

## Secuenciación de Genomas Bacterianos: Herramientas y Aplicaciones

**BU-ISCIII**

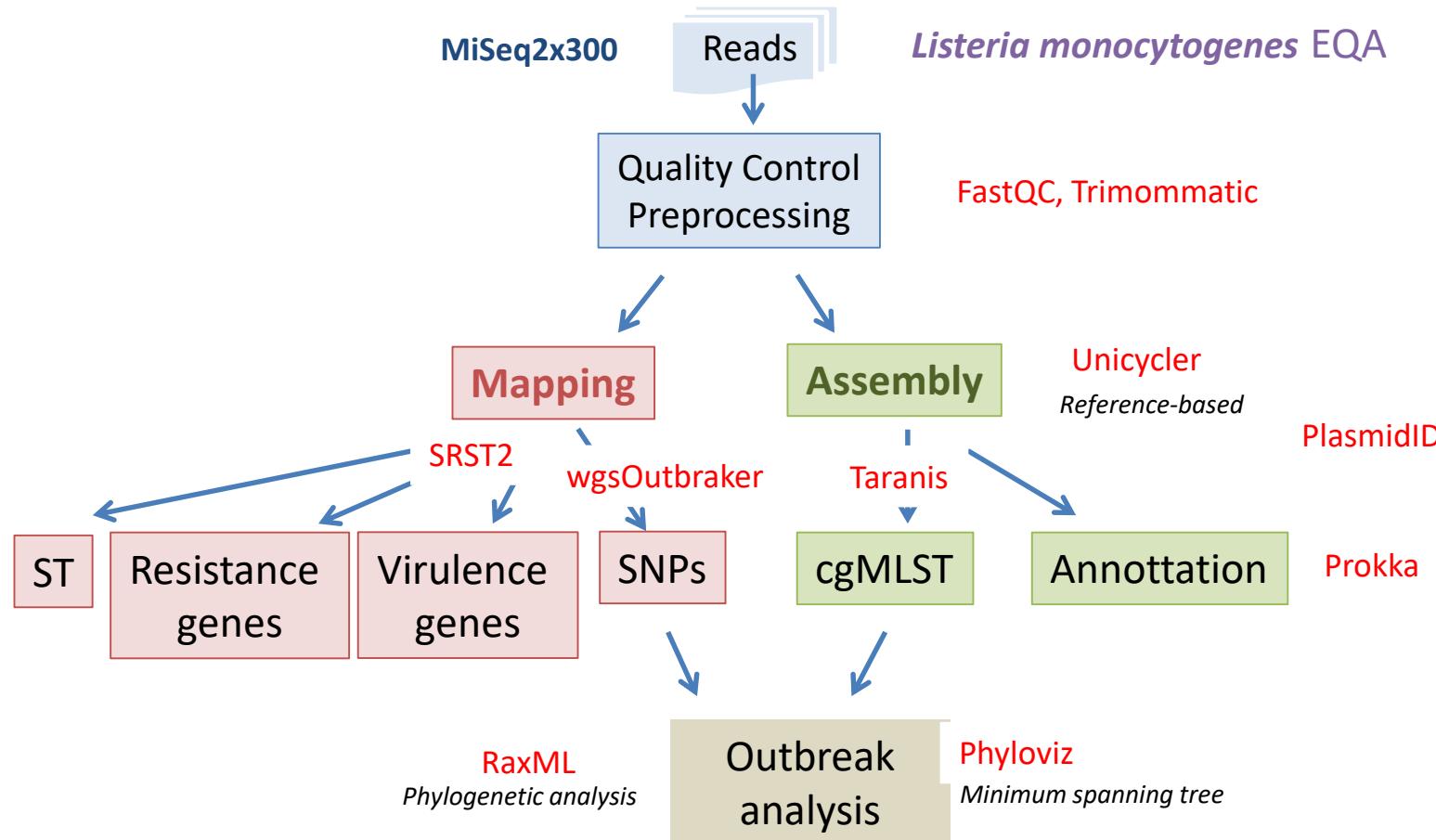
**Unidades Centrales Científico Técnicas - SGSAFI-ISCIII**

24 al 28 octubre 2022, 4<sup>a</sup> Edición  
Programa Formación Continua, ISCIII

# Learning aims and outcomes

- Understand some principles behind NGS and its applications to whole genome sequencing.
- Know the format files generated in NGS data analysis and the workflow analysis.
- Understand the uses of WGS in: specie, antimicrobial resistance genes and virulence factor genes identification, and for typing.
- Outbreak characterization based on SNPs or gene by gene approaches.

# Training workflow



# Teachers

- **Sara Monzón Fernández**, Biotecnóloga y Bioinformática (Analista de datos). Titulado Superior Especialista OPIS. Responsable técnico BU-ISCIII
- **Sarai Varona Fernández**, Bioquímica y Bioinformática (Analista de Datos). Contrato Titulado Superior asociado a proyecto (2021-2022)
- **Isabel Cuesta**, Dra Biología, Bioinformática (Científico de Datos). Científico Titular de OPIS. Coordinador BU-ISCIII

## Session 1.1 - Secuenciación masiva de genomas bacterianos: situación actual

Isabel Cuesta

BU-ISCIII

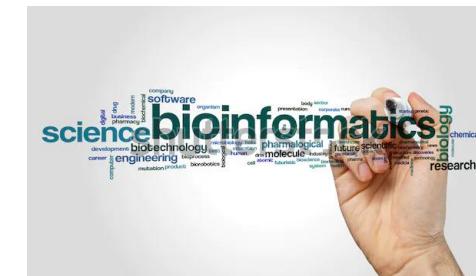
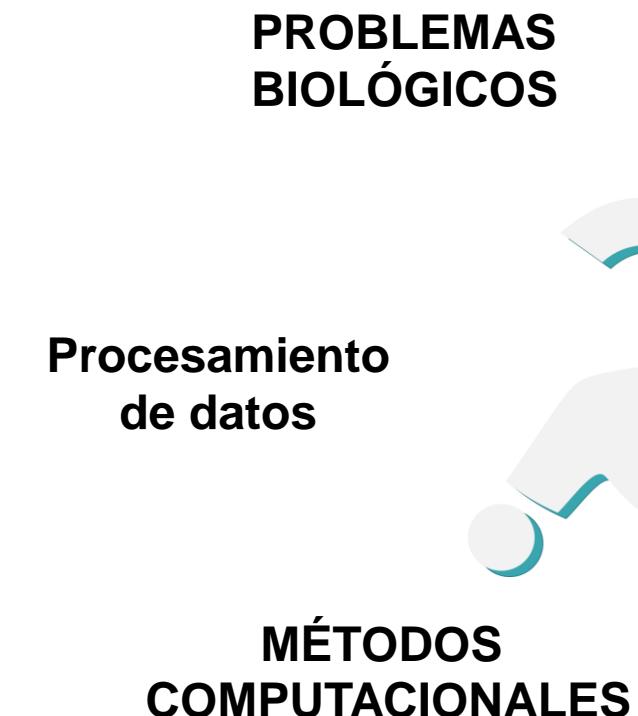
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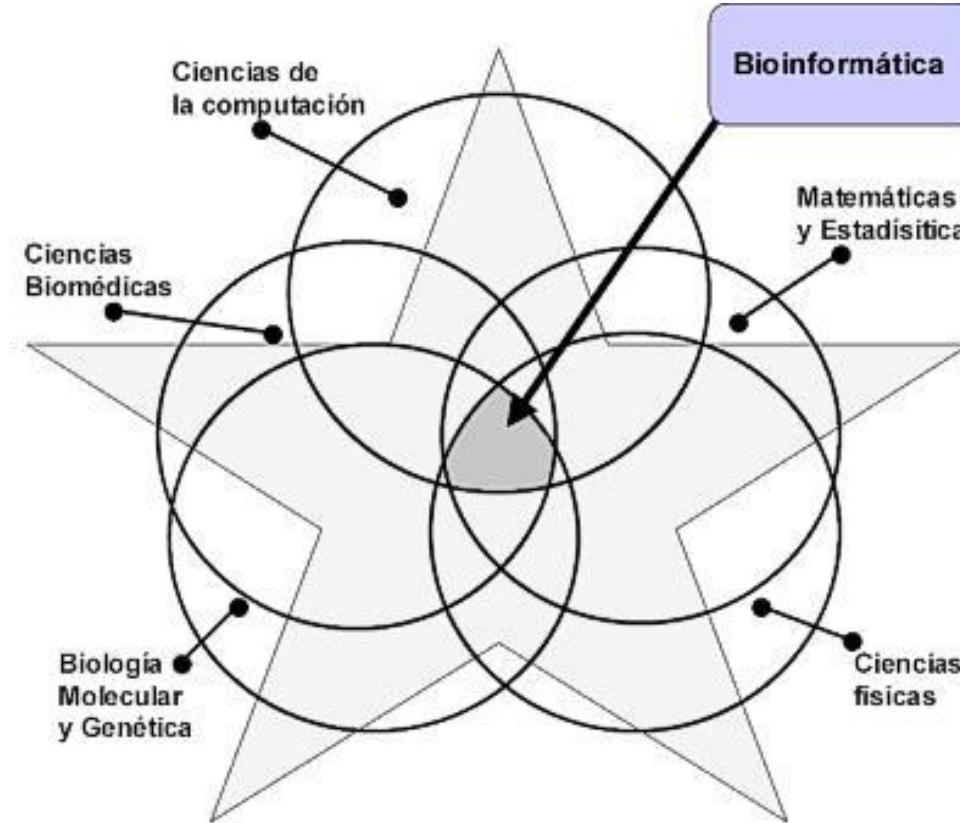
# Index

- BU-ISCIII
- High throughput sequencing platforms update
- Bacterial genome sequencing, brief history
- Advantages of WGS
- Use of WGS in Europe
- Sequencing concepts, library preparation aspects, bioinformatics analysis

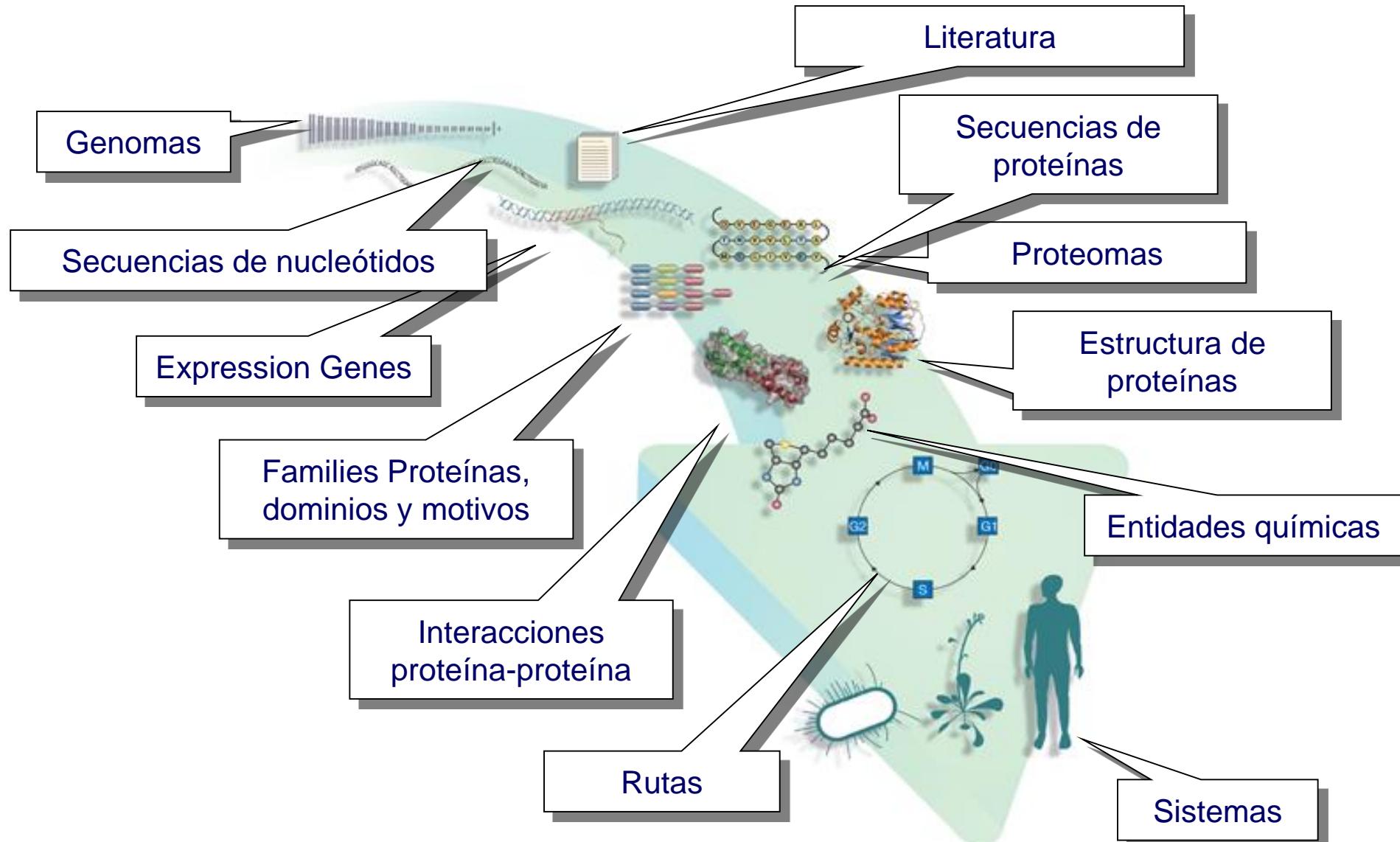
# Qué es la Bioinformática?



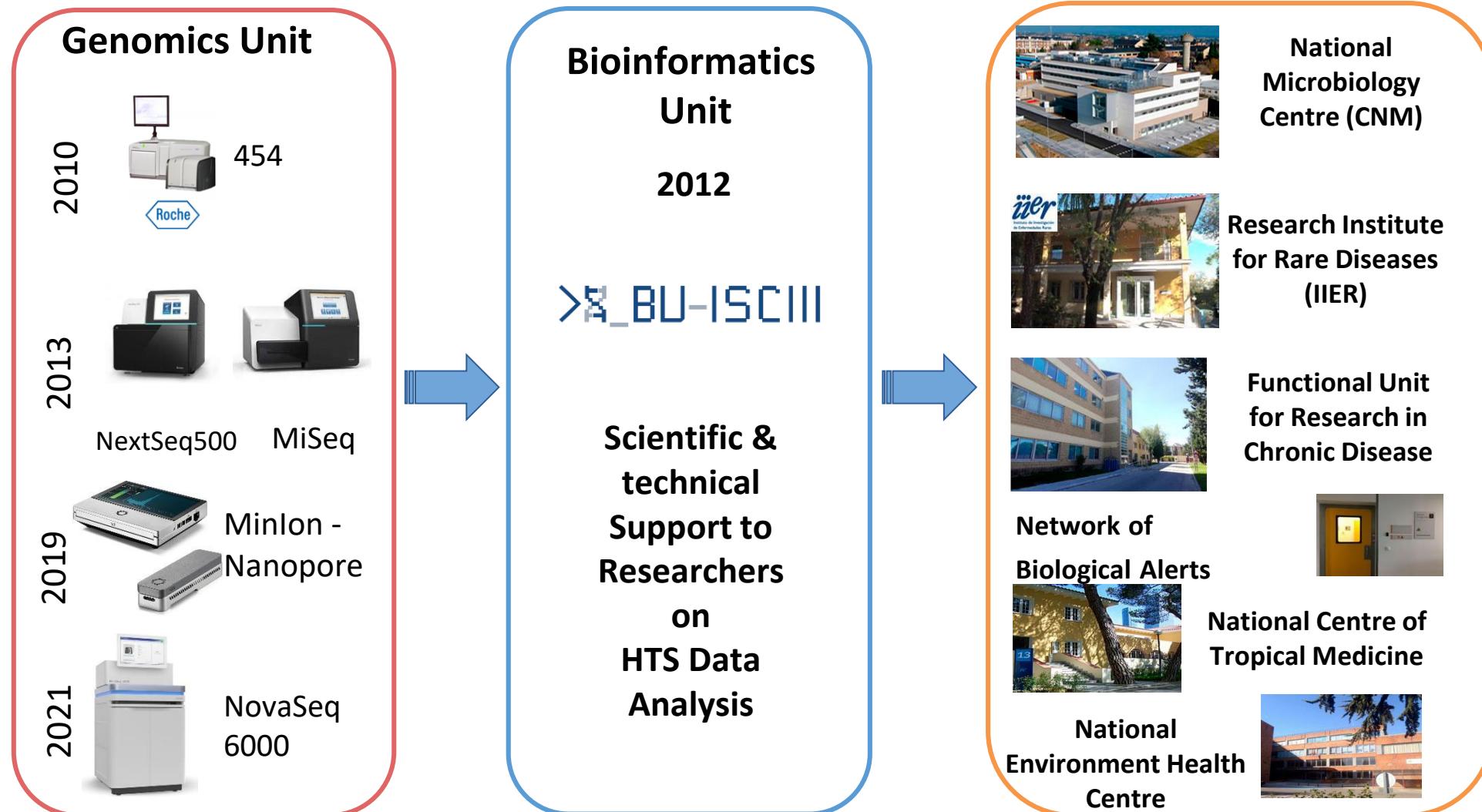
# Bioinformática es multidisciplinar



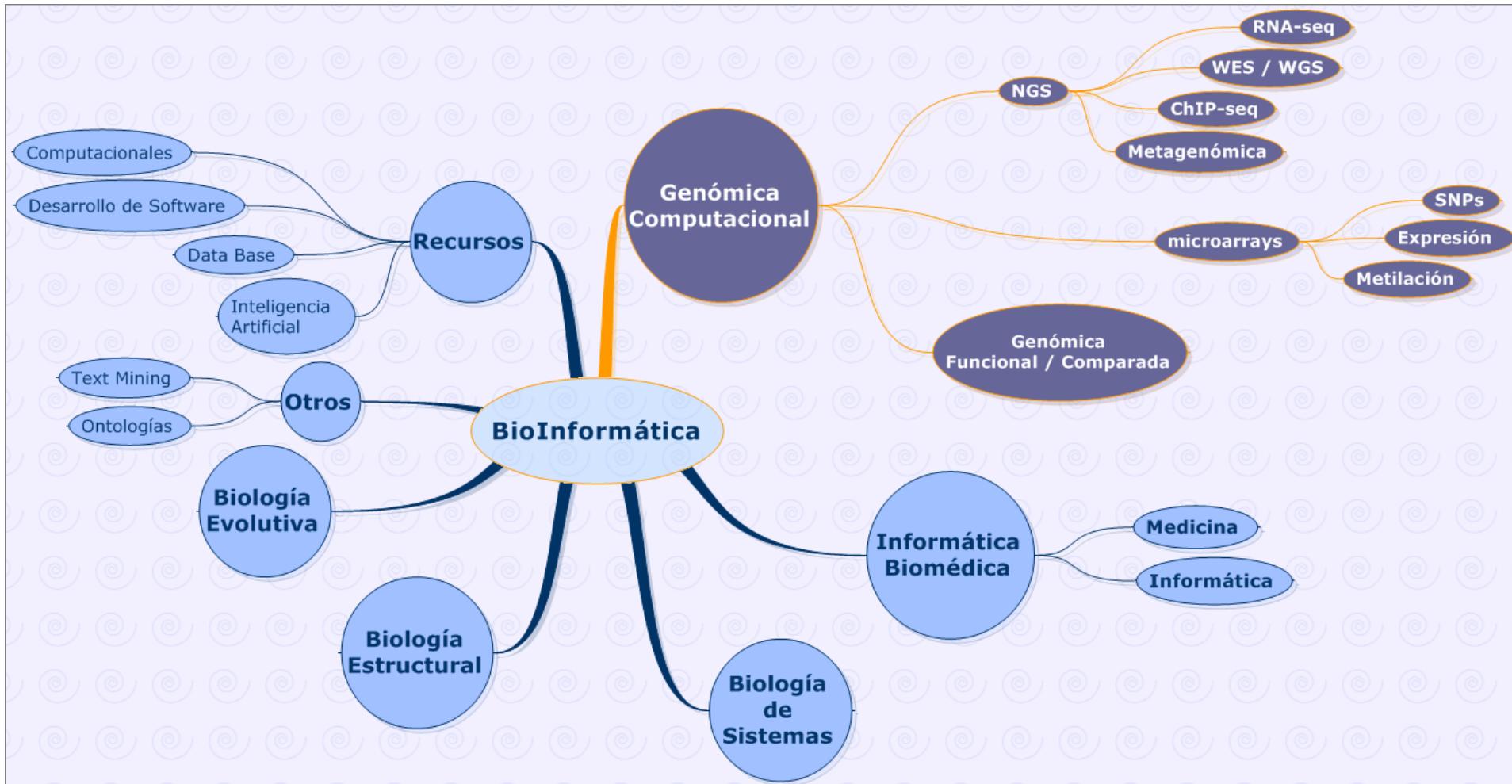
# Tipos de datos dan idea de la dimensión de la Bioinformática



# Por qué nace BU-ISCIII?



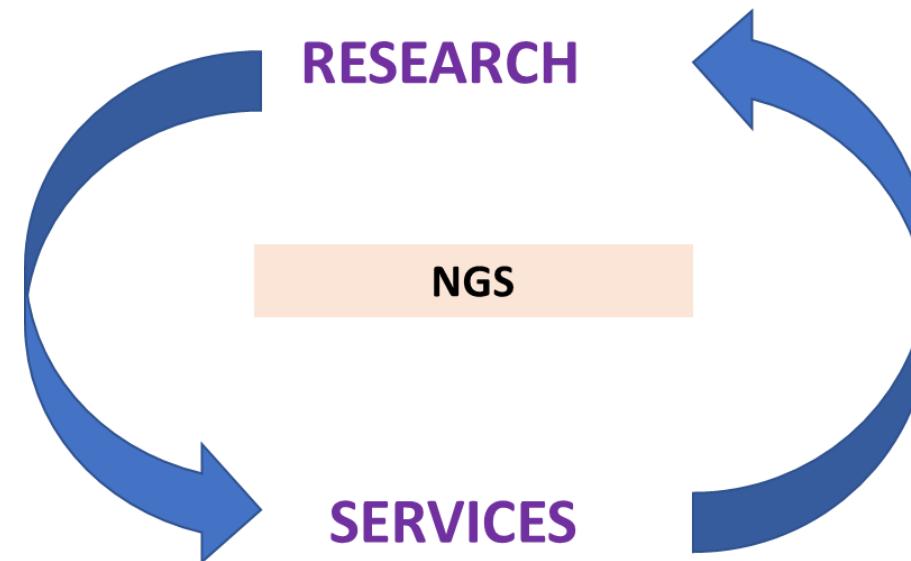
# BU-ISCIII Mission - Activities



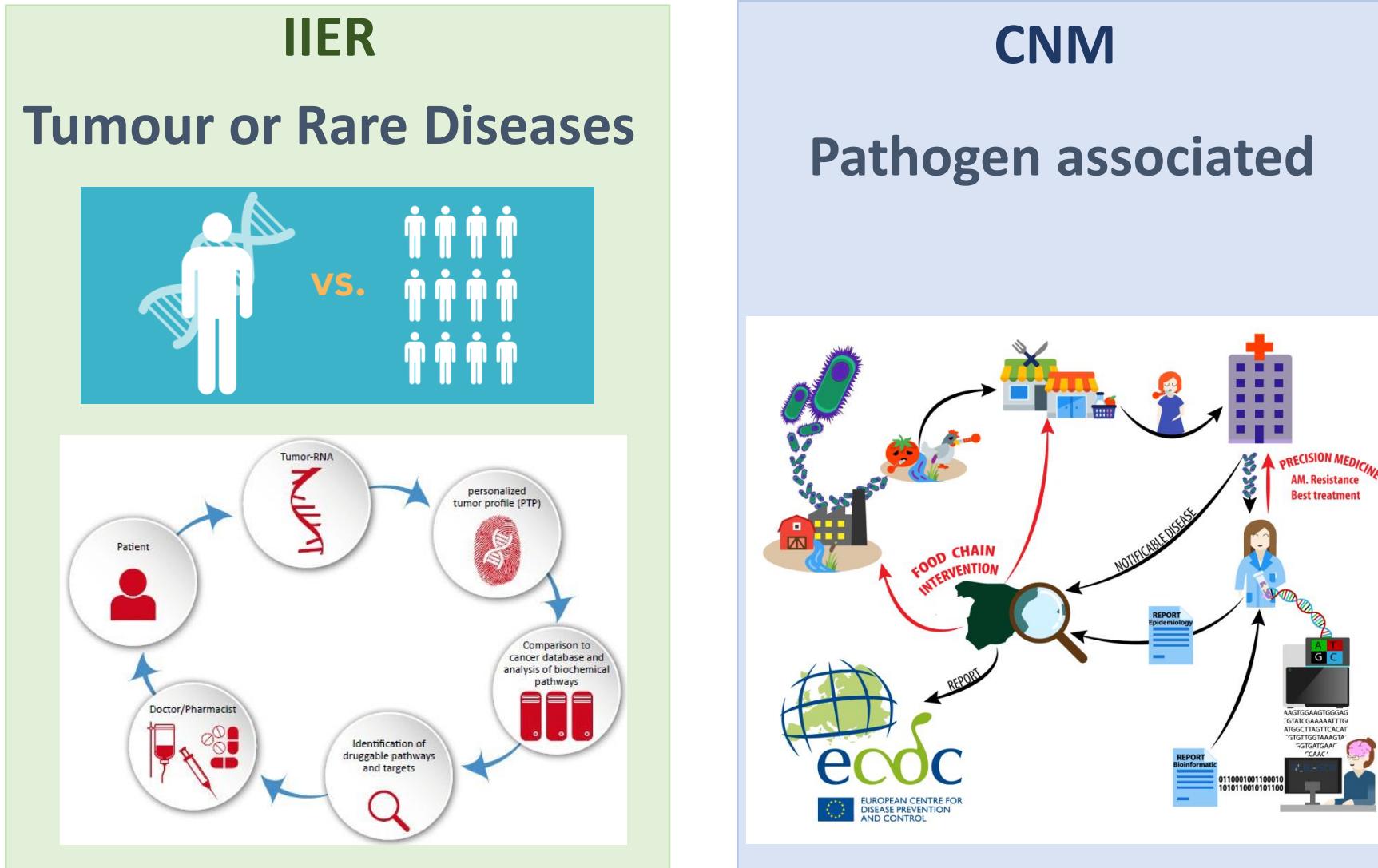
# Bioinformatics Unit Activities

- Identify biological problems (PI / Groups) that could be target of NGS
- Early adopters: establish collaboration with.
- Be strategic providing transversal solutions → reusable tools

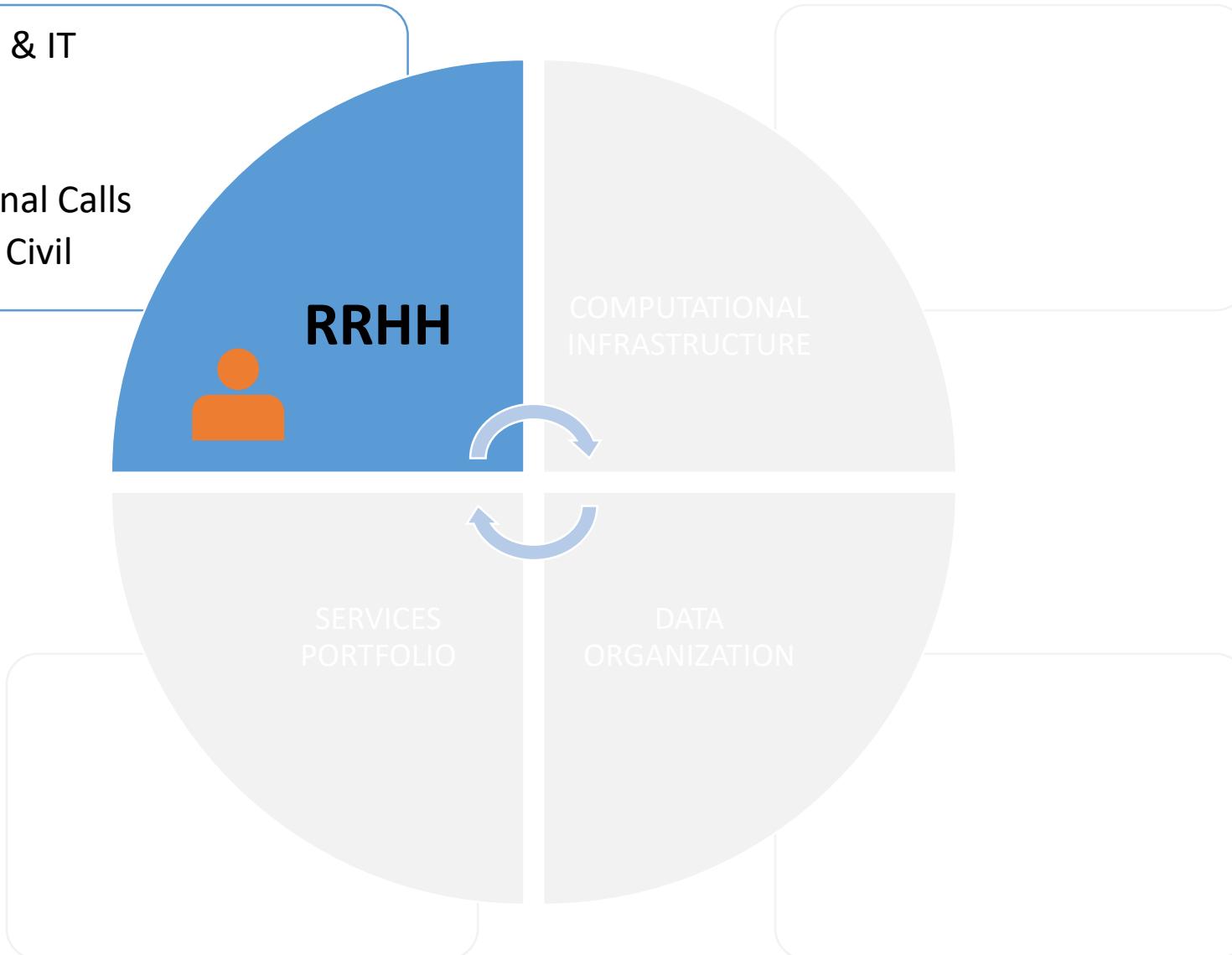
Provide scientific and technical solutions for using NGS in the diagnostic routine or research activity from different ISCIII labs



# Clinical Bioinformatics - Precision Medicine



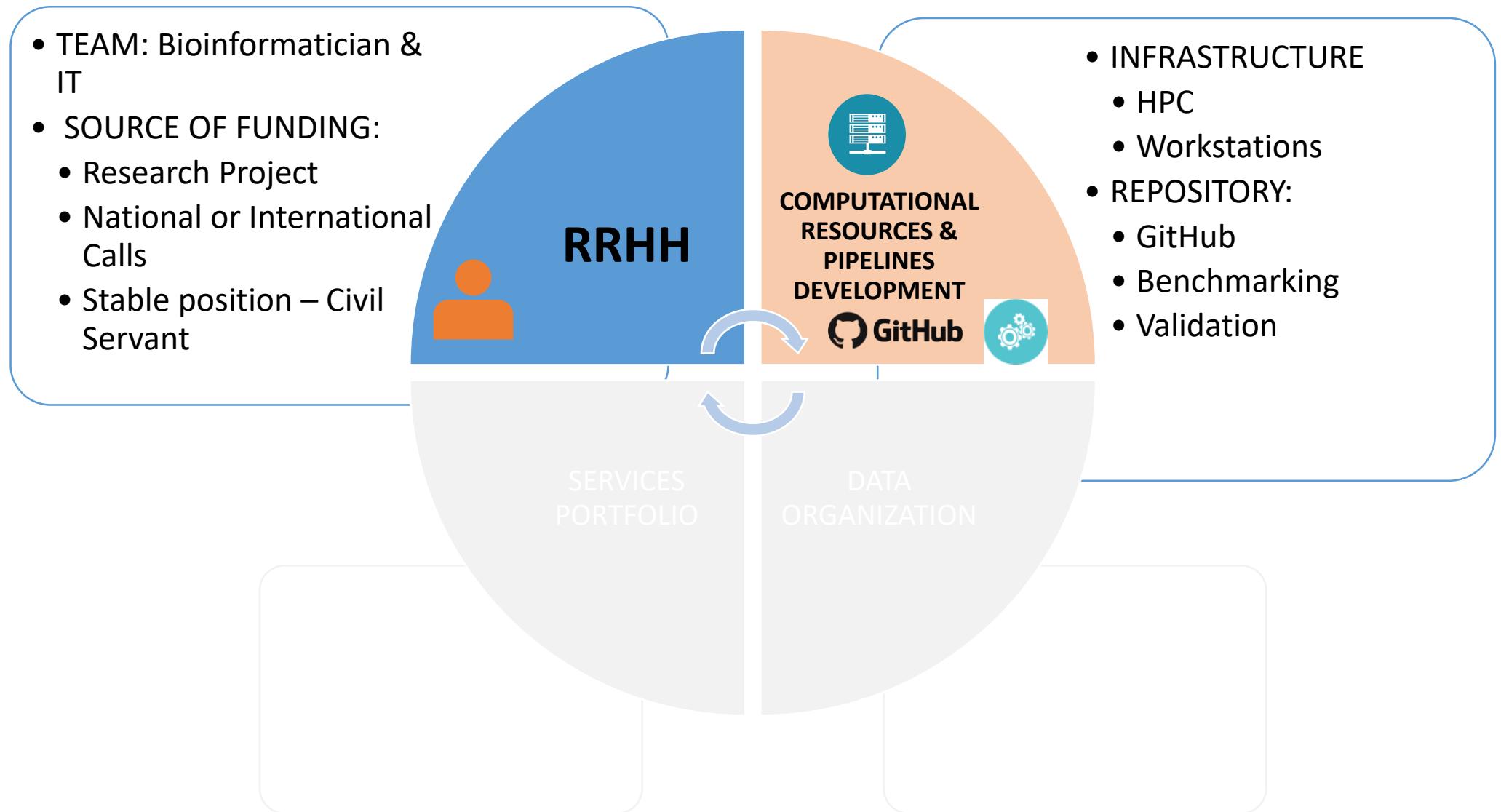
- TEAM: Bioinformatician & IT
- SOURCE OF FUNDING:
  - Research Project
  - National or International Calls
  - Permanent position – Civil Servant



# Human resources

> BU-ISCIII

	Disciplina	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Isabel Cuesta	Dr. Biología Molecular							CIENTIFICO TITULAR OPIS					
Sara Monzón	Biotecnología	TFM	CIBERER			PTA MINECO			T.SUPERIOR OPIS				
Bruno Lobo	Administrador Sistemas			PROYECTO		PTA MINECO			SISTEMAS				
Jorge de la Barrera	Informática			PTA FIS									
Miguel Juliá	Matemáticas							PTA MINECO					
José Luis García	Telecomunicaciones							PROYECTO					
Pedro Sola	Biología					ANTIBIOTICOSU.				ANTIBIOTICOSU.			
Sarai Varona	Bioquímica									PROYECTO (2022)			
Luis Chapado	Telecomunicaciones						COLABORADOR						
Erika Kvalem	Biotecnología										PROYECTO COVID19		
Alberto Lema	Biología									1 año			
Luis Aranda	FP Informática									oct 2021 al 2022			
Guillermo Gorines	Biología										ESTANCIA		
Personal (número)		2	4	4	5	5	4	6	6	6	8	8	2



# Computational Resources

- IT support: establish agreement with IT department including permission for using Linux.



Workstations (5), 4cores, 64Gb, 8TB  
Server, 4-quad, 120Gb, 16TB

## Data Centre (CPD-ISCIII)



HPC 320 cores, 8TB RAM, 10Gbps.  
2 flexible and scalable storages,  
NetApp, 70 TB and 250TB

- Analysis pipelines reproducibility

nextflow



Singularity containers  
Admin support & environment independency  
Sharing code easier

GitHub

<https://github.com/BU-ISCIII>

# Bioinformatic Analysis: Software validation - ECDC EQAs

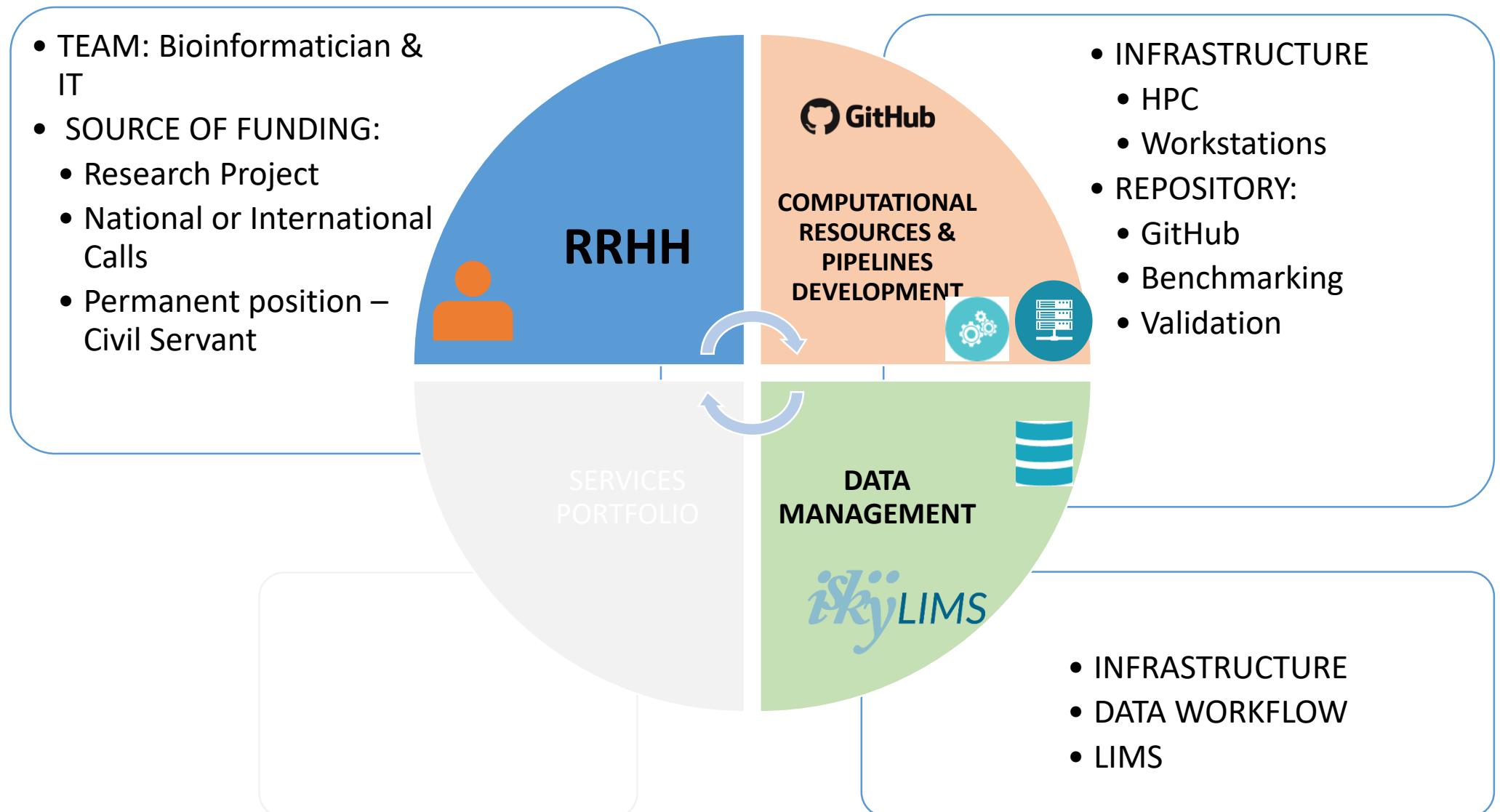
**Table 5. Results of allele-based cluster analysis**

Lab ID	Approach	Allelic calling method	Allele based analysis			
			Assembler	Scheme	Difference within cluster	Difference outside cluster
EQA provider	BioNumerics	Assembly- and mapping-based	SPAdes	Applied Math (cgMLST/Pasteur)	0-3	24-1112
19	BioNumerics	Assembly- and mapping-based	SPAdes	Applied Math (cgMLST/Pasteur)	0-3	25-1120
35	SeqSphere	Assembly-based only	Velvet	Ruppitsch (cgMLST)	0-2	16-1065
70	SeqSphere	Assembly-based only	Velvet	Ruppitsch (cgMLST)	0-2	16-1062
105*	SeqSphere	Assembly-based only	SPAdes v 3.80	Ruppitsch (cgMLST)	0-1*	23-812
129	SeqSphere	Assembly-based only	Velvet			
135	SeqSphere	Assembly-based only	CLC Genomic Workbench 10			
141	SeqSphere	Assembly-based only	SPAdes 3.9.0			
142	Inhouse	Assembly-based only	SPAdes			
144	SeqSphere	Assembly-based only	Velvet			

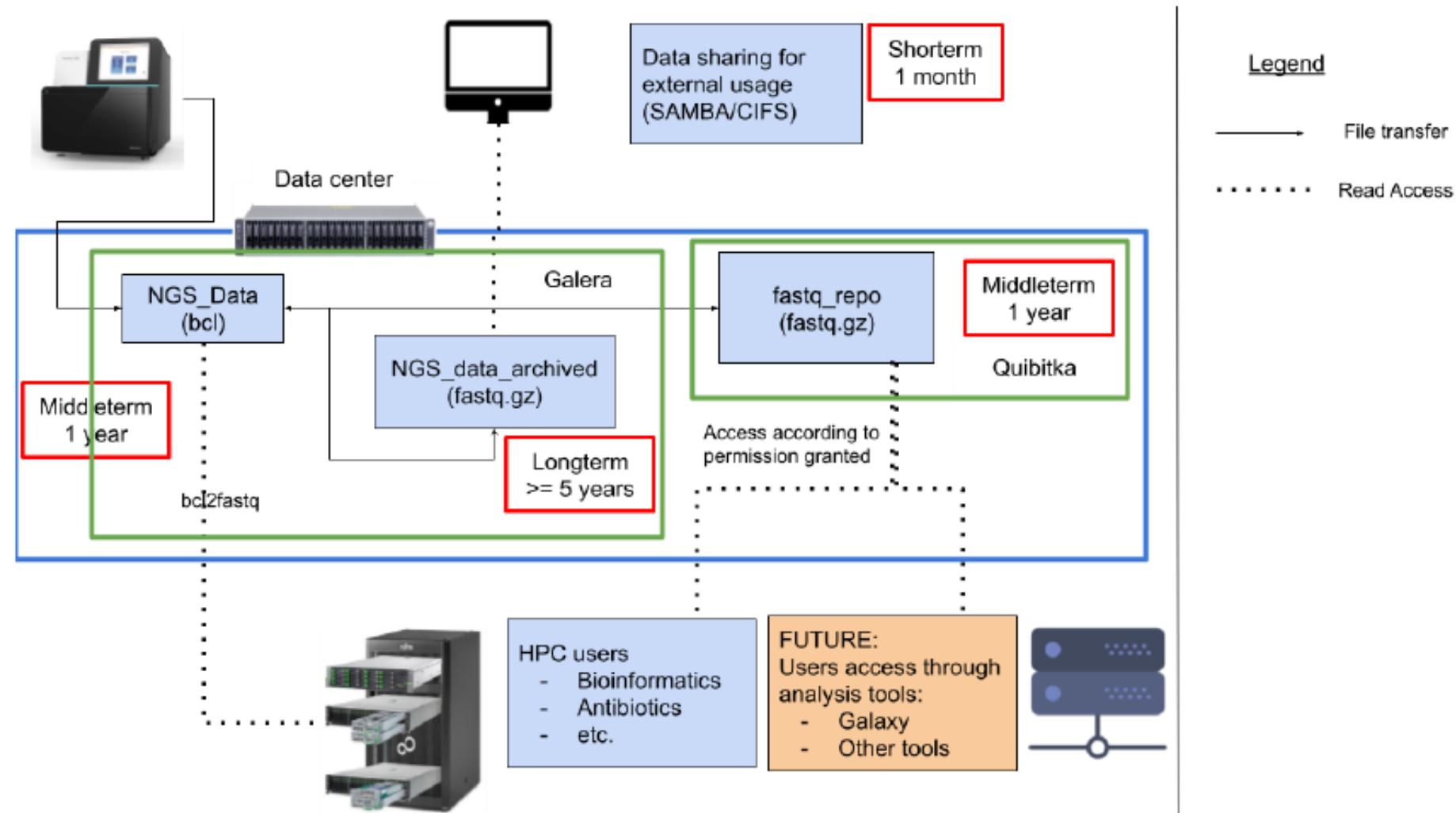
**Table 4. Results of SNP-based cluster analysis**

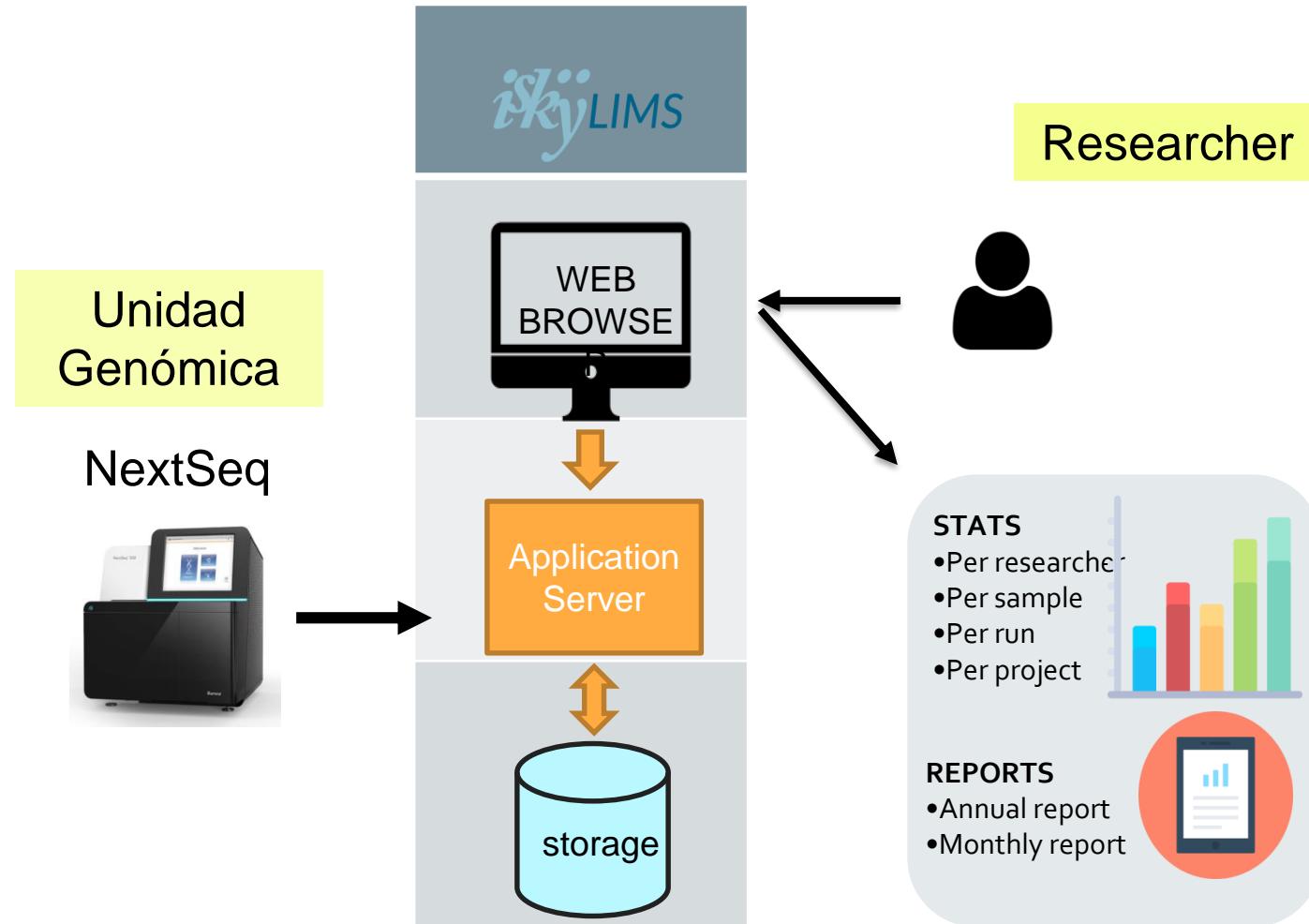
Lab ID	SNP-based						
	Approach	Reference	Read mapper	Variant caller	Assembler	Distance within cluster	Distance outside cluster
Provider	Reference-based	ST6 (REF4)	BWA	GATK		0-3	38-71
19*	Reference-based	ST6 ID 2362	BWA	GATK		0-4	43-81
56	Assembly-based			ksnp3	SPAdes	0-57*	561-591 (6109)
105	Reference-based	ST6 J1817	Bowtie2	VARSCAN 2		0-2*	22-42 (1049)
108	Reference-based	In-house strain resp ST	CLC assembly cell v4.4.2	CLC assembly cell v4.4.2		0-2	37-72
142*	Reference-based	Listeria EGDe (cc9)	CLC Bio	CLC Bio		0-1219	1223-2814 (8138)
146	Reference-based	ST6 ref. CP006046 ST1 ref. F2365 ST213/ST382 no ref.	BWA	In-house		0-358	

**Fifth external quality assessment scheme for Listeria monocytogenes typing**

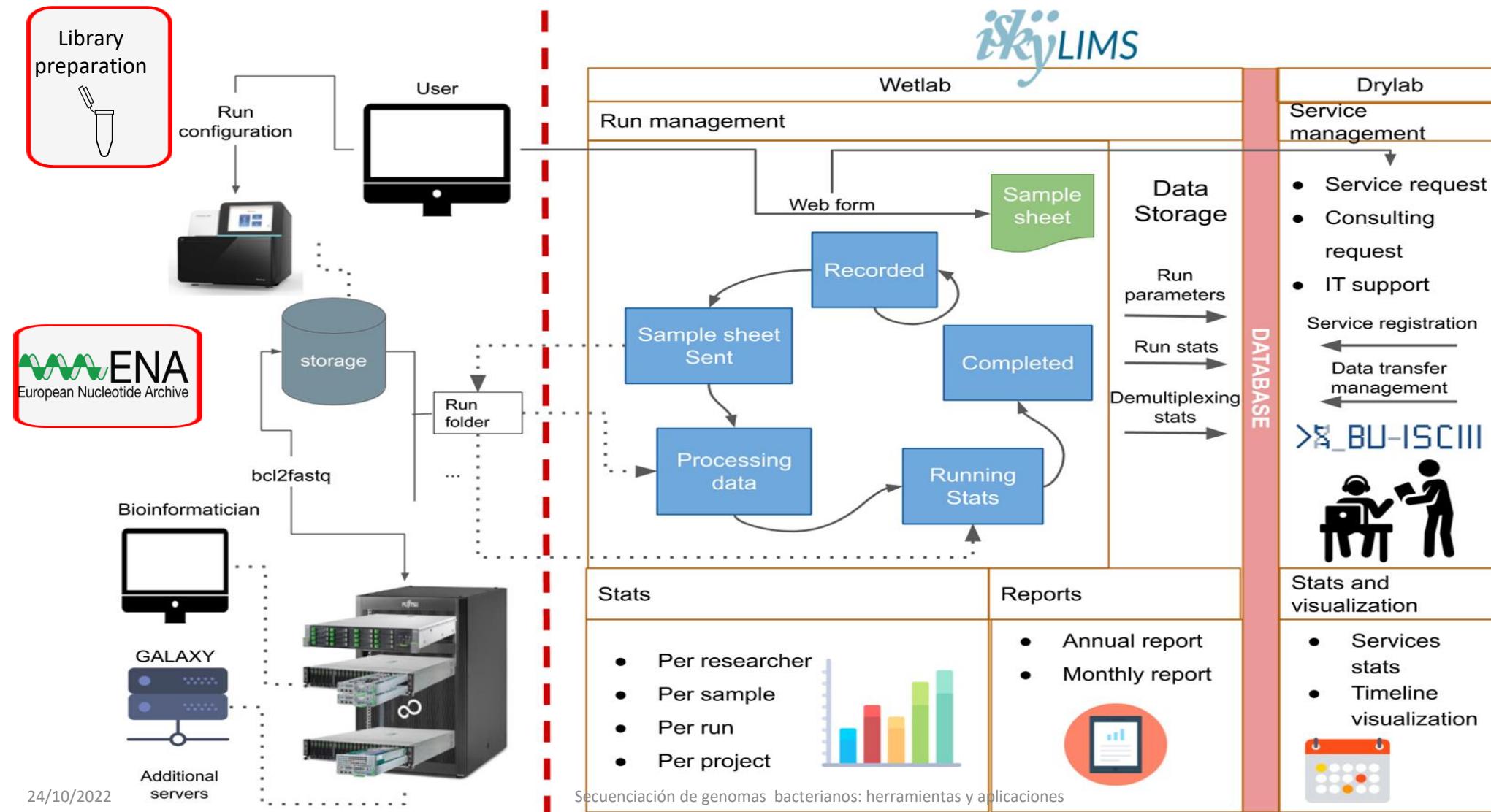


# Infrastructure and data management





# Infrastructure and data management: LIMS



## SERVICIOS DE LA UNIDAD

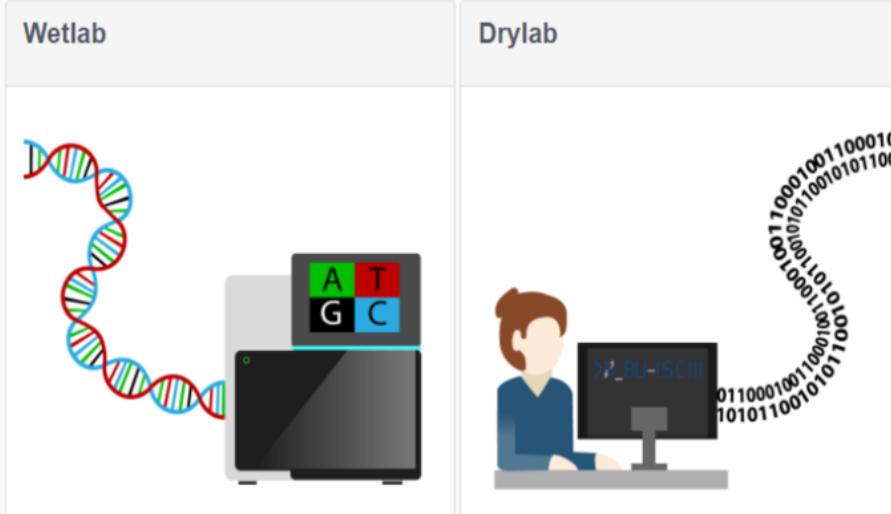


<https://iskylims.isciii.es/>



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 icuesta  ..... [Login](#)



Logos



Connect



## Links

- Contact
  - Getting started
  - FAQs

## Sitemap

- iSkyLIMS home
  - Drylab page
  - Wetlab page

<https://iskylims.isciii.es/>

 smonzon [Logout](#) [My account](#)

## BioInformatics

### iSkyLIMS: DryLab

#### Welcome

This section will allow you to check BU-ISCIII service activity. Available processes are request new services, collaborations, counseling and infrastructure. You will be able to check the status of your ongoing services.



#### Services ongoing and queued

Under construction. This will be a table with services ongoing or queued

#### Timeline of services

Under construction. Kind of diagram with services dates.



smonzon

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## Service Request Form

Form for requesting internal service to Bioinformatic Unit

### Sequencing Data

**User's projects\***

BMartinez20161213  
EXOMAS\_ND\_20170303  
EXOMAS\_ND\_20170327\_RE  
EXOMAS\_ND\_20170228

**Run specifications****File extension****Sequencing platform**

# SERVICES REQUEST

[HOME](#)[SERVICES REQUEST](#)[COUNSELING REQUEST](#)[INFRASTRUCTURE REQUEST](#)

- Genomic Data Analysis
  - Download and quality analysis
    - Data download
    - Sequence quality analysis
    - Sequence pre-processing (quality filtering)
  - Next Generation Sequencing data analysis
    - DNAseq: Exome sequencing (WES) / Genome sequencing (WGS) / Target sequencing
      - Trio/family variant calling pipeline
      - Variant calling and annotation pipeline
      - Microbial: Whole genome outbreak analysis pipeline
      - Microbial: wgMLST
      - Microbial: MLST + virulence + AMR + plasmid analysis
      - Microbial: Assembly + automatic annotation
      - Microbial: plasmidID pipeline - strain plasmid characterization
    - RNAseq: Transcriptome sequencing
      - miRNA-Seq pipeline
      - mRNA-Seq pipeline
    - Amplicon sequencing (Deep sequencing)
      - Low frequency variant detection
      - Viral: assembly and minor variants detection
  - Metagenomics
    - 16S taxonomic profiling
    - Shotgun metagenomics profiling
    - Shotgun metagenomics - Virus genome reconstruction
  - CHIP-SEQ
    - Peak detection and annotation

# SERVICES REQUEST



**Service Description**

**Service description file\***  
 No file selected.

**Service Notes\***

# COUNSELING REQUEST



## Service selection

### Available Services \*

- Bioinformatics consulting and training
  - Bioinformatics analysis consulting
  - In-house and outer course organization
  - Student training in collaboration: Master thesis, research visit,...

## Service Description

### Service description file\*

No file selected.

### Service Notes\*

# INFRASTRUCTURE REQUEST



HOME SERVICES REQUEST PIPELINES MANAGE SERVICES



bioinfoadm

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## Requesting Services Application

Infrastructure Request Service form.

User support

- Installation and support of bioinformatic software on Linux OS (Not available)
- Installation and access to Virtual machines in the Unit server
- Code snippets development
- OT-2 robots (Not available)

Service Notes

Submit your Request

# Infrastructure and data management

 [HOME](#) [RUN PREPARATION](#) [SEARCH](#) [STATISTICS](#) [REPORTS](#)

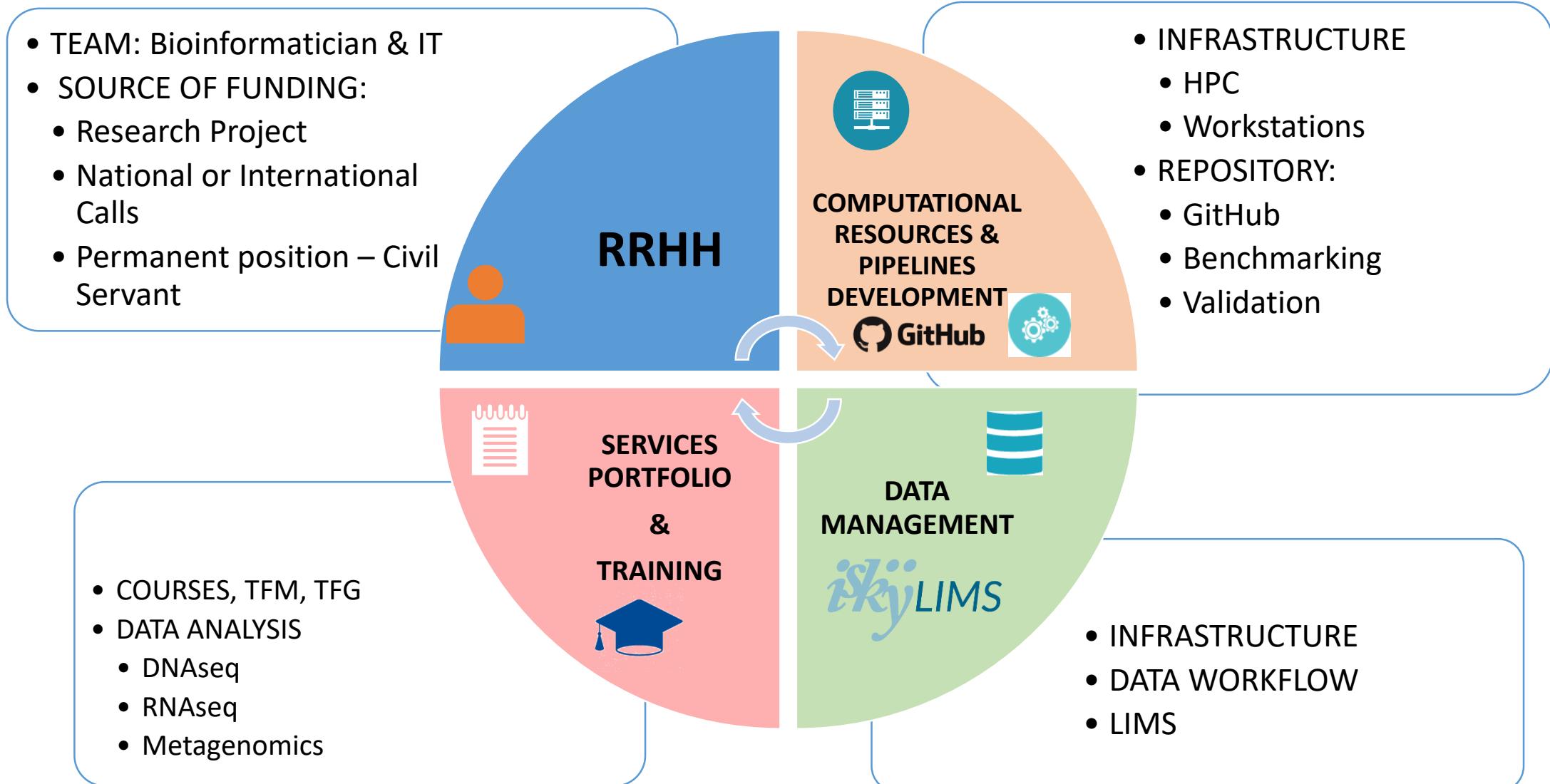
 bioinfoadm [Logout](#) [My account](#)

## Statistics results for Investigator rabad

Projects using the sequencer NS500454 :

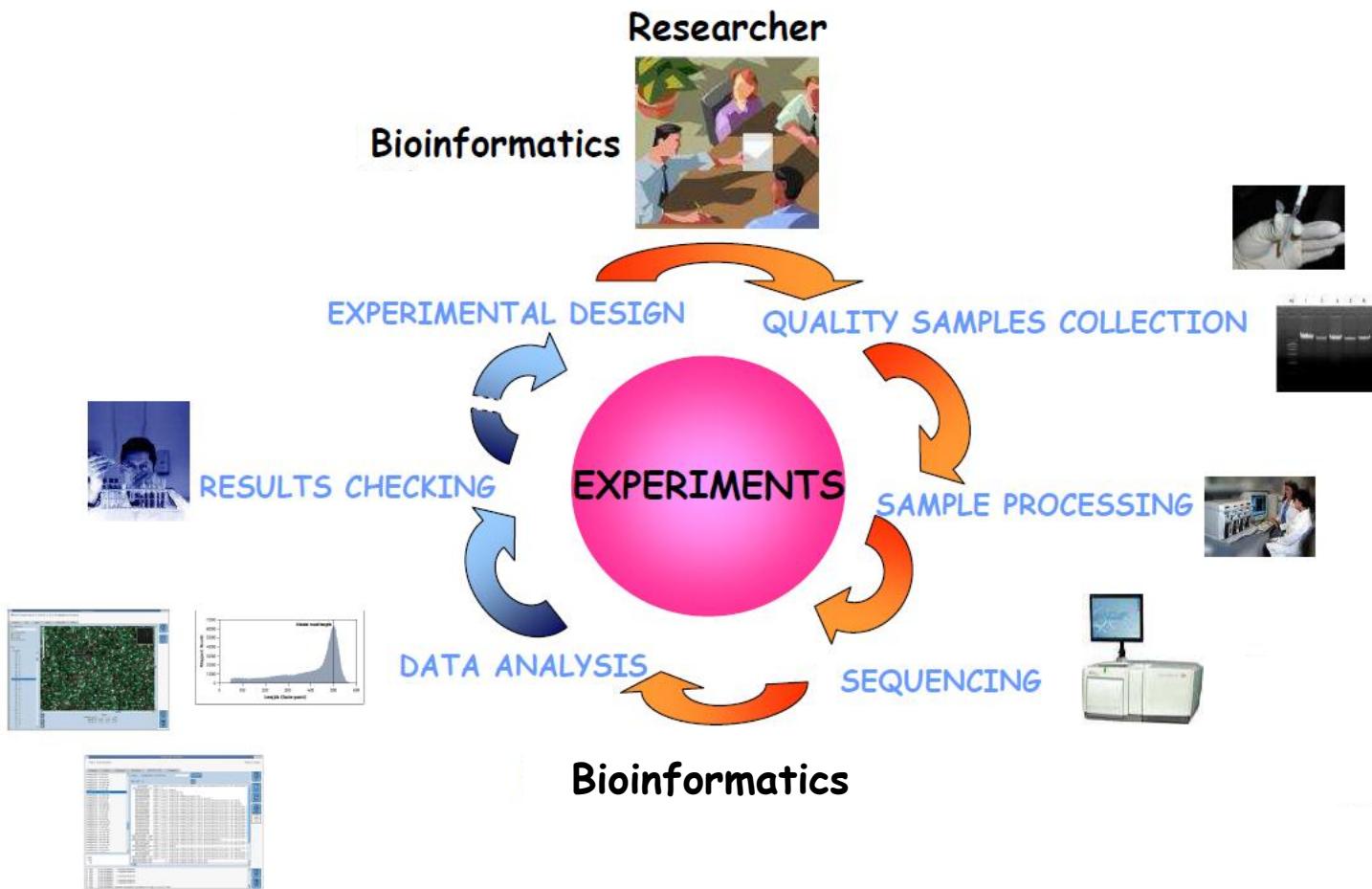
[Export Table To Excel](#)

Project name	Date	Library Kit	Samples	Cluster PF	Yield Mb	% Q> 30	Mean	Sequencer ID
NextSeq_CNM_191_20191004_RAbad	No Date	Nextera DNA CD Indexes (96 Indexes plated)	48	149,441,968	45,876	89.98	33.70	NS500454
NextSeq_CNM_166_20190528b_Rabad	No Date	Nextera XT v2 Set B	96	139,317,411	43,016	89.58	33.72	NS500454
NextSeq_CNM_166_20190528a_Rabad	No Date	Nextera XT v2 Set A	82	102,267,350	31,623	89.26	33.65	NS500454
NextSeq_CNM_150_20190218B_RAbad	No Date	Nextera XT v2 set B	20	17,335,577	5,352	86.77	33.17	NS500454
NextSeq_CNM_150_20190221A_RAbad	No Date	Nextera XT v2 Set A	96	127,755,164	39,595	85.28	32.86	NS500454
NextSeq_CNM_166_20190528c_Rabad	No Date	Nextera XT v2 Set C	96	152,945,860	47,264	89.38	33.68	NS500454
NextSeq_CNM_170_20190620_RAbad	No Date	IDT-ILMN Nextera UD Index Set A for Nextera DNA FI	47	131,012,486	39,671	90.74	33.94	NS500454
NextSeq_CNM_171_20190624_RAbad	No Date	IDT-ILMN Nextera UD Index Set A for Nextera DNA FI	47	140,488,964	42,597	89.61	33.72	NS500454



- **GENÓMICA COMPUTACIONAL: ANÁLISIS DE DATOS MASIVOS**  
Técnicas de secuenciación masiva (NGS)
- **ASESORIA Y FORMACIÓN EN BIOINFORMÁTICA**  
Orientación en el análisis bioinformático  
Organización de cursos internos y externos
- **SOPORTE A USUARIOS**  
Generación y acceso a máquinas virtuales que contienen software bioinformático, ubicadas en los servidores de la Unidad

# Workflow en NGS



# Services Portfolio

		QC	Assembly	Reference based Mapping	Variant calling	Annotation	Pipelines
<b>DNAseq</b>	<b>HUMAN</b>						
	WES Target -Panels	Report html		(Bam file)	(Vcf file)	Desease model (Vcf file annotated)	.Trio / family .Tumor .Pampu caller
<b>mRNA</b>	<b>MICROBIAL</b>						
	WGS Amplicon	Report html	<i>De novo</i> / Reference (fasta file)	MLST, Resistance g, Virulence g	SNPs Phylogenetic analysis	Structural Functional	.WGSOutbraker .Plasmid ID
<b>RNAseq</b>	<b>mRNA</b>	RSQC Report html	<i>De novo</i> (fasta file)	Transcripts coverage / expression	Variants (Vcf file)	Transcripts annotation	mRNA seq
	<b>miRNA</b>						miRNA seq
<b>Metagenomics</b>	<b>16S taxonomic profile</b>	Report html	<i>De novo</i>	Green genes DB		species diversity	Qiime
	<b>Shotgun</b>			Genome Ref Seq		Pathogen / Genome coverage	PikaVirus

# Galaxy

172.23.2.60

Galaxy

Analyze Data Workflow Shared Data Visualization Help Login or Register Using 0 bytes

Tools search tools Get Data Send Data Collection Operations Text Manipulation Filter and Sort Join, Subtract and Group Convert Formats Extract Features Fetch Sequences Fetch Alignments Statistics Graph/Display Data MyTools IRMA NGS Data Quality Check Workflows All workflows

Welcome to our Galaxy platform!

This galaxy server has been built and is maintained by the Bioinformatics Unit of Instituto de Salud Carlos III in order to give an user friendly enviroment to run limited bioinformatic tools and data analysis. Contact us if you are interested in the service and want to take an introductory course to the use this platform.

> BU-ISCIII

THIS IS A PROTOTYPE. If you find any bugs please report them to [mjuliam@isciii.es](mailto:mjuliam@isciii.es)

Take an interactive tour: Galaxy UI History Scratchbook

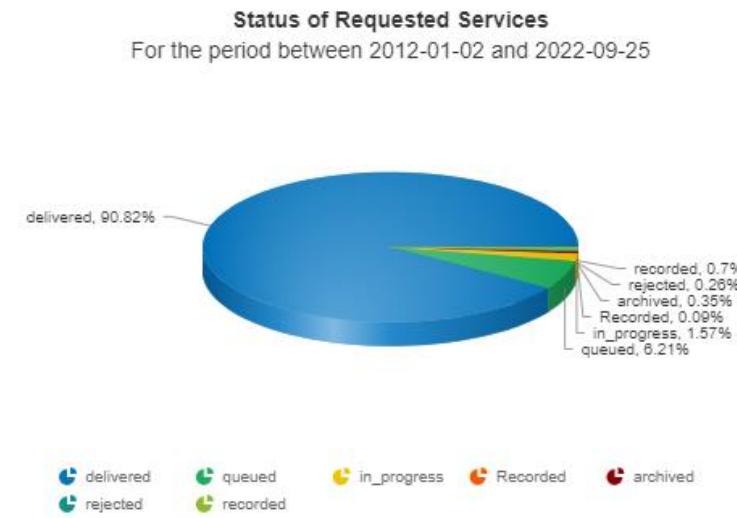
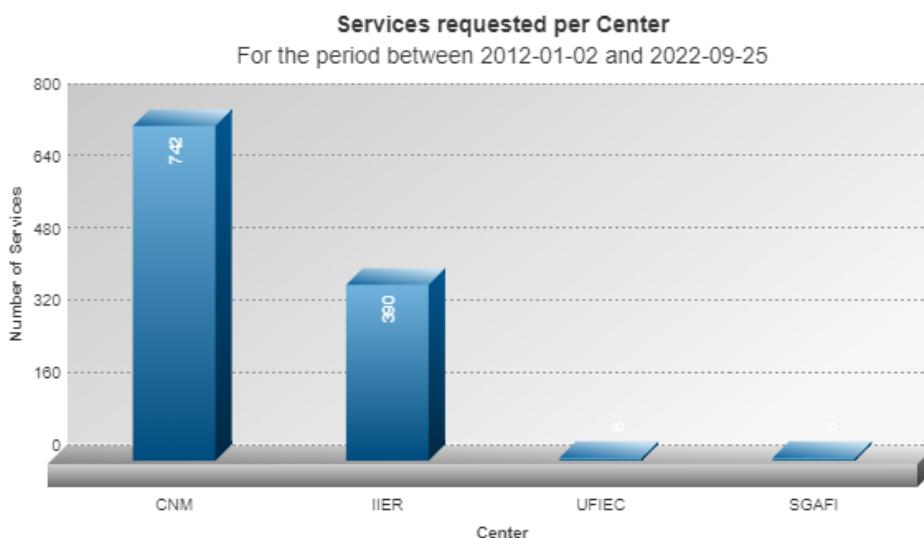
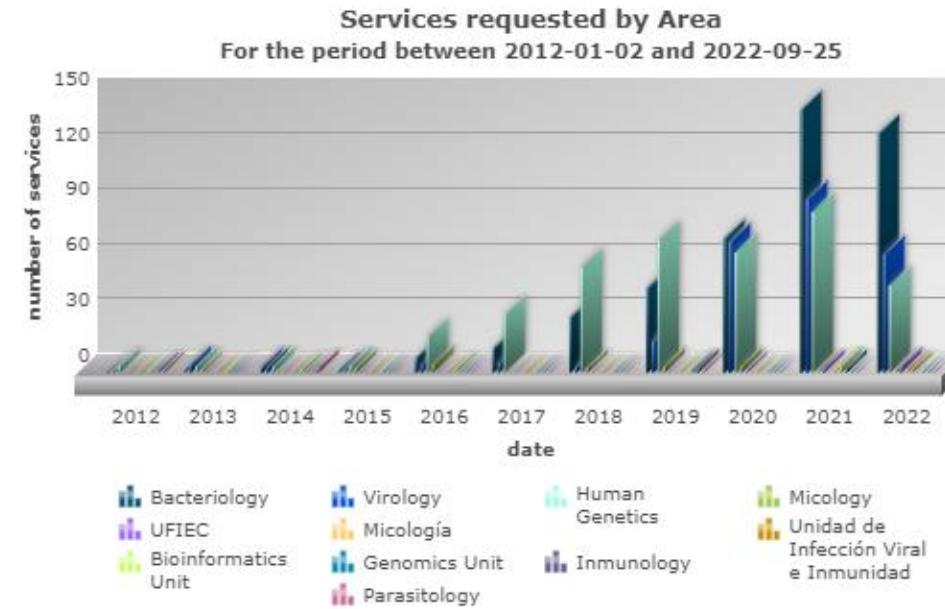
Galaxy is an open platform for supporting data intensive research. Galaxy is developed by [The Galaxy Team](#) with the support of [many contributors](#). If you use this platform to analyse your data, remember to cite both Galaxy Project and this server.

The [Galaxy Project](#) is supported in part by [NHGRI](#), [NSF](#), [The Huck Institutes of the Life Sciences](#), [The Institute for CyberScience at Penn State](#), and [Johns Hopkins University](#).

History search datasets Unnamed history (empty) This history is empty. You can [load your own data](#) or get data from an external source

# Number of services: 2012 – 2022

10  
AÑOS



# Training

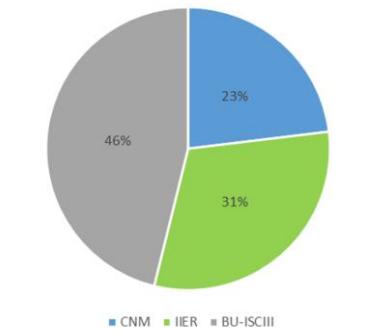
## Courses

ISCIII

Introduction to massive sequencing data analysis, 2013-2022 (9 editions)

Secuenciación de genomas bacterianos: herramientas y aplicaciones, 2018-2022 (4 editions)

Análisis de genomas virales a través de la plataforma Galaxy, 2022 (2 edition)

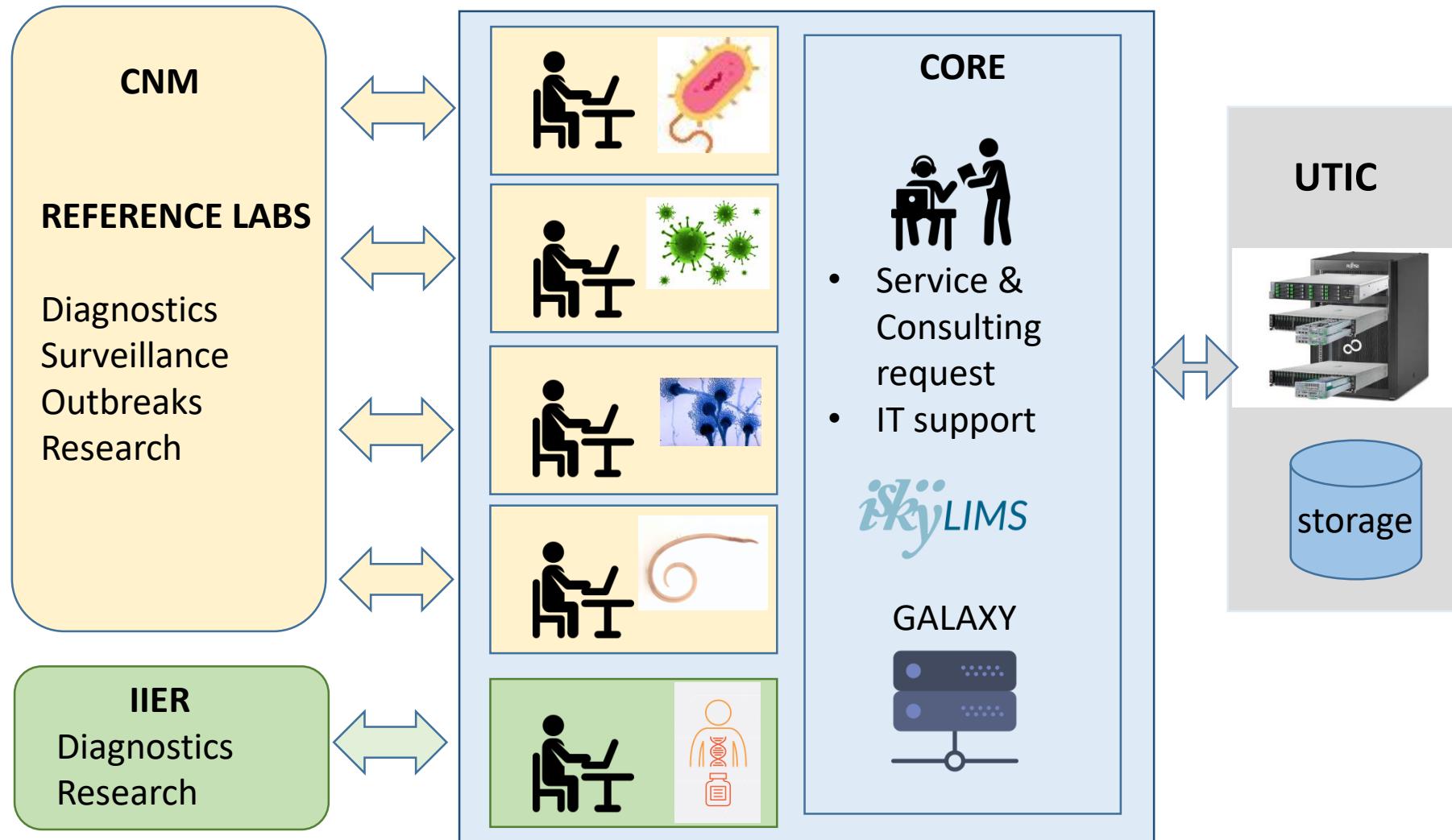


## Master & Grade Students

- Bioinformática y Biología Computacional ENS-ISCIII
- Bioinformática aplicada a la Medicina Personalizada ENS-ISCIII
- Bioinformática UAM
- Genética y Biología Molecular UAM
- Microbiología aplicada a la salud pública e investigación en enfermedades infecciosas, U. Alcalá de Henares
- Sciences in Omics Data Analysis, Universidad de VIC, U. Central de Cataluña
- Master Virología, Complutense University

## Hospitals Students

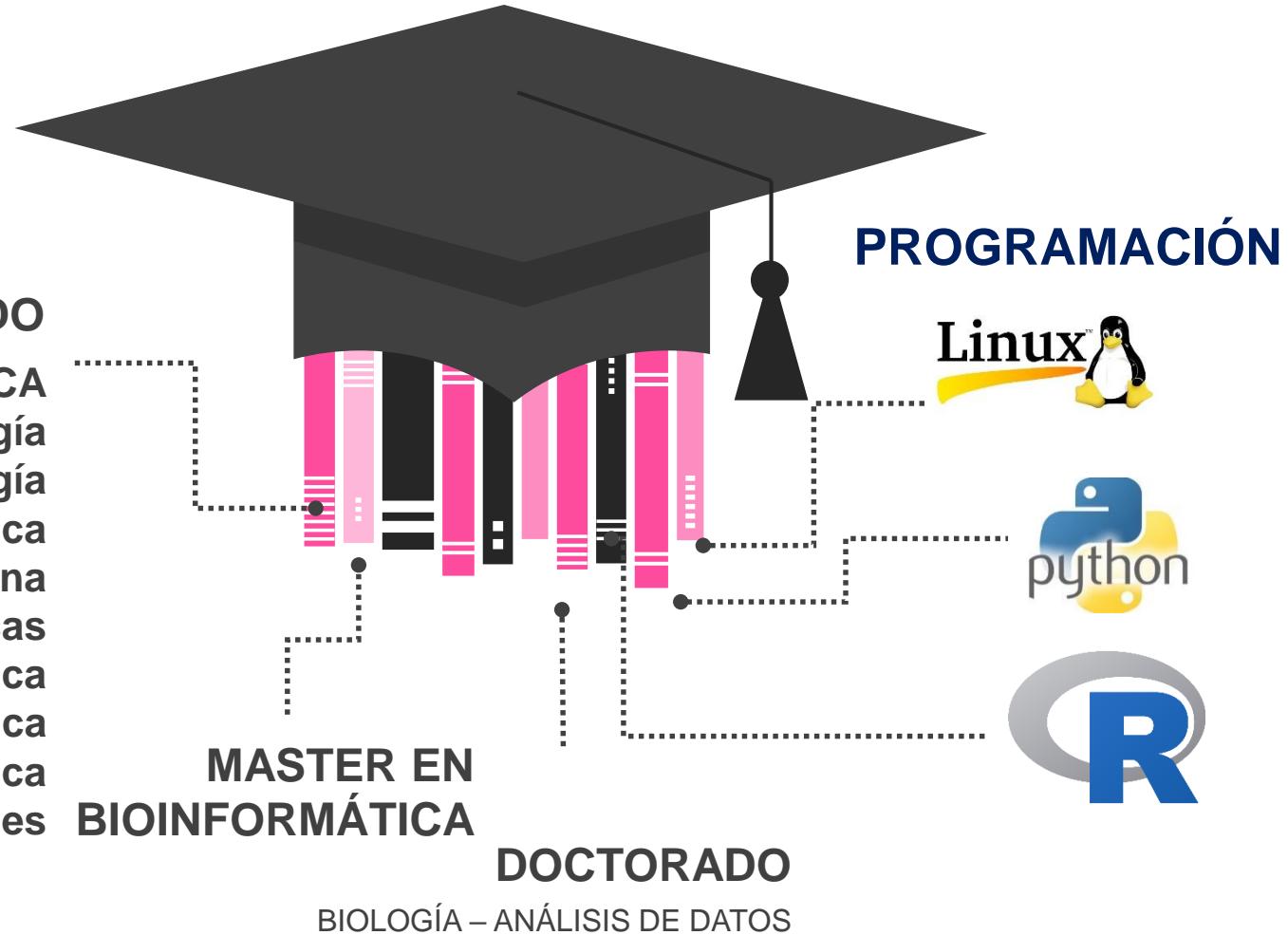
# Roadmap: BU-ISCIII Model



# FORMACIÓN EN BIOINFORMÁTICA

Universidad  
Barcelona.

**GRADO**  
**BIOINFORMÁTICA**  
Biología  
Biotecnología  
Bioquímica  
Medicina  
Matemáticas  
Química  
Física  
Informática  
Telecomunicaciones



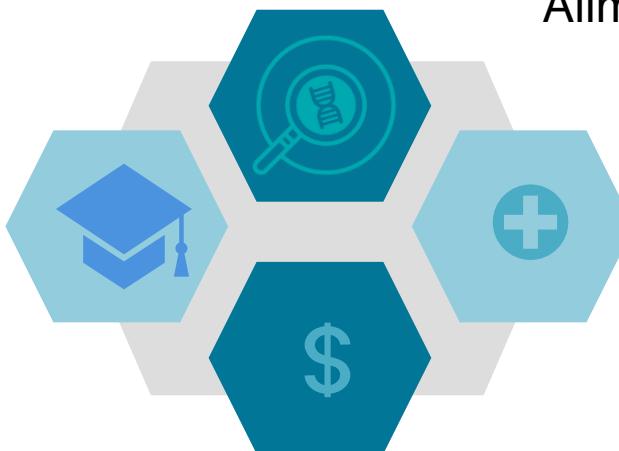
# ¿Dónde trabaja un Bioinformático?



## UNIVERSIDAD

Biociencias  
Informática

## CENTRO DE INVESTIGACIÓN



## EMPRESA

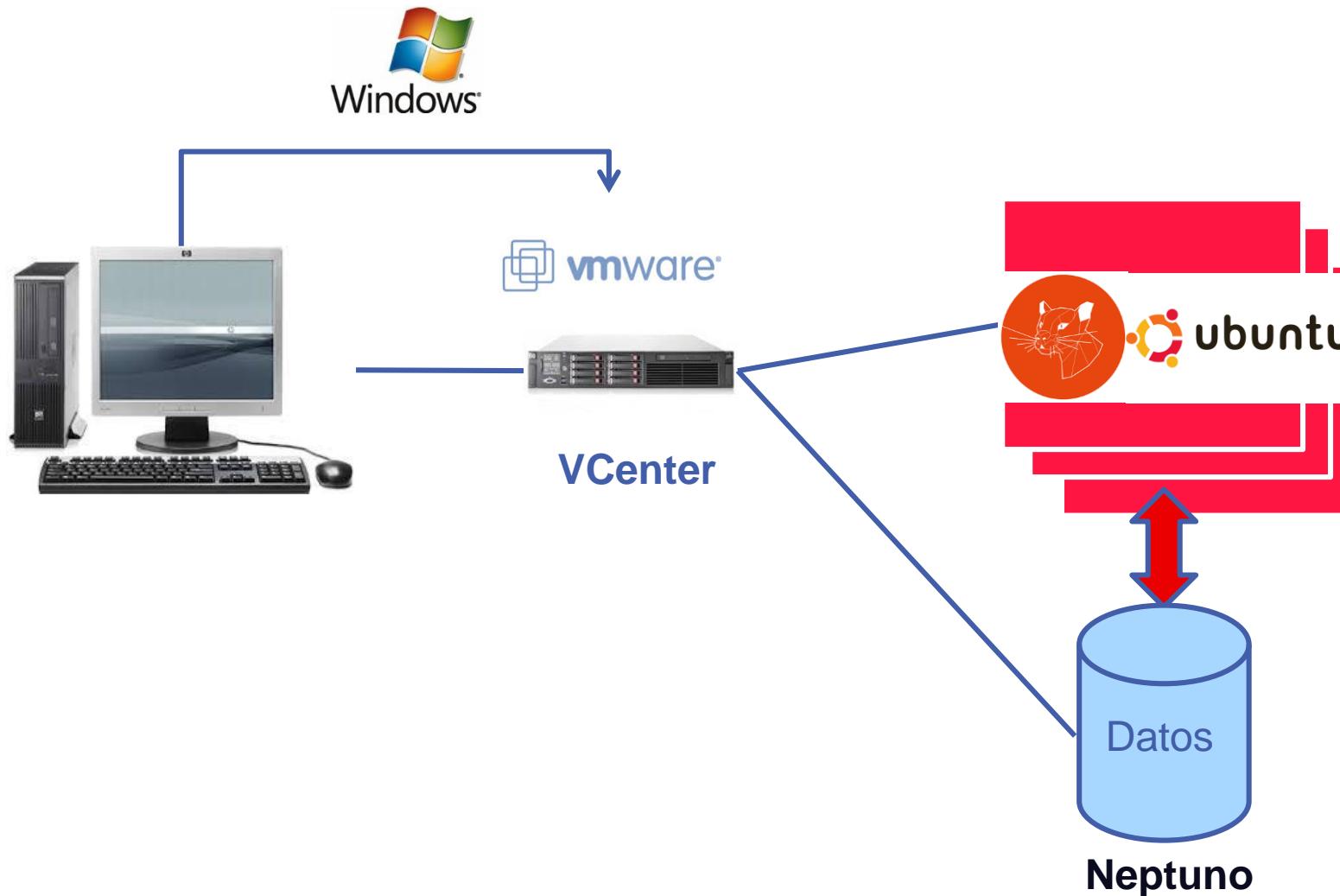
Bioinformática  
Genética  
Genómica

Biomedicina  
Agricultura  
Alimentación

## HOSPITAL

**BIOINFORMÁTICO CLÍNICO**  
Genética  
Oncología  
Cardiología

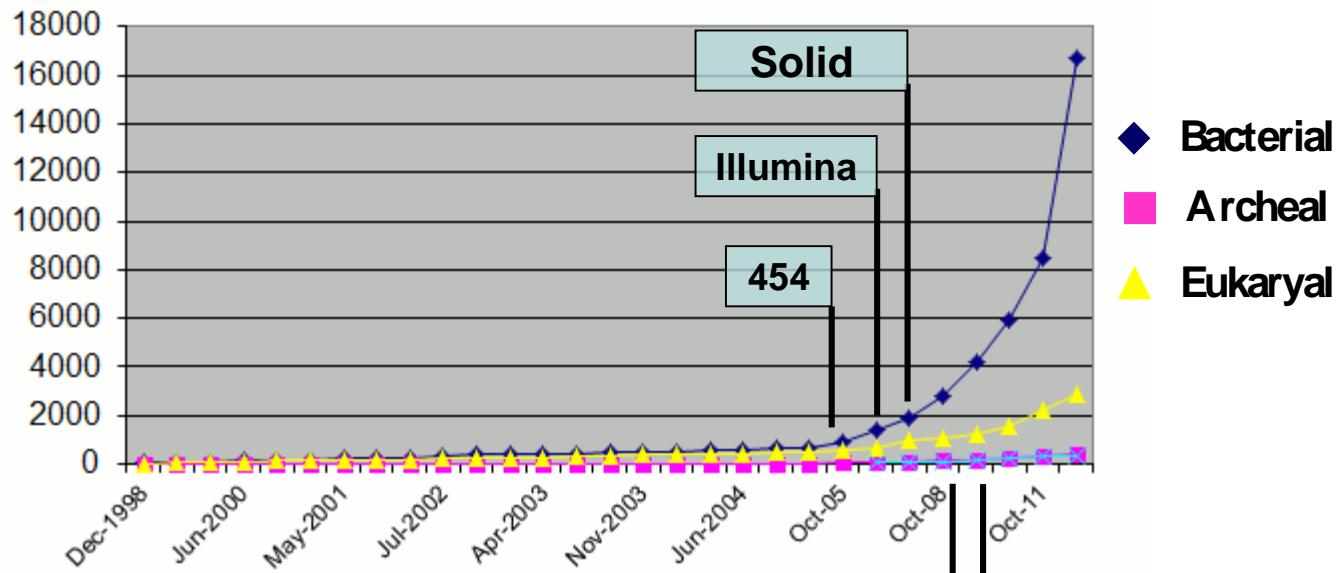
# Recursos Informáticos para el curso



# Genomics Revolution Era



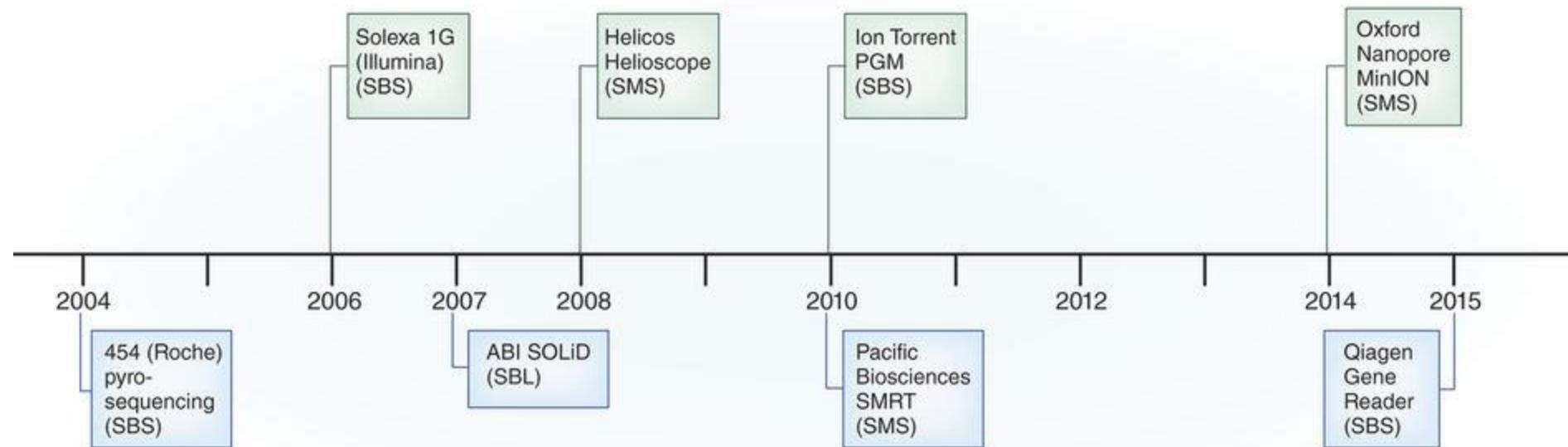
Genome Projects on GOLD according to Phylogenetic Groups ©  
October 2012 - 20327 Projects



Source: <http://www.genomeonline.org>

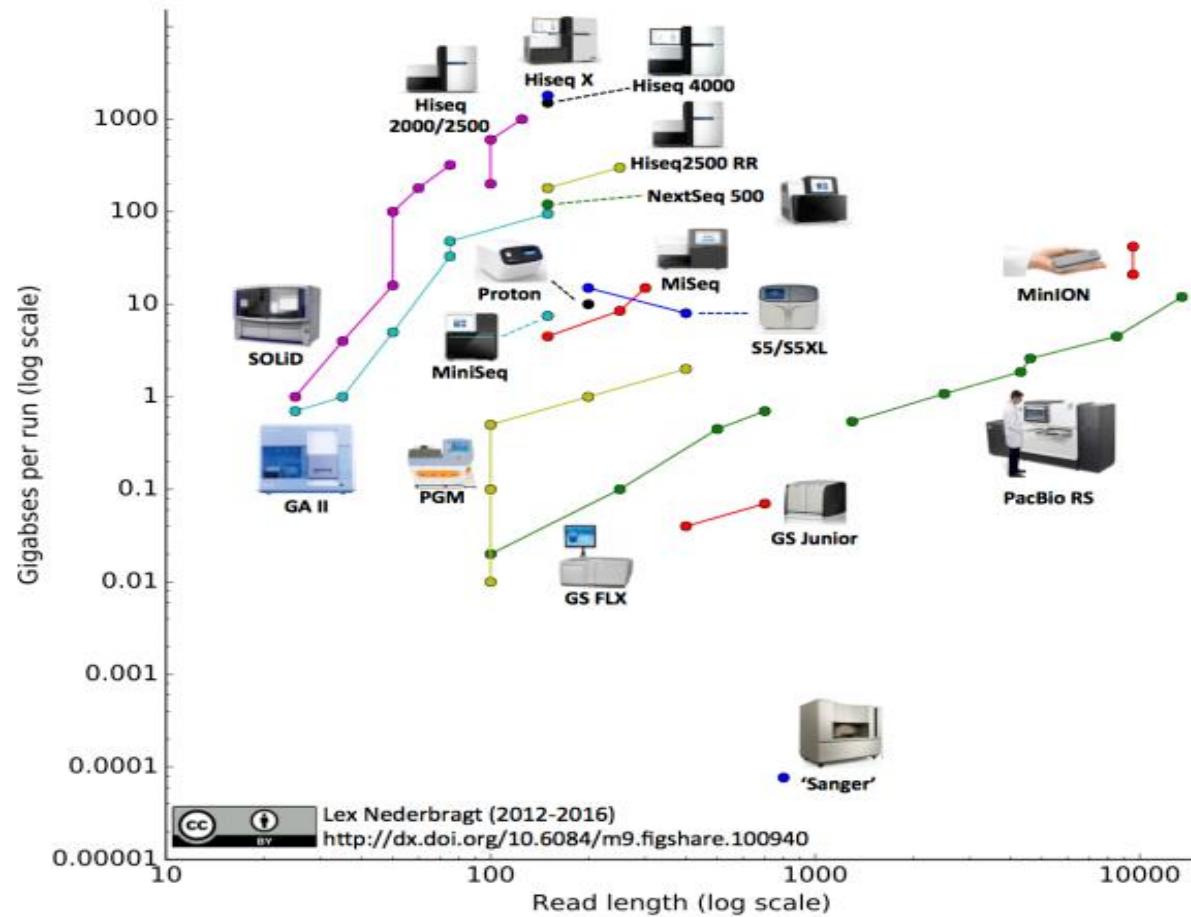


# DNA sequencing technologies 2006-2016



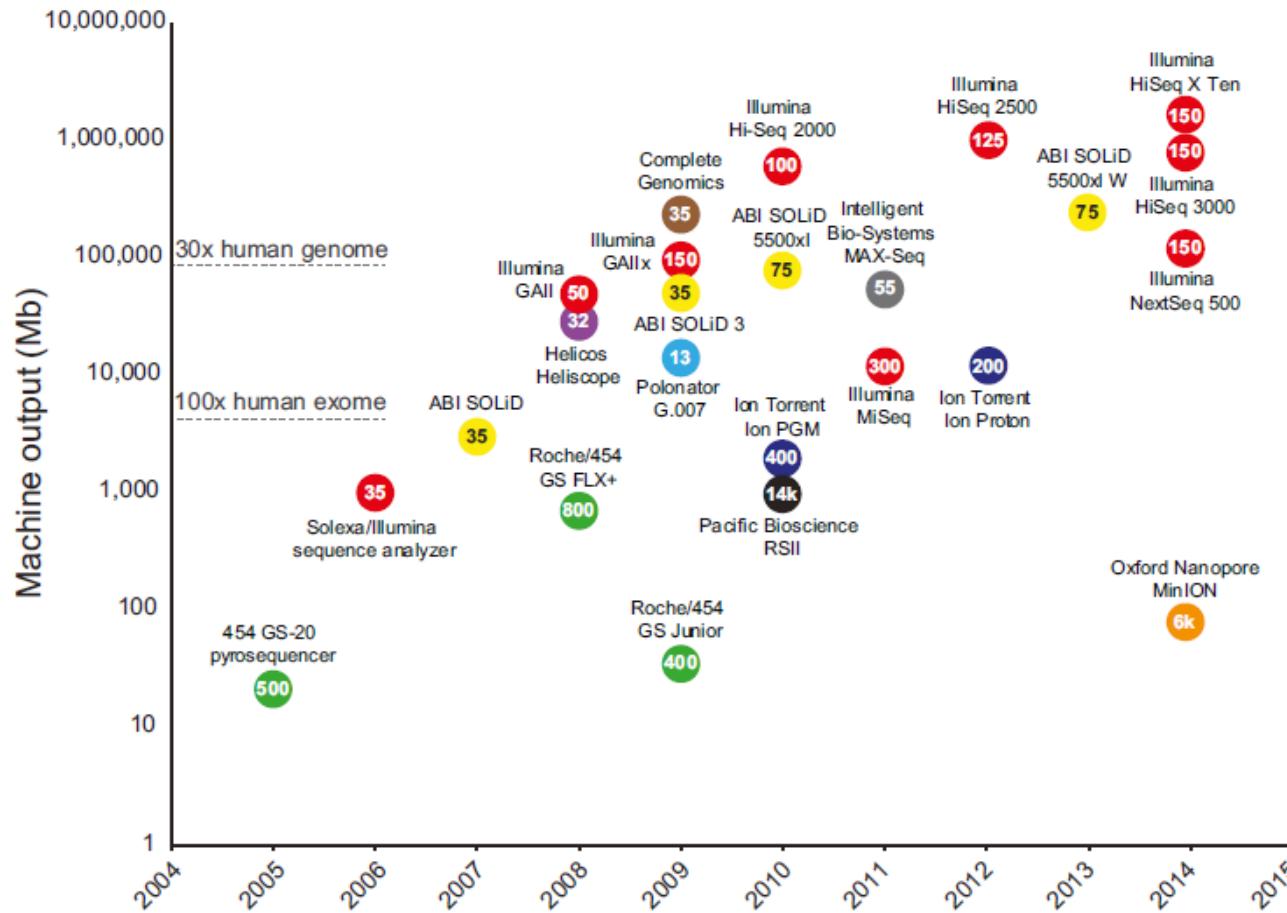
Mardis, Nature Protocols 2017

# High-Throughput Sequencing Technologies



<https://flxlexblog.wordpress.com/>

# High-Throughput Sequencing Technologies

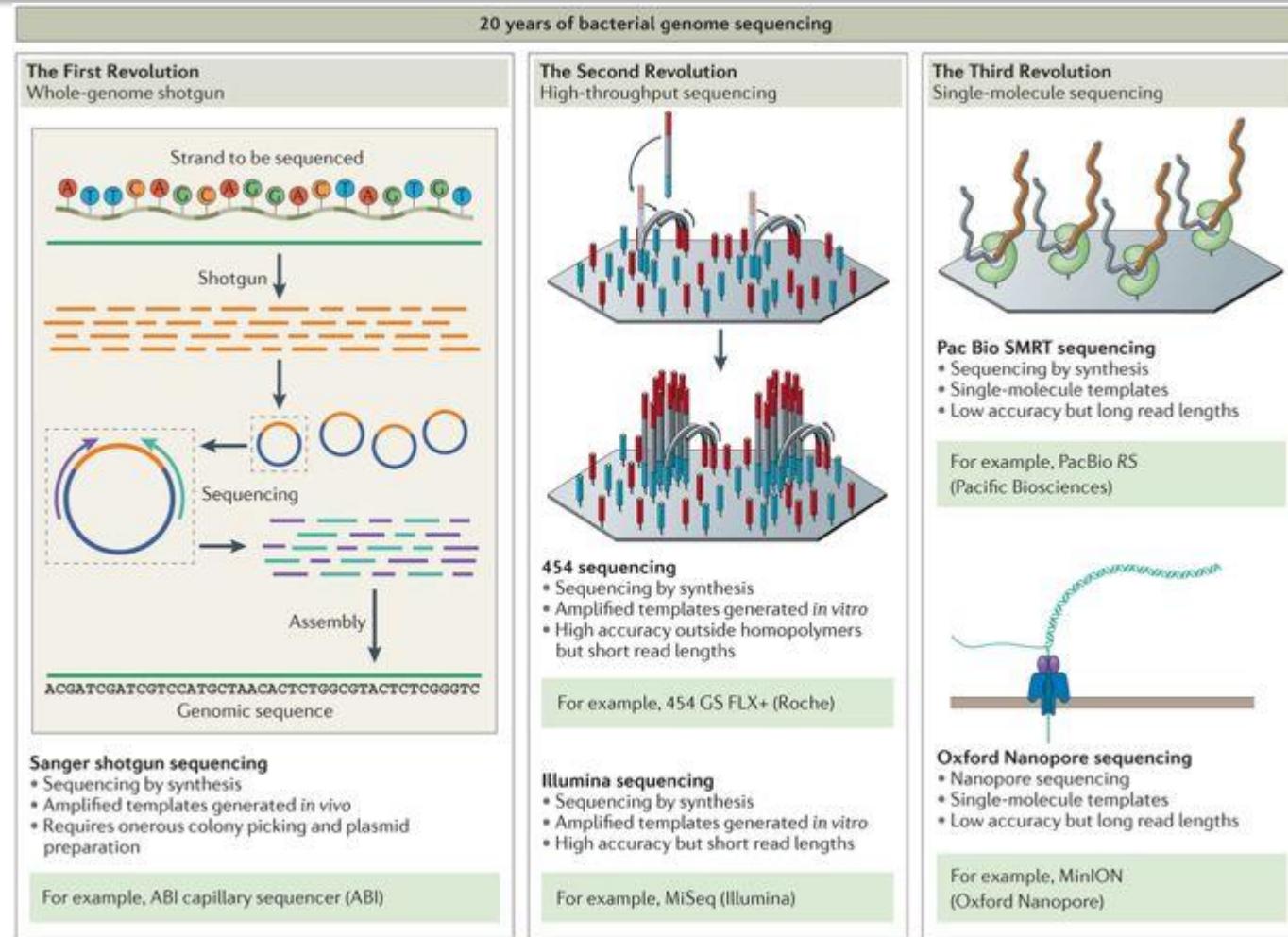


Numbers inside data points denote current read lengths.  
Sequencing platforms are color coded.

Reuter et al., Mol Cell 2015

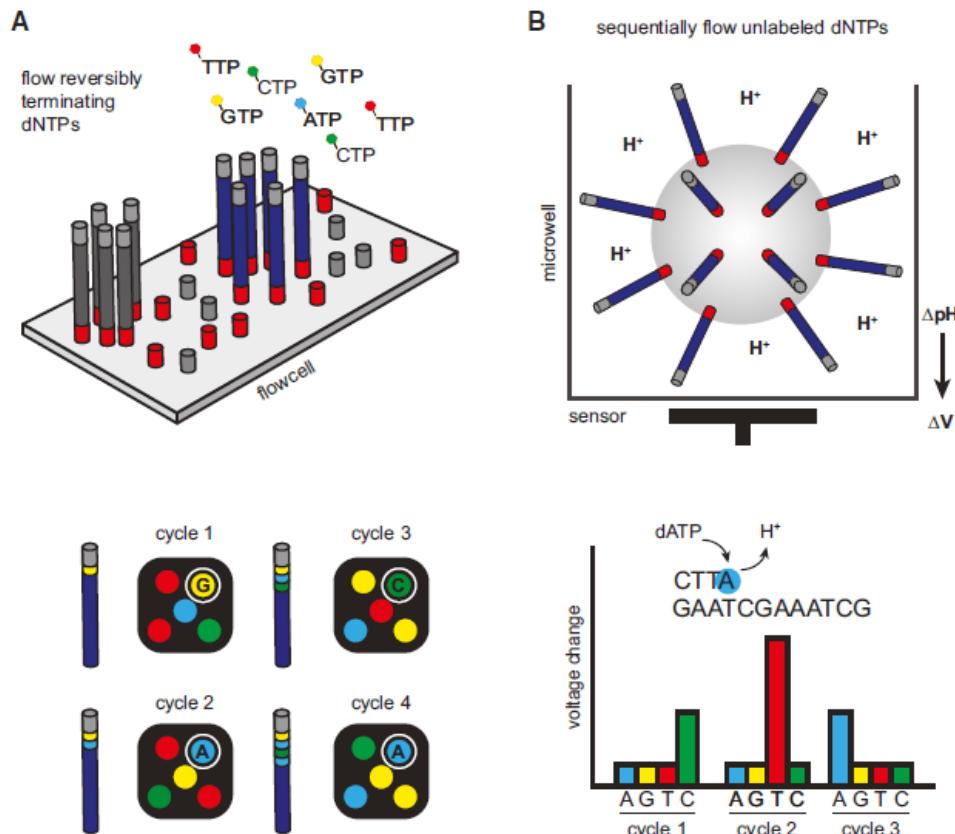
# High-Throughput Sequencing Technologies

The three revolutions in sequencing technology that have transformed the landscape of bacterial genome sequencing



Nature Reviews | Microbiology

# The Second-generation Sequencing Technologies



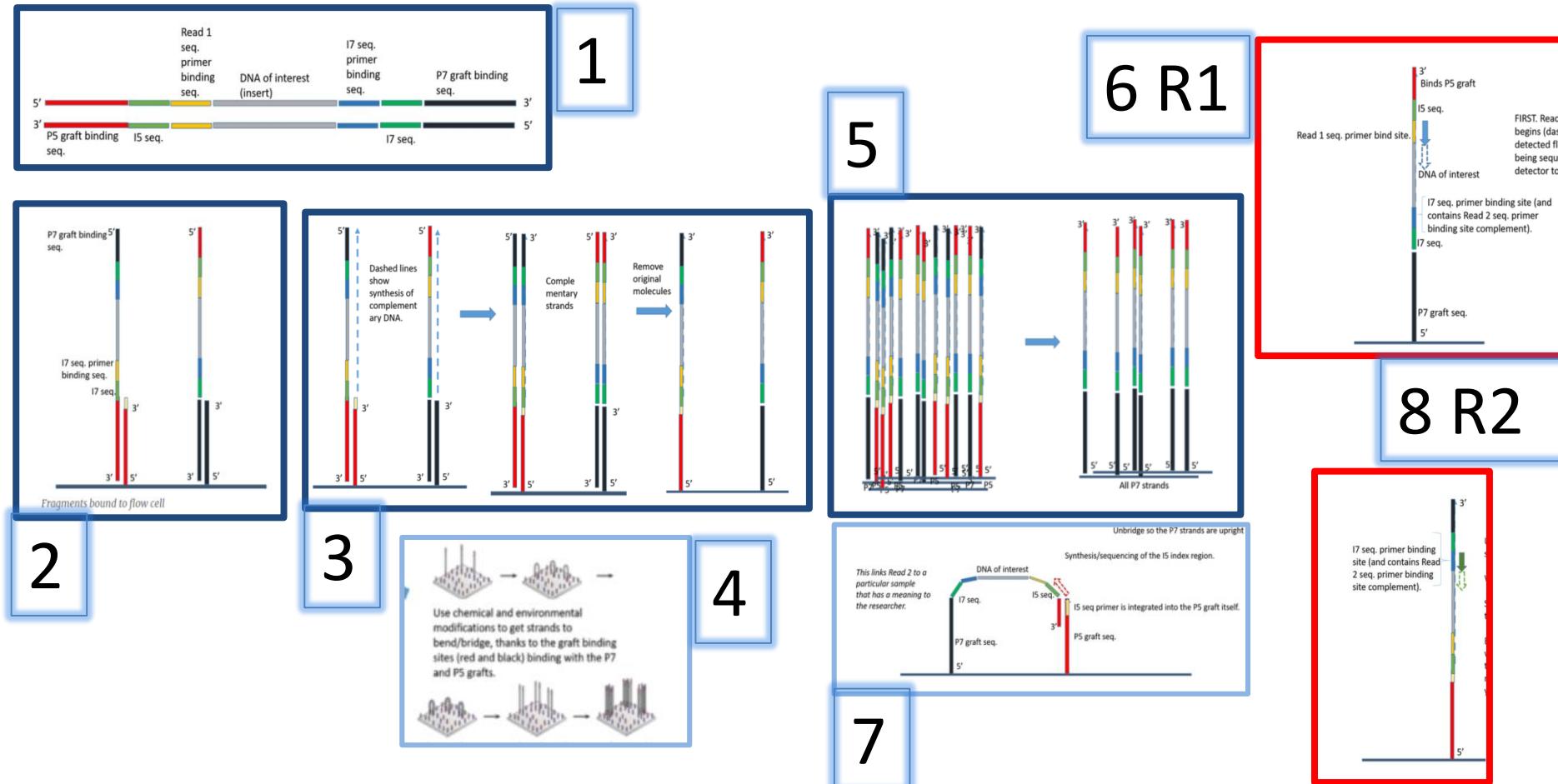
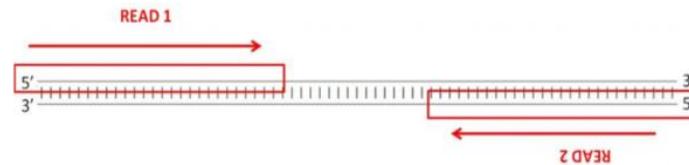
## Clonal Amplification-Based Sequencing Platforms

(A) Illumina's four-color reversible termination sequencing method.

(B) Ion Torrent's semiconductor sequencing method.

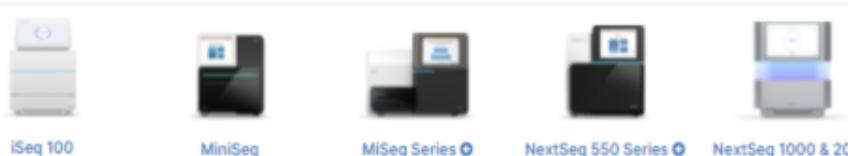
Reuter et al., Mol Cell 2015

# Illumina sequencing



<https://kscbioinformatics.wordpress.com/2017/02/13/illumina-sequencing-for-dummies-samples-are-sequenced/>

# Illumina Benchtop Sequencers



Popular Applications & Methods	iSeq 100	MiniSeq	MiSeq Series	NextSeq 550 Series	NextSeq 1000 & 2000
Large Whole-Genome Sequencing (human, plant, animal)					
Small Whole-Genome Sequencing (microbe, virus)	●	●	●	●	●
Exome & Large Panel Sequencing (enrichment-based)				●	●
Targeted Gene Sequencing (amplicon-based, gene panel)	●	●	●	●	●
Single-Cell Profiling (scRNA-Seq, scDNA-Seq, oligo tagging assays)				●	●
Transcriptome Sequencing (total RNA-Seq, mRNA-Seq, gene expression profiling)				●	●
Targeted Gene Expression Profiling	●	●	●	●	●
miRNA & Small RNA Analysis	●	●	●	●	●
DNA-Protein Interaction Analysis (ChIP-Seq)			●	●	●
Methylation Sequencing				●	●
16S Metagenomic Sequencing		●	●	●	●
Metagenomic Profiling (shotgun metagenomics, metatranscriptomics)				●	●
Cell-Free Sequencing & Liquid Biopsy Analysis				●	●

## Benchtop Sequencer Sheds Light on Ebola Outbreak

Local scientists use the iSeq 100 Sequencing System to analyze transmission patterns and trace the origin of an Ebola outbreak in the Democratic Republic of the Congo.

[Read Article ▶](#)

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

# Illumina Production-Scale Sequencers



NextSeq 550 Series

NextSeq 1000 & 2000

NovaSeq 6000

Popular Applications & Methods	Key Application	Key Application	Key Application
Large Whole-Genome Sequencing (human, plant, animal)			●
Small Whole-Genome Sequencing (microbe, virus)	●	●	●
Exome & Large Panel Sequencing (enrichment-based)	●	●	●
Targeted Gene Sequencing (amplicon-based, gene panel)	●	●	●
Single-Cell Profiling (scRNA-Seq, scDNA-Seq, oligo tagging assays)	●	●	●
Transcriptome Sequencing (total RNA-Seq, miRNA-Seq, gene expression profiling)	●	●	●
Chromatin Analysis (ATAC-Seq, ChIP-Seq)	●	●	●
Methylation Sequencing	●	●	●
Metagenomic Profiling (shotgun metagenomics, metatranscriptomics)	●	●	●
Cell-Free Sequencing & Liquid Biopsy Analysis	●	●	●

## Optimized NGS Sample Tracking and Workflows

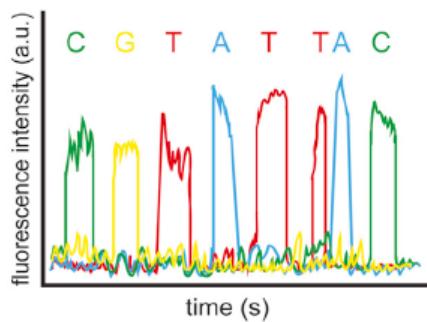
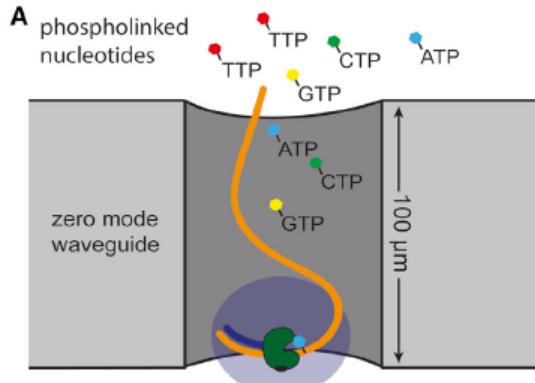
See how a Laboratory Information Management System (LIMS) enabled this large genomics lab to standardize lab procedures and cope with increasing sample volumes from diverse clients.

[Read Case Study >](#)

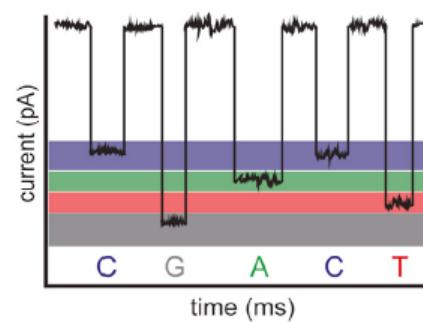
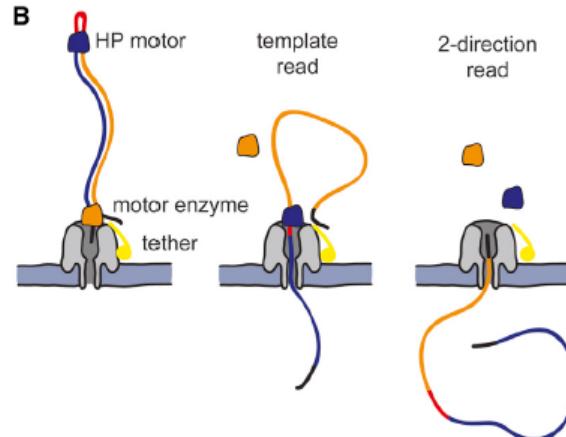
Run Time	12-30 hours	11-48 hours	-13 - 38 hours (dual SP flow cells) -13-25 hours (dual S1 flow cells) -16-36 hours (dual S2 flow cells) -44 hours (dual S4 flow cells)
Maximum Output	120 Gb	330 Gb*	6000 Gb
Maximum Reads Per Run	400 million	1.1 billion*	20 billion
Maximum Read Length	2 × 150 bp	2 × 150 bp	2 × 250**

# The Third-generation Sequencing Technologies

## Single Molecule Sequencing Platforms



Pacific Bioscience's SMRT sequencing



Oxford Nanopore's sequencing

Reuter et al., Mol Cell 2015

# PacBio sequencing and its applications

Rhoads & Au, Gen Prot Bioinf 2015



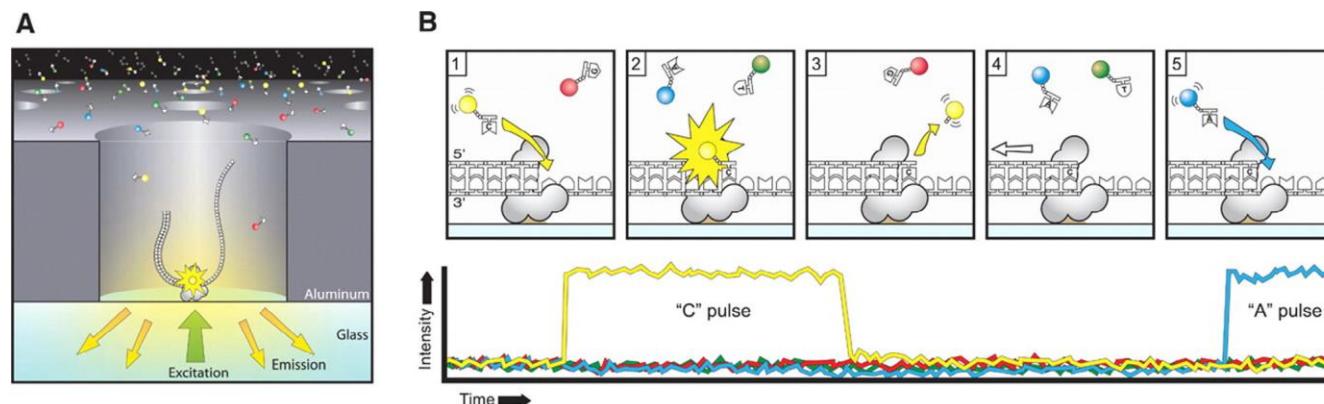
**SMRTbell template:** is a closed, single-stranded circular DNA that is created by ligating hairpin adaptors to both ends of a target dsDNA

**Sequencing by light pulses:** The replication processes in all ZMWs of a SMRTcell are recorder by a movie of light pulses, and the pulses corresponding to each ZMW can be interpreted to be a sequence of bases (**continuous long read, CLR**).

Both strands can be sequenced multiple times (passes) in a single CLR. CLR can be split to multiple reads (subreads) and CCS is the consensus sequence of multiple subreads



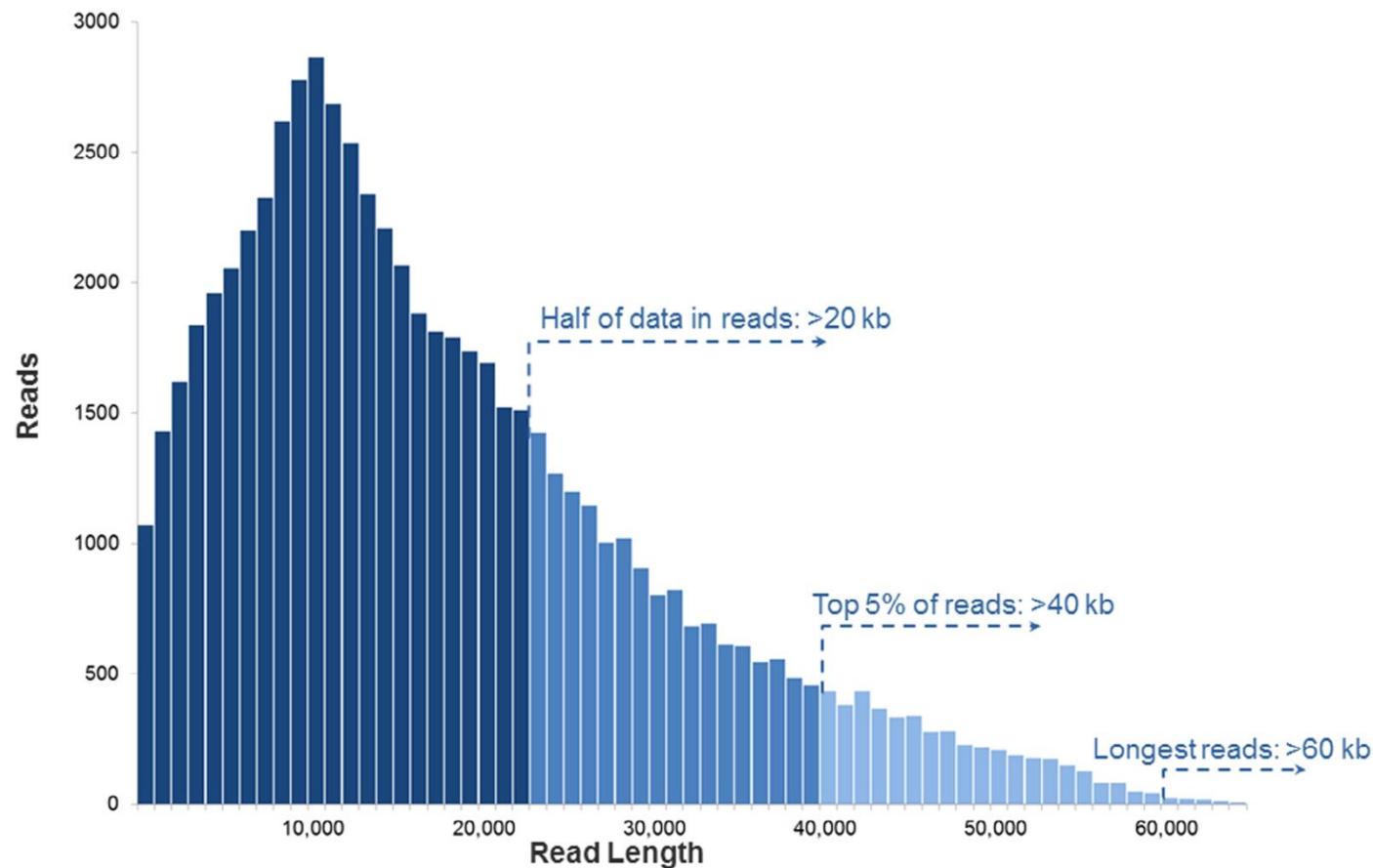
**A single SMRT cell:** this contains 150000 ZMWs (zero-mode waveguide). A SMRTbell diffuses into a ZMW. Approx 35000 -75000 ZMWs produce a read in a run lasting 0,5-4h resulting in 0,5-1Gb.



# PacBio sequencing and its applications

Rhoads & Au, Gen Prot Bioinf 2015

**PacBio RS II read length distribution** using P6-C4 chemistry. Data are based on a 20kb size-selected E. coli library using a 4-h movie. A SMRTcell produces 0,5-1 billion bases.



# PacBio sequencing and its applications

Rhoads & Au, Gen Prot Bioinf 2015

Table 2 *De novo* genome assemblies using hybrid sequencing or PacBio sequencing alone

Species	Method	Tools	SMRT cells	Coverage	Contigs	Achievements	Ref.
<i>Clostridium autoethanogenum</i>	PacBio	HGAP	2	179×	1	21 fewer contigs than using SGS; no collapsed repeat regions ( $\geq 4$ using SGS)	[7]
<i>Potentilla micrantha</i> (chloroplast)	PacBio	HGAP, Celera, minimus2, SeqMan	26	320×	1	6 fewer contigs than with Illumina; 100% coverage (Illumina: 90.59%); resolved 187 ambiguous nucleotides in Illumina assembly; unambiguously assigned small differences in two $> 25$ kb inverted repeats	[33]
<i>Escherichia coli</i>	PacBio	PBcR, MHAP, Celera, Quiver	1	85×	1	4.6 CPU hours for genome assembly (10× improvement over BLASR)	[31]
<i>Saccharomyces cerevisiae</i>	PacBio	PBcR, MHAP, Celera	12	117×	21	27 CPU hours for genome assembly (8× improvement over BLASR); improved current reference of telomeres	[31]
<i>Arabidopsis thaliana</i>	PacBio	PBcR, MHAP, Celera	46	144×	38	1896 CPU hours for genome assembly	[31]
<i>Drosophila melanogaster</i>	PacBio	PBcR, MHAP, Celera, Quiver	42	121×	132	1060 CPU hours for genome assembly (593× improvement over BLASR); improved current reference of telomeres	[31]
<i>Homo sapiens</i> (CHM1hert)	PacBio	PBcR, MHAP, Celera	275	54×	3434	262,240 CPU hours for genome assembly; potentially closed 51 gaps in GRCh38; assembled MHC in 2 contigs (60 contigs with Illumina); reconstructed repetitive heterochromatic sequences in telomeres	[31]
<i>Homo sapiens</i> (CHM1tert)	PacBio	BLASR, Celera, Quiver	243	41×	N/A (local assembly)	Closed 50 gaps and extended into 40 additional gaps in GRCh37; added over 1 Mb of novel sequence to the genome; identified 26,079 indels at least 50 bp in length; cataloged 47,238 SV breakpoints	[32]
<i>Melopsittacus undulatus</i>	Hybrid	PBcR, Celera	3	5.5× PacBio + 15.4× 454 = 3.83× corrected	15,328	1st assembly of $> 1$ Gb parrot genome; N50 = 93,069	[34]
<i>Vibrio cholerae</i>	Hybrid	BLASR, Bambus, AHA	195	200× PacBio + 28× Illumina + 22× 454	2	No N's in contigs; 99.99% consensus accuracy; N50 = 3.01 Mb	[30]
<i>Helicobacter pylori</i>	PacBio	HGAP, Quiver, PGAP	8 per strain	446.5× average among strains	1 per strain	1 complete contig for each of 8 strains; methylation analysis associated motifs with genotypes of virulence factors	[35]

Note: N50, the contig length for which half of all bases are in contigs of this length or greater; MHC, major histocompatibility complex; SV, structural variation.

# PacBio sequencing and its applications

Rhoads & Au, Gen Prot Bioinf 2015

## Advantage

Closes gaps and completes genomes due to longer reads

Identifies non-SNP SVs

## Achievements

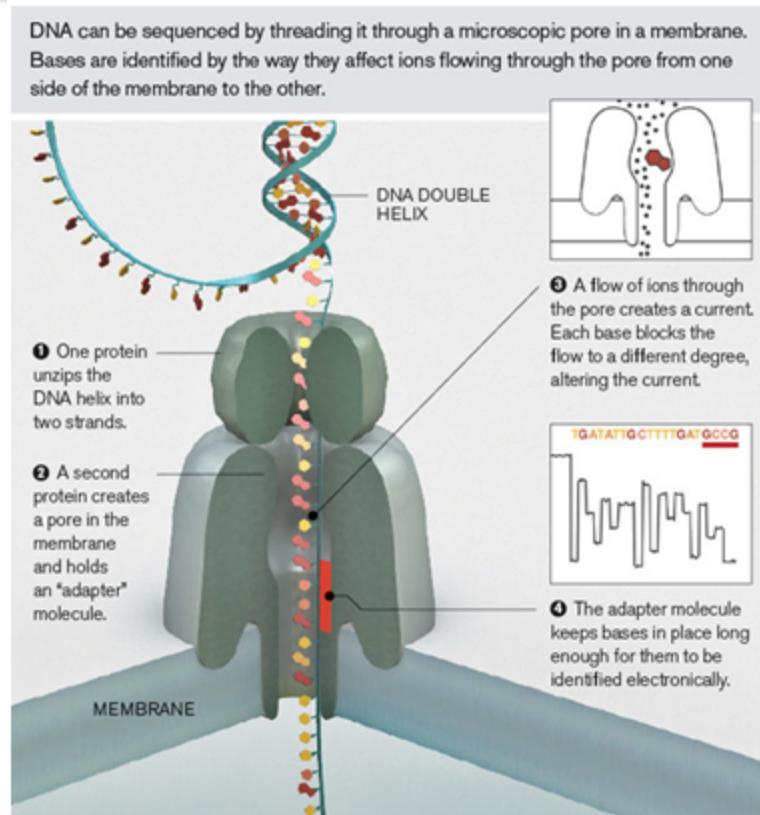
Produced highly-contiguous assemblies of bacterial and eukaryotic genomes

Discovered STRs (short tandem repeats)

## Limitations

Both strands can be sequenced several times if the lifetime of the polymerase is long enough.

# Nanopore-based fourth-generation DNA sequencing technology. ONT, Oxford Nanopore Technologies



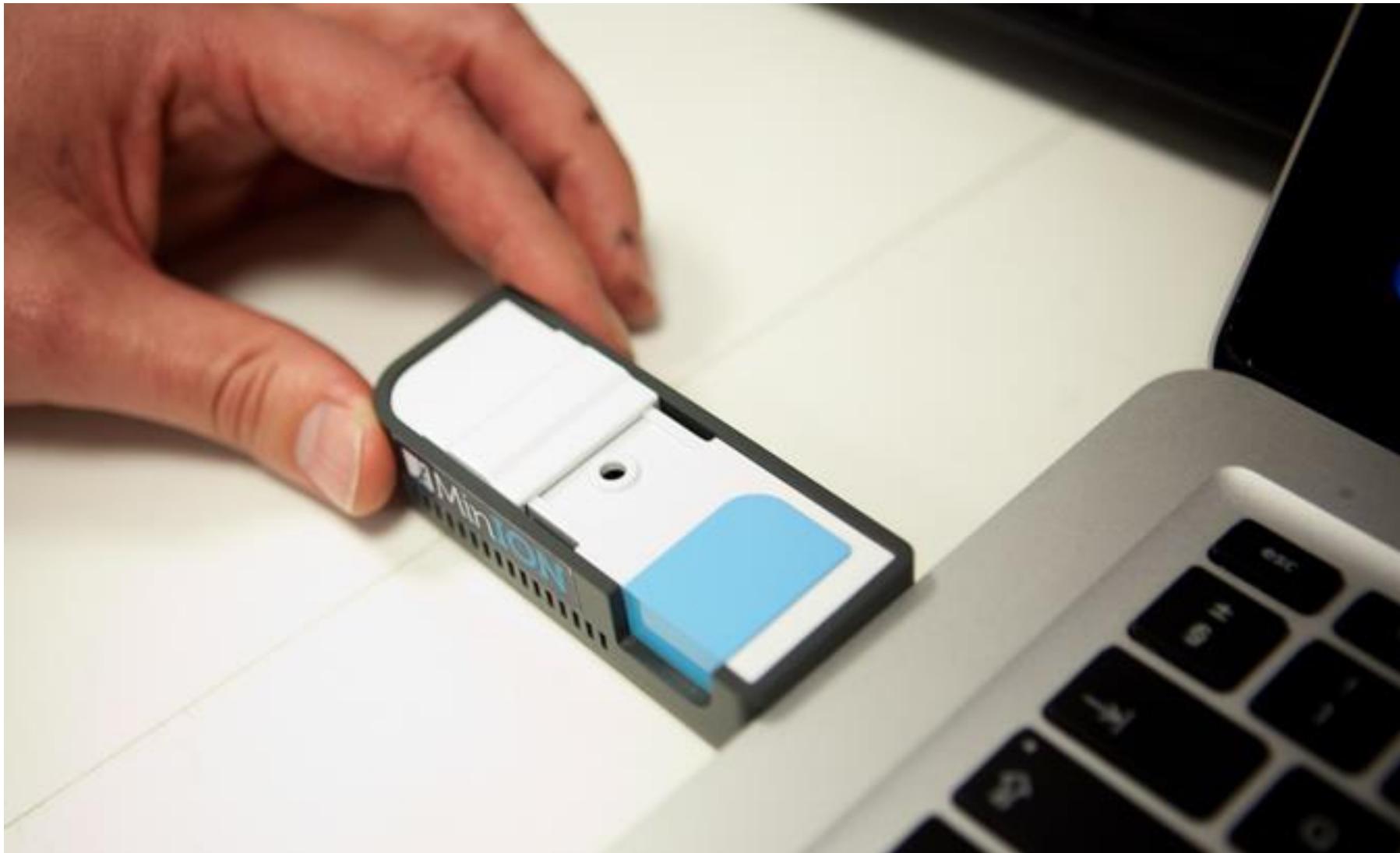
'Strand sequencing' is a technique that passes intact DNA polymers through a protein nanopore, sequencing in real time as the DNA translocates the pore.

Nanopore sequencing also offers, for the first time, direct RNA sequencing, as well as PCR or PCR-free cDNA sequencing.

<https://nanoporetech.com/applications/dna-nanopore-sequencing>

Feng et al , Gen Prot Bioinf 2015

# MinIon, OXFORD NANOPORE



<https://nanoporetech.com/news/movies#movie-24-nanopore-dna-sequencing>

# Oxford Nanopore Technologies, MinION



The MinION is a portable sequencer; flow cells contain up to 512 nanopore sensors.

The Oxford Nanopore system processes the reads that are presented to it rather than generating read lengths. Sample-prep dependent, the longest read reported by a MinION user to date is >1 Mb.

Long reads confer many advantages, including simpler assembly and in the analysis of repetitive regions, phasing or CNVs.



For MinION / GridION  
**Flongle**

Adapter to enable small, rapid nanopore sequencing tests, for mobile or desktop sequencers



**MinION Mk1B**

Your personal nanopore sequencer, putting you in control



**MinION Mk1C**

Your personal nanopore sequencer including compute and screen, putting you in control



**GridION Mk1**

Higher-throughput, on demand nanopore sequencing at the desktop, for you or as a service



**PromethION 24/48**

Ultra-high throughput, on-demand nanopore sequencing, for you or as a service

	Flongle	MinION Mk1B	MinION Mk1C	GridION Mk1	PromethION 24	PromethION 48
Number of channels per flow cell	126	512	512	512	3000	3000
Number of flow cells per device	1	1	1	5	24	48
Price per flow cell	\$90	\$900 - \$475	\$900 - \$475	\$900 - \$475	\$2000 - \$625	\$2000 - \$625
Run time	1 min - 16 hours	1 min - 72 hours	1 min - 72 hours	1 min - 72 hours	1 min - 72 hours	1 min - 72 hours
Yields in field are dependent on sample and preparation methods. Users can get outputs in the following ranges per flow cell when utilising the latest chemistries and protocols	1 - 2 Gb	10 - 30 - 50 Gb	10 - 30 - 50 Gb	10 - 30 - 50 Gb	100 - 200 - 300 Gb	100 - 200 - 300 Gb
Price per Gb for different flow cell yields (yields vary according to sample and preparation methods)	@1 - 2 Gb \$90 per flow cell: \$90 - 45	@10 - 30 - 50 Gb \$900 per flow cell: \$90 - 30 - 18 \$790 per flow cell: \$79 - 26 - 16 \$675 per flow cell: \$68 - 23 - 14 \$500 per flow cell: \$50 - 17 - 10 \$475 per flow cell: \$48 - 16 - 9.5	@10 - 30 - 50 Gb \$900 per flow cell: \$90 - 30 - 18 \$790 per flow cell: \$79 - 26 - 16 \$675 per flow cell: \$68 - 23 - 14 \$500 per flow cell: \$50 - 17 - 10 \$475 per flow cell: \$48 - 16 - 9.5	@10 - 30 - 50 Gb \$900 per flow cell: \$90 - 30 - 18 \$790 per flow cell: \$79 - 26 - 16 \$675 per flow cell: \$68 - 23 - 14 \$500 per flow cell: \$50 - 17 - 10 \$475 per flow cell: \$48 - 16 - 9.5	@100 - 200 - 300 Gb \$1,600 per flow cell: \$16 - 8 - 5 \$1,120 per flow cell: \$11 - 6 - 4 \$940 per flow cell: \$9 - 5 - 3.1 \$680 per flow cell: \$7 - 3.4 - 2.3 \$625 per flow cell: \$6 - 3 - 2	@100 - 200 - 300 Gb \$1,600 per flow cell: \$16 - 8 - 5 \$1,120 per flow cell: \$11 - 6 - 4 \$940 per flow cell: \$9 - 5 - 3.1 \$680 per flow cell:

# Library preparation



Oxford Nanopore has developed VolTRAX – a small device designed to perform library preparation automatically, so that a user can get a biological sample ready for analysis, hands-free. VolTRAX is designed as an alternative to a range of lab equipment, to allow consistent and varied, automated library prep options.

## VolTRAX V2 Starter Pack

\$8,000.00

VolTRAX V2 is designed to automate all laboratory processes associated with Nanopore Sequencing from sample extraction to library preparation.

# MinIT, Analysis



Eliminating the need for a dedicated laptop for nanopore sequencing with MinION.  
\$2400

## MinIT Specifications:

Pre-installed software: Linux OS, MinKNOW, Guppy, EPI2ME

Bluetooth and Wi-Fi enabled; you can control your experiments using a laptop, tablet or smartphone

fastq or fast5 files are written to Onboard storage: 512 GB SSD

Processing: GPU accelerators (ARM processor 6 cores, 256 Core GPU), 8 GB RAM.

Small footprint, 290g

1 x USB 2.0 port, 1 x USB 3.0 port and 1 x Ethernet port (1 Gbit capacity)

MinIT has now been replaced by the MinION Mk1C, which combines the real-time, portable sequencing of MinION, with powerful integrated compute, a high-resolution touchscreen, and full connectivity.

# SmidgION, Mobile analysis



Oxford Nanopore has now started developing an even smaller device, SmidgION.

**potential applications** may include remote monitoring of pathogens in a breakout or infectious disease; the on-site analysis of environmental samples such as water/metagenomics samples, real time species ID for analysis of food, timber, wildlife or even unknown samples; field-based analysis of agricultural environments, and much more.

# PacBio sequencing and its applications

Rhoads & Au, Gen Prot Bioinf 2015

## 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 3730×1	1st	600–1000	0.001	96	0.5–3 h	500	[14,18–21]
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	$2 \times 125$	0.1	$8 \times 10^9$ (paired)	7–60 h	0.03	[9,16,26]
Illumina HiSeq 2500 (Rapid Run)	2nd	$2 \times 250$	0.1	$1.2 \times 10^9$ (paired)	1–6 days	0.04	[9,16,26]
SOLiD 5500×1	2nd	$2 \times 60$	5	$8 \times 10^8$	6 days	0.11	[14,24]
PacBio RS II: P6-C4	3rd	$1.0\text{--}1.5 \times 10^4$ on average	13	$3.5\text{--}7.5 \times 10^4$	0.5–4 h	0.40–0.80	[5,12,15]
Oxford Nanopore MinION	3rd	$2\text{--}5 \times 10^3$ on average	38	$1.1\text{--}4.7 \times 10^4$	50 h	6.44–17.90	[22,23]

# Characteristics, strengths and weaknesses of commonly used sequencing platforms

**Table 2**

Characteristics, strengths and weaknesses of commonly used sequencing platforms

Platform \ Instrument	Throughput range (Gb) <sup>a</sup>	Read length (bp)	Strength	Weakness
<i>Sanger sequencing</i>				
ABI 3500/3730	0.0003	Up to 1 kb	Read accuracy and length	Cost and throughput
<i>Illumina</i>				
MiniSeq	1.7–7.5	1×75 to ×150	Low initial investment	Run and read length
MiSeq	0.3–15	1×36 to 2×300	Read length, scalability	Run length
NextSeq	10–120	1×75 to 2×150	Throughput	Run and read length
HiSeq (2500)	10–1000	×50 to ×250	Read accuracy, throughput,	High initial investment, run
NovaSeq 5000/6000	2000–6000	2×50 to ×150	Read accuracy, throughput	High initial investment, run
<i>IonTorrent</i>				
PGM	0.08–2	Up to 400	Read length, speed	Throughput, homopolymers <sup>c</sup>
S5	0.6–15	Up to 400	Read length, speed,	Homopolymers <sup>c</sup>
Proton	10–15	Up to 200	Speed, throughput	Homopolymers <sup>c</sup>
<i>Pacific BioSciences</i>				
PacBio RSII	0.5–1 <sup>b</sup>	Up to 60 kb	Read length, speed (Average 10 kb, N50 20 kb)	High error rate and initial
Sequel	5–10 <sup>b</sup>	Up to 60 kb	Read length, speed (Average 10 kb, N50 20 kb)	High error rate
<i>Oxford Nanopore</i>				
MINION	0.1–1	Up to 100 kb	Read length, portability	High error rate, run length,

<sup>a</sup> The throughput ranges are determined by available kits and run modes on a per run basis. As an example of a 15-GB throughput, thirty-five 5-MB genomes can be sequenced to a minimum coverage of 40× on the Illumina MiSeq using the v3 600 cycle chemistry.

<sup>b</sup> Per one single-molecule real-time cell.

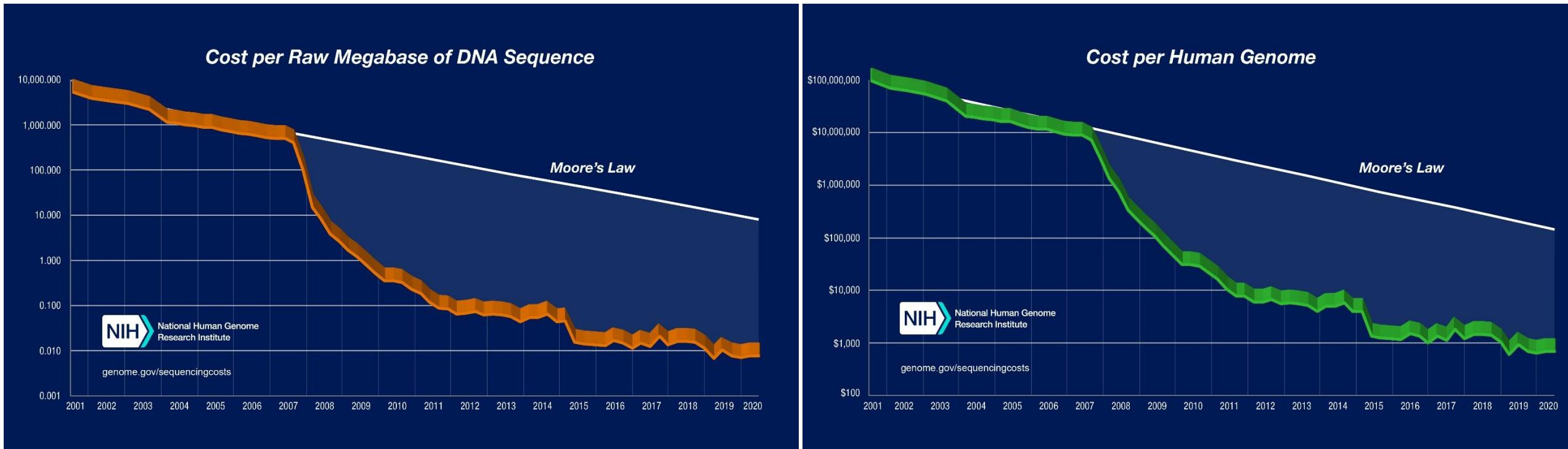
<sup>c</sup> Results in increased error rate (increased proportion of reads containing errors among all reads) which in turn results in false-positive variant calling.

Besser et al., Clin Micr Infect, 2018

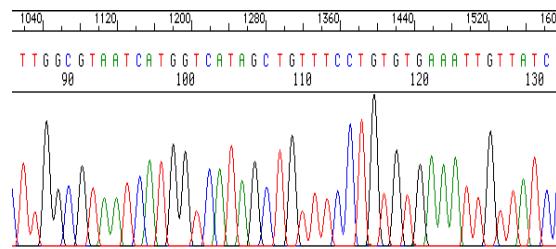
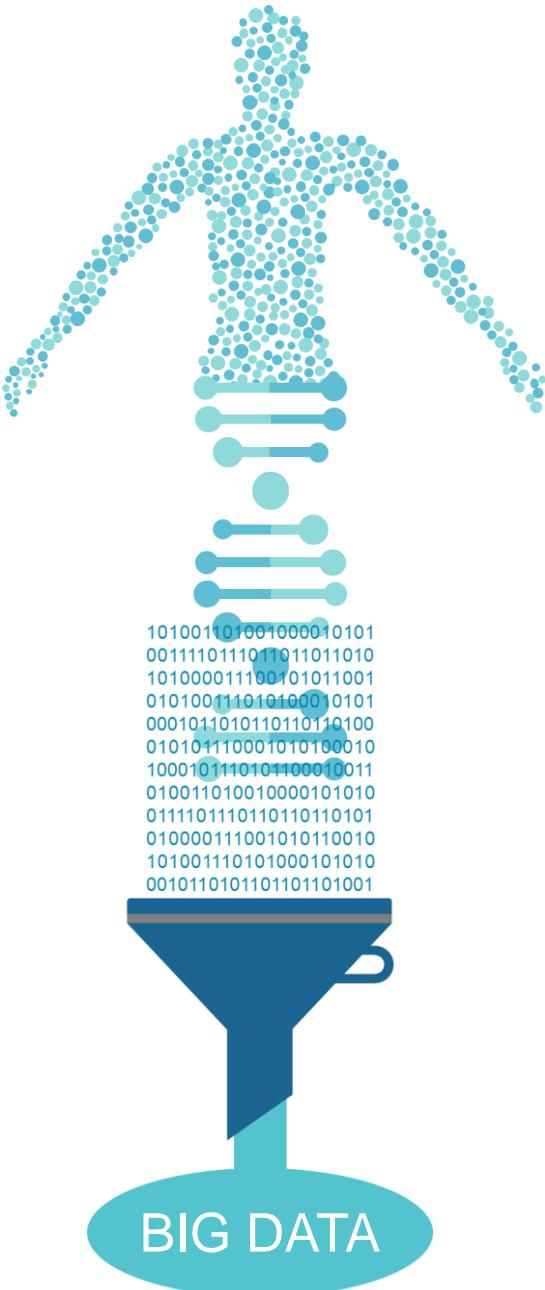
# NGS PLATFORMS, main characteristics

- Numero de bases que secuencia
- Numero lecturas → aplicaciones
- Longitud de las lecturas -→ importante para las aplicaciones ensamblado genomas, de illumina a PacBio
- Error de la base → Corrección con profundidad de lectura
- Formato fichero salida
- Software dedicado, universal fastq

# Coste actual de la secuenciación



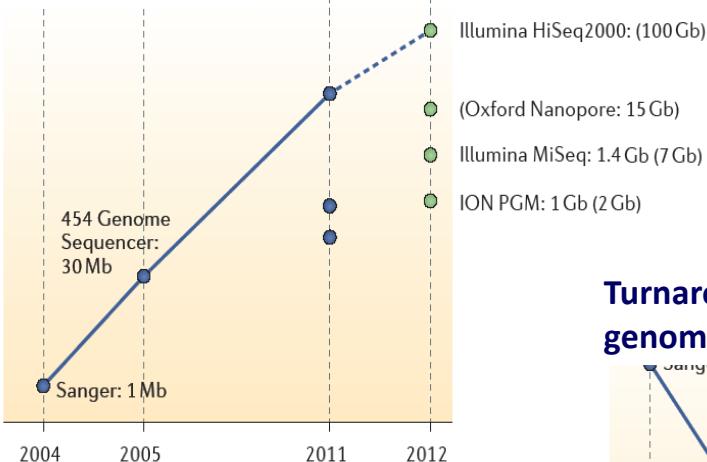
# BIG DATA



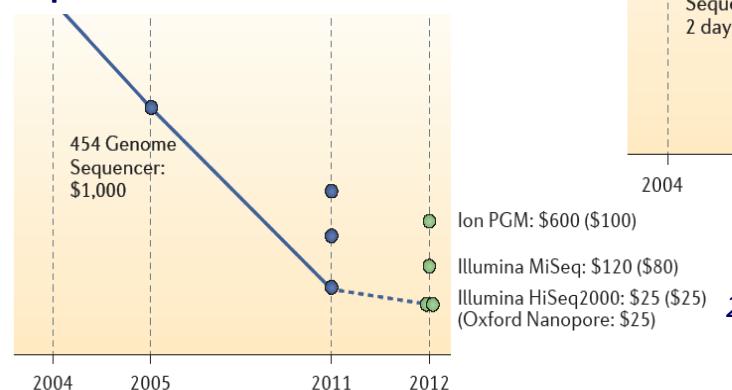
```
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AGAAAAAAACCTTGANGGAAAAAAATCAGACATTTCAGAGGTGGAAGGCAAACGTGAAACAAAATAATTCA  
+  
DGGGEDHHHGGGFE#CBACBCA<?HHHHBHHHHHHHHHEFEGGGGGG/GGDDDGHFHGFCHFHHEHEH8  
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:3082:1029 1:N:0:ACAGTG  
GTAATAACAGACTGANATCACAAAGGATGCTGGAAACAAACCTTAAAGATAAGCTTGGATAAGCTTCTTATT  
+  
B:B:BB:=55177#55877<775EDD>E=B?BBBBGGGDAG@>GGGGGG@EEEEBE>GGGGGGAAA?<@  
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+  
BC??A66: )74781#????;452.27'64(8,851DG8GB#????#????#????#????#????#  
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+  
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+  
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+  
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```

# Sequencing platforms in Microbiology

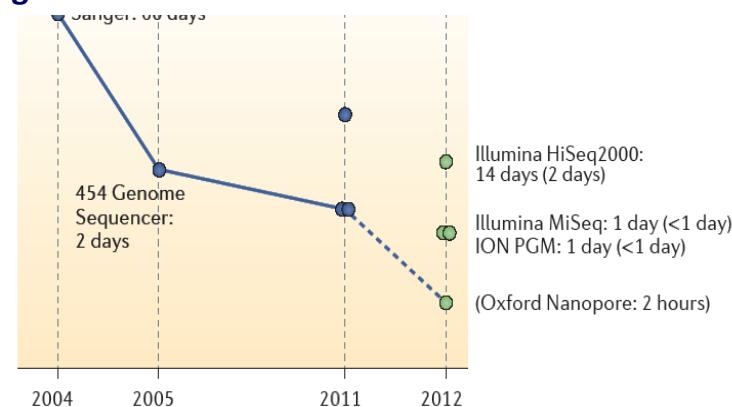
Raw daily output



Cost per Mb assembled sequence



Turnaround time: bacterial genome

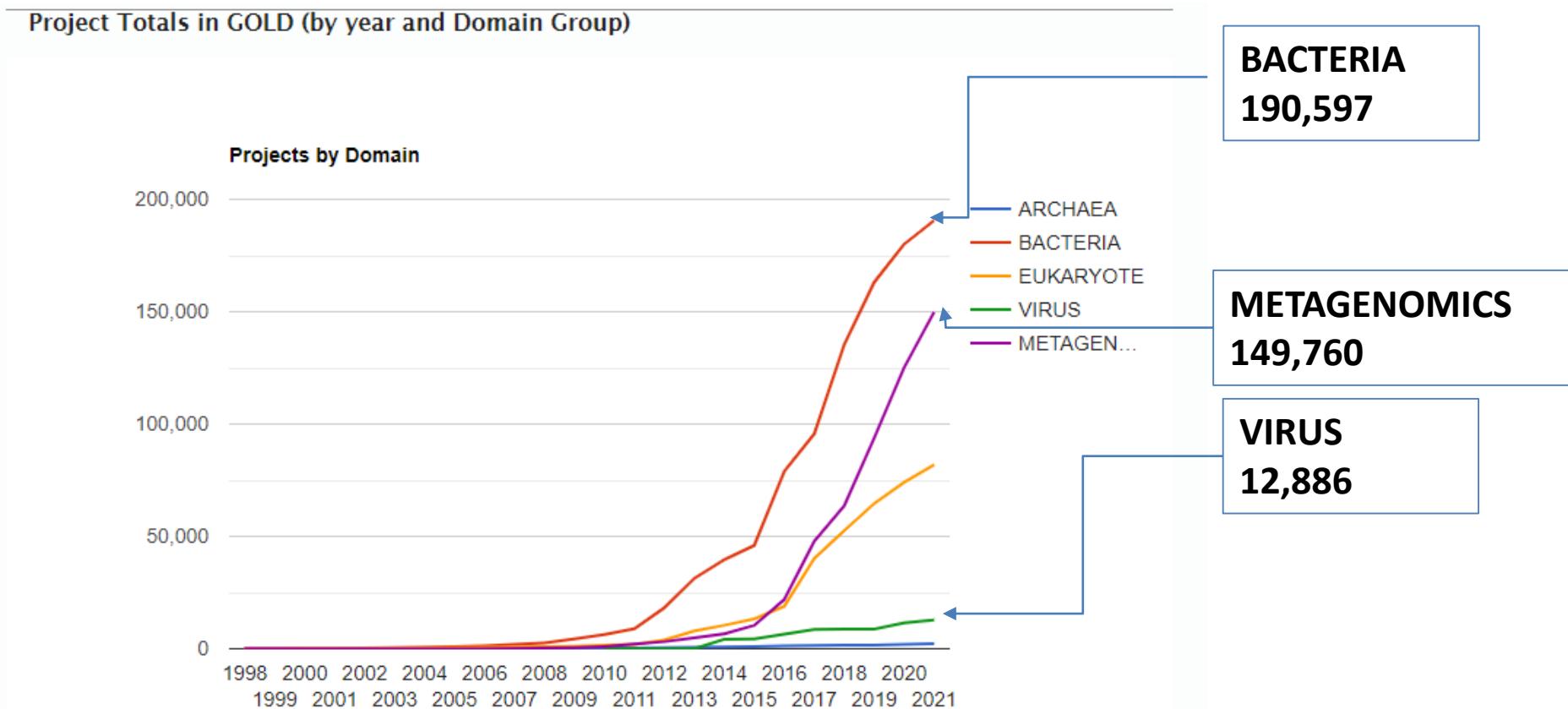


2

# Sequencing projects

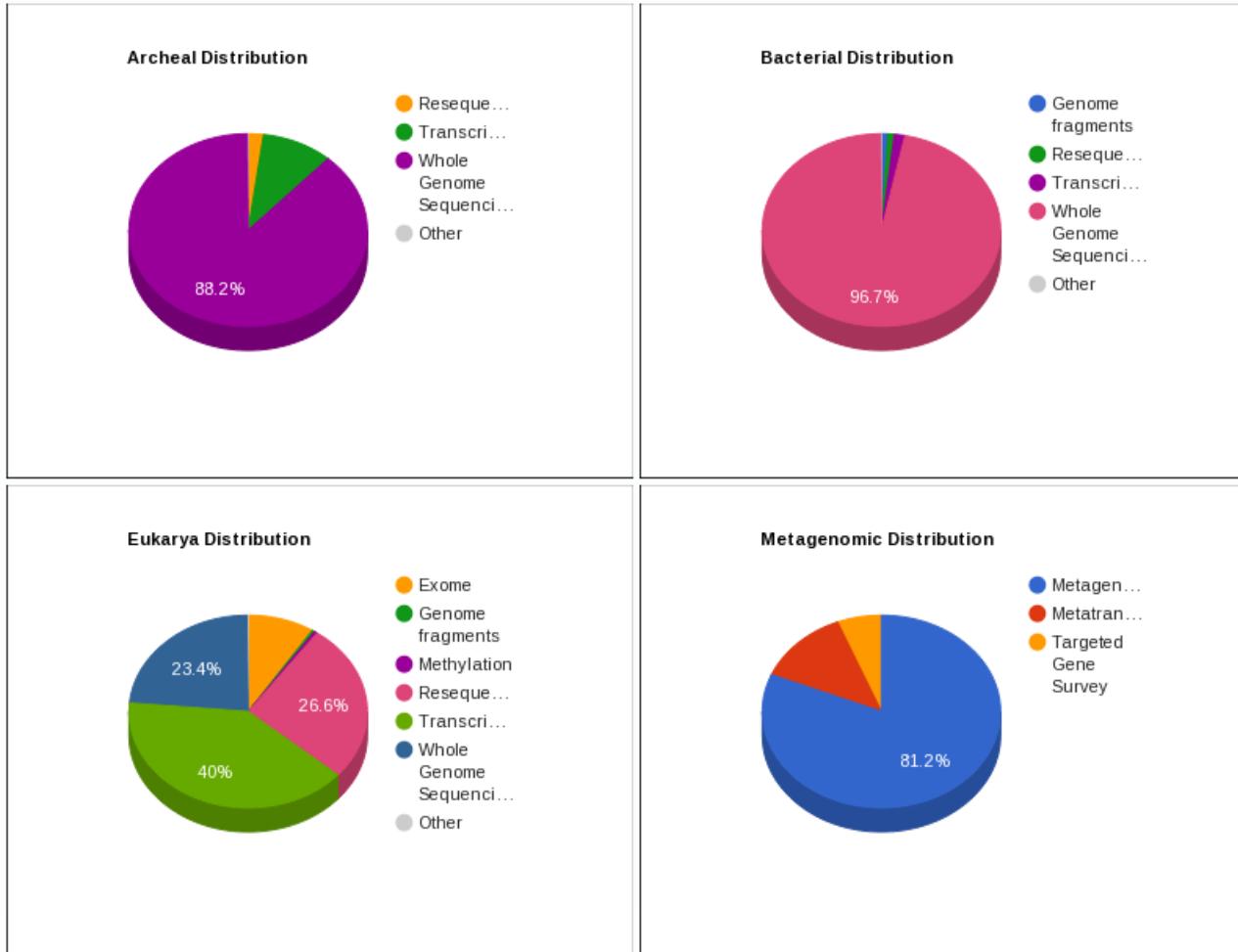
<https://gold.jgi.doe.gov/>

## GOLD, Genome Online DataBase

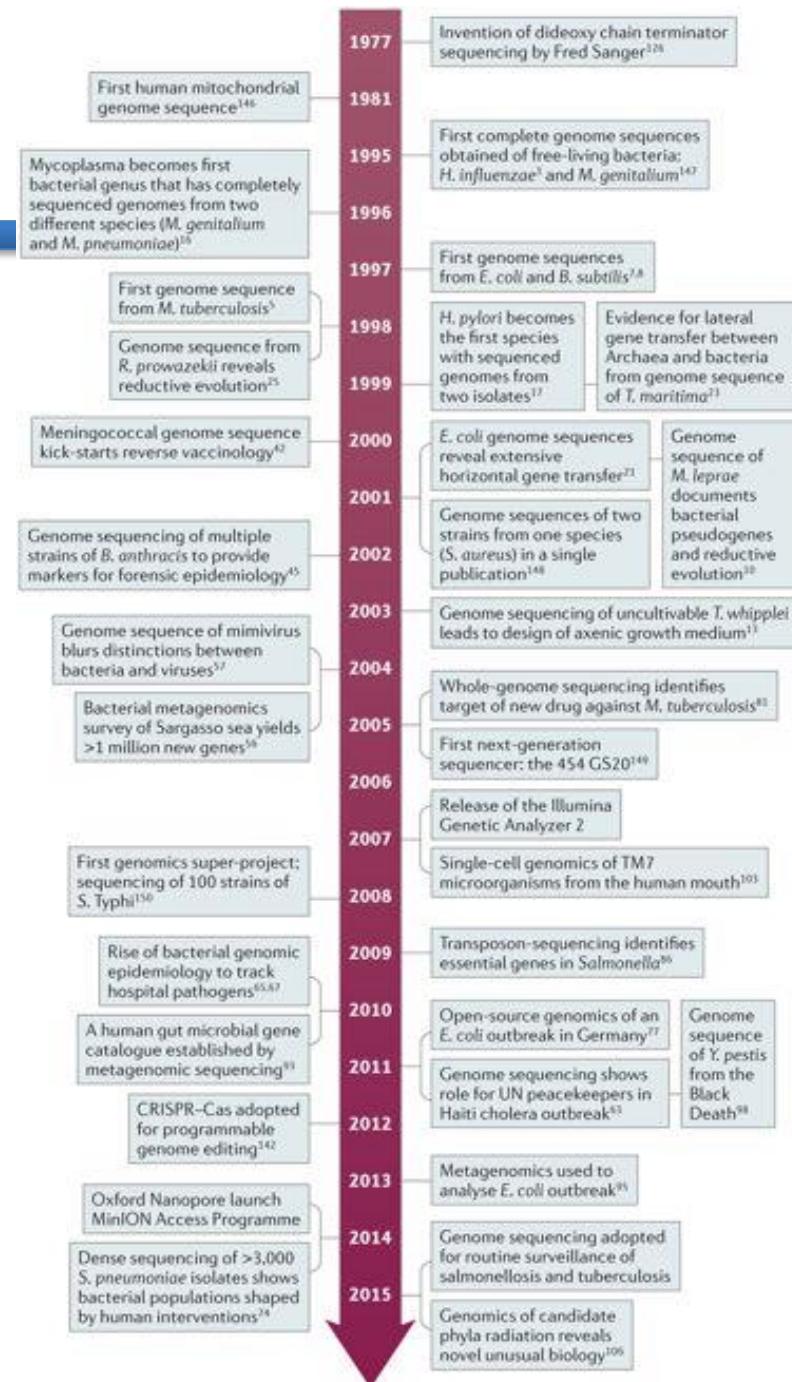


# Organisms distribution of Sequencing projects

GOLD Project Distributions

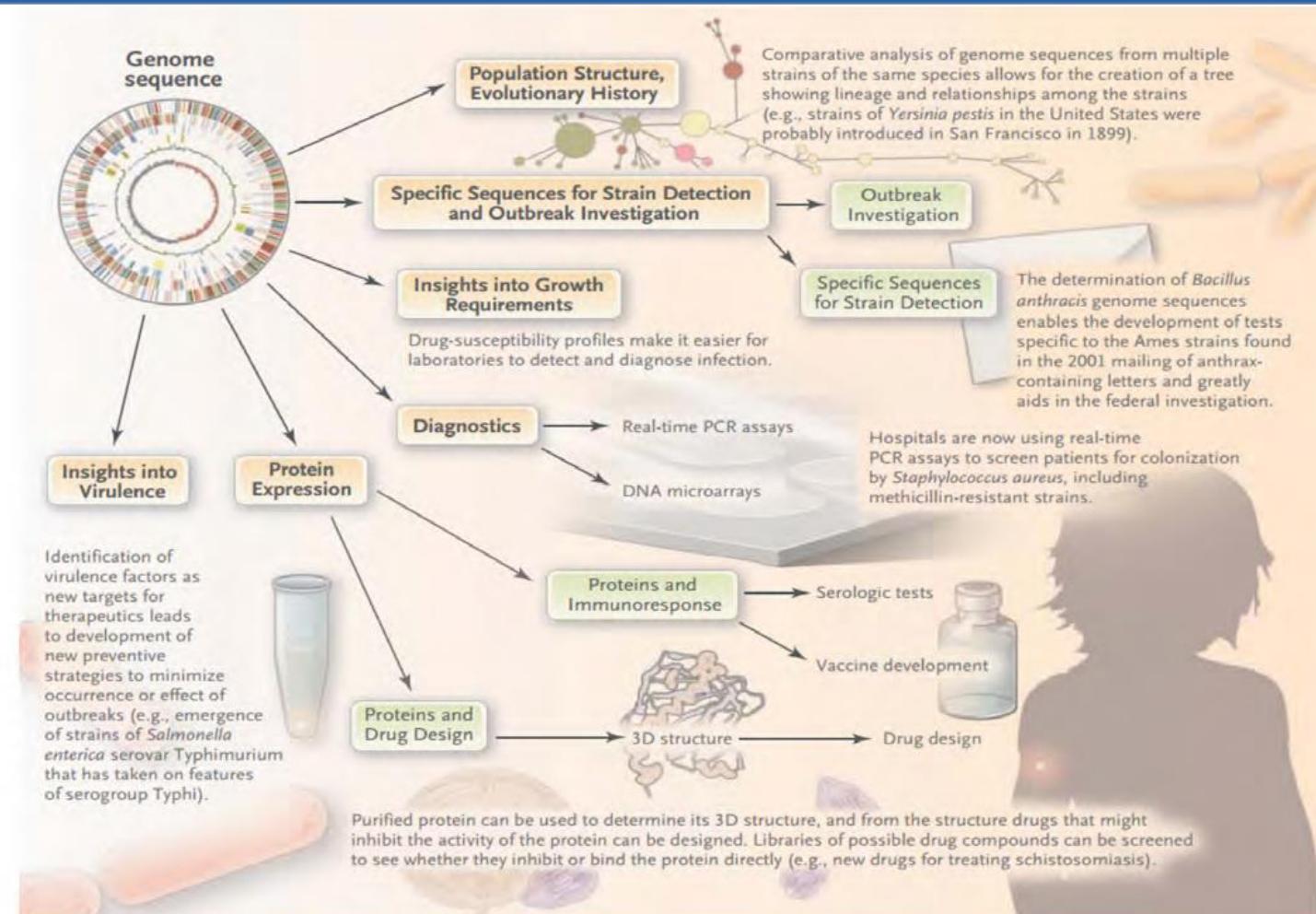


# Brief history of the major events that have shaped the sequencing and analysis of bacterial genomes in the past two decades



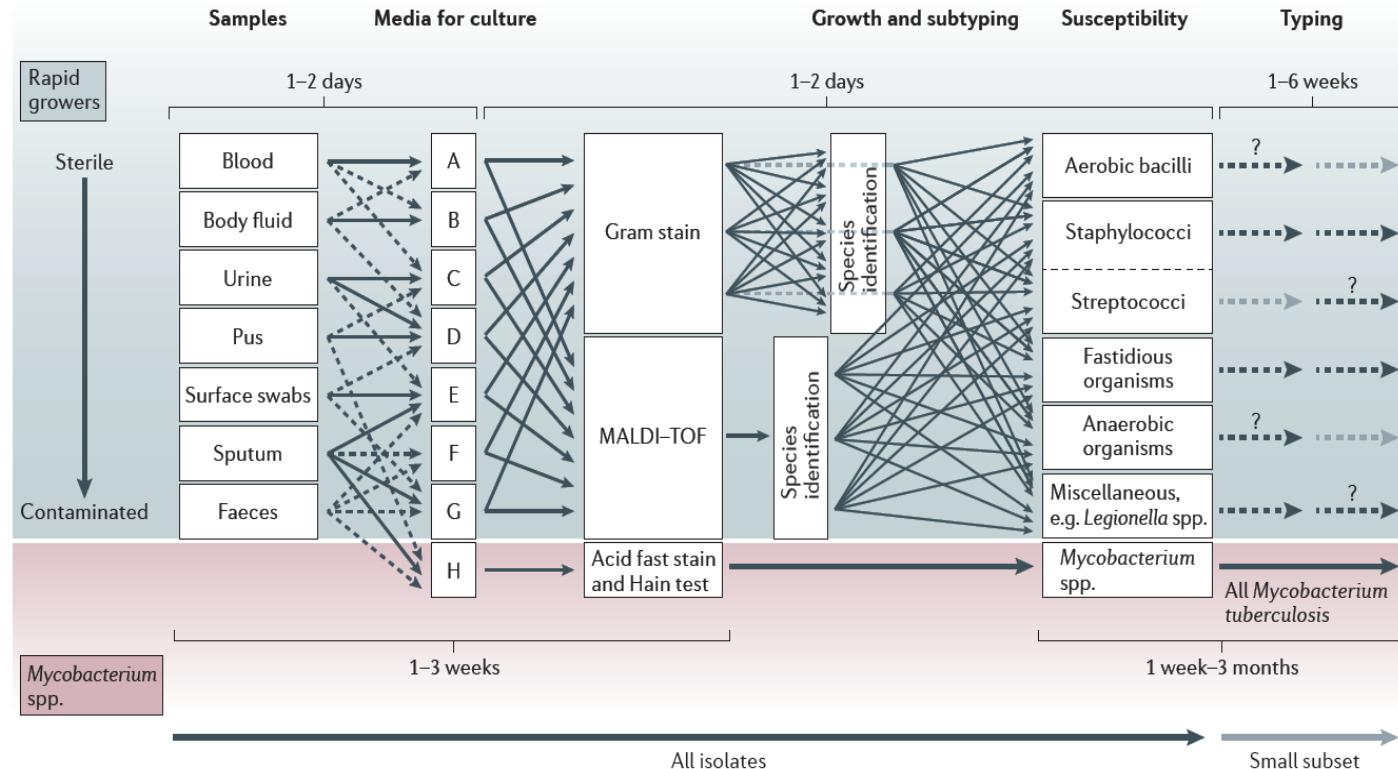
# Use of microbial genomics for tool development

Report from The American Academy of Microbiology, 2015



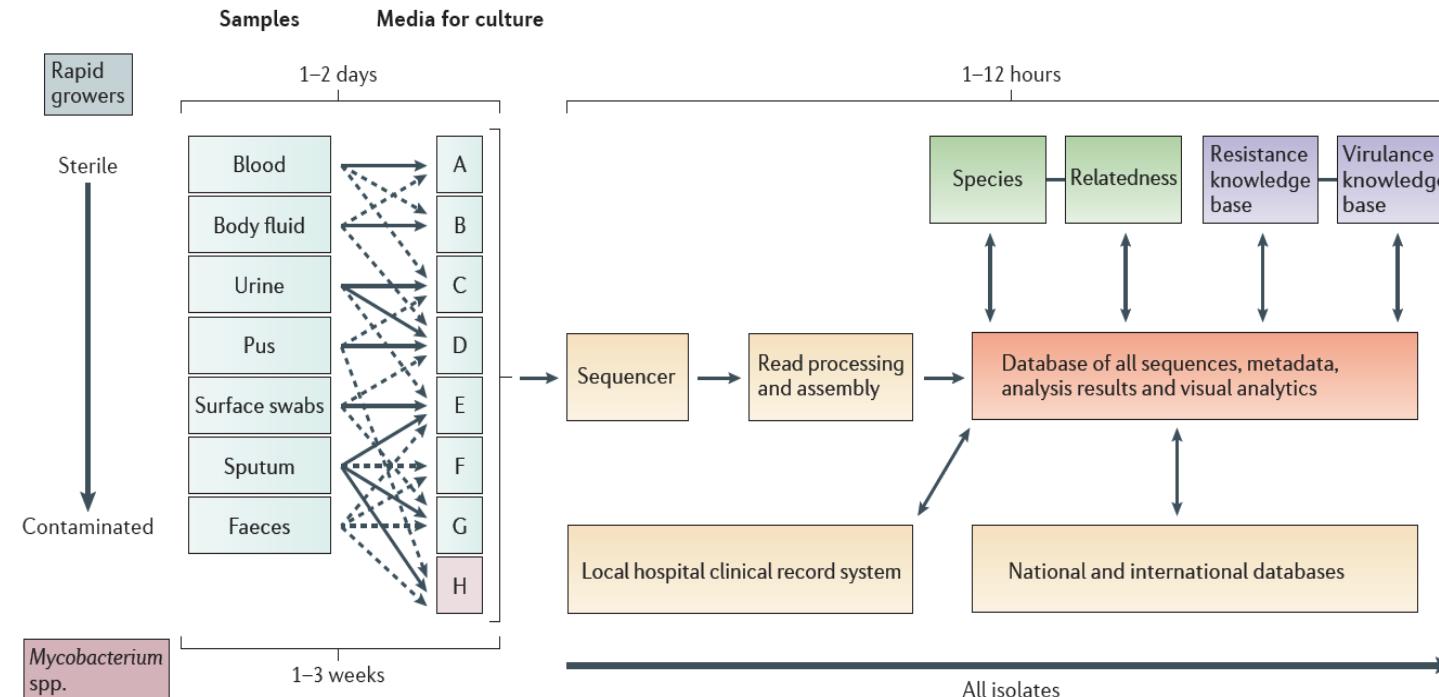
# Workflow for processing samples for bacterial pathogens

Didelot et al., Nature Genet Review 2012, 13:601-612



Ongoing developments in DNA-sequencing technologies are likely to affect the diagnosis and monitoring of all pathogens, including viruses, bacteria, fungi and parasites.

# The diagnostic and clinical applications of bacterial WGS

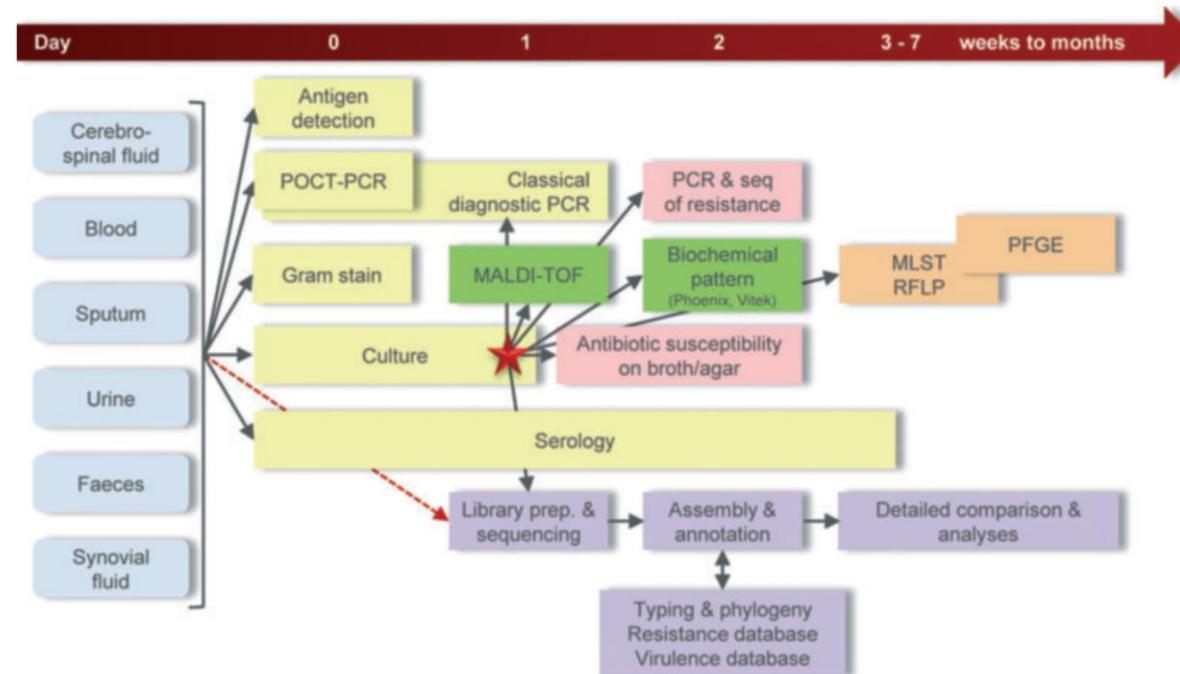


Didelot et al., Nature Genet Review 2012, 13:601-612

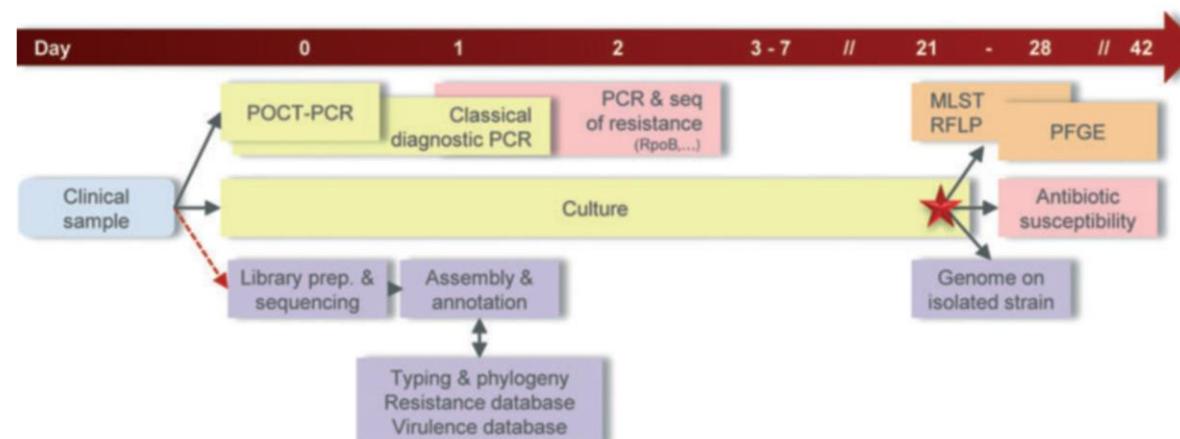
# Schematic representation of the timeline for the processing of clinical samples with classical pathogens

Bertelli and Greub, Clin Microb and Infect, 2013

(a) classical  
pathogens



(b) slow-growing  
bacteria such as  
*Mycobacterium*  
*tuberculosis*



## Foodborne outbreak identification “Crisis del pepino”

2011

Mayo

- 24 Primera muerte en Alemania  
26 Alemania acusa a los pepinos españoles  
30 Prohibición de importaciones de verduras de España y Alemania  
31 Laboratorios alemanes desmienten oficialmente que los pepinos españoles sean el foco de infección

Junio

- 10 Resolución de la crisis

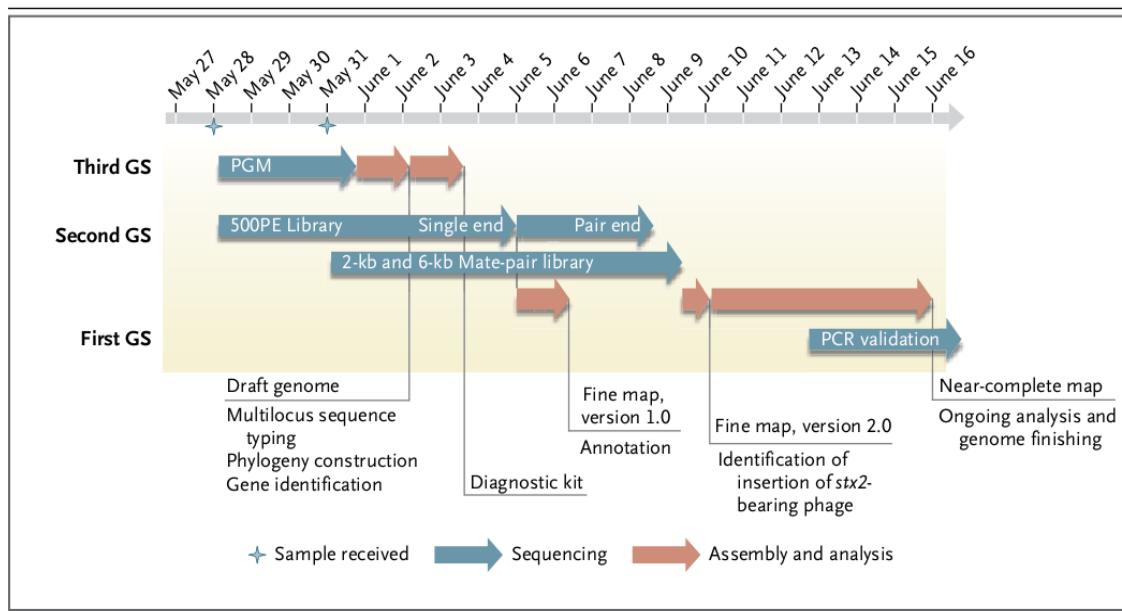
Causado por la toxi-infección de Escherichia coli enterohemorrágica (EHEC) (*Escherichia coli* O104:H4)

Muerte: 32 personas en Alemania, 1 Suecia y 1 Francia y 2263 infectados en 12 países de Europa.

Crisis Política y Económica Europa:  
Alto impacto en la Economía Europea, mayor afectación en la Española

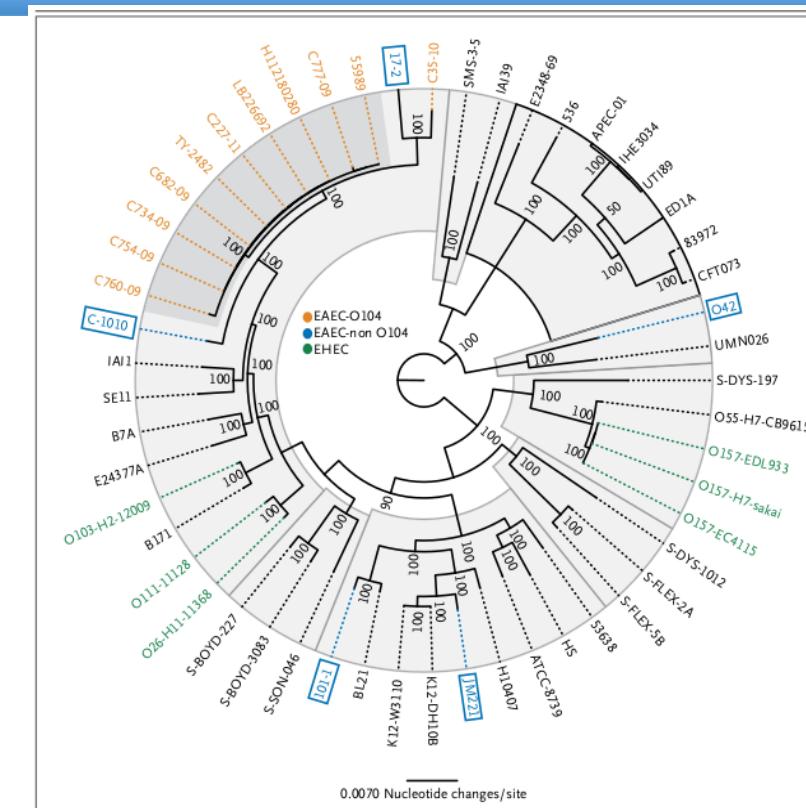


# Foodborne outbreak identification “Crisis del pepino”



**Figure 1.** Timeline of the Open-Source Genomics Program.

After receiving the first batch of DNA samples on May 28, 2011, sequencing runs with the use of the Ion Torrent Personal Genome Machine (PGM) and Illumina (small-insert library) were initiated simultaneously. On May 31, the second batch of DNA was received and used for Illumina large-insert sequencing. An assembly of the Ion Torrent reads was released on June 2, which enabled subsequent analyses (multilocus sequence typing, phylogenetic analysis, and genome comparisons). Errors in the Ion Torrent data were corrected with the use of later Illumina data, and a high-quality draft genome sequence was created. GS denotes generation of sequencing technology. The symbols at May 28 and May 31 in the timeline indicate the arrival of DNA samples.



**Figure 2.** Phylogenetic Comparisons of 53 *Escherichia coli* and *Shigella* Isolates.

Genomic sequences were compared with the use of 100 bootstrap calculations, as described by Sahl et al.<sup>35</sup> The species-based phylogeny was inferred with the use of 2.56 Mbp of the conserved core genome. The O104:H4 isolates are shown in orange, the reference enteroaggregative *E. coli* (EAEC) isolates in blue, and the enterohemorrhagic *E. coli* isolates in green. (The classification of the other strains is shown in Fig. 4 and Table 4 in the Supplementary Appendix.) The O104:H4 isolates cluster into a single clade (dark gray); in contrast, the reference EAEC isolates are extremely divergent and are represented throughout the phylogeny.

# The idea that via a One Health approach infectious diseases could be better controlled and prevented



# ECDC roadmap and international commitment

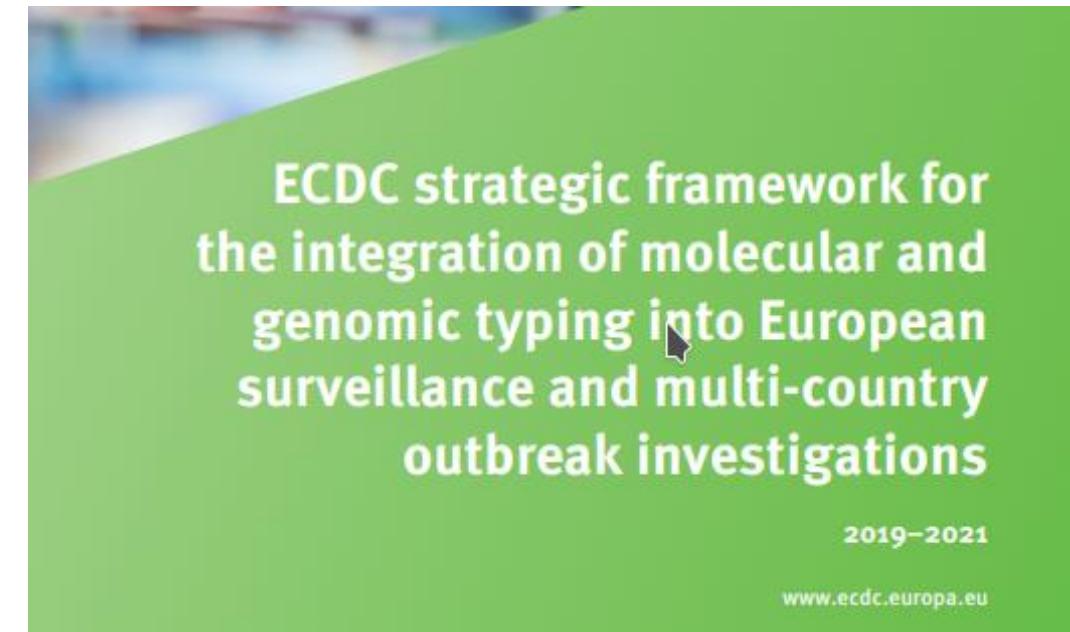


EUROPEAN CENTRE FOR  
DISEASE PREVENTION  
AND CONTROL



Version 2.1, 2016–2019

[www.ecdc.europa.eu](http://www.ecdc.europa.eu)



ECDC strategic framework for  
the integration of molecular and  
genomic typing into European  
surveillance and multi-country  
outbreak investigations

2019–2021

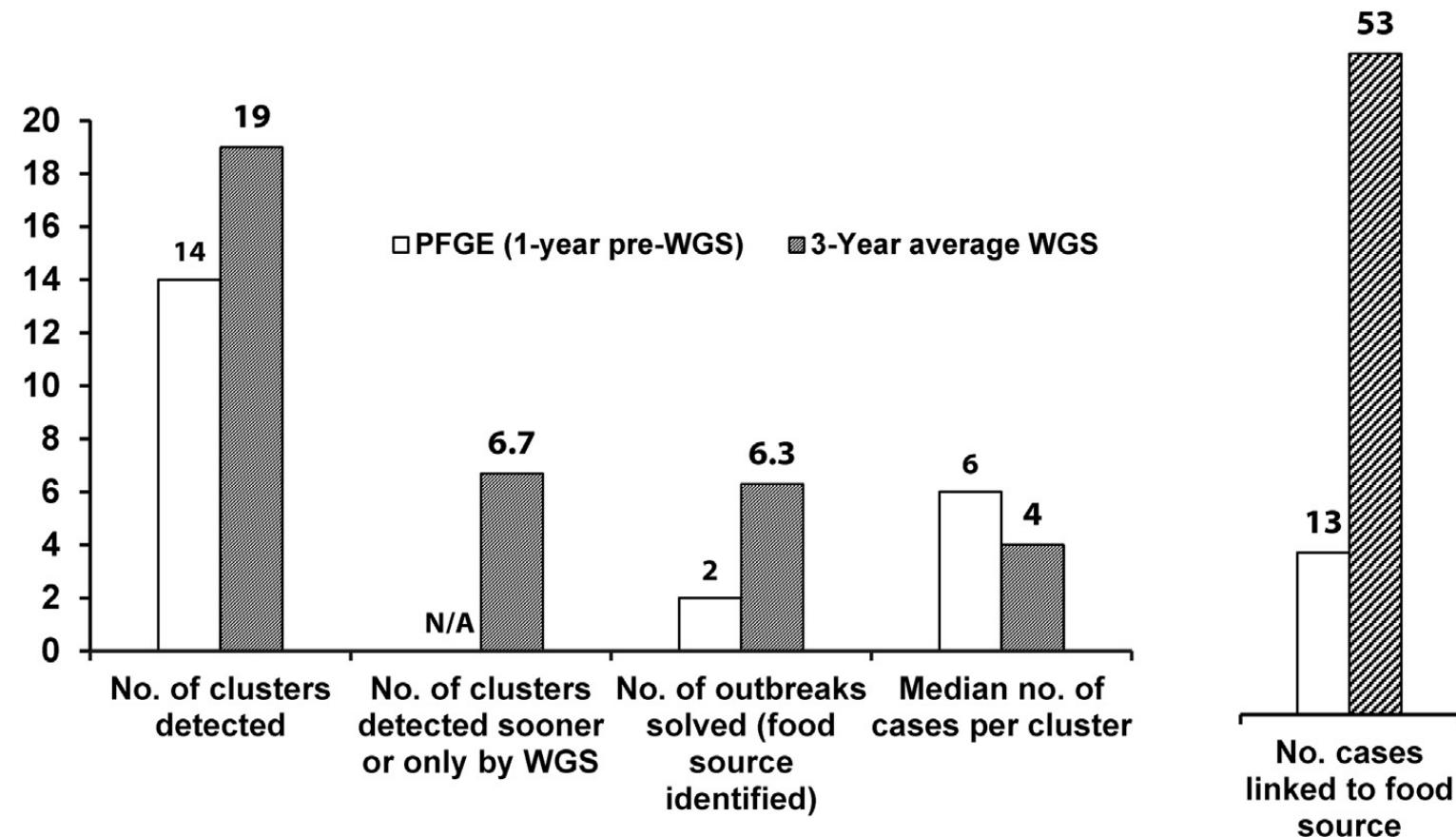
[www.ecdc.europa.eu](http://www.ecdc.europa.eu)

- **Operationalisation of EU-wide WGS-based surveillance systems in the near term:** start implementation of WGS-based surveillance for *Listeria monocytogenes*, *Neisseria meningitidis*, Carbapenemase-producing *Enterobacteriaceae* and antibiotic-resistant *Neisseria gonorrhoeae*; 2018

## Early data from surveillance of listeriosis in the USA

Besser et al., Clin Micr Infect, 2018

The number of outbreaks detected increased 36% after implementation of real-time WGS based surveillance, and likewise the number of solved outbreaks increased more than three-fold

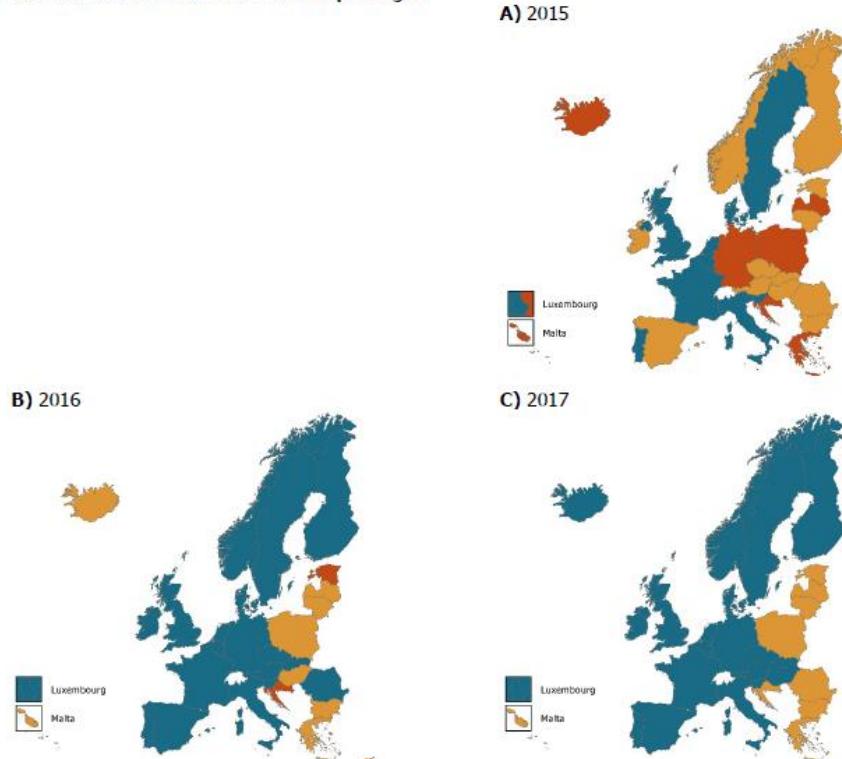


## ECDC technical report: Monitoring the use of wgs in infectious disease surveillance in Europe 2015-2017

**WGS provides higher resolution and accuracy** than classical molecular typing methods, such as PFGE or MLVA, contributing to a better understanding of infectious disease and drug resistance transmission patterns and thereby improving the effectiveness of interventions for their control.

# ECDC technical report: Monitoring the use of wgs in infectious disease surveillance in Europe 2015-2017

**Figure 1.** National public health reference laboratories use of WGS-based typing for national surveillance of at least one human pathogen



**Figure 3. Number of EU/EEA countries using WGS-based typing as first or second-line method for routine surveillance and outbreak investigations in National Public Health Reference Laboratories by disease group and pathogen, 2017**

**Foodborne pathogens**  
*Listeria monocytogenes*  
*Salmonella enterica*  
*Shiga toxin-producing E. coli (STEC)*

**Antimicrobial-resistant pathogens**  
Carbapenemase-producing *Enterobacteriaceae* (CPE)  
Antibiotic-resistant *Neisseria gonorrhoeae*  
Multidrug-resistant *Mycobacterium tuberculosis*

**Vaccine-preventable pathogens**  
Invasive *Neisseria meningitidis*  
Human Influenza virus

Number of countries by use of WGS-typing in 2017 or being planned by 2019, per pathogen

4	8	3	6	9		
4	10		9	2	5	
7	8		5	2	8	
2	4	12	2	3	7	
1	10	7	6	1	5	
10	7	3	4	6		
4	9	2	8	7		
6	14	2	5	3		

#### Legend

- No information
- No, it is not used for public health operations or planned by 2019
- No, it is not used for public health operations or planned by 2019
- Only for outbreak investigations
- Routine surveillance and outbreak investigations - Second-line typing
- Routine surveillance and outbreak investigations - First-line typing

# ECDC technical report: Monitoring the use of wgs in infectious disease surveillance in Europe 2015-2017

**Table 1.** Number of EU/EEA countries with one or more national public health reference laboratories having access to next-generation sequencing (NGS) technologies for routine public health operations, by technology and instrument used, 2017

NGS technology	Instrument	Foodborne pathogens		Antimicrobial-resistant pathogens		Vaccine-preventable diseases			
		<i>L. monocytogenes</i>	<i>S. enterica</i>	STEC	CPE	AR-N. gonorrhoeae	MDR-TB	<i>N. meningitidis</i>	Human Influenza virus
Illumina	HiSeq series	3	3	3	2	1	2	3	1
	HiSeq X series						1		
	MiniSeq	1	2	3	2		4		1
	MiSeq series	12	10	7	7	10	7	13	7
	NextSeq	2	2	2	3	1	3	2	2
Ion Torrent	S4								1
	S5	1	1	1	1		1		
	S5 XL								1
	PGM		1	1		1		1	1
	Proton							1	1
Oxford Nanopore Technologies	MinION	1	1	2			1	1	1
Pacific Biosciences-PacBio	PacBio RS II	1		2					
Other not specified	-	3	2	2	1	1	1	1	

**Table 2.** Bioinformatics tools used by the National Public Health Reference Laboratories using WGS-based typing for surveillance and outbreak investigations of foodborne pathogens, July 2017\*

	Number of EU/EEA countries		
Tools used for sequence analysis	<i>L. monocytogenes</i> (n=14)	<i>S. enterica</i> (n=7)	STEC (n=9)
Commercial software	9	4	4
Open source software	4	3	5
In-house suite of customised tools	4	2	2

\* Not mutually exclusive

# ECDC technical report: Monitoring the use of wgs in infectious disease surveillance in Europe 2015-2017

**Table 3. Number of EU/EEA countries using WGS-based typing for surveillance and outbreak investigations in the national public health reference laboratories and respective typing scheme, sampling frame, bioinformatics analysis, and raw data storage practice by pathogen, 2017**

	2017	Foodborne pathogens			Antimicrobial resistant pathogens			Vaccine preventable pathogens	
		<i>L. monocytogenes</i>	<i>S. enterica</i>	STE C	CPE	<i>AR-N. gonorrhoeae</i>	MDR TB	Human influenza virus	<i>N. meningitidis</i>
Number of countries using WGS for routine surveillance and outbreak investigations		14	7	9	10	6	10	8	15
Typing scheme									
First-line WGS	9	5	8	7	5	6	3	7	
Second-line WGS	5	2	1	3	1	4	5	8	
Sampling frame									
Continuous comprehensive	12	6	8	7	2	9	-	15	
Sentinel/ subset of case samples	2	1	1	3	4	1	8	-	
Bioinformatic analysis *									
cgMLST	12	6	5	6	4	5	-	12	
SNP	7	5	5	5	2	7	-	5	
Resistome prediction	4	5	7	8	4	6	-	3	
wgMLST	5	3	3	2	2	2	-	2	
Virulome/ mobilome prediction	4	2	9	5	1	-	-	1	
MLST prediction	12	6	8	3	-	-	-	2	
Serogroup prediction	7	6	9	1	-	-	-	2	
NG-MAST	-	-	-	-	3	-	-	-	
Speciation	-	1	1	-	-	3	-	1	
Hemagglutinin and neuraminidase sequence prediction	-	-	-	-	-	-	4	-	
Phylogenetic relationship	-	1	1	1	-	-	7	1	
Identification of specific point mutations	-	1	1	-	-	-	6	1	
rMLST	-	-	-	-	-	-	-	5	
MLST+porA VR1 and VR2+fetA	-	-	-	-	-	-	-	12	
Vaccine antigen prediction	-	-	-	-	-	-	-	9	
Other not specified	-	-	-	1	-	3	3	1	
Raw sequence data storage *									
Dedicated closed database(s)	13	5	7	10	6	10	6	12	
Publicly available database(s)	1	2	2	-	1	1	2	3	

\* Not mutually exclusive

## ECDC technical report: Monitoring the use of wgs in infectious disease surveillance in Europe 2015-2017

### Conclusions

This emerging mainstream practice should enable **pan-European WGS-derived data exchange in the medium-term**, subject to **harmonisation** of sequence analysis pipelines for output compatibility, agreement on international WGS derived type nomenclature and development of **secure and efficient international data sharing** and management platforms.

Current bottlenecks mainly relate to development of expertise in **epidemiological-WGS data integrative analysis** and access to user-friendly international nomenclature

## Skills needed to translate WGS data into public health action



Bioinformatician	Epidemiologist	Microbiologist
Algorithms for genome mapping, assembly and comparisons	Epidemiology of communicable diseases	Microbiological diagnostics
Inferences from genomic data	Statistical analysis	Subtyping of pathogens
Genomic data handling and processing	Case-control studies	Pathogen genomics and evolution
Genome data visualization and integration	Health data linkage	Access to culture collections with epidemiological context
	Risk assessment and communication	

# WHO, landscape paper: wgs for foodborne disease surveillance

FIGURE 2.1

Challenges of coordinating WGS for integrated food chain surveillance



# Spanish National Microbiology Center (CNM)

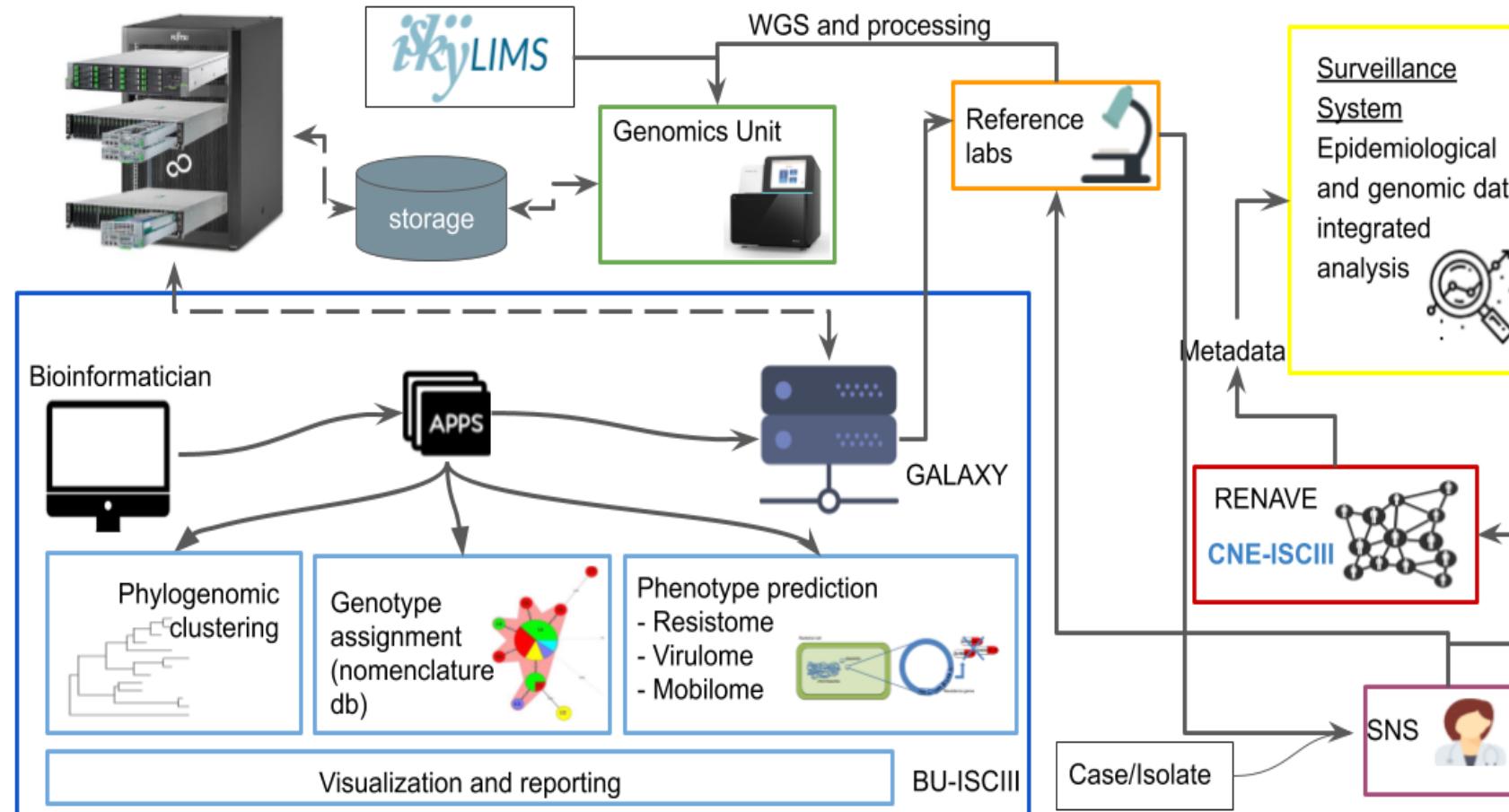


Mission: Provide support to the National Health System and the different Spanish Regions in the diagnosis and control of infectious diseases. In order to fulfill this mission it acts as Reference center offering a series of scientific activities:

- Diagnosis
- **Surveillance** →
- Infectious diseases research
- Training

Outbreak research:  
Molecular source  
detection

# Bioinformatics Unit preparedness



Adapted and extended from Expert opinion on whole genome sequencing for public health surveillance. ECDC  
26/09/2022

II Jornada UCCTS - ISCIII

# Andalusian Listeria Outbreak

**Actualización de información sobre el brote de intoxicación alimentaria causado por *Listeria monocytogenes*.**

Publica: Agencia Española Seguridad alimentaria y Nutrición  
Fecha: 29 agosto 2019  
Sección: Seguridad Alimentaria

Jueves 29 de agosto de 2019, 12.00 horas

## ACTUALIZACIÓN EN RELACIÓN CON LA DISTRIBUCIÓN DE PRODUCTOS RELACIONADOS CON LA ALERTA.

La Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) recomienda a las personas que tengan en su domicilio algún producto de la marca "La Mechá" se abstengan de consumirlo. Si se dispone del producto se debe devolver al punto de compra y, de no ser posible, desecharlo.

## Brote de listeriosis: sube el número de afectados y se apunta a la falta de higiene en la carne como causa

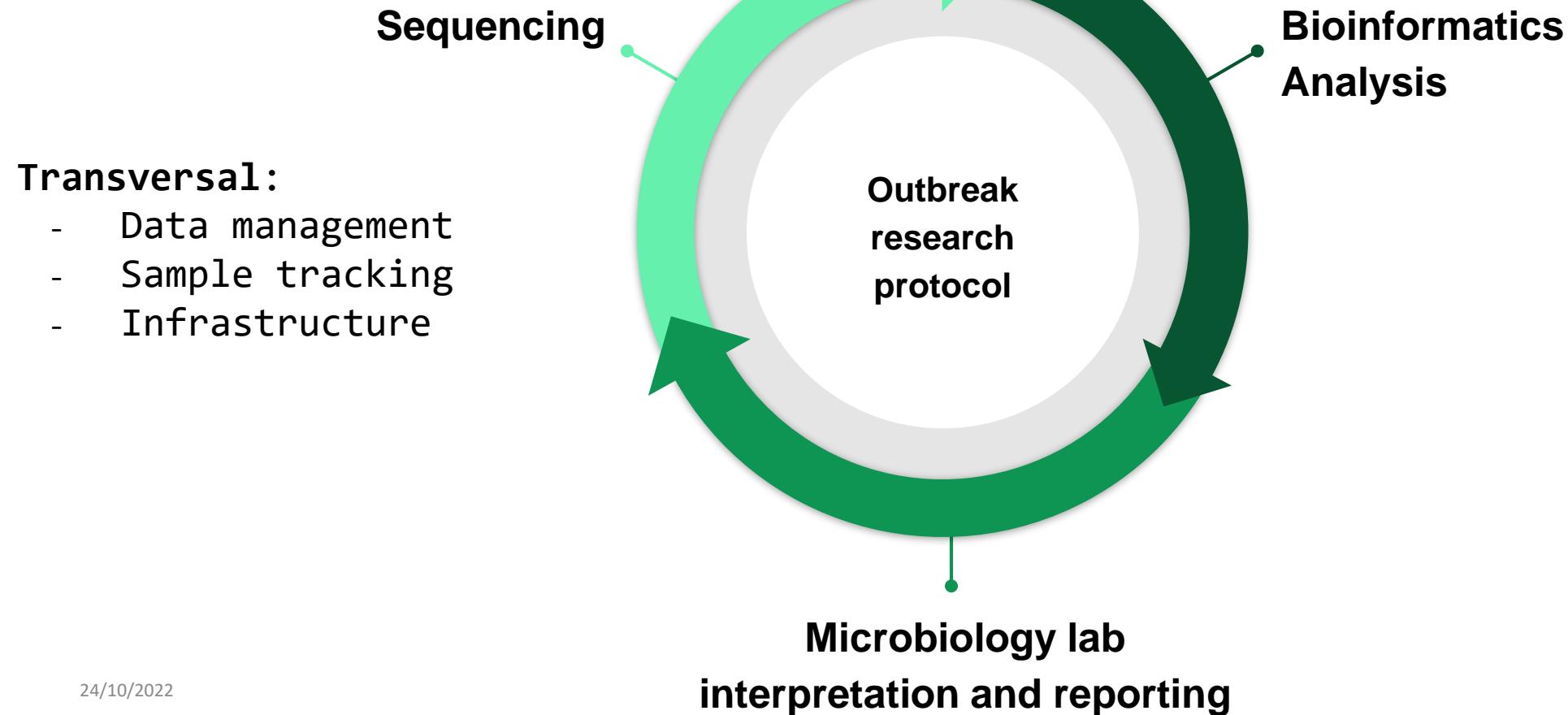
EFE 25.08.2019

- Tres nuevos casos, en Sevilla y Cádiz, dejan el número de personas afectadas en Andalucía en 192.
- [La carne con listeria de la marca blanca se vendió en los municipios de Sevilla.](#)
- La empresa que vendió la marca blanca de Magrudis dice que cumple los protocolos.



- Meat “La Mechá”. Margulis S.L.
- 250 cases related.
- Meat “"La Montanera del Sur". INCARYBE S.L”, suspicion. (Cádiz)
- Meat “Sabores de Paterna” (Málaga)

# Andalusian Listeria Outbreak

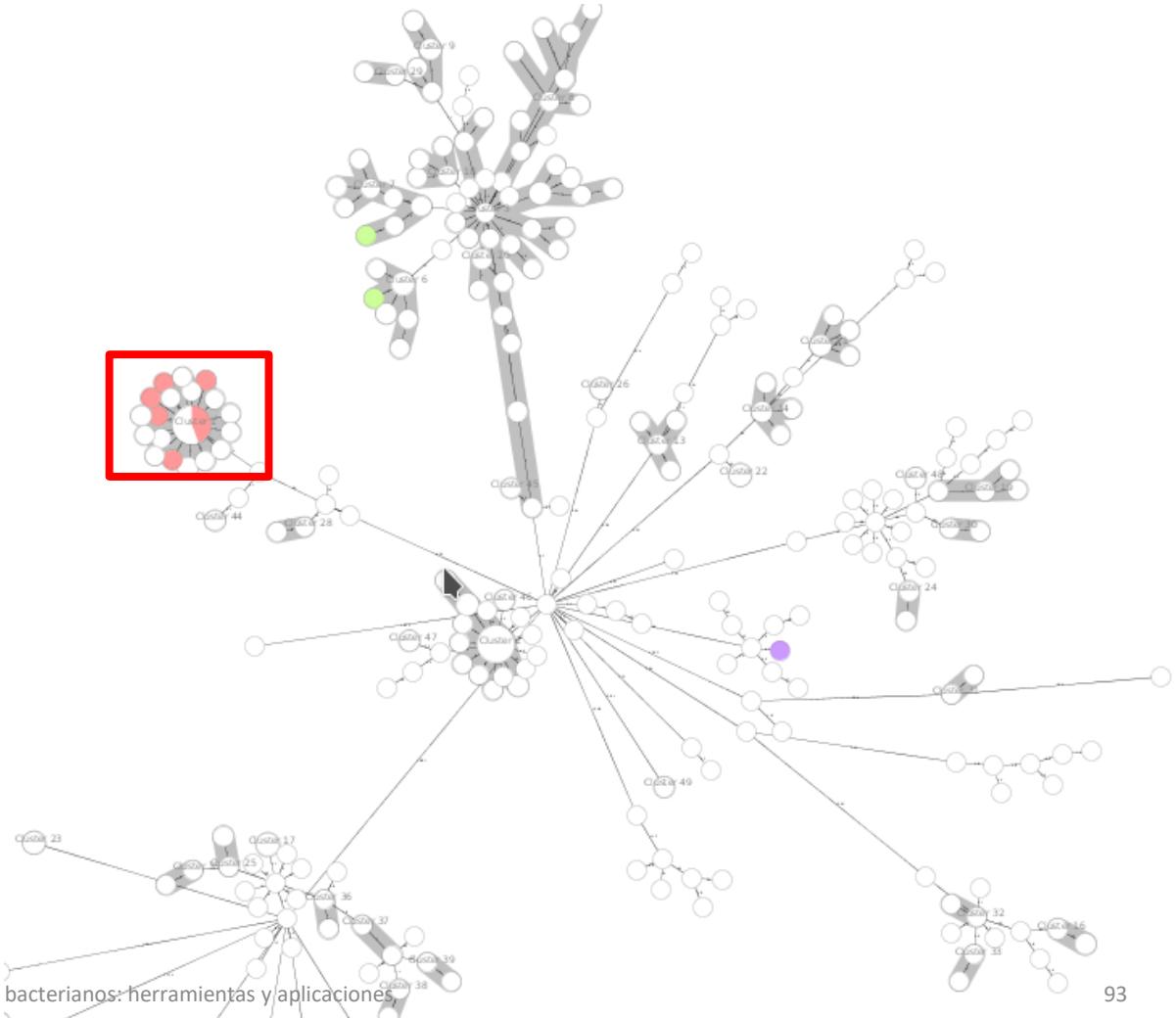


# Andalusian Listeria Outbreak

- 625 listeria samples already sequenced
- 258 suspected to be related to the outbreak (mid august to mid september)

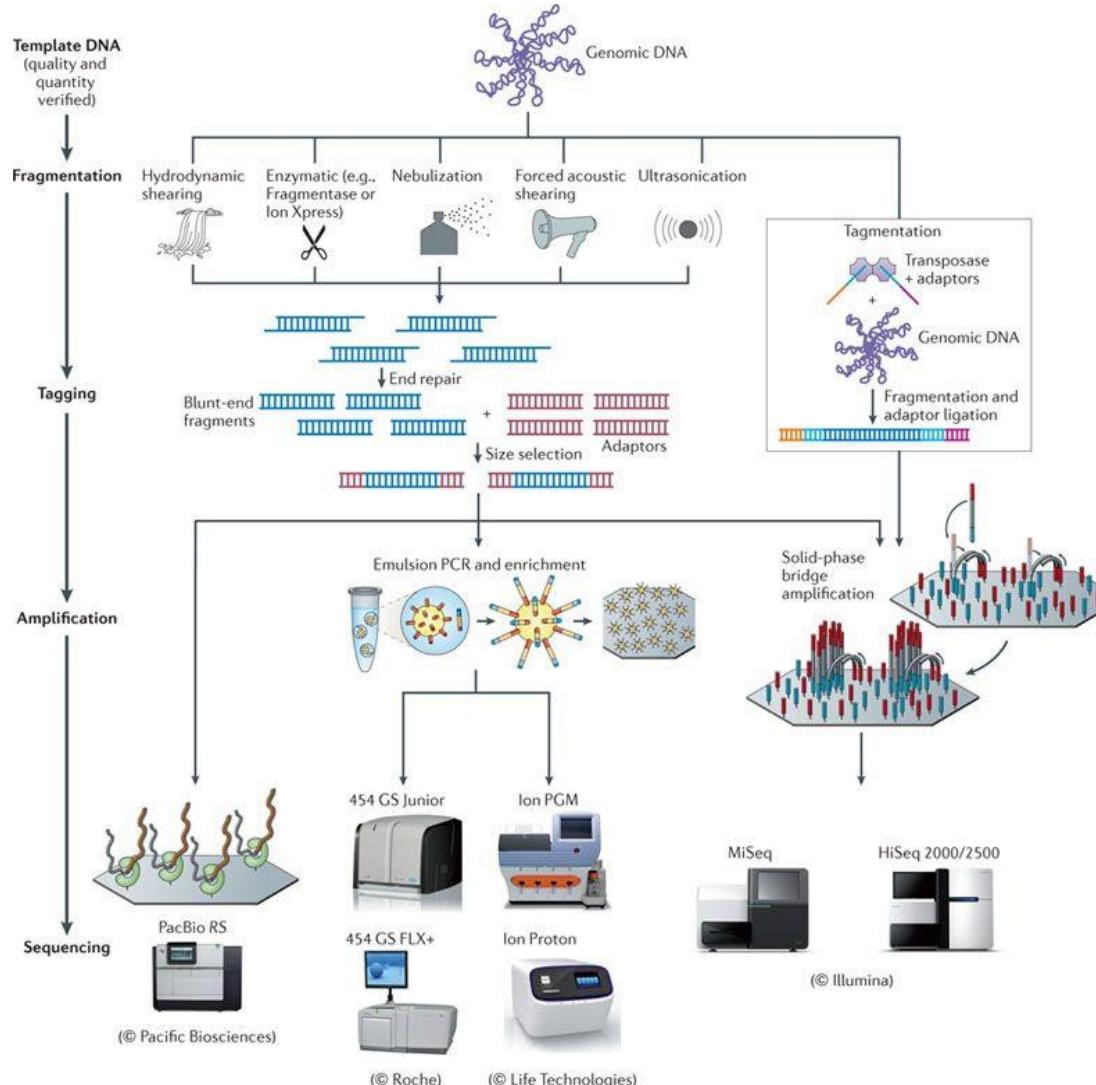
## Results:

- 233 related to the outbreak, confirmed to be caused by the meat “La Mechá”
- 25 sporadic cases not related to the outbreak.



# High-throughput sequencing platforms

&gt;§\_BU-ISCIII



Nature Reviews | Microbiology Loman et al, 2012

## PREPARACIÓN LIBRERÍA, estrategias

### SECUENCIACIÓN GENOMA, EXOMA, TRANSCRIPTOMA

1. Sin amplificación
2. Amplificación con PCR
3. Sondas captura

- Tamaño de fragmento
- Longitud de la lectura
- Single o Paired-end
- Número de bases por muestra
- Profundidad de cobertura x

### SECUENCIACIÓN GENOMAS

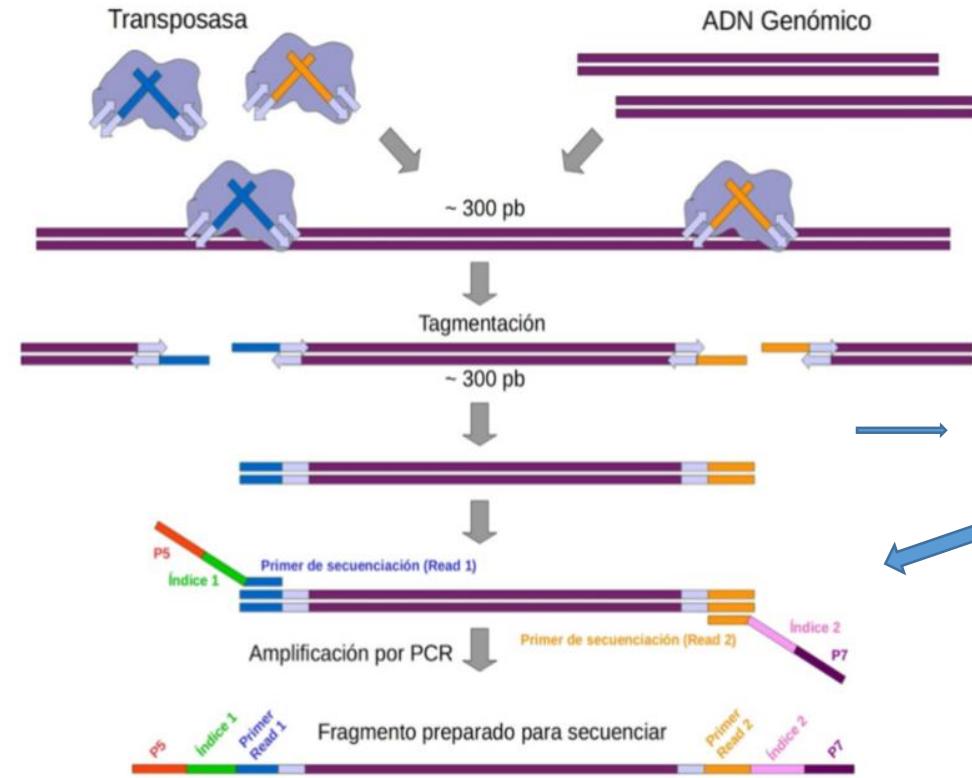
1. Metagenómica

### IDENTIFICACIÓN MICROORGANISMOS

1. Metataxonomía

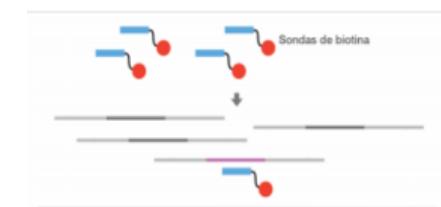
## PREPARACIÓN LIBRERÍA

### ENZIMÁTICA FÍSICA



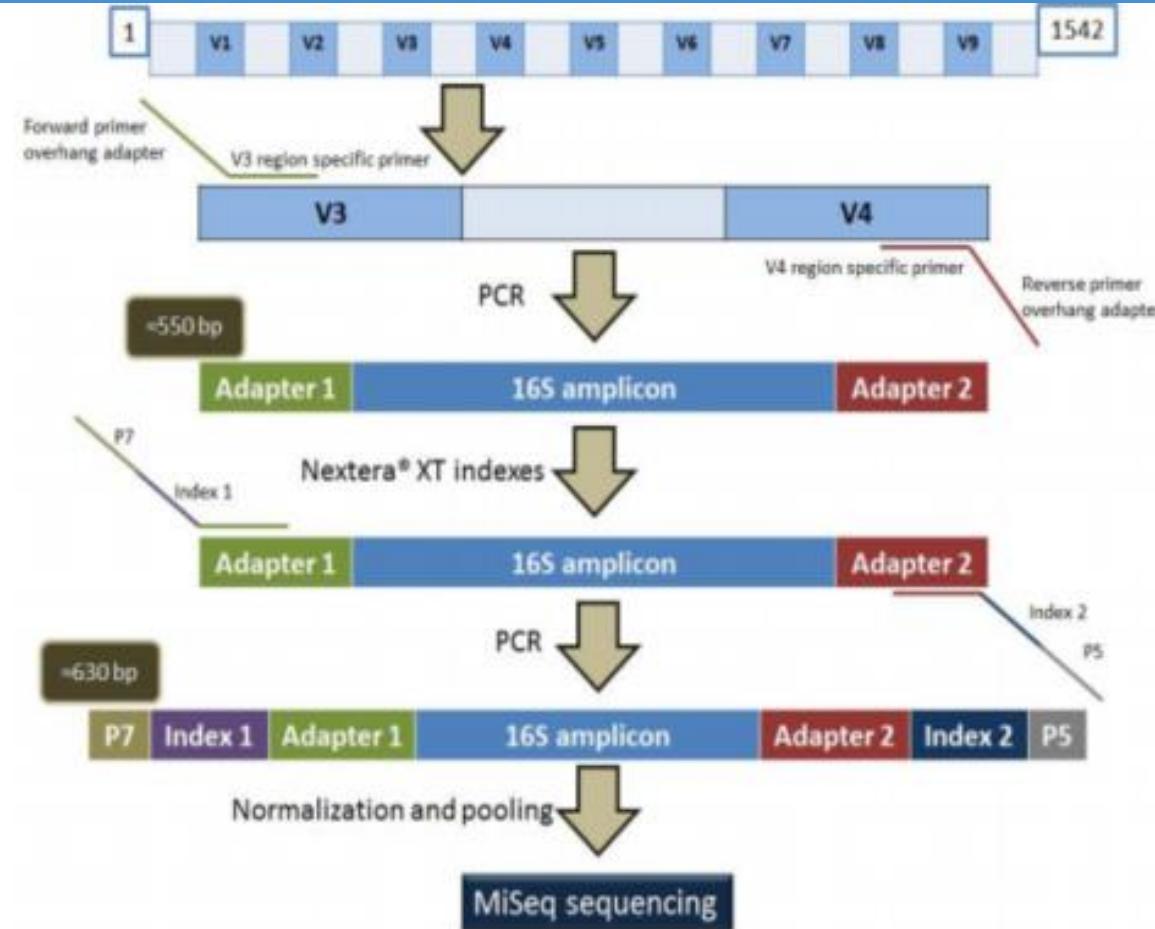
RNA → cDNA

ENRIQUECIMIENTO:  
PCR  
CAPTURA SONDAS



Guia Práctica Genómica [https://www.uv.es/varnau/GM\\_Cap%C3%ADtulo\\_2.pdf](https://www.uv.es/varnau/GM_Cap%C3%ADtulo_2.pdf)

## PREPARACIÓN LIBRERÍA, rRNA 16S, caracterización microbiota



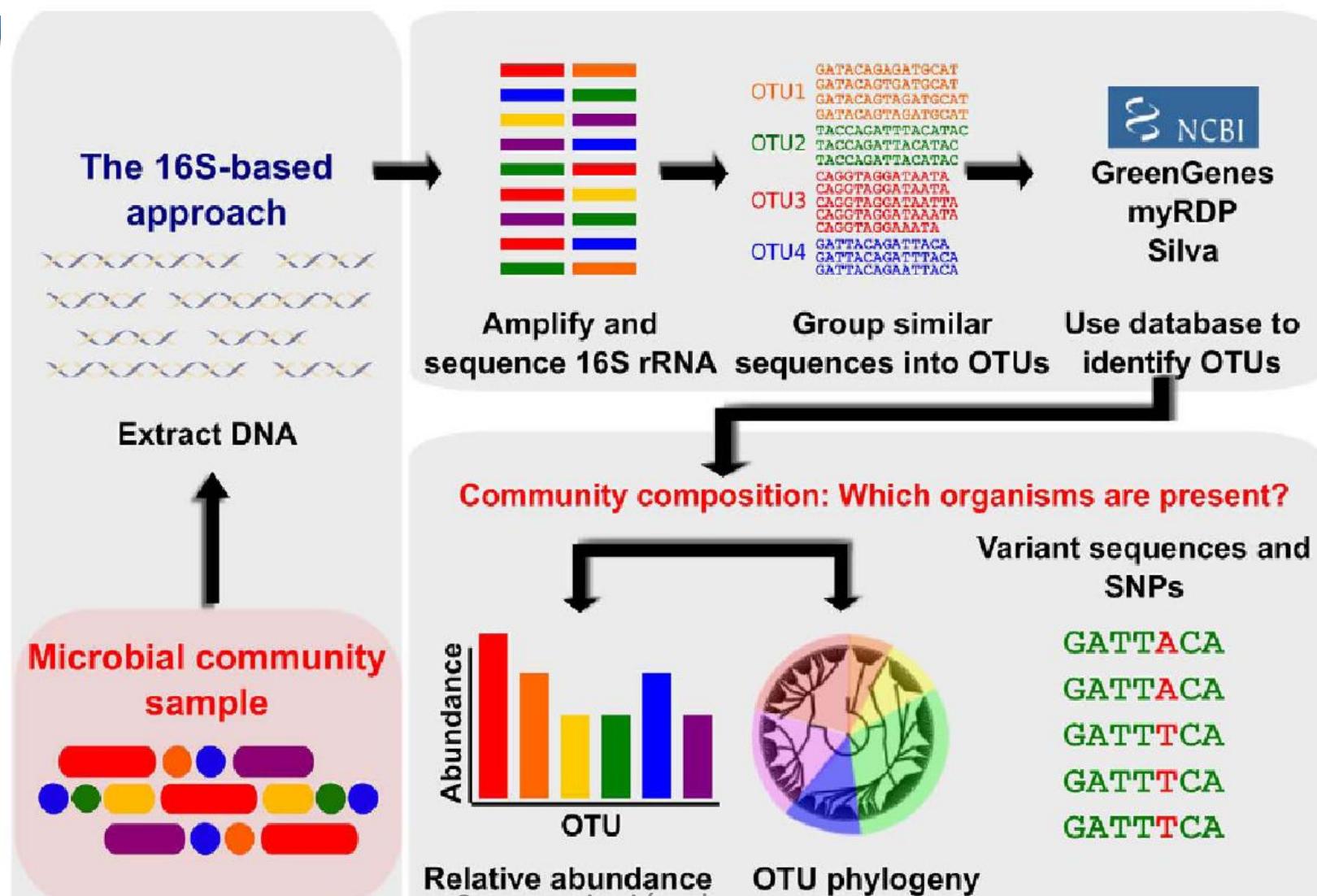
# Metataxonomics vs Metagenomics (16S vs Shotgun)

	Metagenetics	Metagenomics
<b>Amplified sequence</b>	Marker regions	Whole genome
<b>Computing time</b>	Usually short	Usually long
<b>Taxonomic composition</b>	Yes	Yes
<b>New pathogen detection</b>	No	Yes
<b>Genome coverage information</b>	No	Yes

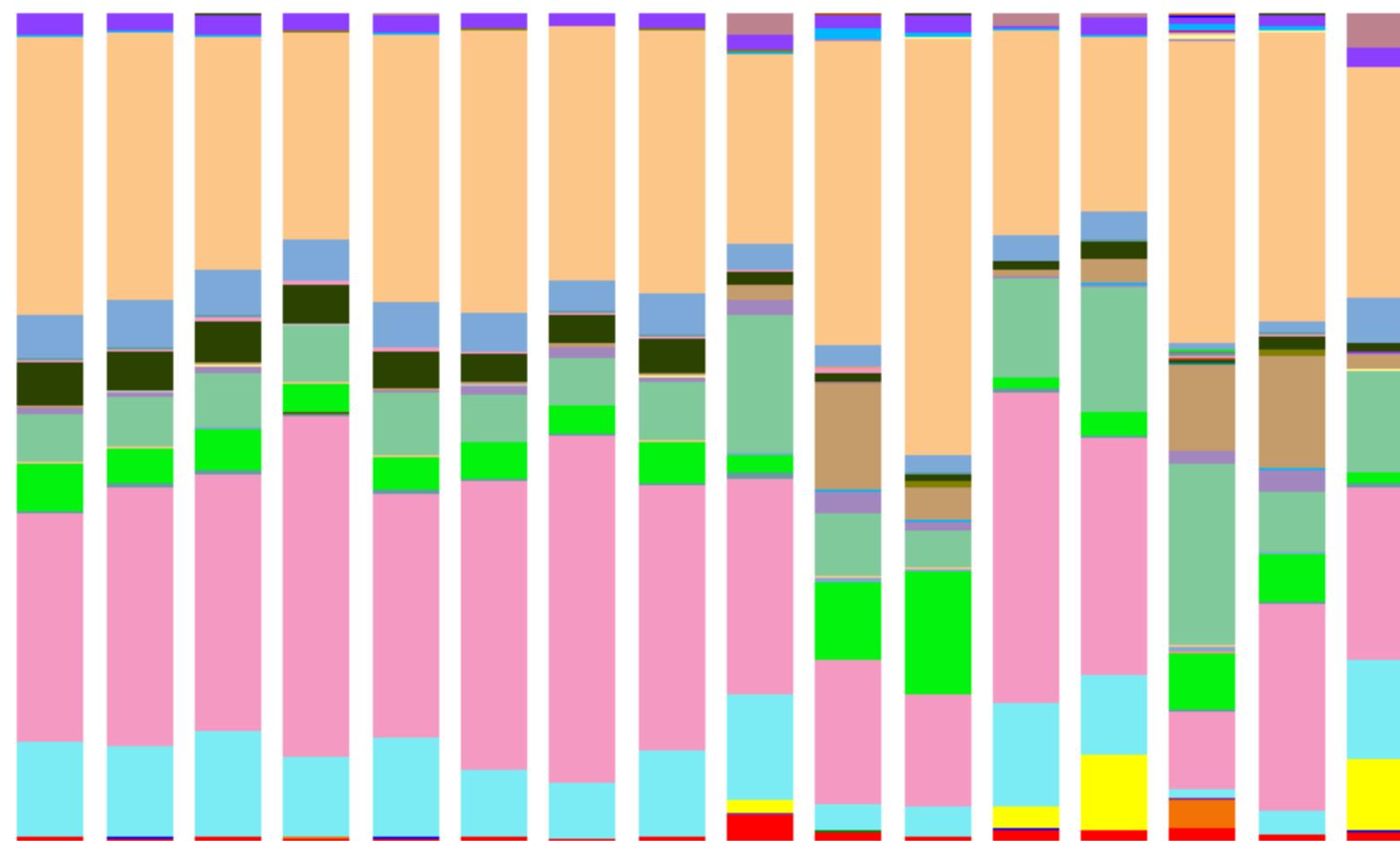
## Secuenciación de genomas

Secuenciación de genomas bacterianos: herramientas y aplicaciones

# Metataxonomics



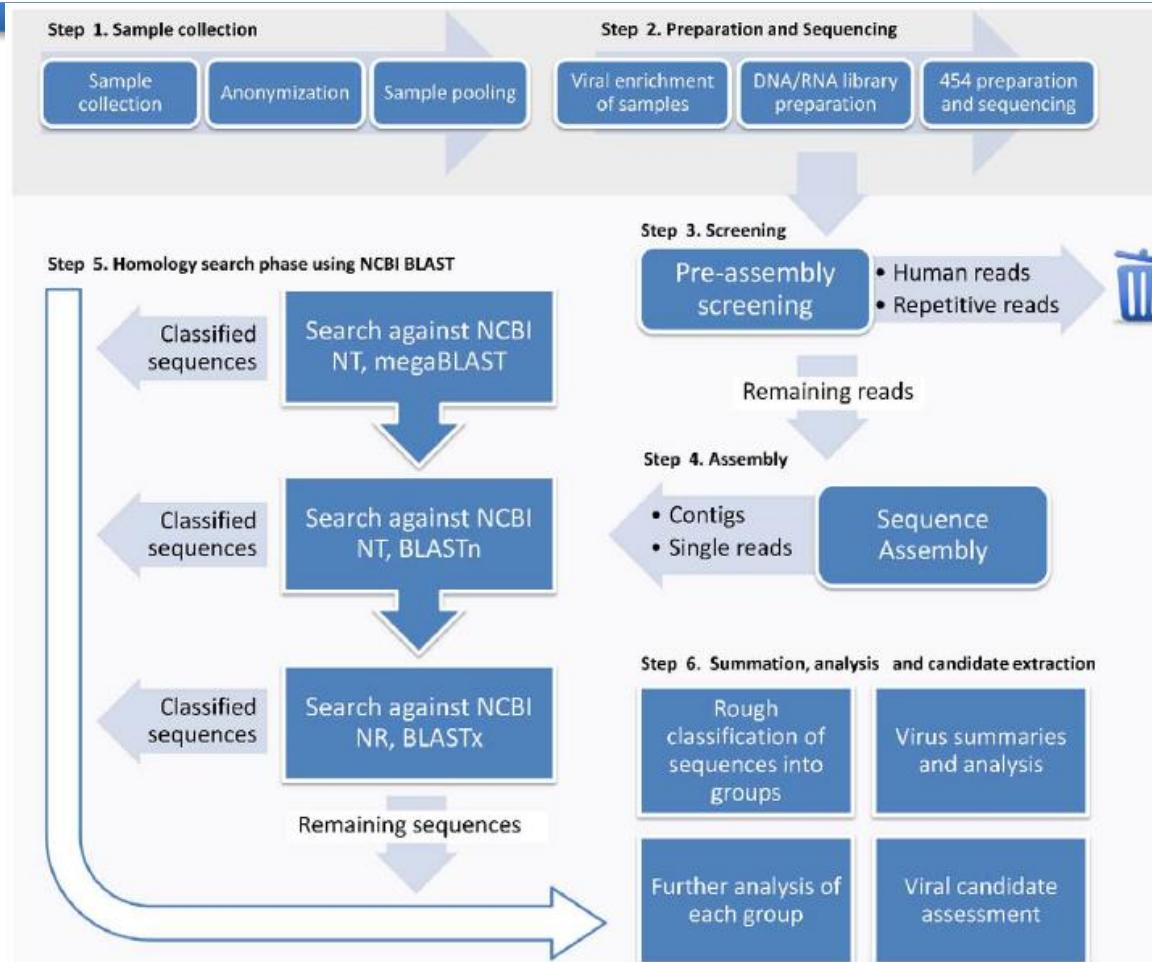
# Taxonomy summary (i.e. phylum level)



## Problemas:

- Raros en el genoma (< 0.1%)
- Los trozos similares dificultan el ensamblado correcto de lecturas pequeñas
- No todos los rRNA se amplifican en la misma medida con los *primers* universales
- Especies con diversas copias de sus genes rRNA
- **No se conoce un umbral fijo de similitud que separe especies**
- **Tendencia a producirse quimeras en la PCR**

# Metagenómica, pipeline de análisis

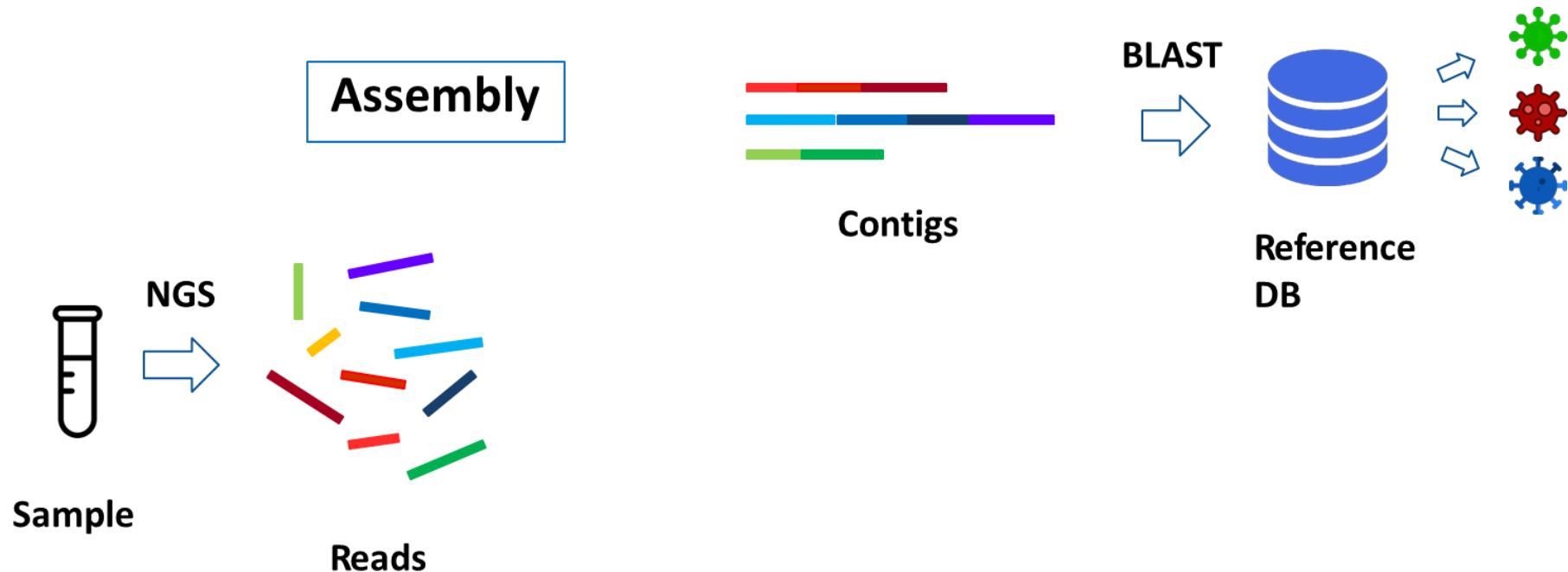


Lysholm et al., Plos One 2012:7,2, e30875

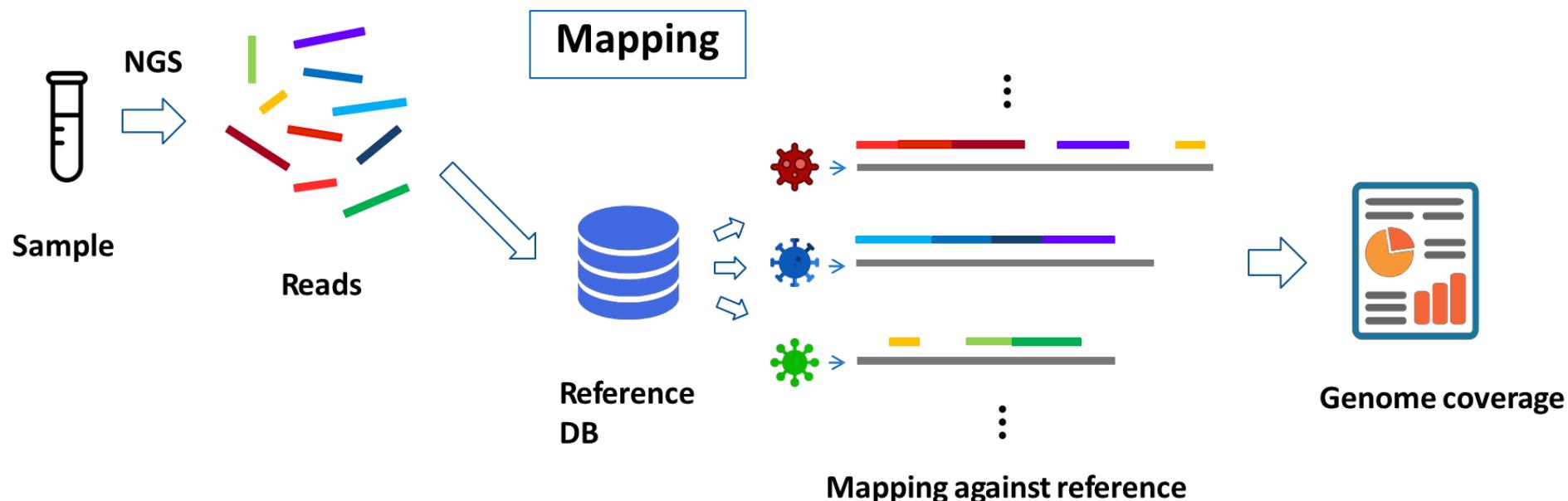
Secuenciación de genomas

Secuenciación de genomas bacterianos: herramientas y aplicaciones

# Metagenomic analysis approaches



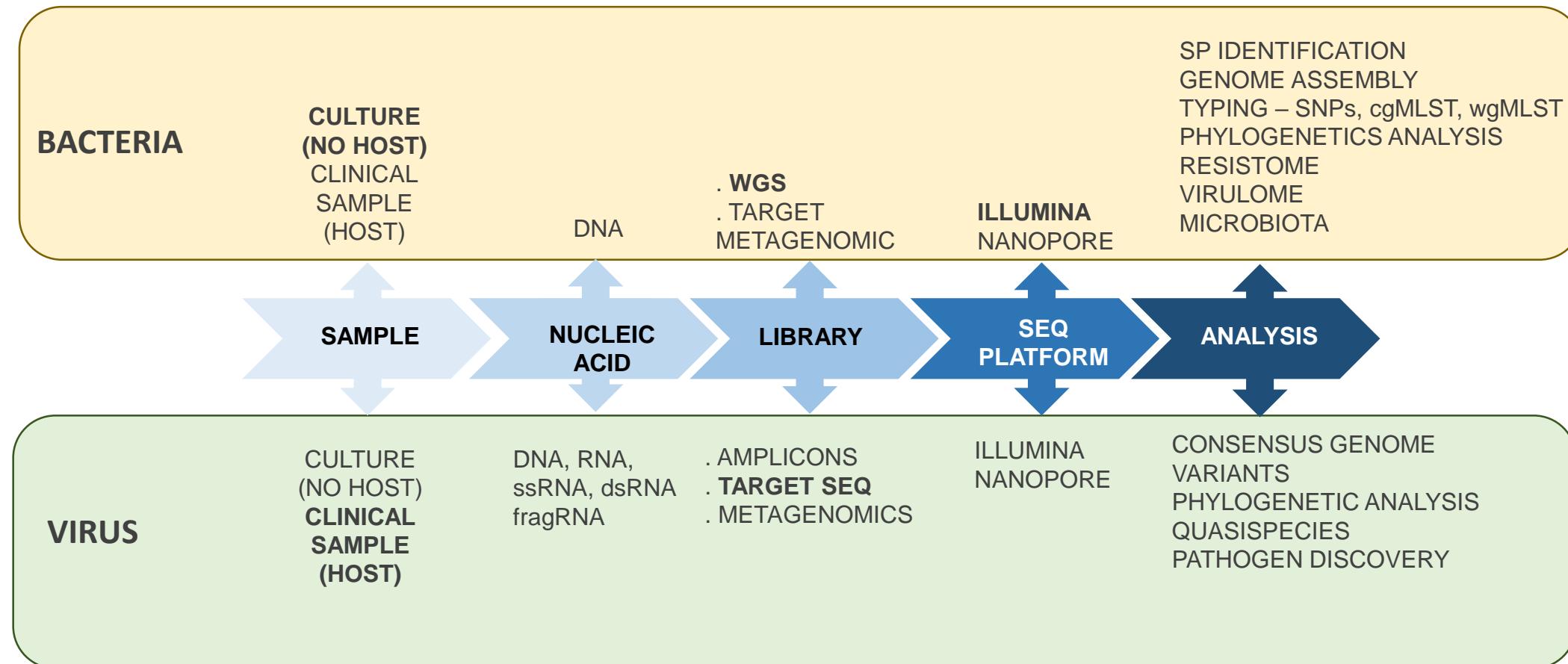
# Metagenomic analysis approaches



# Metataxonomics vs Metagenomics (16S vs Shotgun)

Software	Organism	Genetic portion used		Binning algorithm used			Genome coverage	Novel pathogen discovery
		Genetic markers	Whole Genome	Clustering	Mapping	Assembly		
Mothur	Bacteria	X		X			No	No
QIIME	Bacteria	X		X		X	No	No
MEGAN	Bacteria		X			X	No	No
Platypus	Bacteria		X		X		No	No
SURPI	Virus		X			X	No	Yes
Virus-TAP	Virus		X			X	No	Yes
VIP	Virus		X		X		No	Yes
Pathosphere	Virus, Bacteria, Eukarya		X			X	No	Yes

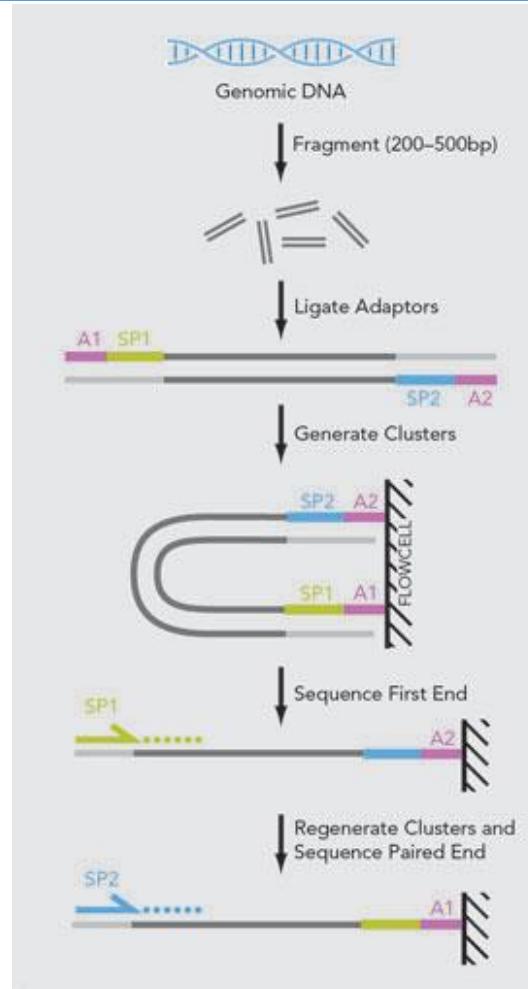
# Bacterial and viral Genome Sequencing



# Bioinformatics analysis in microbial genomics

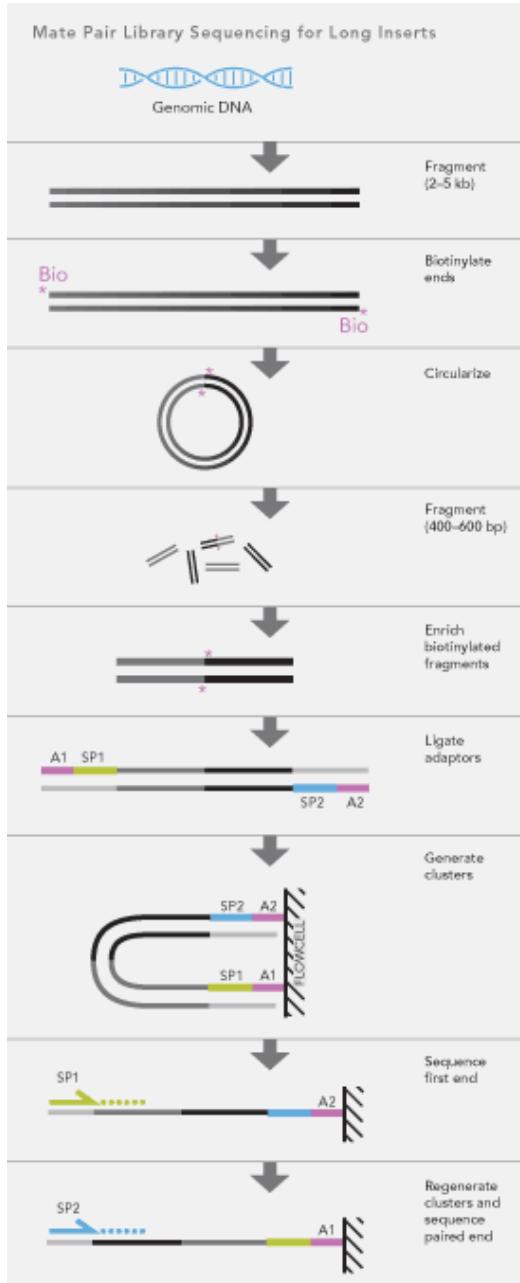
- SPECIE IDENTIFICATION
  - WGS - Kmers analysis
  - TARGET METAGENOMIC, rRNA - MICROBIOTA
- ASSEMBLY GENOME
  - de NOVO or REFERENCE -BASED
  - cgMLST, wgMLST - MINIMUM SPANING TREE
  - METAGENOMIC - HOMOLOGY -BASED
- VARIANT CALLING
  - REFERENCE GENOME SELECTION
  - HAPLOID GENOME
  - LOW FREQUENCY VARIANT - QUASISPECIES
  - SNPs MATRIX - PHYLOGENETIC ANALYSIS
- STRUCTURAL AND FUNCTIONAL ANNOTATION
  - RESISTOME, VIRULOME, SEQUENCE-TYPE

# Que es Pair-end?



**Secuenciación de un fragmento (bp)**

**Modificación de single-read DNA,  
Leyendo por ambos extremos, forward y reverse**



Mate Pair library preparation is designed to generate short fragments that consist of two segments that originally had a separation of several kilobases in the genome. Fragments of sample genomic DNA are end-biotinylated to tag the eventual mate pair segments. Self-circularization and refragmentation of these large fragments generates a population of small fragments, some of which contain both mate pair segments with no intervening sequence. These Mate Pair fragments are enriched using their biotin tag. Mate Pairs are sequenced using a similar two-adapter strategy as described for paired-end sequencing.

# Que es Mate-pair?

**Secuenciación de dos fragmentos separados kb.**

**Util:**  
**Secuenciación de un Genoma de novo**  
**Finalizar un genoma**  
**Detección de variantes estructurales**

## Sequencing terms

### Depth of coverage

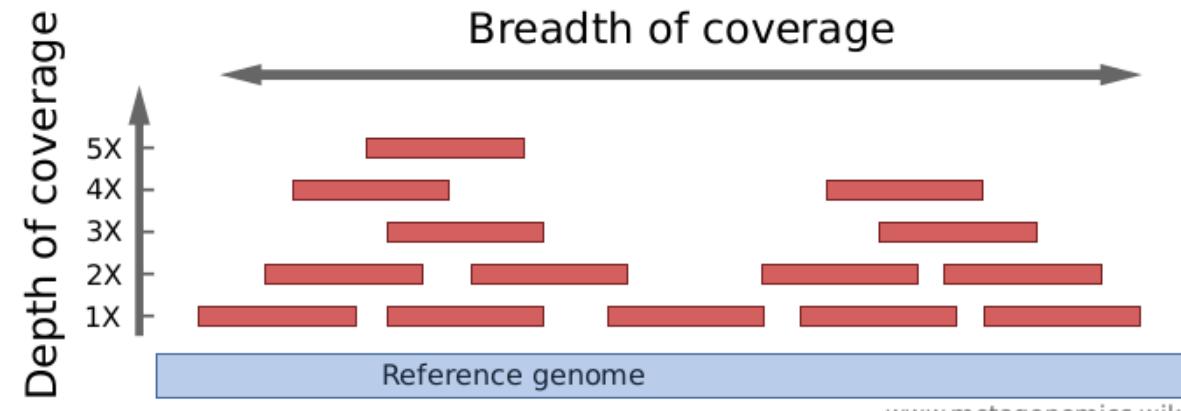
How strong is a genome "covered" by sequenced fragments (short reads)?

Per-base coverage is the average number of times a base of a genome is sequenced. The coverage depth of a genome is calculated as the number of bases of all short reads that match a genome divided by the length of this genome. It is often expressed as 1X, 2X, 3X,... (1, 2, or, 3 times coverage).

### Breadth of coverage

How much of a genome is "covered" by short reads? Are there regions that are not covered, even not by a single read?

Breadth of coverage is the percentage of bases of a reference genome that are covered with a certain depth. For example: 90% of a genome is covered at 1X depth; and still 70% is covered at 5X depth.



# Calculo de cobertura: número de lecturas

## Estimating Sequencing Runs

### Coverage Equation

The Lander/Waterman equation is a method for computing coverage<sup>1</sup>.

The general equation is:

$$C = LN / G$$

- C stands for coverage
- G is the haploid genome length
- L is the read length
- N is the number of reads

So, if we take one lane of single read human sequence with v3 chemistry, we get

$$C = (100 \text{ bp}) * (189 \times 10^6) / (3 \times 10^9 \text{ bp}) = 6.3$$

This tells us that each base in the genome will be sequenced between six and seven times on average.

**Sequencing Coverage Calculator**

Support Center: Sequencing Coverage Calculator

Application or product: Whole-Genome Sequencing

Coverage: 100 x

Duplicates: 2 %

Genome or region size (in million bases): 3300 Mb

Total read length (e.g. 200 for 2x100): 600 cycles

Benchtop Sequencers      Production-Scale Sequencers

iSeq       NextSeq 500/550

MiSeq       NovaSeq 6000

MiSeq / MiSeq Dx in RUO mode       HiSeq 3000/4000

NextSeq 500/550       HiSeq 1500/2500 Rapid Run

Clusters

Output per unit (flow cell or lane)

Exceeds maximum read length?

Number of units per sample (flow cell or lane)

Samples per unit (flow cell or lane)

Comments

Products

Support Center: Sequencing Coverage Calculator

Thank you for using the Illumina coverage estimator.

The results were calculated based on: **coverage needed**. Explain the estimations

	MiSeq	MiSeq	MiSeq	MiSeq
Run type	v3 Reagents	v2 Reagents	v2 Nano Reagents	v2 Micro Reagents
Clusters	25,000,000 per flow cell	15,000,000 per flow cell	1,000,000 per flow cell	4,000,000 per flow cell
Output per unit (flow cell or lane)	15,000,000,000 per flow cell	9,000,000,000 per flow cell	600,000,000 per flow cell	2,400,000,000 per flow cell
Exceeds maximum read length?	Does not exceed maximum of 2x250	Read length exceeds maximum of 2x250	Read length exceeds maximum of 2x250	Read length exceeds maximum of 2x150
Number of units per sample (flow cell or lane)	22,449 flow cells	37,415 flow cells	561,224 flow cells	140,306 flow cells
Samples per unit (flow cell or lane)	-0/flow cell	-0/flow cell	-0/flow cell	-0/flow cell
Comments	Upgraded software: MCS v2.3 or later; MiSeq	Upgraded hardware or from September 2012 and later: MCS v2.0 or later; Reagent Kit v3 (150/600)	Upgraded hardware or from September 2012 and later: MCS v2.0 or later; MiSeq Reagent Kit v2 (300/500)	Upgraded hardware or from September 2012 and later: MCS v2.0 or later; MiSeq Reagent Nano Kit v2 (300/500)
Products	MiSeq Reagent Kit v3	MiSeq Reagent Kits v2	MiSeq Reagent Kits v2	MiSeq Reagent Kits v2

Get the results in a comma-separated values (CSV) report.

[https://emea.support.illumina.com/downloads/sequencing\\_coverage\\_calculator.html](https://emea.support.illumina.com/downloads/sequencing_coverage_calculator.html)

Thanks for your attention!

Questions???