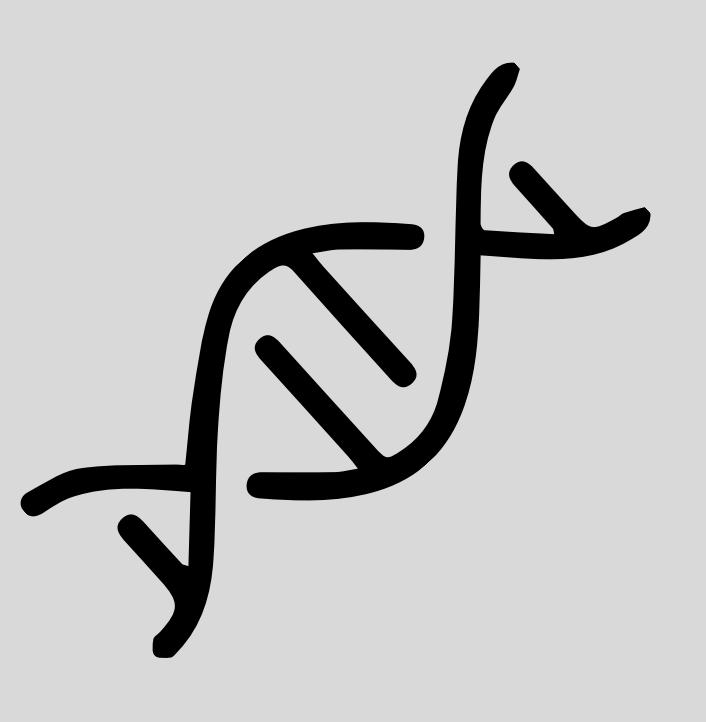


TECNOLOGÍAS DE SECUENCIACIÓN

Laura Natalia González, MSc

Romain Guyot, PhD





SANGER

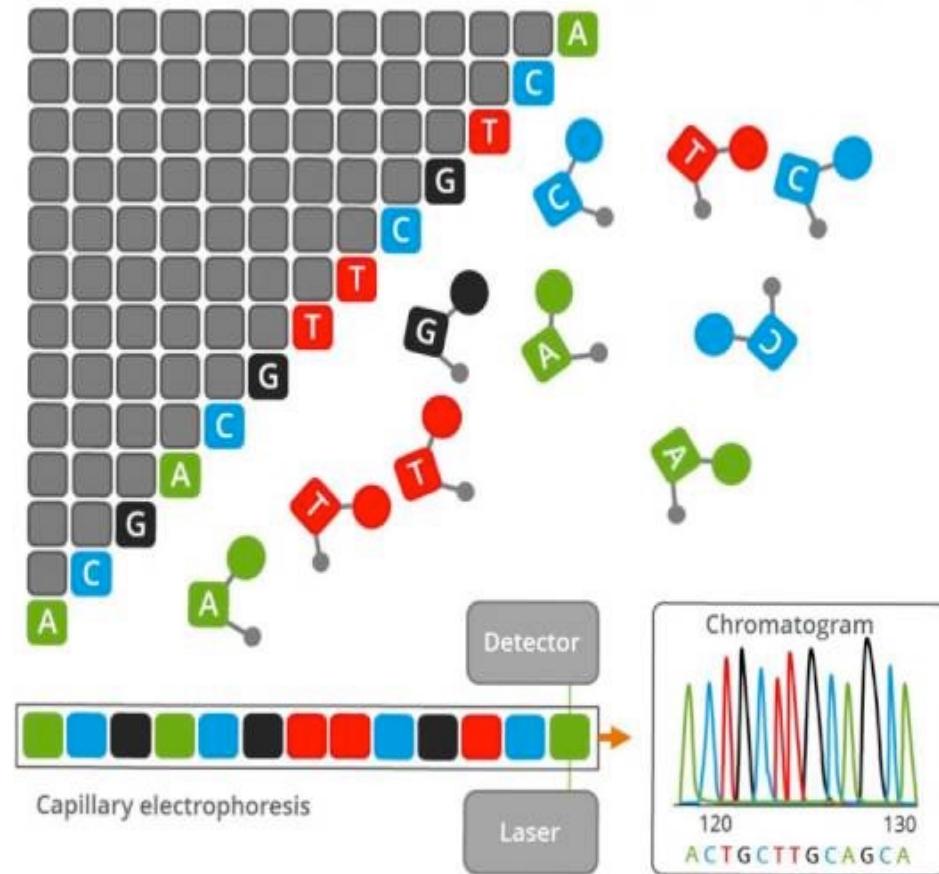
Es similar a la PCR con algunas excepciones

Incluye nucleótidos (A, C, G, T) normales (dNTPs) para la extensión, pero también incluye nucleótidos dideoxy (ddNTPs)

Usa solo un primer y la polimerasa para hacer nuevas copias de ssDNA.

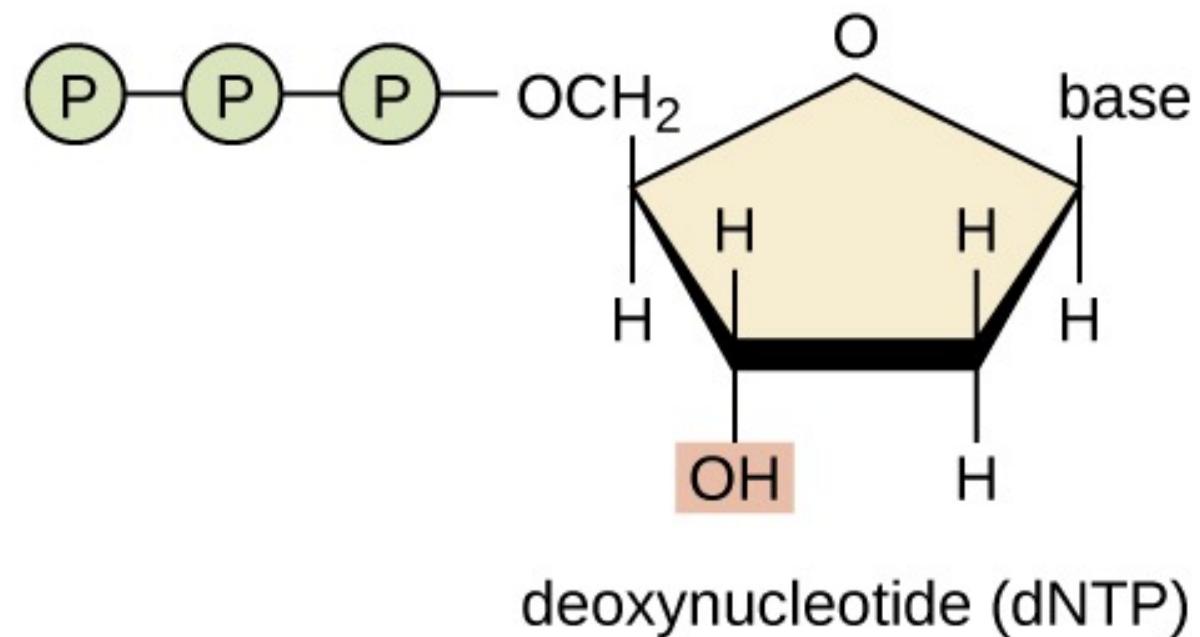
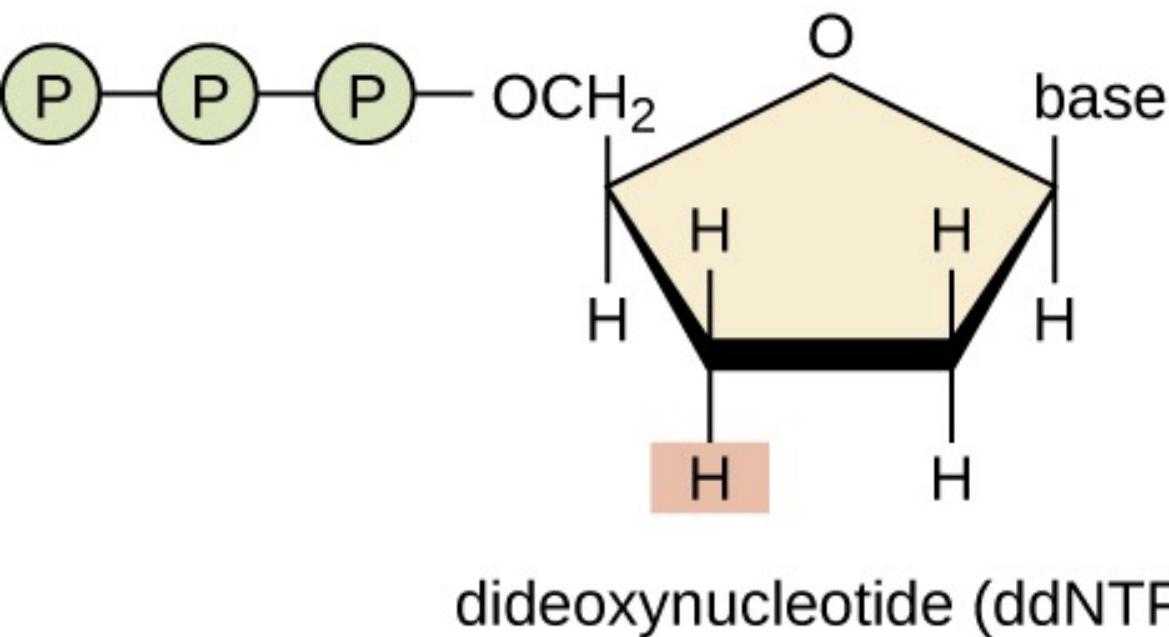
Los ddNTPs están marcados y son terminadores

PCR containing fluorescent, chain-terminating dideoxynucleotide triphosphates

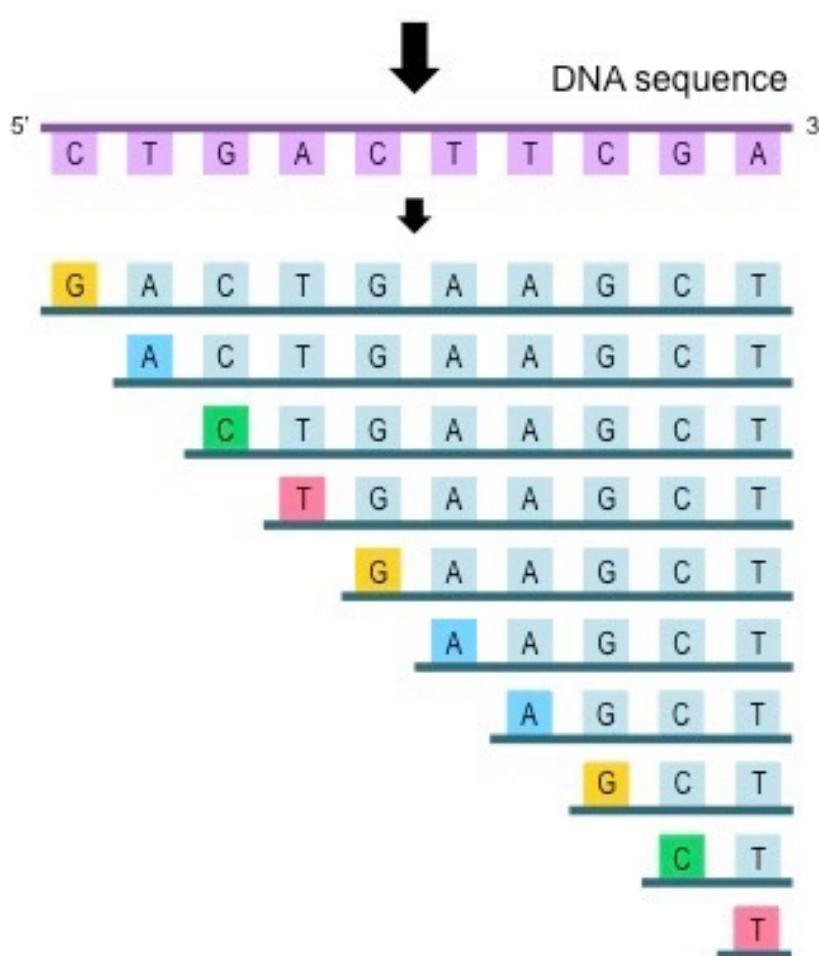
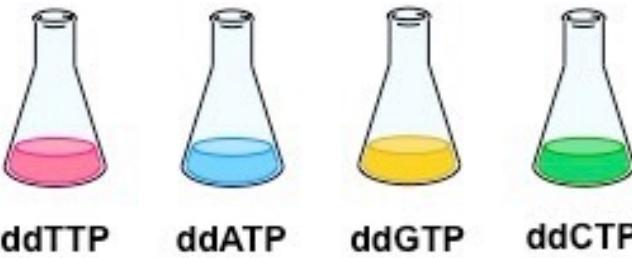


SANGER

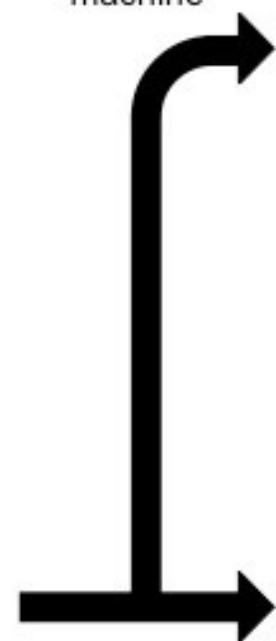
¿Qué es un ddNTP?



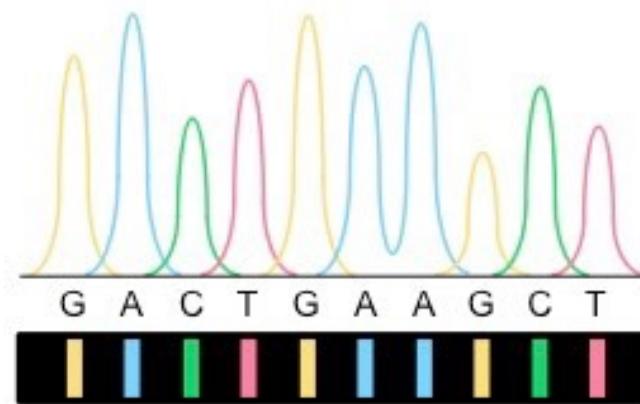
4 × PCR (+ one dideoxynucleotide)



Use a sequencing machine

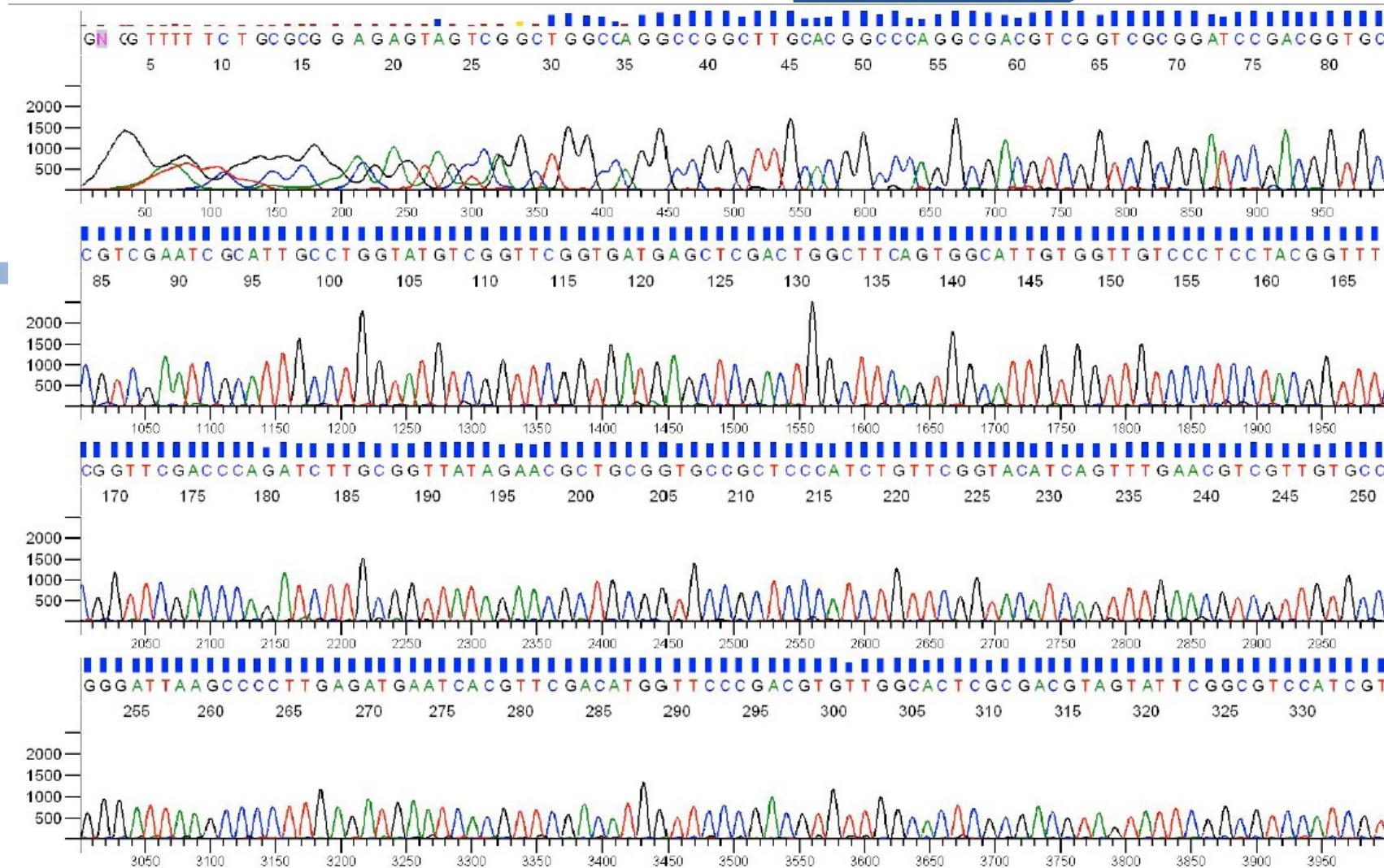


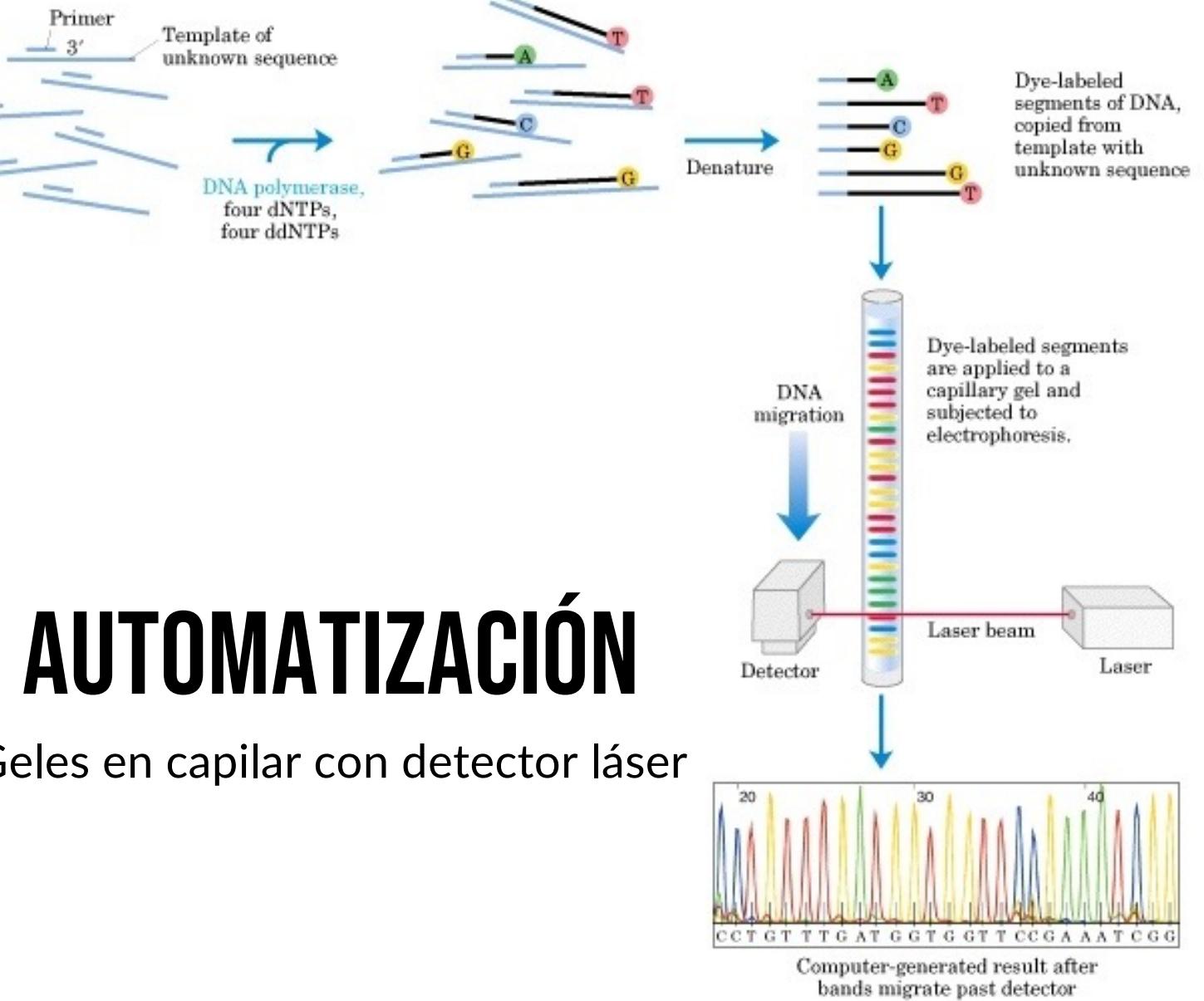
Separate with a gel



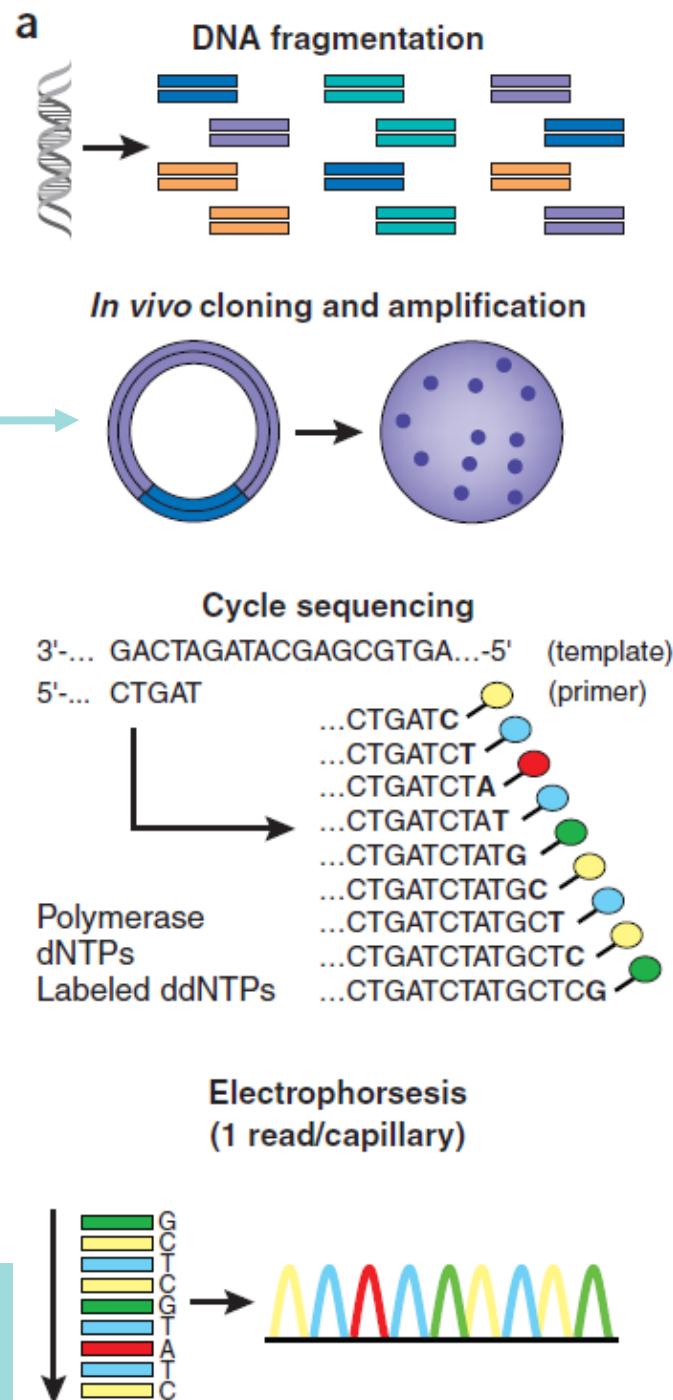
SANGER SEQUENCING OUTPUT

Cada reacción de secuenciación nos da un CROMATOGRAMA, usualmente ~600-1000 bp





Clonación de fragmentos desconocidos para genomas completos



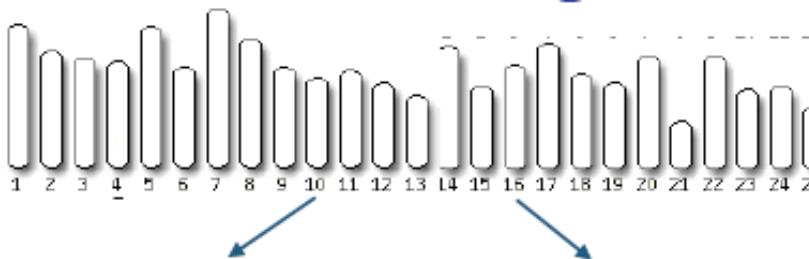


SANGER

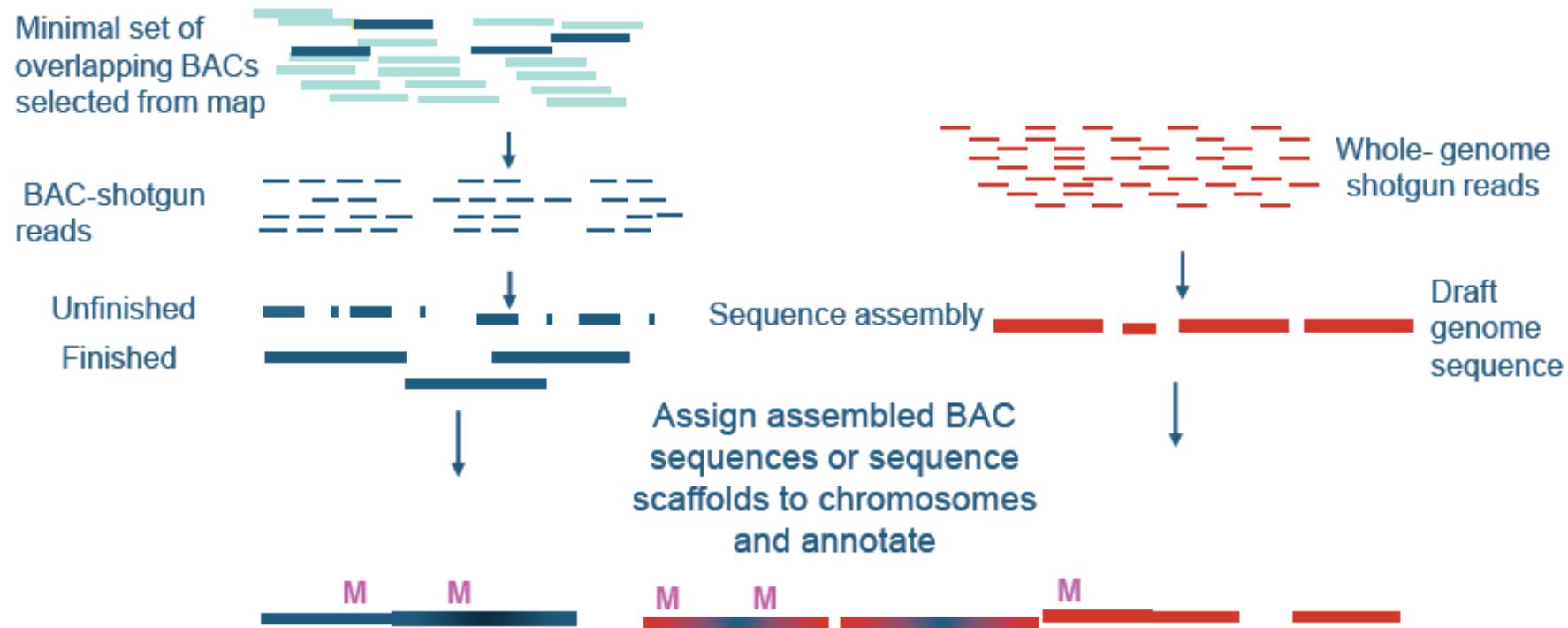
Secuenciación del genoma humano

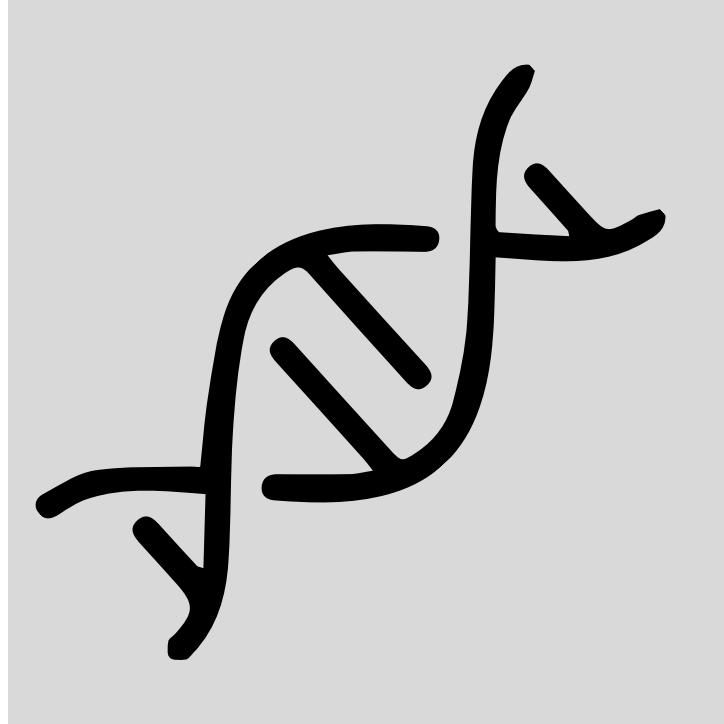
ESTRATEGIAS DE SECUENCIACIÓN DE GENOMA COMPLETO

Jerárquica
Clon por clon
Top down



Whole genome Shotgun



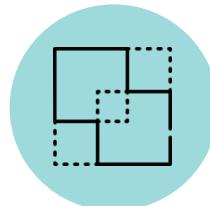


NGS

SECUENCIACIÓN DE NUEVA GENERACIÓN

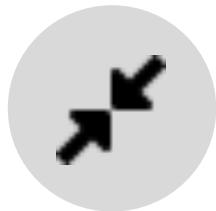
REVOLUCIÓN NGS

La secuenciación se aceleró con los mismos conceptos que aceleraron los circuitos integrados



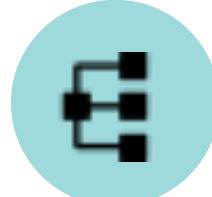
Integración

Muchas más bp de secuencia/tiempo

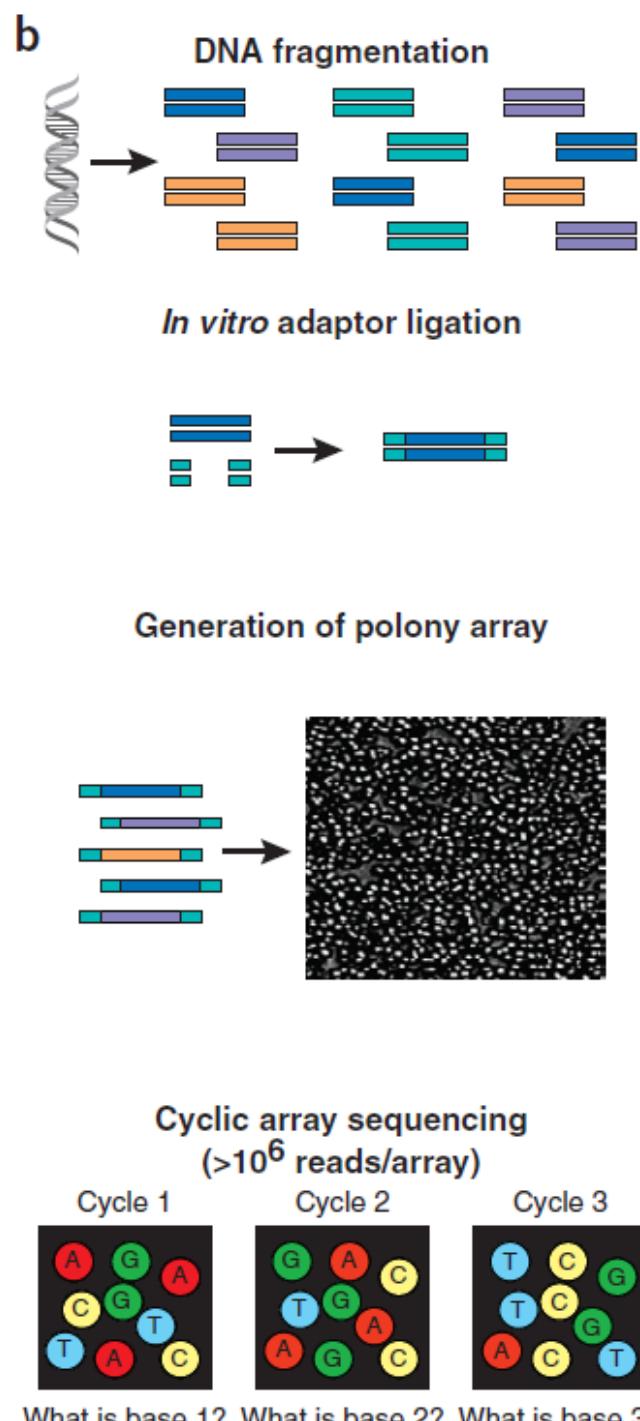


Miniaturización

Sacrificando principalmente la longitud



Paralelización



MÉTODOS DE ARREGLOS CÍCLICOS

1. Fragmentación (sonicación)
2. Ligación de adaptadores (PCR y Seq)
3. Amplificación (PCR en emulsión o Puente)
4. Secuenciación
5. Adición de reactivos de seq.
6. Detección dNTP incorporado
7. Toma de imagen

ILLUMINA (SOLEXA)

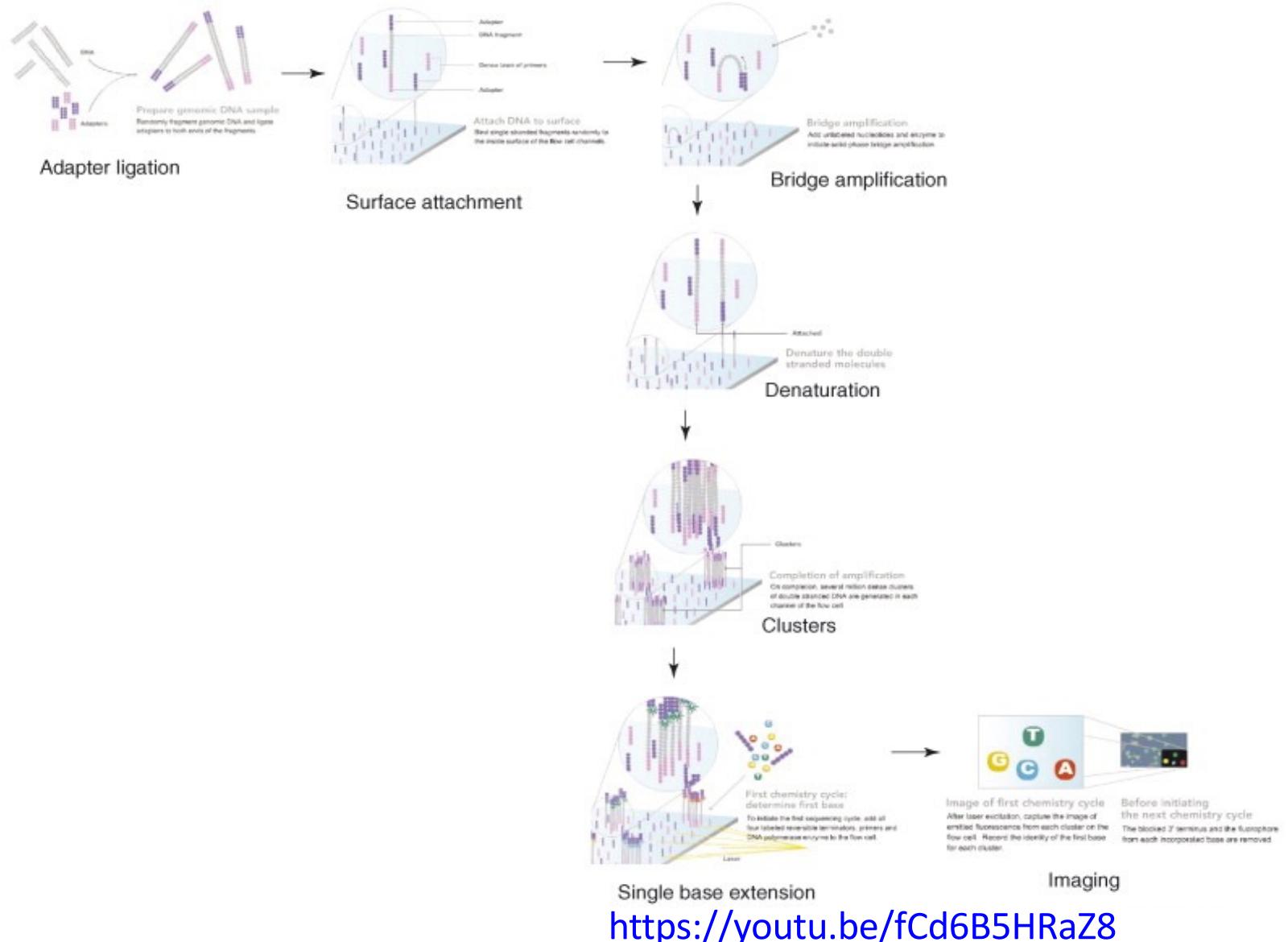
PCR en puente

Secuenciación por síntesis
usando CRT

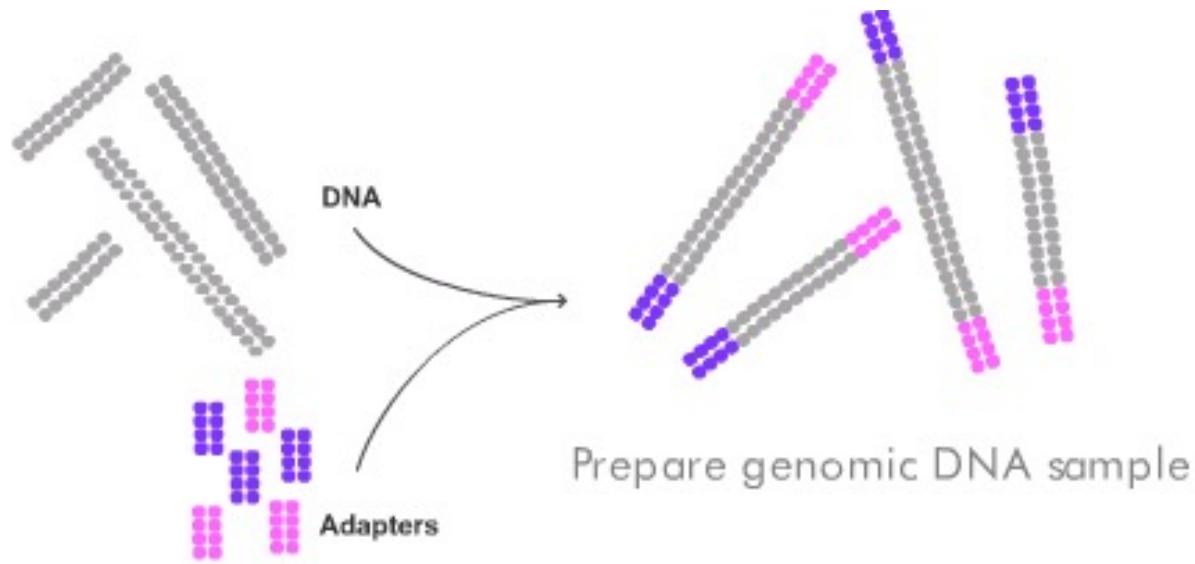
Lecturas muy cortas
max 300bp

Secuenciación en ambos
sentidos (paired-end)

Muy exactas



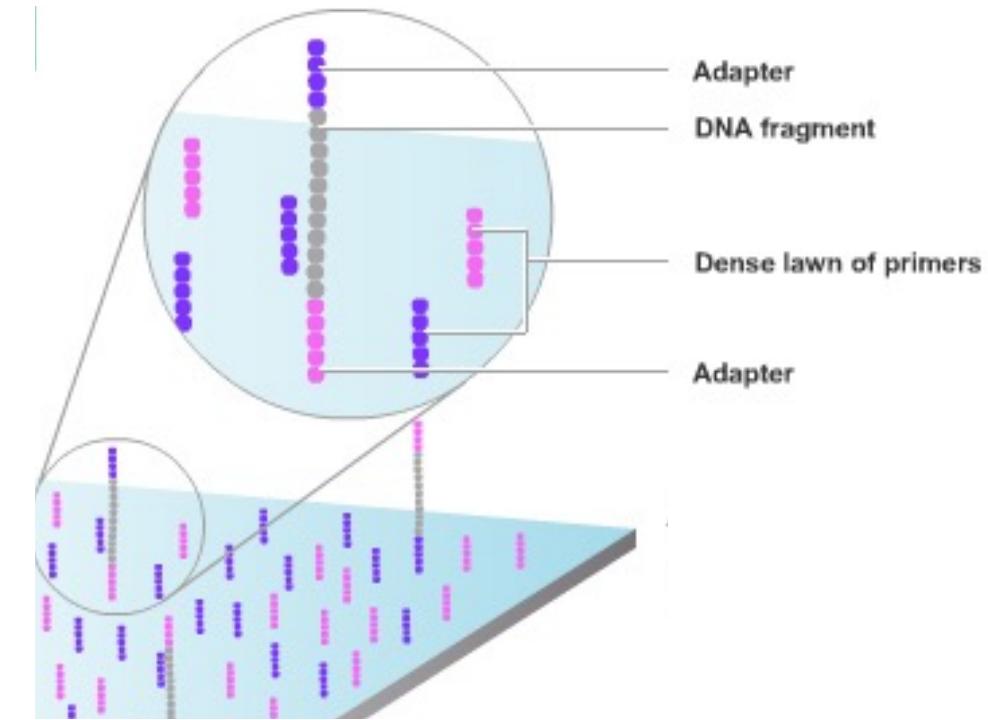
1. FRAGMENTAR DNA



Fragmentar DNA aleatoriamente en fragmentos de tamaño requerido para la librería.

Ligar adaptadores a los fragmentos

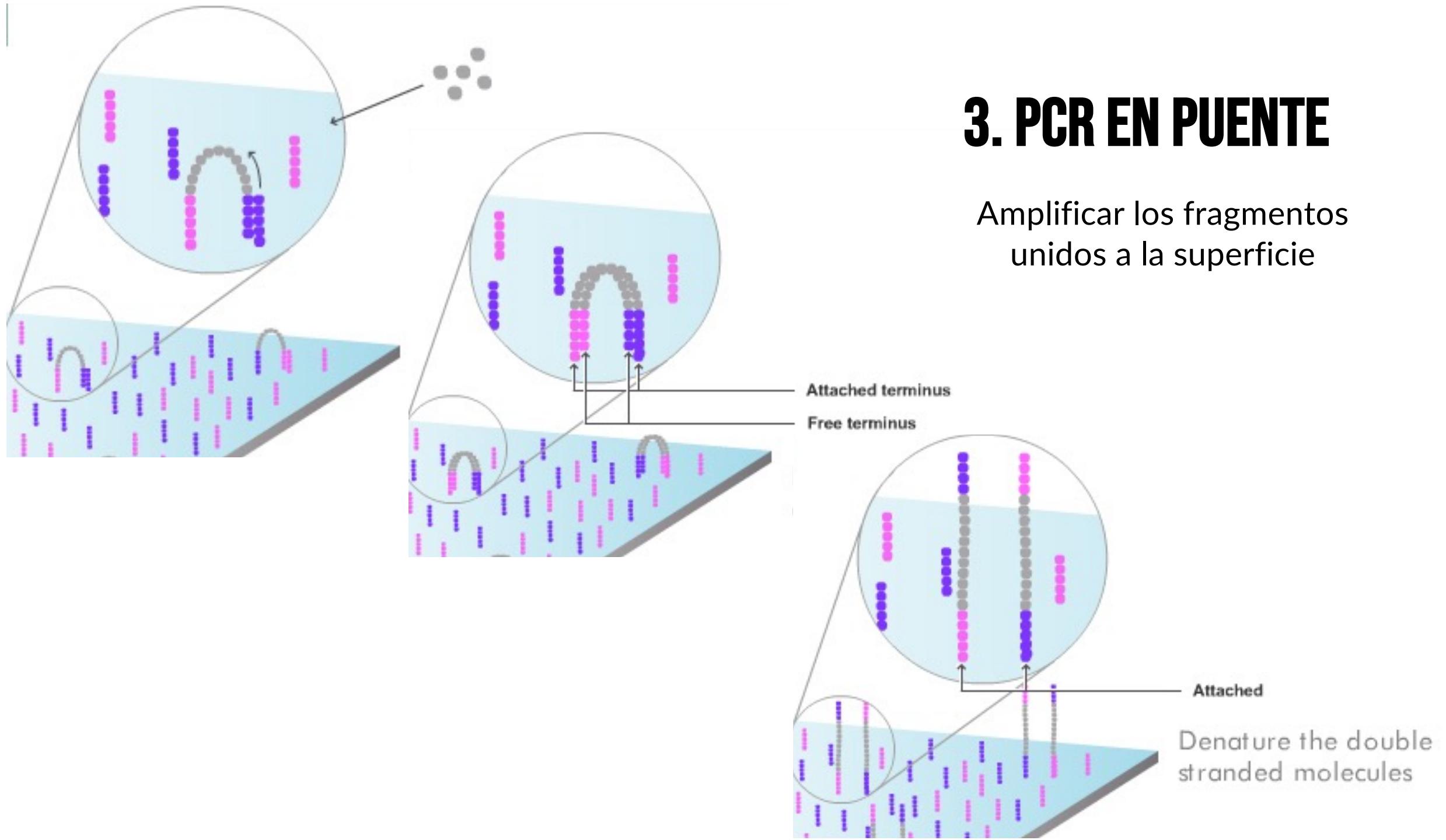
2. UNIR DNA A LA SUPERFICIE

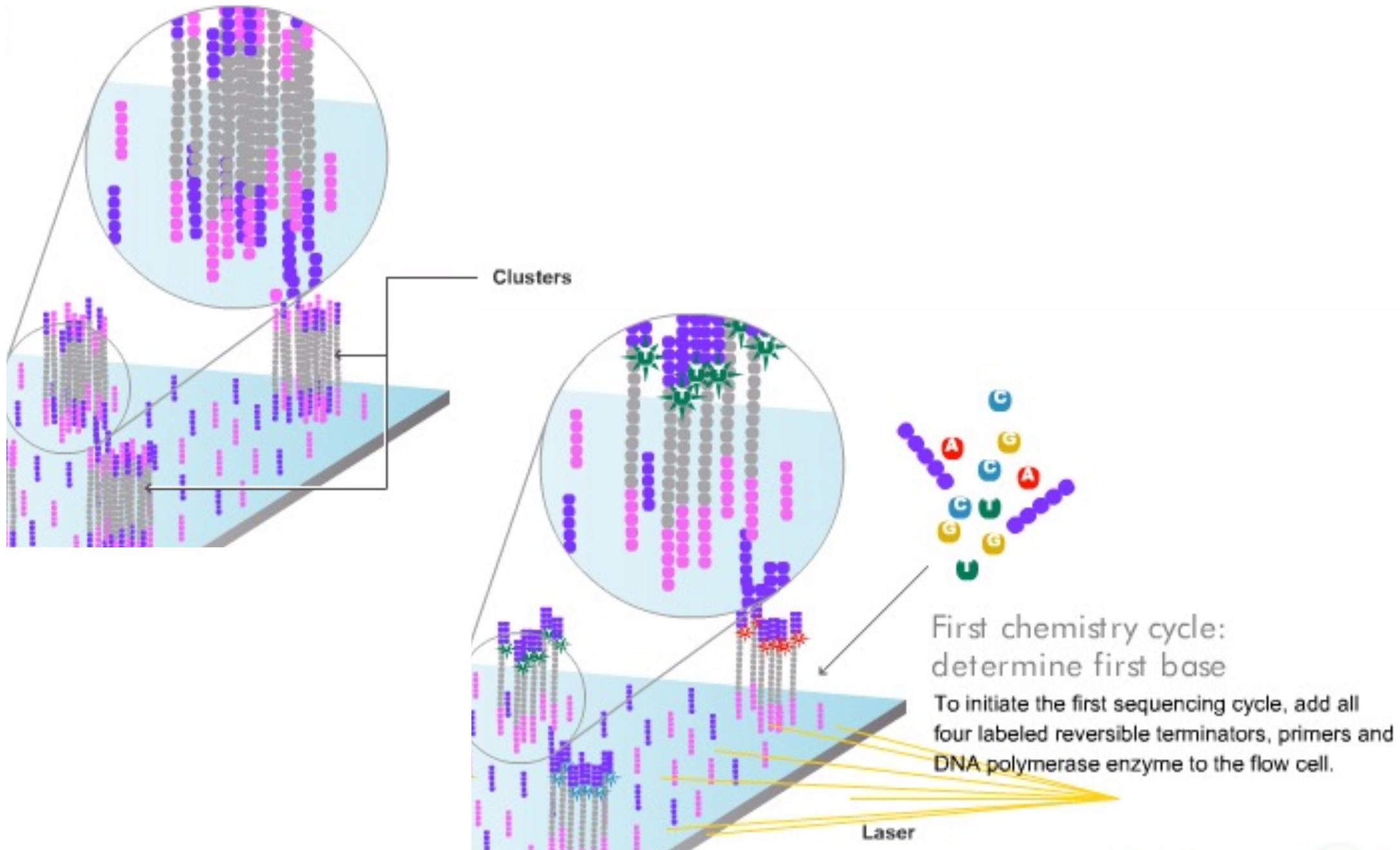


Unir aleatoriamente los fragmentos a la superficie o placa

3. PCR EN PUENTE

Amplificar los fragmentos
unidos a la superficie





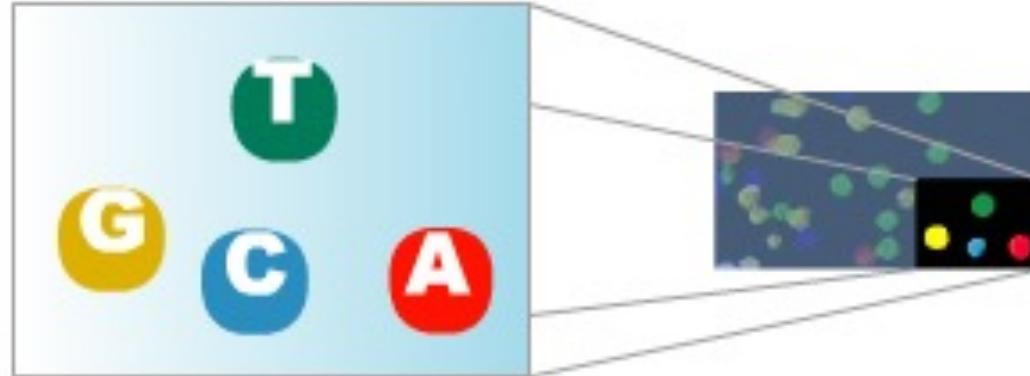
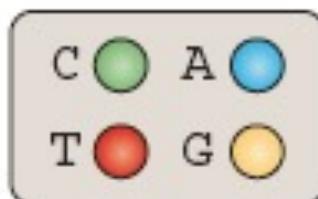


Image of first chemistry cycle

After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.

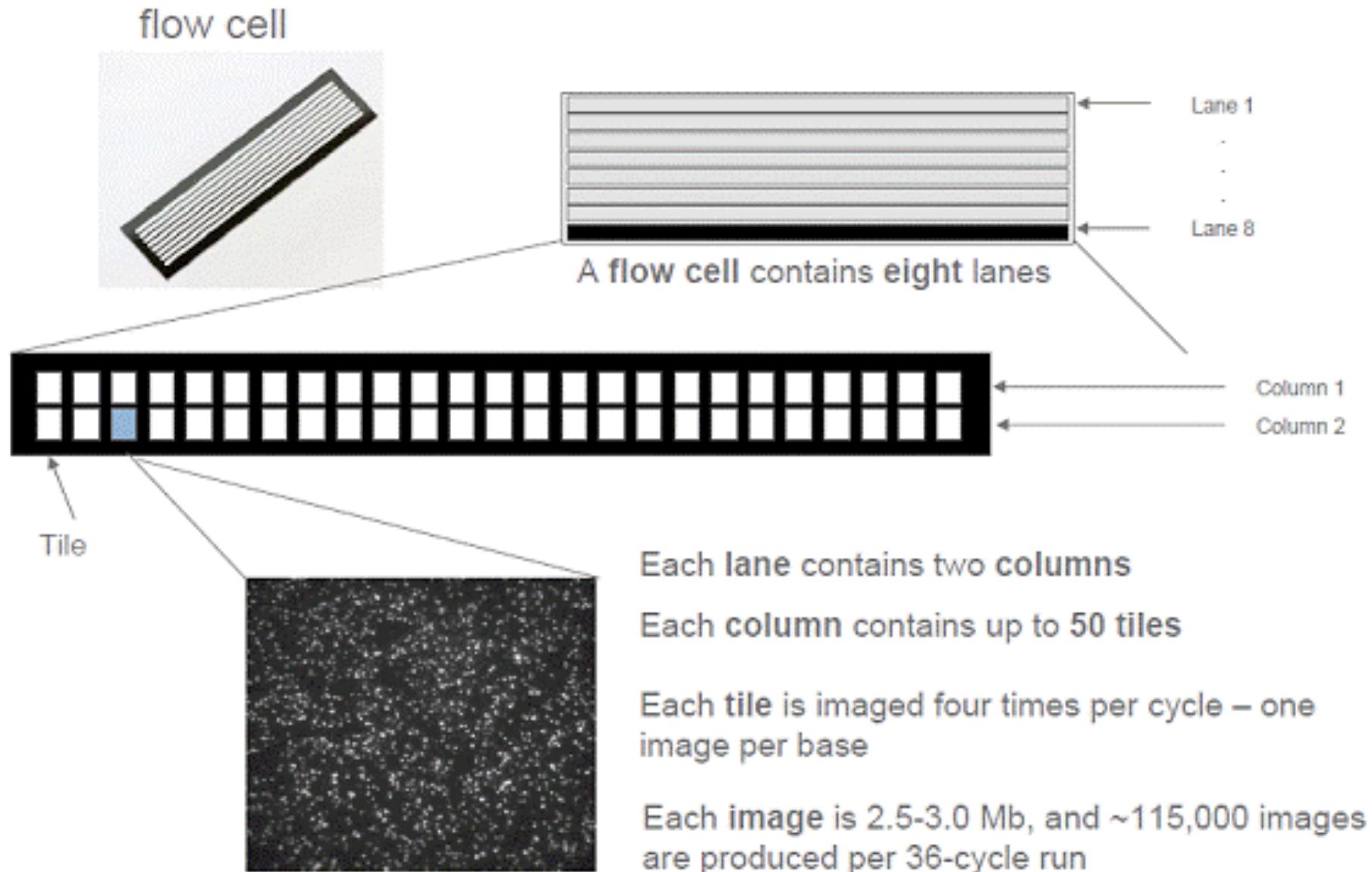
Before initiating the next chemistry cycle

The blocked 3' terminus and the fluorophore from each incorporated base are removed.



Top: CATCGT
Bottom: ccccccc

Technology Overview - GAII



PLATAFORMAS ACTUALES



iSeq 100



MiniSeq



MiSeq Series



NextSeq 550 Series



NextSeq 2000



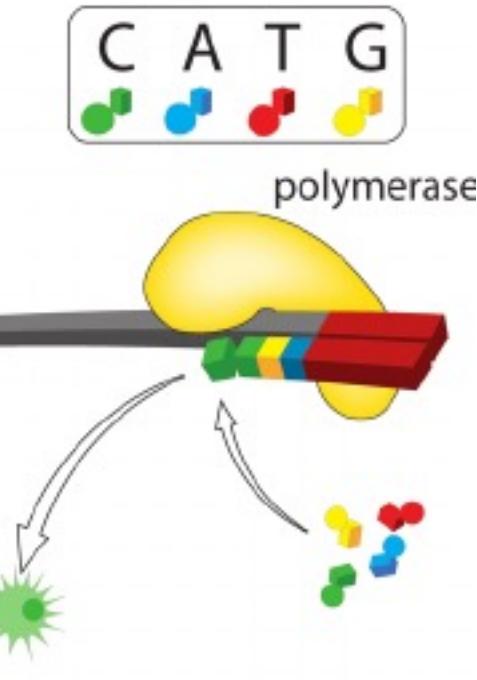
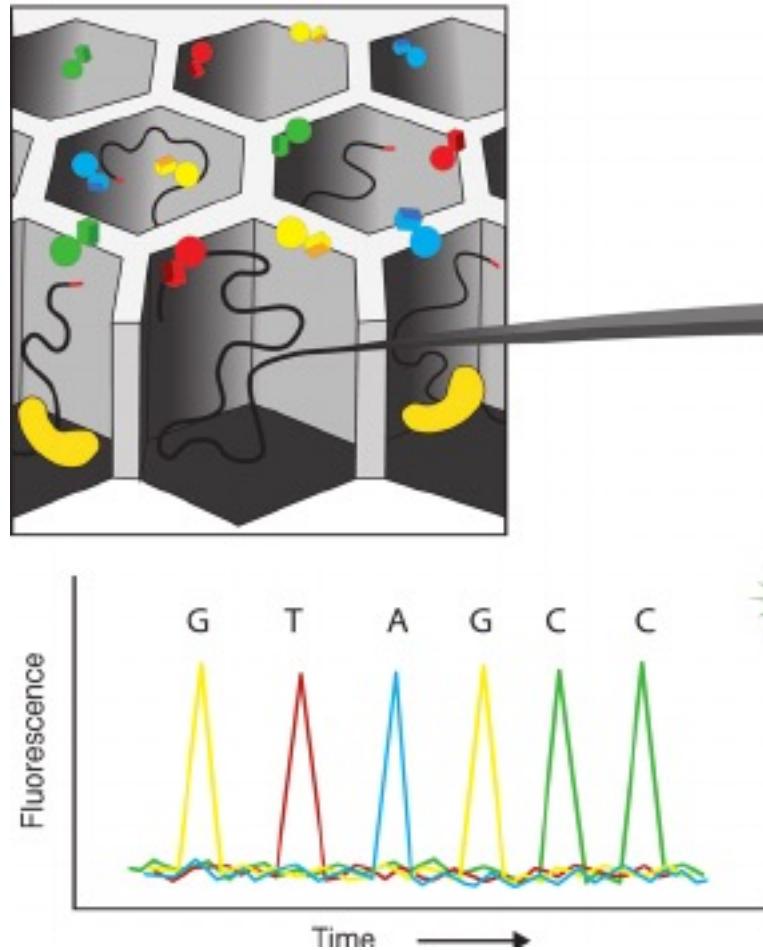
NovaSeq 6000

Run Time	9.5–19 hrs	4–24 hours	4–55 hours	12–30 hours	24–48 hours	~13 - 38 hours (dual SP flow cells)
Maximum Output	1.2 Gb	7.5 Gb	15 Gb	120 Gb	300 Gb*	~13–25 hours (dual S1 flow cells)
Maximum Reads Per Run	4 million	25 million	25 million [†]	400 million	1 billion [*]	~16–36 hours (dual S2 flow cells)
Maximum Read Length	2 × 150 bp	2 × 150 bp	2 × 300 bp	2 × 150 bp	2 × 150 bp	~44 hours (dual S4 flow cells)

<https://www.illumina.com/science/technology/next-generation-sequencing/illumina-sequencing-history/decade-in-sequencing.html>

<https://www.illumina.com/systems/sequencing-platforms.html>

MÉTODOS SIN AMPLIFICACIÓN



1. Fragmentación grande
2. Secuenciación
3. Detección dNTP incorporado
4. Toma de imagen

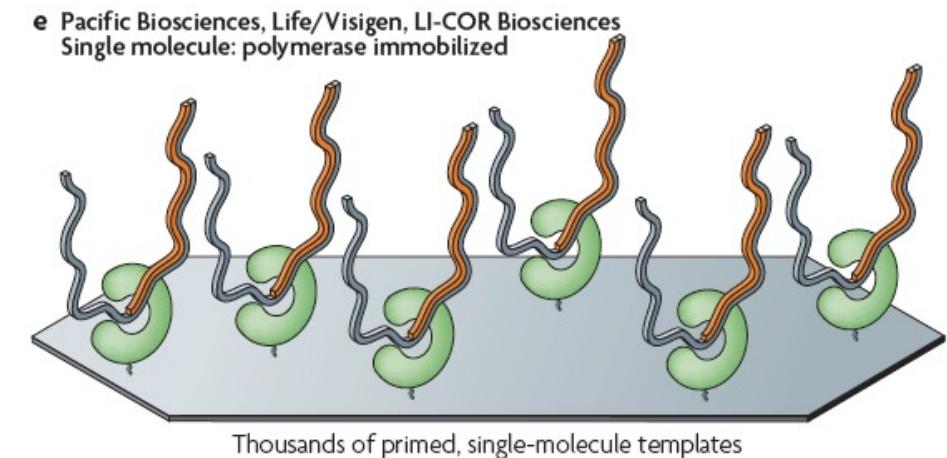
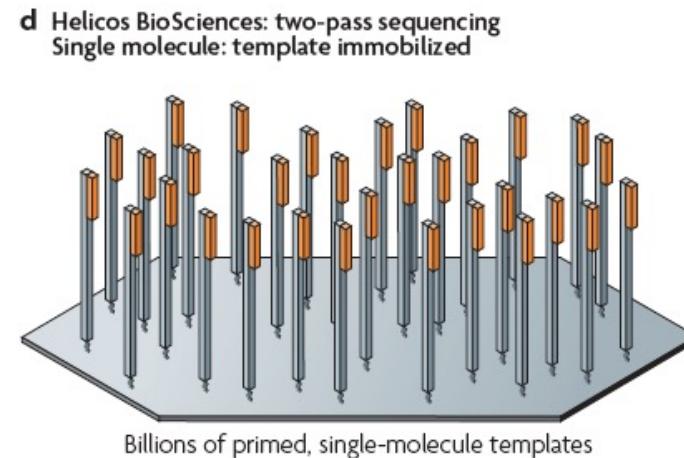
PACIFIC BIOSCIENCES

Single molecule – Real
time - SMRT cells

Inmovilización de la
polimerasa

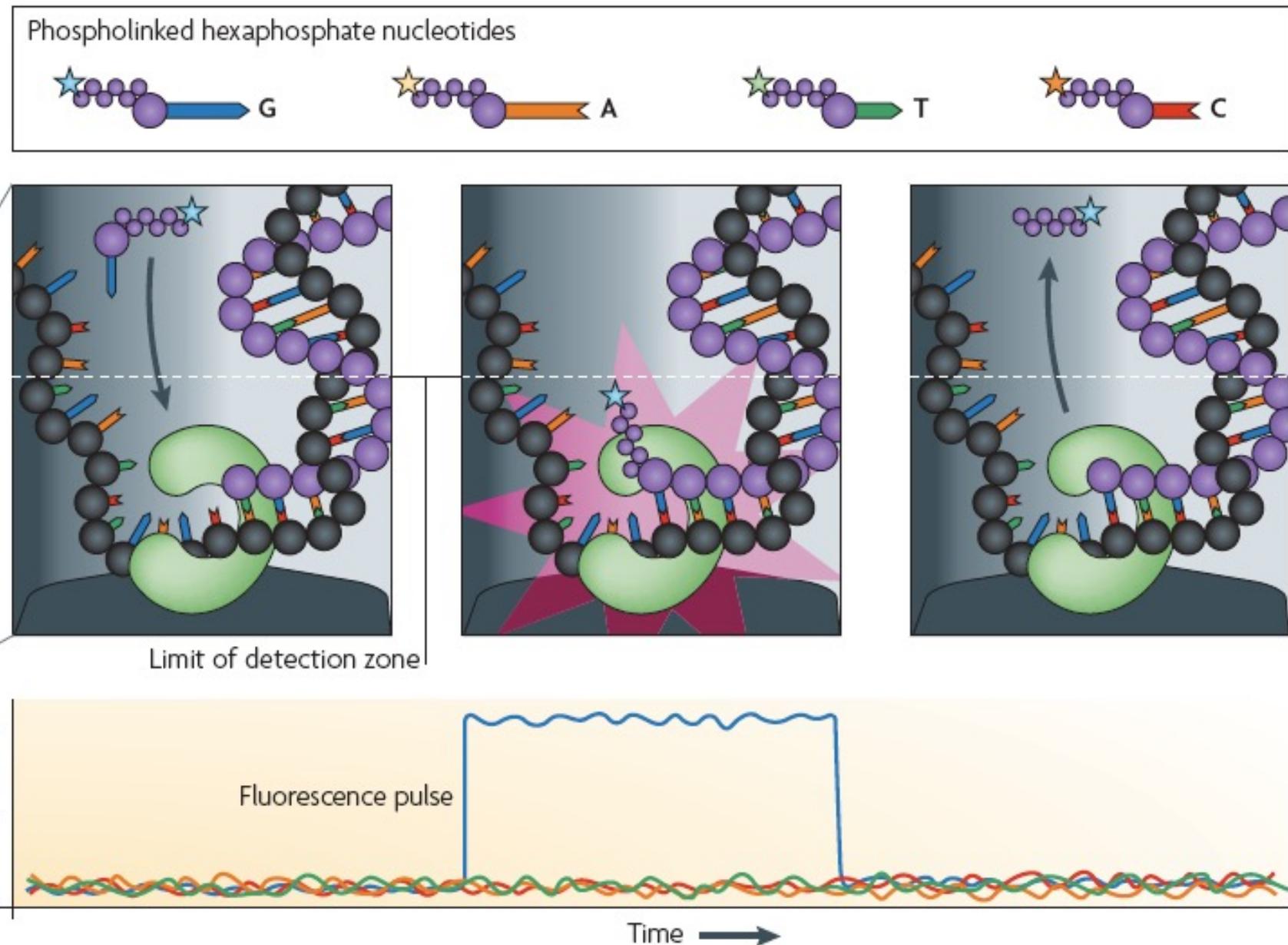
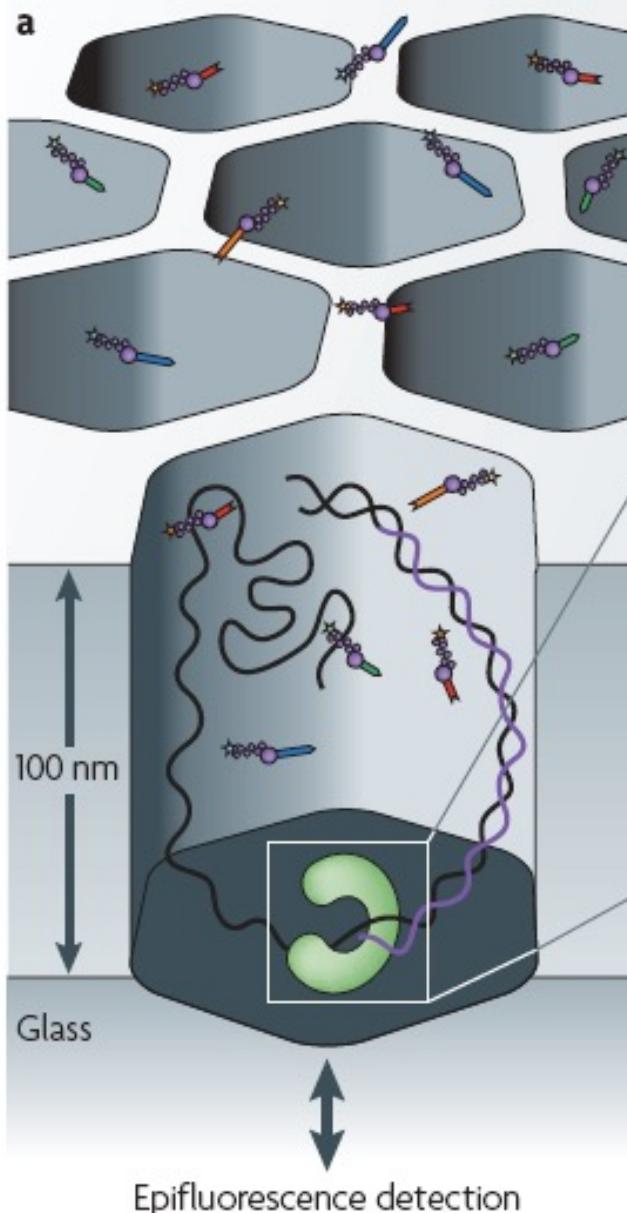
Secuenciación por
síntesis usando dNTP
marcado

Error 1-10%



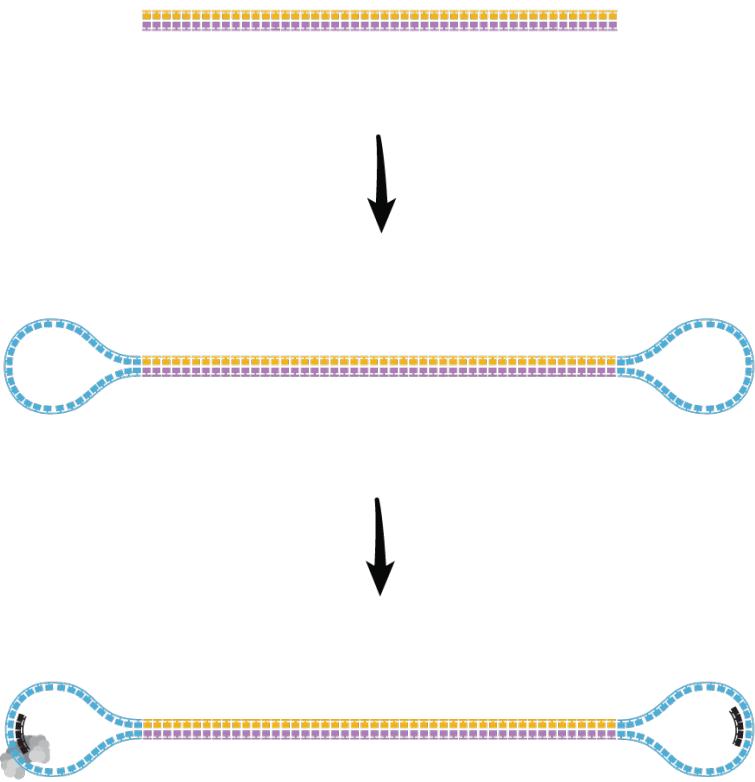
<https://www.youtube.com/watch?v=v8p4ph2MAvI>

https://youtu.be/_ID8JyAbwEo



HIFI READS

Start with high-quality double stranded DNA



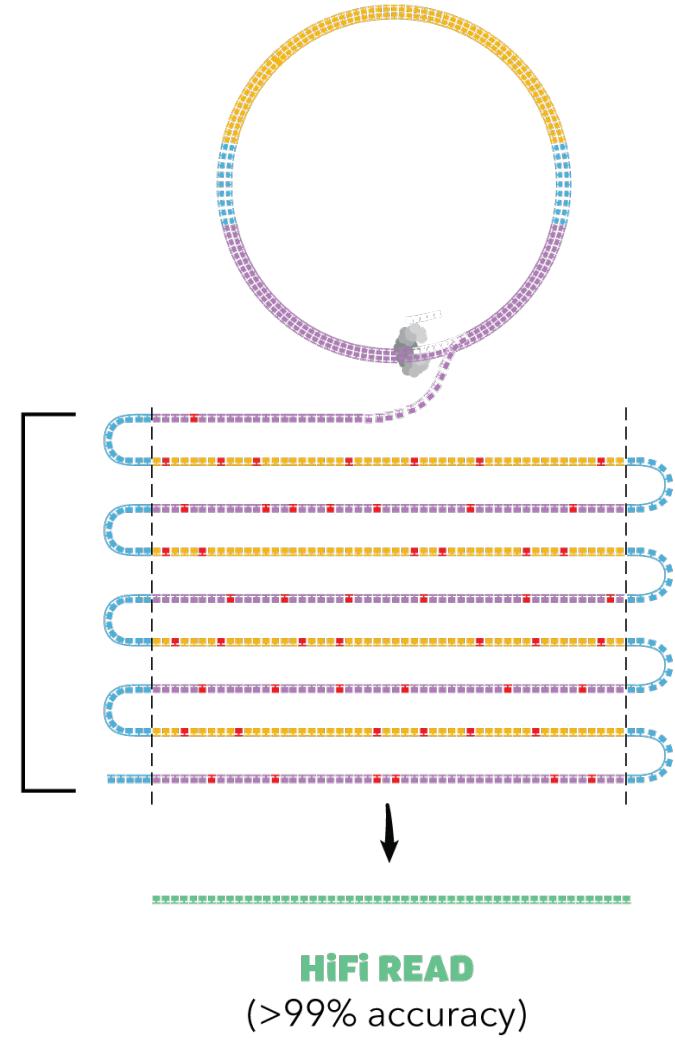
Ligate SMRTbell adapters and size select

Circularized DNA is sequenced in repeated passes

The polymerase reads are trimmed of adapters to yield subreads

Anneal primers and bind DNA polymerase

Consensus is called from subreads



PLATAFORMAS ACTUALES

	Sequel II System	Sequel System
Supported SMRT Cell	SMRT Cell 8M	SMRT Cell 1M
Number of HiFi Reads >99%* Accuracy	Up to 4,000,000	Up to 500,000
Sequencing Run Time per SMRT Cell	Up to 30 hrs	Up to 20 hrs
Recommended Chemistry	2.0	3.0
Instrument Control Software	v9.0	v8.0
SMRT Link	v9.0	v9.0



NANOPORE

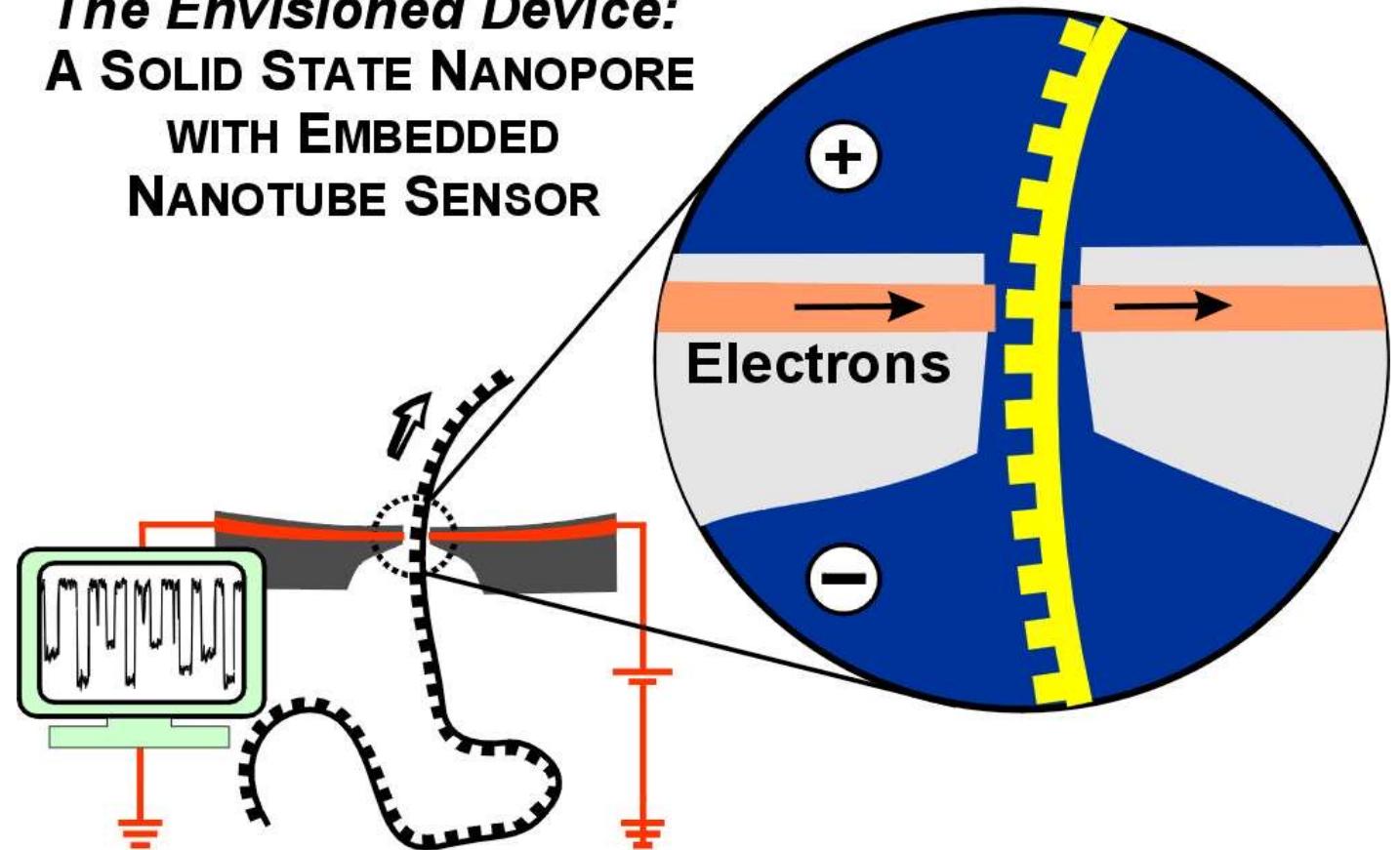
Secuenciación por detección de electrones en canal

Lecturas muy largas
10000-100000pb

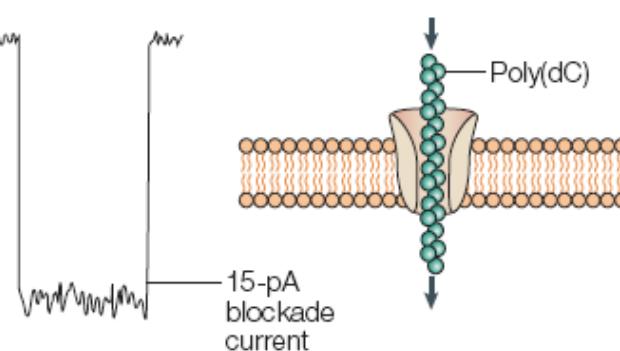
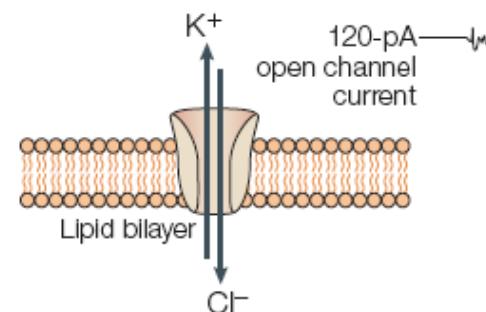
Error entre 10% y 20%

<https://vimeo.com/297106166>

The Envisioned Device: A SOLID STATE NANOPORE WITH EMBEDDED NANOTUBE SENSOR



b



Porina o hemolisina
Mycobacterium stegmatis

The MinION device is a miniaturised single molecule analysis system, designed for single use and to work through the USB port of a laptop or desktop computer



The MinION device is designed to sense from complex samples such as blood/serum



PLATAFORMAS ACTUALES

<https://nanoporetech.com/how-it-works>



Powerful

Get up to 30 Gb data from a single flow cell.



Portable

Sequence anywhere, including at sample source.



Real time

Immediate data streaming for rapid, actionable results.



Unrestricted read length

Generate short to ultra-long (>2 Mb) reads for ultimate experimental flexibility.



Flexible

Run up to five independently addressable Flongle or MiniON Flow Cells.



High throughput

As much as 150 Gb of data – streamed in real time for immediate analysis.



Integrated compute

Powerful onboard data processing and analysis, minimising IT requirements.



Compact

Small footprint, suitable for any lab.



No capital cost

Pay just for consumables.



On-demand sequencing

Run up to 48 independently addressable, high-capacity PromethION Flow Cells.



Ultra-high throughput

Generate terabases of data – streamed in real time for immediate analysis.



Powerful compute

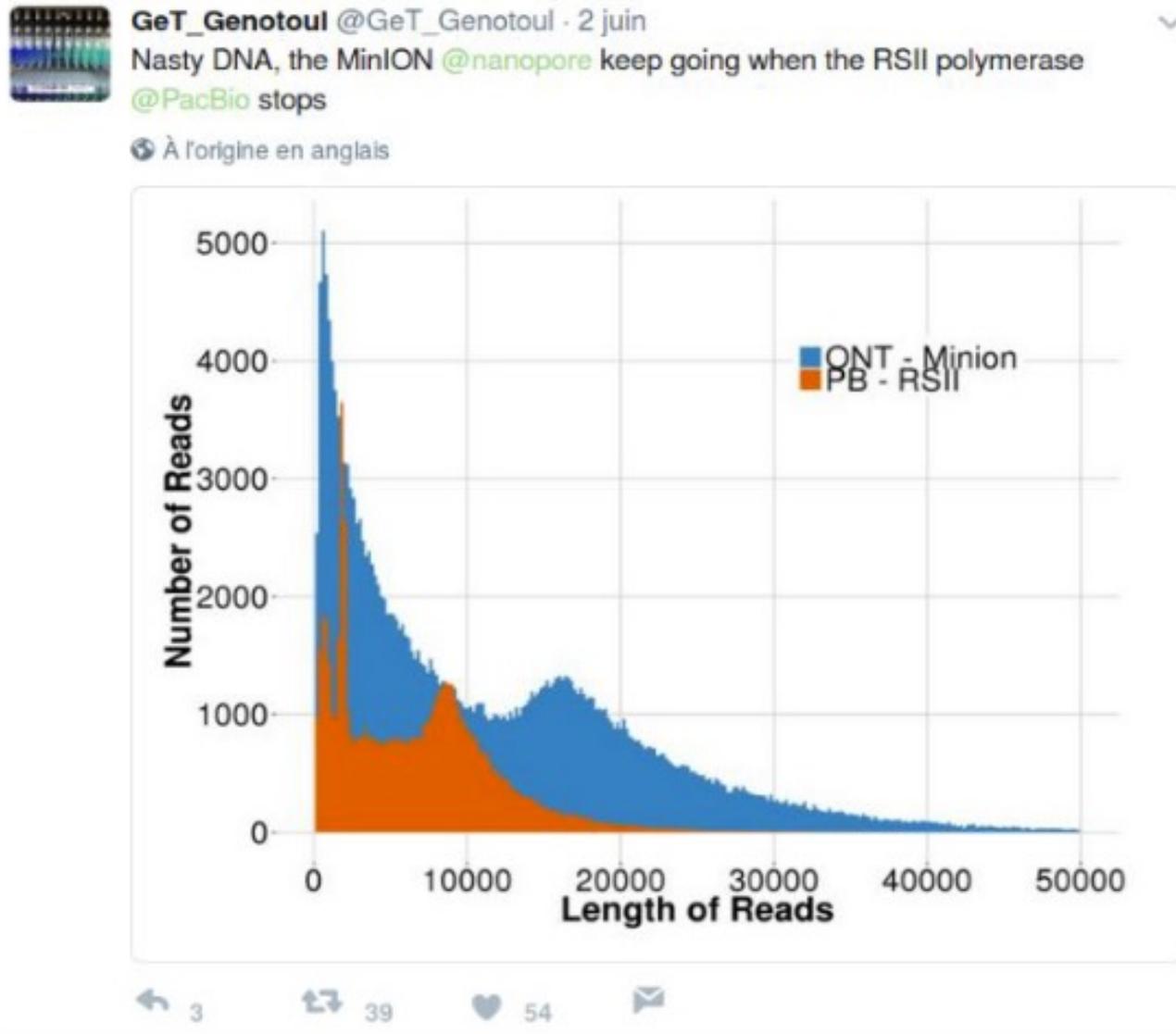
Alleviate data analysis bottlenecks.



Population-scale sequencing

Generate sub-\$1,000 human genomes.

Same sample / RSII vs MinION



SMRT limited by the longevity of the polymerase. A faster polymerase for the Sequel sequencer (chemistry v3, 2018) increased the read lengths to an average 30-kb polymerase read length.

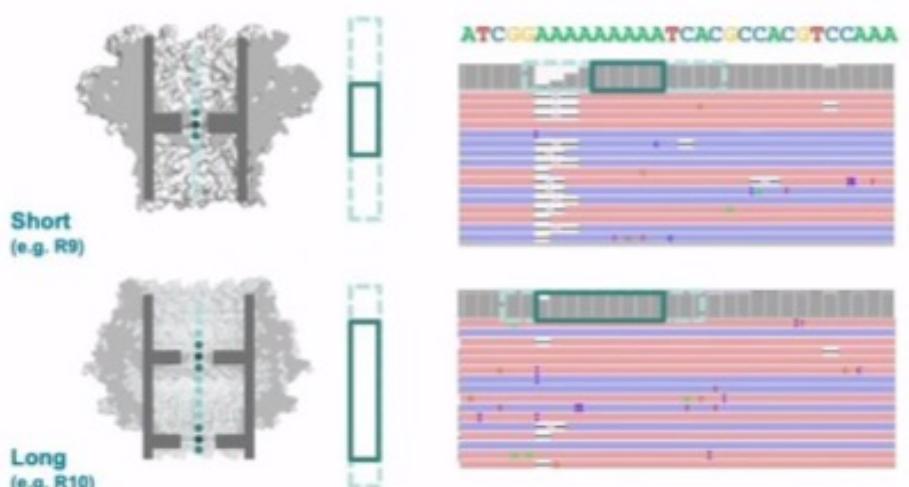
Last upgrades !

Oxford's Nanopores

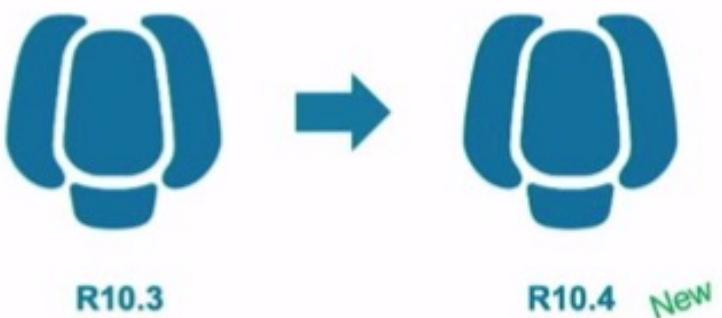
R9 and R10

Short and long "reader heads"

- Length of the main discrimination site ("read head") affects accuracy
- Short read heads allow easier decoding of individual bases (R9 series)
- Longer read heads see more range and are more information rich (R10 series)



© 2021 Oxford Nanopore Technologies Limited.
Oxford Nanopore Technologies products are not intended for use for health assessment or to diagnose, treat, mitigate, cure, or prevent any disease or condition.



Improving the R10 series

- We are continuously seeking to improve our nanopores
- R10 series of pores are still being iterated on – new R10.4 version
 - Extended discrimination profile, more sensing range
 - Higher flow cell yield of nanopores

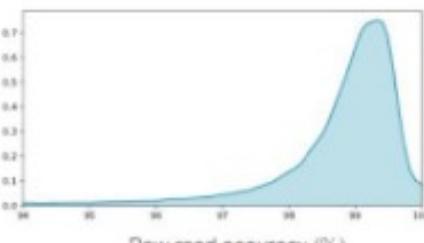


- Tweet

Oxford Nanopore @nanopore

Flow cells using our latest pore — R10.4 — can now be trialled through the expanding Q20+ Early Access Programme, which is now open to all applicants. Find out more about Q20+ and R10.4, and register to take part in the programme, here: bit.ly/3CEUJ1g

Raw read modal 99.3%, >Q20



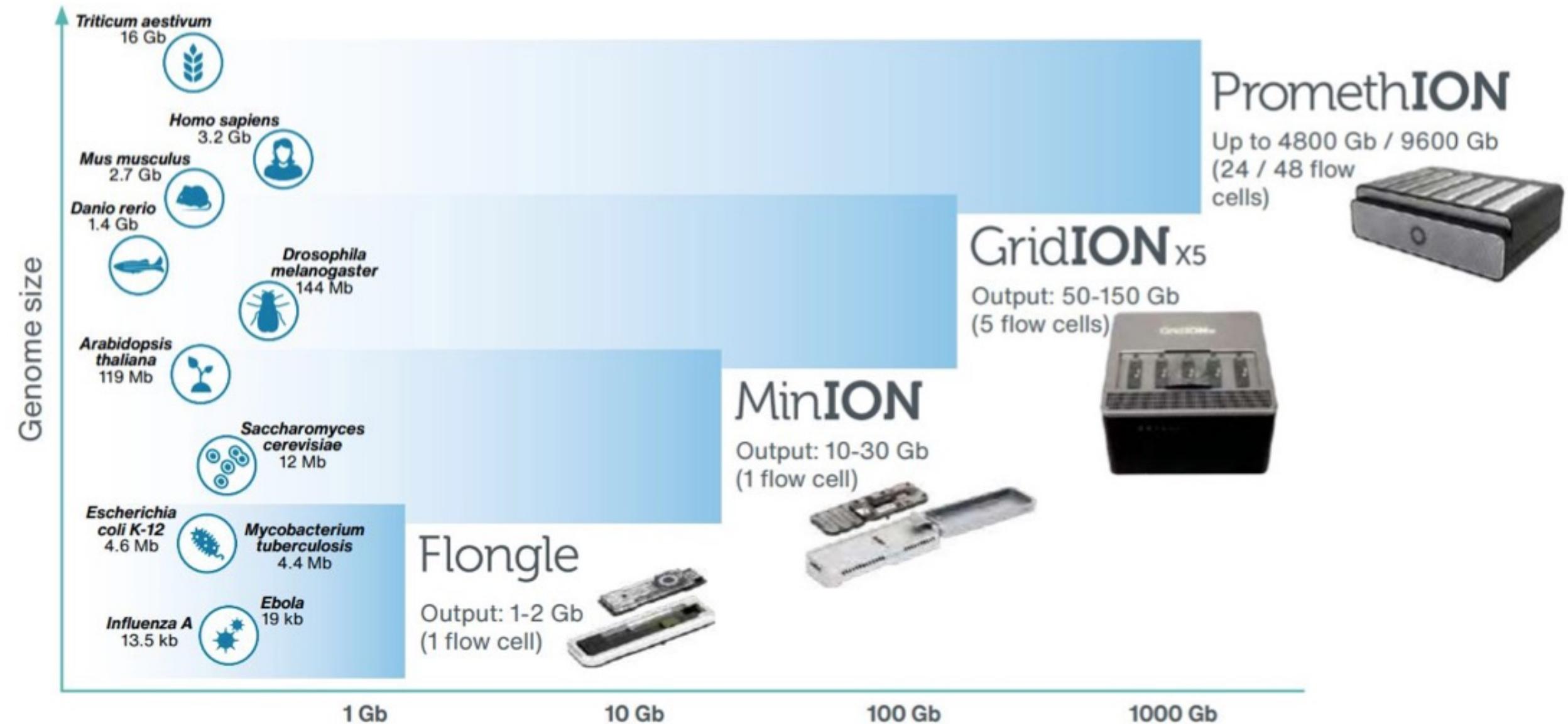
8:30 AM · Sep 23, 2021 · HubSpot

33 Retweets 1 Quote Tweet 62 Likes

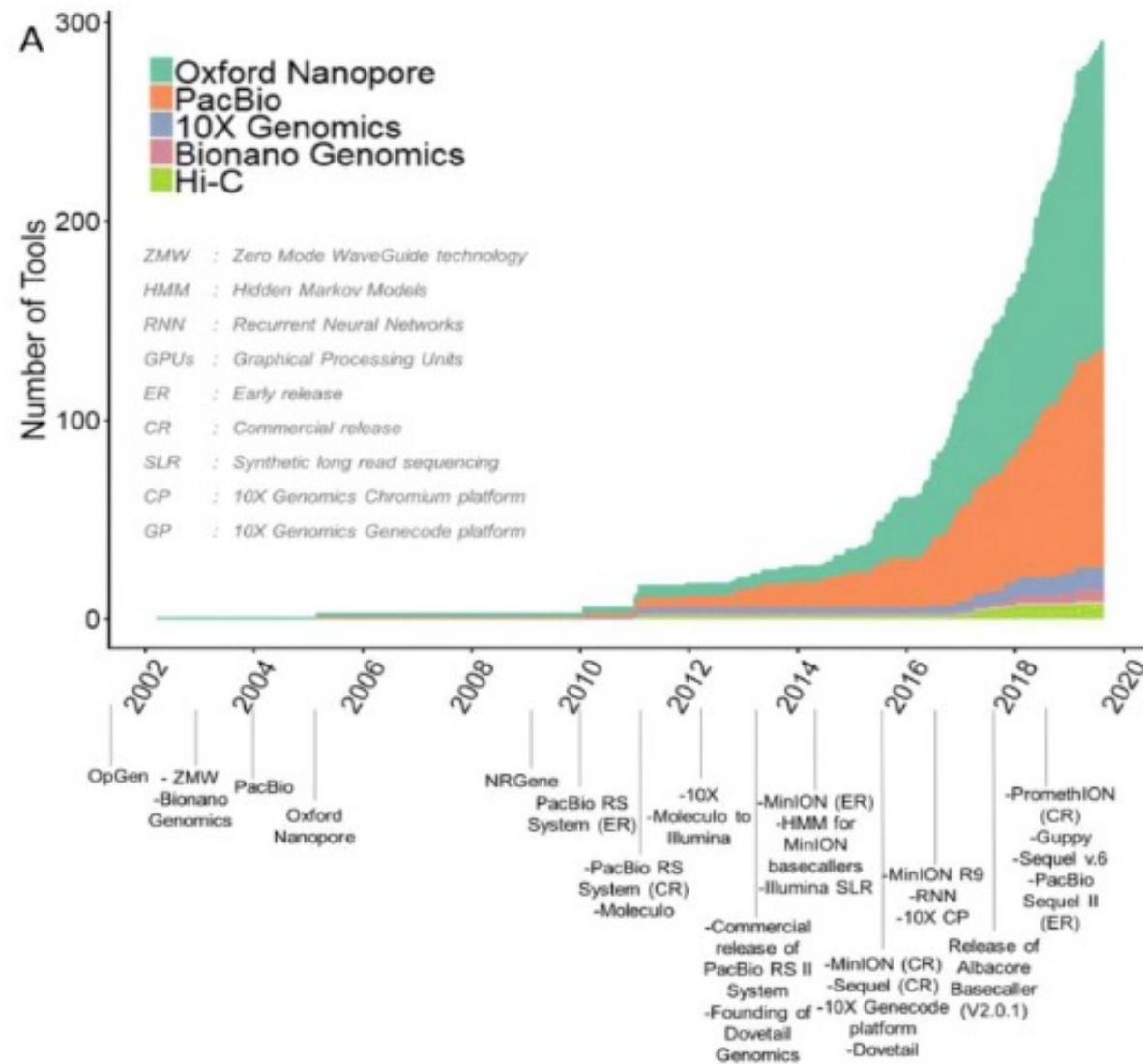


<https://community.nanoporetech.com/posts/q20-early-access-group-br>

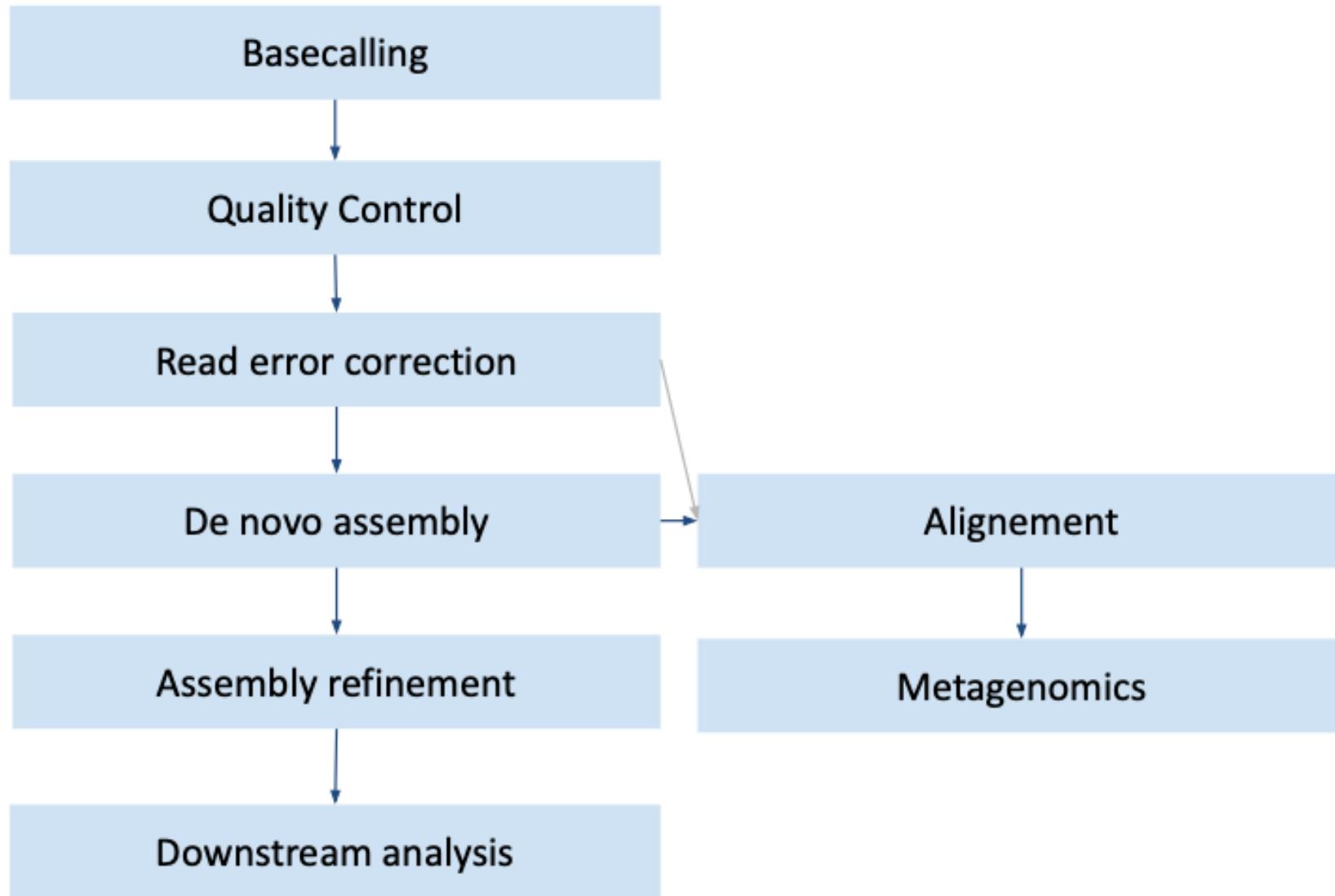
A lot of data !



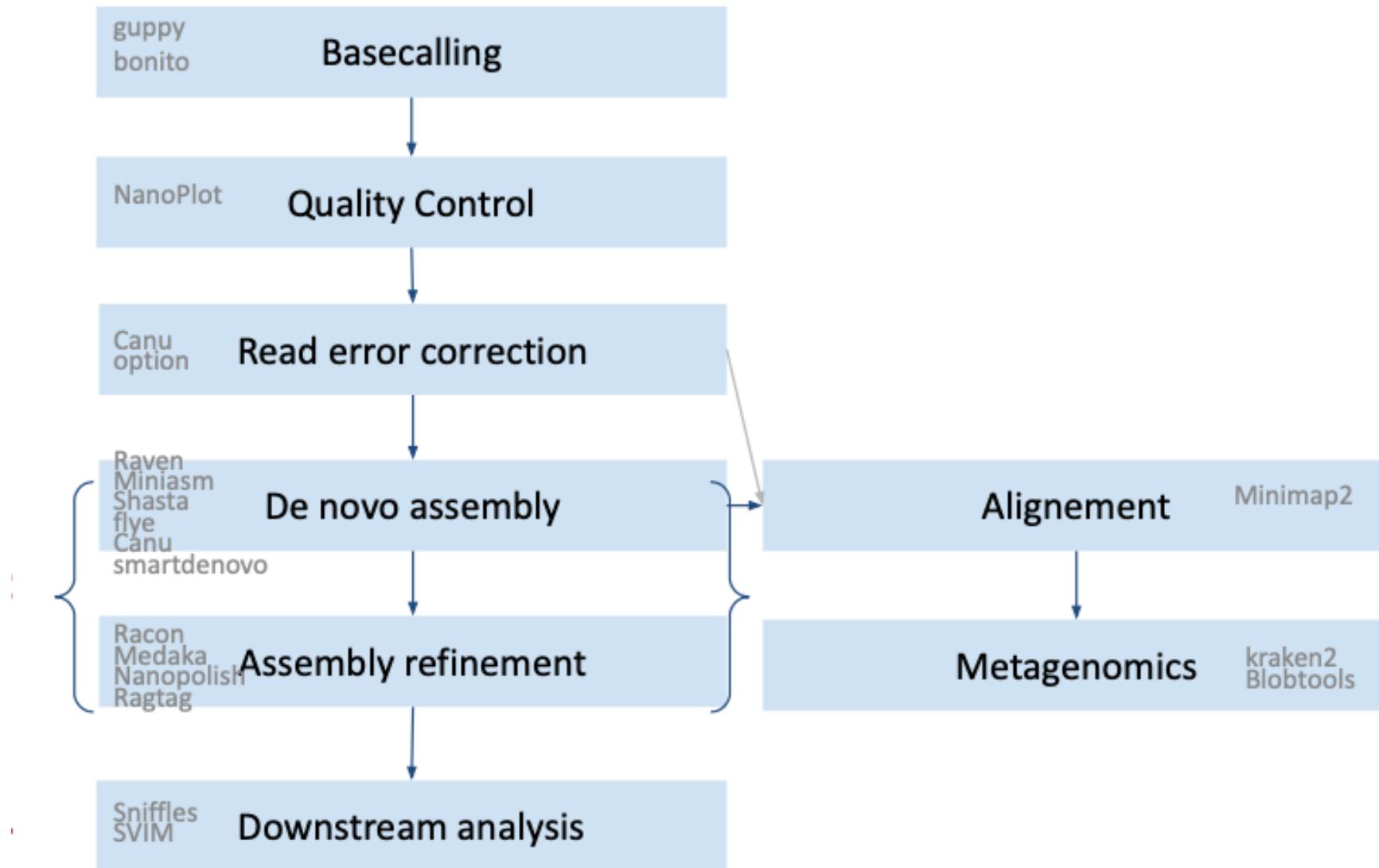
A lot of tools are being developed and upgraded frequently !



Typical long-read analysis pipelines for ONT data



Typical long-read analysis pipelines for ONT data



[Sans titre]

Cliquez pour

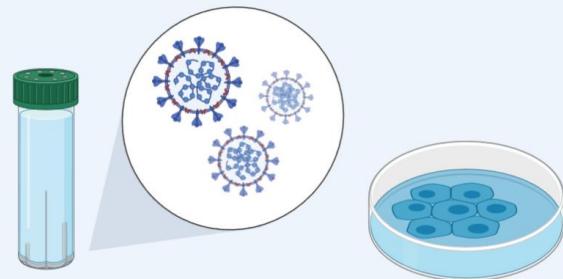
- Cliquez pour ajouter

Pied de page

SARS-CoV-2 sequencing workflow

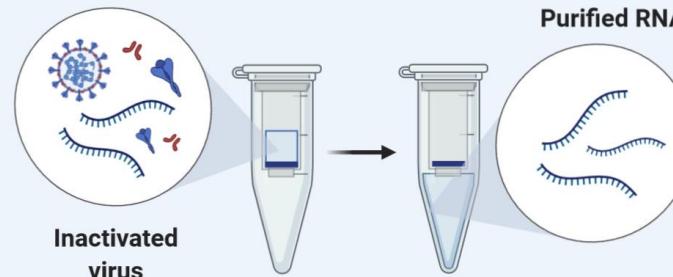
1 Specimens collected

Clinical samples and isolates



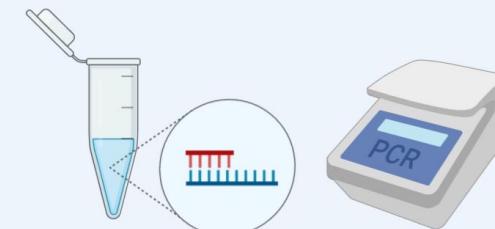
2 Viral RNA extraction

NucleoMag Virus kit (Macherey-Nagel) ~45 min



3 cDNA synthesis and multiplex PCR

ProtoScript II First Strand cDNA Synthesis Kit (NEB) ~25 min
Q5 Hot Start High-Fidelity DNA Polymerase (NEB) ~4h per primer set



4 MinION (ONT) and iSeq100™ (Illumina) library preparation



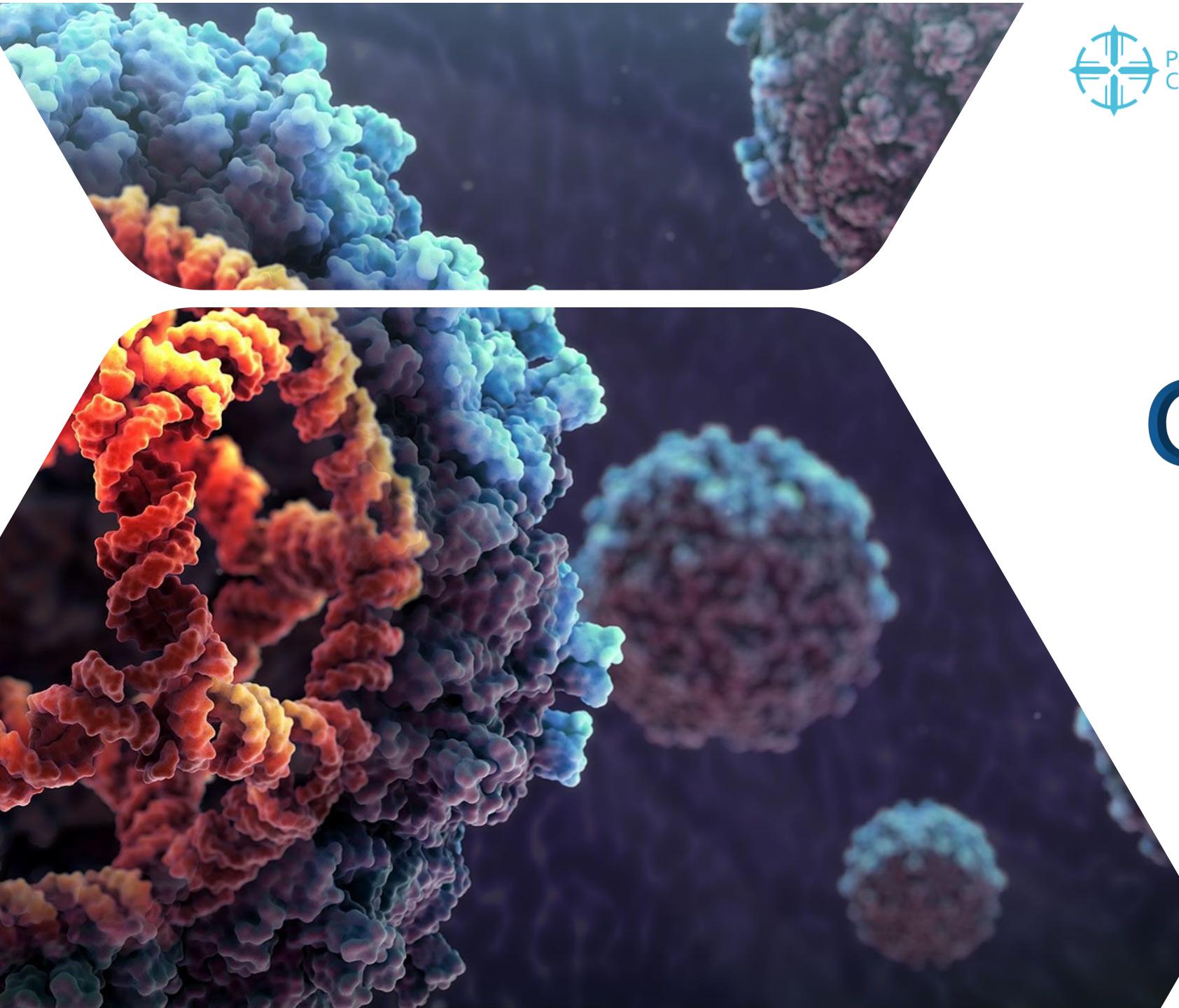
5 MinION and iSeq100™ sequencing



6 Data analysis

MinION/Illumina bioinformatics specific pipelines ~1h





GRACIAS