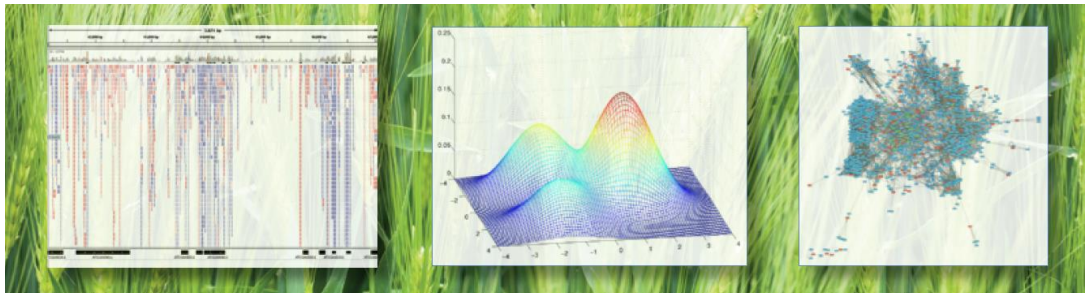
Ecole Chercheur 2017

De l'expression des gènes aux réseaux

Module 1 : High Troughput Sequencing Technologies, available and futures…



SPS

SACLAY PLANT SCIENCES

Mathilde CLEMENT
mathilde.clement@inra.fr

I. What is transcriptomic analysis?

II. DNA Sequencing history

III. Available High Troughtput Sequencing technologies

IV. What is France Génomique?

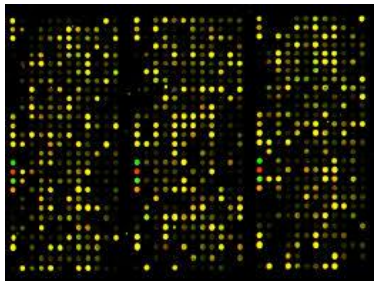
What is a transcriptomic analysis ?

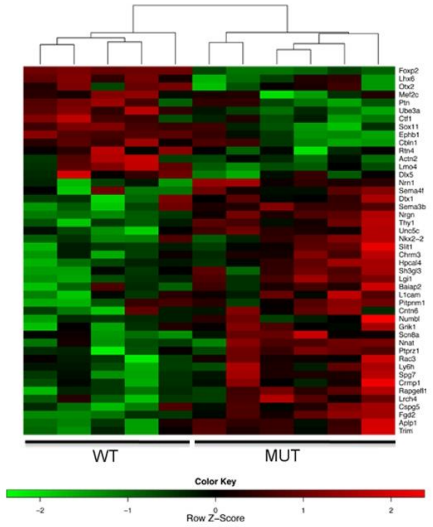
Quantification of the changing expression levels of each transcript during development and under different conditions

What is a transcriptomic analysis ?

Quantification of the changing expression levels of each transcript during development and under different conditions

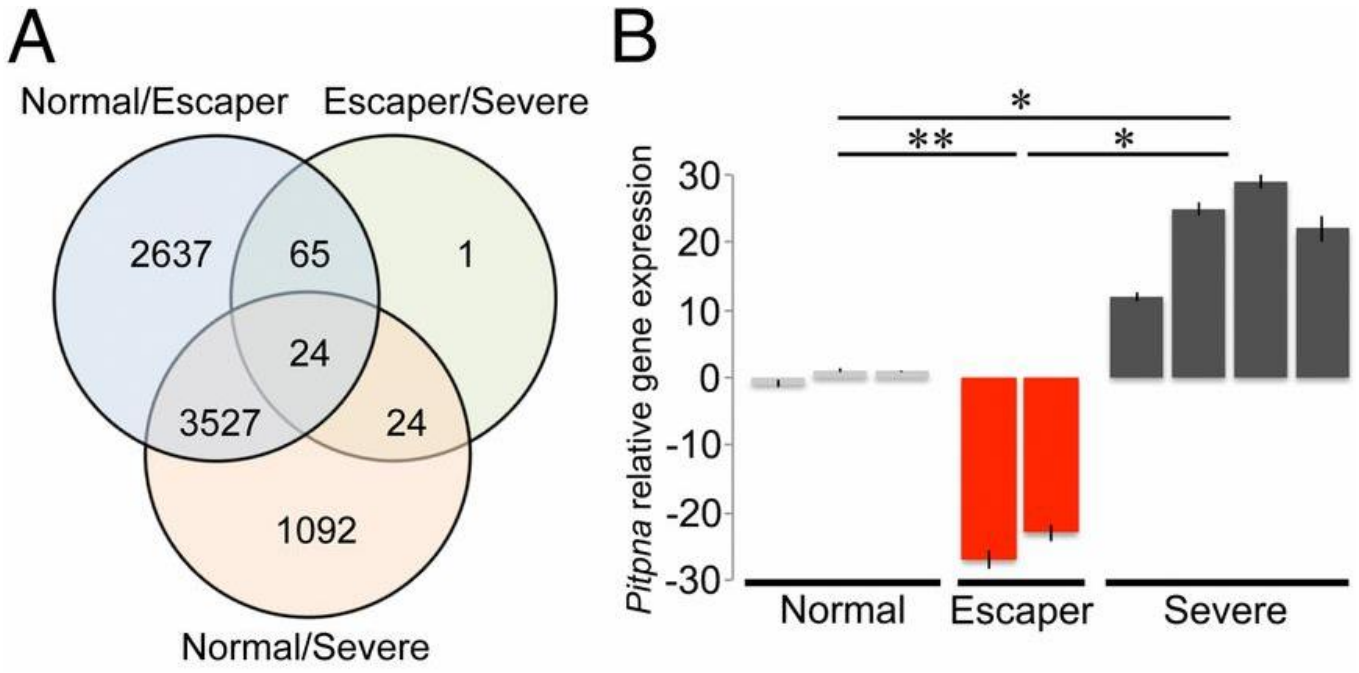
Microarray technology in the mid-1990s





What is a transcriptomic analysis ?

Repression of phosphatidylinositol transfer protein α ameliorates the pathology of Duchenne muscular dystrophy

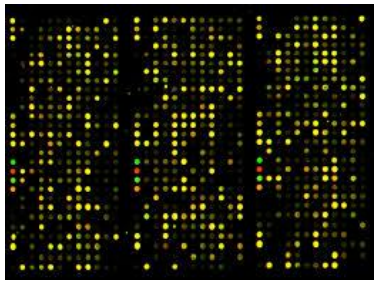


Agilent Technologies
Canine 4 × 44K

Viera et al., (2017) *PNAS*

What is a transcriptomic analysis ?

Limitation of microarray analysis :
Why did we need another technique of gene expression analysis?



- **Arrays had to be designed and manufactured for each species**
- **Detection of only « known » genes**
- **No detection of genes weakly differentially expressed**

What is a transcriptomic analysis ?

Limitation of microarray analysis :
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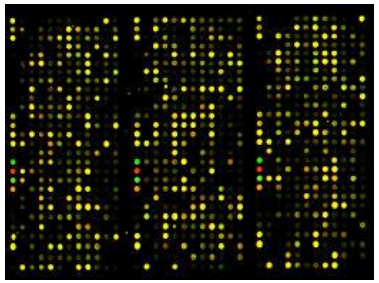
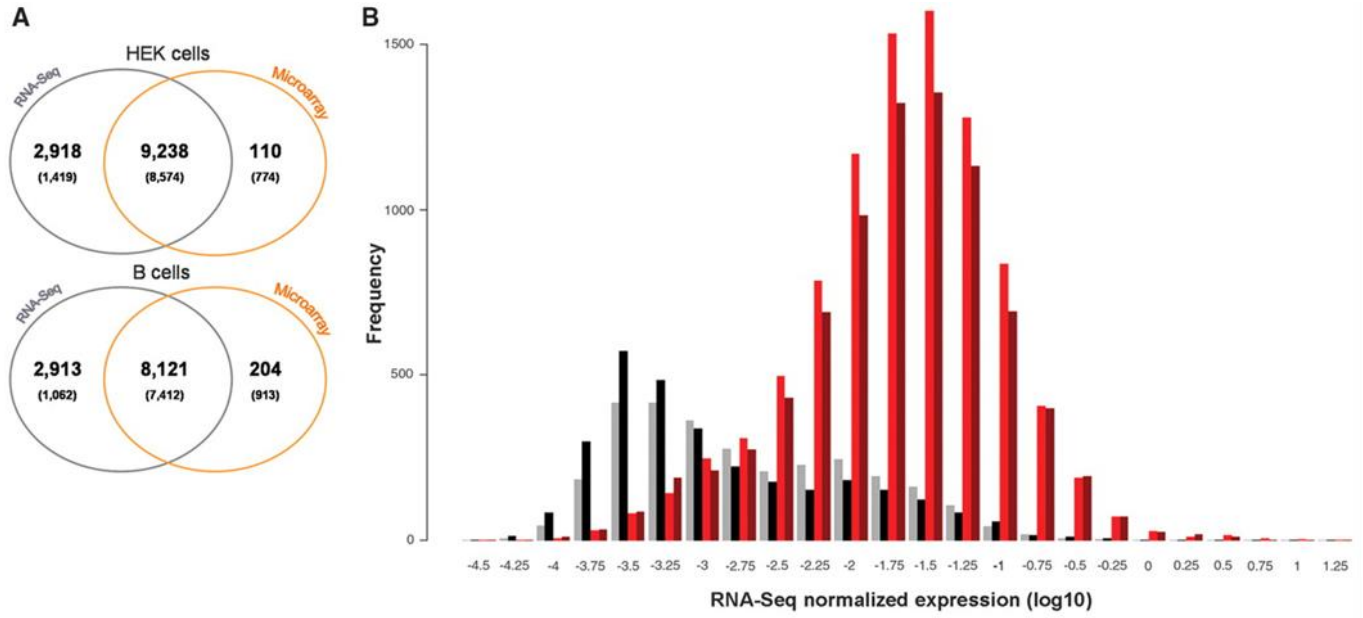


- **Arrays had to be designed and manufactured for each species**
- **Detection of only « known » genes**
- **No detection of genes weakly differentially expressed**

In 2008 , the first RNA-Seq protocol appering
- measuring transcriptomes at base-par resolution
- using essentially the same protocol for any species
- minimal noise level

What is a transcriptomic analysis ?

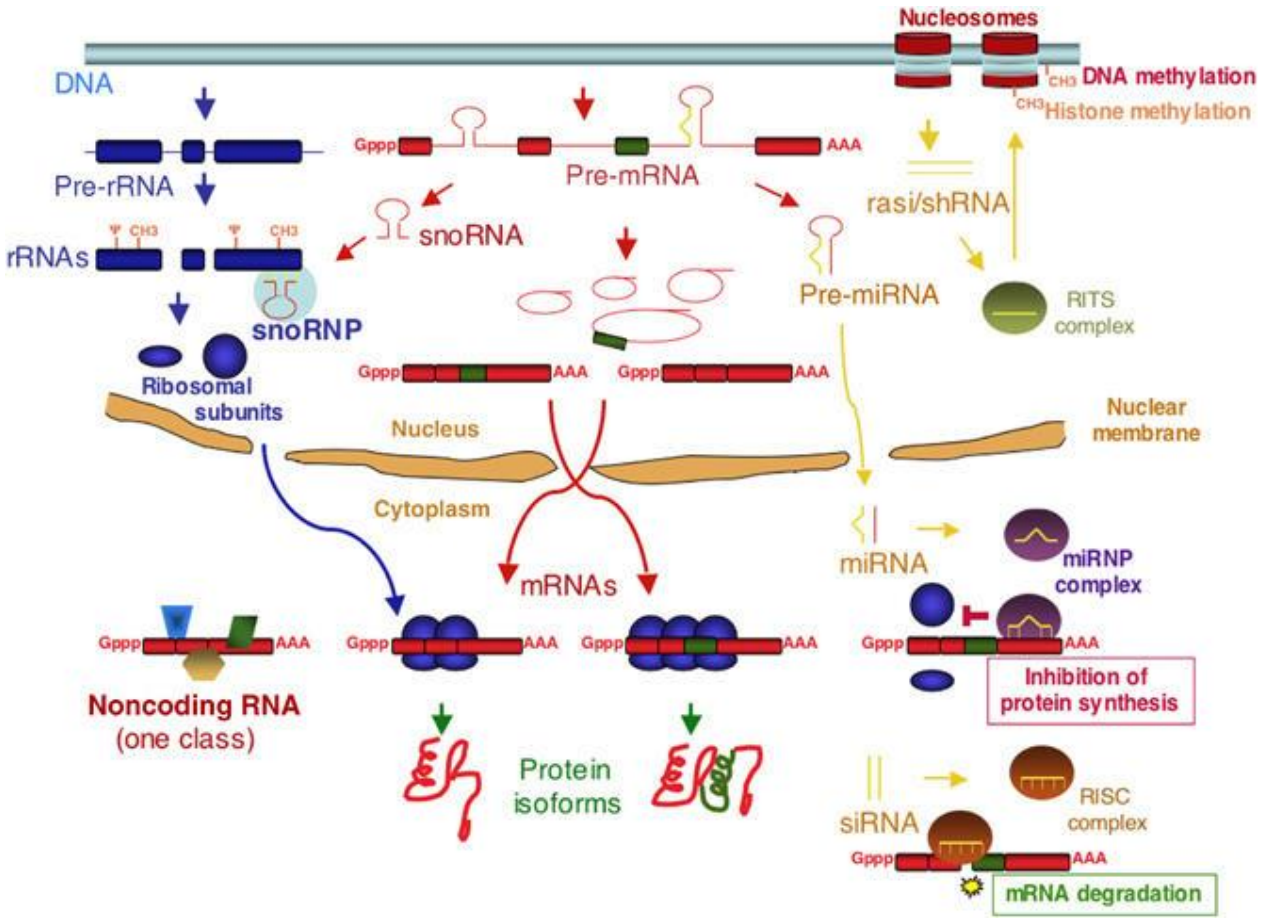


Sultan et al., (2008) Sciences

RNA-Seq can detect 25% more genes than can microarrays

What is a transcriptomic analysis ?

An overview of the eukaryotic transcriptome…



Mendes Soares LM & Valcarcel J. *EMBO J* (2006) **25**, 923-931

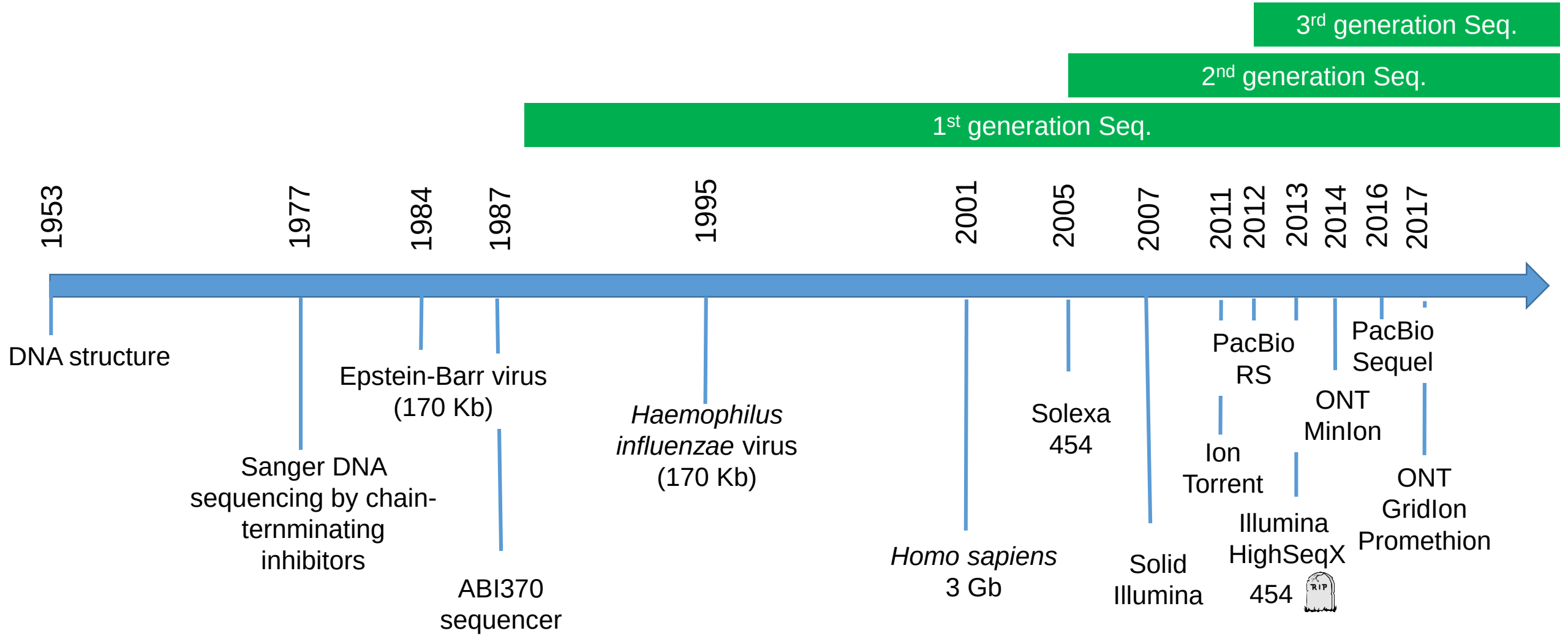
What is a transcriptomic analysis ?

An overview of the eukaryotic transcriptome…

- Catalogue all species of transcript, including mRNAs, non-codin RNAs and small RNAs;
- Determine the transcriptional structure of genes, in terms of their start sites, 5’ and 3’ ends, splicing patterns and other post-transcriptional modifications;
- and to quantify the changing expression levels of each transcript during development and under different conditions

DNA Sequencing History

Sequencing over the Ages



DNA Sequencing History

1st generation sequencing _ Sanger sequencing

http://dailym.ai/1f1XeTB

Frederick Sanger

13 Aug 1918 – 19 Nov 2013

Won the Nobel Prize for Chemistry in **1958** and **1980**. Published the dideoxy chain termination method or "Sanger method" in 1977

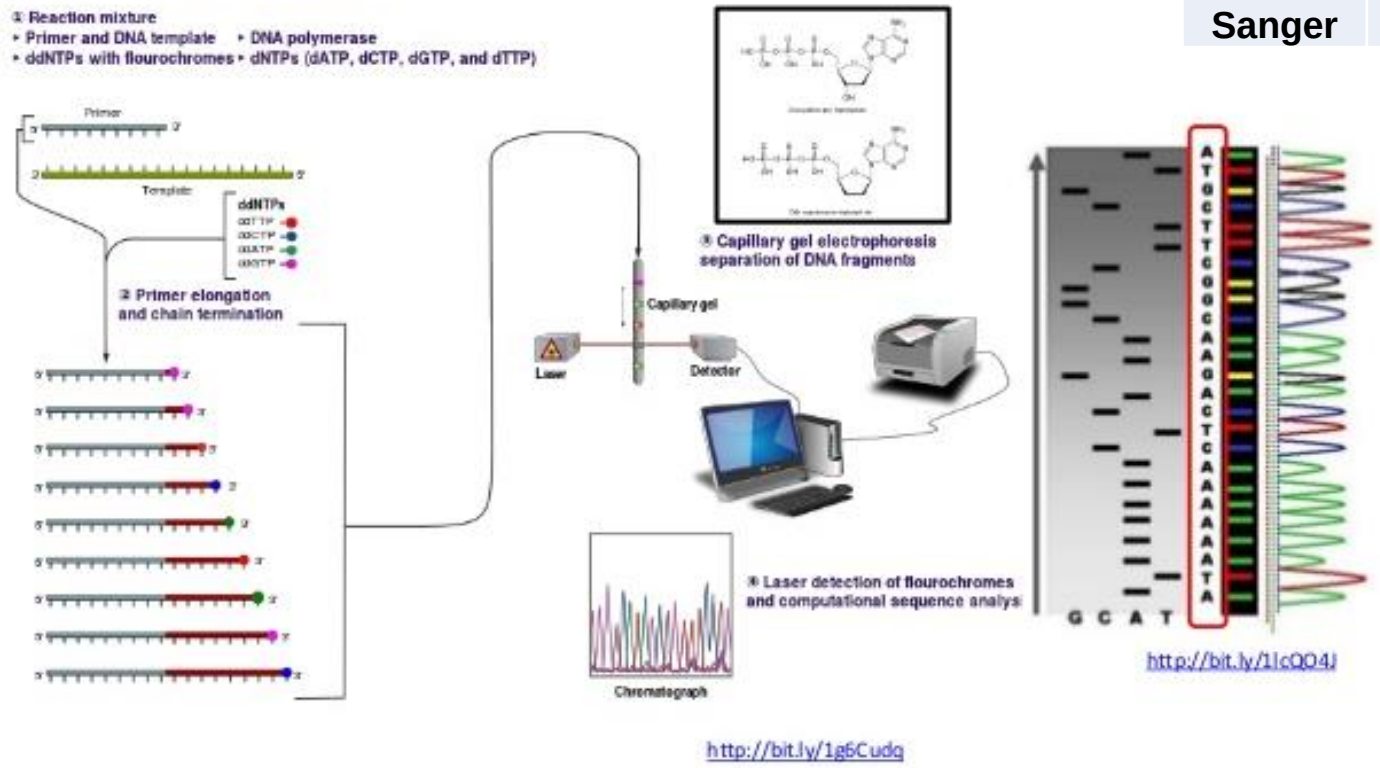
DNA sequencing with chain-terminating inhibitors

F. SANGER, S. NICKLEN, AND A. R. COULSON

DNA Sequencing History

1st generation sequencing _ Sanger sequencing

Platform	Reads per run	Read length	Bases per run (gigabases)
ABI Sanger	96	800	0.0000768



The methods relies on the use of (dideoxynucleotide) ddNTP which will terminate the polymarization

DNA Sequencing History

1st generation sequencing _ Sanger sequencing

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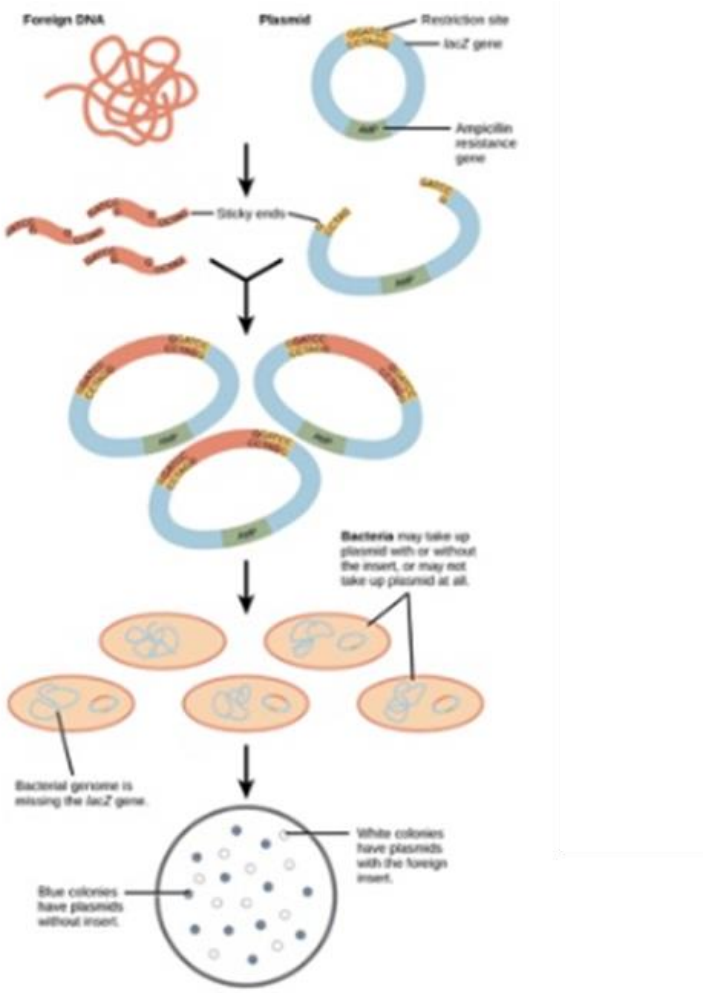

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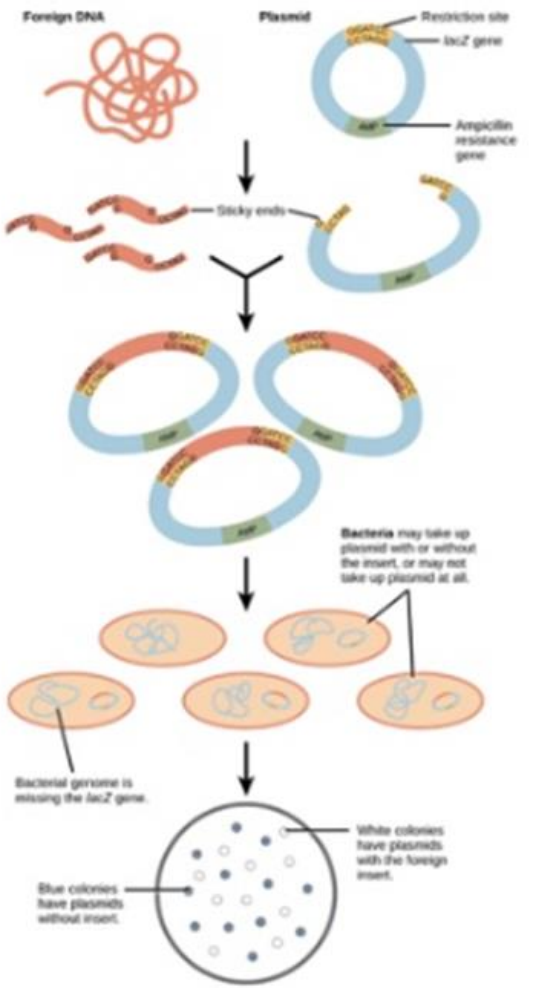
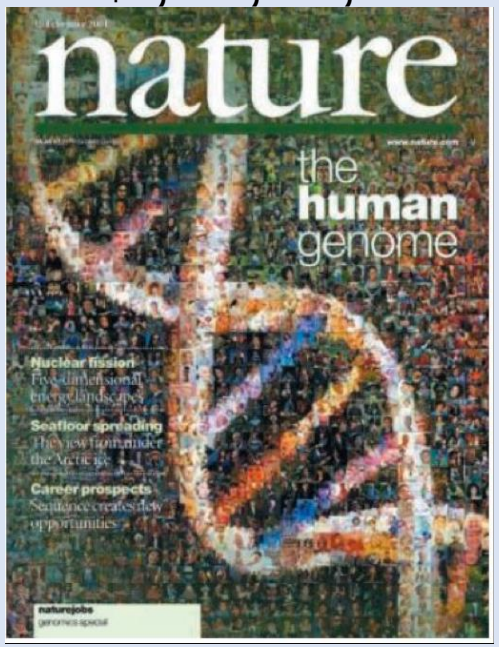


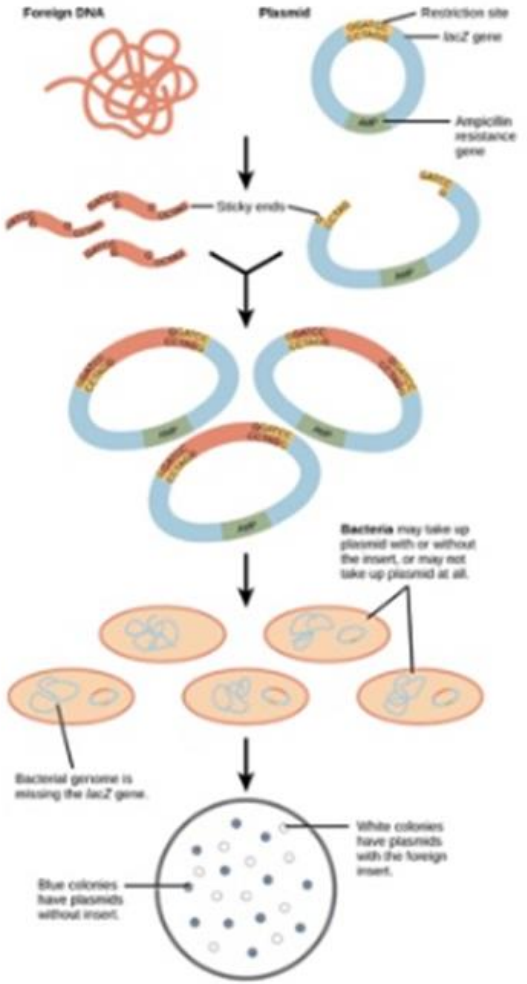
The International Human Genome Consortium
13 years (1990-2003)
~ $3,000,000,000



Nature. 2001 Feb 15;409(6822):860-921.
Initial sequencing and analysis of the human genome.

DNA Sequencing History

1st generation sequencing _ Sanger sequencing

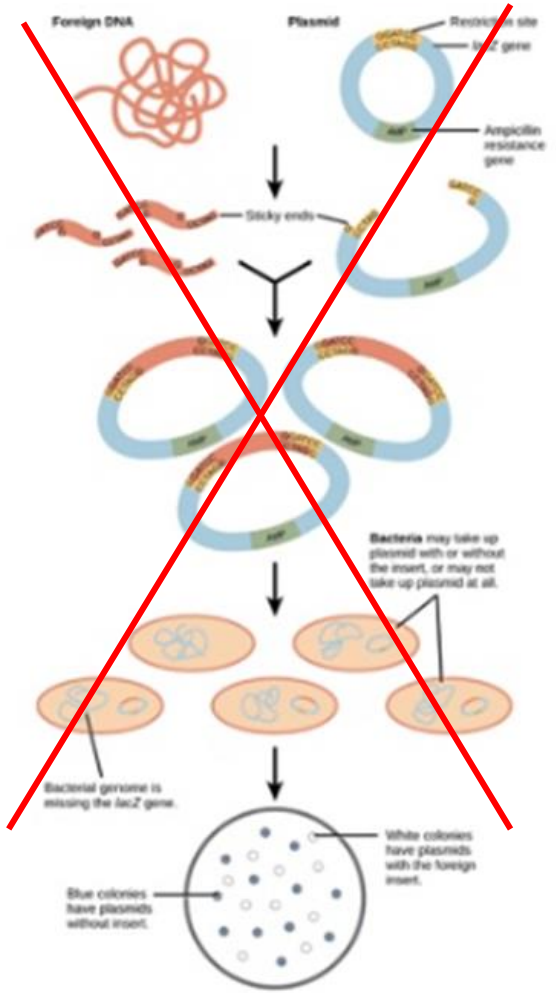


Very high quality sequences (99.999%), 800 bp

Sanger sequencing is not an high throughput technology

DNA Sequencing History

1st generation sequencing _ Sanger sequencing



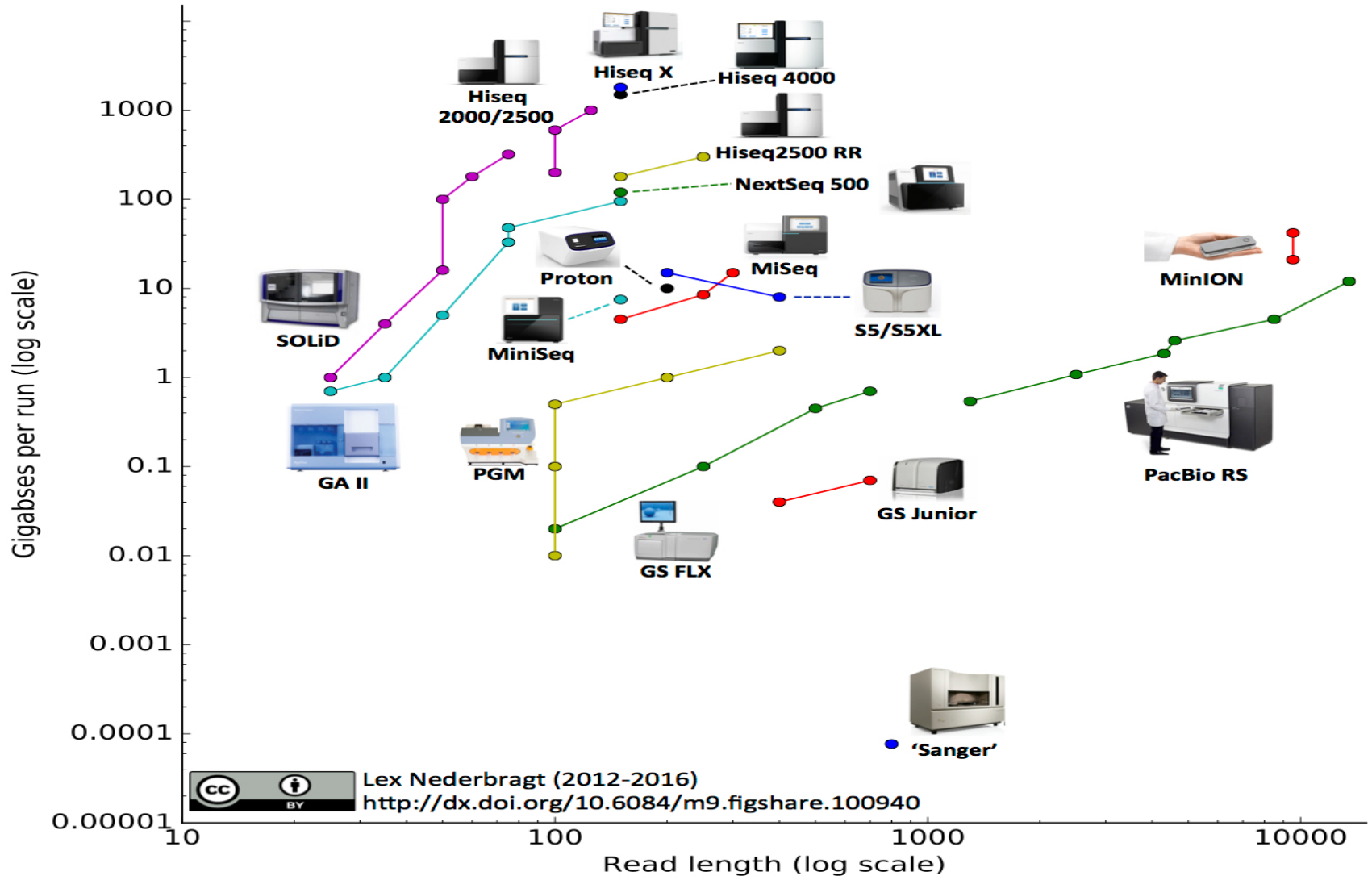
Very high quality sequences (99.999%)

Sanger sequencing is not an high throughput technology

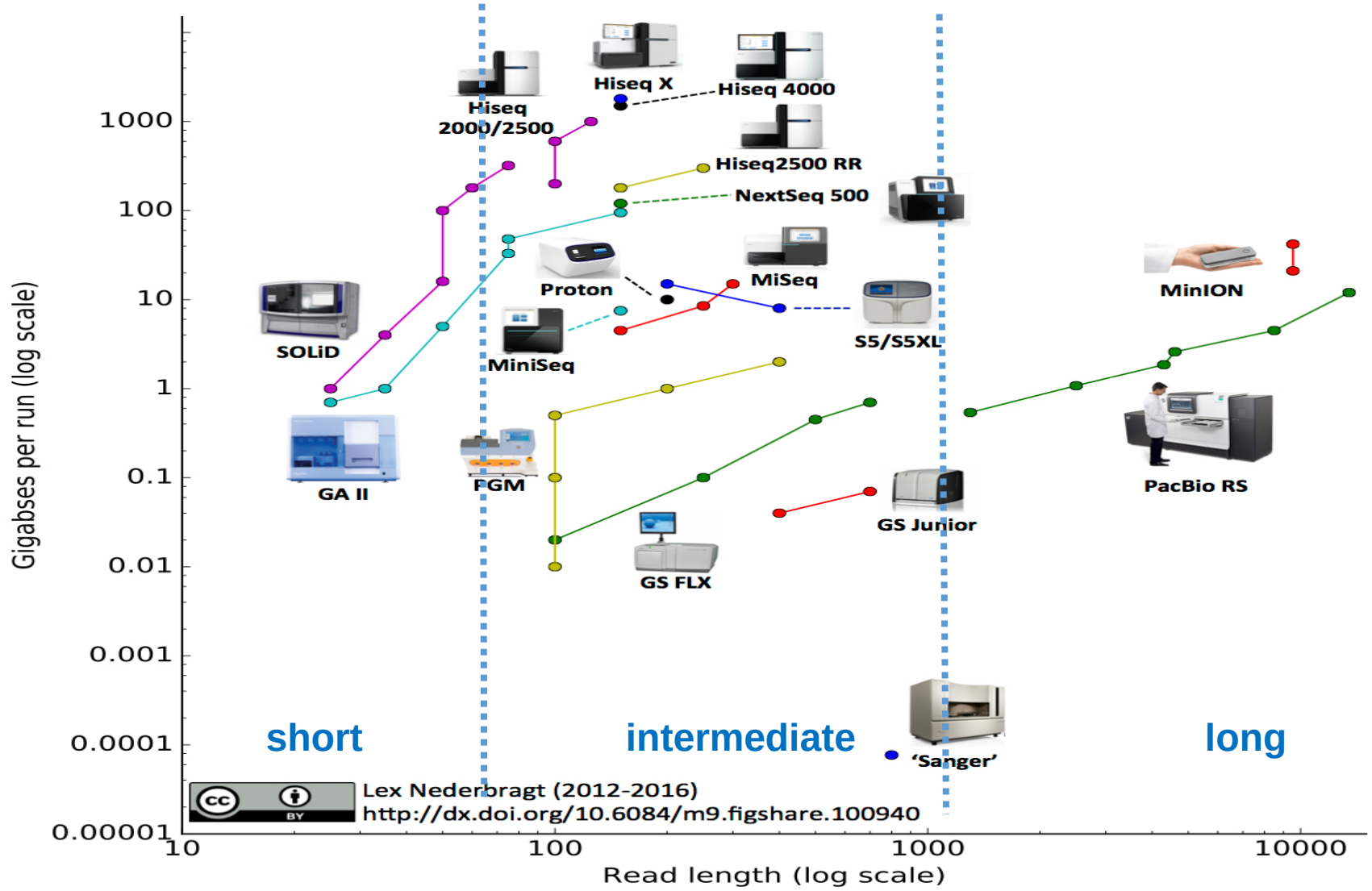
Massive parallel sequencing
No need to clone sequences and performe libraries of plasmids in bacteria

2nd /3rd generation sequencing technologie_Next Generation Sequencing_NGS

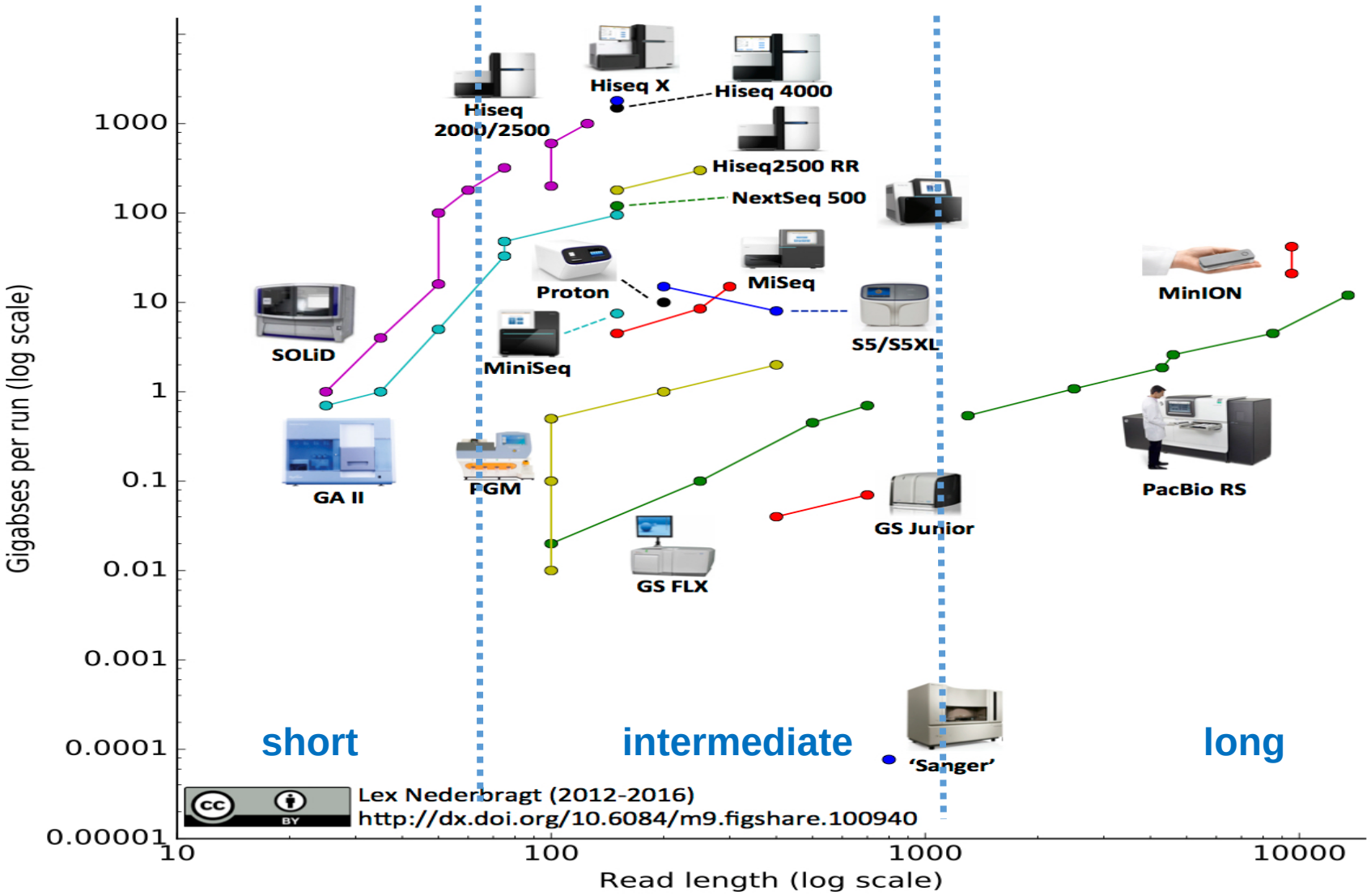
Available High Throughput Sequencing Technologies…



Available High Throughput Sequencing Technologies…



Available High Throughput Sequencing Technologies…



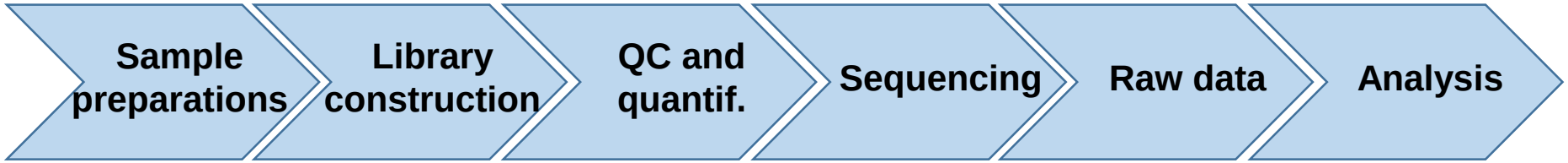
….the arms race…

Human genome project:
13 years (1990-2003)
~ $3,000,000,000

With Illumina XTen platform (2014):
< 1 week
~$1,000

Available High Throughput Sequencing Technologies…

The general principle of NGS is to sequence several DNA fragments in the same sample



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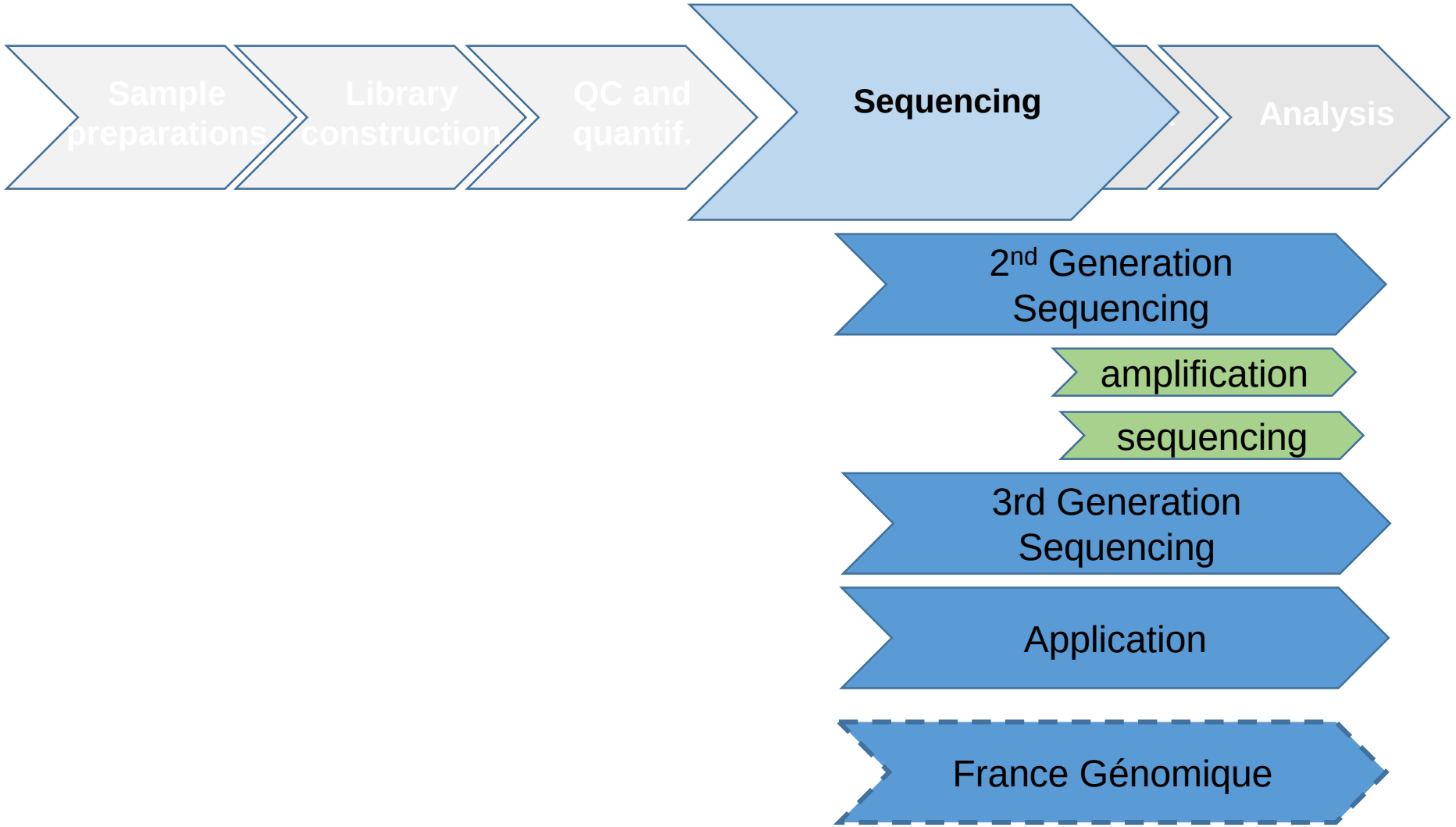


Amplification

Library amplification is required to obtain a sufficient signal from the sequencer.
Two types of amplification:

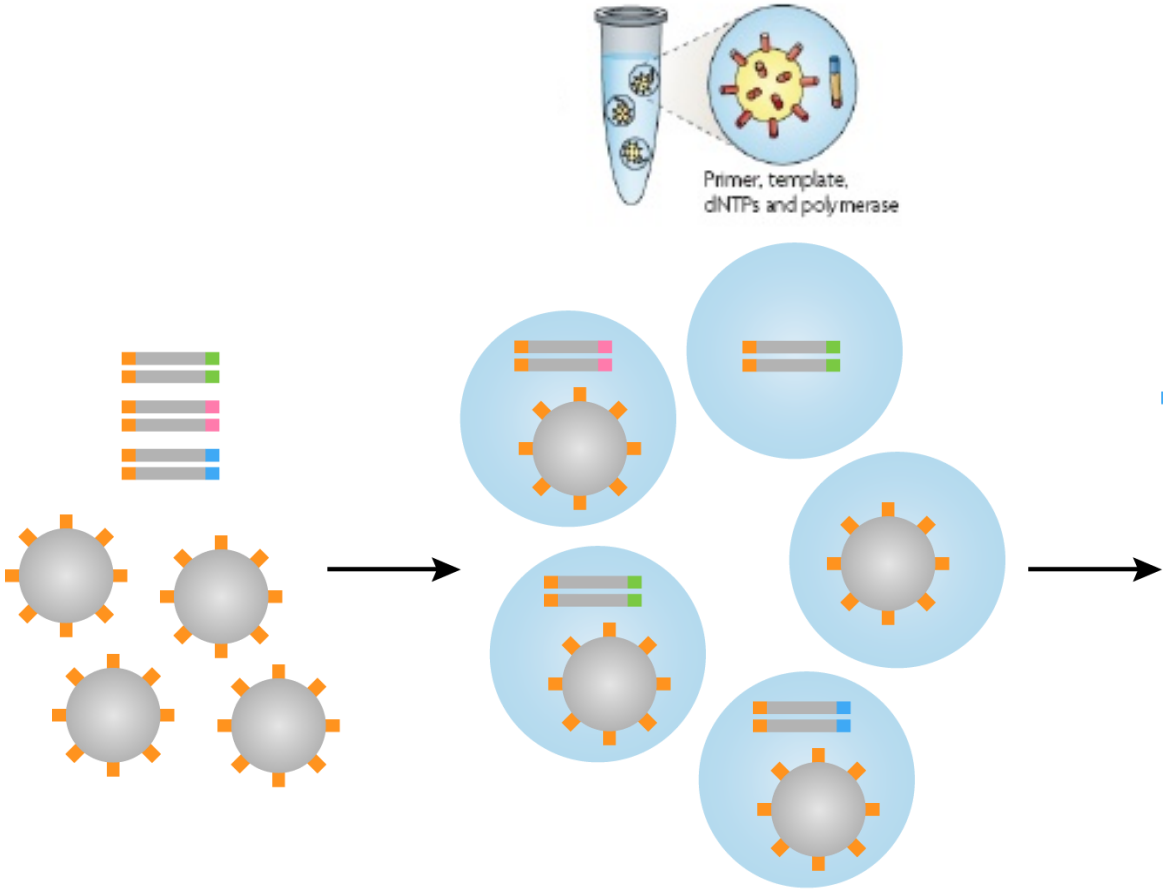
1- the oil/ water emulsion _ePCR_emulsion PCR
Roche 454, SOLID, Ion PGM

2- immobilisation on solid phase with an oligonucleotide (primer) and bridge amplification
Illumina

2nd Generation sequencing

Amplification

1- Emulsion PCR (Roche 454, SOLID, Ion PGM)



2nd Generation sequencing

Amplification

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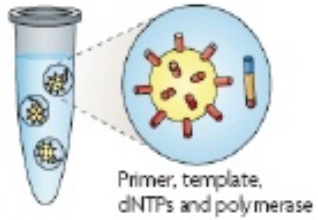
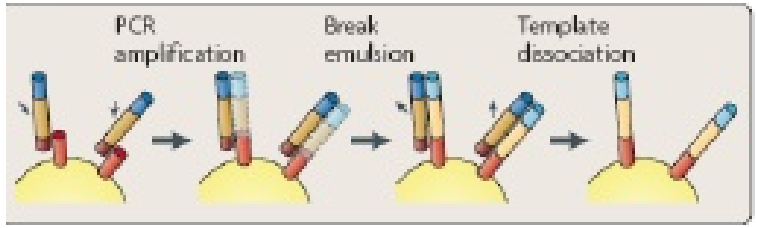
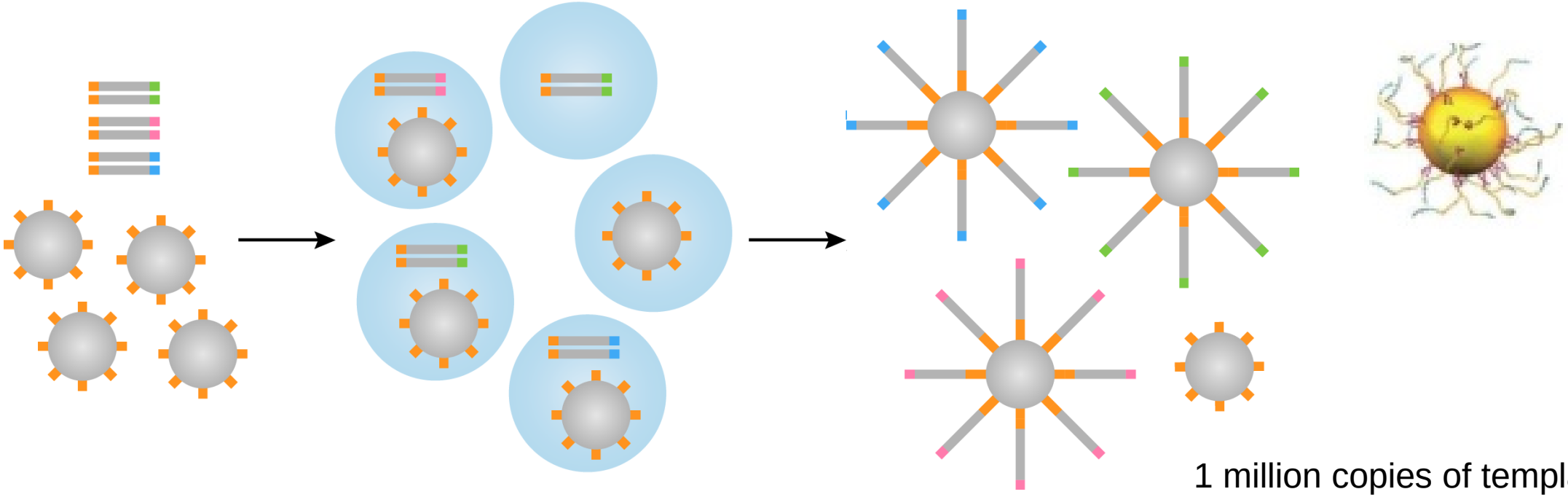




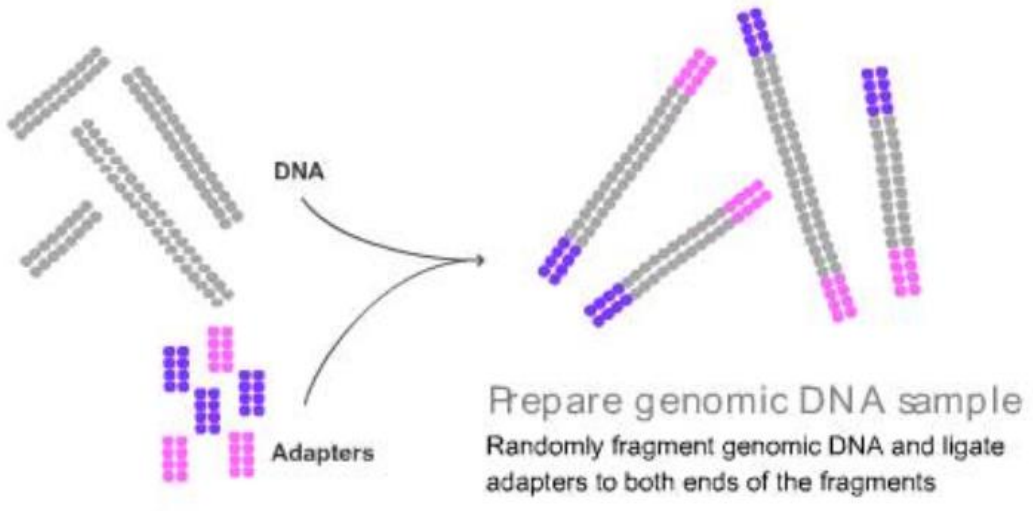


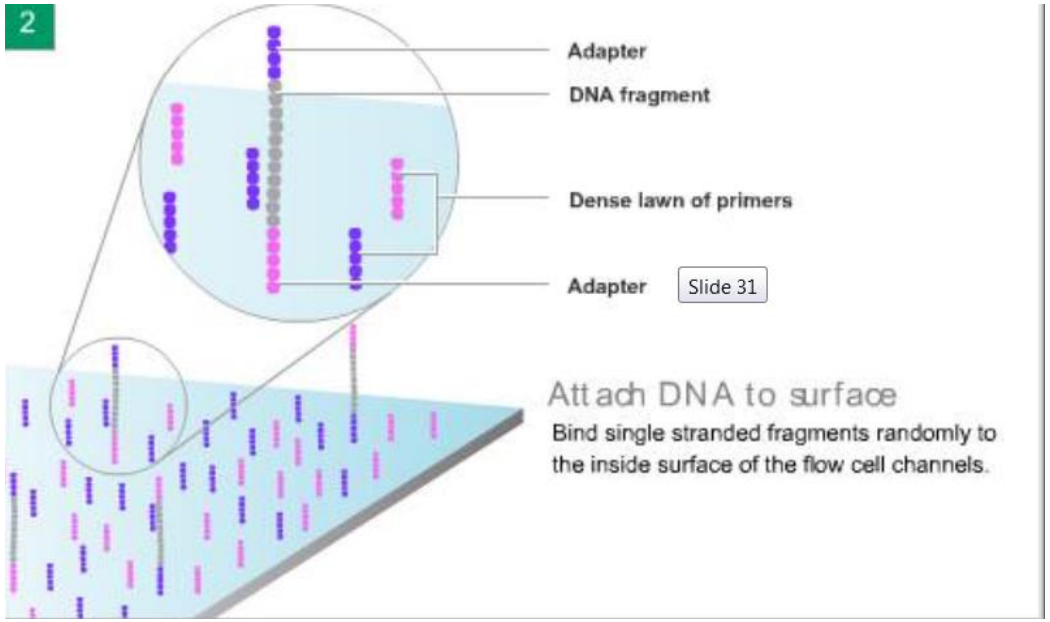
1 million copies of template/bead

2nd Generation sequencing

Amplification

1- immobilisation on solid phase with an oligonucleotide (primer) and bridge amplification (Illumina)





2nd Generation sequencing

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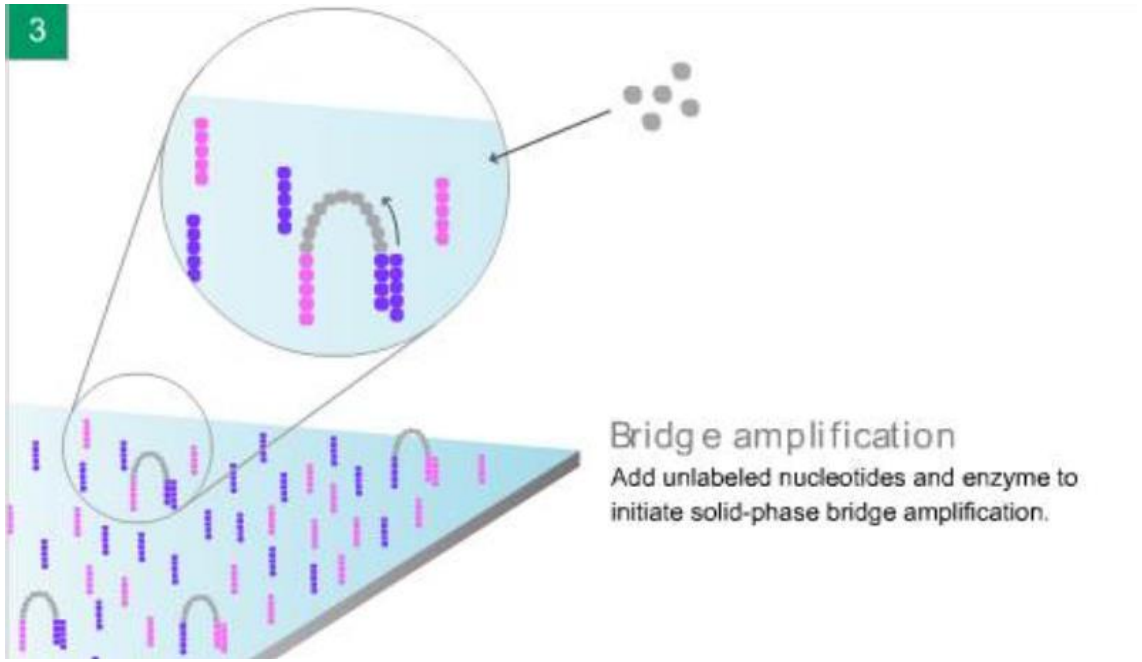

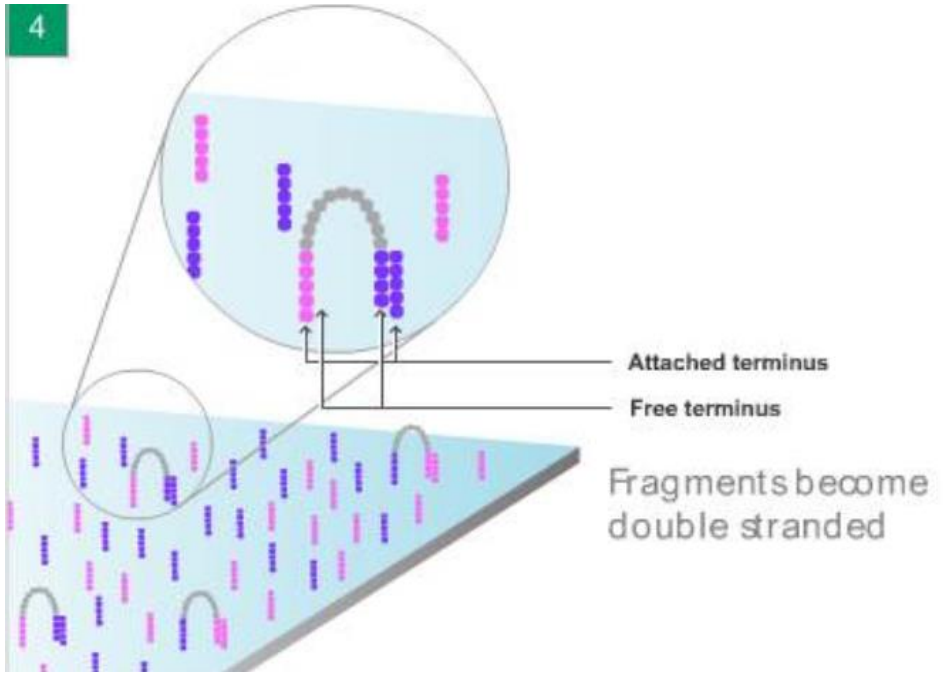

2nd Generation sequencing

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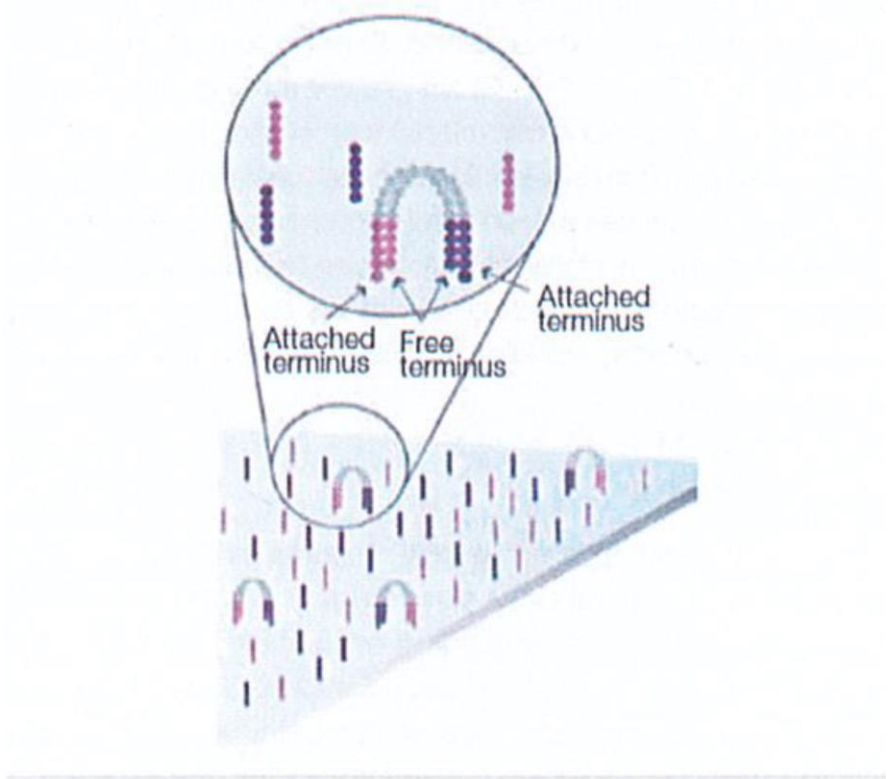
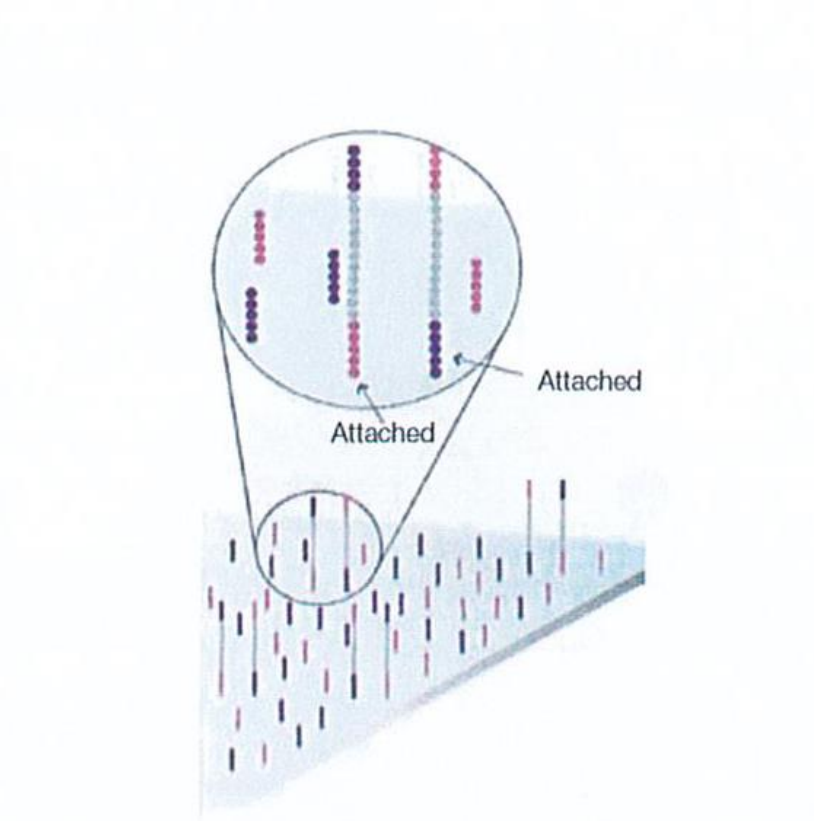


The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

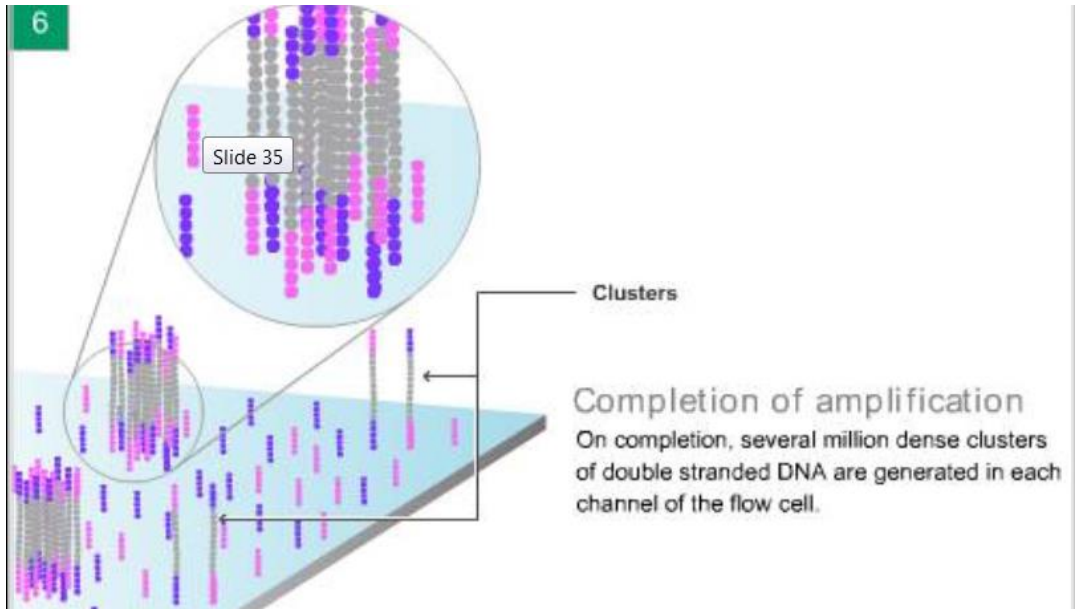


Denaturation leaves single-stranded templates anchored to the substrate.

2nd Generation sequencing

Amplification

1- immobilisation on solid phase with an oligonucleotide (primer) and bridge amplification (Illumina)



Sequencing

Several types of sequencing dependent on the sequencer.

1- Pyrosequencing
Roche 454

2- sequencing by ionic detection
Ion PGM_Ion Proton_IonS5 by Ion Torrent / Thermo Fisher

3- sequencing by ligation
SOLID

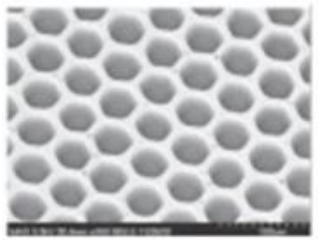
4- sequencing by four-color reversible termination
Illumina

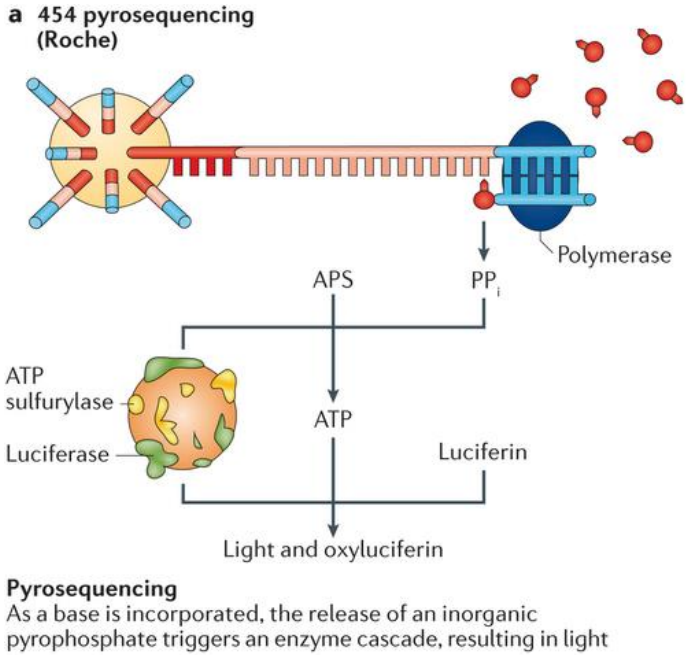
2nd Generation sequencing

Sequencing

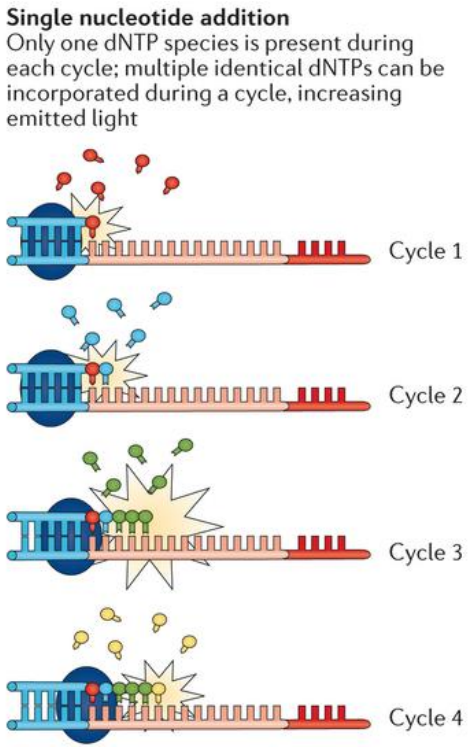
1- Pyrosequencing _ Roche 454

Platform	Label	Reads per run	Read length (mode or average)	Bases per run (gigabases)
ABI Sanger	ABI Sanger 3730xl	96	800	0.0000768
454	**454 GS FLX**	**1 million**	**700**	**0.7**





Pyrosequencing
As a base is incorporated, the release of an inorganic pyrophosphate triggers an enzyme cascade, resulting in light



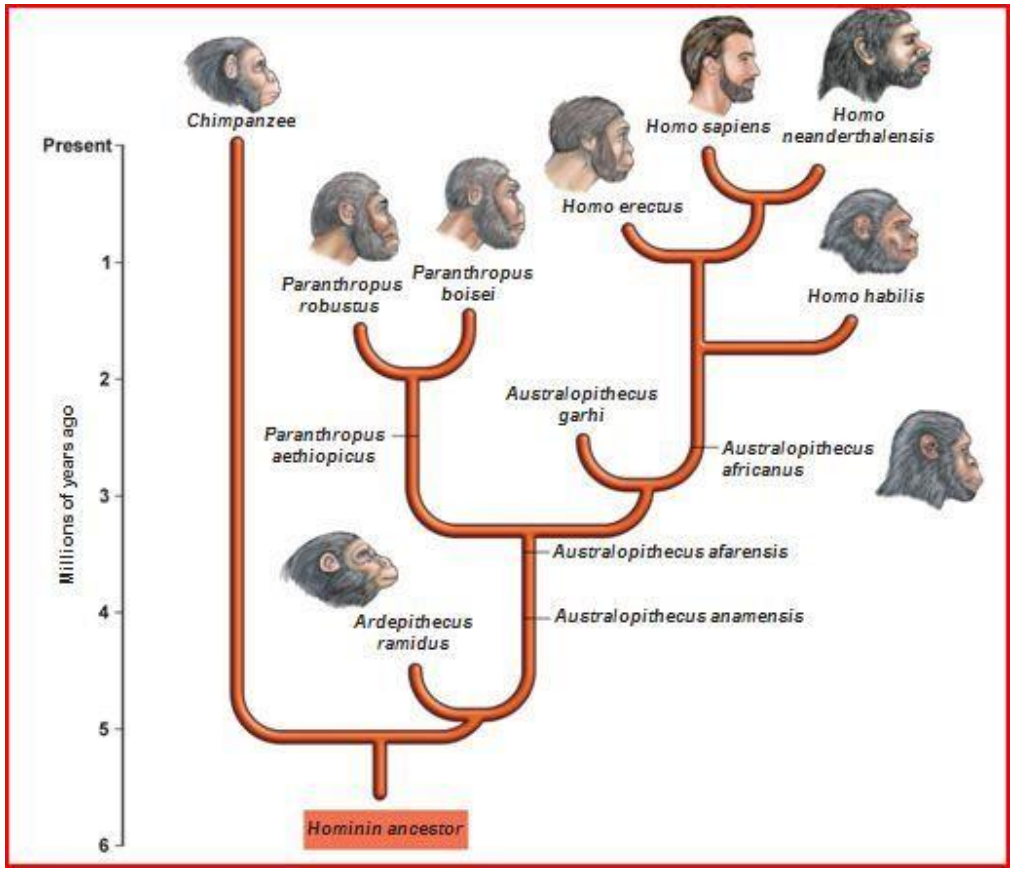
Roche

450,000 $

2nd Generation sequencing

Sequencing

1- Pyrosequencing _ Roche 454



Analysis of one million base pairs of Neanderthal DNA

Nature **444**, 330-336 (16 November 2006) | doi:10.1038/nature05336

A complete Neandertal mitochondrial genome sequence determined by high-throughput sequencing

Cell. 2008 August 8; 134(3): 416–426 | doi:10.1016/j.cell.2008.06.021

2nd Generation sequencing

Sequencing

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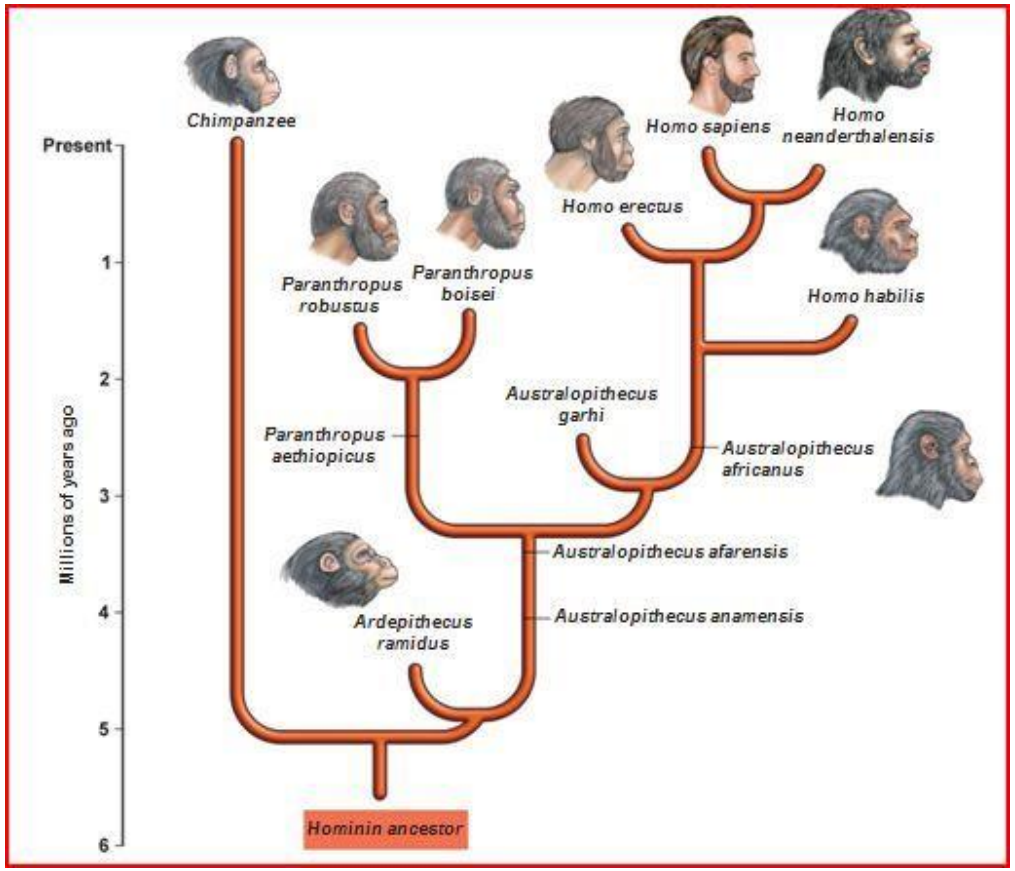

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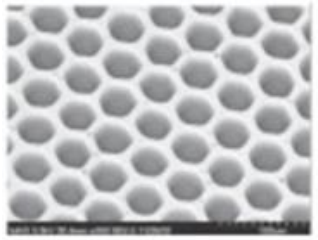
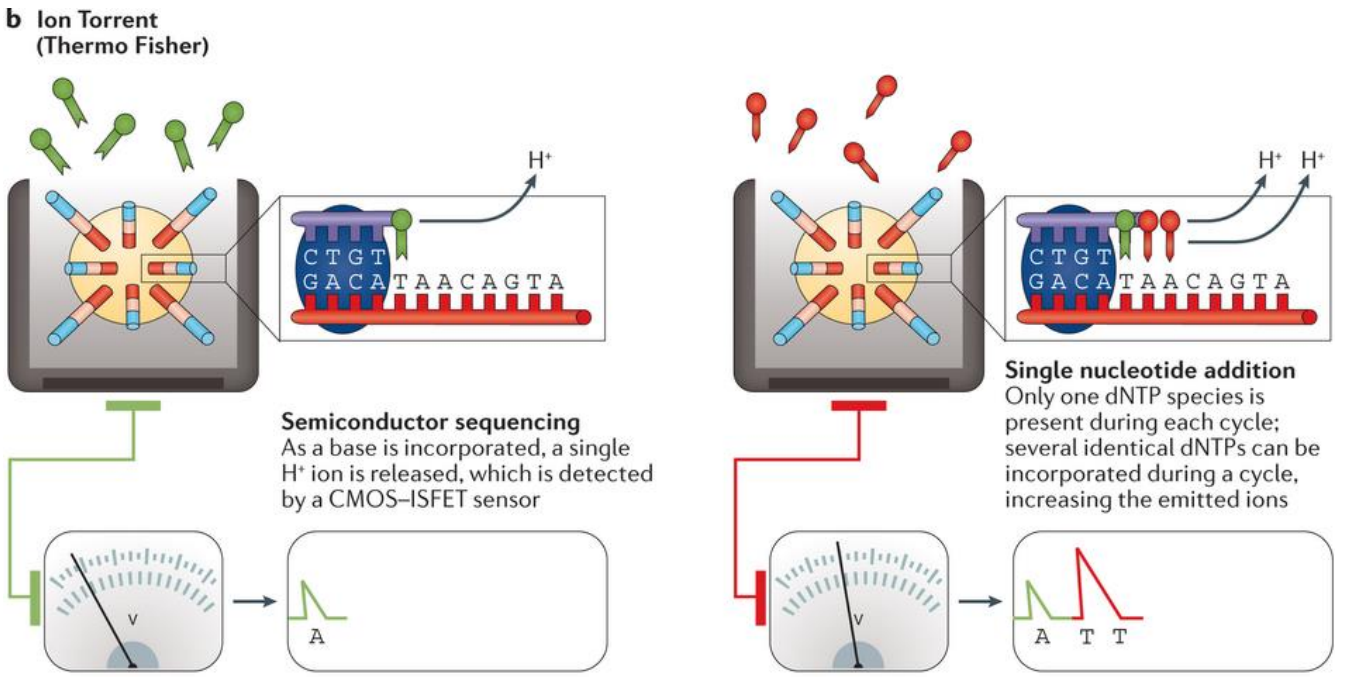
2nd Generation sequencing

Sequencing

2- Sequencing by ionic detection _Ion Torrent

Platform	Reads per run	Read length (mode or average)	Bases per run (gigabases)
ABI Sanger	96	800	0.0000768
454	1 millions	700	0.7
Ion Torrent	**75 millions**	**200**	**15**





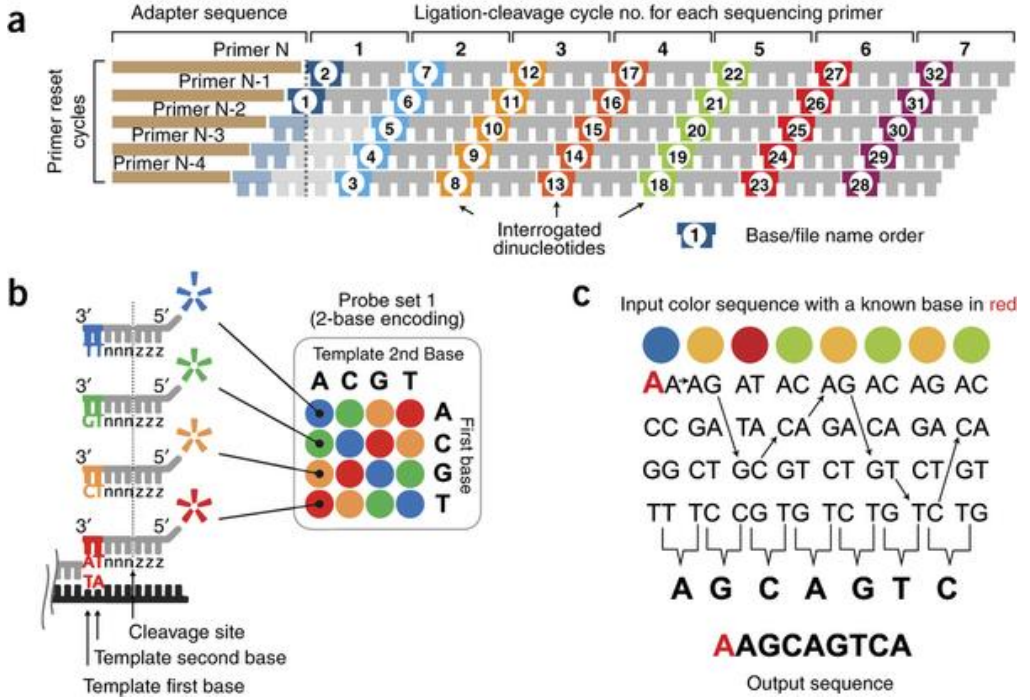
49- 65,000$

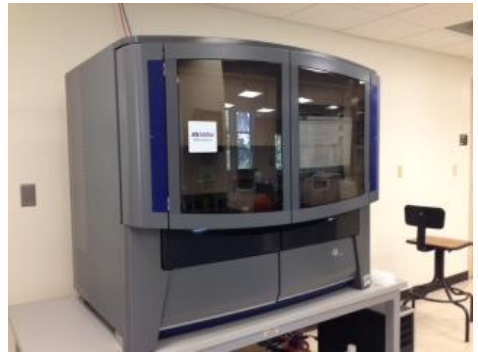
2nd Generation sequencing

Sequencing

3- Sequencing by ligation

Platform	Reads per run	Read length (mode or average)	Bases per run (gigabases)
ABI Sanger	96	800	0.0000768
454	1 millions	700	0.7
IonTorrent	75 millions	200	15
SOLiD	**3 billions**	**75**	**320**



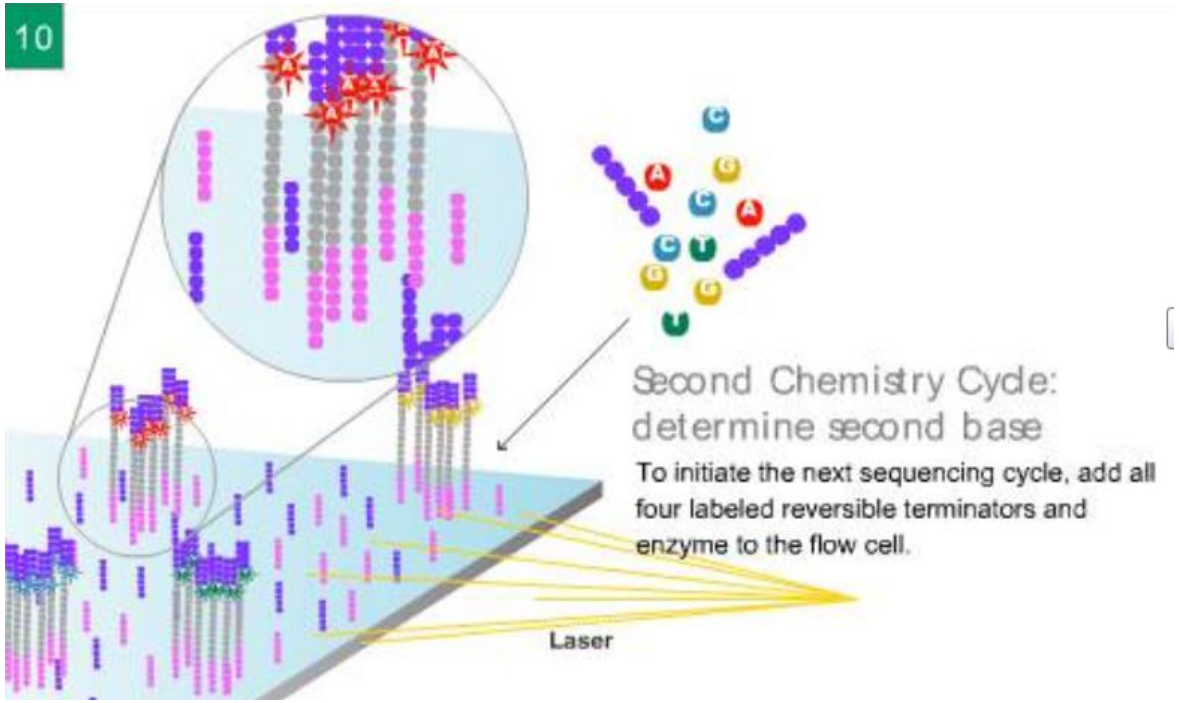


251,000 $

Sequencing

illumina®

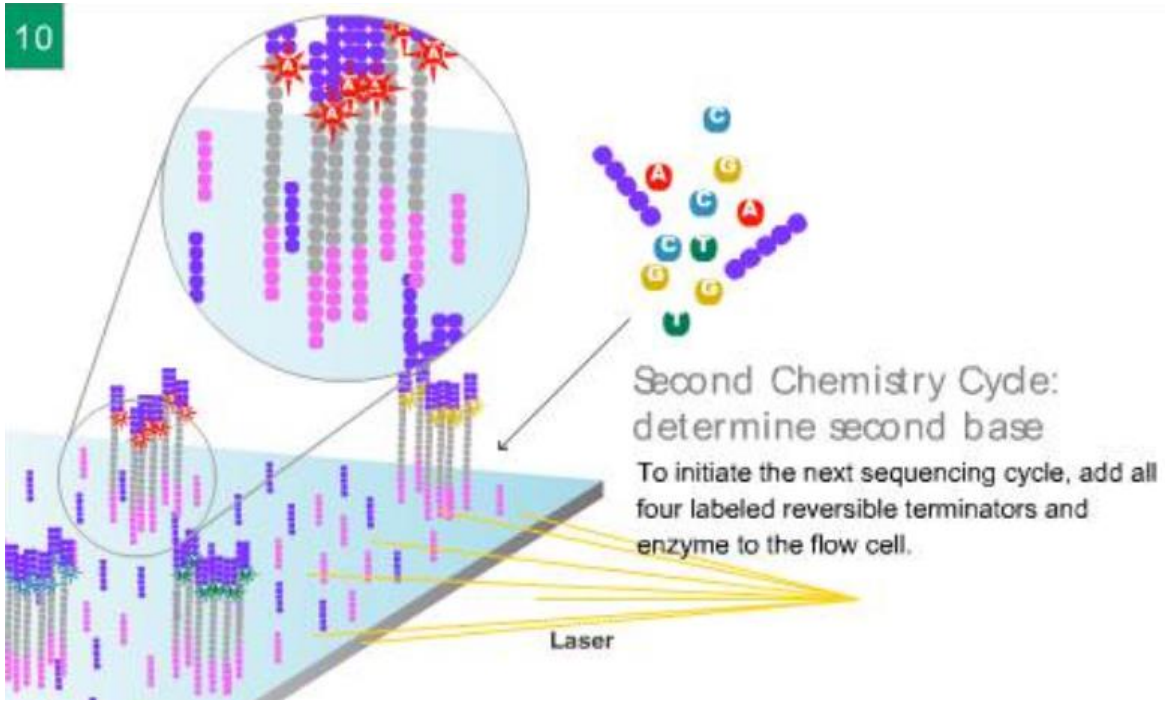
3- Sequencing by four-color reversible termination_Illumina



Sequencing

illumina®

3- Sequencing by four-color reversible termination_Illumina



Sequencing

illumina®

3- Sequencing by four-color reversible termination_Illumina

9

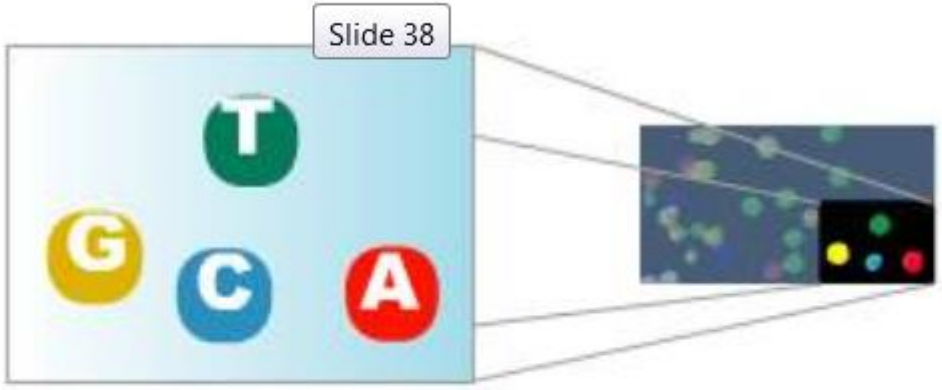


Image of first chemistry cycle

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

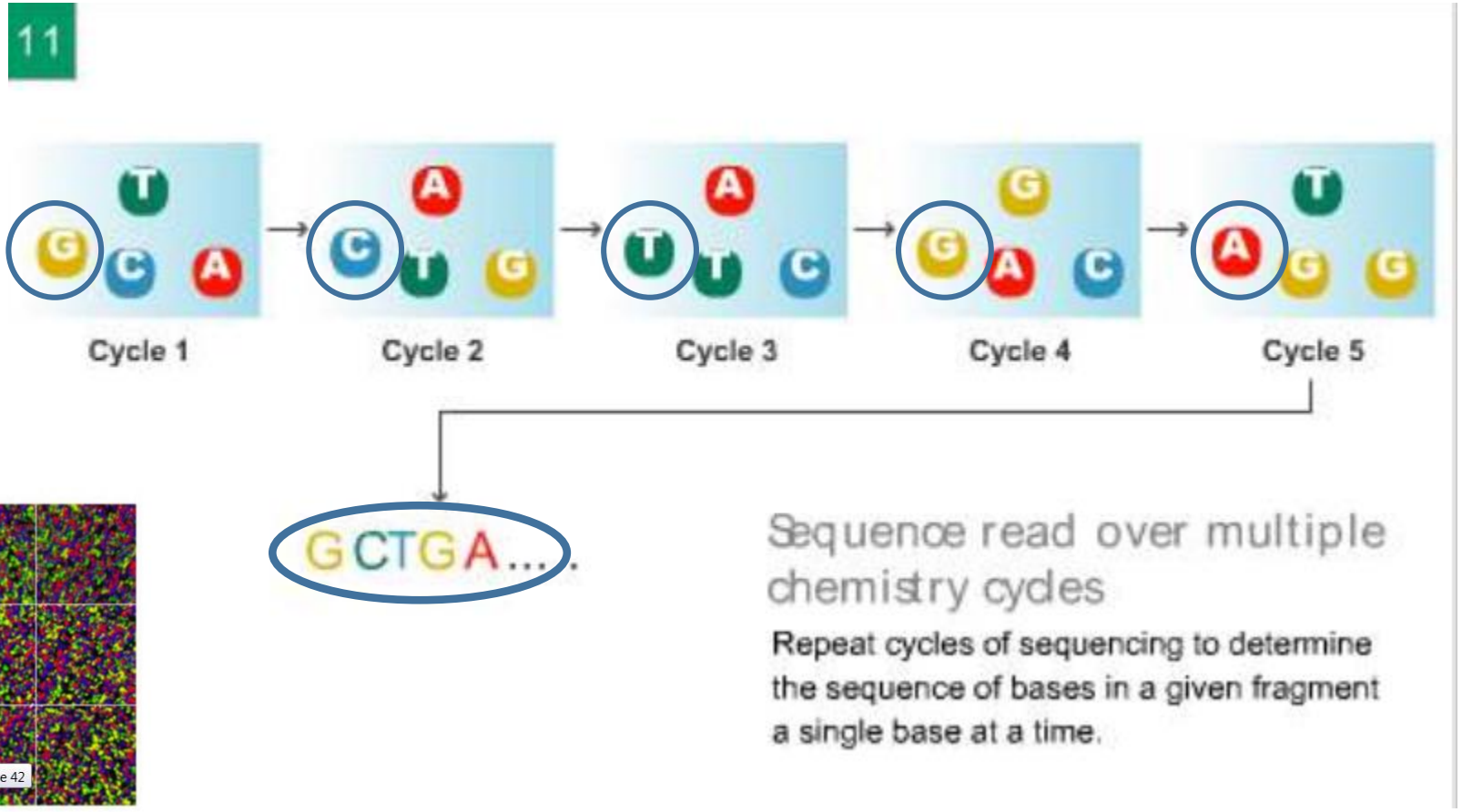
The blocked 3' terminus and the fluorophore from each incorporated base are removed.

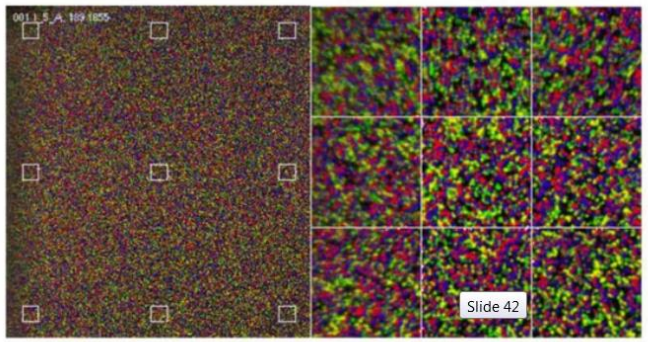
2nd Generation sequencing

Sequencing

illumina®

3- Sequencing by four-color reversible termination_Illumina





2nd Generation sequencing

Sequencing

illumina®

Platform	Reads per run	Read length (mode or average)	Bases per run (gigabases)
ABI Sanger	96	800	0.0000768
454	1 millions	700	0.7
IonTorrent	75 millions	200	15
SOLiD	3 billions	75	320
Illumina	**600 millions to 6 milliards**	**100 to 300**	**7.5 to 2 000**

MiniSeq System

Up to 7.5 Gb

MiSeq Series ⊕

Up to 15 Gb

NextSeq Series ⊕

Up to120 Gb

NextSeq Series ⊕

Up to120 Gb

HiSeq Series ⊕

Up to 750 Gb

HiSeq X Series†

Up to 800 Gb

NovaSeq Series ⊕

Up to 2 Tb

2nd Generation sequencing

Sequencing

illumina®

Platform	Reads per run	Read length (mode or average)	Bases per run (gigabases)
ABI Sanger	96	800	0.0000768
454	1 millions	700	0.7
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Illumina	**600 millions to 6 milliards**	**100 to 300**	**7.5 to 2 000**

MiniSeq System

50,000 $

MiSeq Series

99,000 $

NextSeq Series

250,000 $

NextSeq Series

250,000 $

HiSeq Series

690,000 $-
900,000 $

HiSeq X Series†

1,000,000 $
(X10= 10,000,000 $)

NovaSeq Series

850,000 $-
985,000 $

2nd Generation sequencing

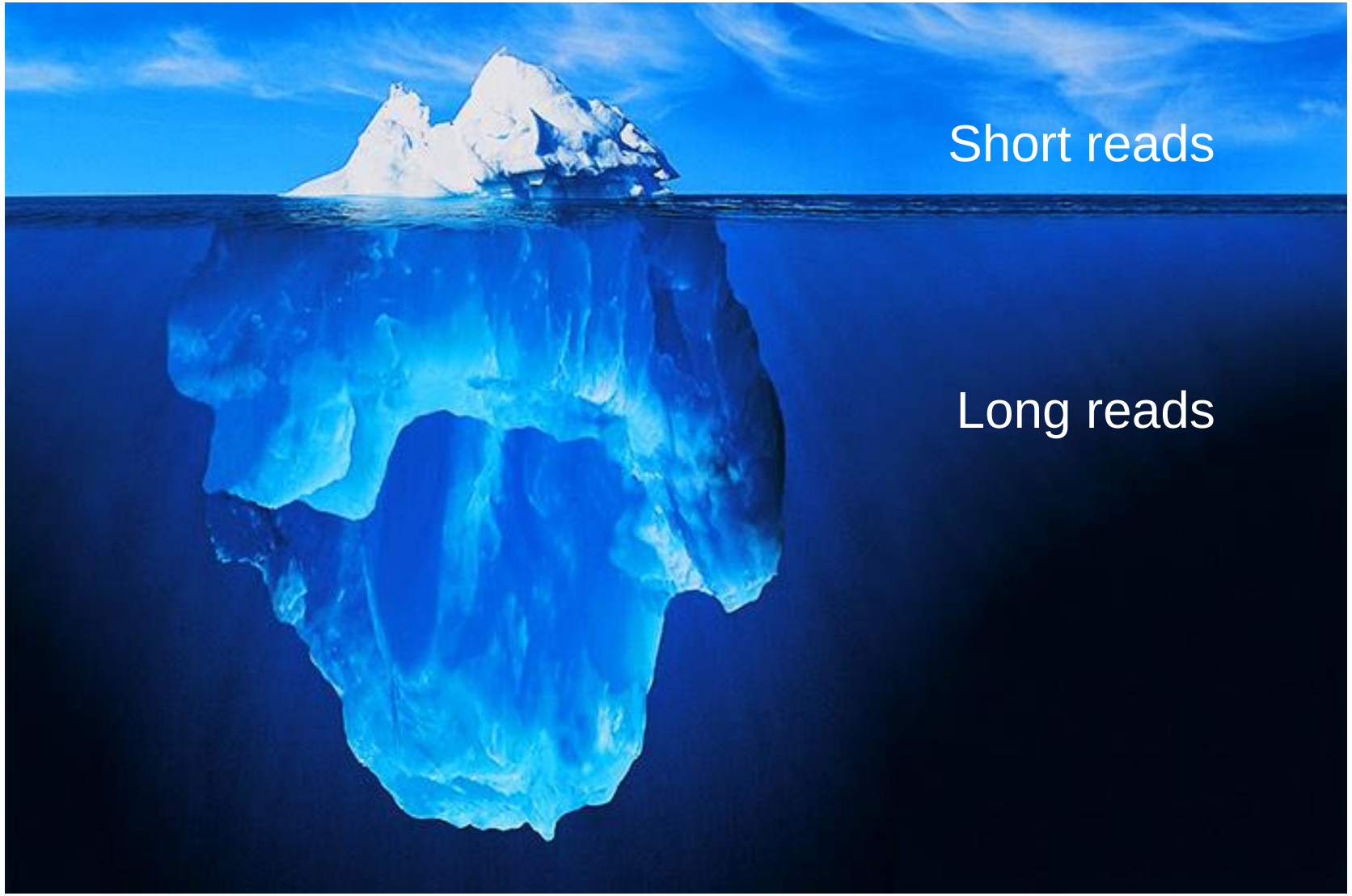
+Advantages

- High throughput technology
- Very good quality of the sequences, error rate< 0.1 %

-Limitations

- Process of reverse transcription
- Secondary structure of RNA
- PCR-biais
- Sequences with high AT do not amplify as well as GC-normal one
- Short reads

2nd Generation sequencing



3rd Generation sequencing

Since 2012, a new cohort of techniques has been developed:

- using single molecule sequencing
- single real time sequencing
- removing the need for clonal amplification.

This :

- reduces errors caused by PCR
- simplifies library preparation
- and, most importantly, gives a much higher read length

PACIFIC BIOSCIENCES®

Oxford NANOPORE Technologies®

3rd Generation sequencing

Single molecule Long Read Sequencing

3rd Generation sequencing

Single molecule Long Read Sequencing

PACIFIC BIOSCIENCES®

Platform	Year	Reads per run	Read length (mode or average)	Bases per run (gigabases)
ABI Sanger	2002	96	800	0.0000768
454	2011	1 millions	700	0.7
SOLiD	2013	3 milliards	75	320
IonTorrent	2015	75000000	200	15
Illumina	2016	600 millions to 6 milliards	100 to 300	7.5 to 2 000
PacBio	**2014**	**660000**	**13500**	**20**

RSII 700,000 $

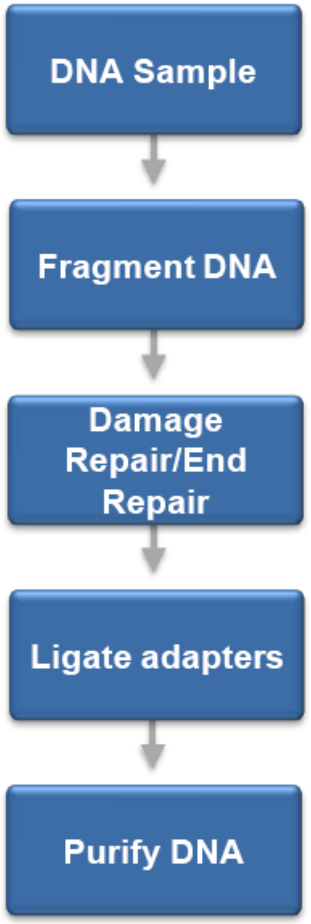
Sequel 350,000 $

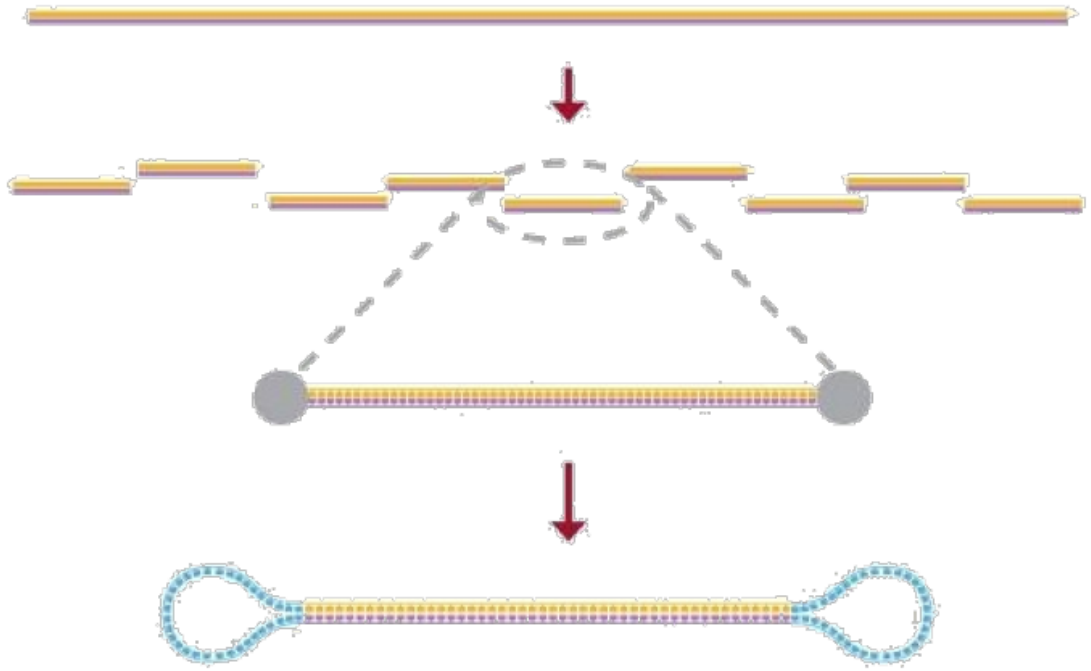
Roche

3rd Generation sequencing

Single molecule Long Read Sequencing

PACIFIC BIOSCIENCES®



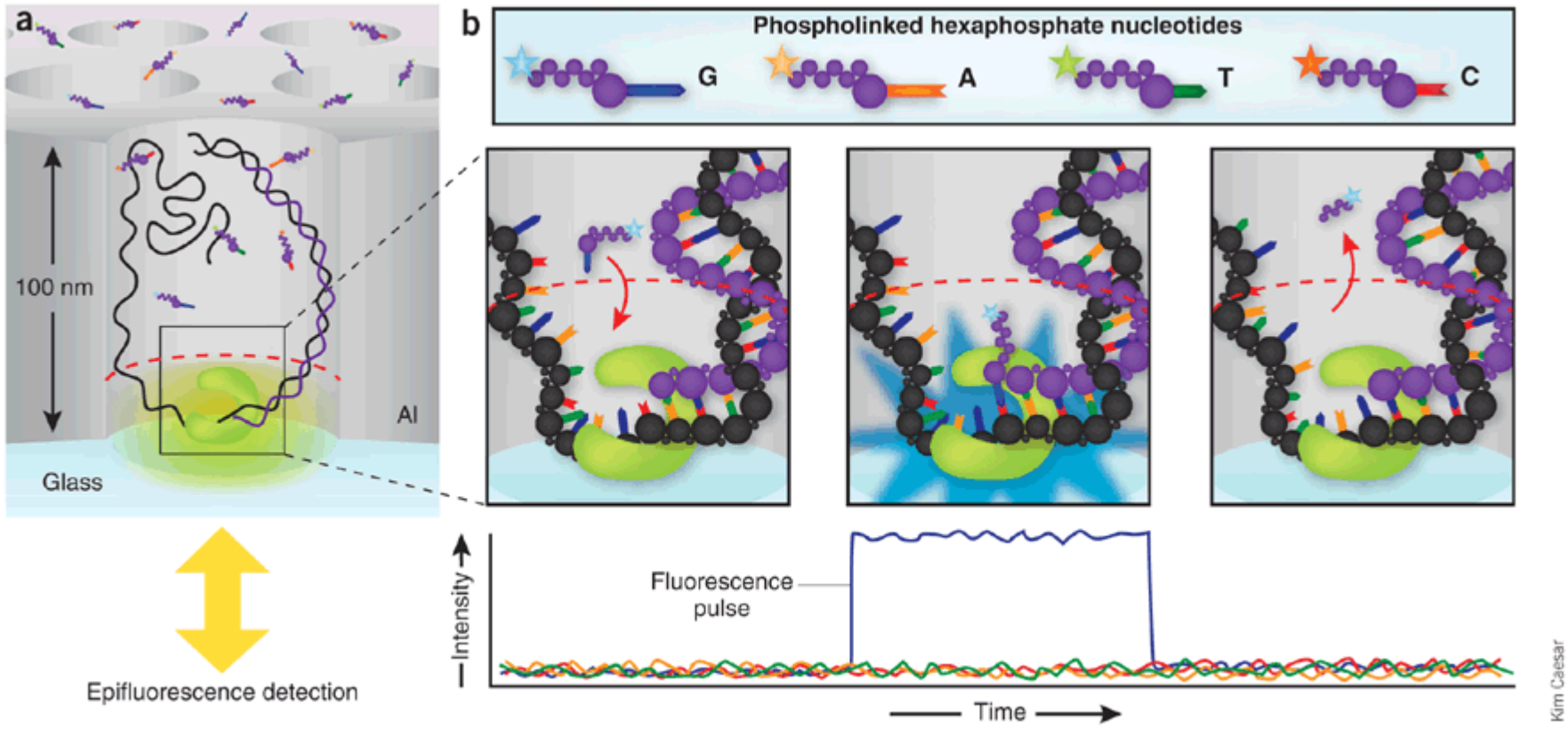


SMRTbell™ Template preparation can be used to create libraries of various insert sizes from 250 bp to 20,000 bp depending on the needs of the application.

3rd Generation sequencing

Single molecule Long Read Sequencing

PACIFIC BIOSCIENCES®



3rd Generation sequencing

Single molecule Long Read Sequencing

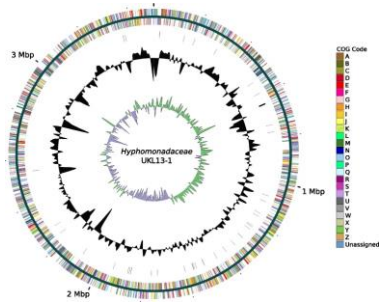
PACIFIC BIOSCIENCES®

Application: **Whole genome**

Jarvis et al. (2017) Nature

Bickhart et al. (2017) Nature genetics

Brozynska et al. (2016) Plant Biotechnology Journal



Driscoll et al. (2017) Standards in Genomic Sciences

Seo et al. (2016) Nature

INRA TOULOUSE/SUNRISE PROJECT

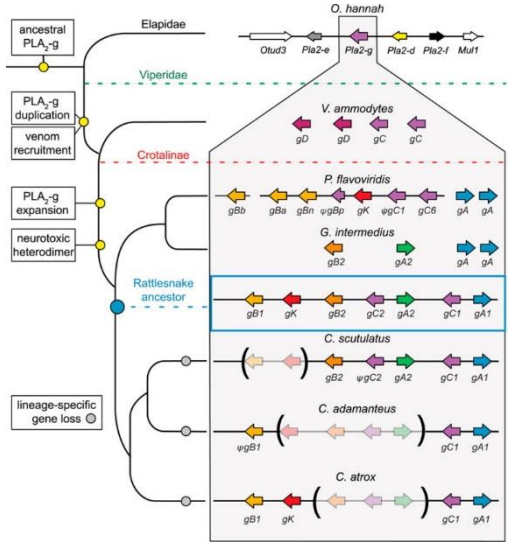
3rd Generation sequencing

Single molecule Long Read Sequencing

PACIFIC BIOSCIENCES®

Application: **Targeted sequencing**

to fully characterize genetic complexity — including structural variation, rare SNPs, indels, copy number variation, microsatellites, haplotypes, and phasing



Evolution of PLA2 related toxins

Dowell et al. (2016) Current Biology

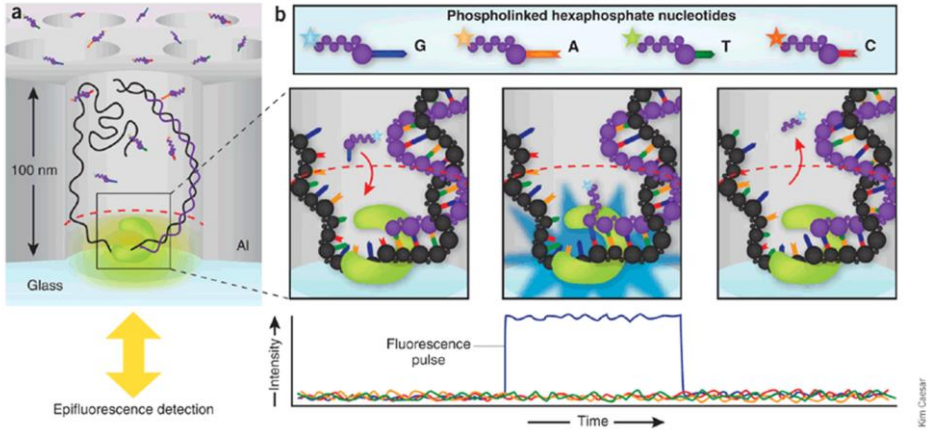
3rd Generation sequencing

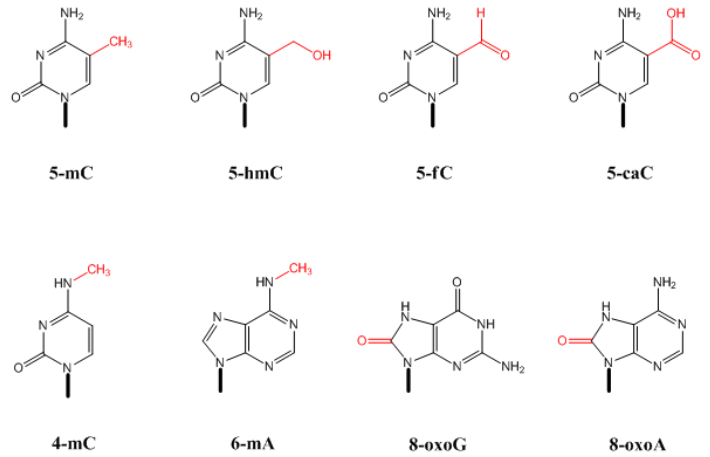
Single molecule Long Read Sequencing

PACIFIC BIOSCIENCES®

Application: **Epigenetics**

Single Molecule, Real-Time (SMRT) Sequencing directly detects epigenetic modifications by measuring kinetic variation during base incorporation. By capturing these modifications simultaneously with sequence data, this method eliminates the need for special sample preparation and additional sequencing.





3rd Generation sequencing

Single molecule Long Read Sequencing

PACIFIC BIOSCIENCES®

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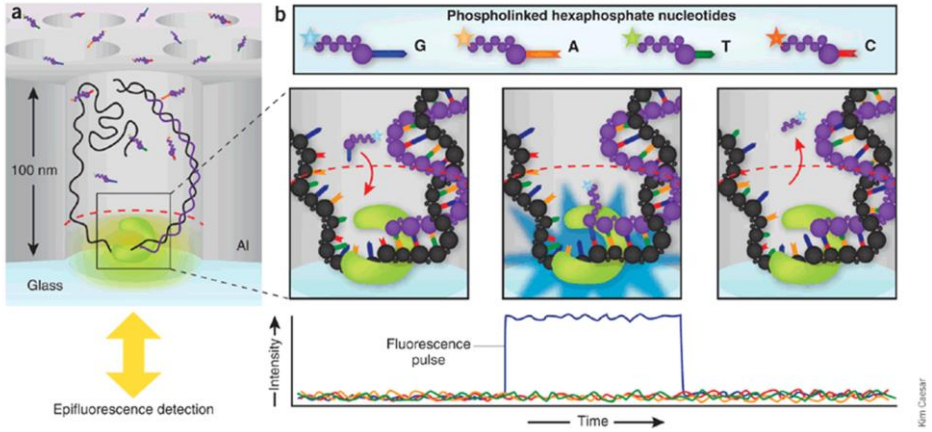
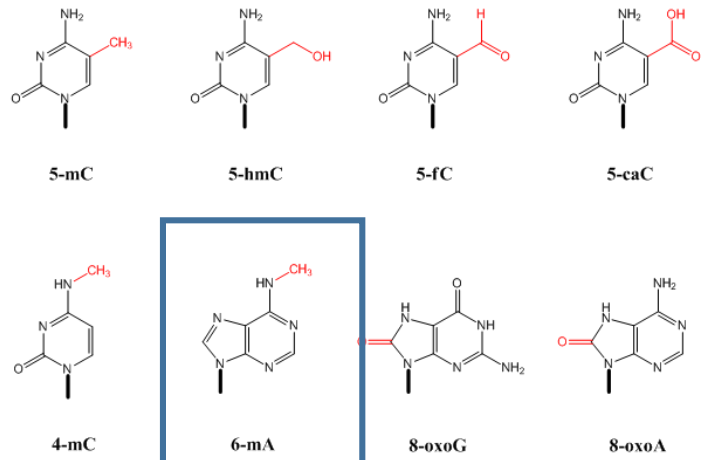




Role in gene activation

Wu et al. (2016) Nature

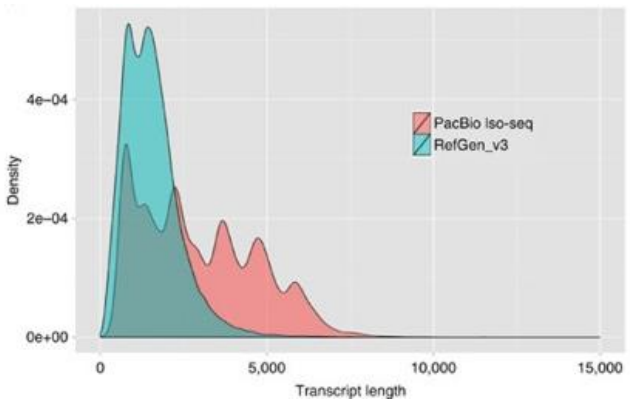
3rd Generation sequencing

Single molecule Long Read Sequencing

PACIFIC BIOSCIENCES®

Application: **RNA Sequencing**

- The isoform sequencing (Iso-Seq) application generates full-length cDNA sequences — from the 5’ end of transcripts to the poly-A tail —transcriptome reconstruction
- The Iso-Seq method generates information without about alternatively spliced exons and transcriptional start sites. It also delivers information about poly-adenylation sites for transcripts up to 10 kb.



Wang et al. (2016) Nature Communications

Workman et al. (2017) *bioRxiv*

3rd Generation sequencing

Single molecule Long Read Sequencing

Oxford NANOPORE Technologies®

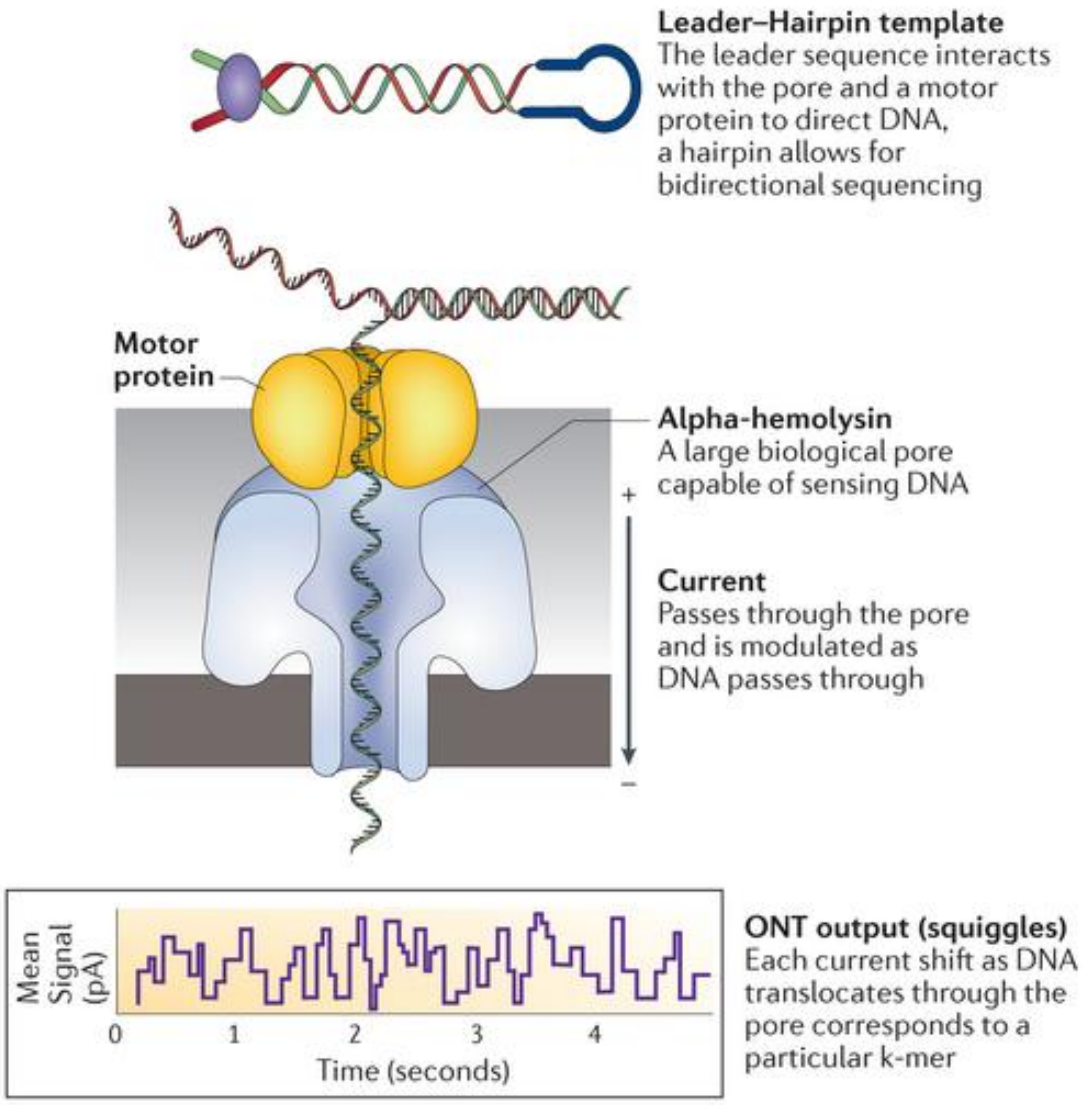
Platform	Reads per run	Read length (mode or average)	Bases per run (gigabases)
ABI Sanger	96	800	0.0000768
454	1 millions	700	0.7
SOLiD	3 milliards	75	320
IonTorrent	75000000	200	15
Illumina	600 millions to 6 milliards	100 to 300	7.5 to 2 000
PacBio	660000	13500	12.000
Oxford Nanopore	**4400000**	**9545**	**42**

MinION 1,000 $

3rd Generation sequencing

Single molecule Long Read Sequencing

Oxford NANOPORE Technologies®



3rd Generation sequencing

Single molecule Long Read Sequencing

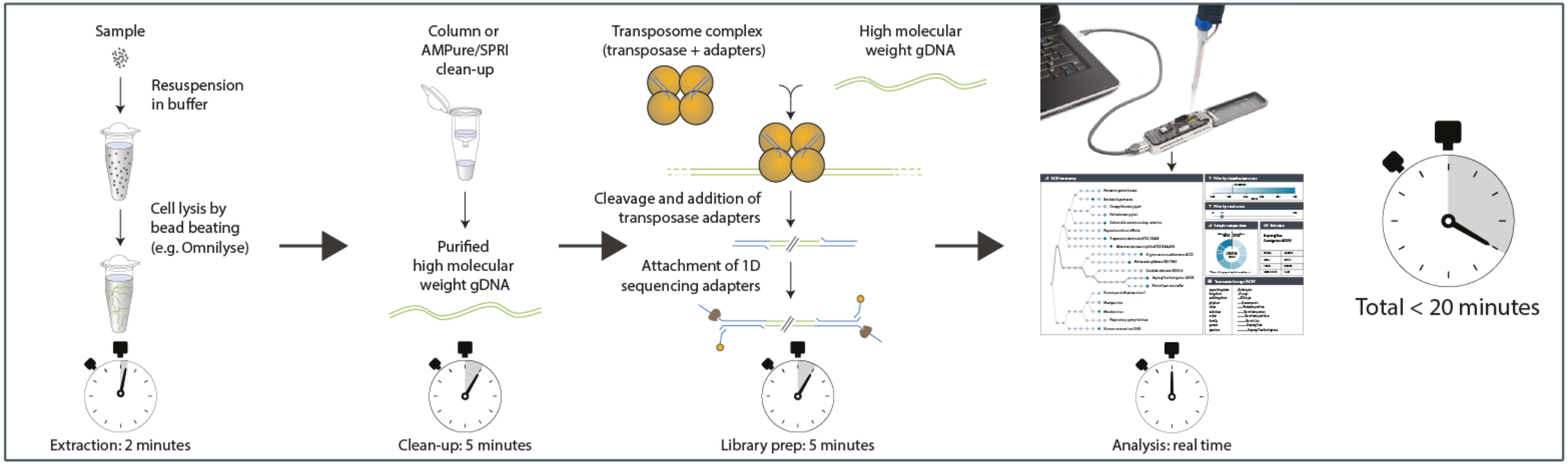
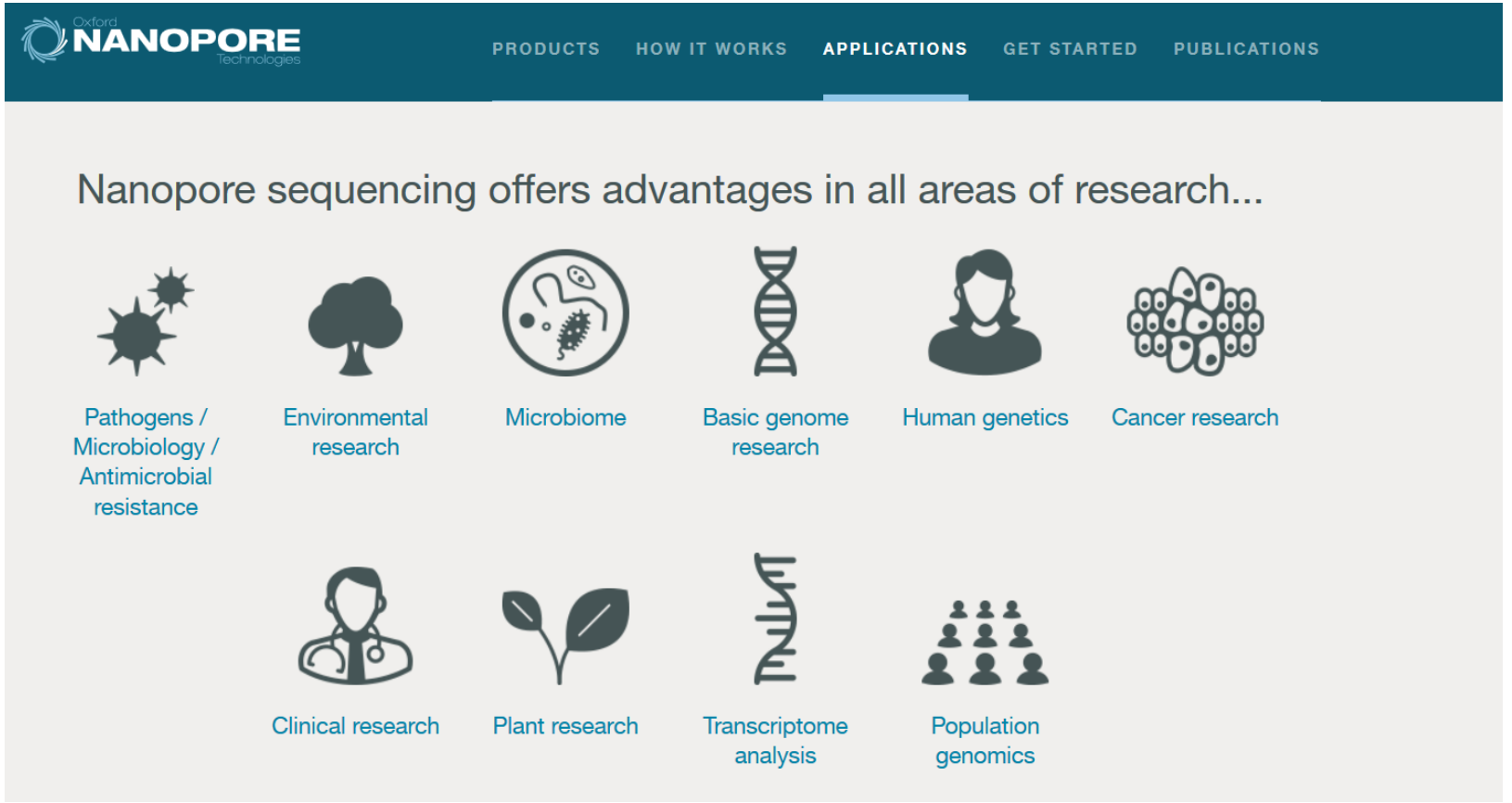


Fig. 1 Identification of bacterial, archaeal, fungal and viral species using rapid gDNA library preparation and the real-time WIMP analysis workflow

3rd Generation sequencing

Single molecule Long Read Sequencing

Oxford NANOPORE Technologies®



3rd Generation sequencing

Single molecule Long Read Sequencing

Oxford NANOPORE Technologies®

Application: **Microbiome**

3rd Generation sequencing

Single molecule Long Read Sequencing

Oxford NANOPORE Technologies®

Application: **Microbiome**

Bork et al., (2015) Science

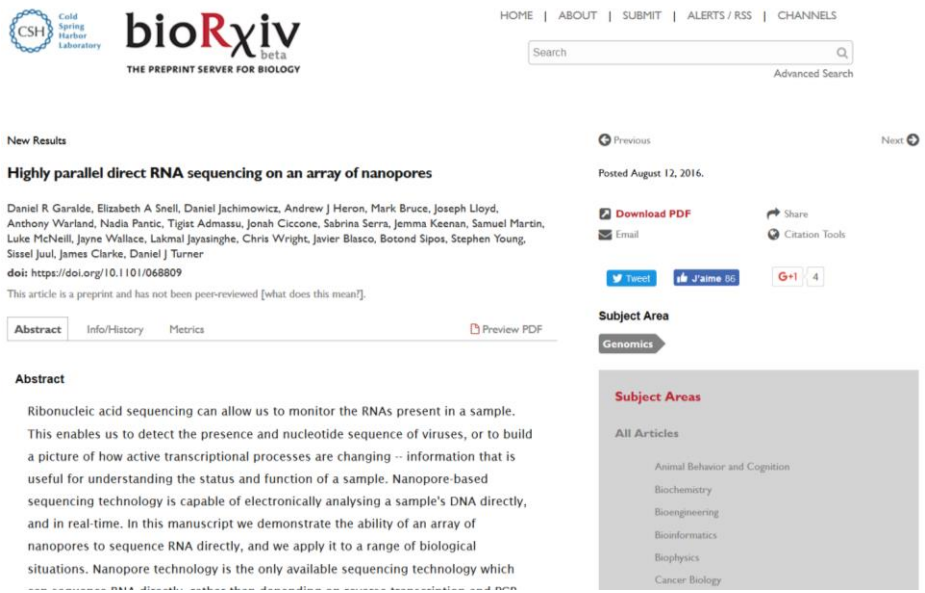
3rd Generation sequencing

Single molecule Long Read Sequencing

Oxford NANOPORE Technologies®

Application: **RNA Sequencing**

Nanopore technology is the only available sequencing technology which can sequence RNA directly



Garalde et al., (2016) bioRxiv

3rd Generation sequencing

Single molecule Long Read Sequencing

Oxford NANOPORE Technologies®

Next….

GridION

MinION X5

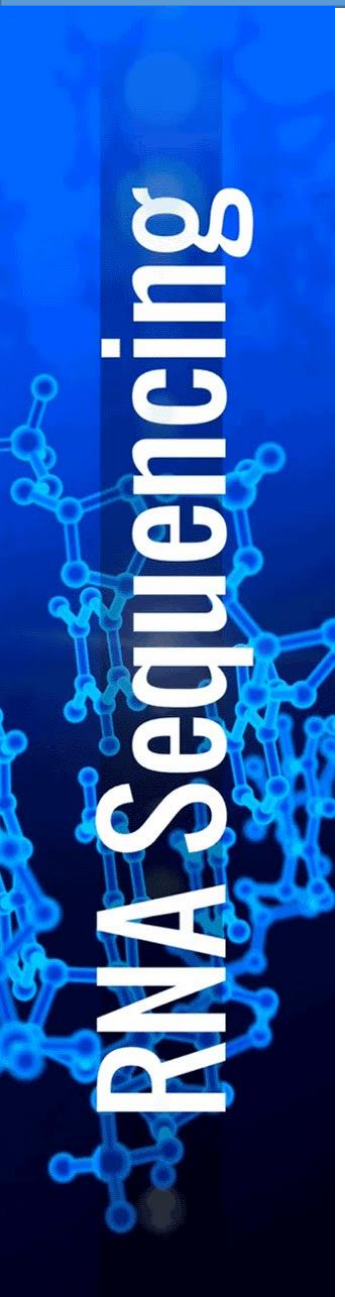
PromethION

MinION X48

SmidgION

Always more….

Application of RNA sequencing



RNA Sequencing provides insight into :

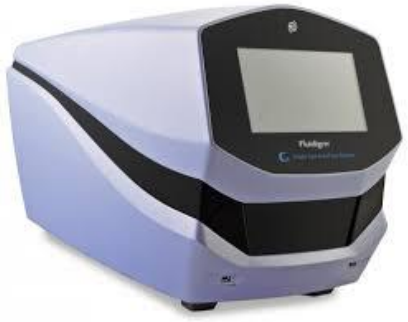
- **gene expression analysis**
- **discovery of novel transcripts**
- **identification of alternatively spliced genes**
- **detection of allele specific expression**
- **de novo RNA sequencing (whithout reference transcriptome)**
- **single cell RNA sequencing**

In addition to polyadenylated messager RNA (mRNA) Transcripts , analysis of différent poppulation of RNAs :

- **Total RNA**
- **Pre-mRNA**
- **Non coding RNA : micro RNA, long ncRNA**
- **tRNA sequencing**

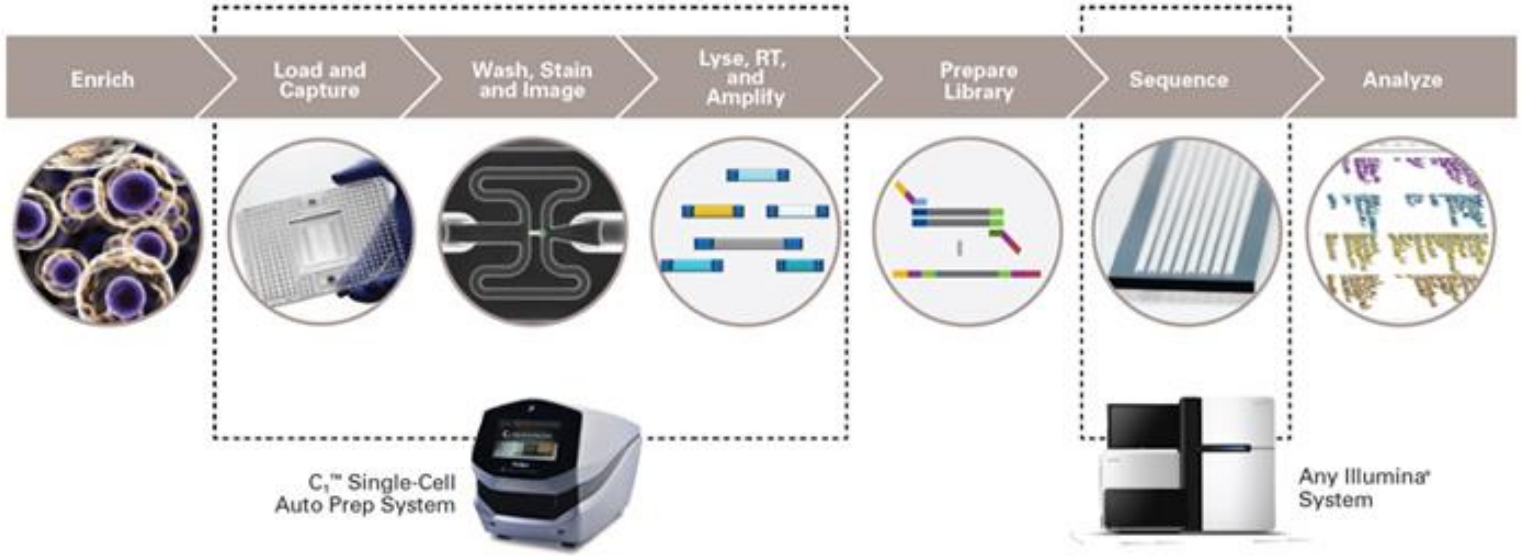
Application of RNA sequencing

Single Cell RNA-Seq



FLUIDIGM®

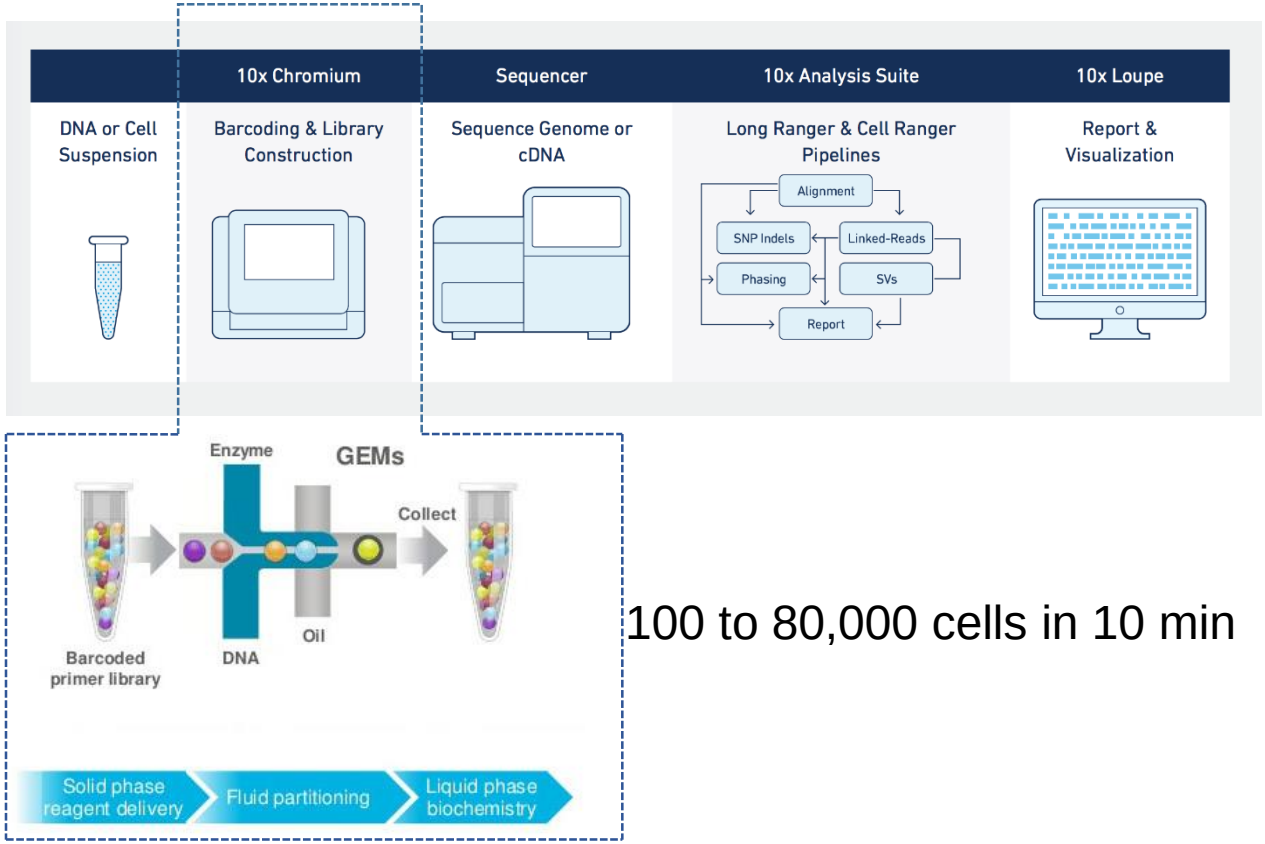
C_1 ™ Single-Cell Auto Prep System
96 cells in 5 min



Application of RNA sequencing

Single Cell RNA-Seq

10x GENOMICS®



100 to 80,000 cells in 10 min

Application of RNA sequencing

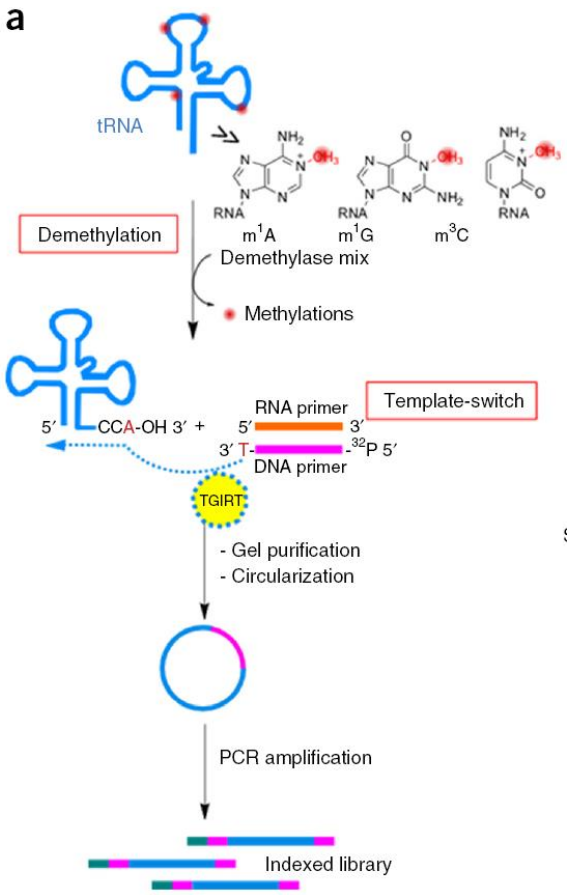
tRNA Sequencing

Efficient and quantitative high-throughput tRNA sequencing

Guanqun Zheng[1,7], Yidan Qin[2,7], Wesley C Clark[1], Qing Dai[3], Chengqi Yi[1,6], Chuan He[1,3–5], Alan M Lambowitz[2,8] & Tao Pan[1,4,8]

Despite its biological importance, tRNA has not been adequately sequenced by standard methods because of its abundant post-transcriptional modifications and stable structure, which and are particularly problematic for reverse transcriptases (RTs), causing cDNA synthesis to stop or incorporate a wrong nucleotide. In mammals, N^1-methyladenosine (m^1A) is present in all tRNAs at position 58, N^3-methylcytosine (m^3C) is present in five tRNAs at position 32 and the variable loop, and N^1-methylguanosine (m^1G) is present in about half of all tRNAs at position 37 or 9. We applied two recombinant enzymes as a mixture to remove these three methylations in human tRNAs. The first was the wild-type enzyme AlkB from *Escherichia coli*, which is known to efficiently demethylate m^1A and m^3C in single-stranded nucleic acids as its DNA and RNA repair function[5,6]. Wild-type AlkB, however, works very poorly on m^1G modification[7]. On the basis of its known three-dimensional structure complexed with nucleic acids, we engineered AlkB to generate a specific mutant, D135S, that

Zheng et al., (2015) Nature Methods



Application of RNA sequencing

Dual RNA-Sequencing

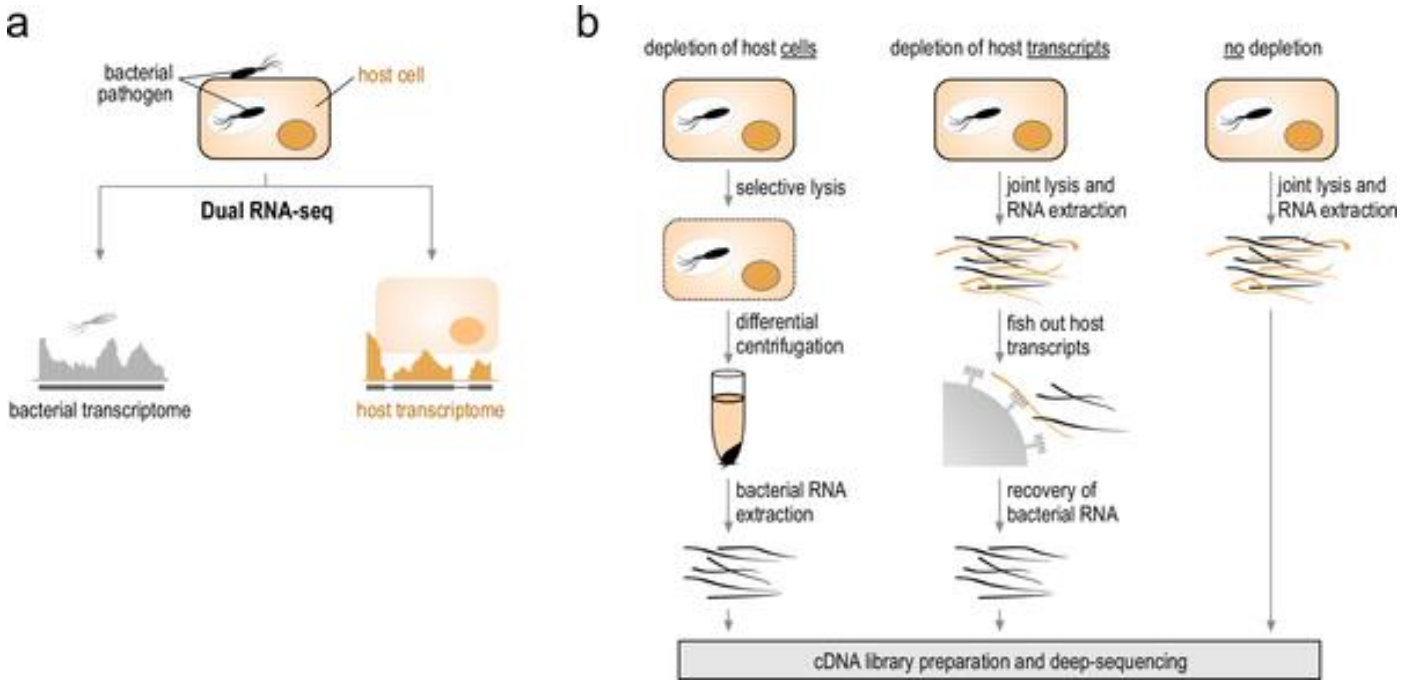
Westermann et al., (2017) Plos Pathogens

PLOS | PATHOGENS

REVIEW

Resolving host–pathogen interactions by dual RNA-seq

Alexander J. Westermann[1☯], Lars Barquist[1☯], Jörg Vogel[1,2*]



Application of RNA sequencing

In situ RNA-Sequencing

Ke et al., (2013) Nature methods

BRIEF COMMUNICATIONS

FISSEQ, Fluorescent In Situ RNA SEQuencing

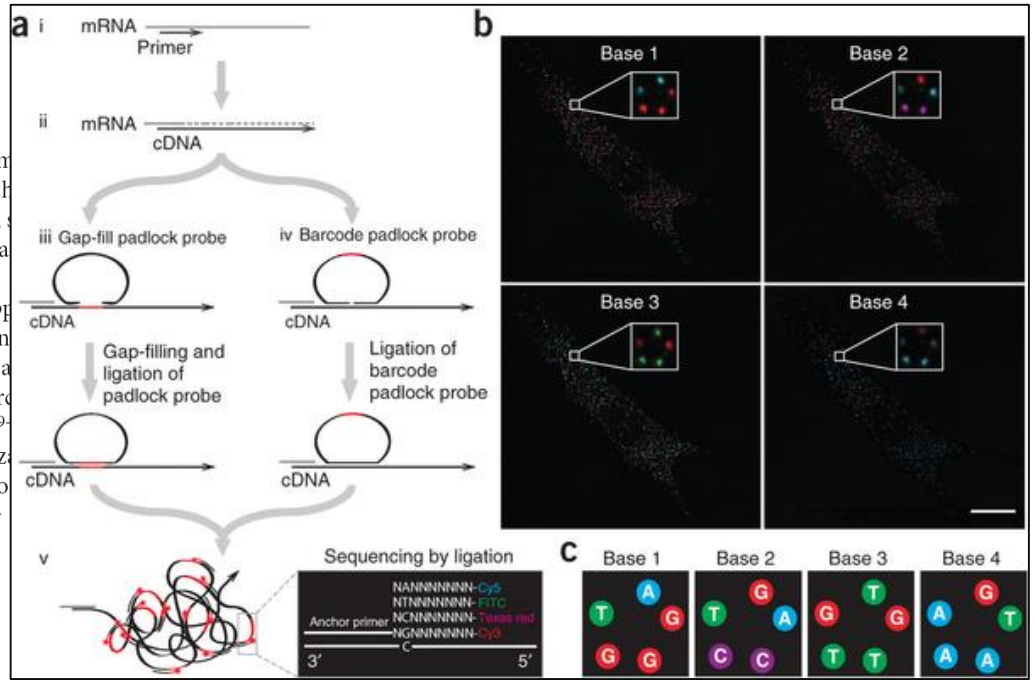
In situ sequencing for RNA analysis in preserved tissue and cells

Rongqin Ke[1,2,5], Marco Mignardi[1,2,5], Alexandra Pacureanu[3], Jessica Svedlund[1], Johan Botling[2], Carolina Wählby[3,4] & Mats Nilsson[1,2]

Tissue gene expression profiling is performed on homogenates

are limited in spatial resolution. Also, because of sam
the collected cells might not reflect the nature of th
compartment targeted for expression profiling. Thus,
single molecules directly in the tissue environment is a
for single-cell analysis technology.

Here we show that sequencing chemistry can be app
for analysis of up to four-base-pair fragments in sin
molecules in the unperturbed context of fixed cells a
Our method is based on padlock probing, rolling-circ
cation (RCA) and sequencing-by-ligation chemistry[9-
RCA in combination with padlock-probe circulariz
tions has been used to produce clonally amplified ro



Conclusions

NGS technologies are now routine part of biological research.

To date more than 14,000 genomes have been deposited within NCBI

In 2013 Schatz and Langmead reported that the world can generated ~15 petabytes per year!!

The new challenge… to provide infrastructure for analyses and storage.

The NGS arm race is not finished…

- -GenapSys (Sigma-Aldrich)
- -Genia (Roche)
- -new nanopore technology with Firefly (Illumina)
- -…

What is France Génomique

A national infrastructure that brings together the capacities and expertise of genomic and bioinformatic plateforms

energie atomique • energies alternatives

What is France Génomique

https://www.france-genomique.org

What is France Génomique

The France Génomique plateforms

What is France Génomique

Technologies, Equipments and Expertise sharing

10X GENOMICS®

illumina®

Oxford NANOPORE Technologies

PACIFIC BIOSCIENCES®

FLUIDIGM®

What is France Génomique

Call for proposals: « Large-scale sequencing projects »

Environmental and agronomical genomics

TARA OCEANS

Plankton diversity

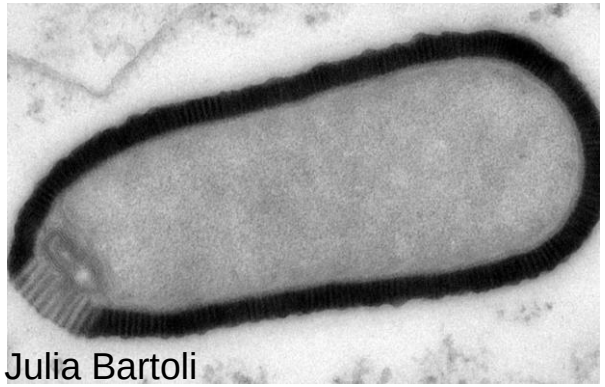
http://oceans.taraexpeditions.org/

Discovery of giant virus

Vostochny Mine, East Siberia

Naymushin / REUTERS

Giant Virus



Julia Bartoli

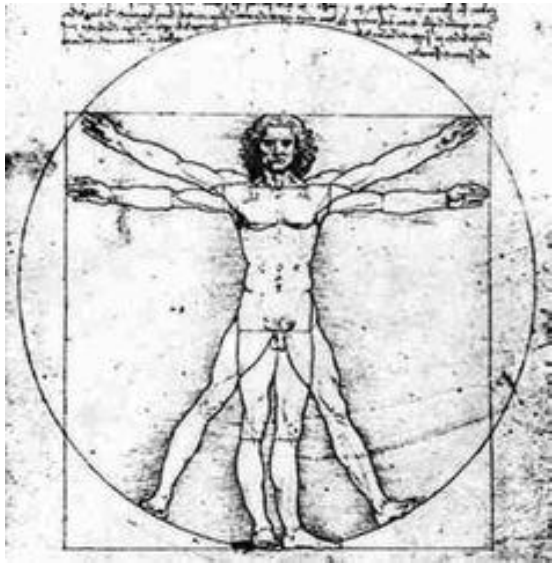
What is France Génomique

Call for proposals: « Large-scale sequencing projects »

Health and Human Genetics

Cancer

Myopathies

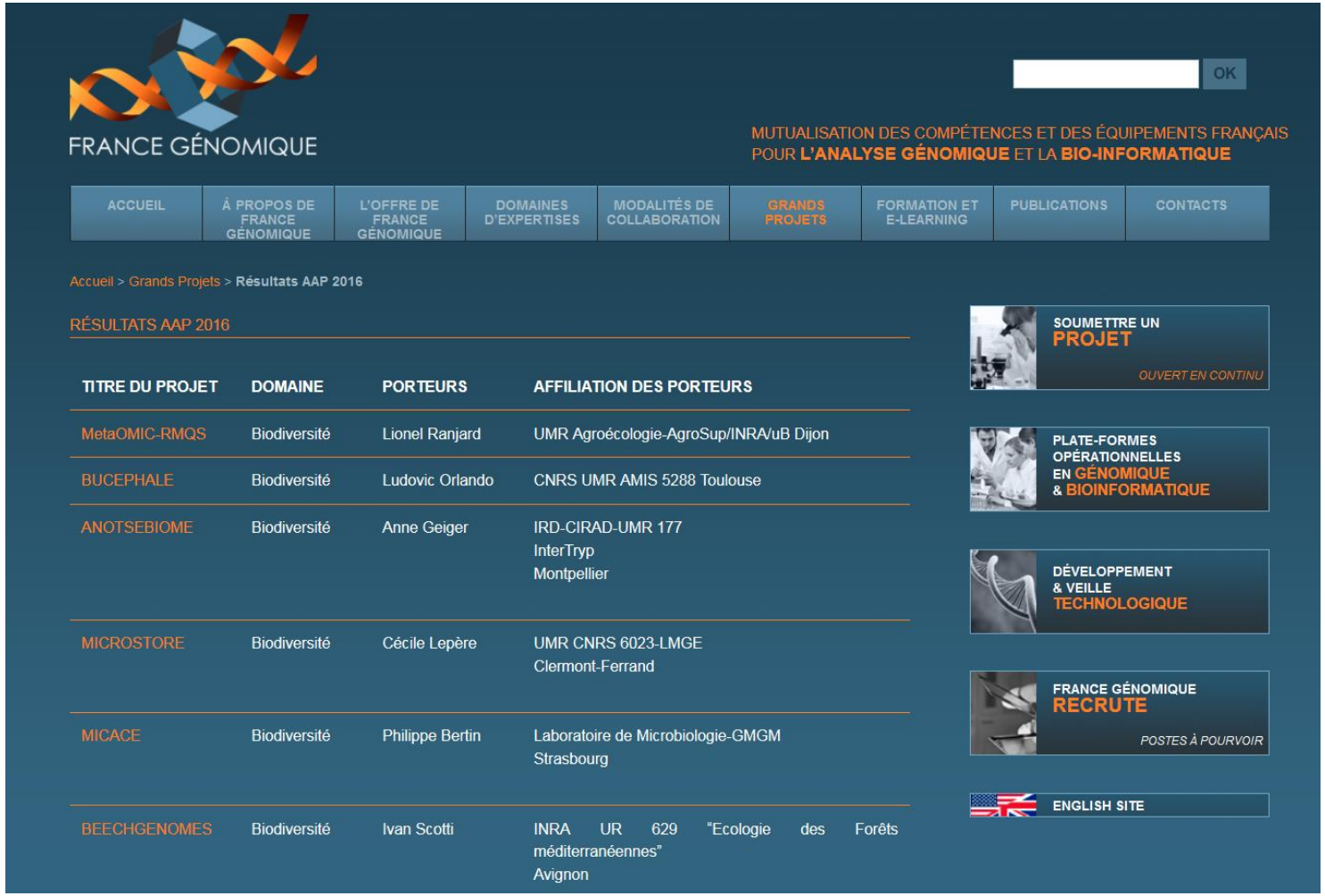


Schizophrenia

Muscular dystrophy

What is France Génomique

Call for proposals: « Large-scale sequencing projects »



FRANCE GÉNOMIQUE

OK

MUTUALISATION DES COMPÉTENCES ET DES ÉQUIPEMENTS FRANÇAIS POUR **L'ANALYSE GÉNOMIQUE** ET LA **BIO-INFORMATIQUE**

ACCUEIL | À PROPOS DE FRANCE GÉNOMIQUE | L'OFFRE DE FRANCE GÉNOMIQUE | DOMAINES D'EXPERTISES | MODALITÉS DE COLLABORATION | GRANDS PROJETS | FORMATION ET E-LEARNING | PUBLICATIONS | CONTACTS

Accueil > Grands Projets > **Résultats AAP 2016**

RÉSULTATS AAP 2016

TITRE DU PROJET	DOMAINE	PORTEURS	AFFILIATION DES PORTEURS
MetaOMIC-RMQS	Biodiversité	Lionel Ranjard	UMR Agroécologie-AgroSup/INRA/uB Dijon
BUCEPHALE	Biodiversité	Ludovic Orlando	CNRS UMR AMIS 5288 Toulouse
ANOTSEBIOME	Biodiversité	Anne Geiger	IRD-CIRAD-UMR 177 InterTryp Montpellier
MICROSTORE	Biodiversité	Cécile Lepère	UMR CNRS 6023-LMGE Clermont-Ferrand
MICACE	Biodiversité	Philippe Bertin	Laboratoire de Microbiologie-GMGM Strasbourg
BEECHGENOMES	Biodiversité	Ivan Scotti	INRA UR 629 "Ecologie des Forêts méditerranéennes" Avignon

SOUMETTRE UN **PROJET** *OUVERT EN CONTINU*

PLATE-FORMES OPÉRATIONNELLES EN **GÉNOMIQUE** & **BIOINFORMATIQUE**

DÉVELOPPEMENT & VEILLE **TECHNOLOGIQUE**

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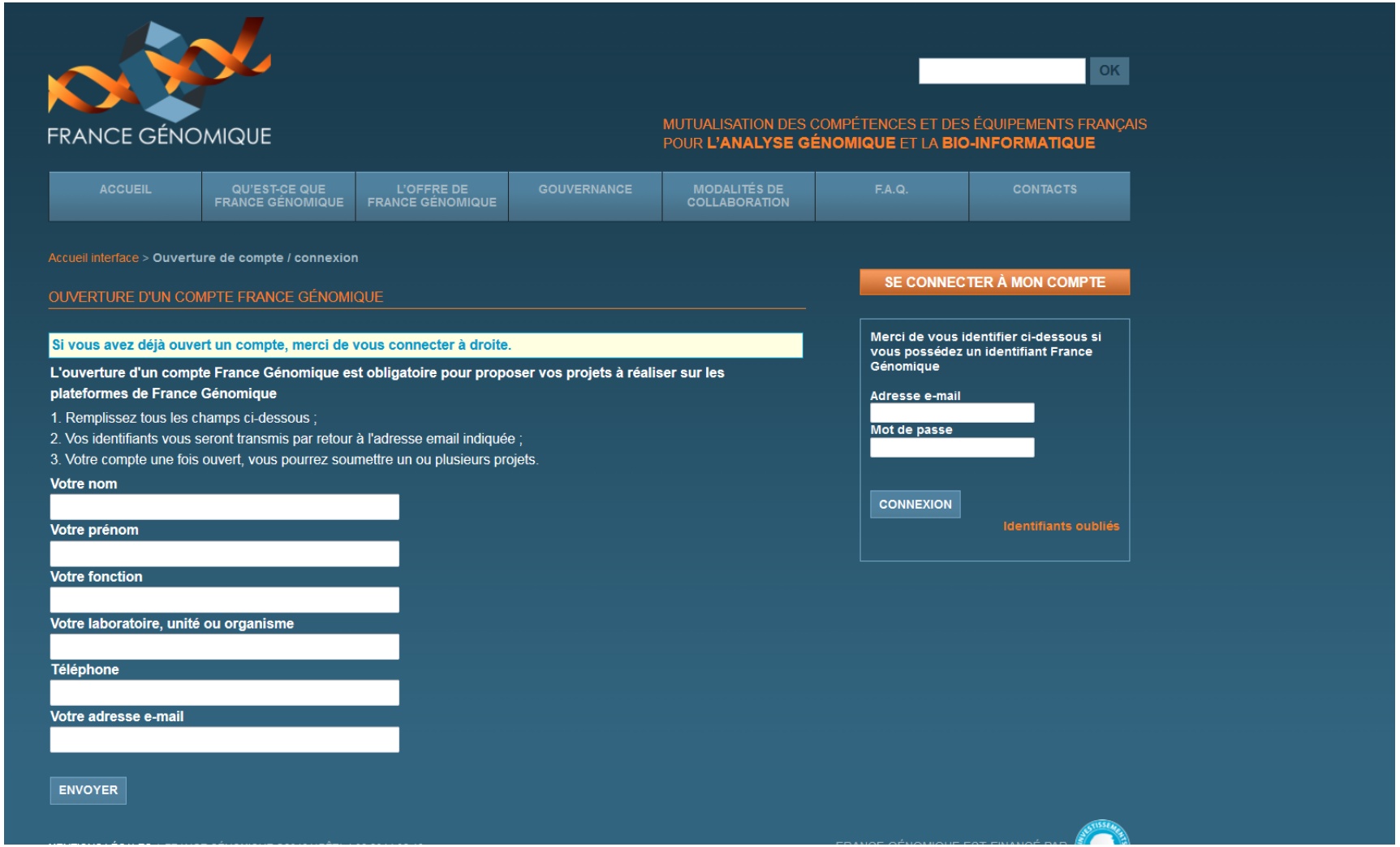
What is France Génomique

Continuous project submission



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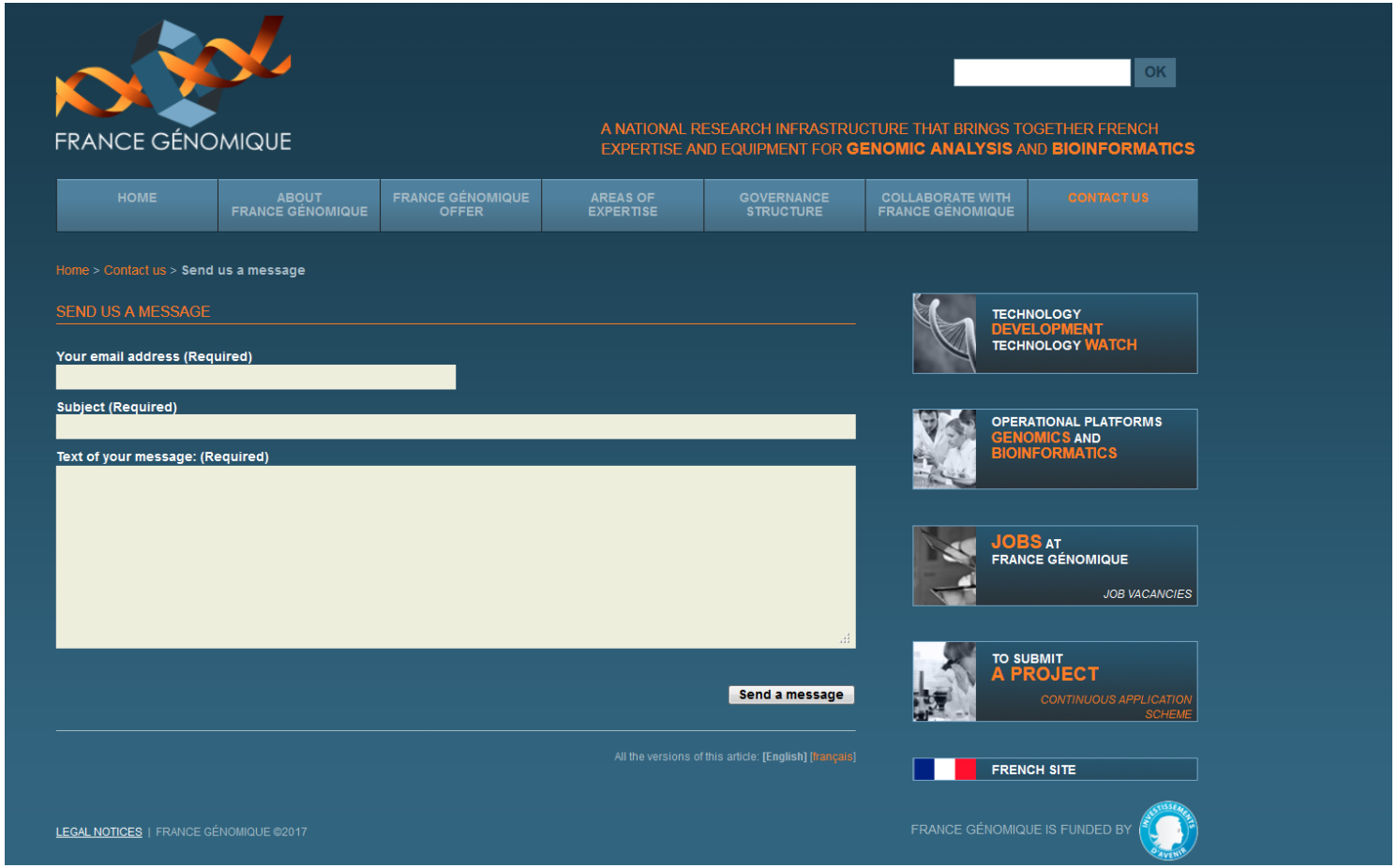
To contact us



@: mathilde.clement@inra.fr

Thank you for your attention!