Illumina sequencing technology

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Illumina sequencing technology

- Introduction to second generation sequencing
- Library preparation
- Amplification
- Sequencing
- Illumina sequencers and throughput
- Other technologies

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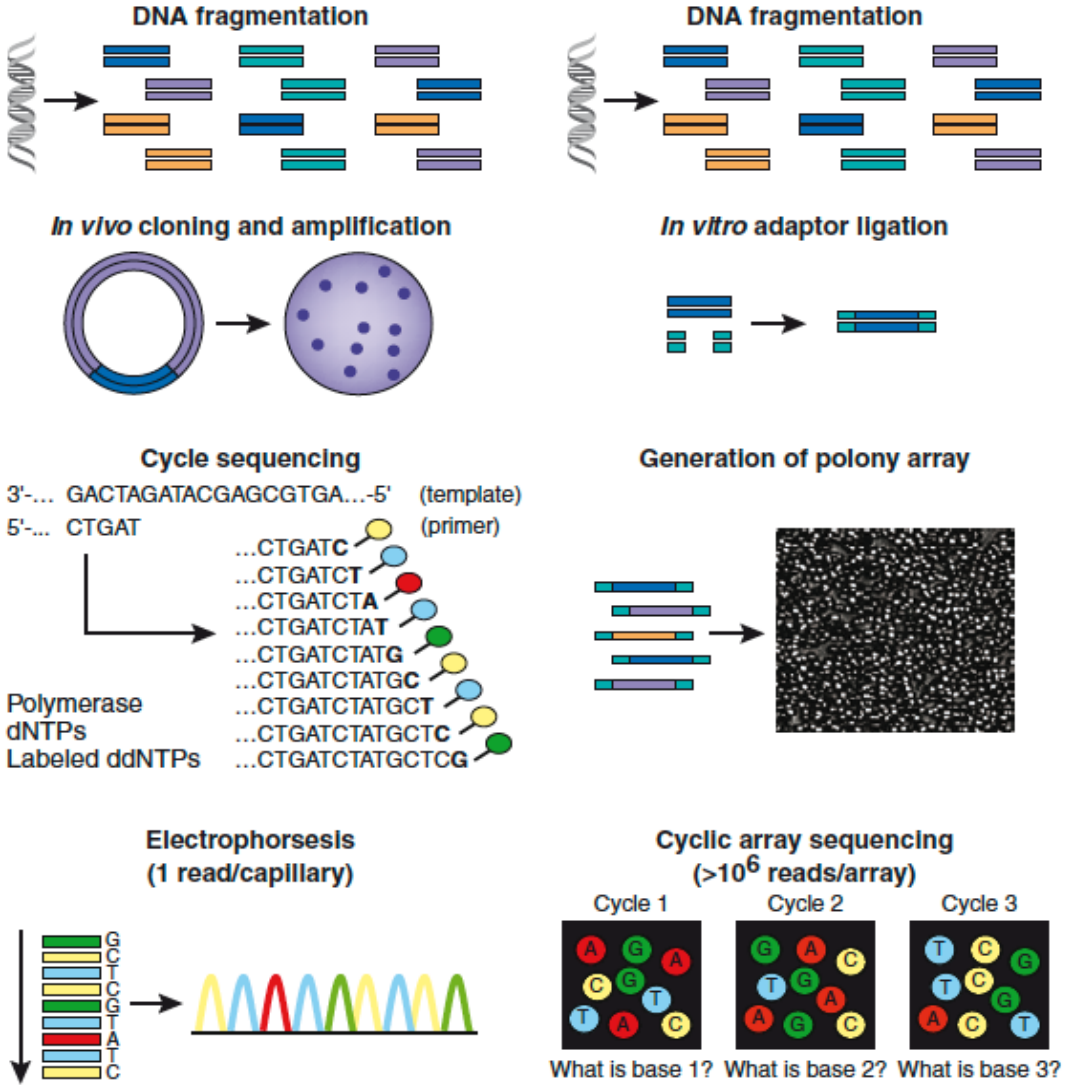
Conventional vs second generation sequencing

Conventional sequencing 2nd generation sequencing



Three main technologies

- SOLiD DNA Sequencer Applied Biosystems
  - Shendure et al., 2005
- Genome Sequencer FLX+ Roche (formerly 454)
  - Margulies et al., 2005
- Illumina (formerly Solexa)
  - Fedurco et al., 2006

➔ Important decrease of

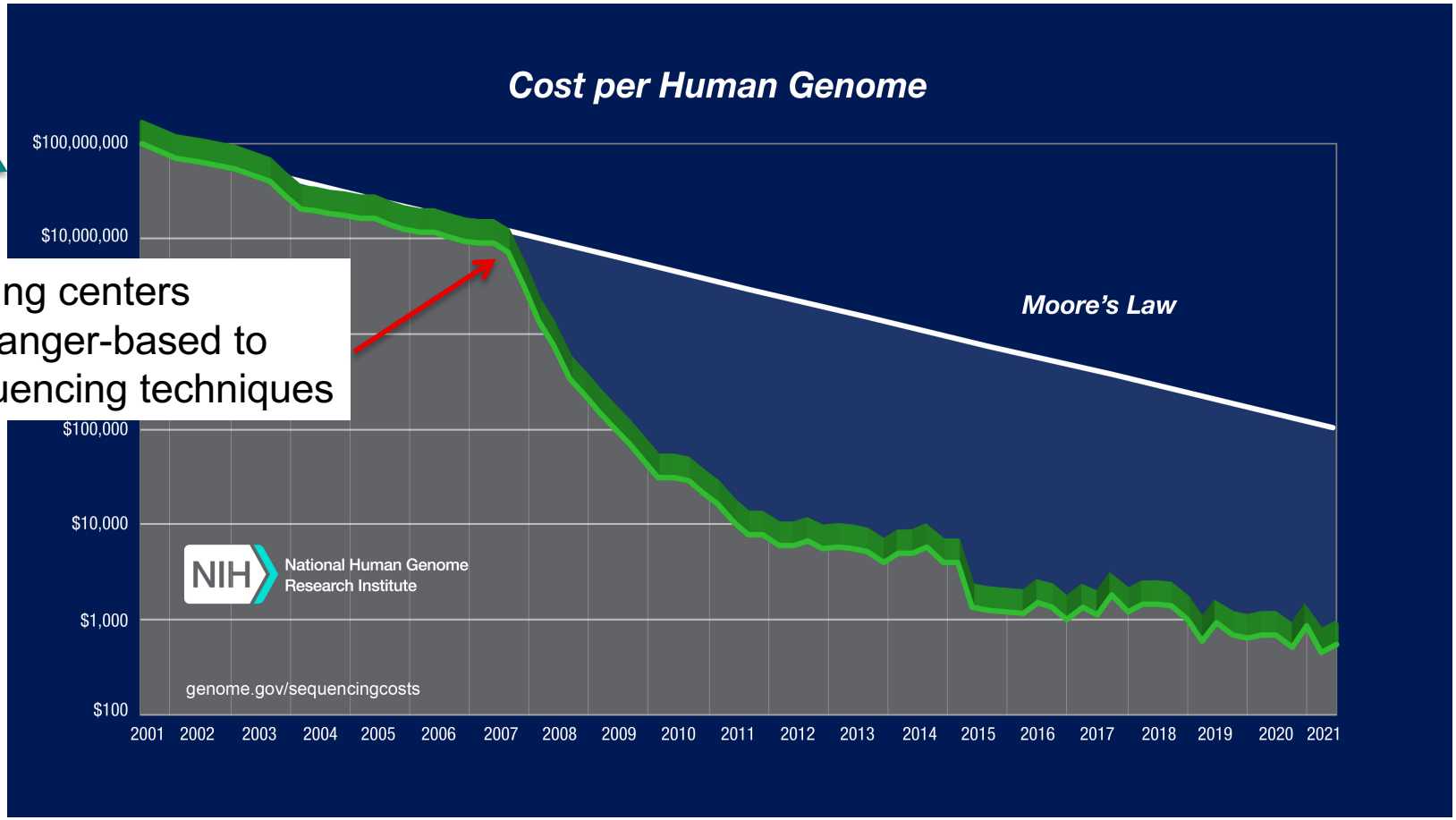
- Cost per base
- Time needed to obtain sequences

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

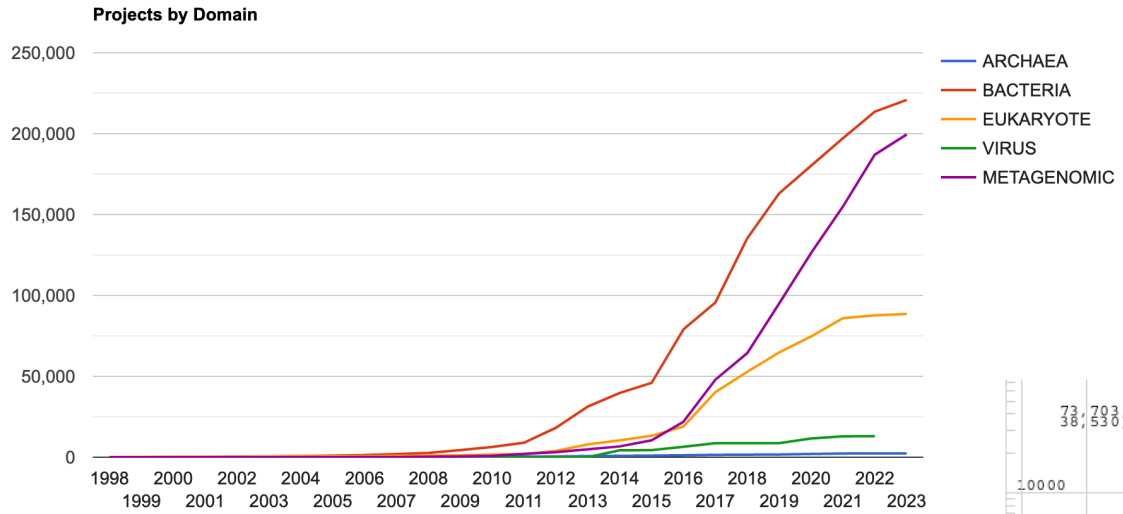


http://www.genome.gov/sequencingcosts/

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

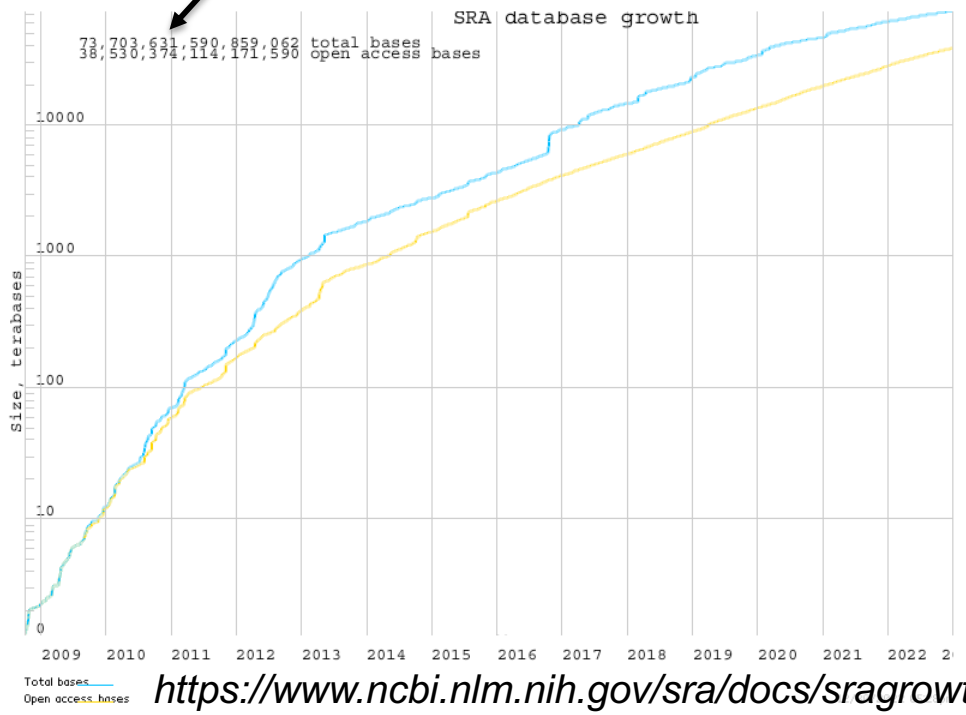
Projects in Genome Online Database



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

Bases in Sequence Read Archive

~ 73,000 Tera bases



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

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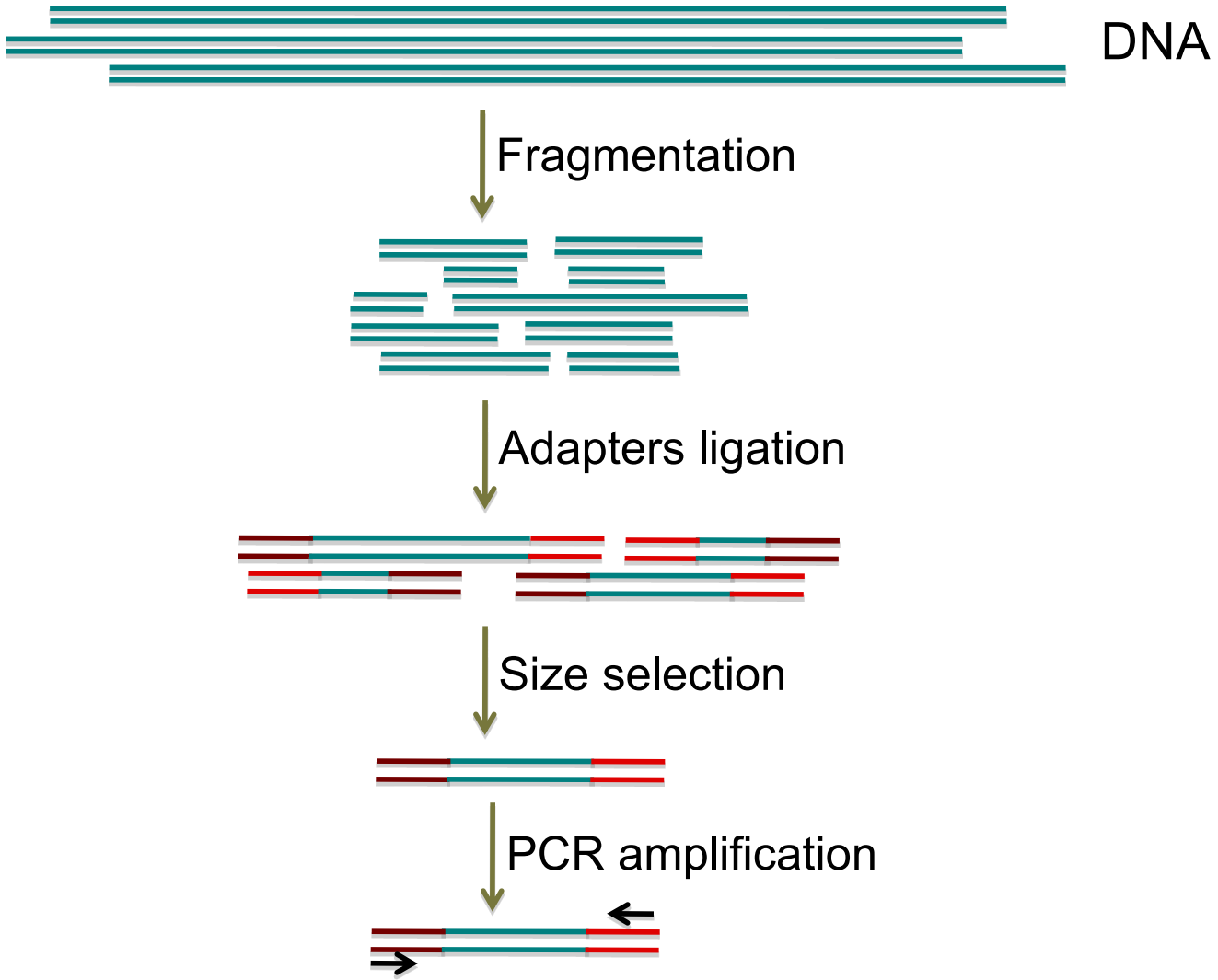
Library preparation



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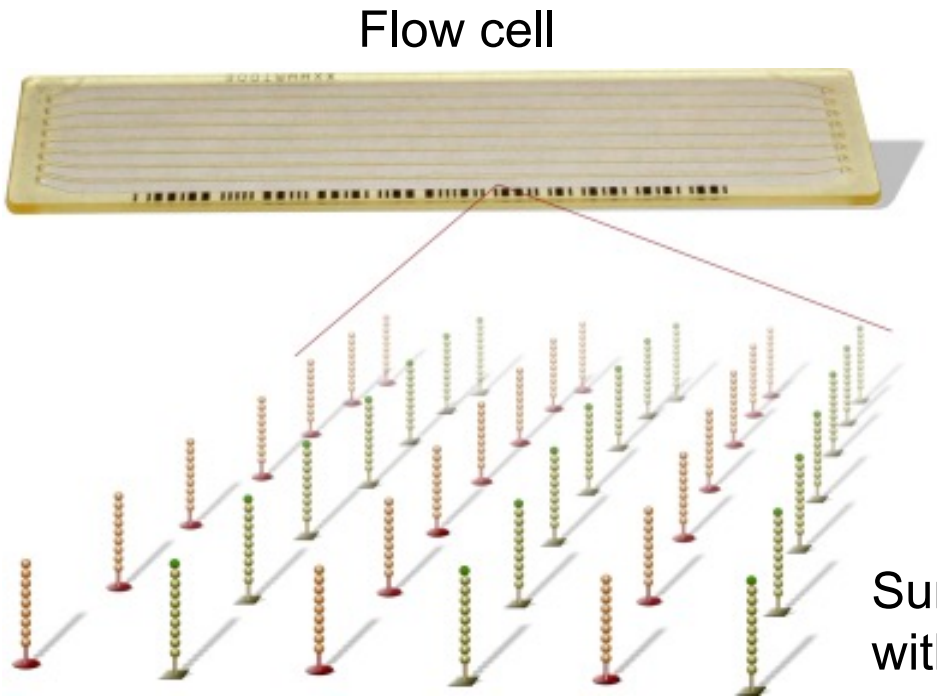
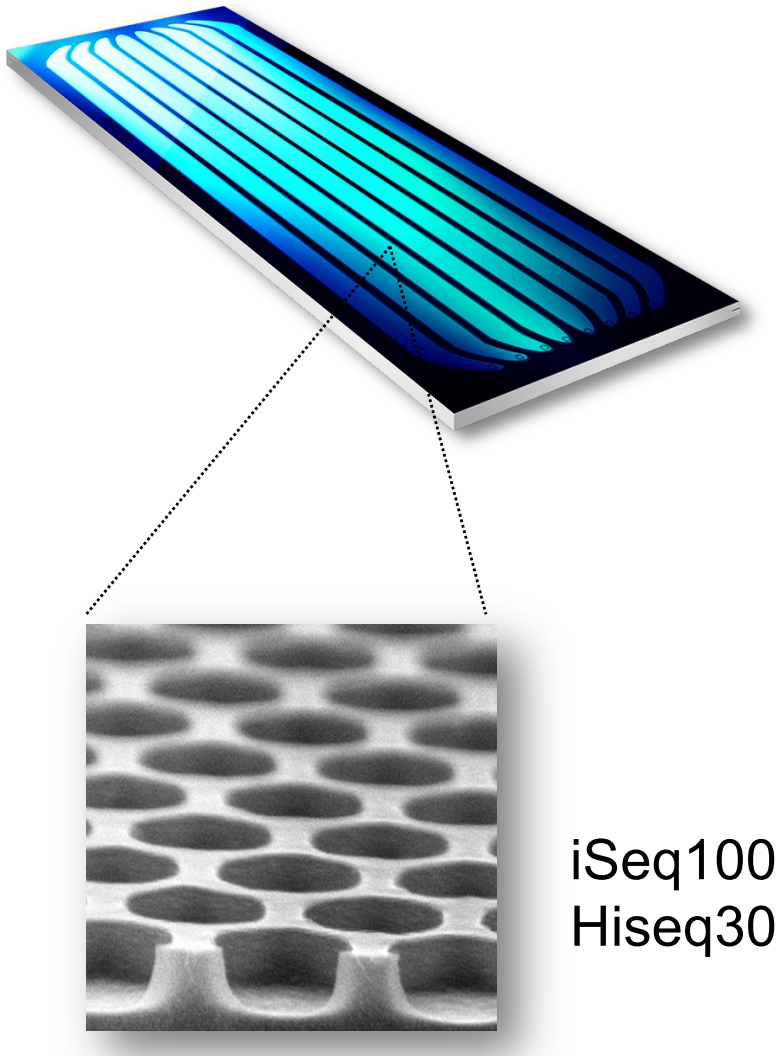
Fixation of DNA fragments on a solid support



https://www.illumina.com

Fixation of DNA fragments on a solid support

Patterned Flow Cell : billions of ordered wells

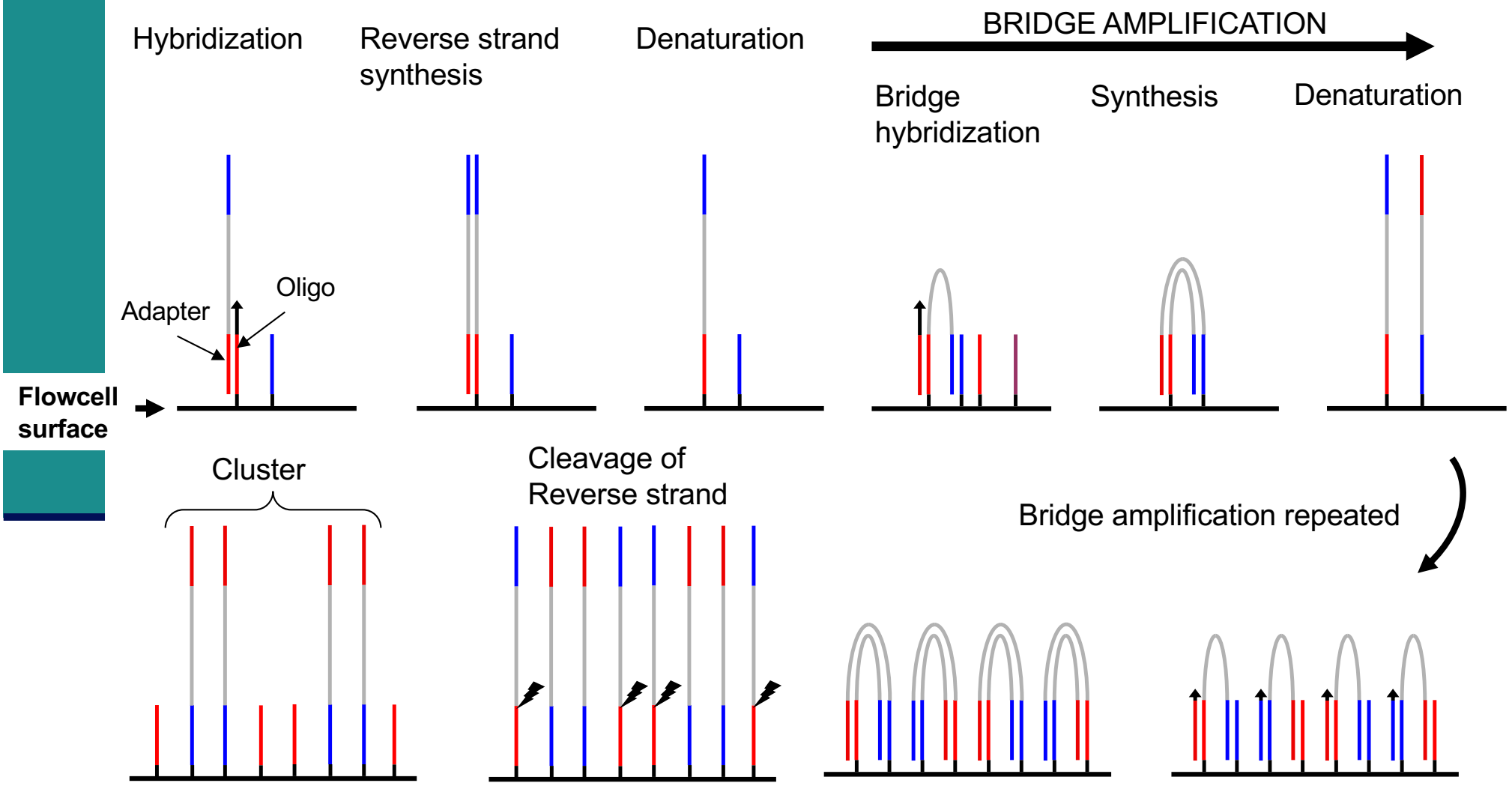


→ More reads
→ Faster run time

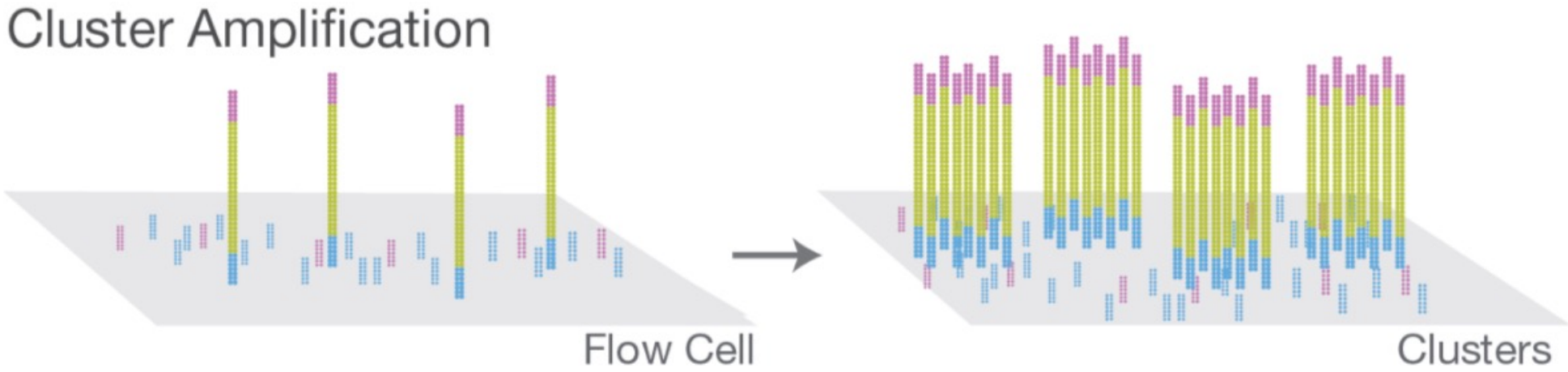
iSeq100, NextSeq1000/2000,
Hiseq3000/4000/X, NovaSeq6000/X sequencers

https://www.illumina.com

Amplification : method



Amplification : result



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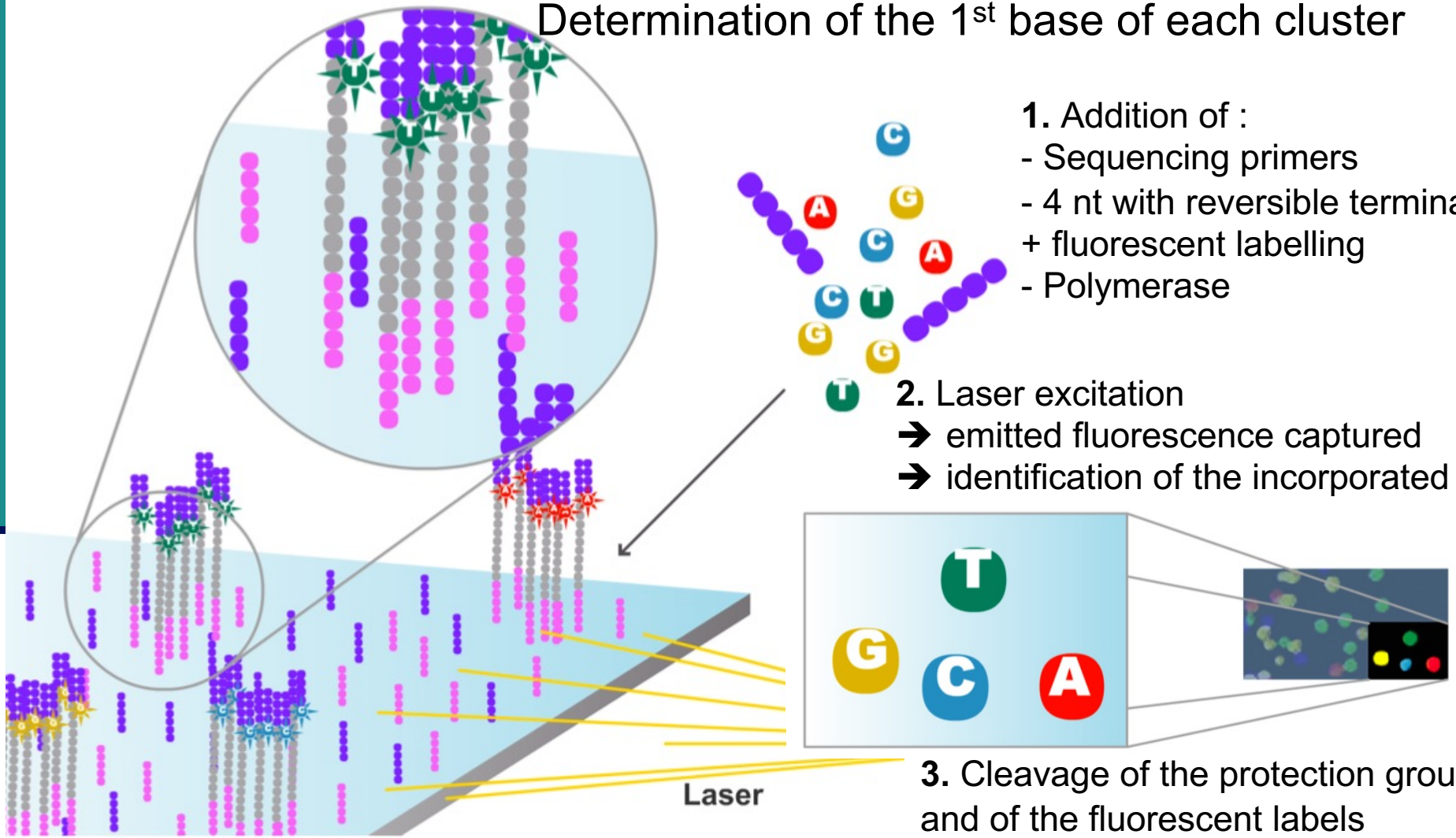
Sequencing by synthesis

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



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

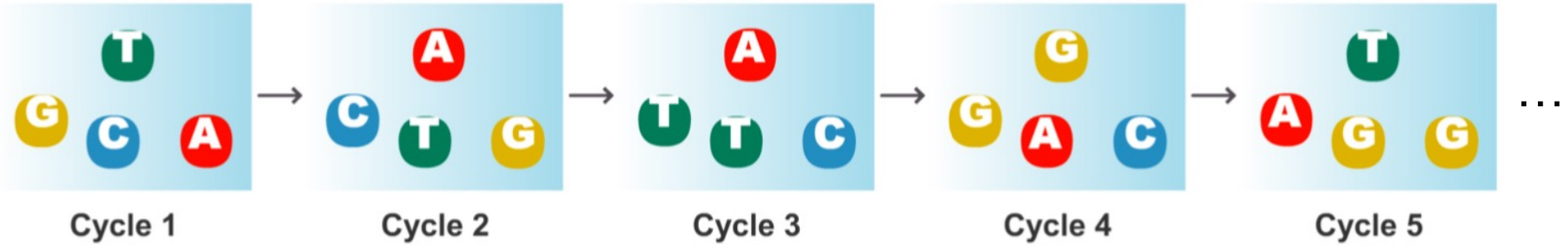
2. Laser excitation
➔ emitted fluorescence captured
➔ identification of the incorporated base

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

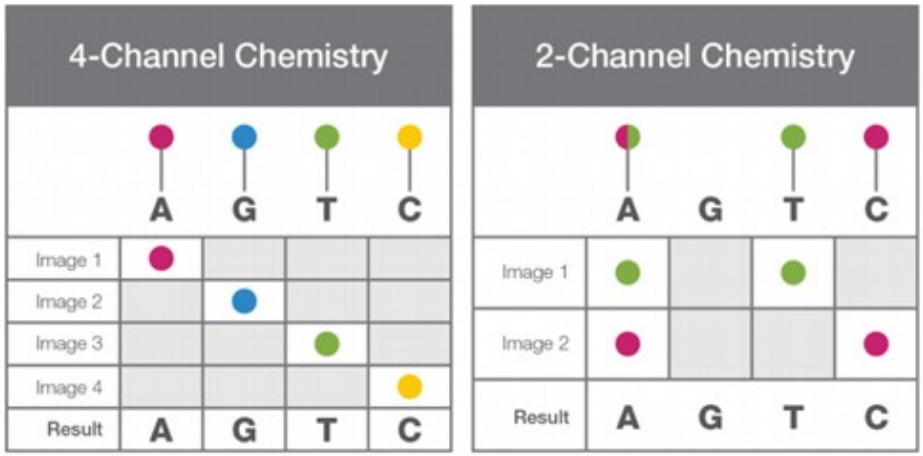
Each lane (top and bottom) is divided into swaths composed of tiles
→ 1 image per tile per color, for each cycle

https://www.illumina.com

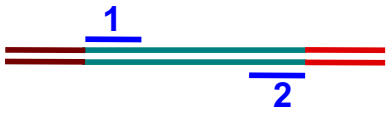
2-channel sequencing by synthesis

- 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

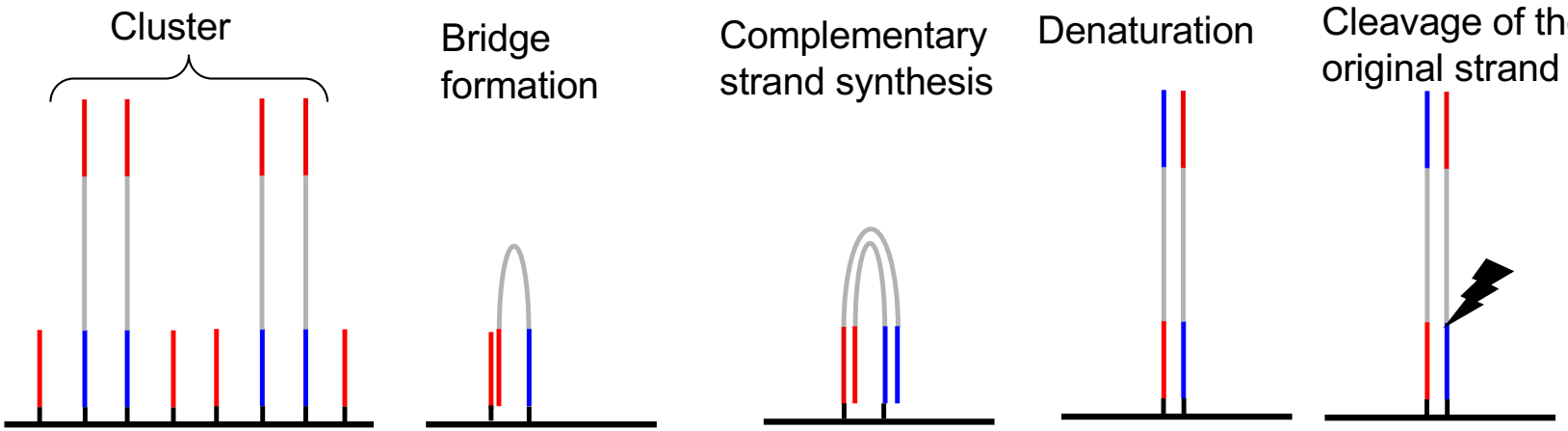


Paired-end sequencing



- 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

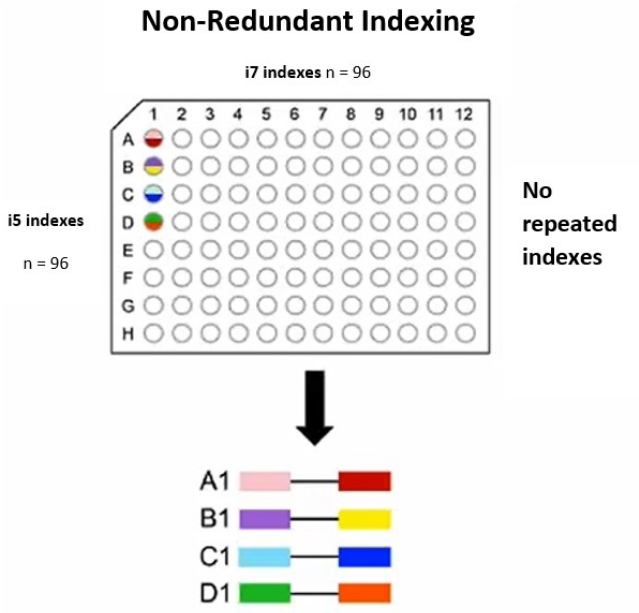
➔ Step performed on the flow cell in the sequencer :
keep the position of clusters
This information allows to link pairs of sequences

Multiplexing

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

- Dual indexing : 2 barcodes

  - Unique dual indexes : distinct index sequences for each of the two indexes



https://support.illumina.com/

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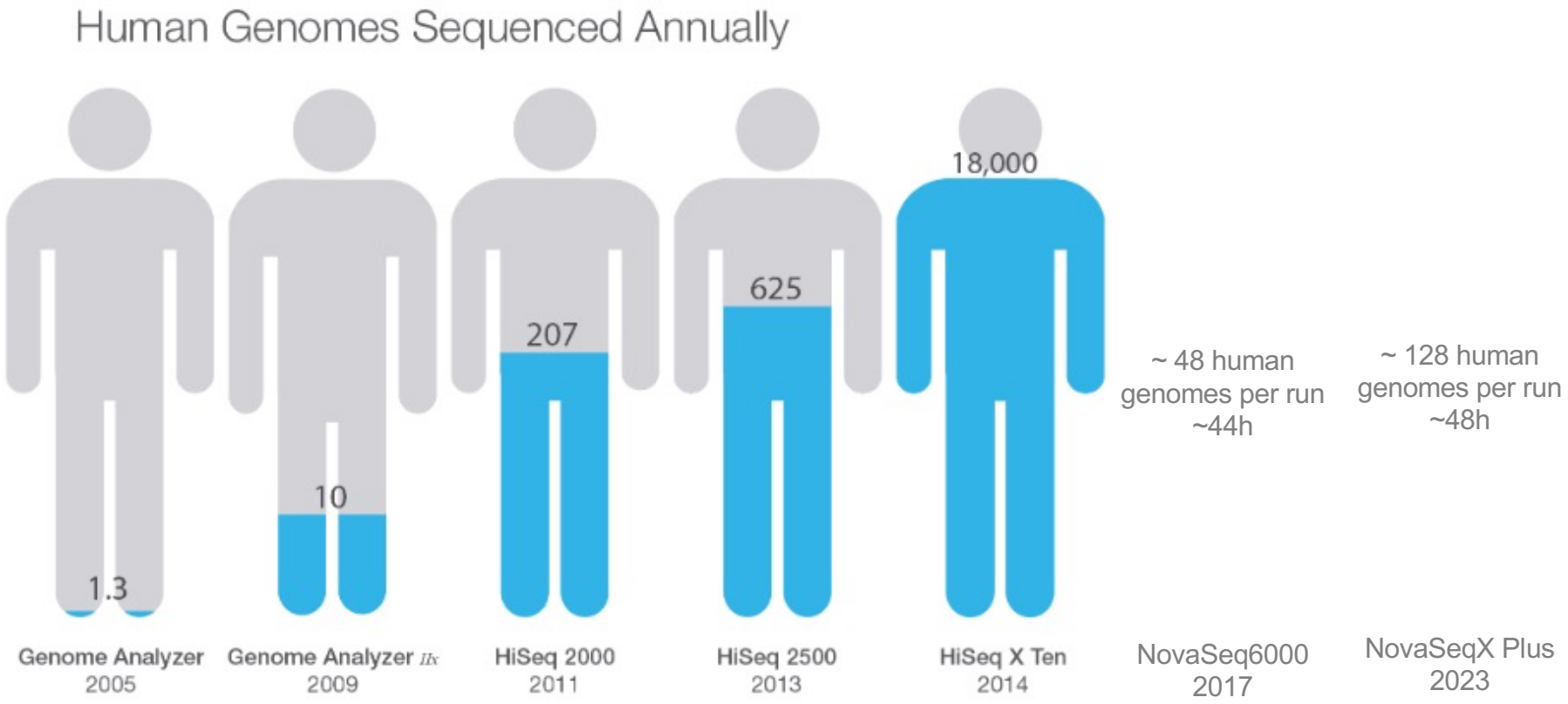
Illumina throughput over time



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

iSeq100 MiniSeq MiSeq NextSeq550 NextSeq1000/2000 NovaSeq6000 NovaSeqX(Plus)

Benchtop sequencers

Production-scale sequencers

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

Illumina sequencers




	iSeq 100	MiniSeq	MiSeq Series ⊕	NextSeq 550 Series ⊕
Run Time	9.5–19 hrs	4–24 hours	4–55 hours	12–30 hours
Maximum Output	1.2 Gb	7.5 Gb	15 Gb	120 Gb
Maximum Reads Per Run	4 million	25 million	25 million †	400 million
Maximum Read Length	2 × 150 bp	2 × 150 bp	2 × 300 bp	2 × 150 bp



	NextSeq 1000 & 2000	NovaSeq 6000 Series ⊕	NovaSeq X Series
Run Time	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)	~13–21 hours (1.5B flow cells‡) ~18–24 hours (10B flow cells‡) ~48 hours (25B flow cells‡)
Maximum Output	360 Gb *	6000 Gb	16 Tb
Maximum Reads Per Run	1.2 billion *	20 billion	26 billion (single flow cells) 52 billion (dual flow cells)
Maximum Read Length	2 × 150 bp	2 × 250 bp**	2 × 150 bp

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

Illumina NextSeq2000

- 3 types of flow cell

Flow cells* name	Maximum number of clusters/flow cells#	Read length options (in bp)			
P1	100 M				300
P2	400 M		100	200	300
P3	1000 M	50	100	200	300

* Each type of flow cell can be used indiscriminately for single read (SR) or paired-end (PE) sequencing : e.g. a 100 bp kit can be used as SR100, PE50, PE25x75 …

100 M clusters = 100 M reads in SR and 2x100 M in PE sequencing

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Other technologies

- Short reads
  - ~ up to 300 bases
  - Genome resequencing, expression and epigenetic analysis
  - MGI, Ultima Genomics, Pacific Biosciences, Element Biosciences, …
- Long reads
  - ~ several kb
  - *De novo* sequencing, structural variation analysis, isoform sequencing
  - Oxford Nanopore Technologies, Pacific Biosciences
  - Longer reads, no amplification, direct RNA sequencing, but lower throughput