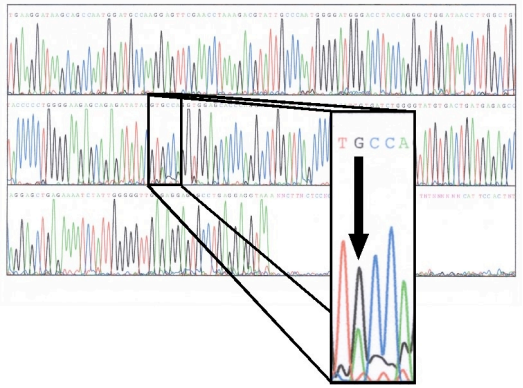
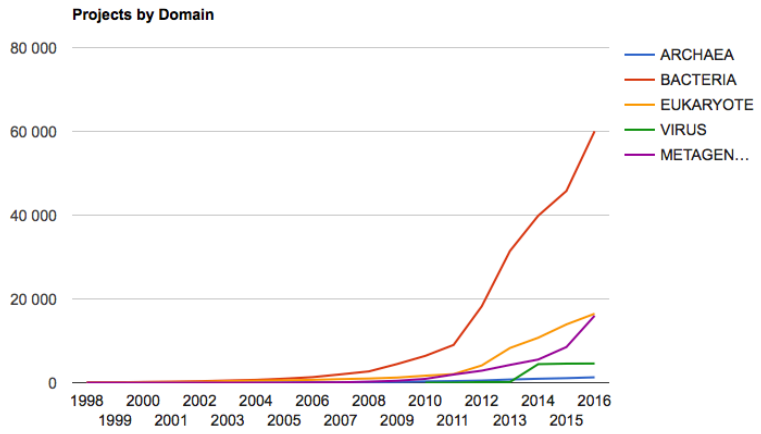
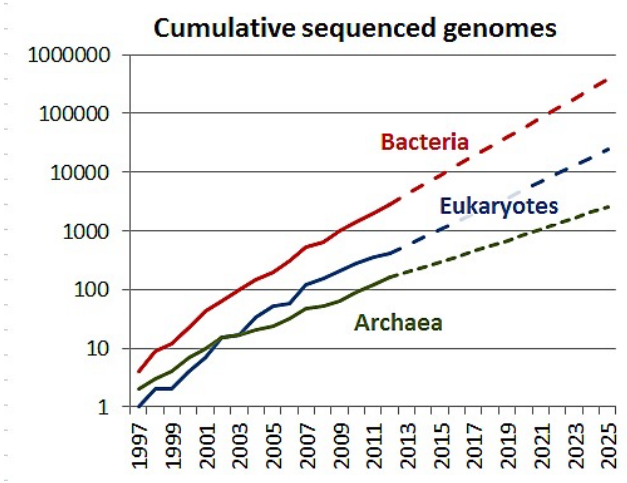
# GENOME SEQUENCING

# Introduction

Sequencing the genomes

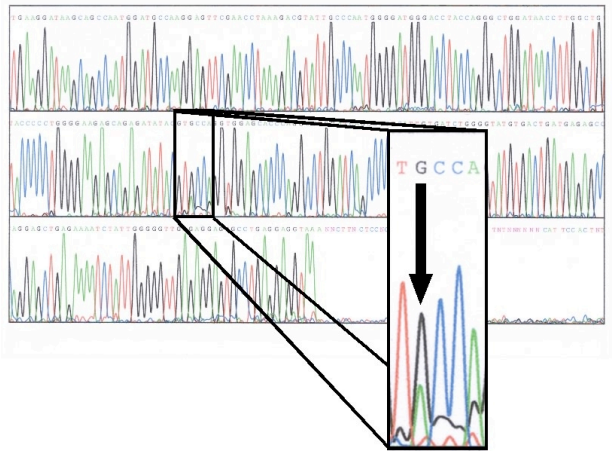


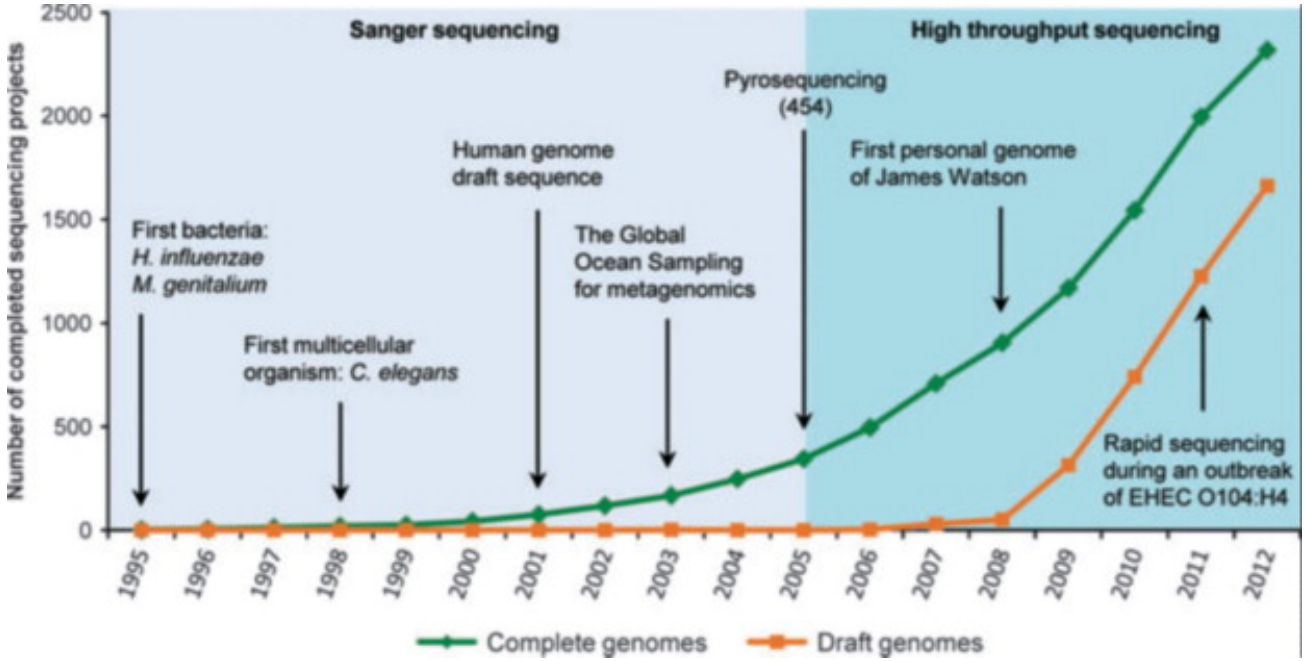



# Introduction

Sequencing the genomes

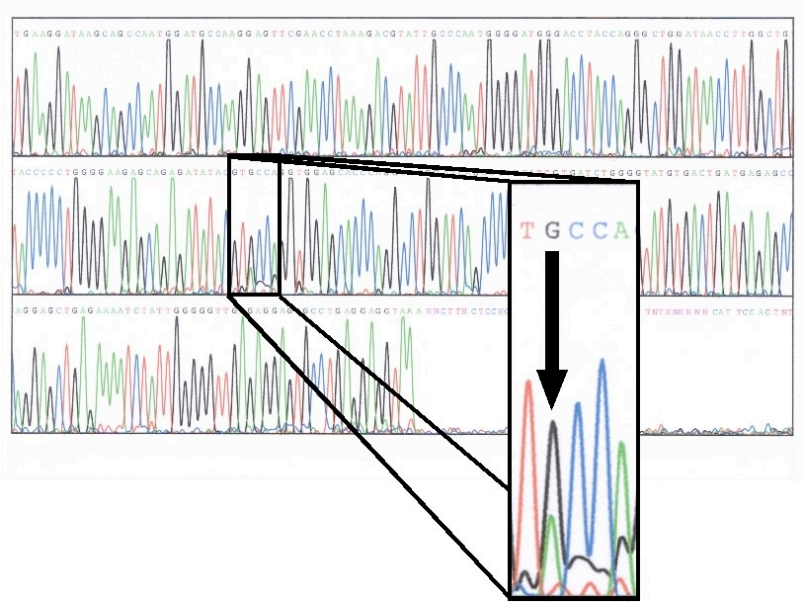



http://www.yourgenome.org/facts/timeline-organisms-that-have-had-their-genomes-sequenced

# Introduction

Sequencing the genomes




**Why sequencing these genomes?**

Complete inventory of the genes of various organisms : # of genes, % of coding genome

Information about the sequence of every polypeptides

Basis for new technologies and medical applications

Global data at the level of the whole cell/tissue (tumor heterogeneity)

New data for evolutionnary and species classification analysis

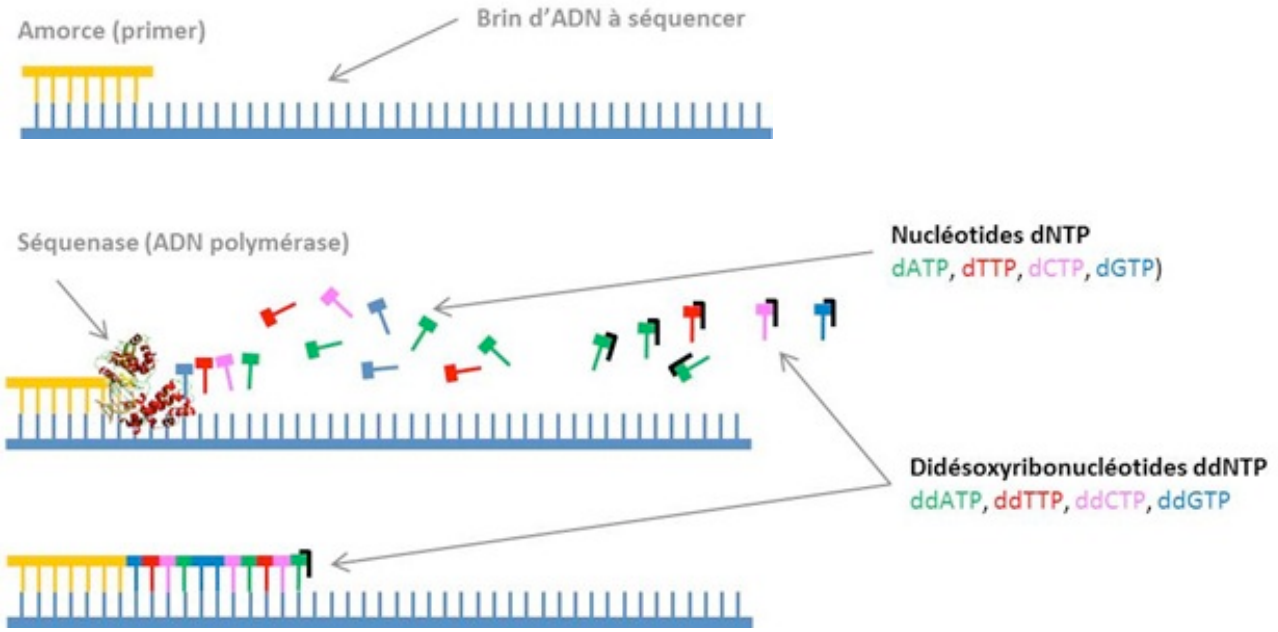
# I. Genome sequencing

## The Sanger Method (1977)

Denaturation
+priming

Polymerization

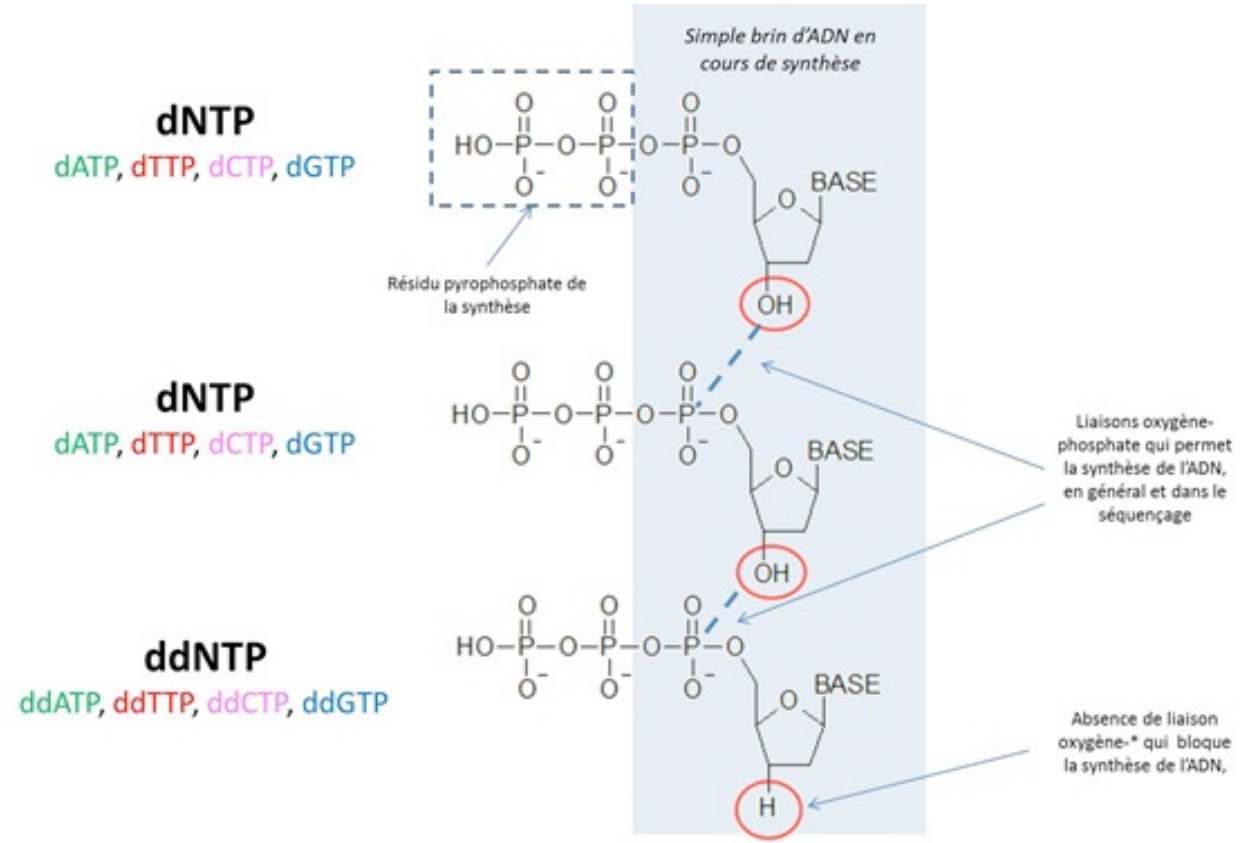
Arrest



# I. Genome sequencing

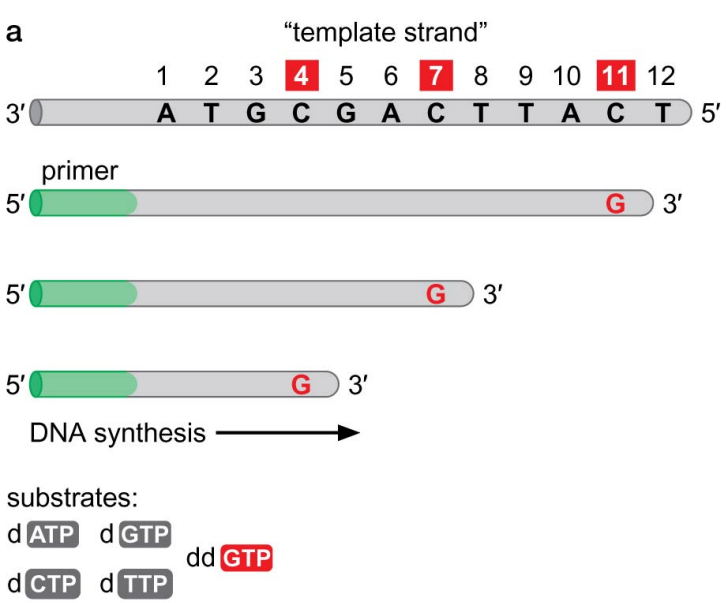
## The Sanger Method (1977)

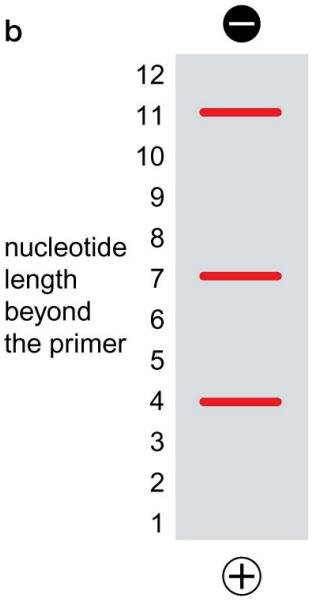
End of polymerization

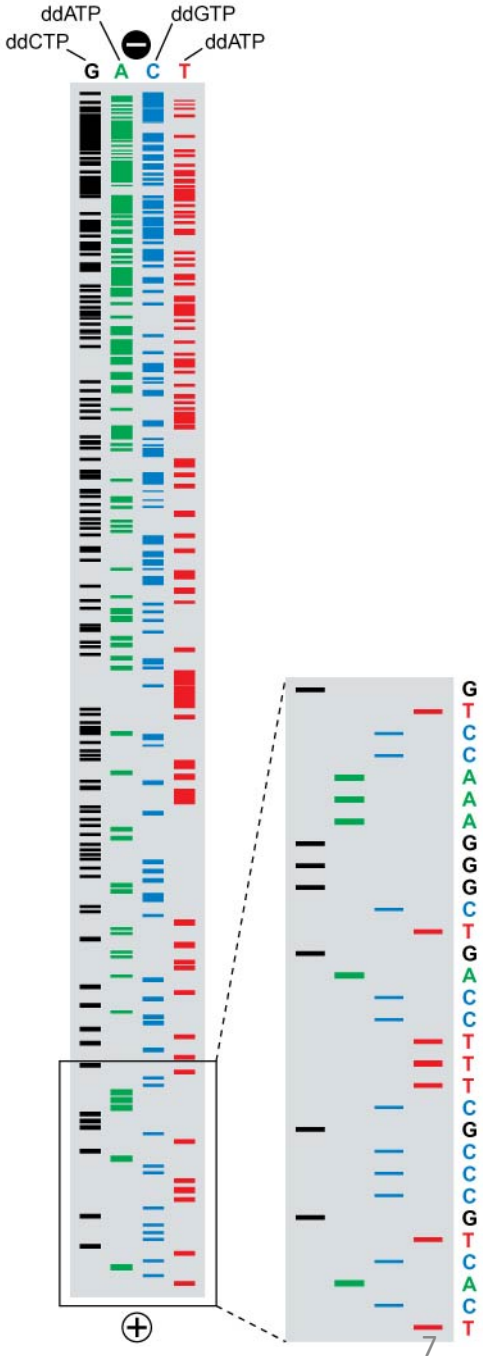


# I. Genome sequencing

## The Sanger Method (1977)

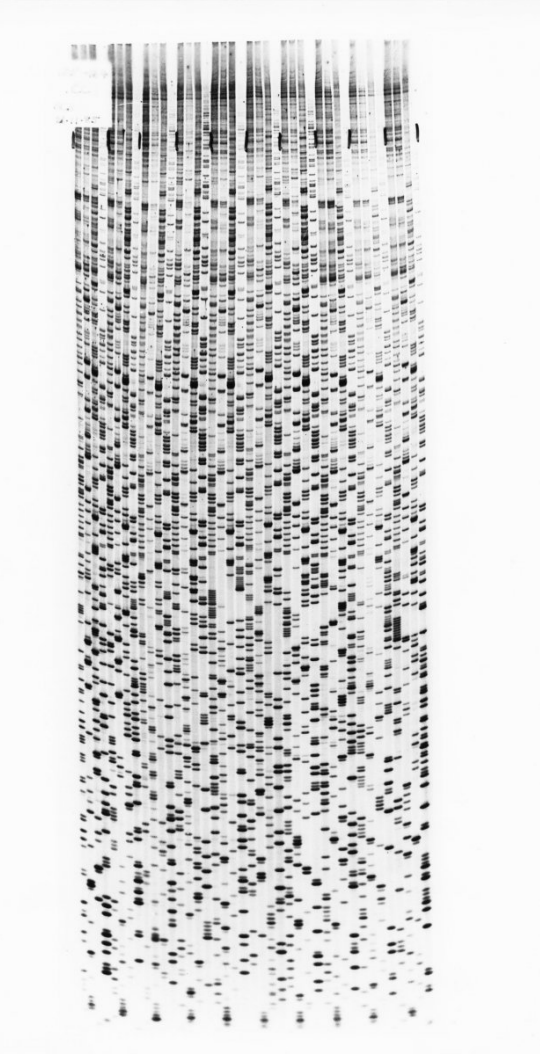


# I. Genome sequencing

## The caveats of the Sanger Method



Technically cumbersome and time consuming (limited samples processing)

High amounts of starting material

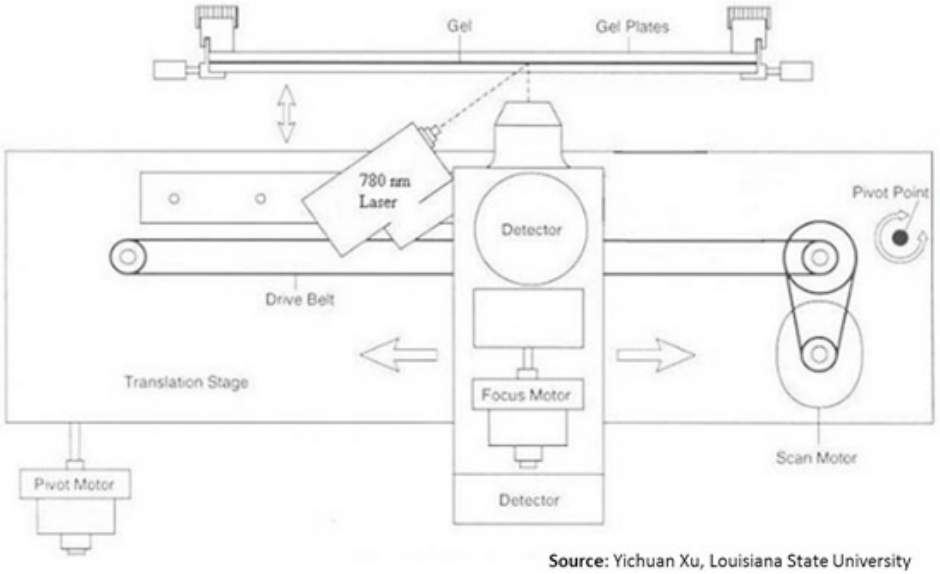
Some knowledge about the sequence is required (primer)

-> Not well-suited for high-throughput analyses (genome sequencing)

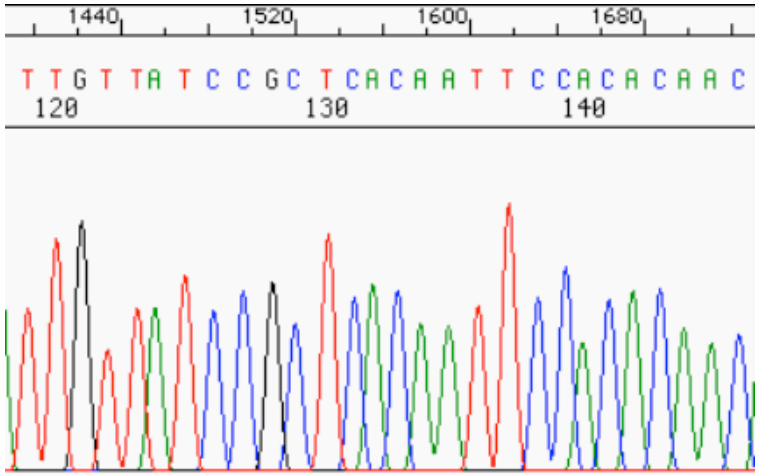
# I. Genome sequencing

The Sanger Method (1977)

1st generation sequencing : Improvement of sequence analysis (fluorescent ddNTP + column electrophoresis)



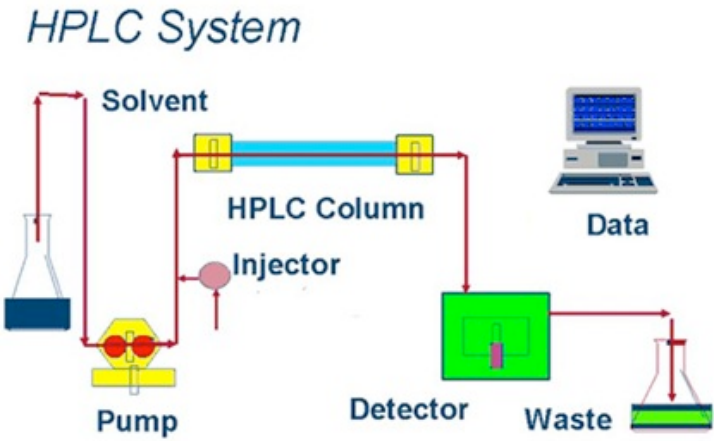

**Source:** Yichuan Xu, Louisiana State University



# I. Genome sequencing

The Sanger Method (1977)

1st generation sequencing : Improvement of sequence analysis (capillary electrophoresis)




Source: College of Arts and Sciences, New Mexico State University

Agilent 1290 Infinity Quaternary LC System

PerkinElmer Flexar FX-15 UHPLC

Nexera UHPLC

Dionex UltiMate 3000 Standard LC Systems

# I. Genome sequencing

1st generation sequencing : Improvement of the methodology

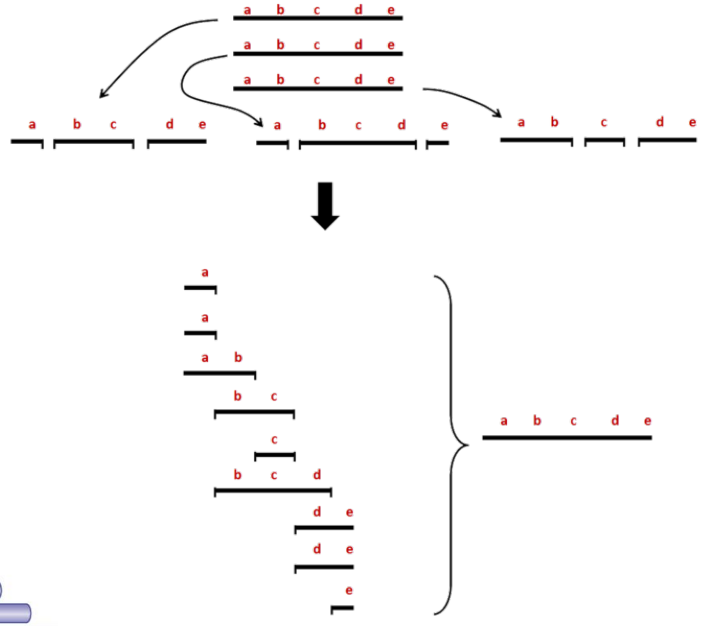
Parallel random sequencing : after fragmentation (shotgun sequencing) and cloning

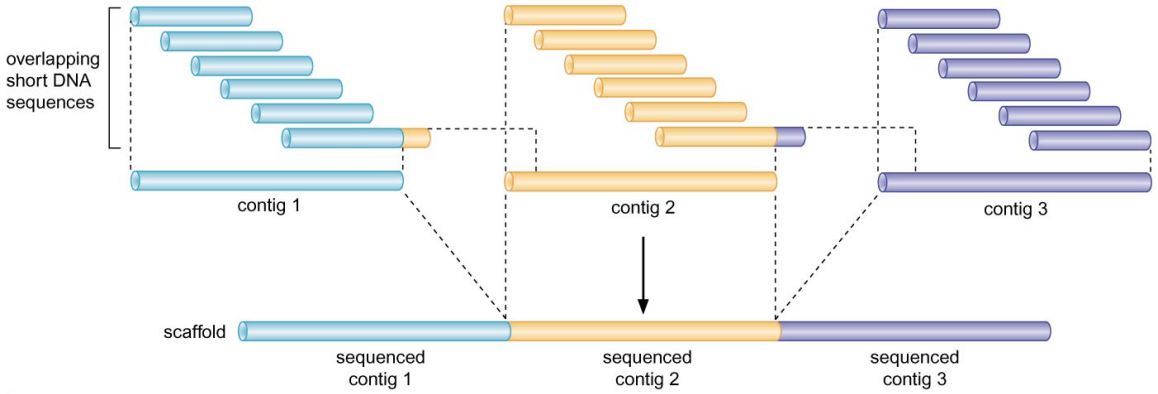
- genomic DNA is fragmented
- cloned to a plasmid vector -> transform

*E. coli*

- a single bacterial colony is picked ->

plasmid DNA isolated



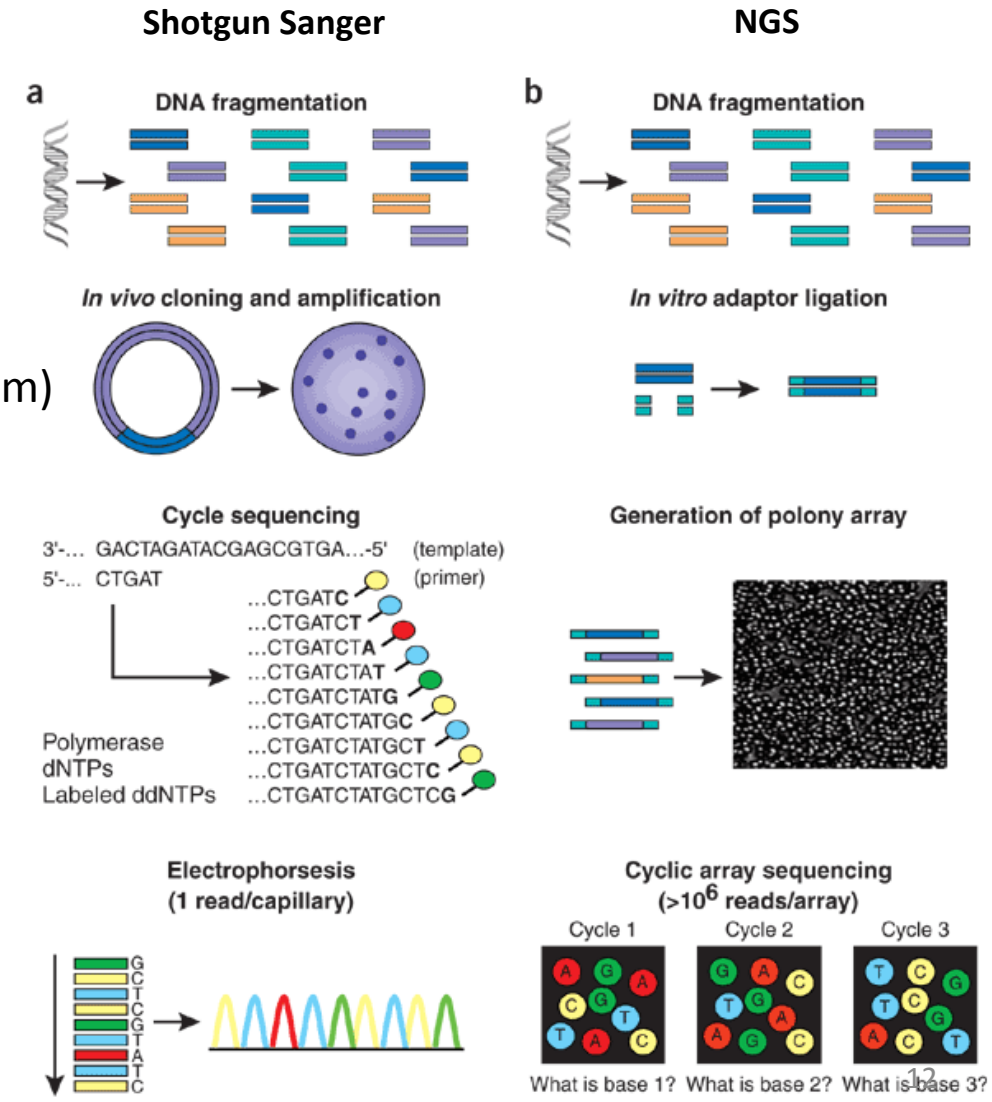



! Redundancy (8x or 20x)

# I. Genome sequencing

2nd generation and next generation (NGS) sequencing : Areas of improvement

- Template preparation
  amplification by PCR (Cell free system)
- Sequencing and imaging
- Data analysis

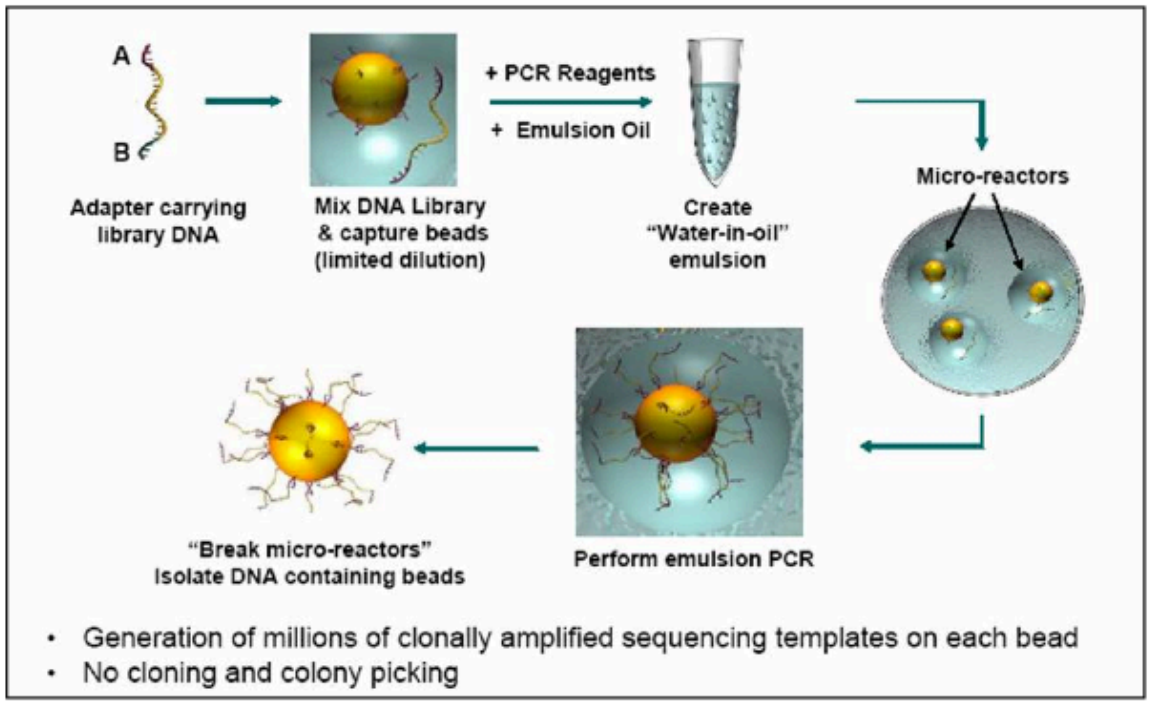


# I. Genome sequencing

2nd generation sequencing/NGS :

Improvement of template preparation : amplification by PCR

Emulsion PCR (emPCR)




Used by 454 (life science), Polonator, Ion torrent and Solid

# I. Genome sequencing

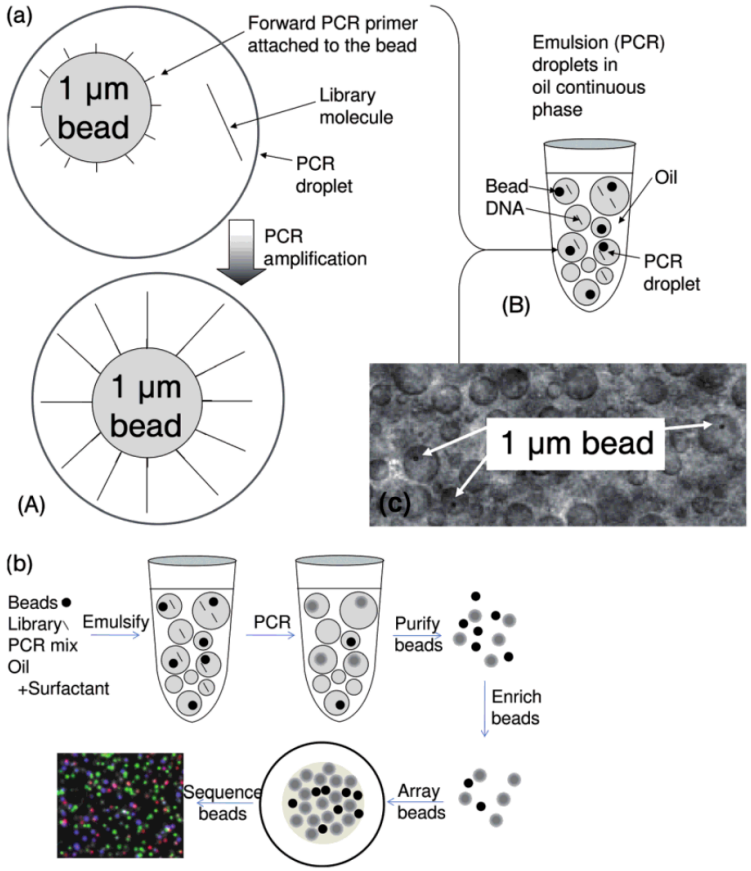
2nd generation sequencing/NGS :

Improvement of template preparation : amplification by PCR

Emulsion PCR (emPCR)

Beads are immobilized on:

Microscope slides (Polonator)
PicoTiterPlates (PTP) (Roche/454)
Microchip sensor (Ion Torrent)

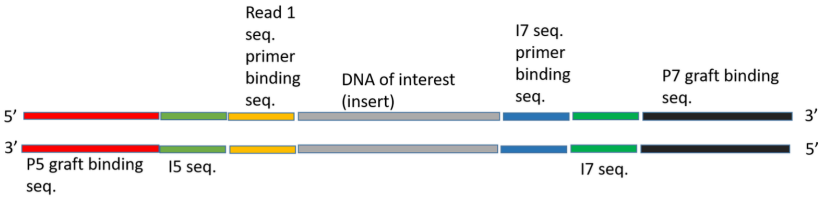


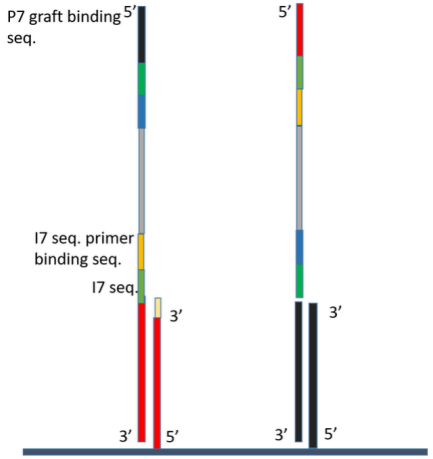
# I. Genome sequencing

2nd generation sequencing :

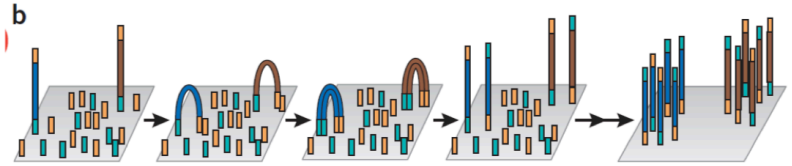
Improvement of template preparation : amplification by PCR

Bridge PCR (illumina plateform)




*Fragments bound to flow cell*




- DNA fragments are flanked with adaptors.
- A flat surface coated with two types of primers, corresponding to the adaptors.
- Amplification proceeds in cycles, with one end of each bridge tethered to the surface.
- Used by Solexa.

## I. Genome sequencing

2nd generation sequencing :

Improvement of template preparation : amplification by PCR

→ Still require large amount of template

→ Sequencing of short length fragments only

→ PCR amplification induces biaises

## I. Genome sequencing

3rd generation sequencing :

Improvement of template preparation : Single Molecule Sequencing (SMRT)

- Requires less starting material
- Provides longer sequence fragments

■ Immobilized on the solid surface by

Primers: Helicos BioSciences
Template: Helicos BioSciences
Polymerase: Pacific Biosciences, Life/Visigen, LI-COR Biosciences

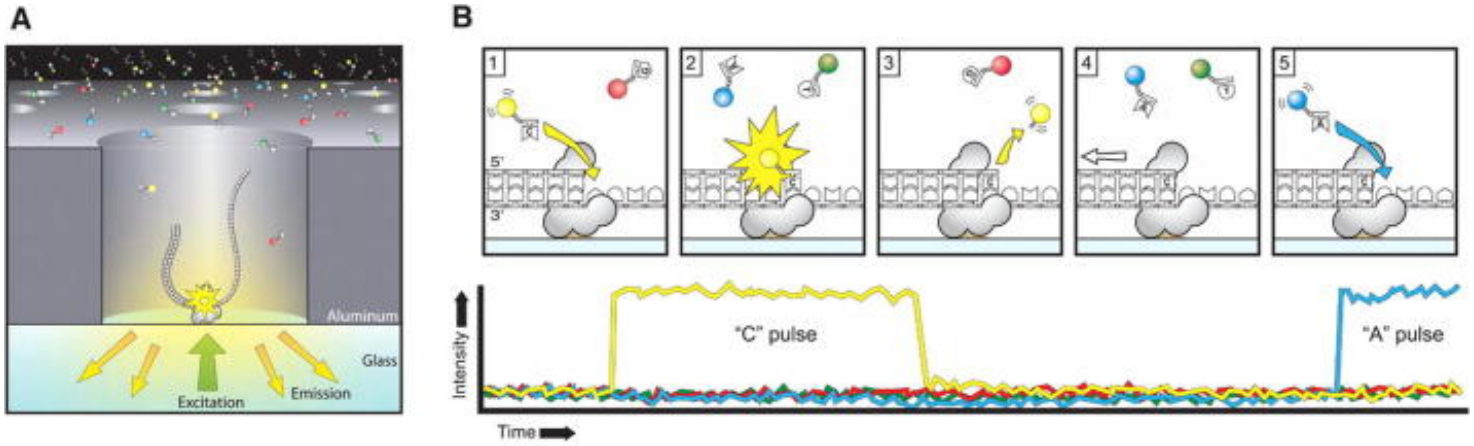
# I. Genome sequencing

3rd generation sequencing :

Improvement of template preparation : Single Molecule Sequencing

SMRT Sequencing Technology : **Single-Molecule** Real-Time Sequencing (3rd generation)

A true continous imaging of dye-labelled nucleotides incorporation




Pacific Biosciences : SMRT : Single Molecule Real-Time Sequencing

# I. Genome sequencing

3rd generation sequencing :

Improvement of template preparation : Single Molecule Sequencing

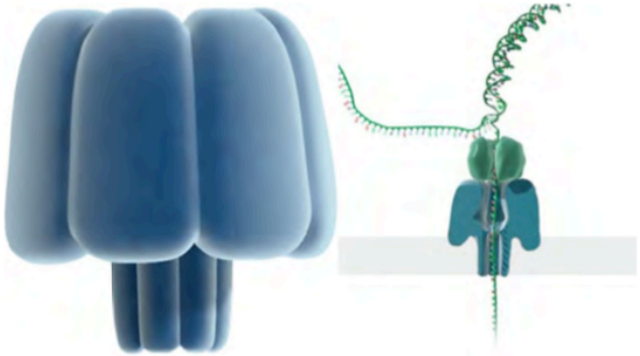
## Oxford Nanopore sequencing


Tether keeps DNA fragment on the membrane leading to a ~20K fold higher DNA concentration close to the pore.

Motor protein unwinds DNA and ratchets it though the pore.

Abasic nucleotides in the hairpin are a recognition point.

Brake protein prevents the motor protein from zipping through the complement strand.

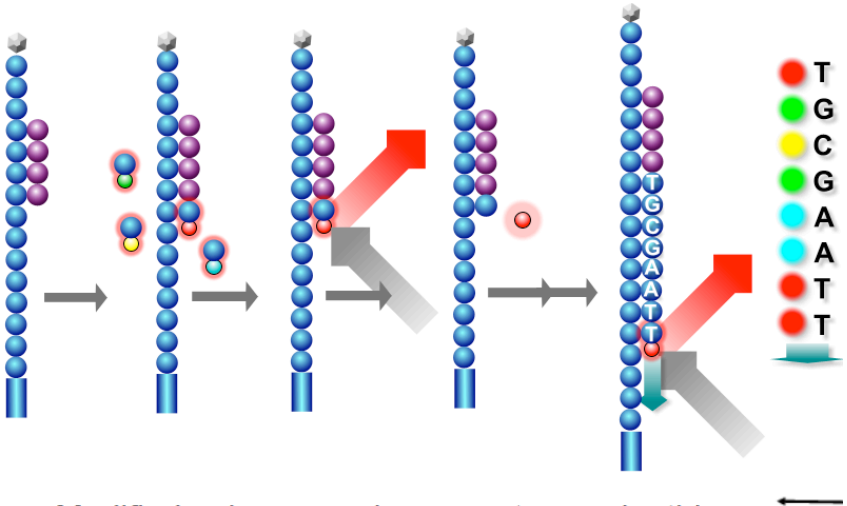


https://www.youtube.com/watch?v=RcP85JHLmnI

# I. Genome sequencing

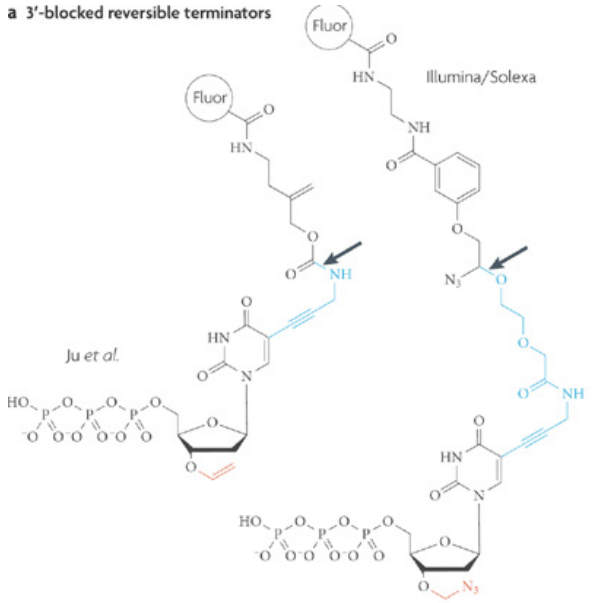
## 2/3rd generation sequencing : real-time sequencing

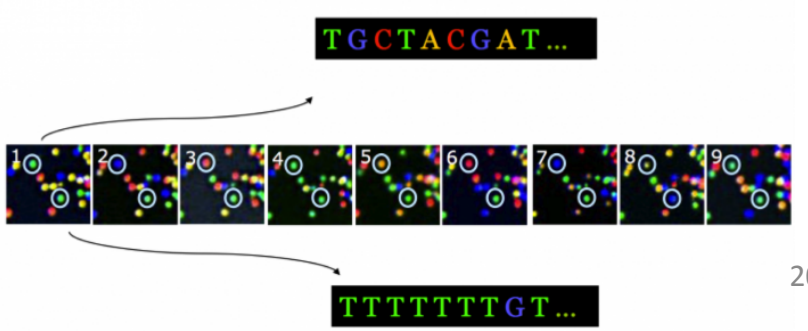
Sequencing **by reversible terminator (illumina/Solexa genome Analyzer)**




Modified polymerase incorporates nucleotides

- after each nucleotide incorporation process stops
- camera reads fluorophore signal (filter for each nucleotide type)
- terminator and labeling is removed and cycle starts again



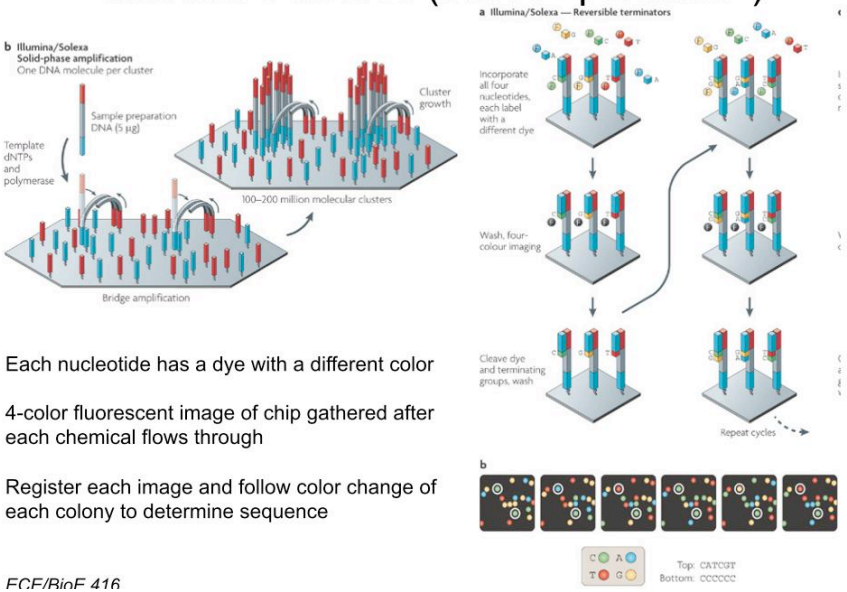
# I. Genome sequencing

2nd generation sequencing : real-time sequencing

Sequencing **by reversible terminator**

**Used in combination with bridge PCR in the illumina/Solexa genome Analyzer**

Illumina Colonies (called “polonies”)



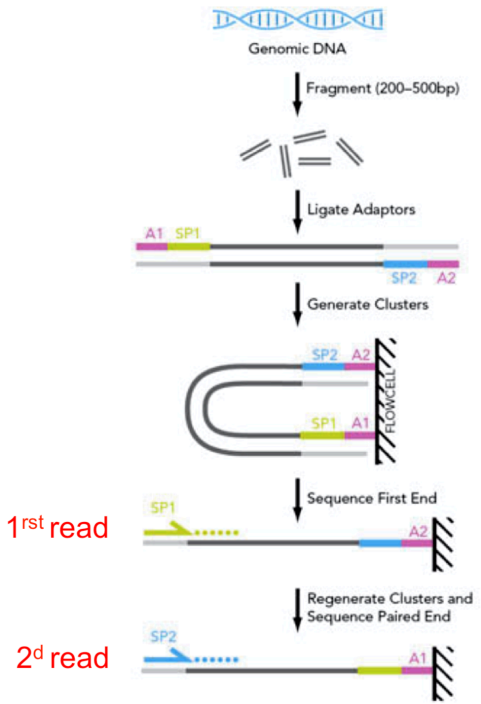

Each nucleotide has a dye with a different color

4-color fluorescent image of chip gathered after each chemical flows through

Register each image and follow color change of each colony to determine sequence

ECE/BioE 416

Paired-end sequencing




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

# I. Genome sequencing

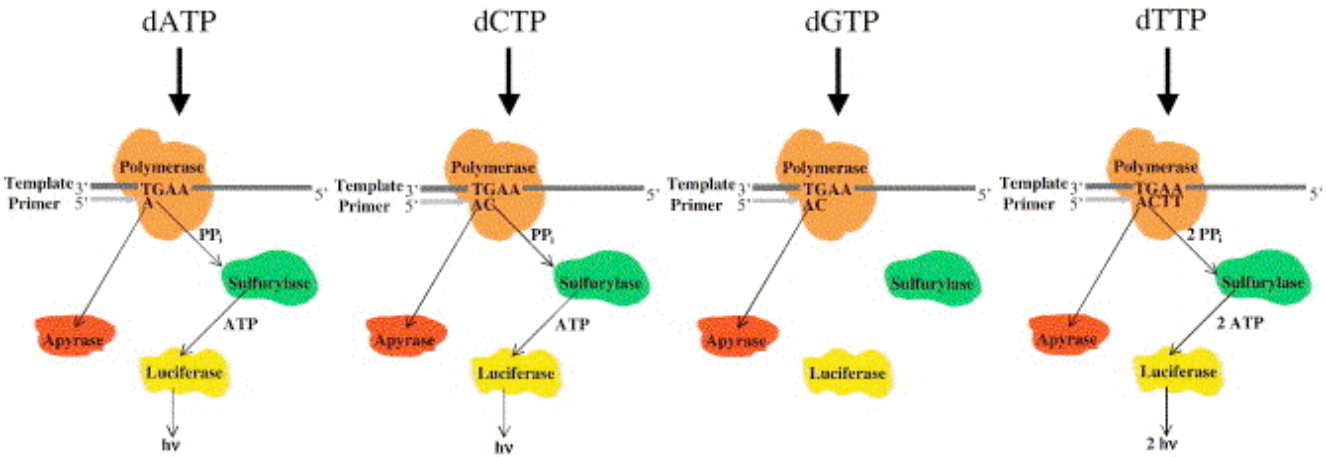
## 2nd generation sequencing : real-time sequencing

Sequencing **by reversible terminator**

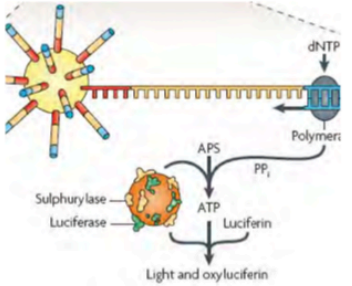
# I. Genome sequencing

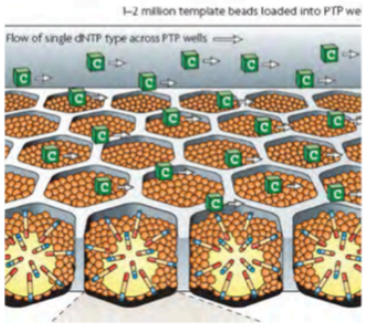
2nd generation sequencing : real-time sequencing

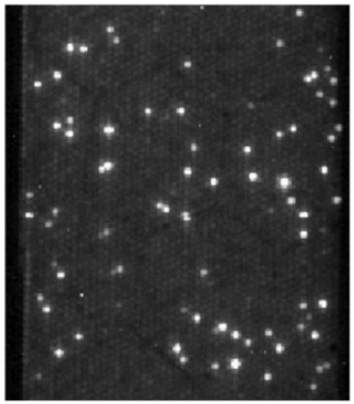
**Pyrosequencing**




**Used in the 454 plateform (Life Sciences)**



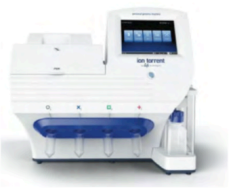



emPCR+Pyrosequencing

# I. Genome sequencing

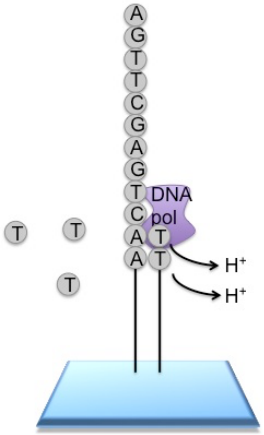
2nd generation sequencing : real-time sequencing

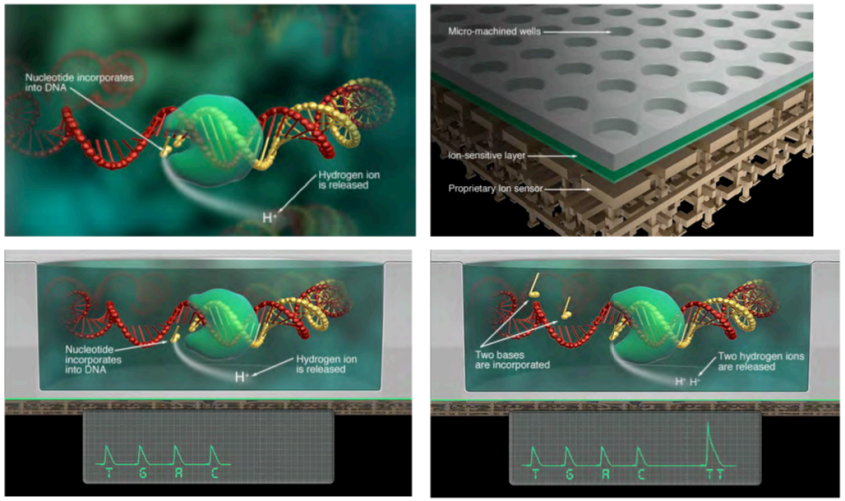


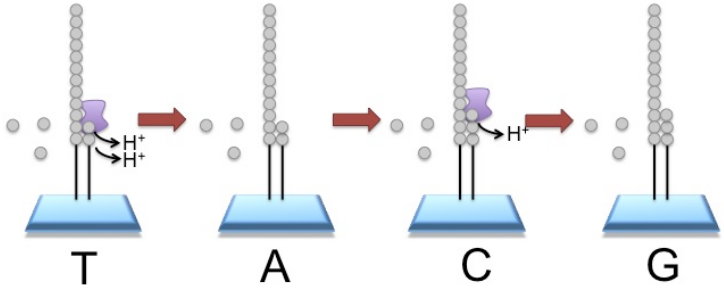
Ion torrent sequencing

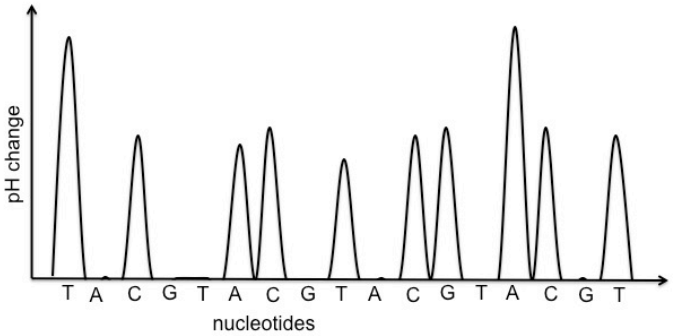
Life Technologies: **Ion Proton & Ion PGM**

**Ion proton sequencing (Ion torrent)**: emPCR + mesurement of changes in pH

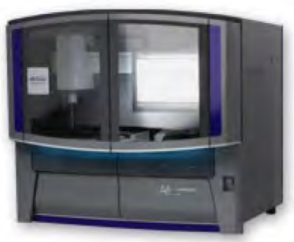


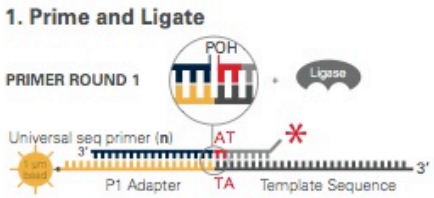
# I. Genome sequencing

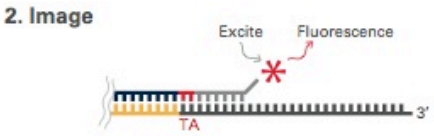


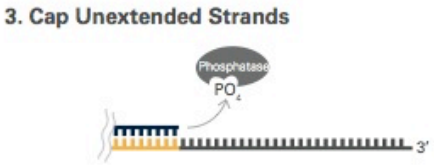
2nd generation sequencing : real-time sequencing

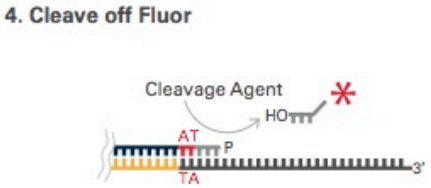
**Ligase-based sequencing (ABI's Solid technology: +emPCR)**

- Difference – DNA ligase
- Hybridization of a fluorescently labelled probe
- SOLiD cycle of 1,2-probe hybridization

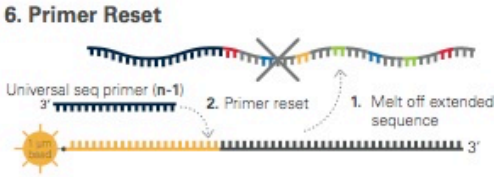



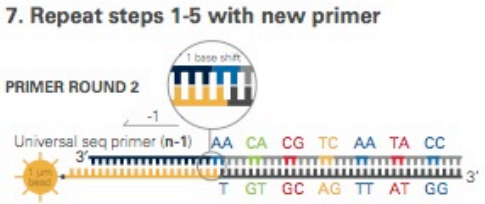







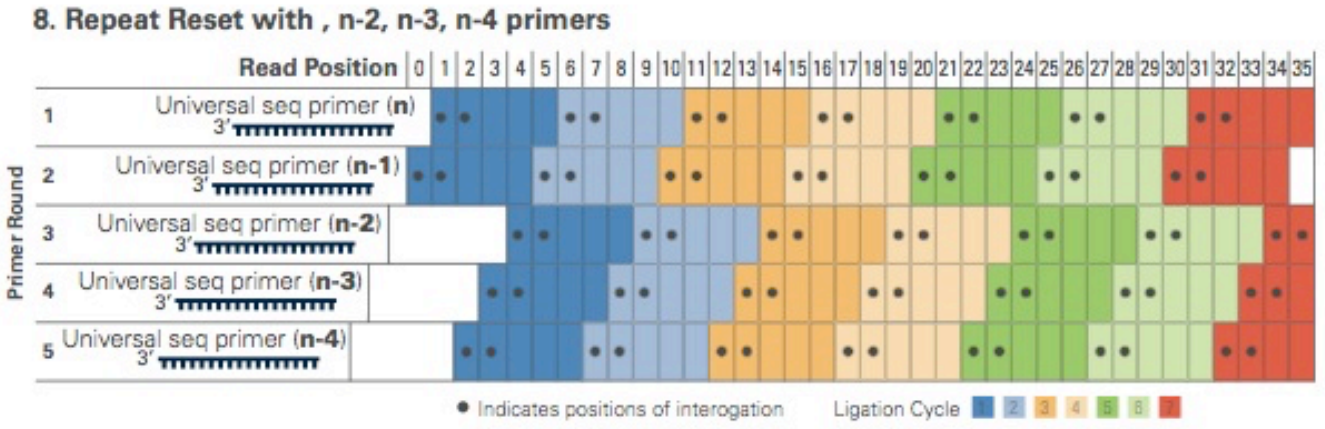



# I. Genome sequencing

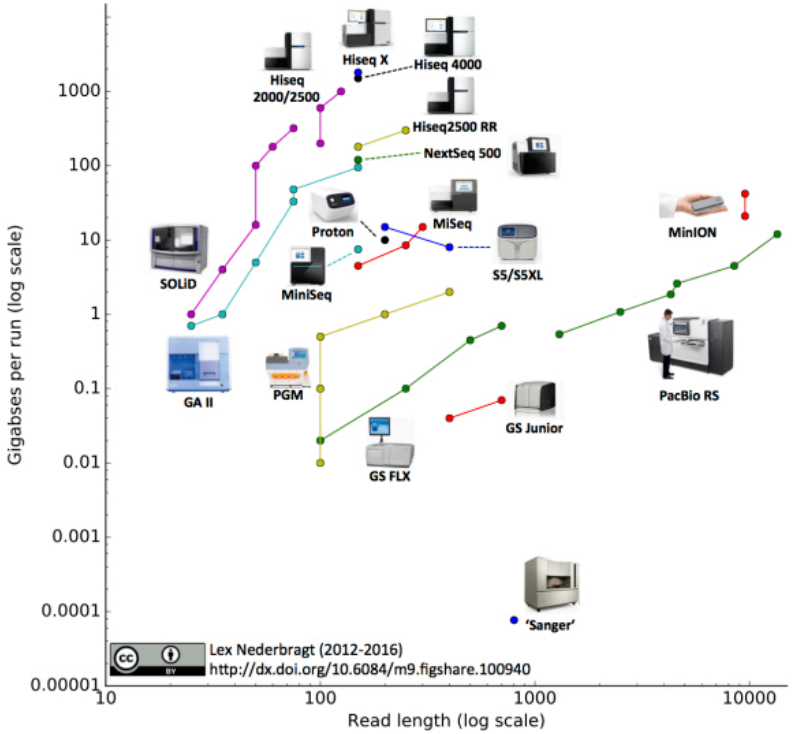
2nd generation sequencing : real-time sequencing

**Ligase-based sequencing**

SOLiD DNA Sequencing Technology (Applied Biosystems)



# II. Genome sequencing : the good choice




Performance comparison of sequencing platforms of various generations

| Method | Generation | Read length (bp) | Single pass error rate (%) | No. of reads per run | Time per run | Cost per million bases (USD) | Refs. |
|---|---|---|---|---|---|---|---|
| Sanger ABI 3730×l | 1st | 600–1000 | 0.001 | 96 | 0.5–3 h | 500 | [14], [18], [19], [20], [21] |
| Ion Torrent | 2nd | 200 | 1 | $8.2 \times 10^7$ | 2–4 h | 0.1 | [15], [25] |
| 454 (Roche) GS FLX+ | 2nd | 700 | 1 | $1 \times 10^6$ | 23 h | 8.57 | [14], [17], [27] |
| Illumina HiSeq 2500 (High Output) | 2nd | 2 × 125 | 0.1 | $8 \times 10^9$ (paired) | 7–60 h | 0.03 | [9], [16], [26] |
| Illumina HiSeq 2500 (Rapid Run) | 2nd | 2 × 250 | 0.1 | $1.2 \times 10^9$ (paired) | 1–6 days | 0.04 | [9], [16], [26] |
| SOLiD 5500×l | 2nd | 2 × 60 | 5 | $8 \times 10^8$ | 6 days | 0.11 | [14], [24] |
| PacBio RS II: P6-C4 | 3rd | $1.0–1.5 \times 10^4$ on average | 13 | $3.5–7.5 \times 10^4$ | 0.5–4 h | 0.40–0.80 | [5], [12], [15] |
| Oxford Nanopore MinION | 3rd | $2–5 \times 10^3$ on average | 38 | $1.1–4.7 \times 10^4$ | 50 h | 6.44–17.90 | [22], [23] |

# II. Genome sequencing : the good choice

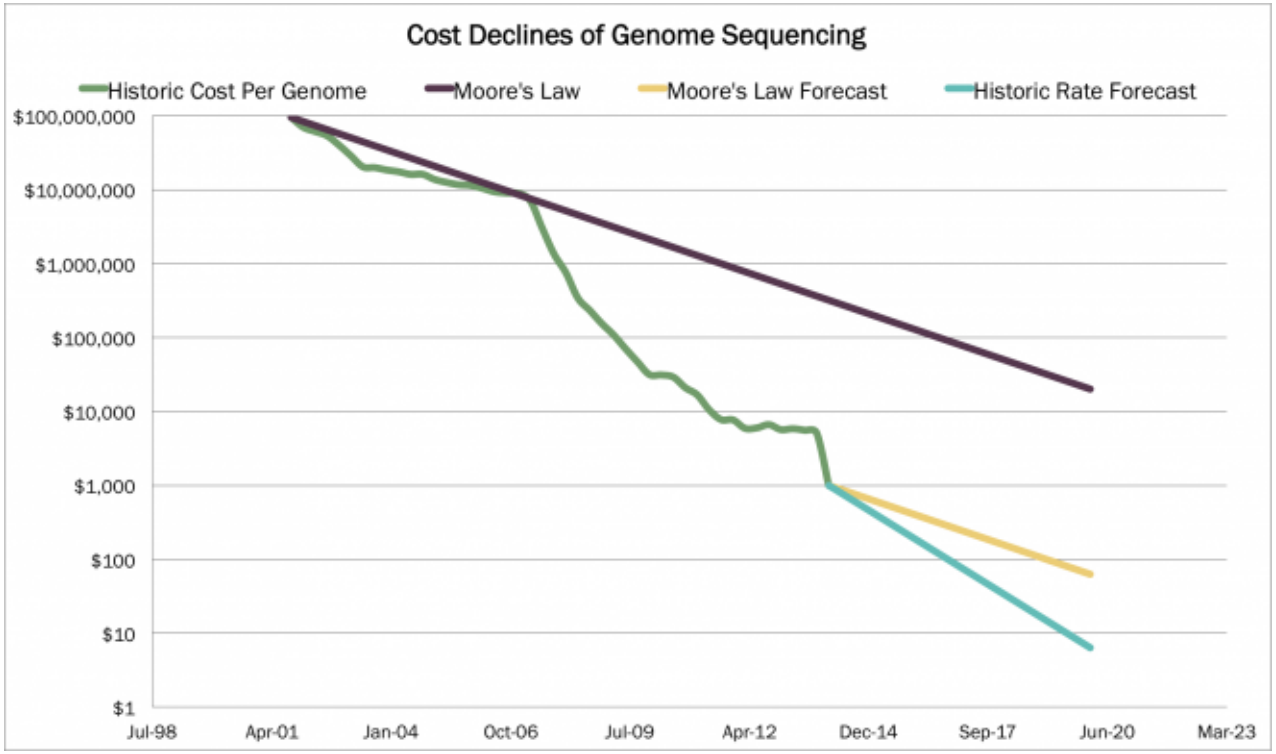
**Number of sequencing machines by country**

| Name | Number of machines |
|---|---|
| United States | 818 |
| China | 200 |
| United Kingdom | 137 |
| Germany | 135 |
| Australia | 79 |
| Canada | 74 |
| Spain | 56 |
| Netherlands | 41 |
| France | 38 |
| Japan | 34 |

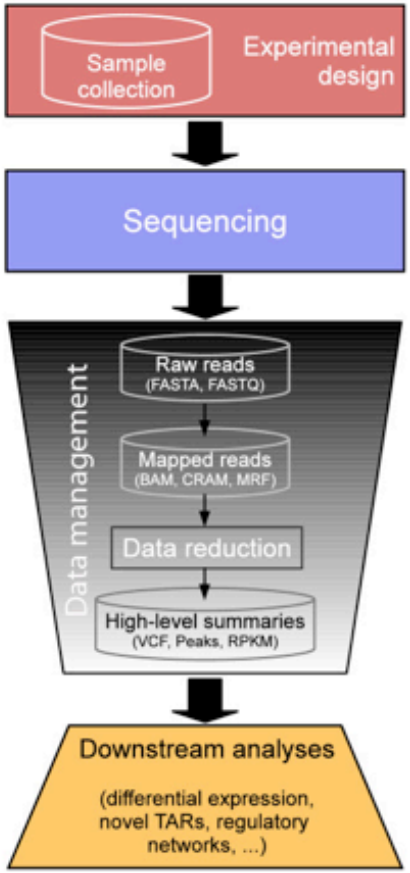
**Centres with platform**

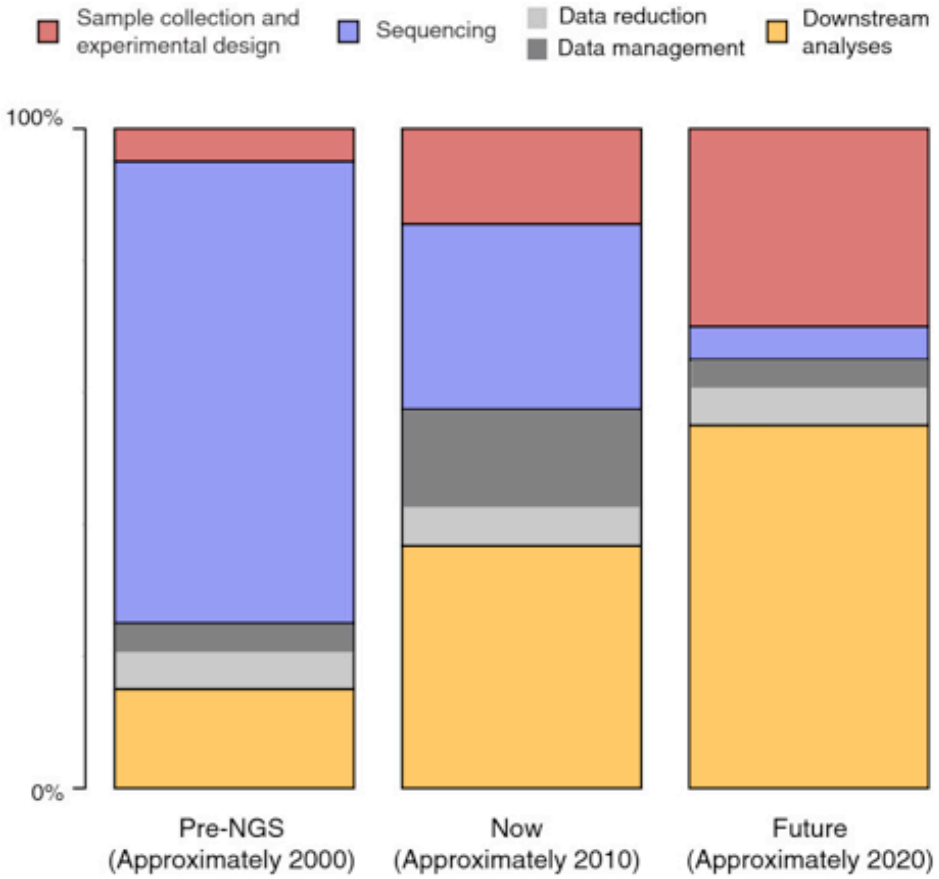
| Name | Number of centres |
|---|---|
| Illumina Genome Analyser 2x | 279 |
| Roche 454 | 265 |
| Illumina HiSeq 2000 | 178 |
| ABI SOLiD | 173 |
| Ion Torrent | 101 |
| Pacific Biosciences | 26 |
| Illumina MiSeq | 23 |
| Polonator | 5 |

# I. Genome sequencing : the price



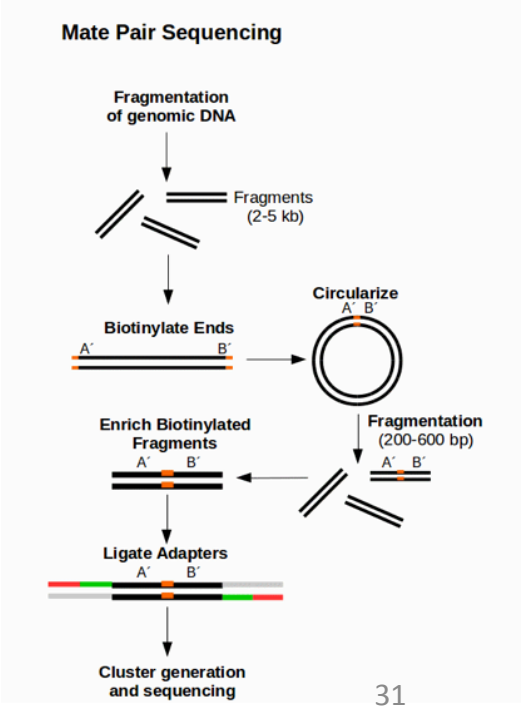
# II. Data analysis



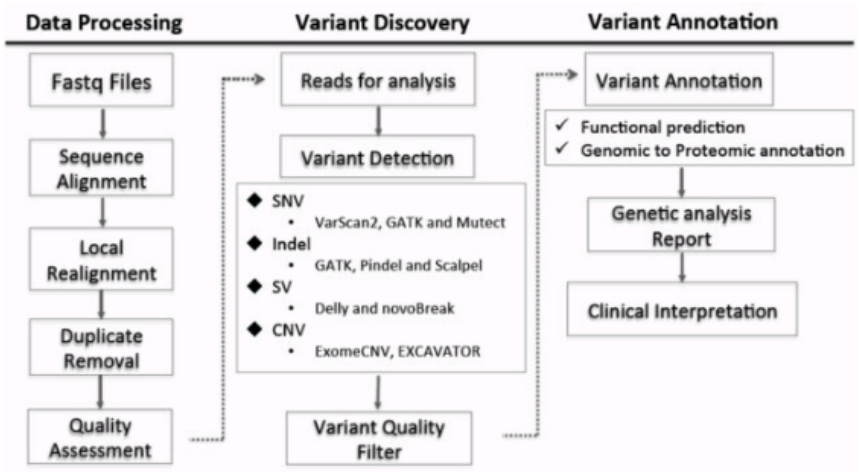
## II. Data analysis

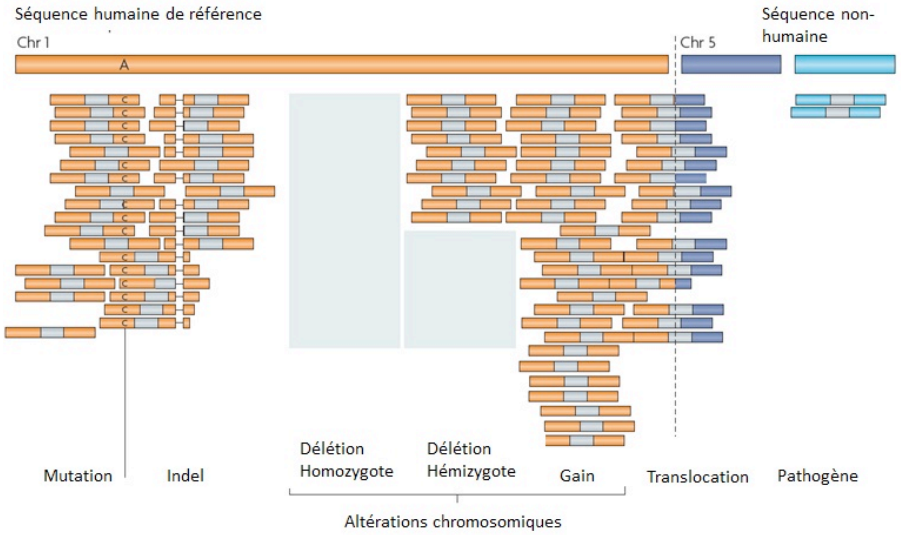
- Bioinformatics tools for:
  - Alignment
  - Base calling/polymorphism detection
  - *De novo* assembly
  - Genome browsing or annotation
- Challenging problems:
  - *De novo* assembly of short reads -> mate-paired libraries required
  - Reads in repetitive regions



# III. NGS Applications

- Genome resequencing: polymorphism and mutation discovery in humans (1000 Genomes Project)



## III. NGS Applications

- Transcriptome sequencing:

  Gene expression

  Alternative splicing

  Transcript annotation