SEPTEMBER
2025

High throughput sequencing

Functional genomic data analysis: transcriptomics

First generation sequencing methods

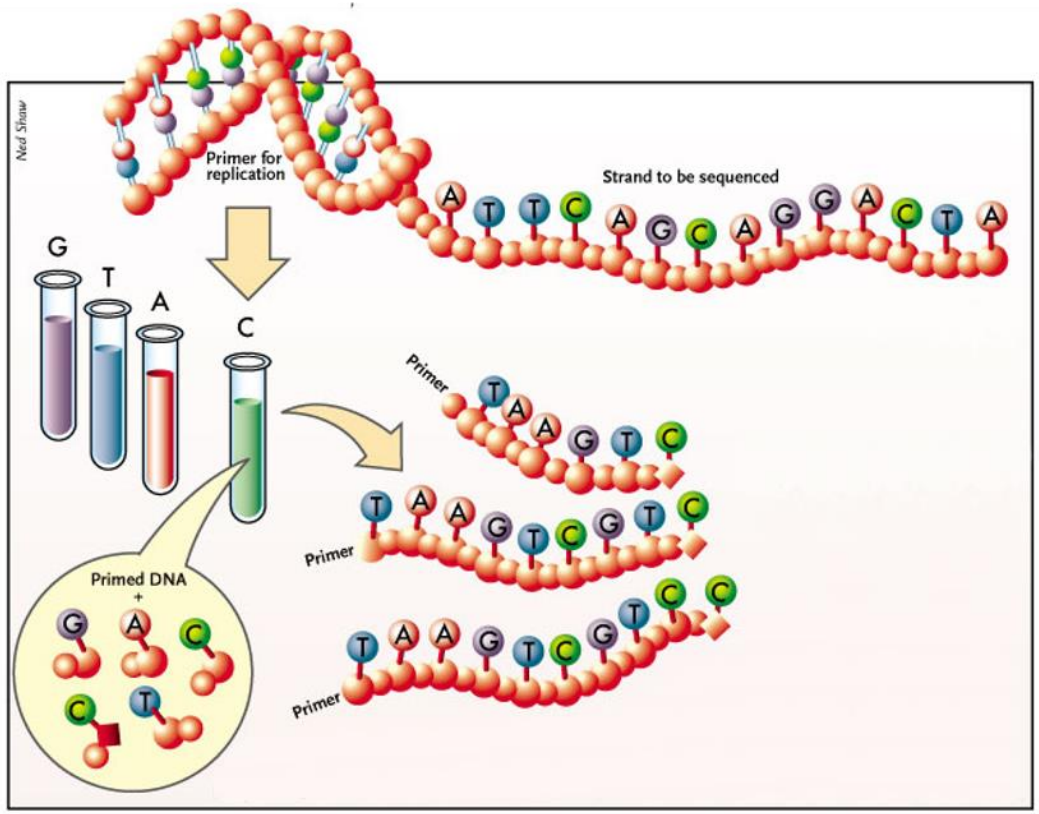
Sanger sequencing by synthesis

Method discovered in 1977 by Frédérick Sanger (nobel price 1980).

DNA polymerisation using a **complementary primer**. Elongation using **thermostable DNA polymerase** (PCR).

Addition of 4 **deoxynucleotides** (dATP, dCTP, dGTP, dTTP) and low concentrations of one of four **dideoxynucleotides** (ddATP, ddCTP, ddGTP ou ddTTP).

These ddNTP once incorporated in the newly synthesized DNA strand, block elongation. Synthesis termination is done by a statistical manner on each possible positions.



Sequence reading

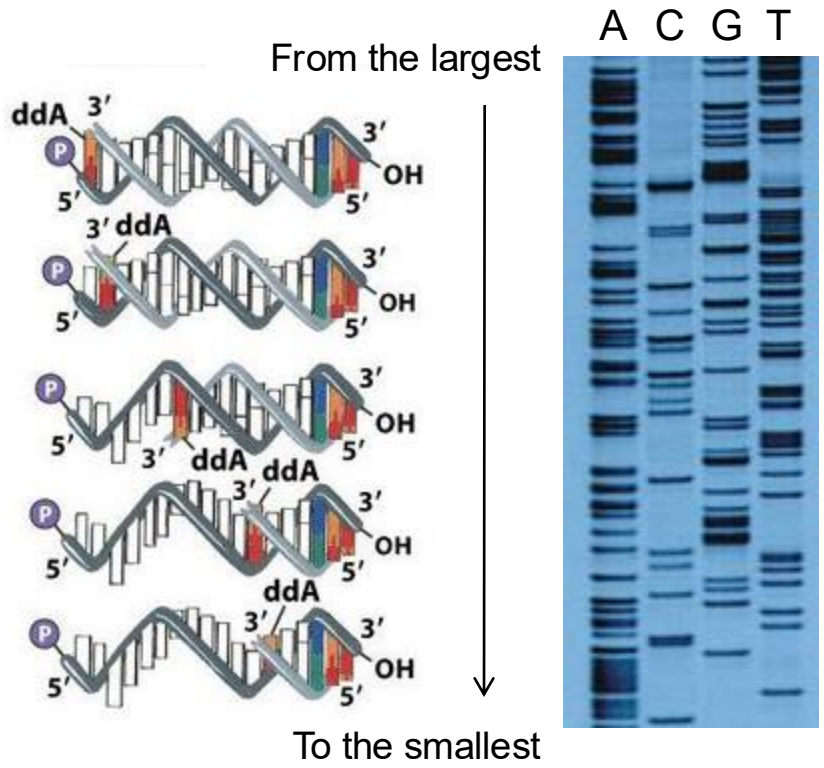
We a get a **mix of DNA fragments** terminating at each position of the sequence.

These fragments are then separated on a DNA **polyacrylamide gel electrophoresis**.

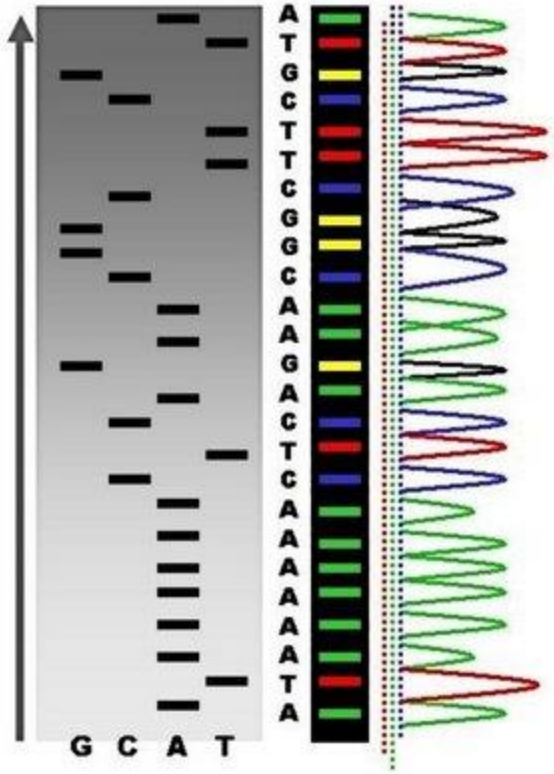
Detection of synthesize fragments is done by the **incorporation of labelling beacon** in the DNA.

At the origin this label was radioactive, attached either on the primer or on the dideoxynucleotide.

Around **1 kb of DNA** by run during **2 days**.
One read by **sample**.



Capillary sequencers

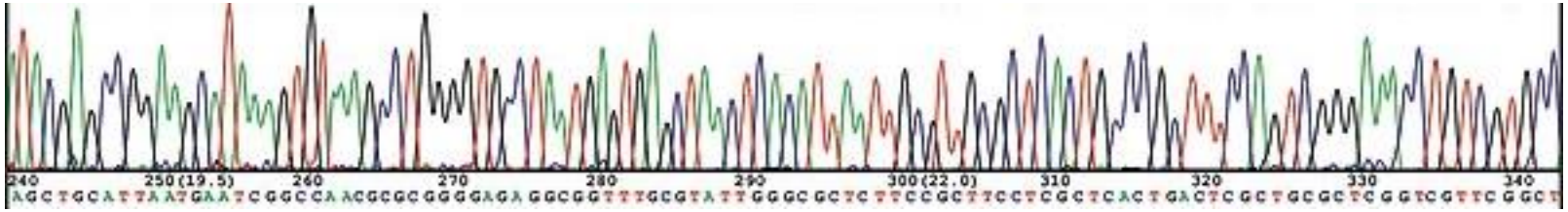


First version in the 90's thanks to the **modification of the radioactive label by a fluorescent one**.

Using **glass capillary of few micron diameters**, on 30 to 50 cm long.

The four nucleotides migrate in the same tube thanks to **four different fluorescent dyes**.

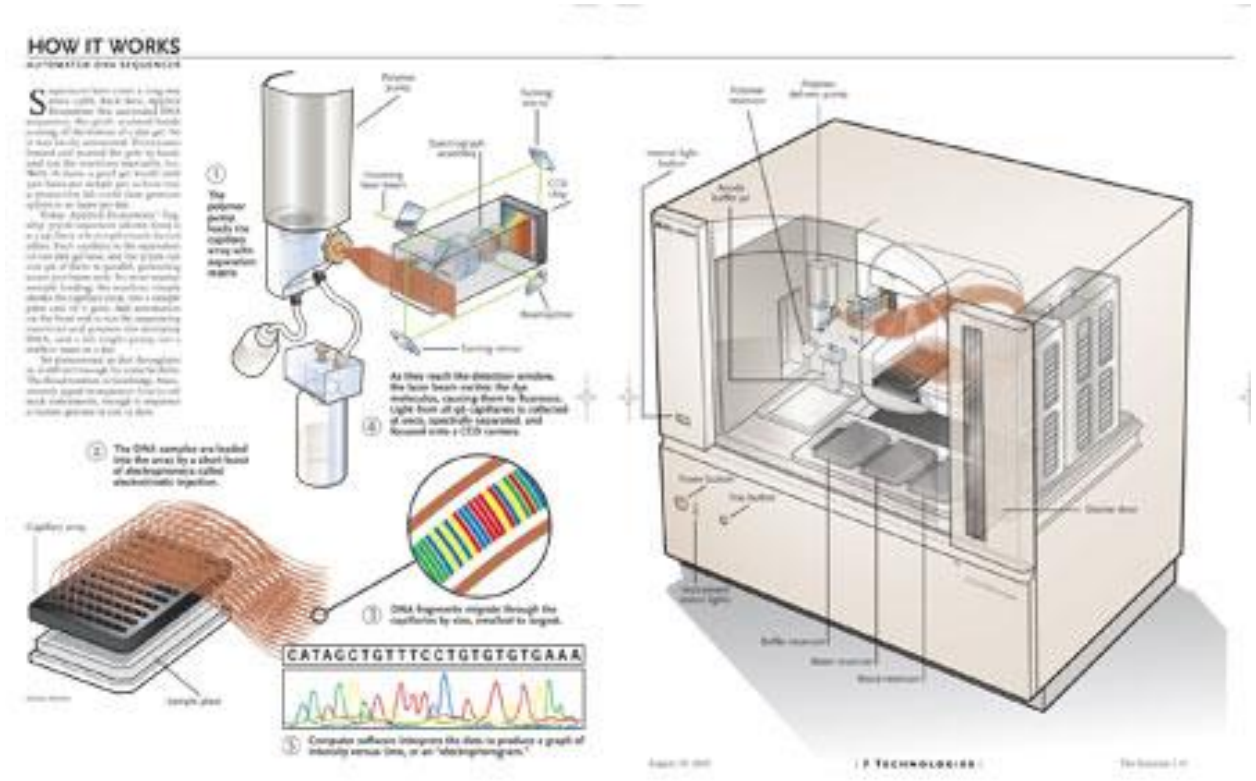
300 kb of DNA by run during **3 hours**. Several hundred sample at a time.



ABI 3730xl DNA Analyzer

96 parallel capillary (up to 50 cm) array.
768 samples, **690 kb** DNA, **3 hours** run.

At the Broad Institute (Cambridge, Massachusetts) **126 devices** were able to sequence **1 human genome** in **12 days**.



From *The Scientist*

Second generation sequencing: high throughput sequencing

The first technologies on the market

Goal: to obtain a huge number of short reads

since 1996

Applied Biosystems
ABI 3730XL
1 Mb / day

January '07 GS20
June '07 GS FLX

Roche / 454
Genome Sequencer FLX
100 Mb / run

January '07

Illumina / Solexa
Genome Analyzer
2,000 Mb (2 Gb) / run

November '07

Applied Biosystems
SOLiD
3,000 Mb (3 Gb) / run

Illumina Genome Analyzer
January 2007

From: Clive Brown <clive.Brown@solexa.com>
Date: Sun, 20 Feb 2005 16:34:46 +0100
To: Nick McCooke <Nick.McCooke@solexa.com>, Tony Smith <Tony
Swerdlow <Harold.Swerdlow@solexa.com>, John Milton <JM.Milto
<Kevin.Hall@solexa.com>, Colin Barnes <Colin.Barnes@solexa.c
<Vincent.Smith@solexa.com>, Klaus Maisinger <Klaus.Maisinger
Conversation: WE'VE DONE IT !!!!
Subject: WE'VE DONE IT !!!!

Tony Cox, Peta and I now agree - having looked at all of the PhiX174 data.

We have re-sequenced our first genome !!!!!!!

DNA library preparation

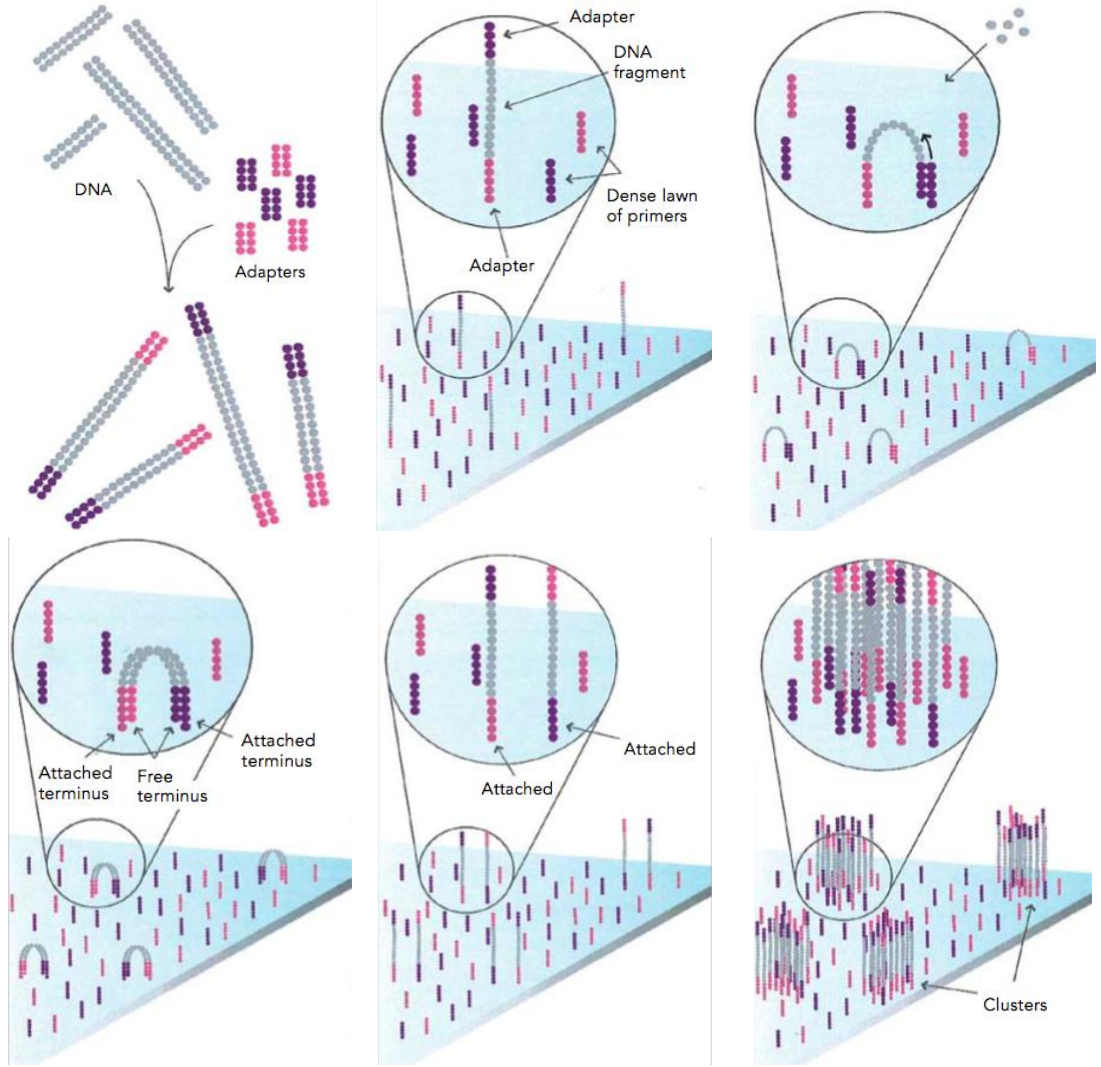
Random DNA fragmentation and size selection.

Ligation of adaptors.

DNA denaturation.

Hybridization of fragments onto the “**flowcell**” surface.

Solid phase bridge PCR.



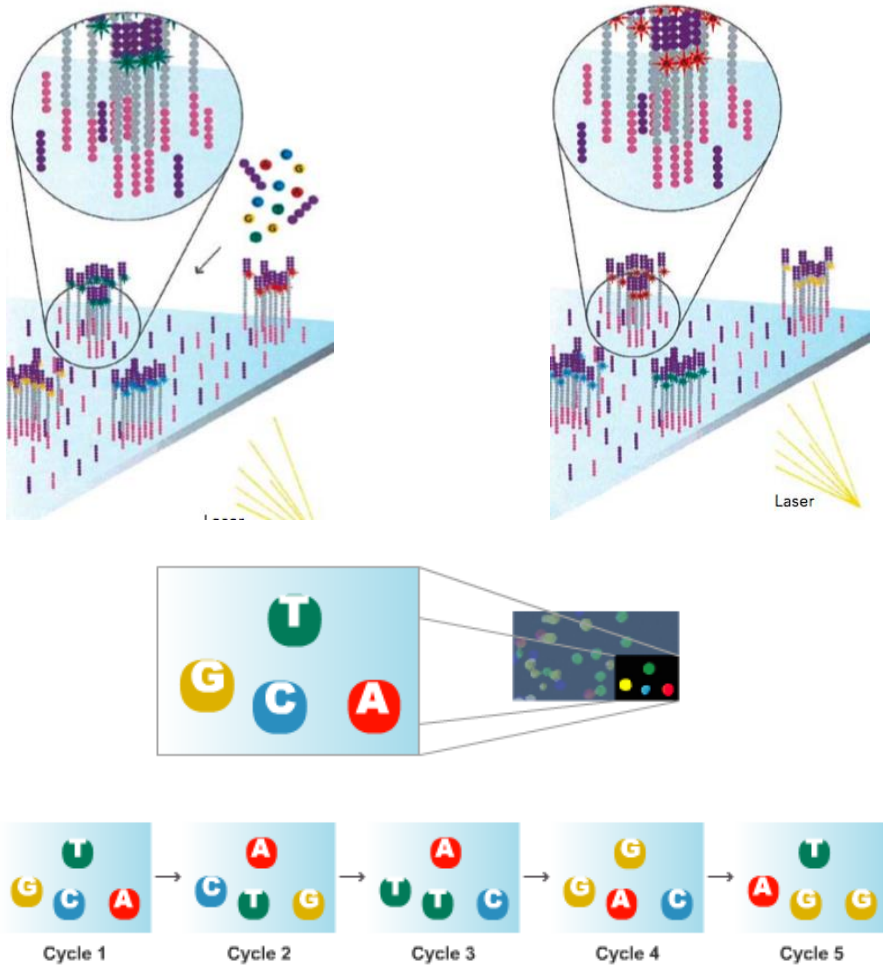
Reversible terminator sequencing

The four **reversible terminators** are added simultaneously.

Laser scanning of the flowcell surface.

Release of the blocking terminator.

Sequencing cycles are repeated one base at a time.

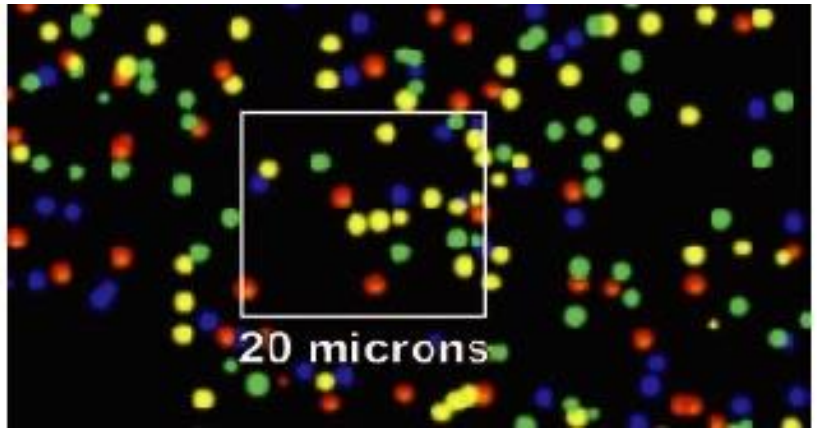


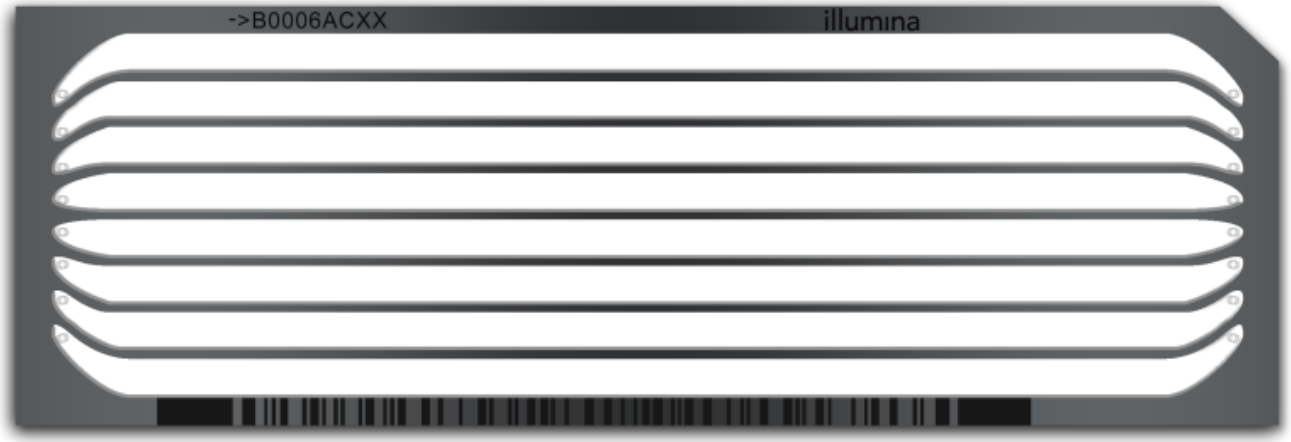
http://www.illumina.com/

Sequence analysis

Scanning at each position for all sequences (reads) in parallel.

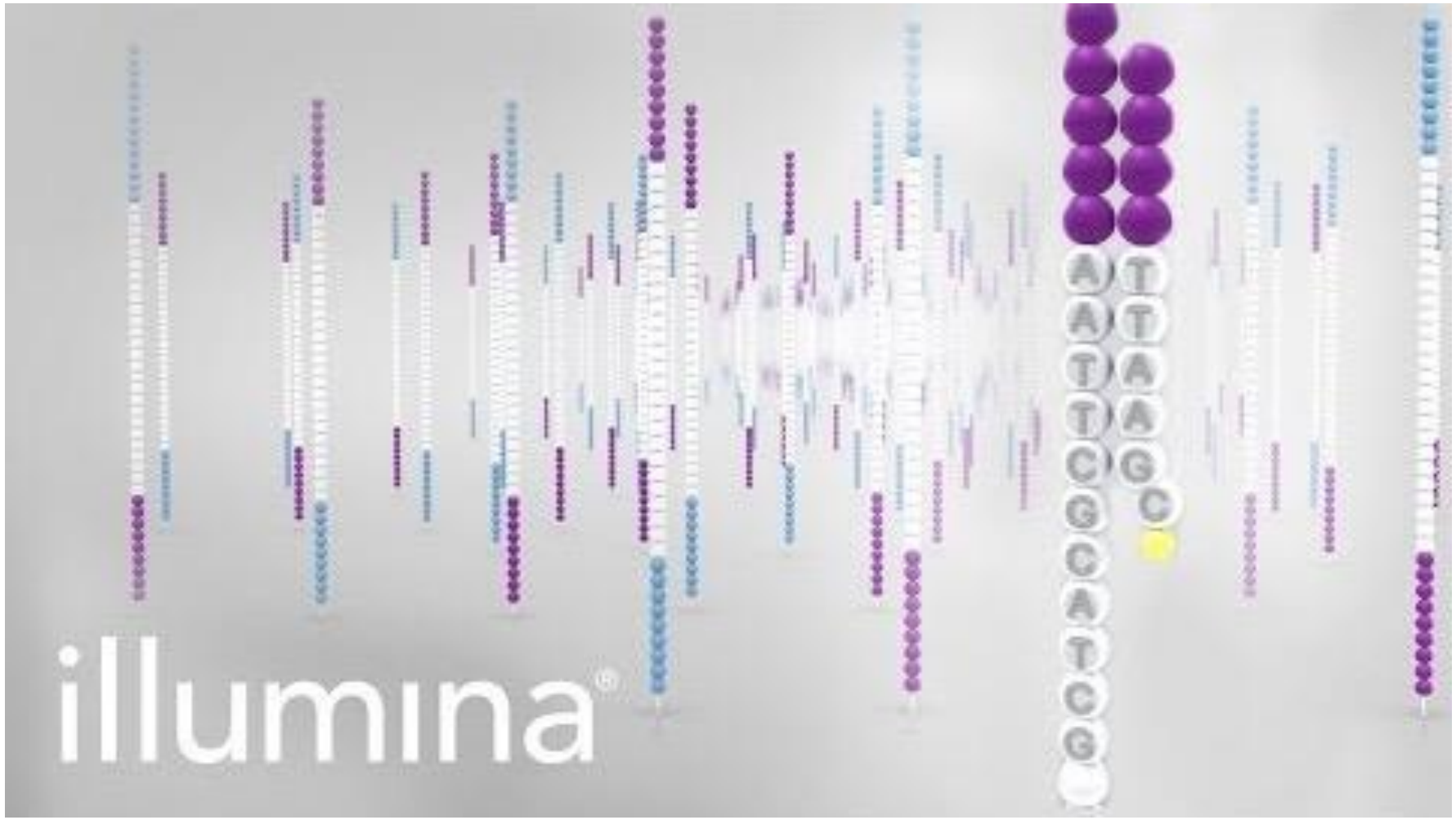
Most of the errors (99%) are sequencing errors (misincorporation).





http://www.illumina.com/

Illumina sequencing by synthesis



https://www.youtube.com/watch?v=fCd6B5HRaZ8

Specifications of the latest Illumina sequencers

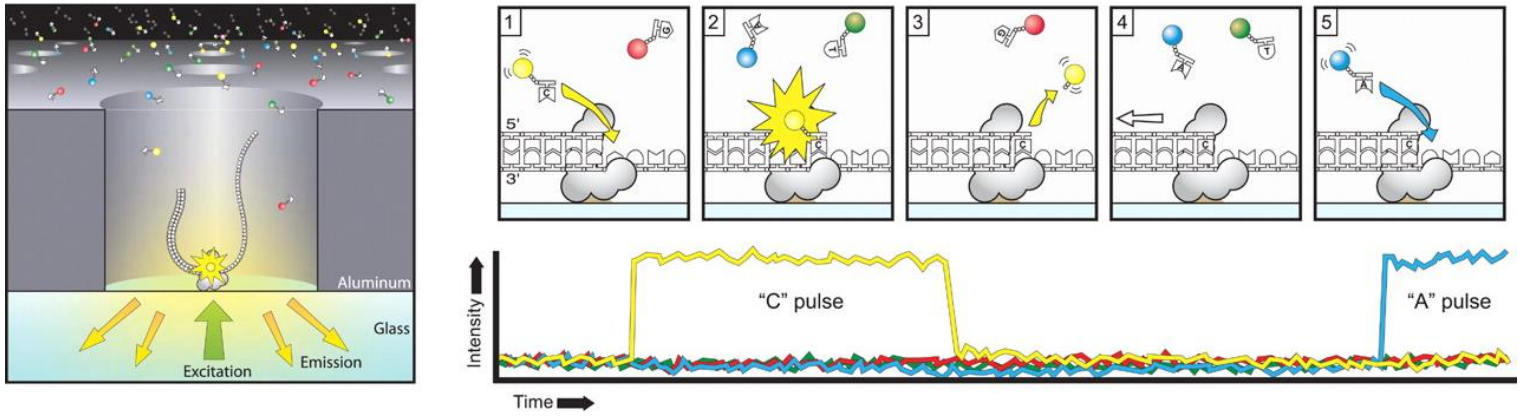
	iSeq 100	MiniSeq	MiSeq	NextSeq 550	NextSeq 2000	NovaSeq 6000	NovaSeq X
Run Time	19 hours	24 hours	56 hours	29 hours	44 hours	44 hours	48 hours
Read length (bp)	2x 150	2x 150	2x 300	2x 150	2x 300	2x 250	2x 150
Read number	8 10^6	50 10^6	50 10^6	800 10^6	3.6 10^9	20 10^9	52 10^9
Ouput	1.2 Gb	7.5 Gb	15 Gb	120 Gb	540 Gb	3,000 Gb	8,000 Gb
Throughput (Gb/day)	1.2	7.5	6	100	270	1,500	4,000

The third generation

Real time and single molecule sequencing

Real time sequencing on **single molecule** thanks to **RNA polymerase immobilisation** in wells.

Each **base incorporation is measure in real time** with a CCD camera under the bottom of the plate.



Eid (2009) *Science*

Revio specifications

25 10^6 reads / SMRT cell
4 SMRT Cells in parallel;

From 15 to 20 kb read length;

Throughput: 90 Gb/SMRT cell, **360 Gb/day**;

Run duration: **24 hours**.

Nanopore technology

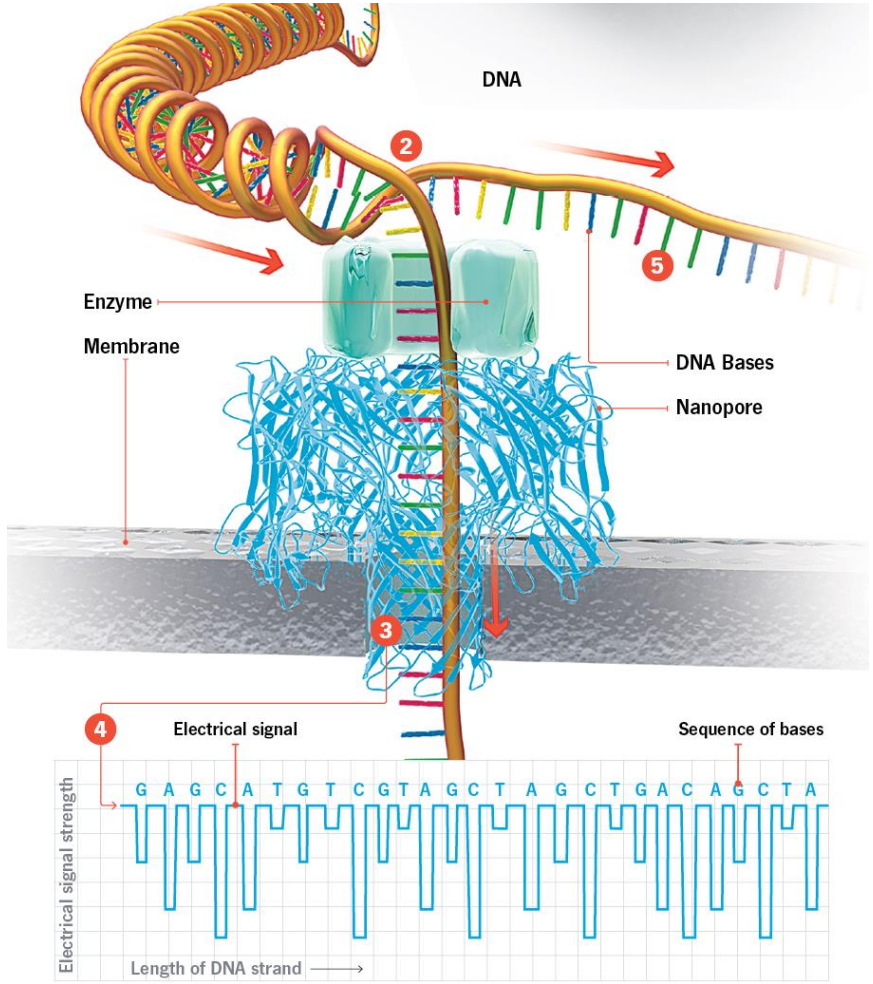
Single molecule detection system by passing single strand nucleic acids through a **nanometric pore**.

Base to base analysis in real time using **electric properties** of the nanopore.

DNA size sequencing of **kilobases.**

No limitation on the acid nucleic type to be detected (**DNA or RNA** even amino acids) including modifications (epigenetics).

No amplification.



Greenwood (2013) *Popular Science*

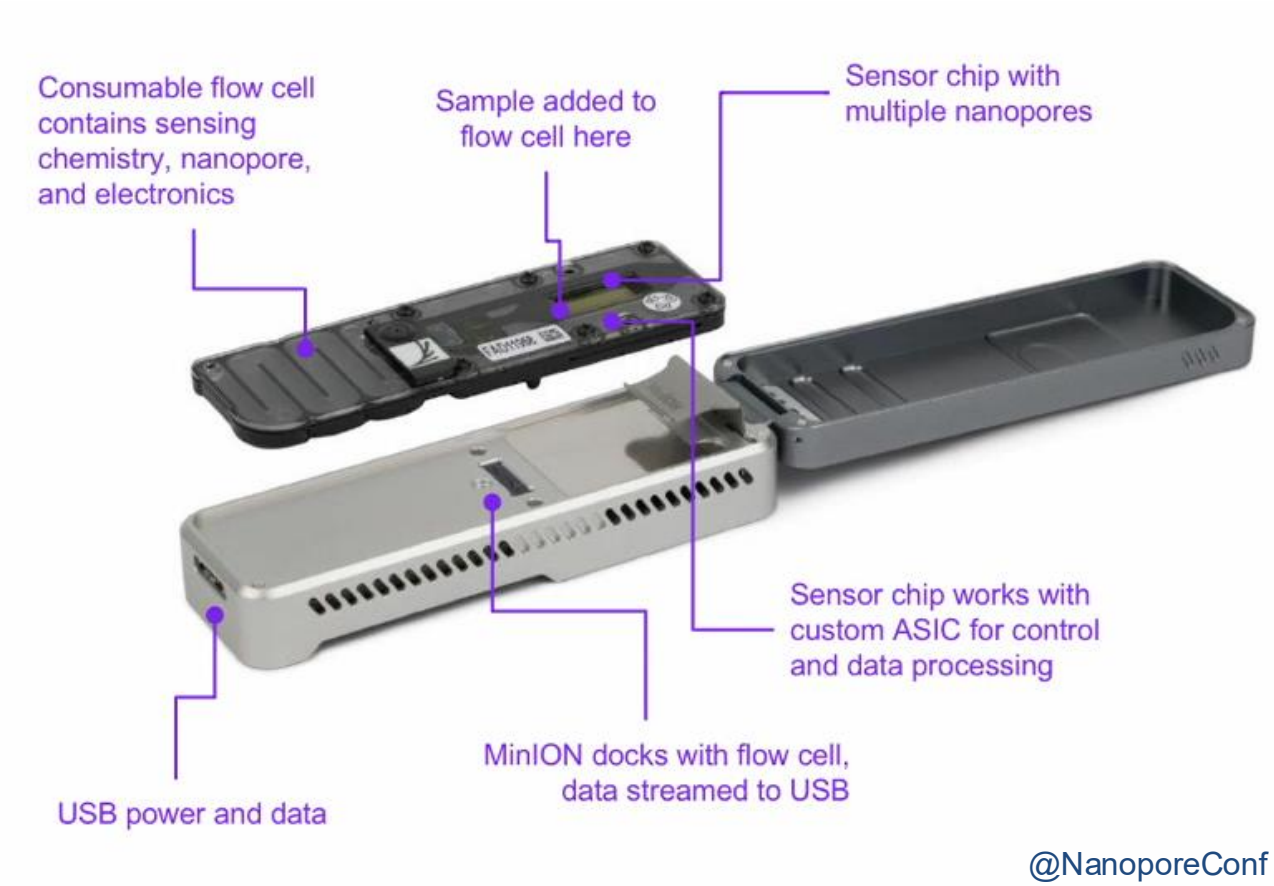
Oxford nanopore technologies

https://www.youtube.com/watch?v=RcP85JHLmnI

The MiniON flow cell

1 flow cell = 1 membrane
with 512 nanopores.

Single molecule sequencing
up to 4 Mb during up to 72 hours.



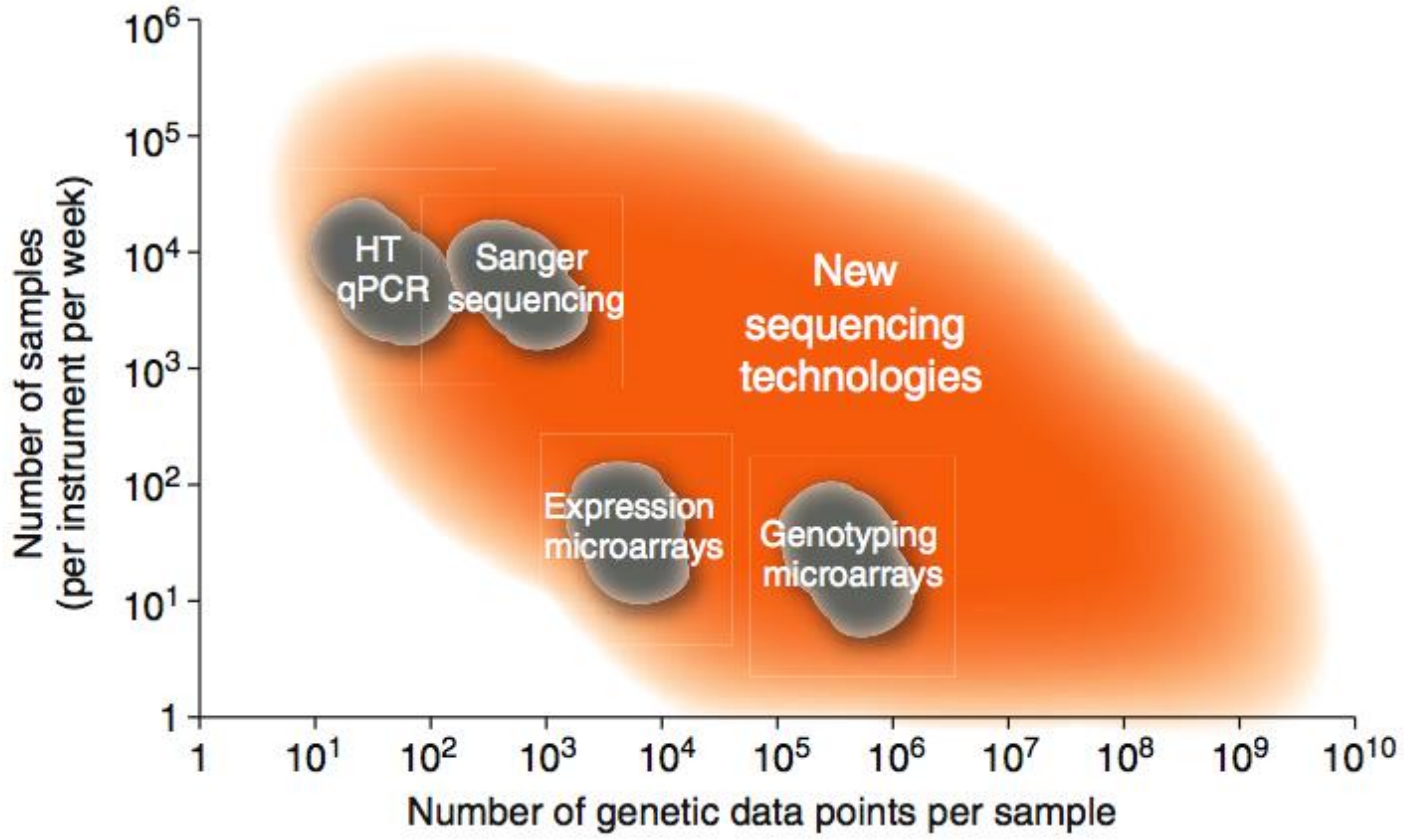
@NanoporeConf

Field genomics: portable sequencer tracks infectious disease outbreaks

Nick Loman using a MinION to sequence the Zika virus in Brazil

Applications

They cover a lot of previous existing techniques



Kahvejian et al. (2008) *Nat. Biotech.*

De novo sequencing

Quicker and cheaper sequencing than Sanger.

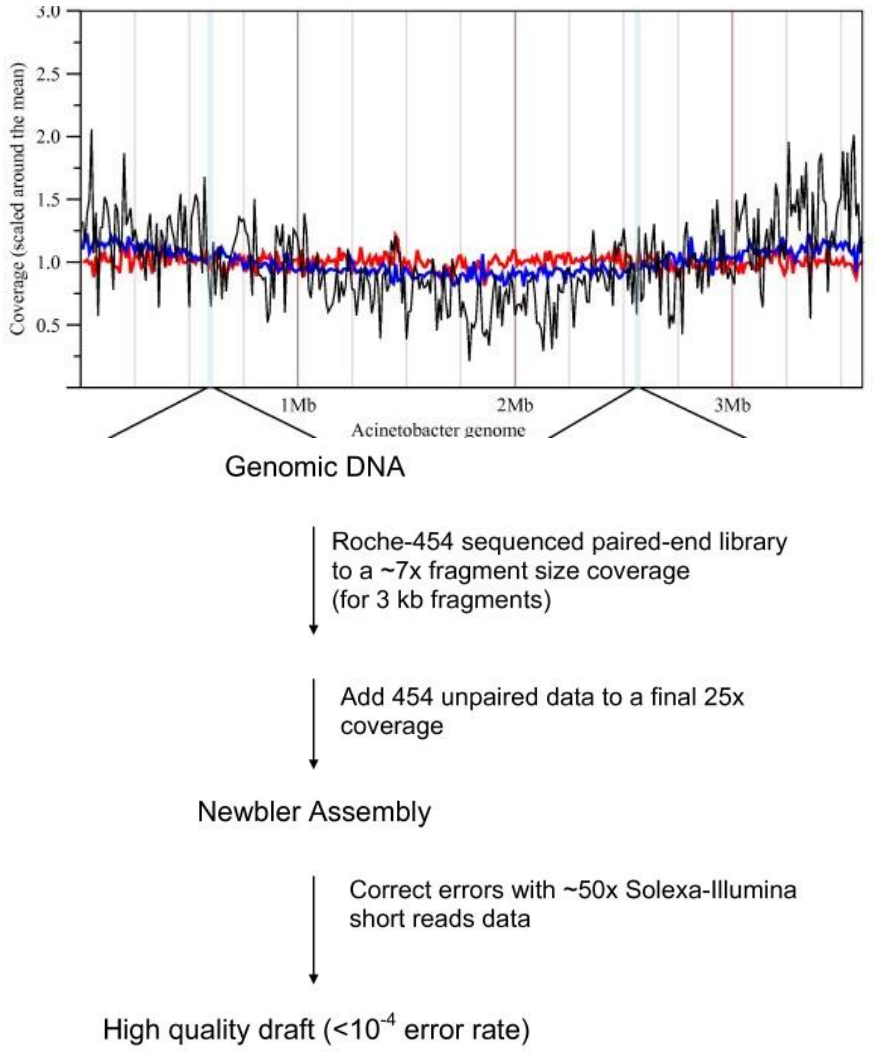
But **reads are smaller**.

Combination of different methods allow to obtain **better quality sequencing drafts**.

=> Combining 454 and Illumina.

Low error rate and **homogenous coverage** due to no cloning biases compared to the Sanger method.

Errors are not same with the two high throughput sequencing methods.



Aury et al. (2008) *BMC Genomics*

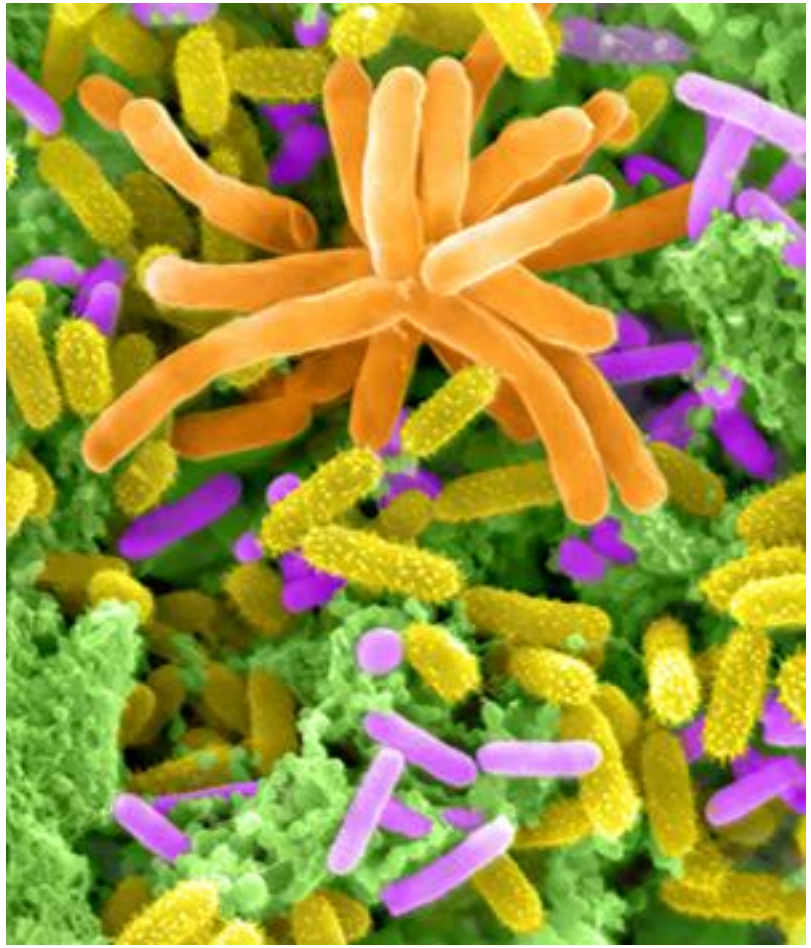
Resequencing applications

Goal: to analyze various genomes compared to a reference one.

Search for polymorphisms and structural variants in populations, mutation identification in biotechnology, organism evolution analyses, cell differentiation along time, ancient DNA discovery…

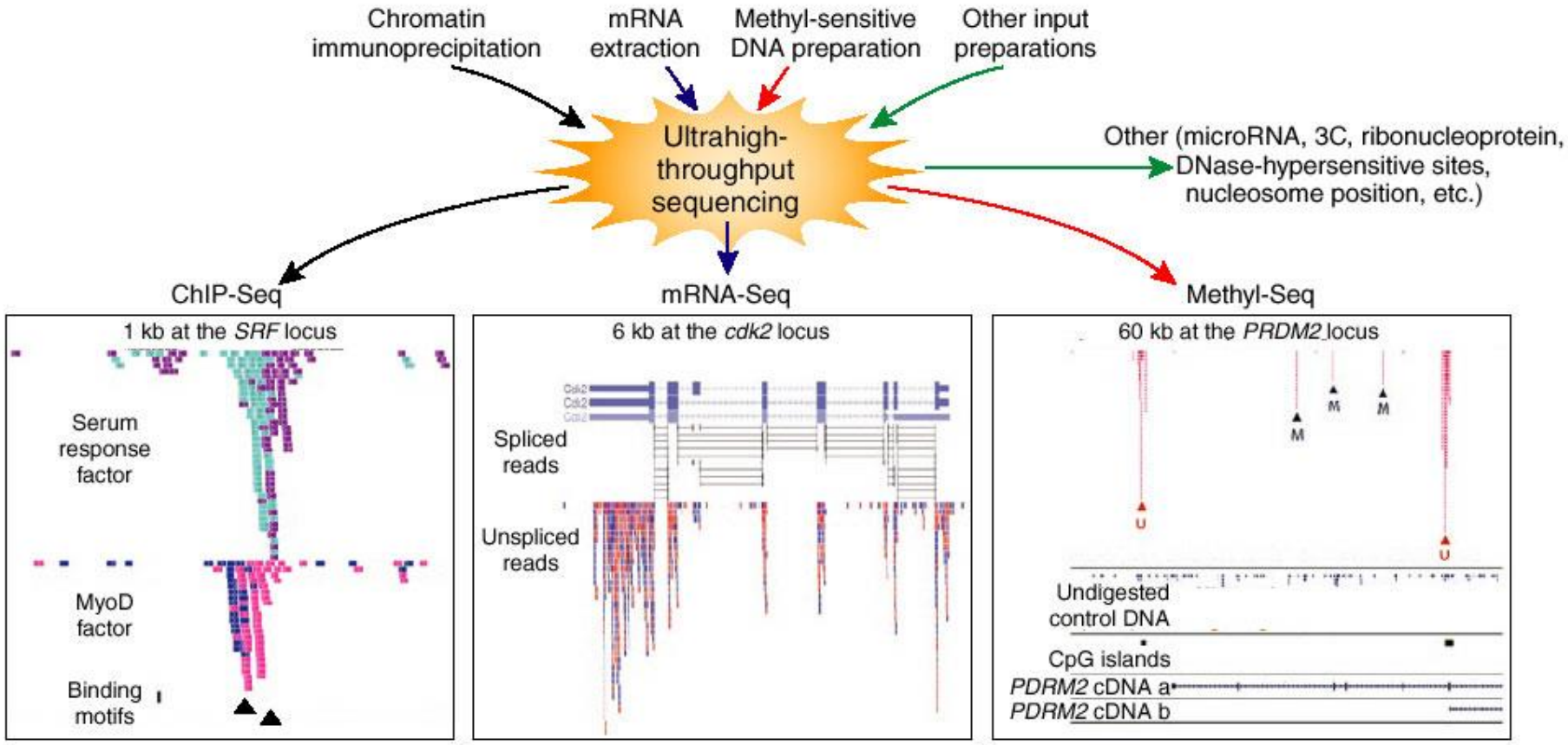
Metagenomics: genome characterisation in samples.

A wide range of applications: characterise pathogen micro-organisms in patient tissues, definition of the species found in environment samples, understand species evolution…



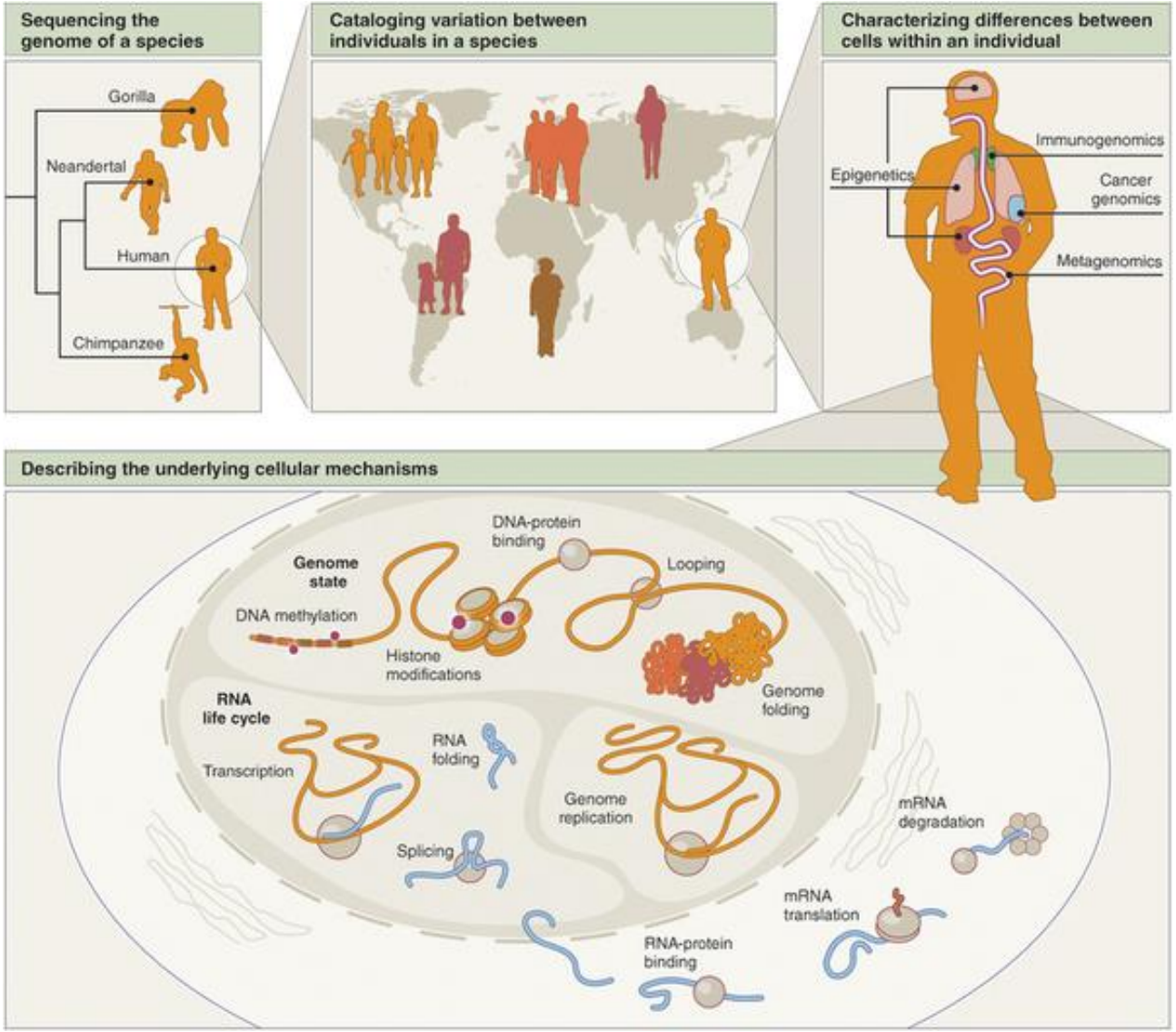
From JGI DOE

Functional genomic applications



Wold et al. (2008) *Nat. Methods*

Evolution

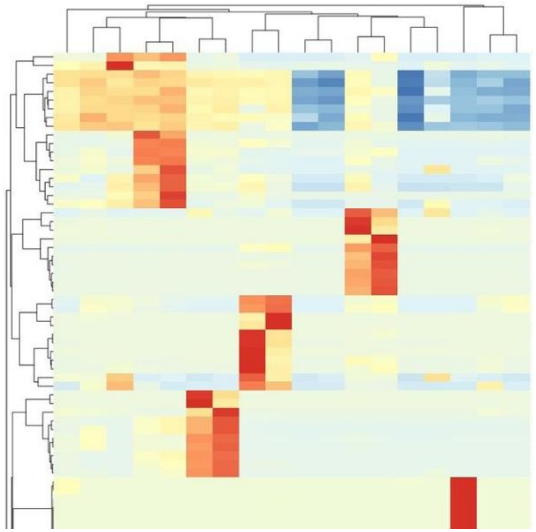


Shendure & Aiden (2012) *Nat. Biotech.*

From bulk to localised single-cell

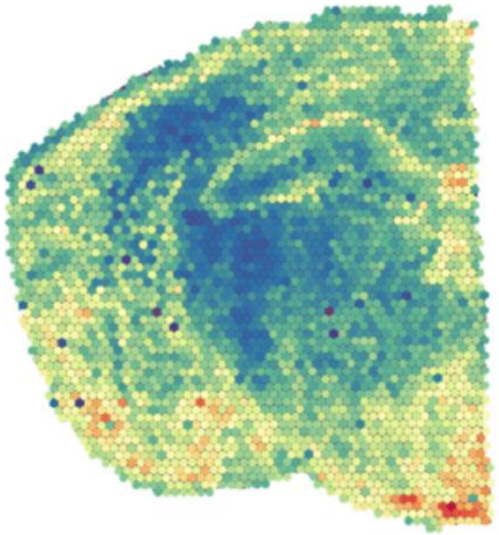
2012 2019

Bulk	Single cell	Spatial
High throughput sequencing Method of the year 2009	Single cell genomics Method of the year 2013	Spatially-resolved transcriptomics Method of the year 2020



Martins et al. (2020)

Han Chen D. (2019)



Jorgensens M. (2021)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