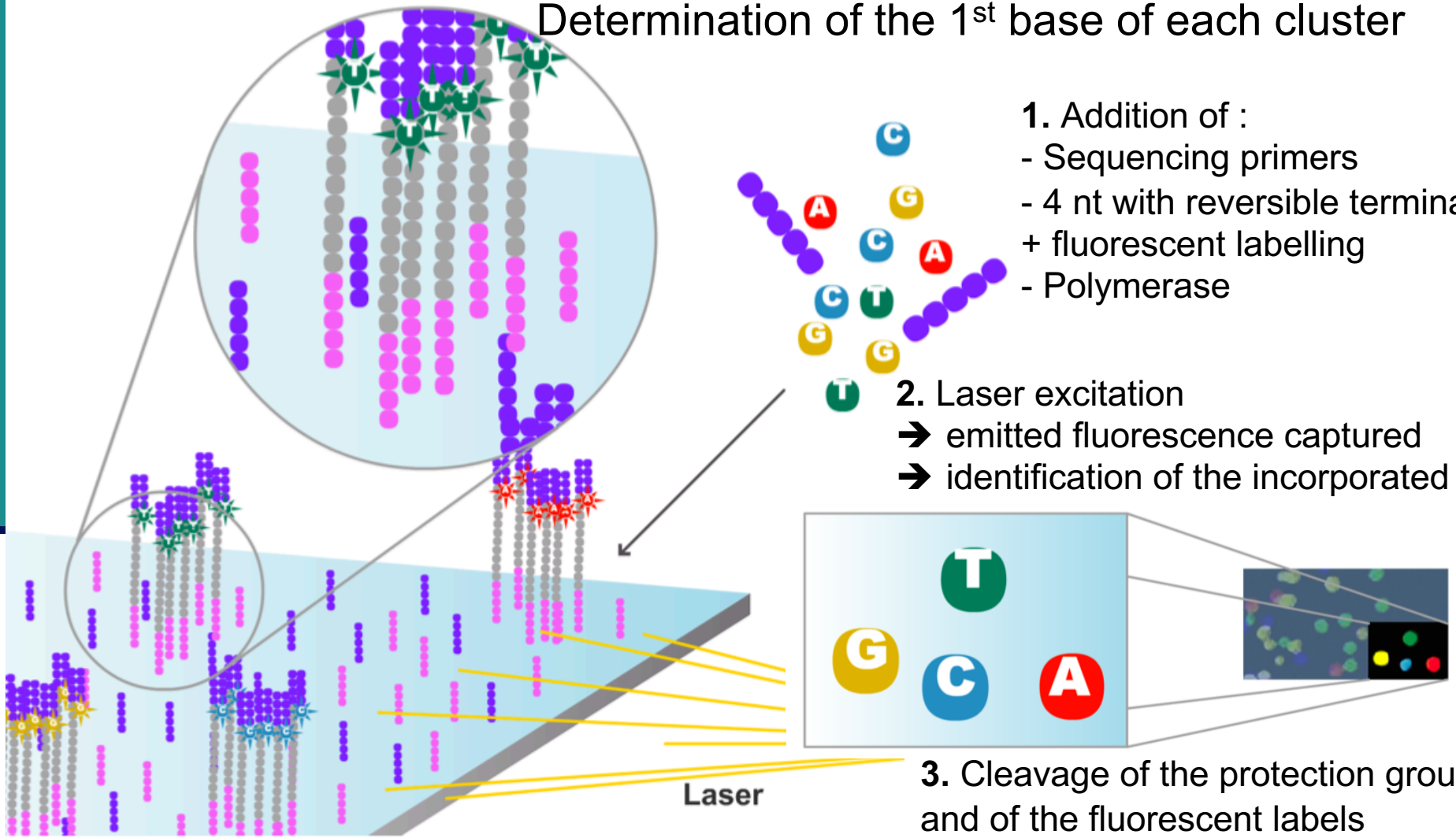
Sequencing by synthesis

First sequencing cycle:
Determination of the 1st base of each cluster



1. Addition of :
- Sequencing primers
- 4 nt with reversible terminators
+ fluorescent labelling
- Polymerase

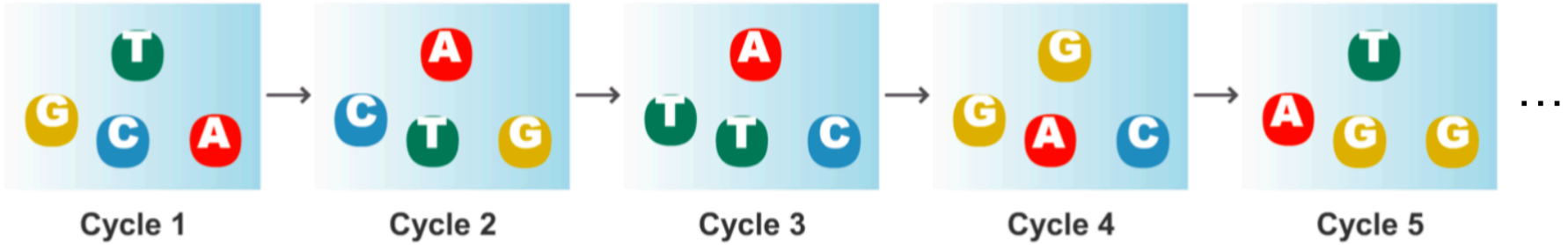
2. Laser excitation
➔ emitted fluorescence captured
➔ identification of the incorporated base

3. Cleavage of the protection groups and of the fluorescent labels

https://www.illumina.com

Sequencing by synthesis

As many sequencing cycles as the number of bases needed in the resulting read



eg cluster 1 = TAAGT…
➔ 1 read

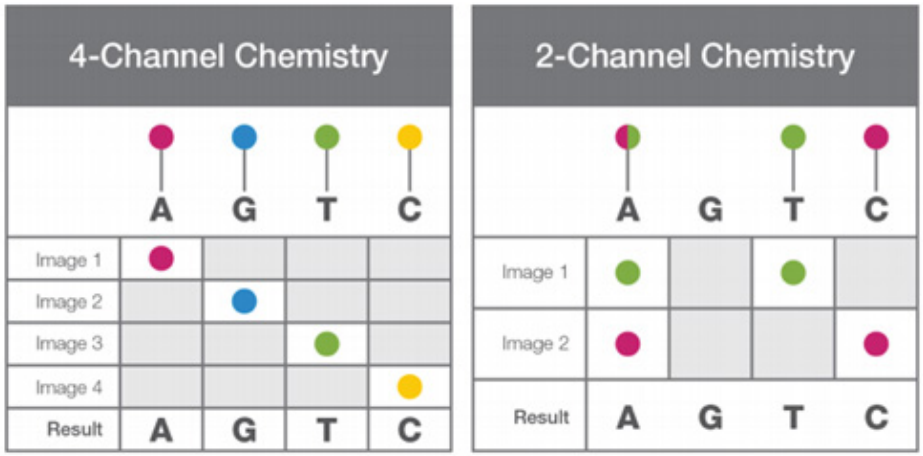
Each lane (top and bottom) is divided into swaths composed of tiles
→ 1 image per tile per color, for each cycle

https://www.illumina.com

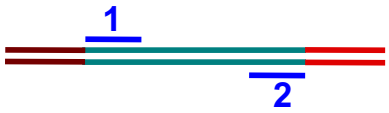
2-channel sequencing by synthesis

- Evolution of the original 4-channel SBS technology
  - 2 fluorescent dyes
  - Only 2 images per cycle instead of 4
  - Accelerates sequencing and data processing times

- Clusters seen in red images → C
- Clusters seen in green images → T
- Clusters observed in both red and green images → A
- Unlabelled clusters → G

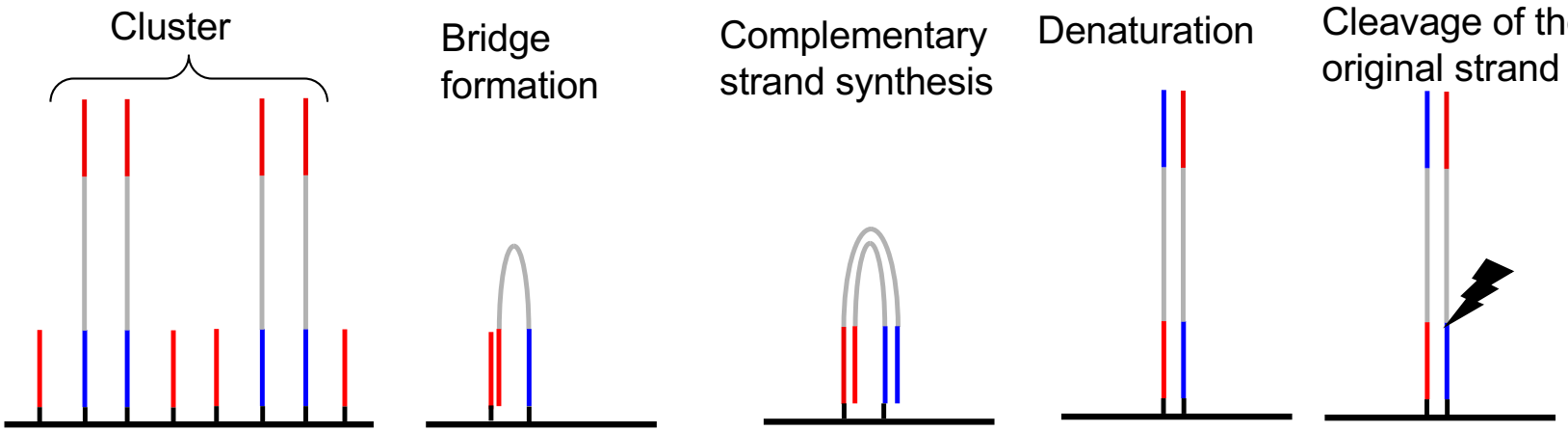


Paired-end sequencing



- Sequencing of read 1 : previously described method
- Then sequencing of read 2 :

For each molecule on the surface of the flow cell :



➔ Sequence the other end of the original molecule

➔ Step performed on the flow cell in the sequencer :
keep the position of clusters
This information allows to link pairs of sequences

Multiplexing

- Add a barcode (index) specific to each sample
- Sequencing of several samples together
- Single indexing : 1 barcode

- Dual indexing : 2 barcodes

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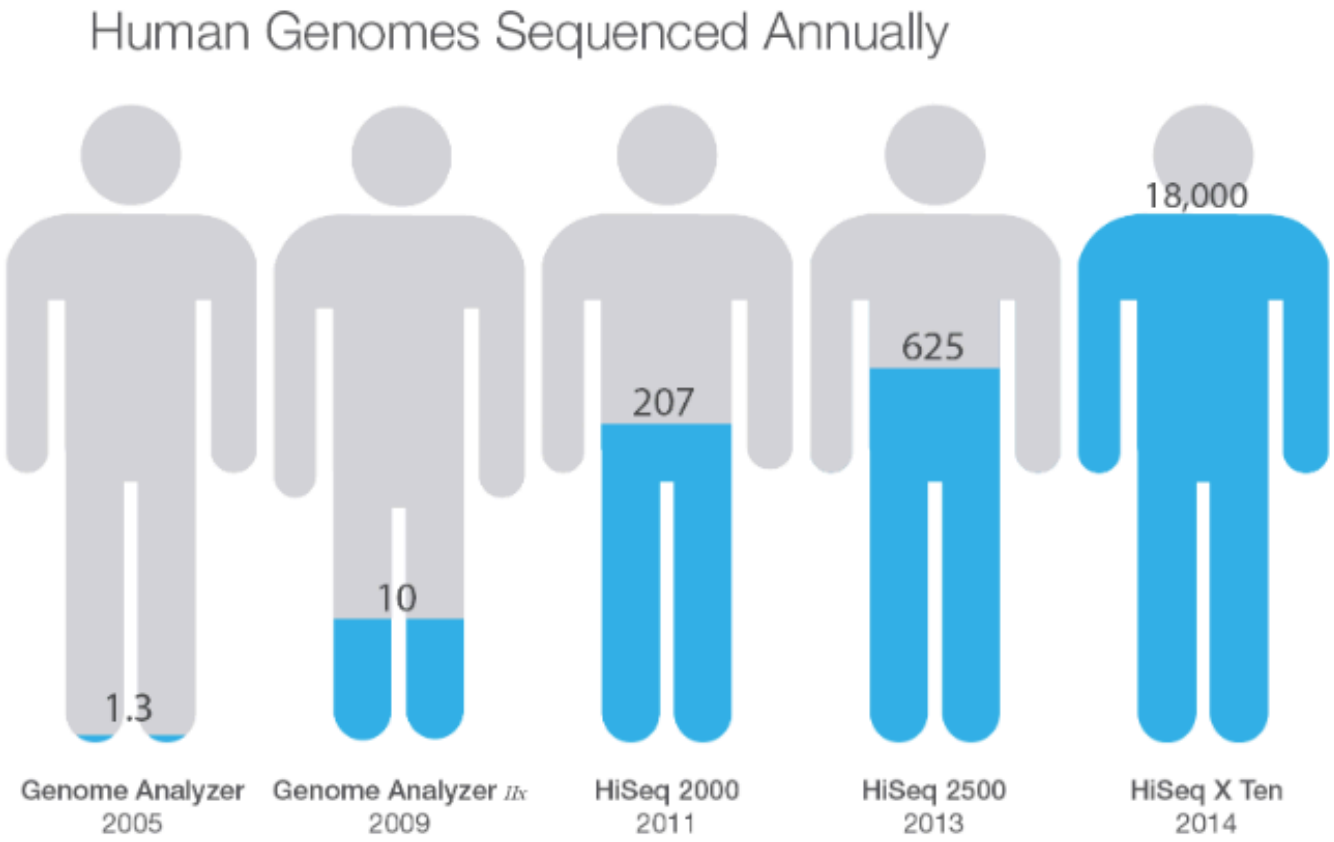
Illumina throughput over time



~ 48 human genomes in 44h

NovaSeq6000
2017

Capacity to sequence a 30X human genome annually
https://www.illumina.com/documents/products/illumina_sequencing_introduction.pdf

Illumina sequencers

Increasing system price and output
Decreasing price per Gb


iSeq 100 System


MiniSeq System


MiSeq Series


NextSeq Series


HiSeq 4000 System


HiSeq X Series‡


NovaSeq 6000 System

Benchtop sequencers

Production-scale sequencers

https://emea.illumina.com/systems/sequencing-platforms.html

Illumina sequencers



	iSeq 100	MiniSeq	MiSeq Series
Run Time	9.5–19 hrs	4–24 hours	4–55 hours
Maximum Output	1.2 Gb	7.5 Gb	15 Gb
Maximum Reads Per Run	4 million	25 million	25 million †
Maximum Read Length	2 × 150 bp	2 × 150 bp	2 × 300 bp

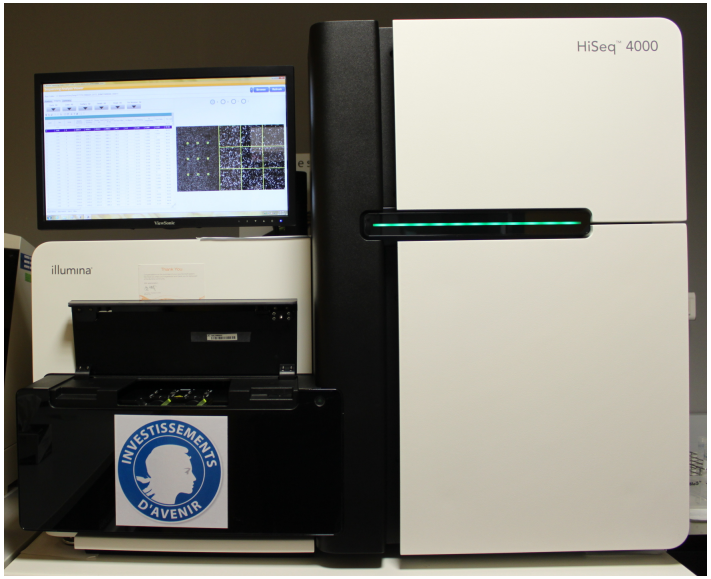


	NextSeq 550 Series	NextSeq 1000 & 2000	NovaSeq 6000
Run Time	12–30 hours	11-48 hours	~13 - 38 hours (dual SP flow cells) ~13–25 hours (dual S1 flow cells) ~16–36 hours (dual S2 flow cells) ~44 hours (dual S4 flow cells)
Maximum Output	120 Gb	330 Gb*	6000 Gb
Maximum Reads Per Run	400 million	1.1 billion*	20 billion
Maximum Read Length	2 × 150 bp	2 × 150 bp	2 × 250**

From https://emea.illumina.com/systems/sequencing-platforms.html

Illumina Hiseq4000 throughput

- Up to 2x150 bp
- ~ 330 million single-end reads per lane
- 1 or 2 flow cells per run
- 8 lanes per flow cell
- Throughput per run with 2 flow cells
  - ~ 5 billion single-end reads
  - ~ 10 billion paired-end reads
  - ~ 1.5 Tbases per 2x150bp run
  - ~ 12 30X human genomes
  - ~ 128 RNA-seq (polyA+) or ChIP-seq (~ 30 million single-end reads)
  - ~ 128 human exomes per 2x100 run (> 60X mean coverage)



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Comparison between different generations of sequencing technologies

- 1st generation
  - Sequencing pre-amplified molecules one by one
- 2nd generation
  - Clonal amplification and sequencing of million of molecules at the same time
  - PCR, RT needed ➔ bias
- 3rd generation
  - Nanopore sequencing, Pacific Biosciences
  - Main improvements
    - Long reads
    - No amplification
    - Direct RNA sequencing
  - Current drawbacks
    - Lower per read accuracy and number of reads than short-read sequencing