SEPTEMBER
2024

# High throughput sequencing

Functional genomic data analysis: transcriptomics

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# First generation sequencing methods

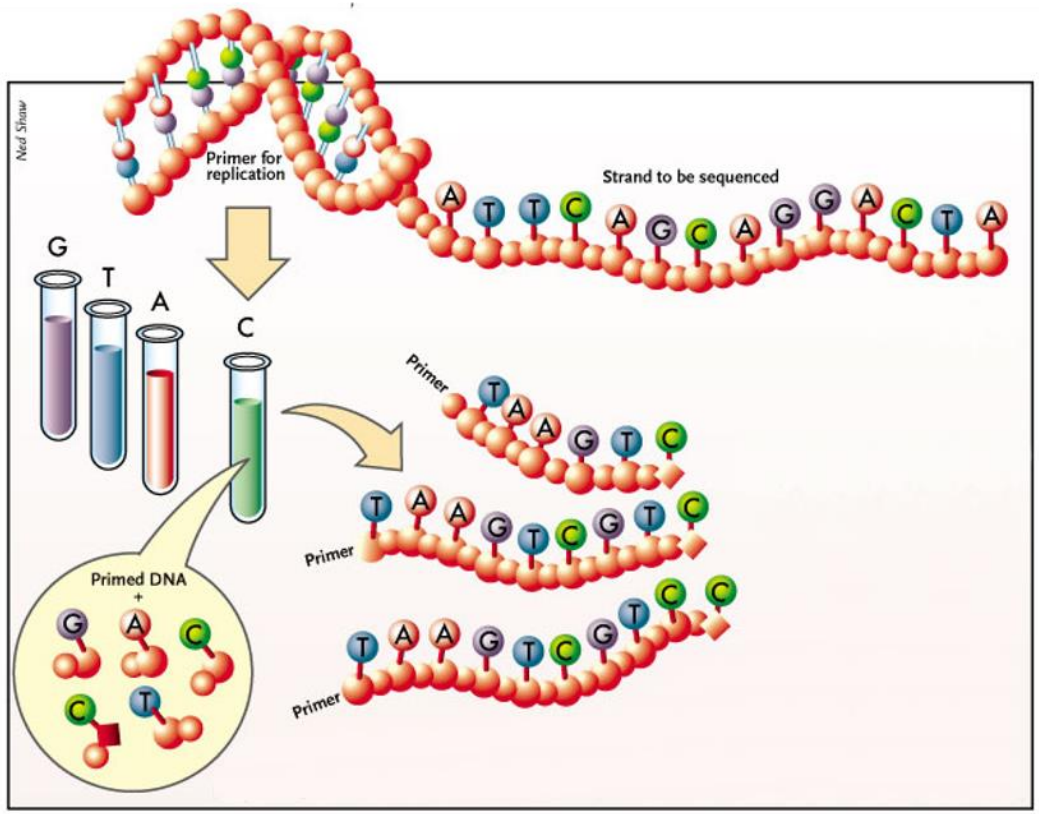
# Sanger sequencing by synthesis

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

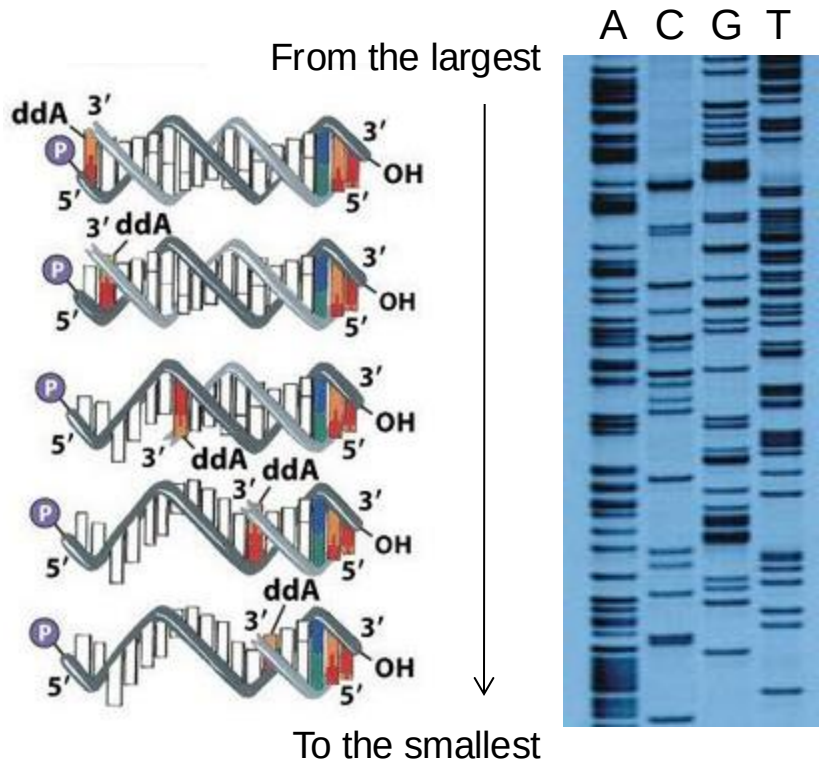
We a get a **mix of DNA fragments** terminating at each position of the sequence.

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

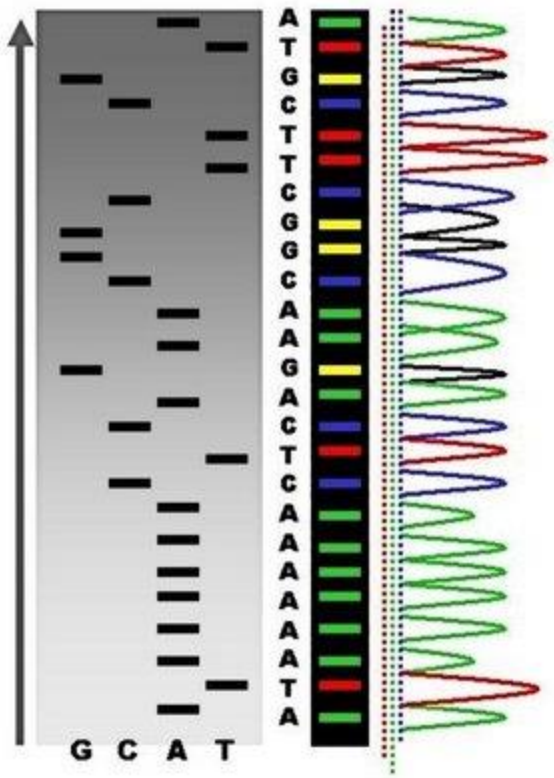
Detection of synthesize fragments is done by the **incorporation of labelling beacon** in the DNA.

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

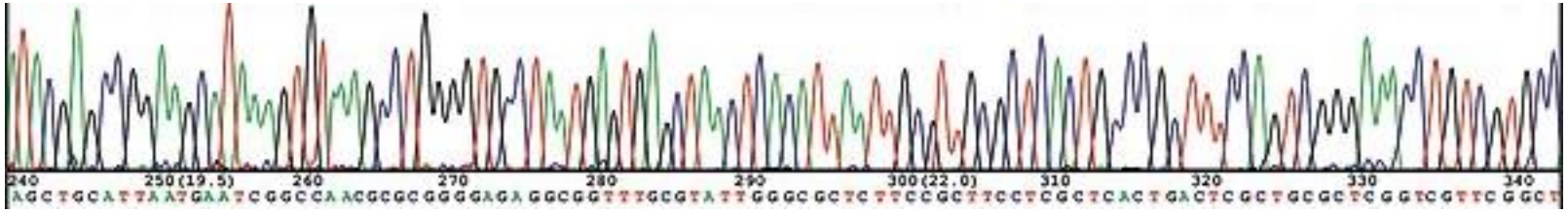



First version in the 90's thanks to the **modification of the radioactive label by a fluorescent one**.

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**.

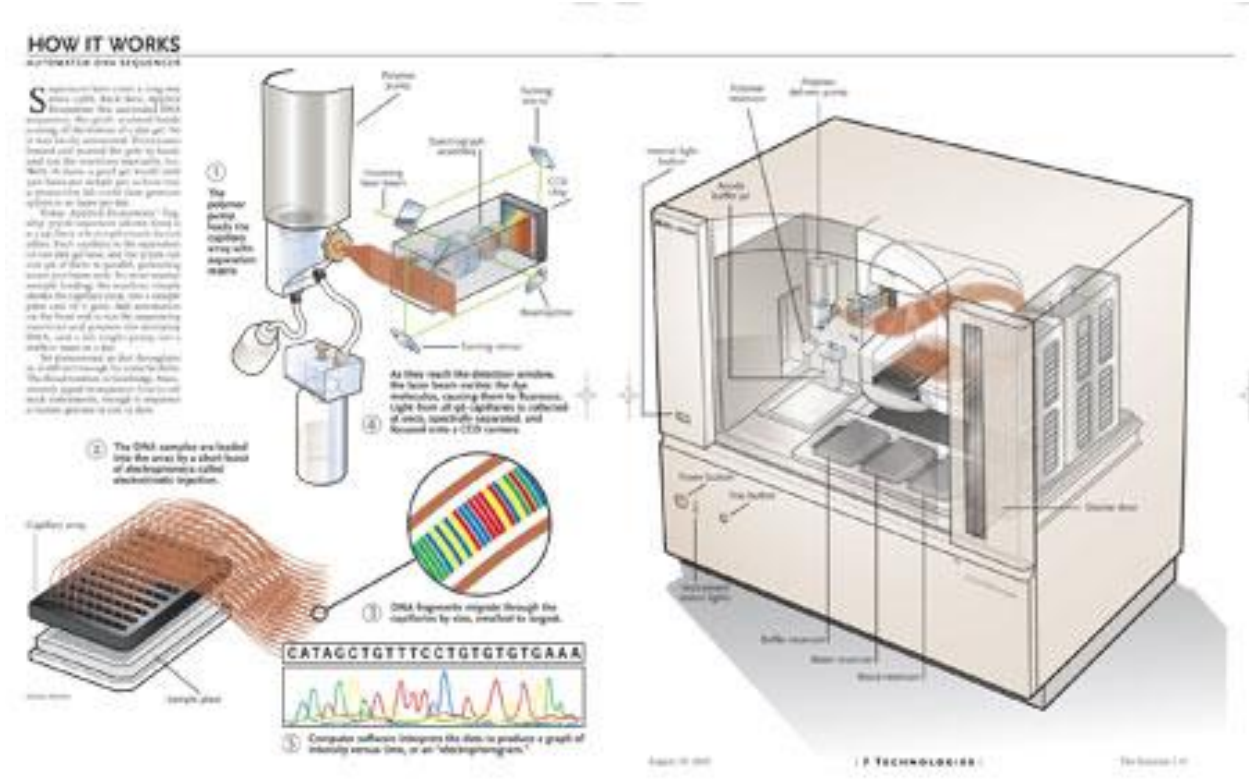
**300 kb of DNA** by run during **3 hours**. Several hundred sample at a time.



# 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

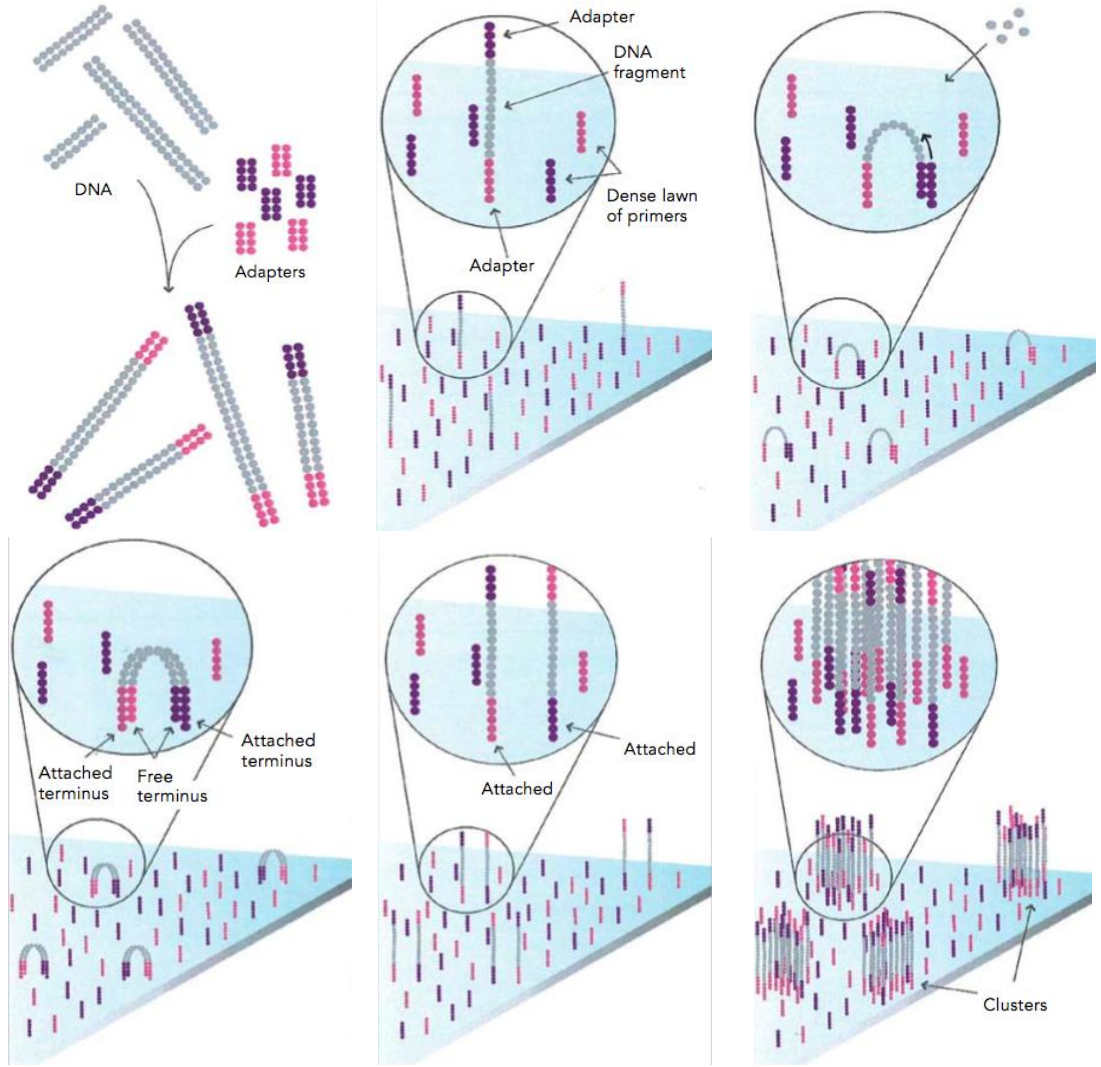
**Random DNA fragmentation** and size selection.

Ligation of adaptors.

DNA denaturation.

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

**Solid phase bridge PCR**.



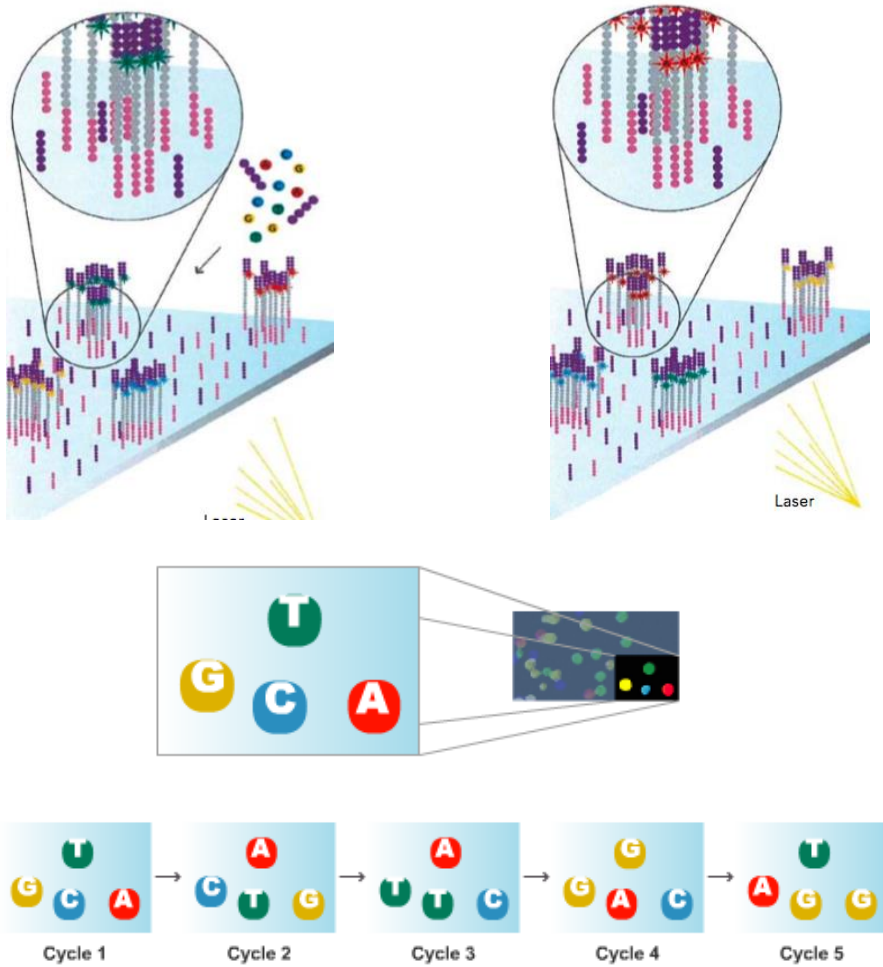
# Reversible terminator sequencing

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.

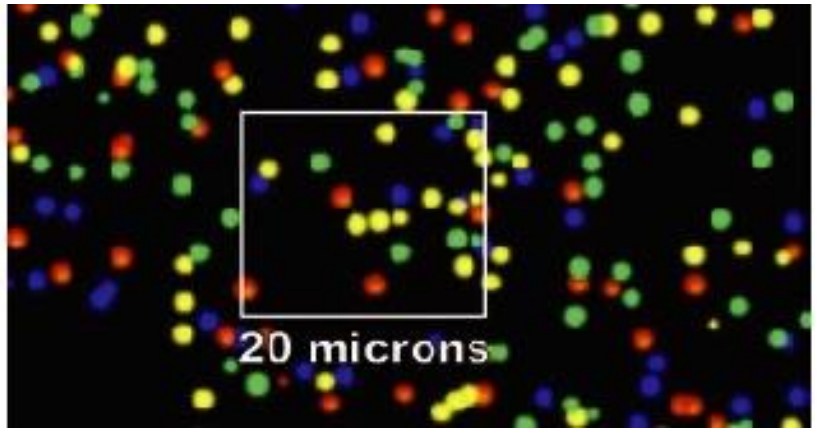


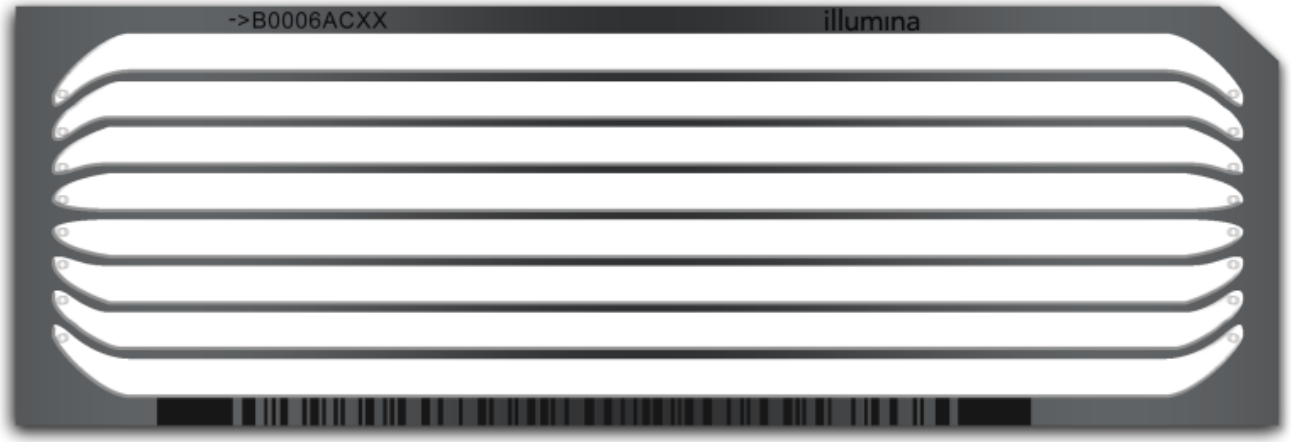

http://www.illumina.com/

# Sequence analysis

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

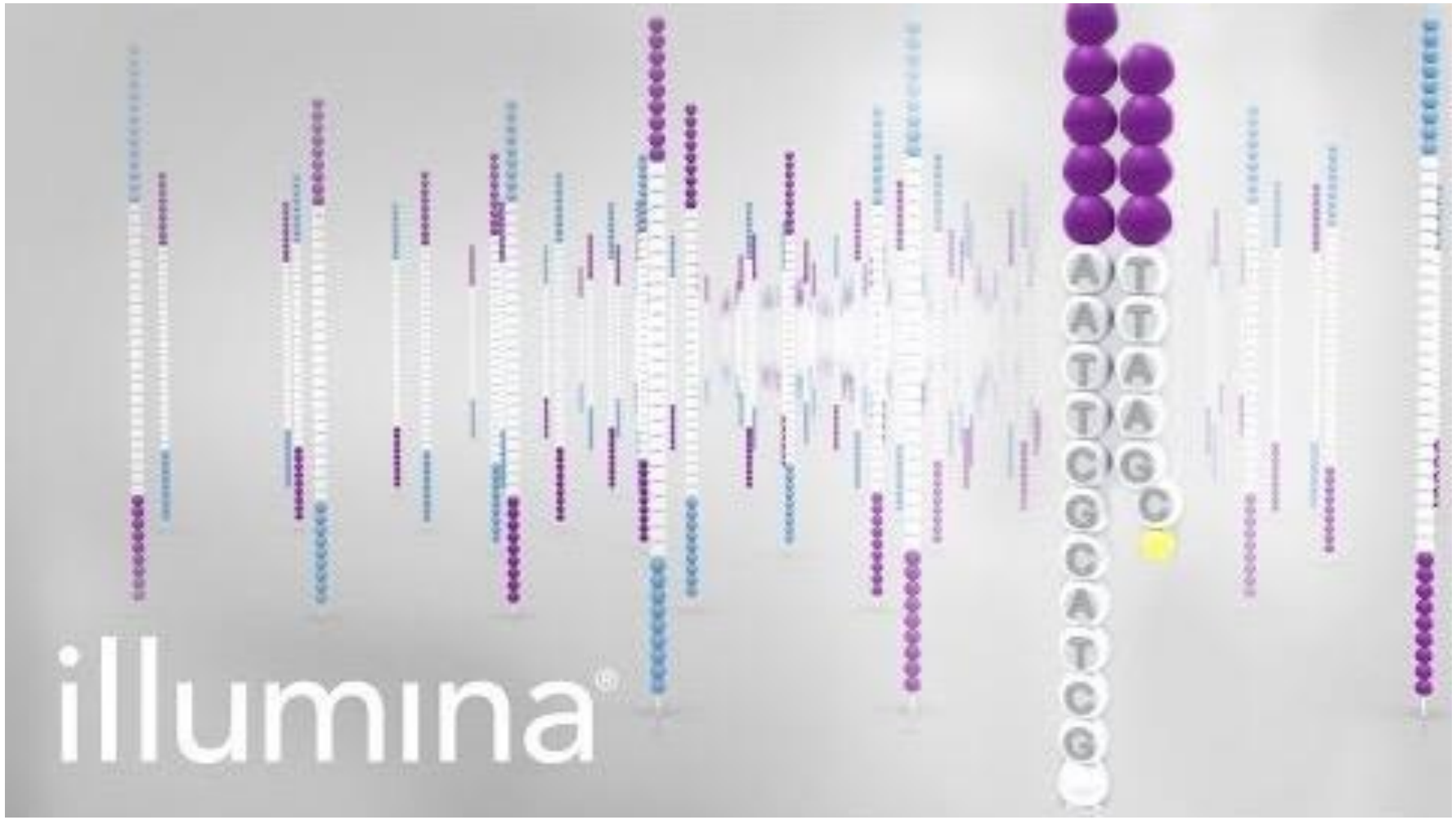
Most of the errors (99%) are sequencing errors (misincorporation).




http://www.illumina.com/

# Illumina sequencing by synthesis




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

# Specifications of the latest Illumina sequencers





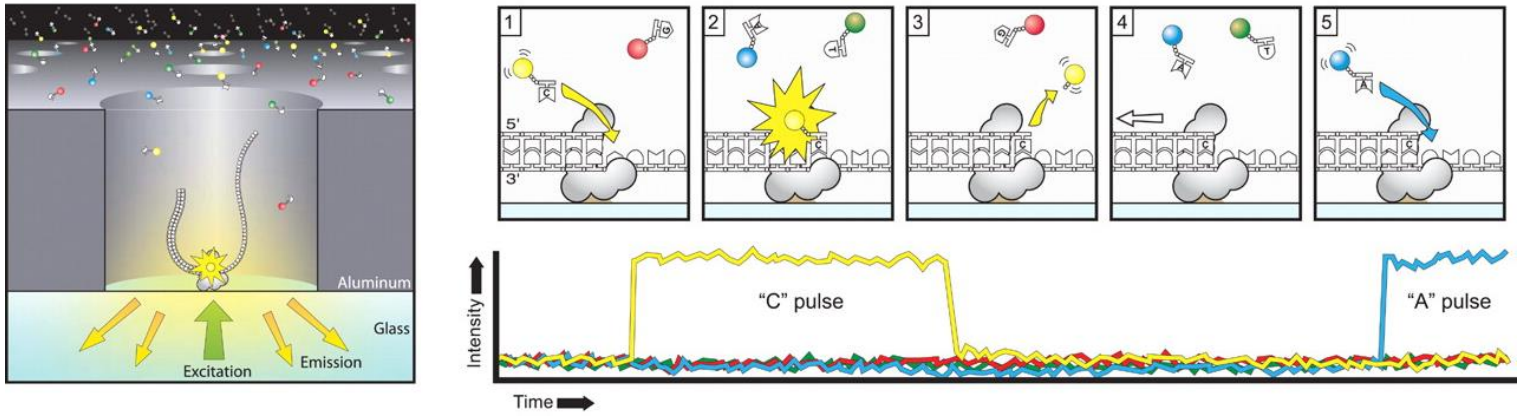

| | MiniSeq | MiSeq | NextSeq 550 | NextSeq 2000 | NovaSeq 6000 | NovaSeq X |
|---|---|---|---|---|---|---|
| Run Time | 24 hours | 56 hours | 29 hours | 48 hours | 44 hours | 48 hours |
| Read length (bp) | 2x 150 | 2x 300 | 2x 150 | 2x 150 | 2x 150 | 2x 150 |
| Read number | 50 $10^6$ | 50 $10^6$ | 800 $10^6$ | 2.4 $10^9$ | 20 $10^9$ | 52 $10^9$ |
| Ouput | 7.5 Gb | 15 Gb | 120 Gb | 360 Gb | 3.000 Gb | 8.000 Gb |
| Throughput | 7 Gb/day | 7 Gb/day | 100 Gb/day | 150 Gb/day | 1.500 Gb/day | 4.500 Gb/day |

# The third generation

# Real time 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

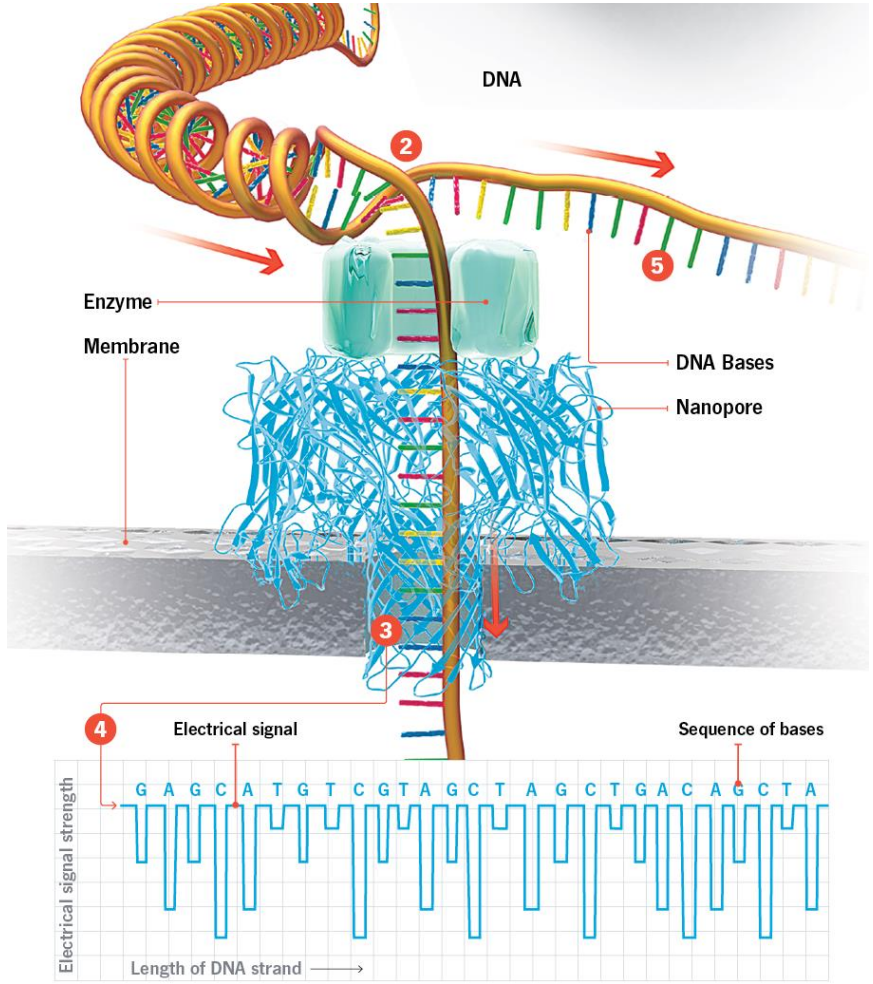
**Single molecule detection** system by passing single strand nucleic acids through a **nanometric pore**.

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*

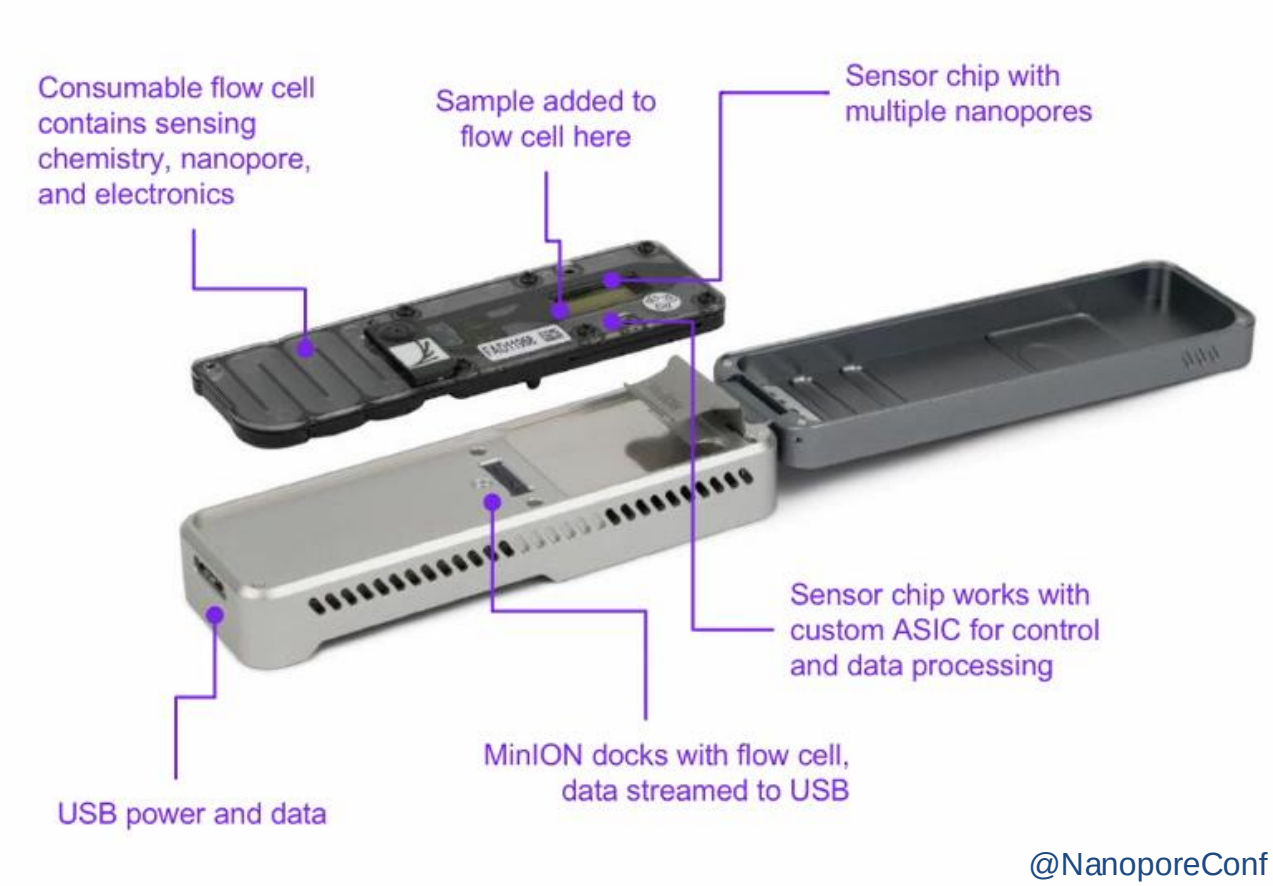
# Oxford nanopore technologies

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.



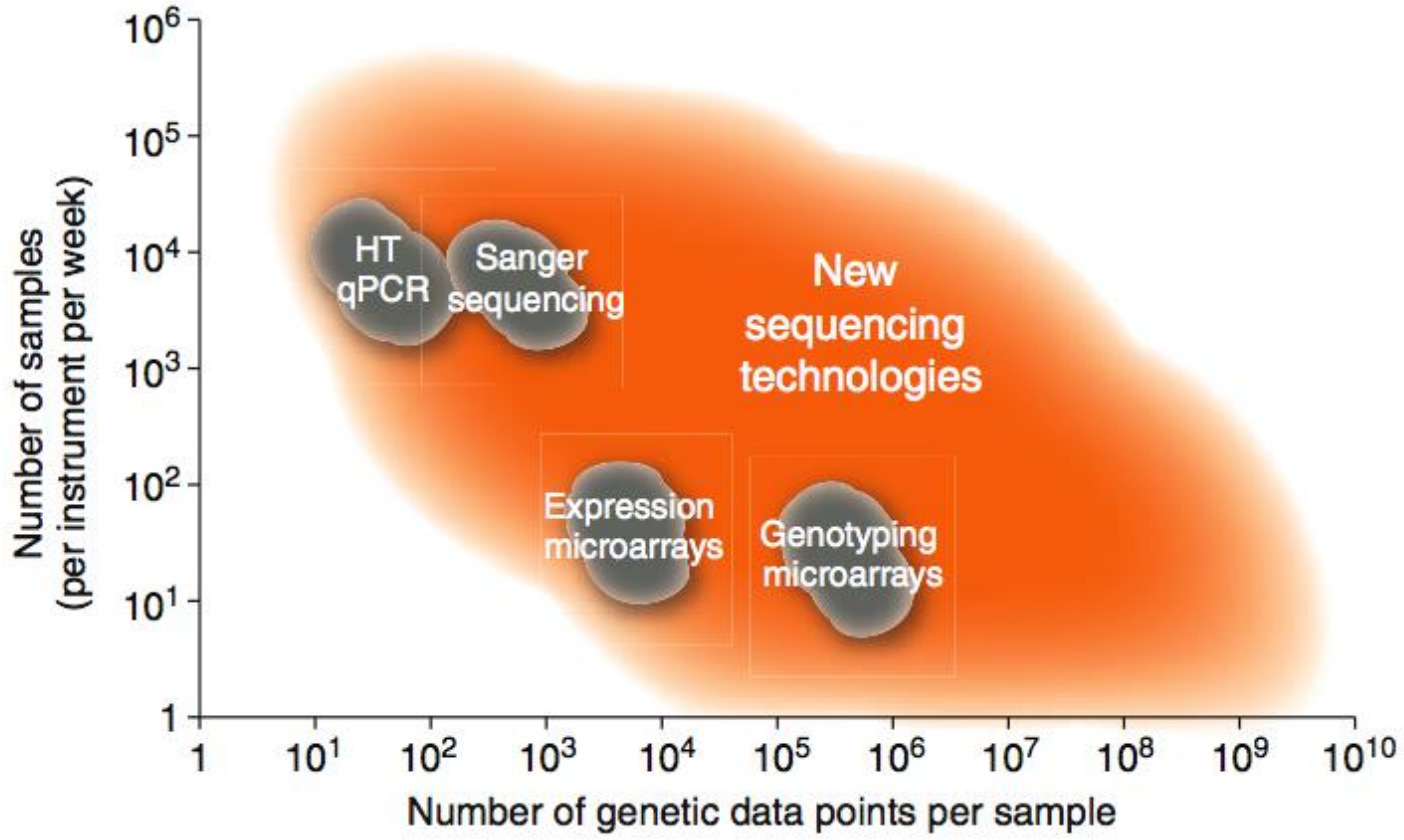

@NanoporeConf

# 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.

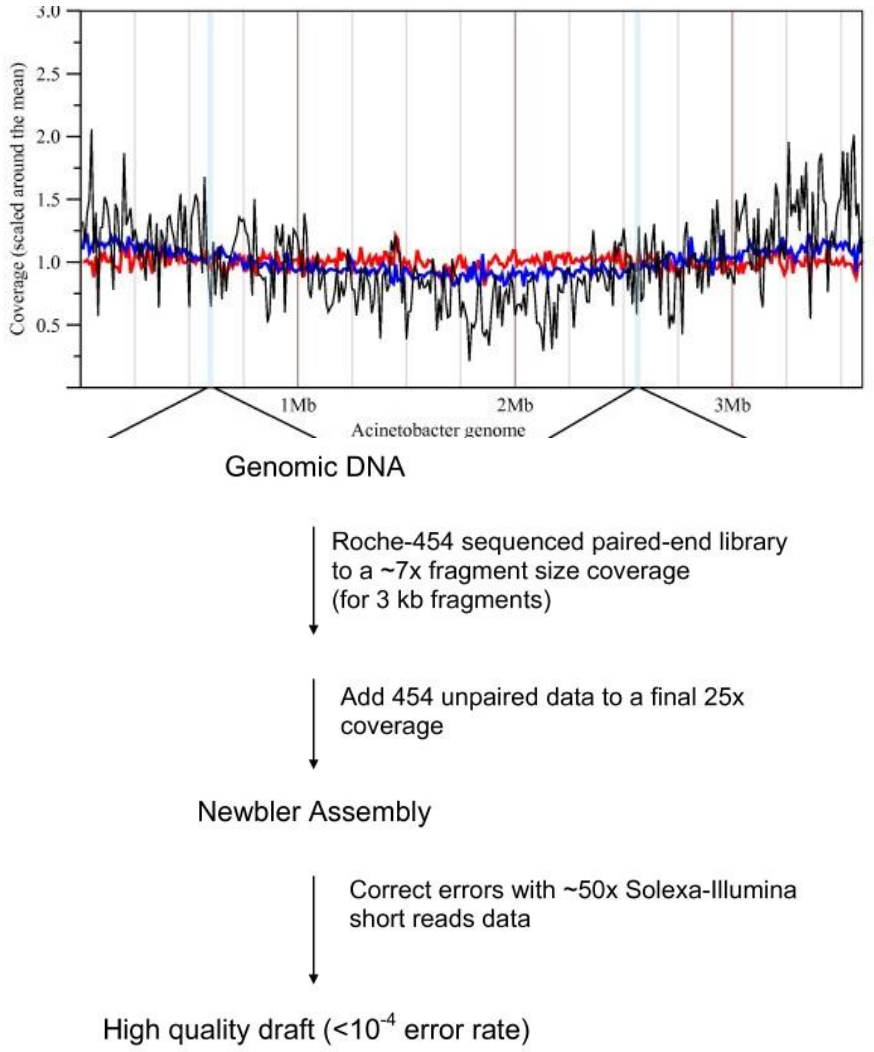
But **reads are smaller**.

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*

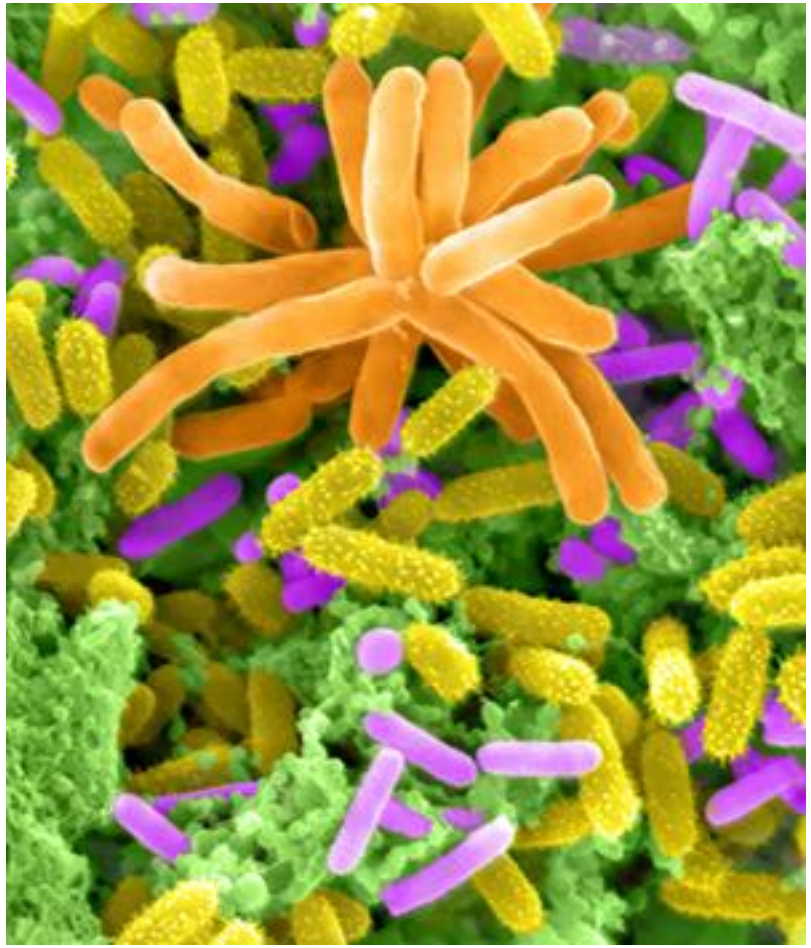
# Resequencing applications

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…

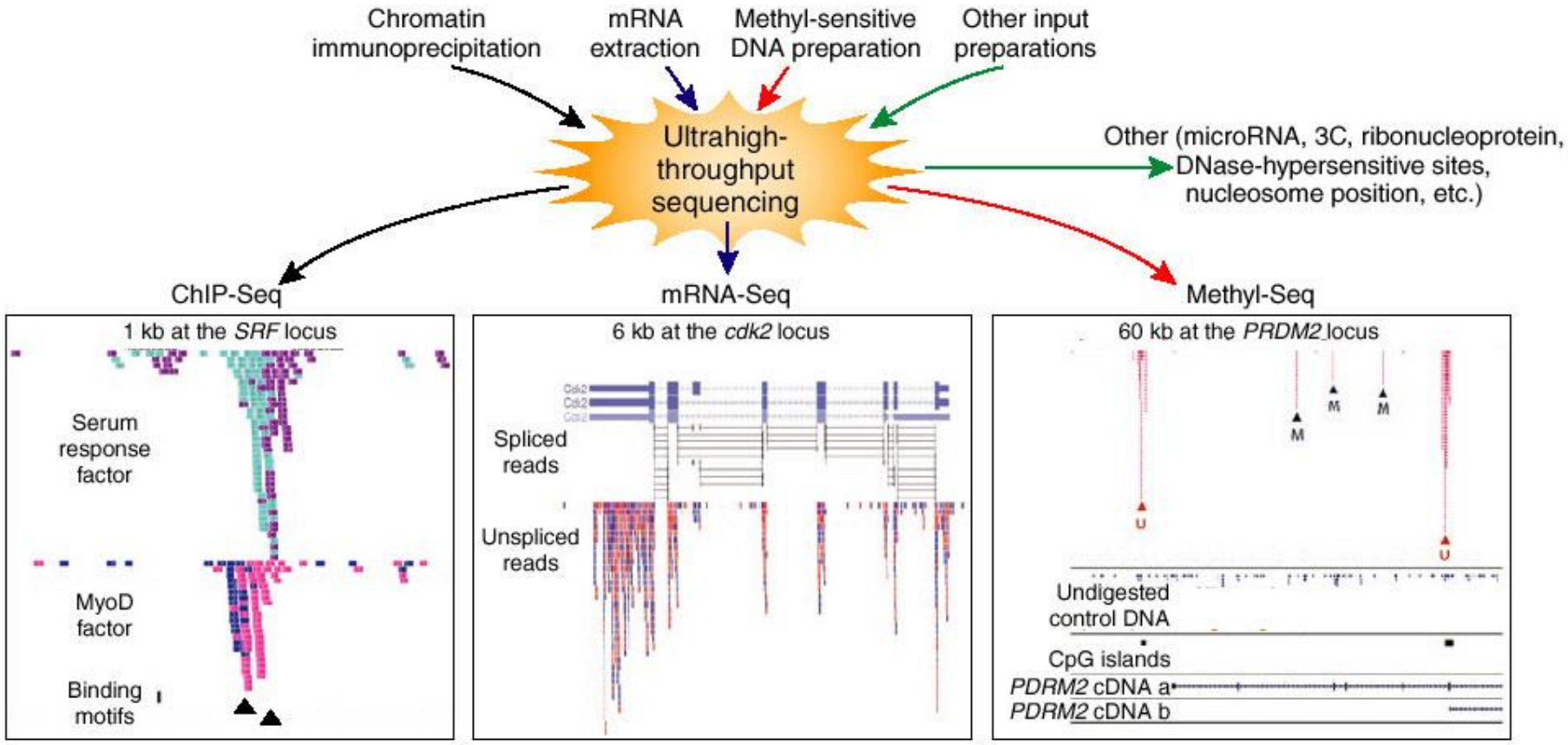
**Metagenomics**: genome characterisation in samples.

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



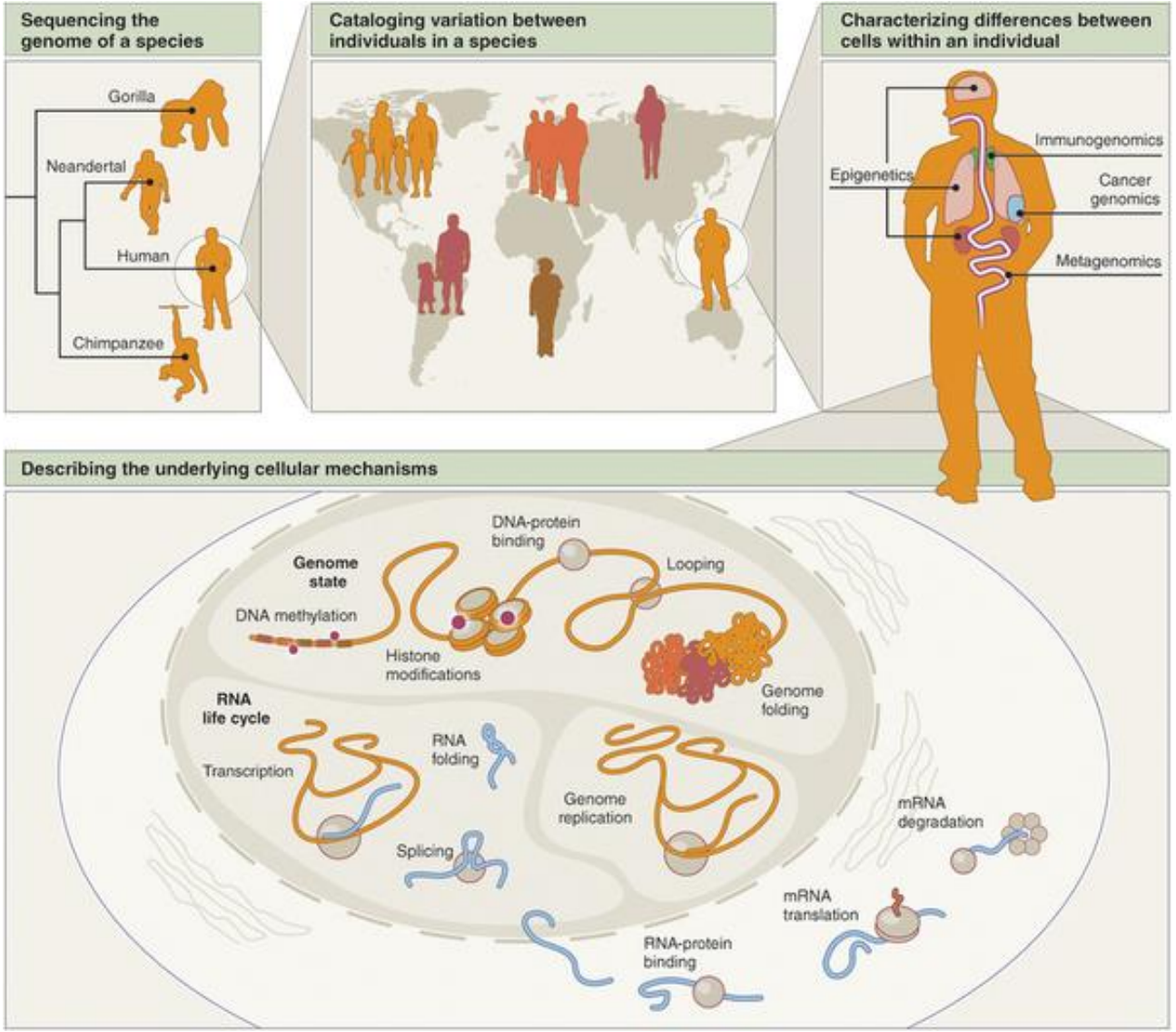
From JGI DOE

# Functional genomic applications




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

# Perspectives

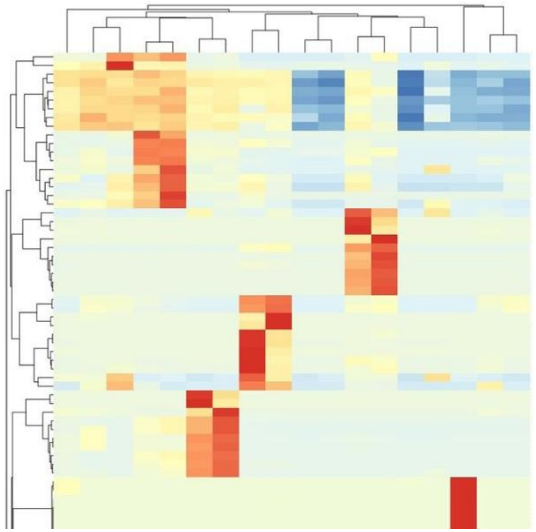



Shendure & Aiden (2012) *Nat. Biotech*.

# From bulk to localised single-cell

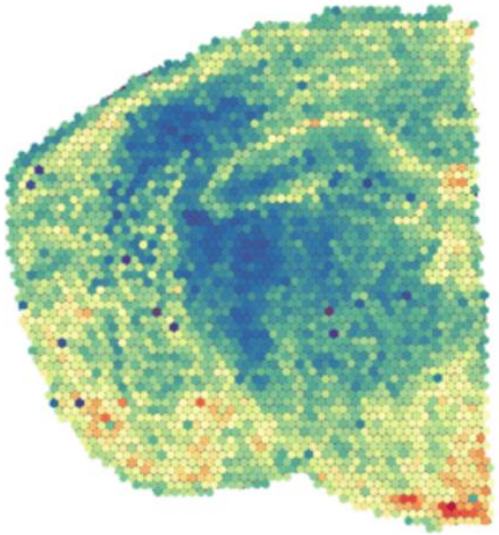
2012 2019

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



Martins et al. (2020)

Han Chen D. (2019)



Jorgensens M. (2021)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