MODULO 5: Introducción al análisis de datos de secuenciación masiva y sus aplicaciones en Microbiología

Isabel Cuesta

17 Enero 2023

UAH - ISCIII

BU-ISCIII

Unidades Centrales Científico Técnicas – SGSAFI-ISCIII

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- BU-ISCIII
- Conocer las aplicaciones de la secuenciación masiva en microbiología y los tipos de análisis de datos.
- Conocer los ficheros generados por plataformas como Illumina, y evaluar su calidad.
- Aprender a usar de Galaxy, herramienta web que permite el manejo y análisis de datos procedentes de técnicas de secuenciación masiva.
- Reconstruir la secuencia consenso del genoma de SARS-CoV-2 e identificar las mutaciones y variantes asociadas.
- Ensamblar genomas secuenciados con plataforma Illumina y analizar la calidad del ensamblado.

BIONINFORMATICS UNIT (BU-ISCIII)

- **Sara Monzón,** Biotecnóloga y Bioinformática (Analista de datos). Titulado Superior Especialista OPIS. Responsable técnico BU-ISCIII
- **Sarai Varona,** 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 U. Bioinformática (BU-ISCIII)

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- Que es la Bioinformática. BU-ISCIII

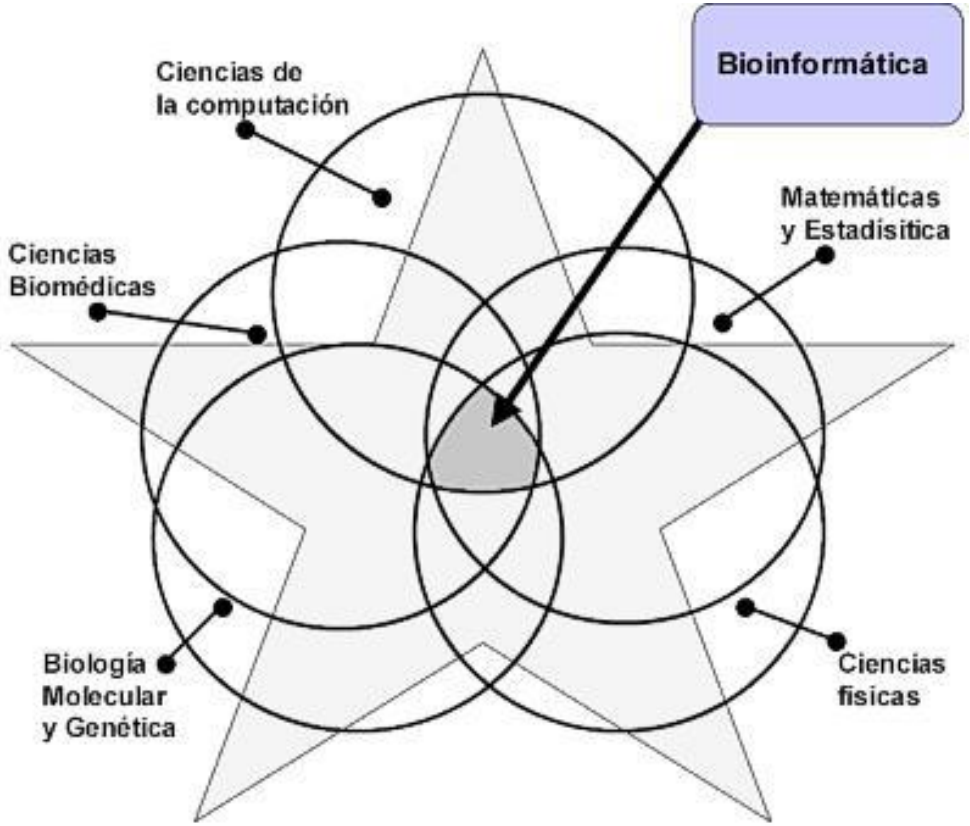
Qué es la Bioinformática?

PROBLEMAS BIOLÓGICOS

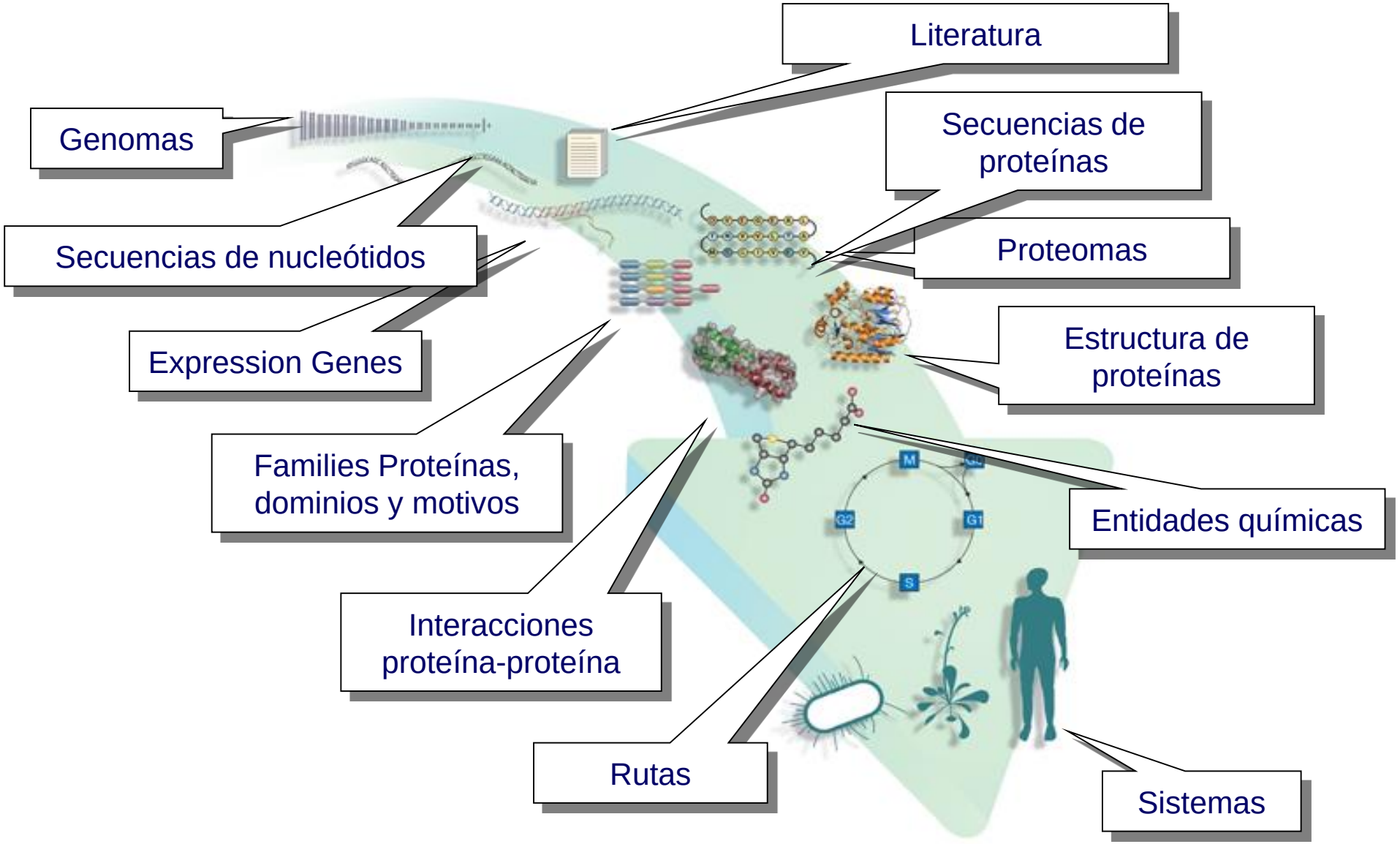
Procesamiento de datos

MÉTODOS COMPUTACIONALES

Bioinformática es multidisciplinar



Tipos de datos dan idea de la dimensión de la Bioinformática

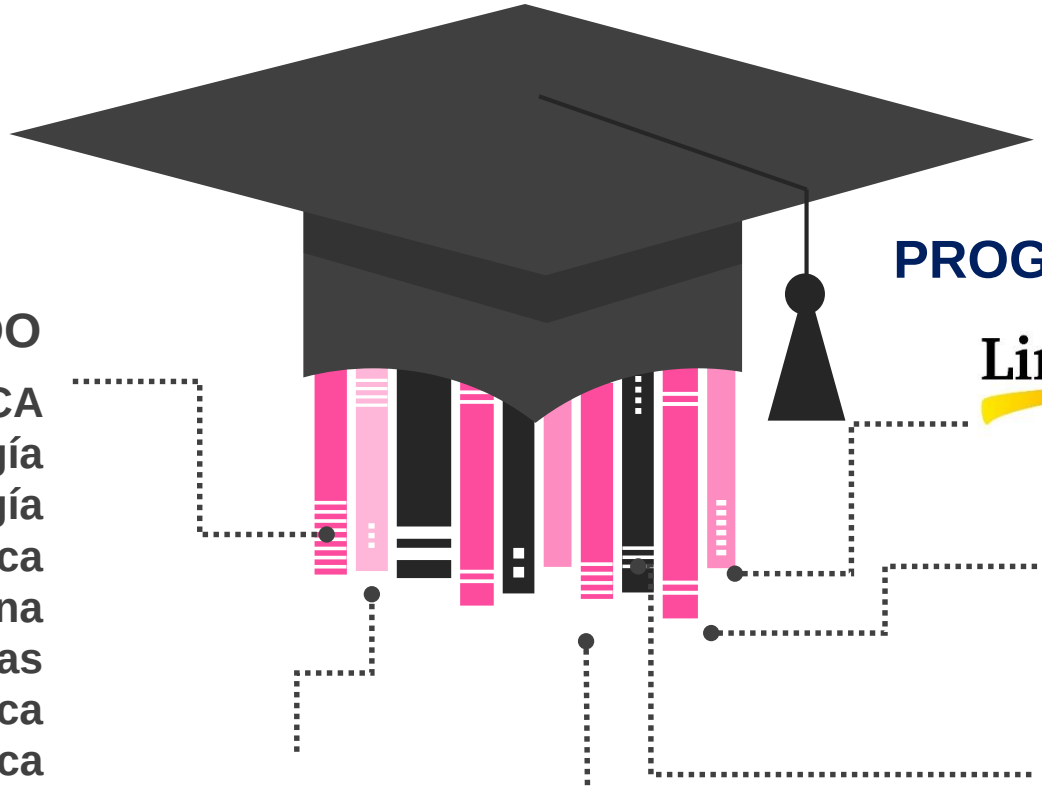


FORMACIÓN EN BIOINFORMÁTICA

Universidad Barcelona.

GRADO

BIOINFORMÁTICA
Biología
Biotecnología
Bioquímica
Medicina
Matemáticas
Química
Fisica
Informática
Telecomunicaciones



MASTER EN BIOINFORMÁTICA

PROGRAMACIÓN

Linux

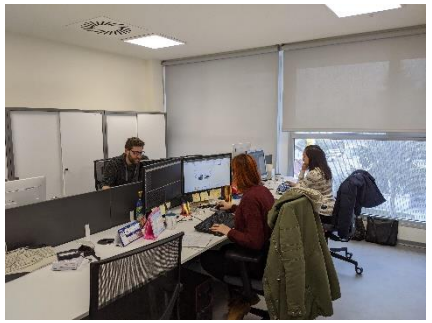
python

R

DOCTORADO

BIOLOGÍA – ANÁLISIS DE DATOS

¿Dónde trabaja un Bioinformático?

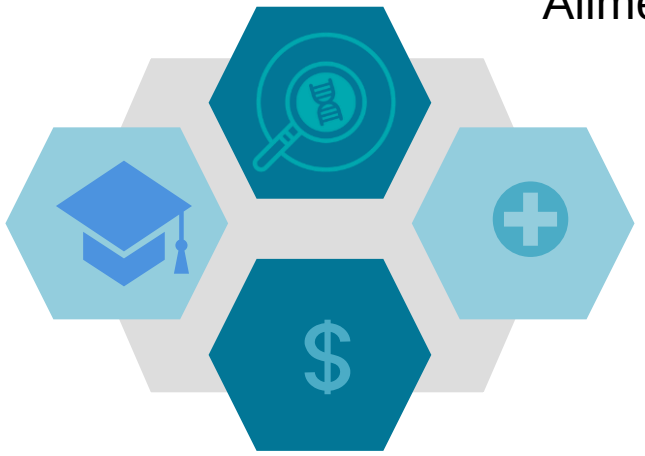


CENTRO DE INVESTIGACIÓN

Biomedicina
Agricultura
Alimentación

UNIVERSIDAD

Biociencias
Informática



HOSPITAL

BIOINFORMÁTICO CLÍNICO
Genética
Oncología
Cardiología

EMPRESA

Bioinformática
Genética
Genómica

¿Por qué nace BU-ISCIII?

10 AÑOS

Genomics Unit

2010

454

2013

NextSeq500 MiSeq

2019

MinIon - Nanopore

2021

NovaSeq 6000

Bioinformatics Unit

2012

>_BU-ISCIII

Scientific & technical Support to Researchers on HTS Data Analysis

National Microbiology Centre (CNM)

Research Institute for Rare Diseases (IIER)

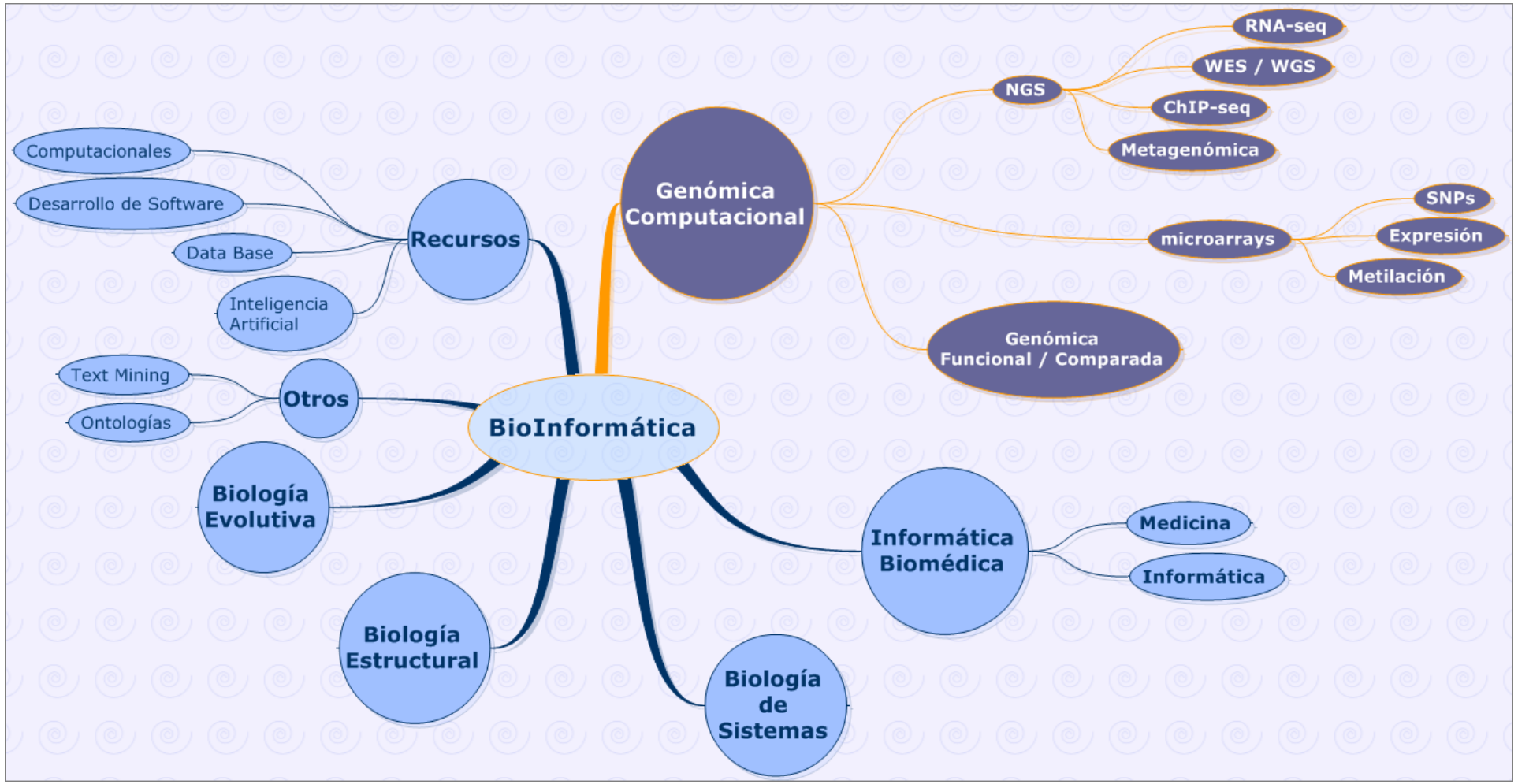
Functional Unit for Research in Chronic Disease

Network of Biological Alerts

National Centre of Tropical Medicine

National Environment Health Centre

BU-ISCIII Mission - Activities



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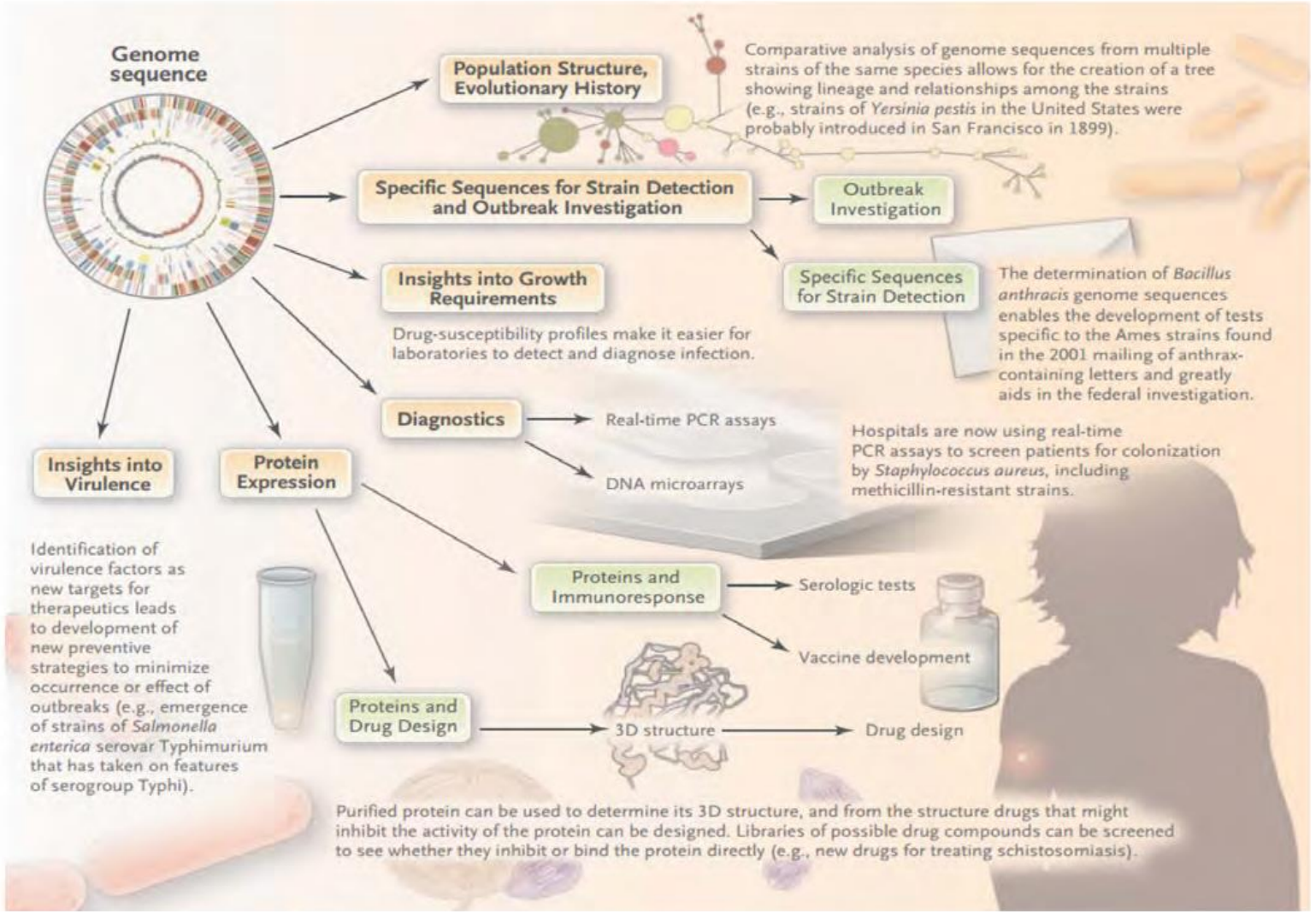
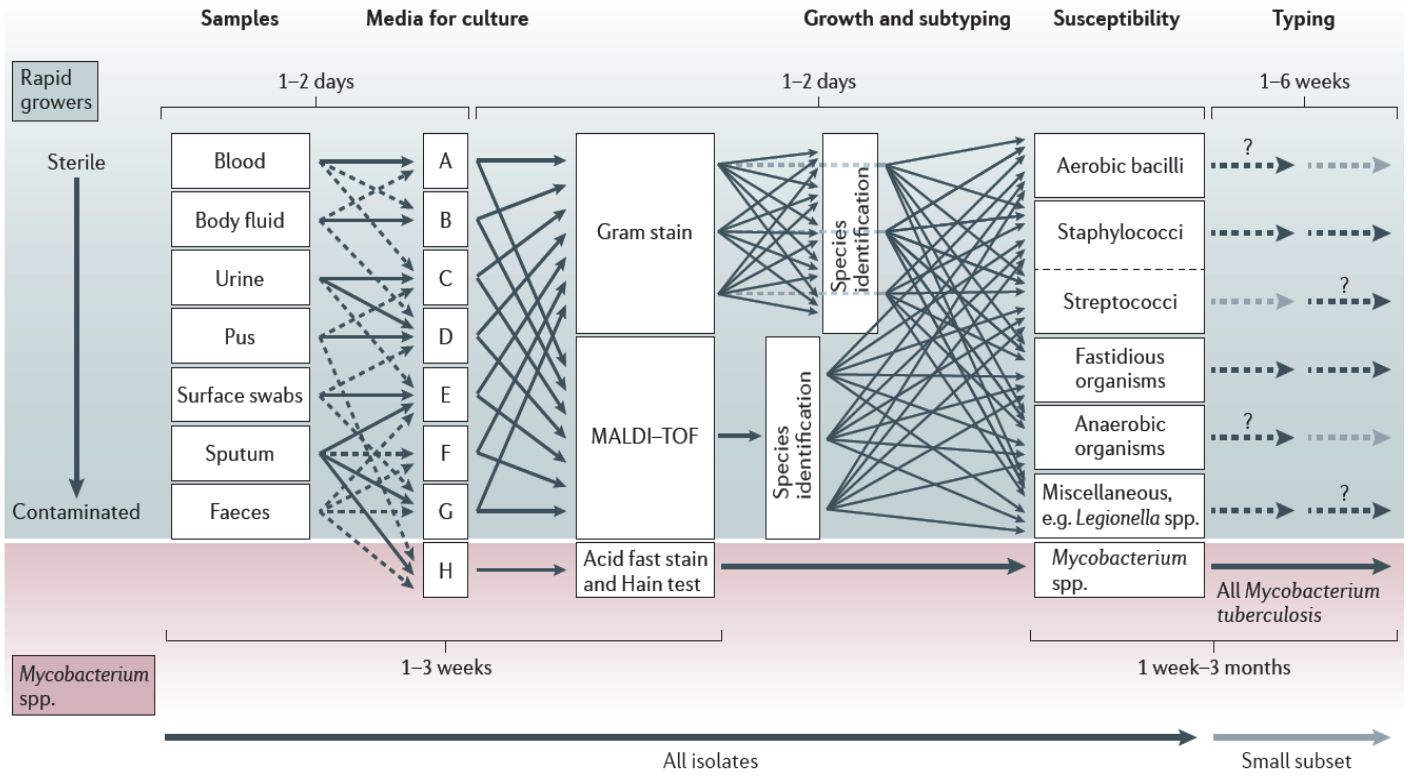
Use of microbial genomics for tool development

Report from The American Academy of Microbiology, 2015



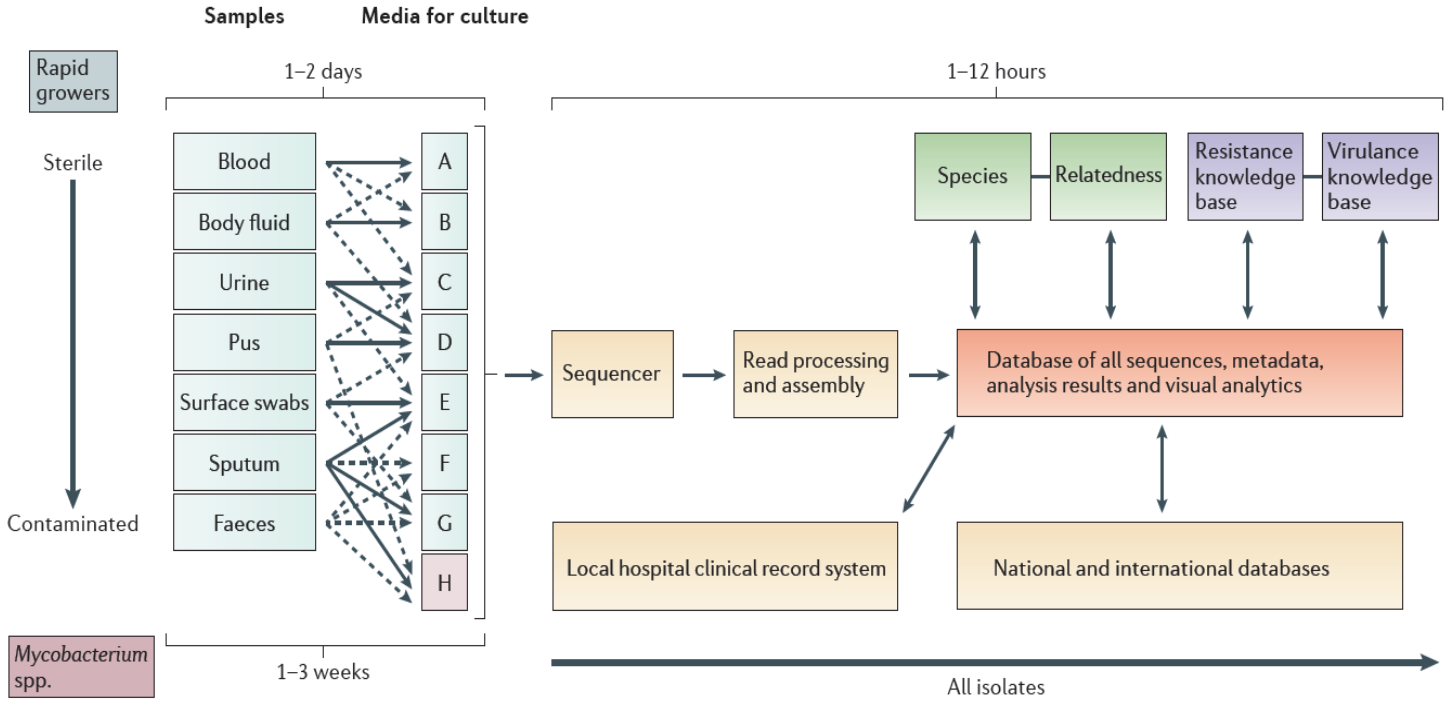
Workflow for processing samples for bacterial pathogens

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

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

Secuenciación Genoma

华大基因 BGI

Universität Hamburg

Universitätsklinikum Hamburg-Eppendorf

Bertelli and Greub, Clin Microb and Infect, 2013

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 d afectados y se apunta a la falta de higiene en la carne como causa

EFE 25.08.2019

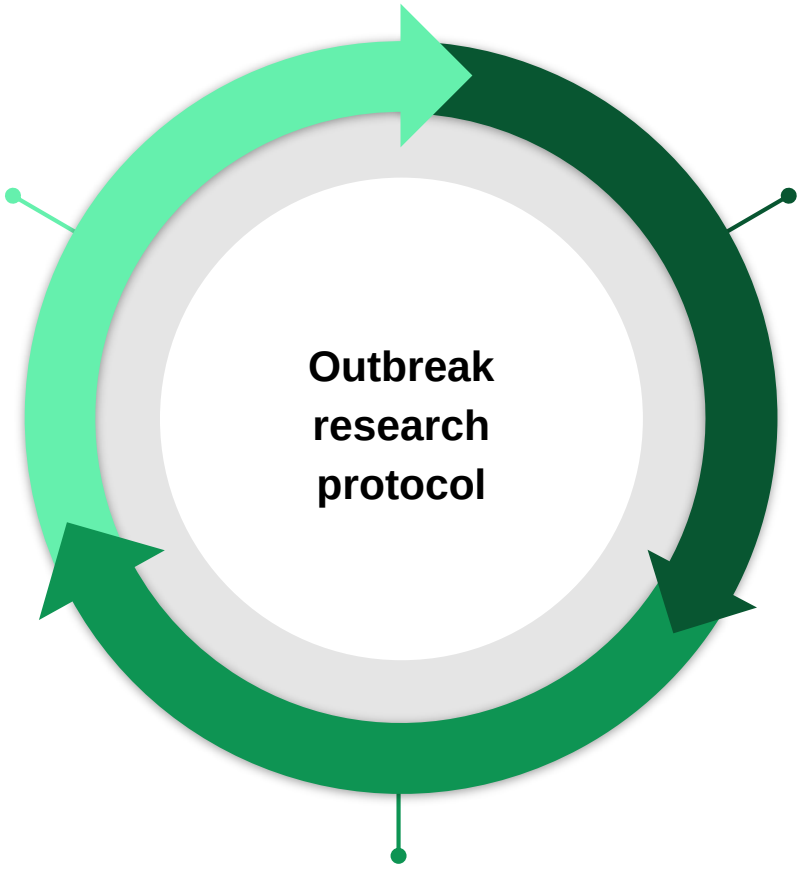
- Tres nuevos casos, en Sevilla y Cádiz, dejan el número de personas afectadas en Andalucía en 192.
- La carne con listeria de la marca blanca se vendió en los municipios de Sevilla.
- La empresa que vendió la marca blanca de Magrudis dice que cumple los protocolos.

- 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

Transversal:

- Data management
- Sample tracking
- Infrastructure

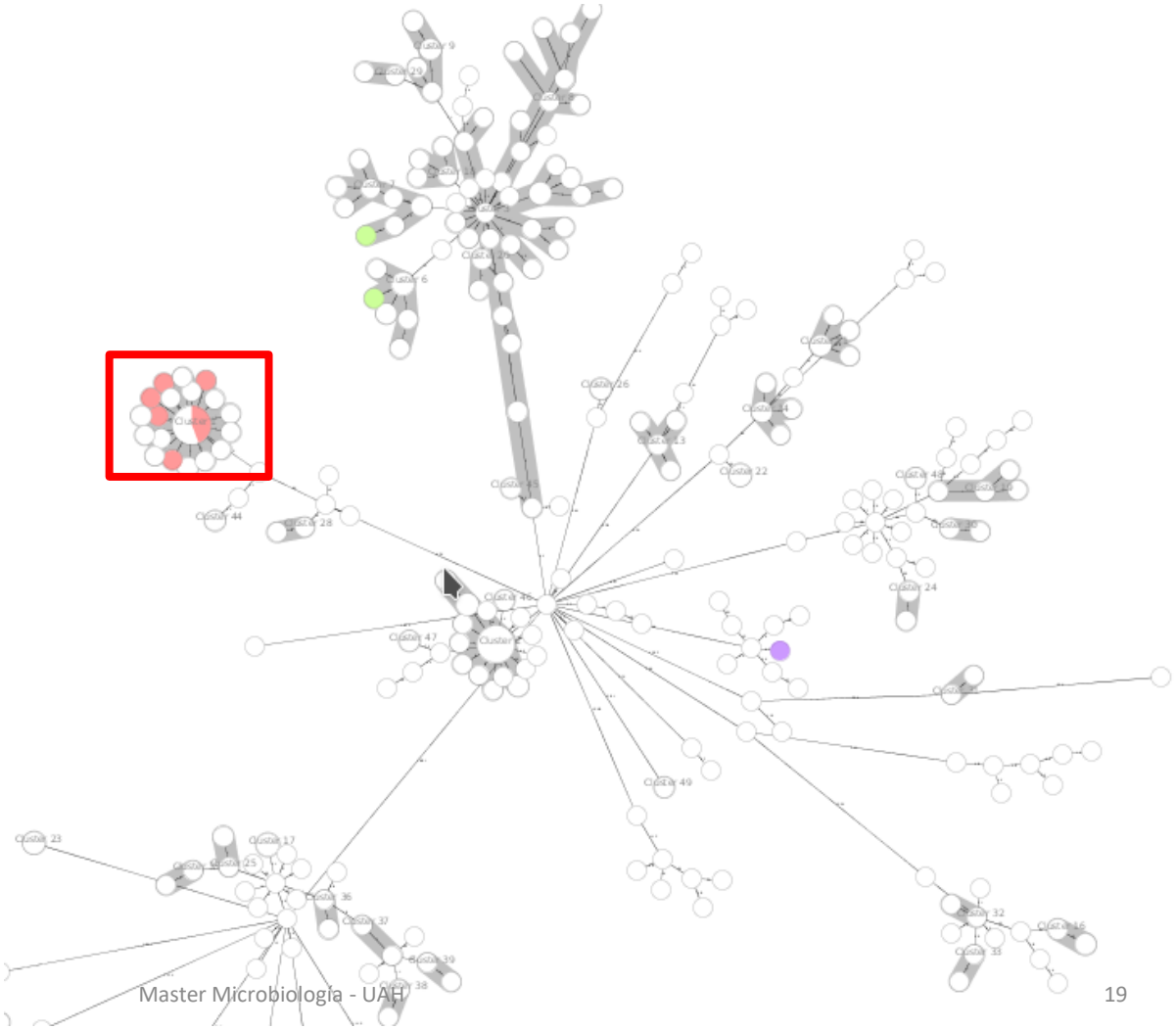


Andalusian Listeria Outbreak

- 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.



Pathogen discovery: new virus – SARS-CoV-2

Deep Meta-Transcriptomic Sequencing

bronchoalveolar lavage fluid (BALF)

Meta-transcriptomic library

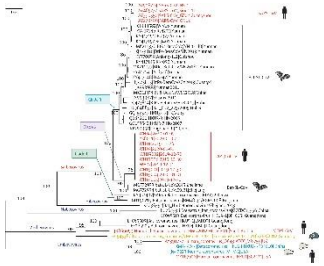
2x150 MiniSeq 56,565,928 sequences reads

De novo-assembled - Megahit

384,096 Contigs

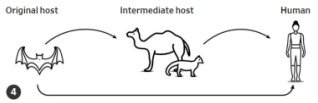
Screened for potential aetiological agents

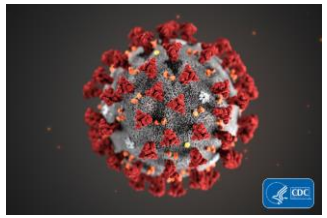
The longest 30,474 nt



89.1% identity

Closely related to a bat SARS-like coronavirus





Wu et al., Nature 2020

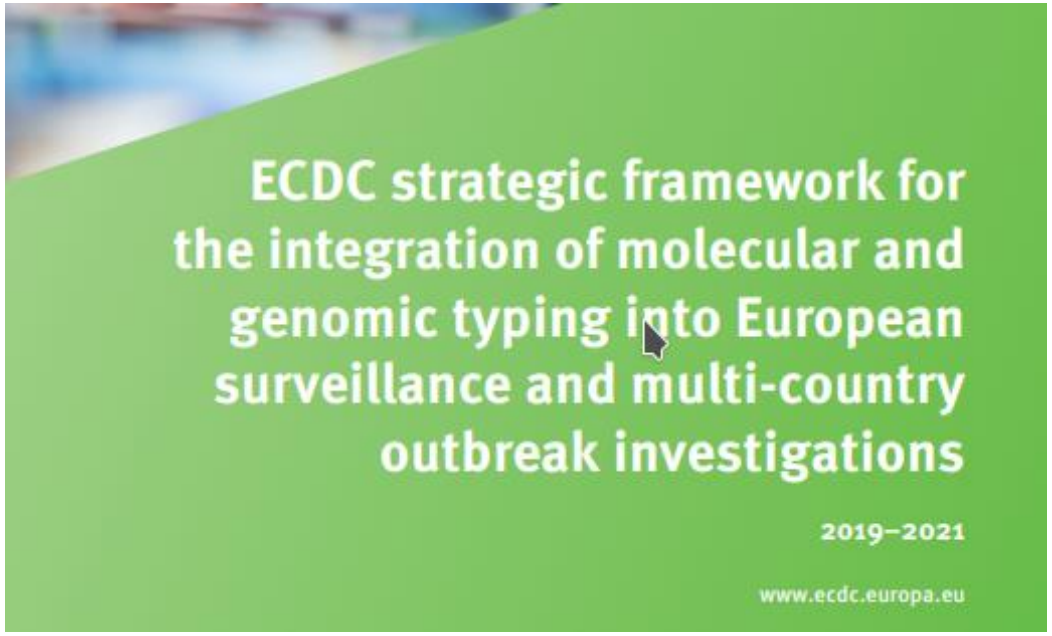
One Health approach, *infectious diseases could be better controlled and prevented*

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** → Outbreak research: Molecular source detection
- Infectious diseases research
- Training

ECDC roadmap and international commitment



- **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

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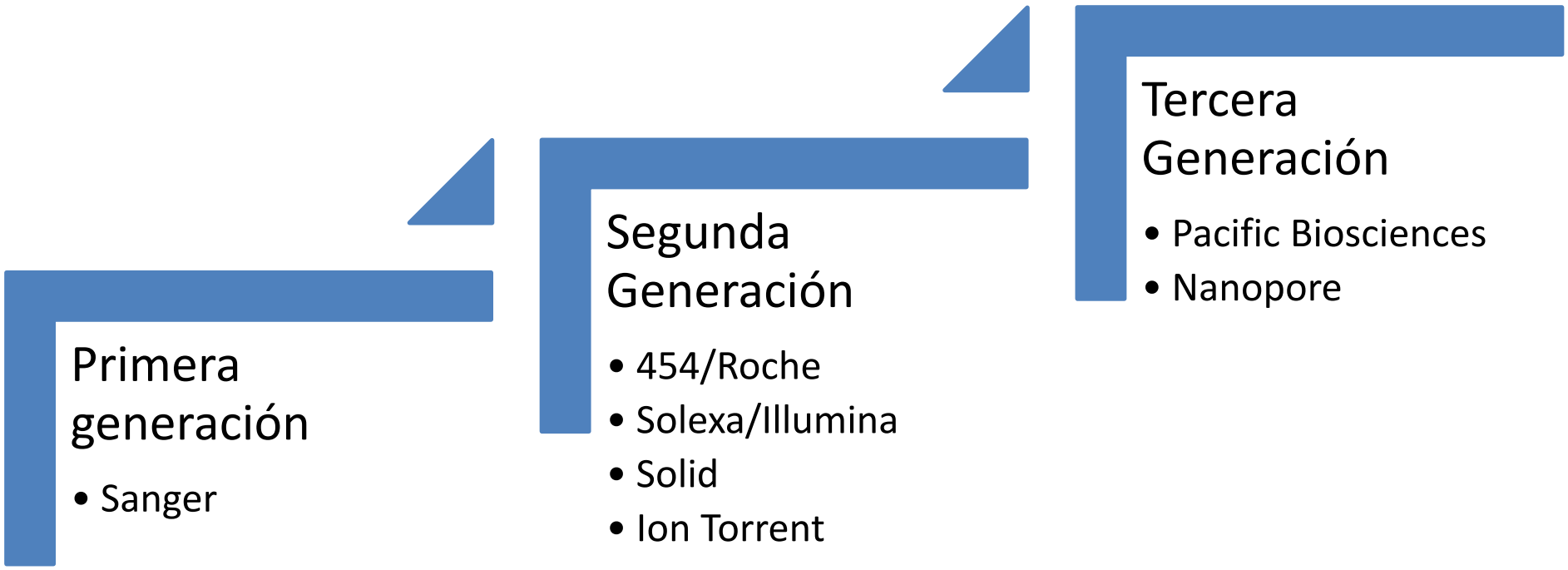
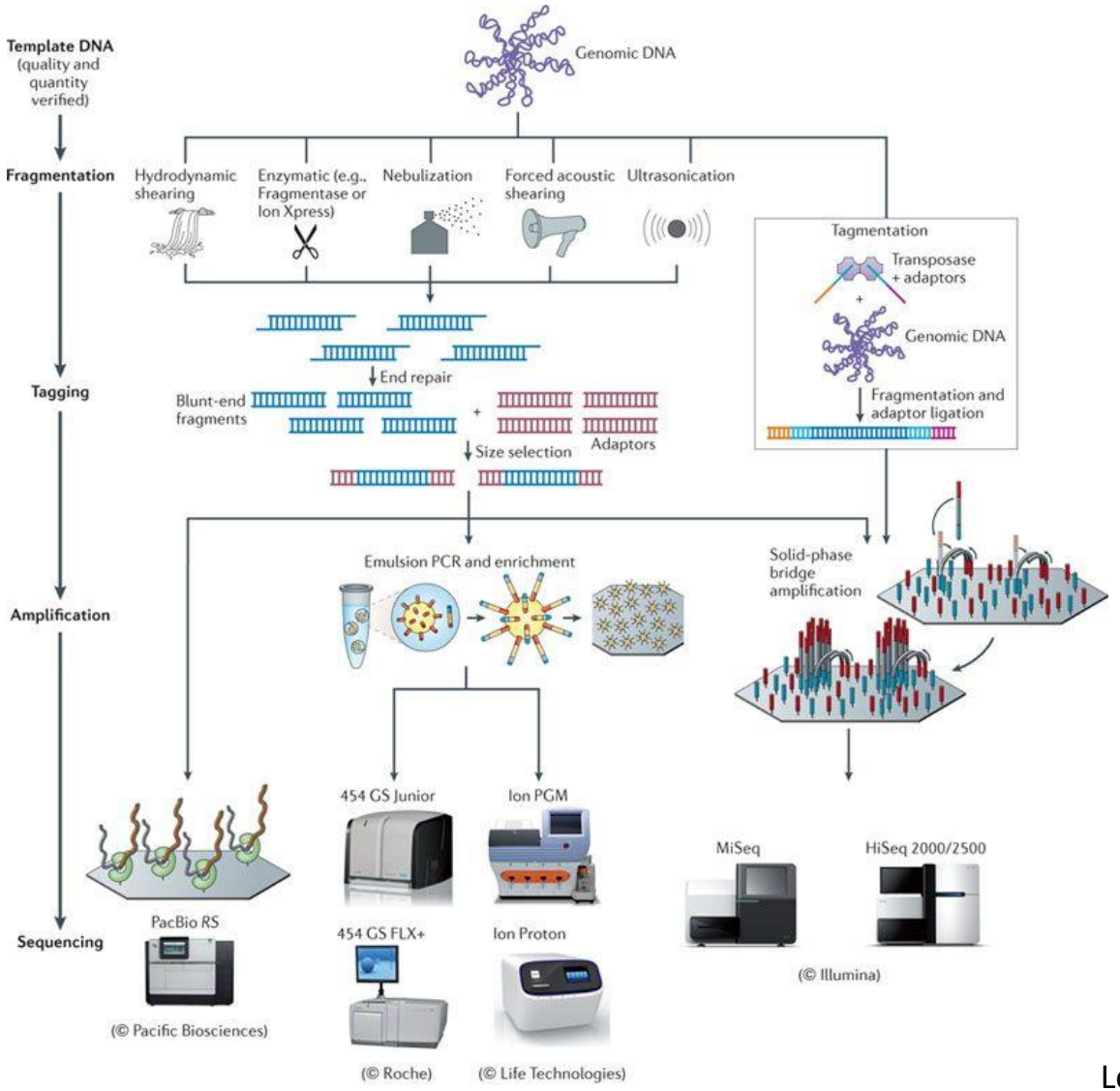
- Tipos de análisis de datos.

DNA sequencing technologies 2006-2023

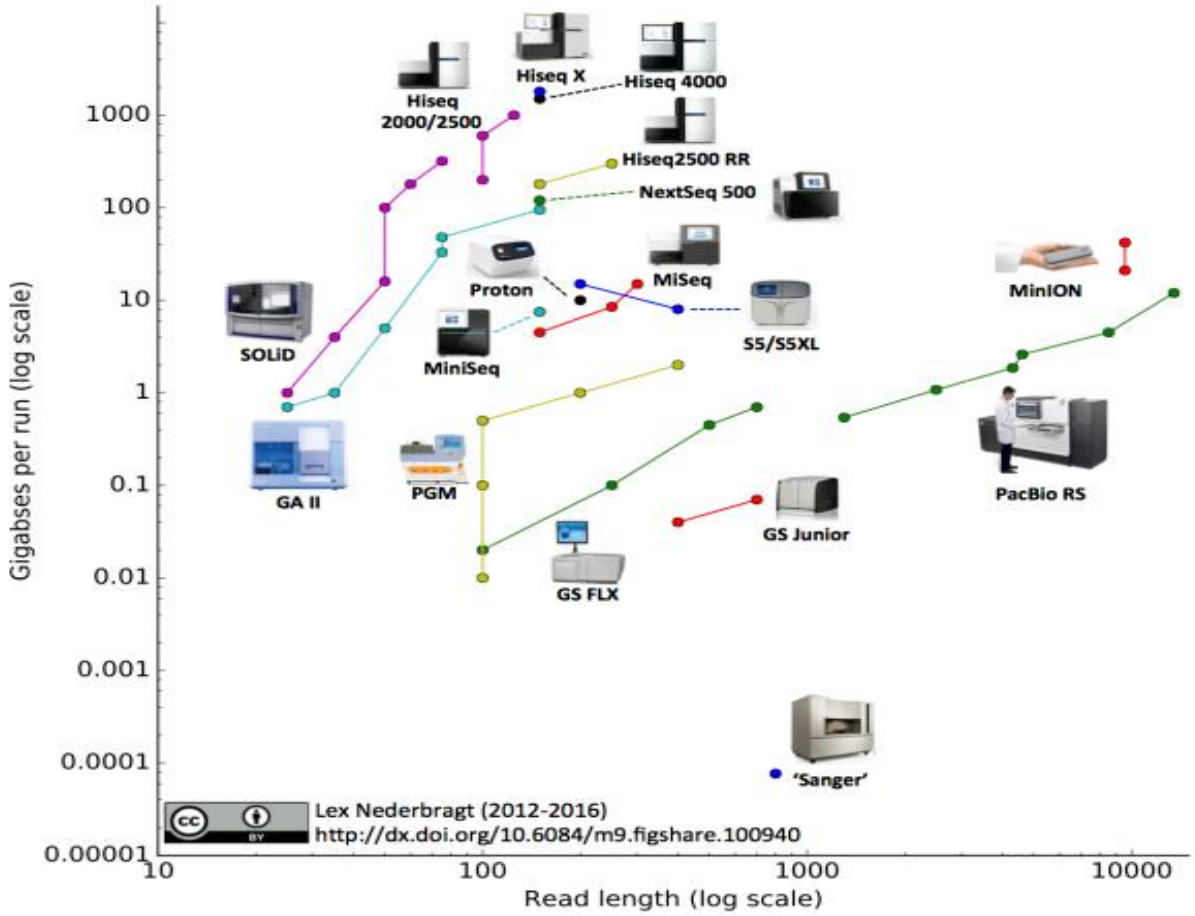


High-throughput sequencing process



Loman et al, 2012

High-Throughput Sequencing Technologies



https://flxlexblog.wordpress.com/

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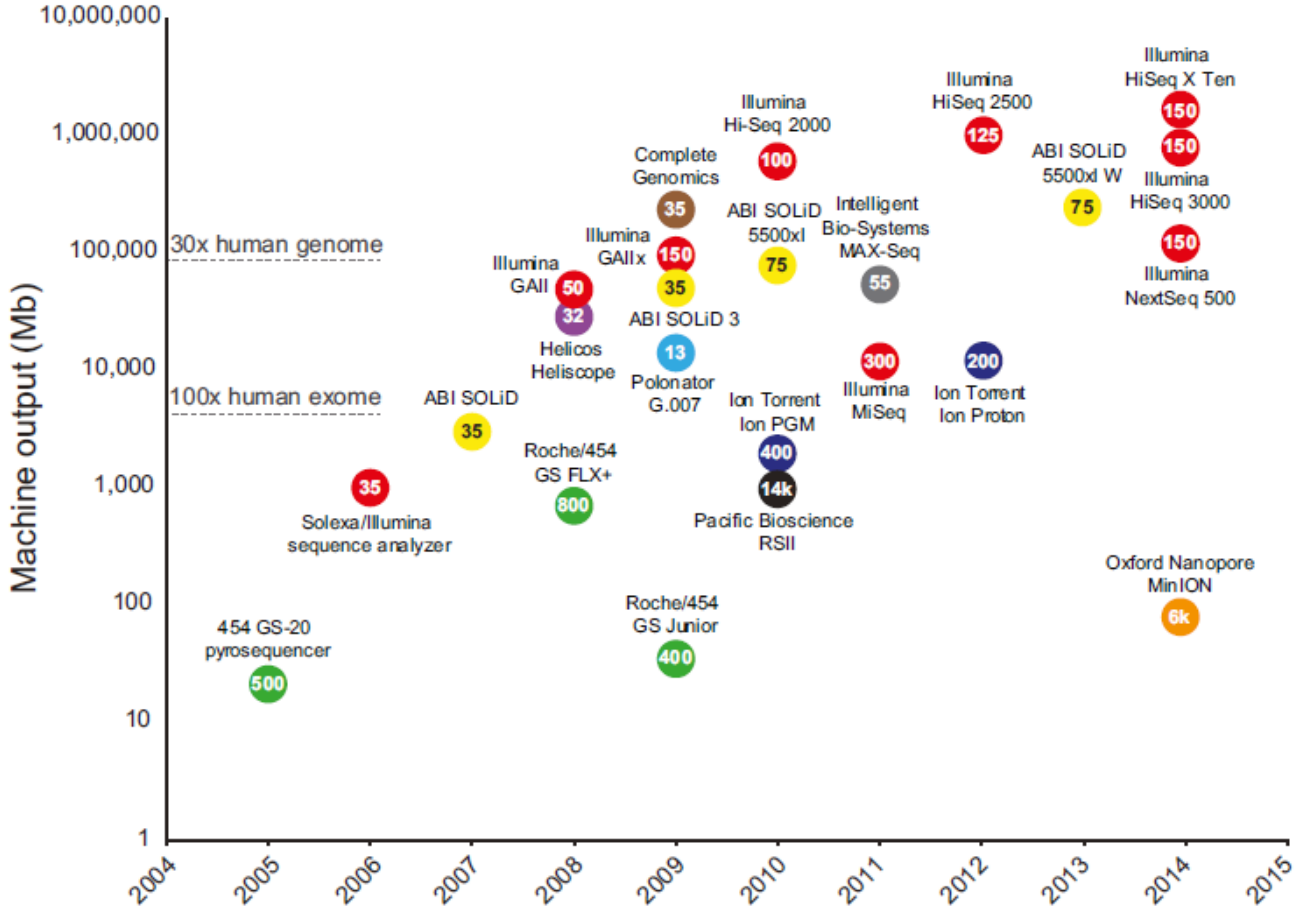
High-Throughput Sequencing Technologies



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

Reuter et al., Mol Cell 2015

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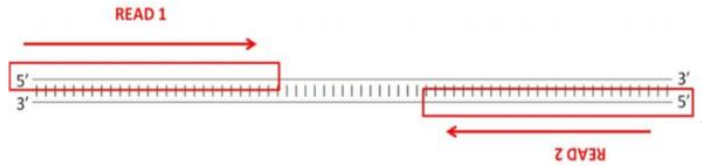
Master Microbiología - UAH

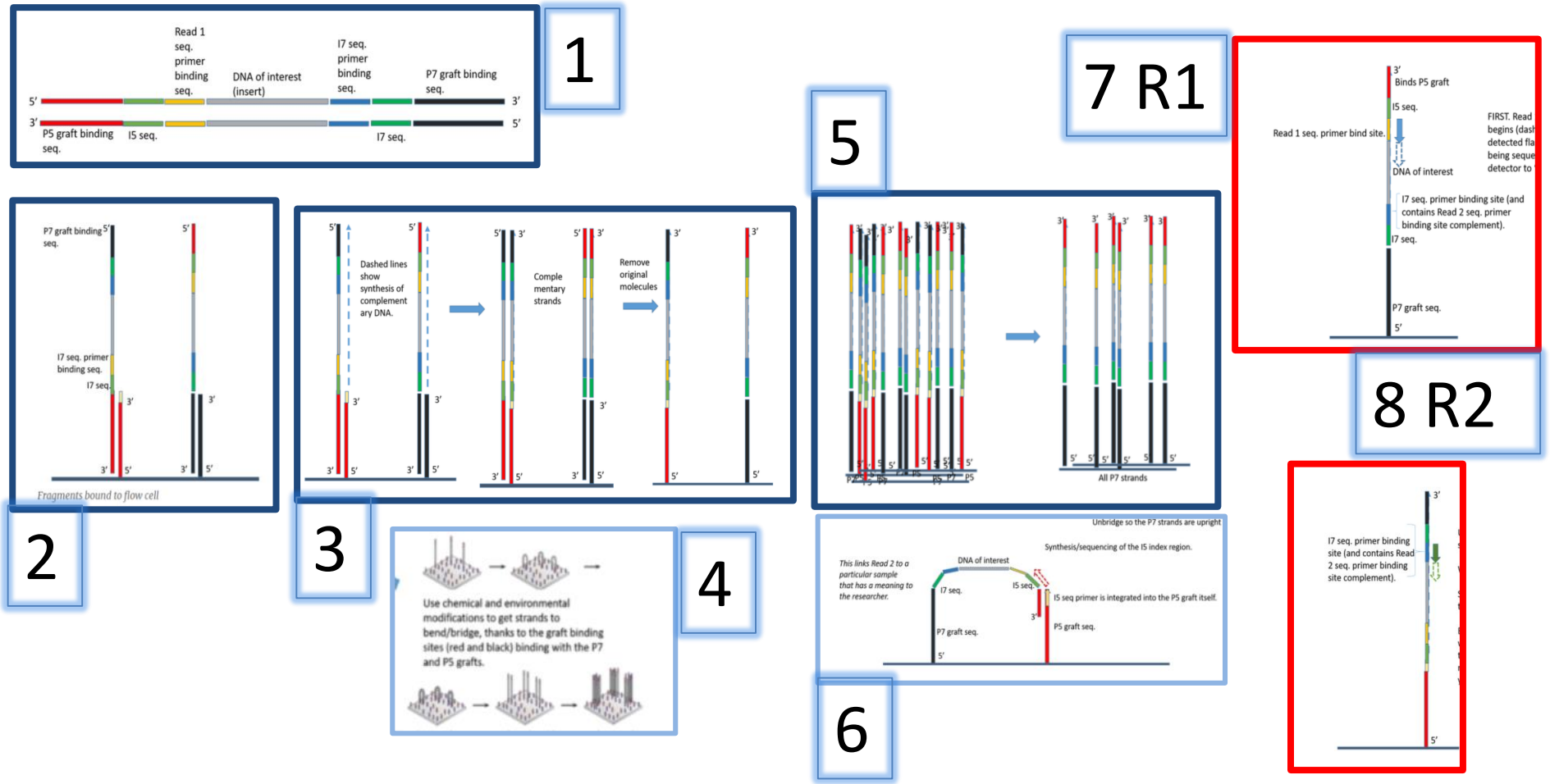
NGS PLATFORMS, main characteristics

- 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

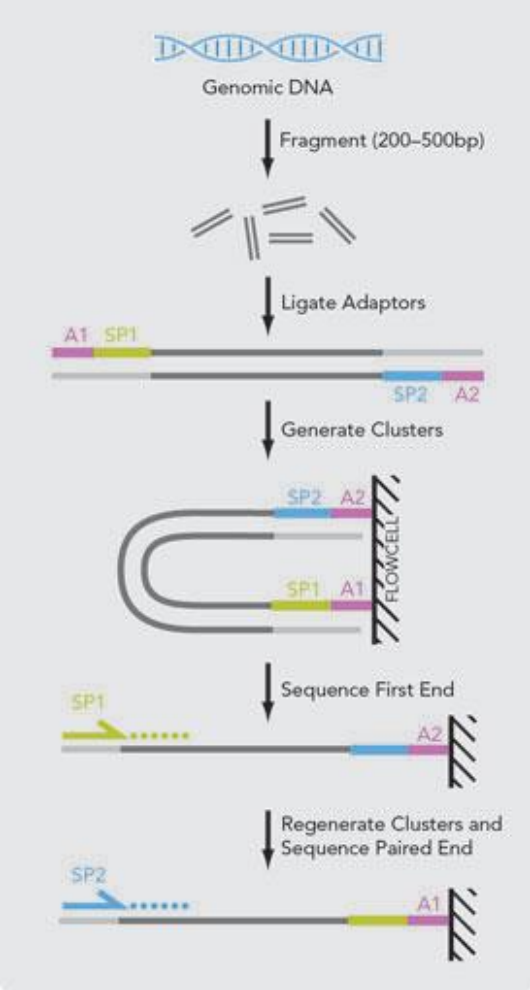
Illumina sequencing





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

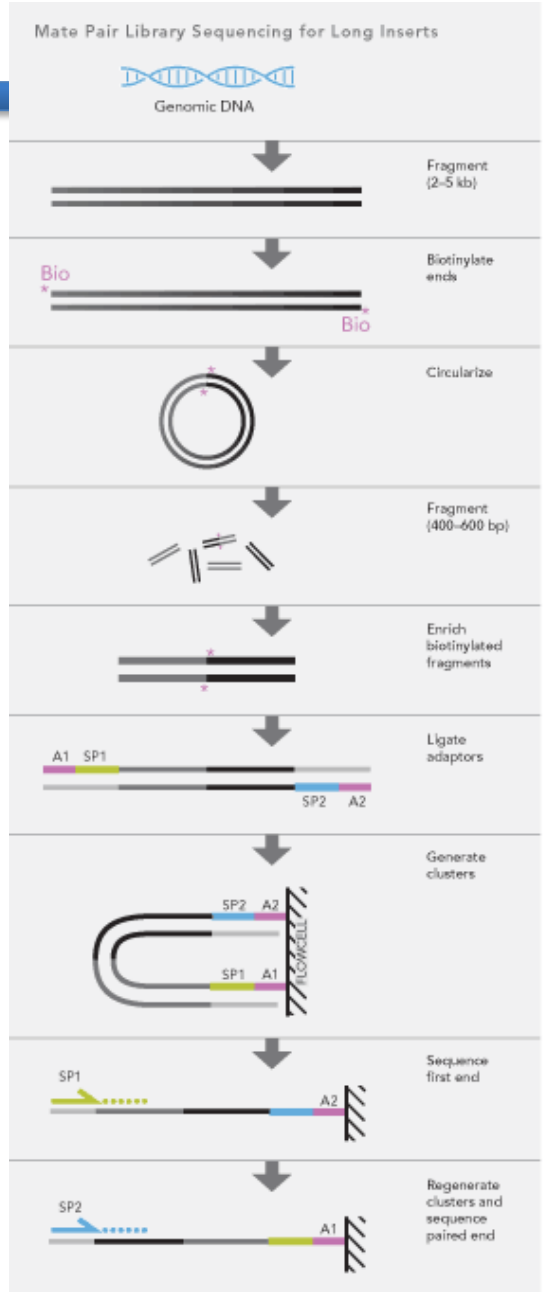
Que es Pair-end?



Secuenciación de un fragmento (bp)

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

Que es Mate-pair?



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.

Secuenciación de dos fragmentos separados kb.

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

Sequencing terms

Breadth of coverage

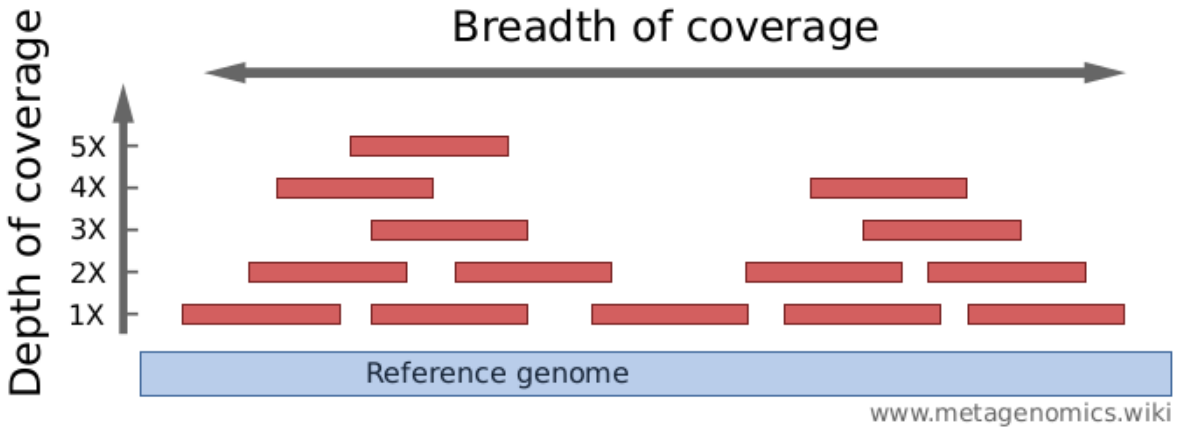
How much of a genome is "covered" by short reads? Are there regions that are not covered, even not by a single read?

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.

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).



Depth of coverage and genome coverage

Depth of coverage

$$\text{the sequencing coverage} = \frac{\text{the number of total reads} \times \text{the read length}}{\text{the length of target sequence or genome}}$$

Genome coverage

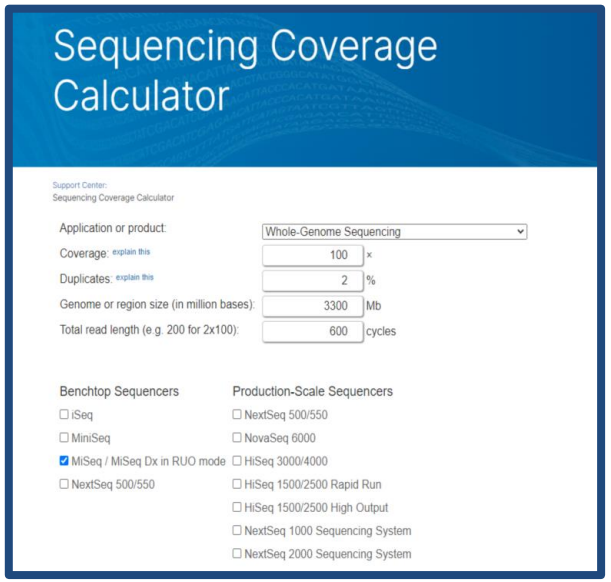
% length sequence genome

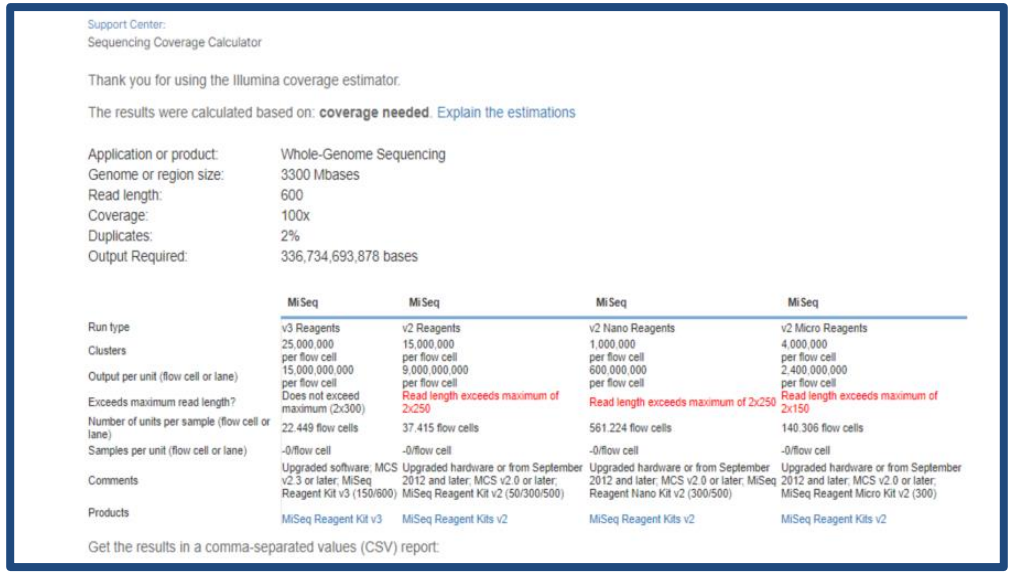
Increase number of raw reads

- For the low-frequency variants
- For assembly (also read lenght)

Calculo de cobertura: número de lecturas

Total output required = region size * coverage / ((1-duplicates/100) * on target/100)





https://emea.support.illumina.com/downloads/sequencing_coverage_calculator.html

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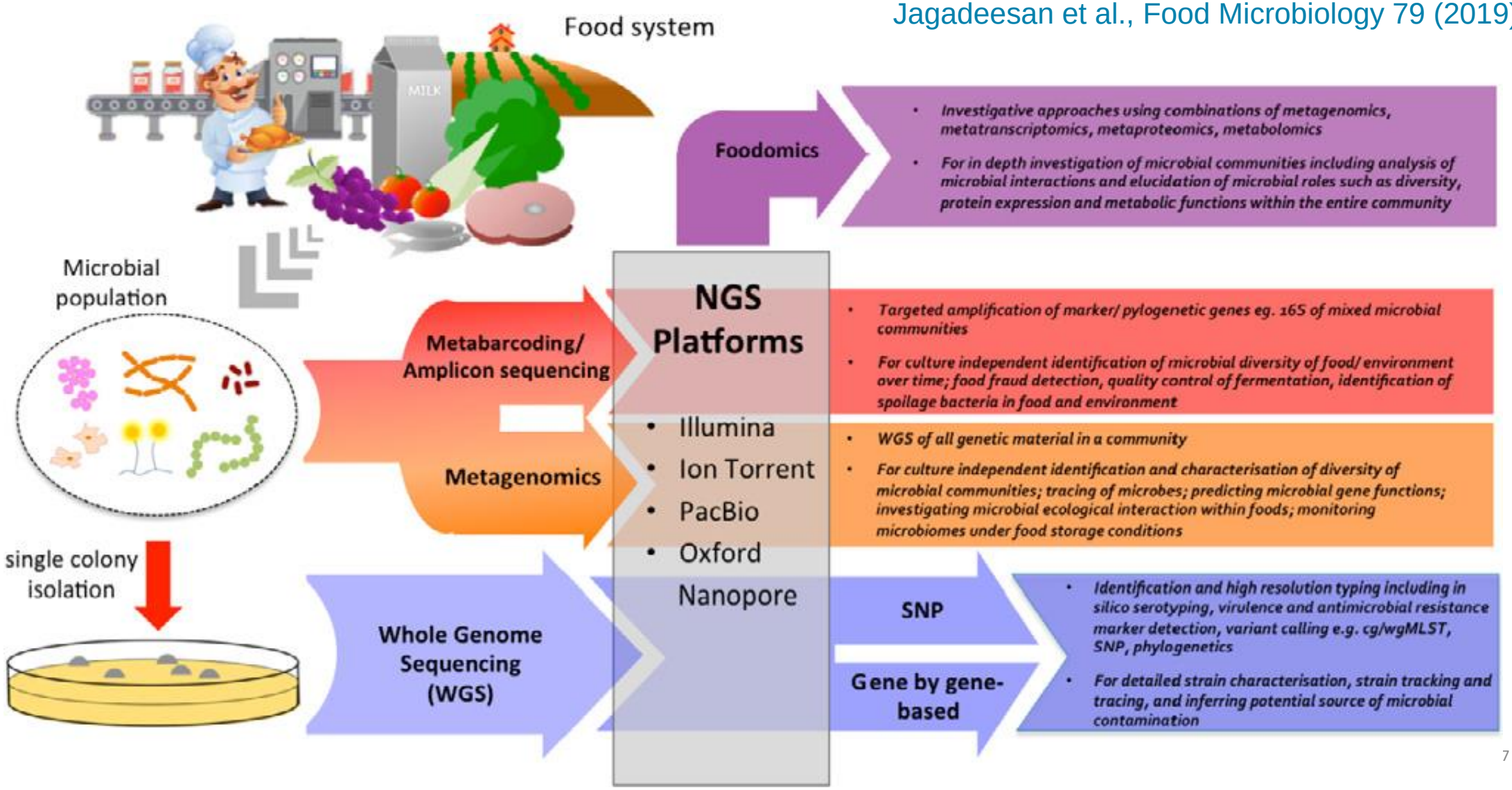
- Conocer las aplicaciones de la secuenciación masiva en microbiología
- Repaso conceptos secuenciación masiva
- Estrategias basadas en preparación de librería
- Tipos de análisis de datos.

Estrategias basadas en preparación de librería

Jagadeesan et al., Food Microbiology 79 (2019) 96–115



Estrategias basadas en preparación de librería

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

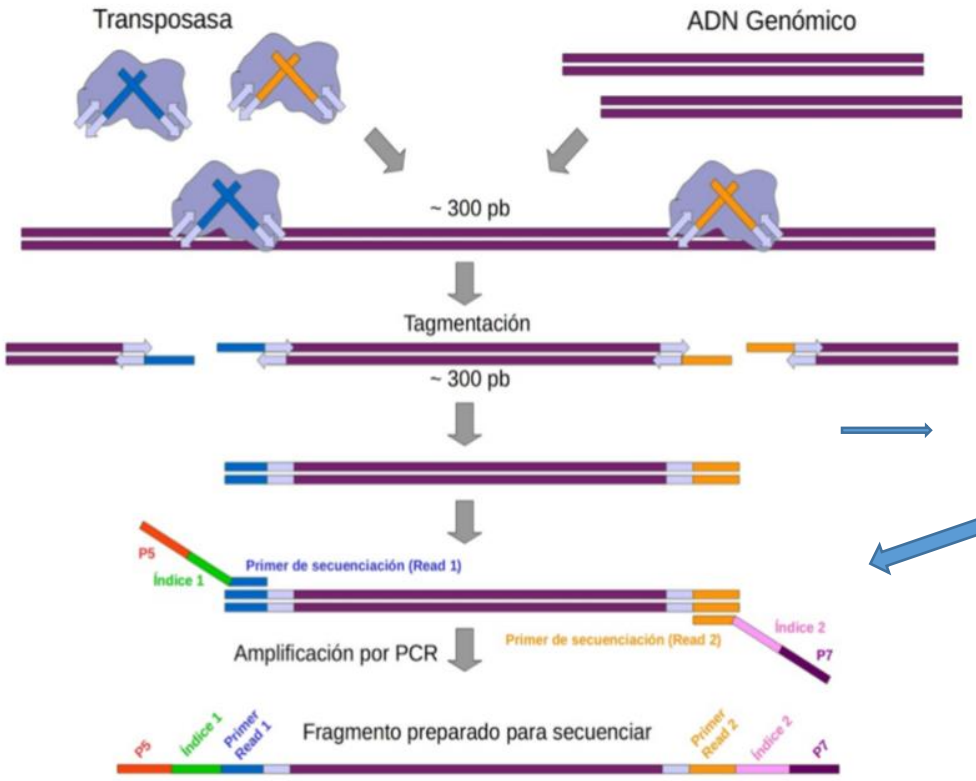
1. Metagenómica

IDENTIFICACIÓN MICROORGANISMOS

1. Metataxonomía

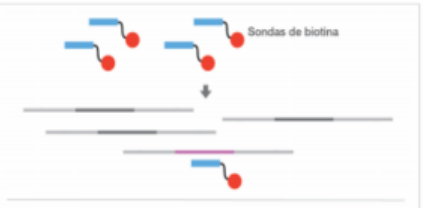
Estrategias basadas en preparación de librería

ENZIMÁTICA
FÍSICA



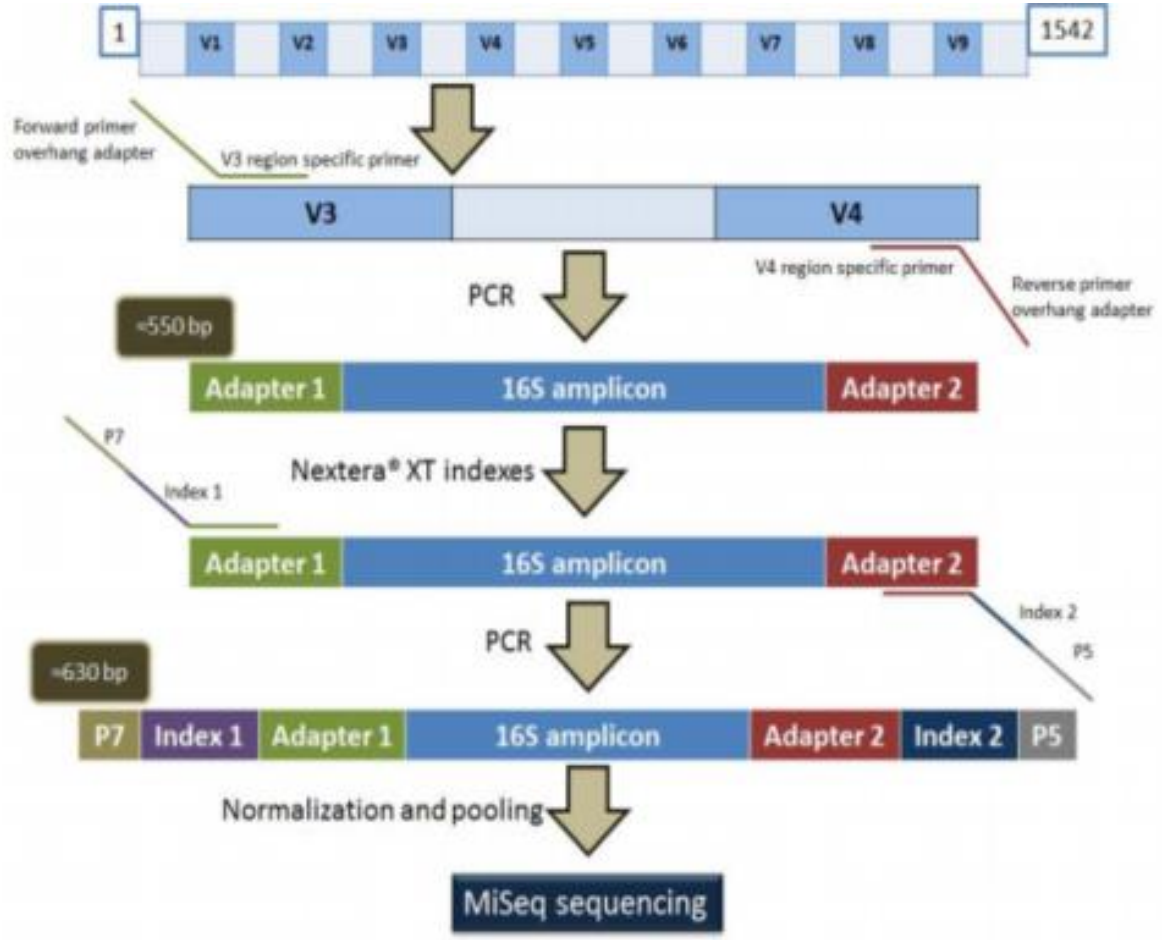
RNA -> cDNA

ENRIQUECIMIENTO:
PCR
CAPTURA SONDAS



Guia Práctica Genómica https://www.uv.es/varnau/GM_Cap%C3%ADtulo_2.pdf

PREPARACIÓN LIBRERÍA, rRNA 16S, caracterización microbiota



Main Steps of Viral Genome Sequencing by NGS or HTS

- Nucleic acid amplification
- Library preparation
- High throughput sequencing platforms
- Data analysis

Viral Genome Sequencing

CLINICAL SAMPLE

NGS

CULTURE

ENRICHMENT TECHNIQUES

↑↑ HOST & VIRUS reads ↓

NGS

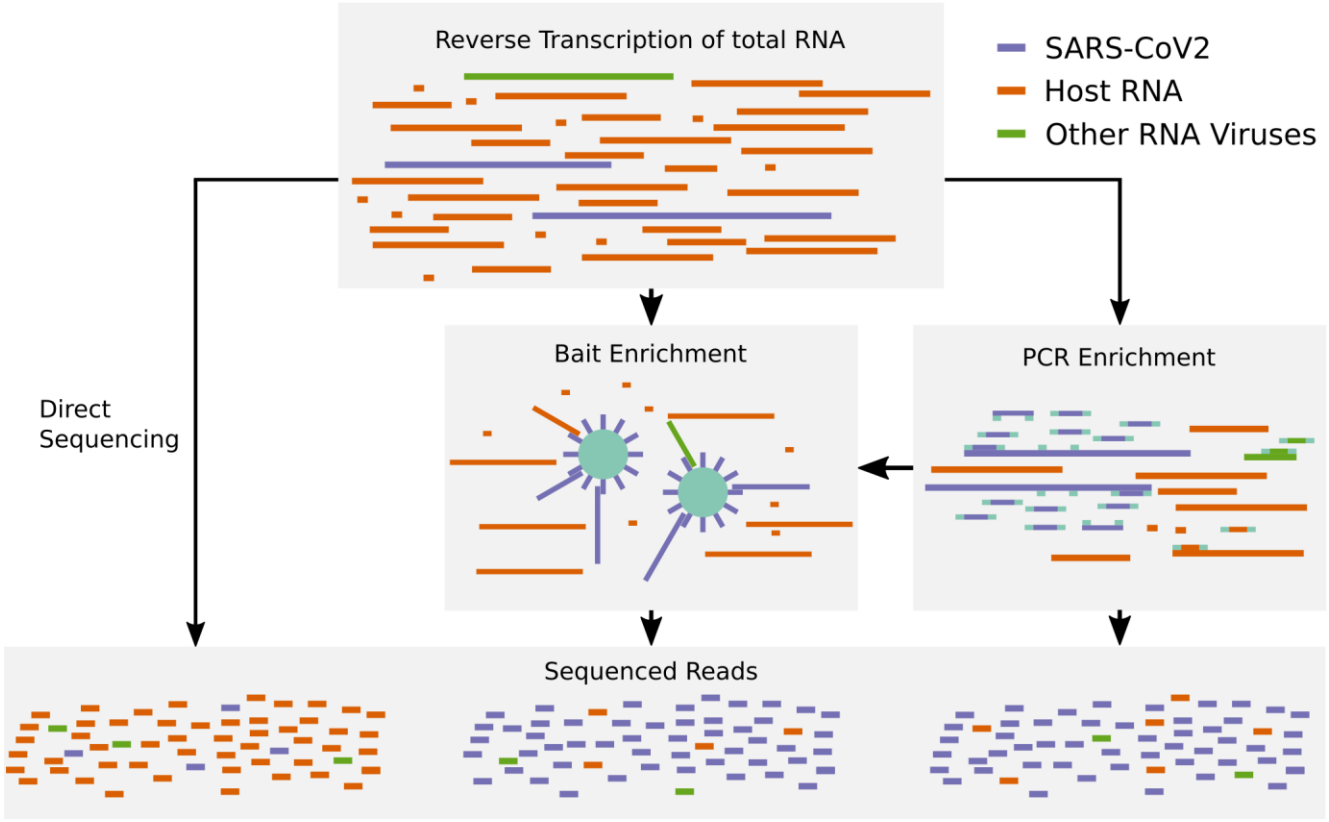
True presence of a VIRUS

↓ Host & **VIRUS** reads ↑↑

Viral Genome Coverage

NGS needs a cutoff to determine the true presence of a pathogen versus carry-over or contamination between specimens or other non-specific reads.

Enrichment Techniques

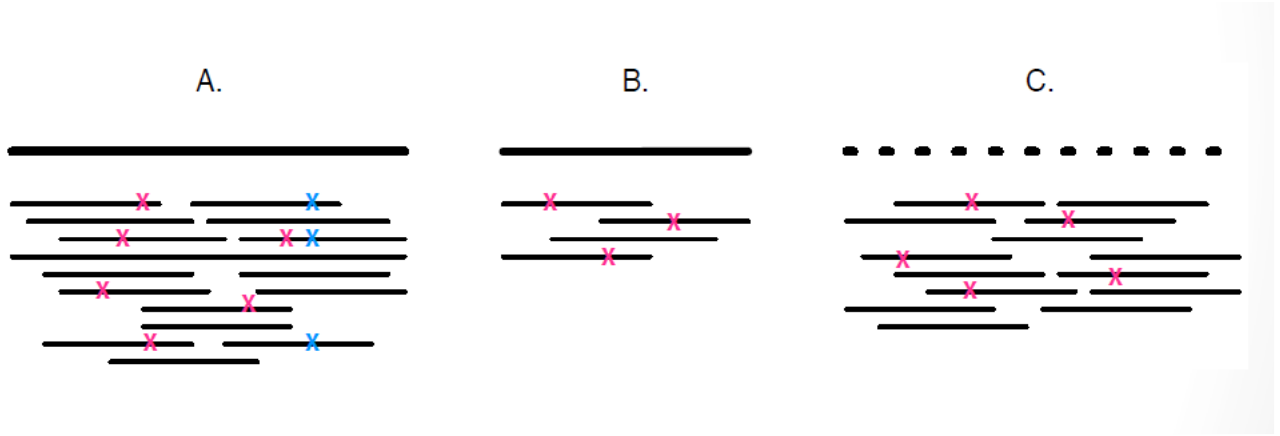


Index

- BU-ISCIII
- Conocer las aplicaciones de la secuenciación masiva en microbiología
- Repaso conceptos secuenciación masiva
- Estrategias basadas en preparación de librería
- Tipos de análisis de datos.

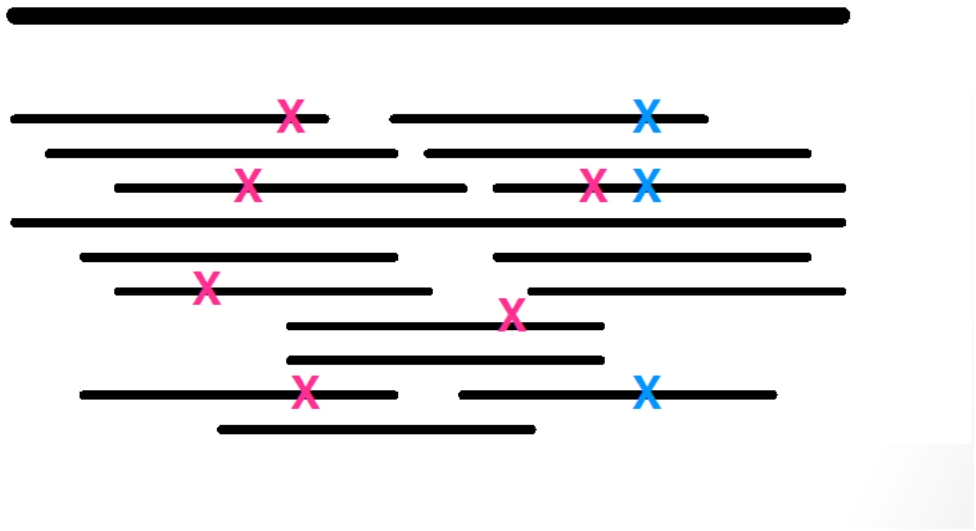
Básicamente tres problemas

Resecuenciación, Conteo y ensamblado



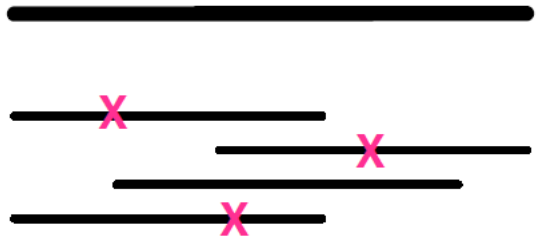
Resecuenciación

Conocemos el genoma, genoma de referencia, y queremos identificar variaciones SNPs (azul), en un background de errores (rosa). Obtenemos secuencia genoma consenso.



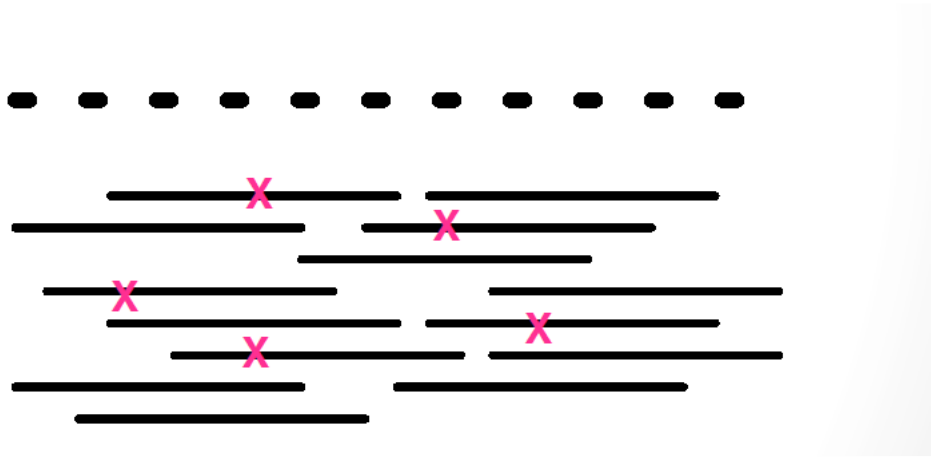
Conteo

Número de lecturas de un gen (amplicón) o mRNA (RNAseq). Equivalente a expresión en Microarrays.



Ensamblado

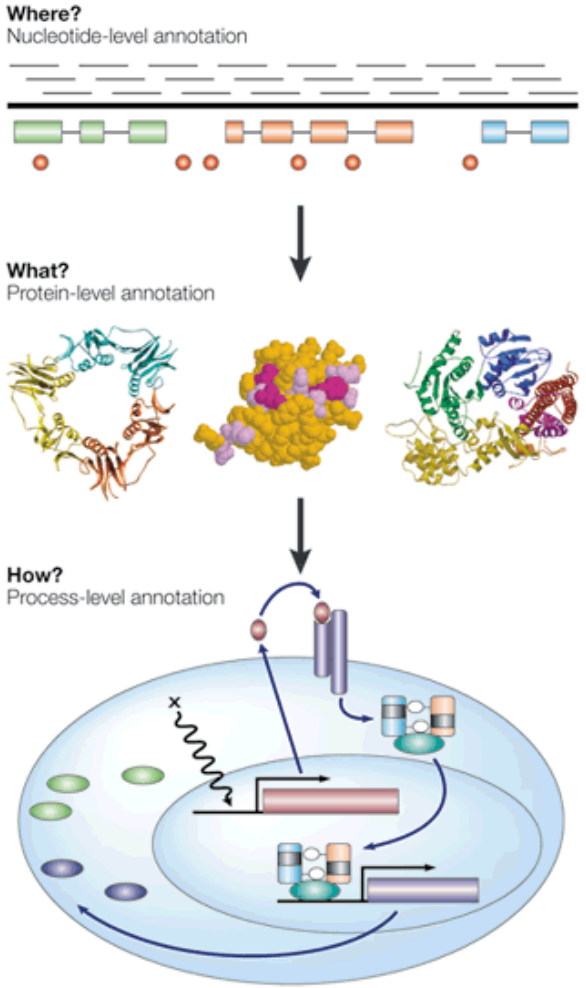
No hay genoma de referencia y lo construimos de novo



Anotación

Genome annotation is the process of attaching biological (and positional) information to sequences. It consists of three main steps:

- identifying portions of the genome that do not code for proteins
- Identifying coding elements on the genome, a process called gene prediction
- attaching biological information to these elements

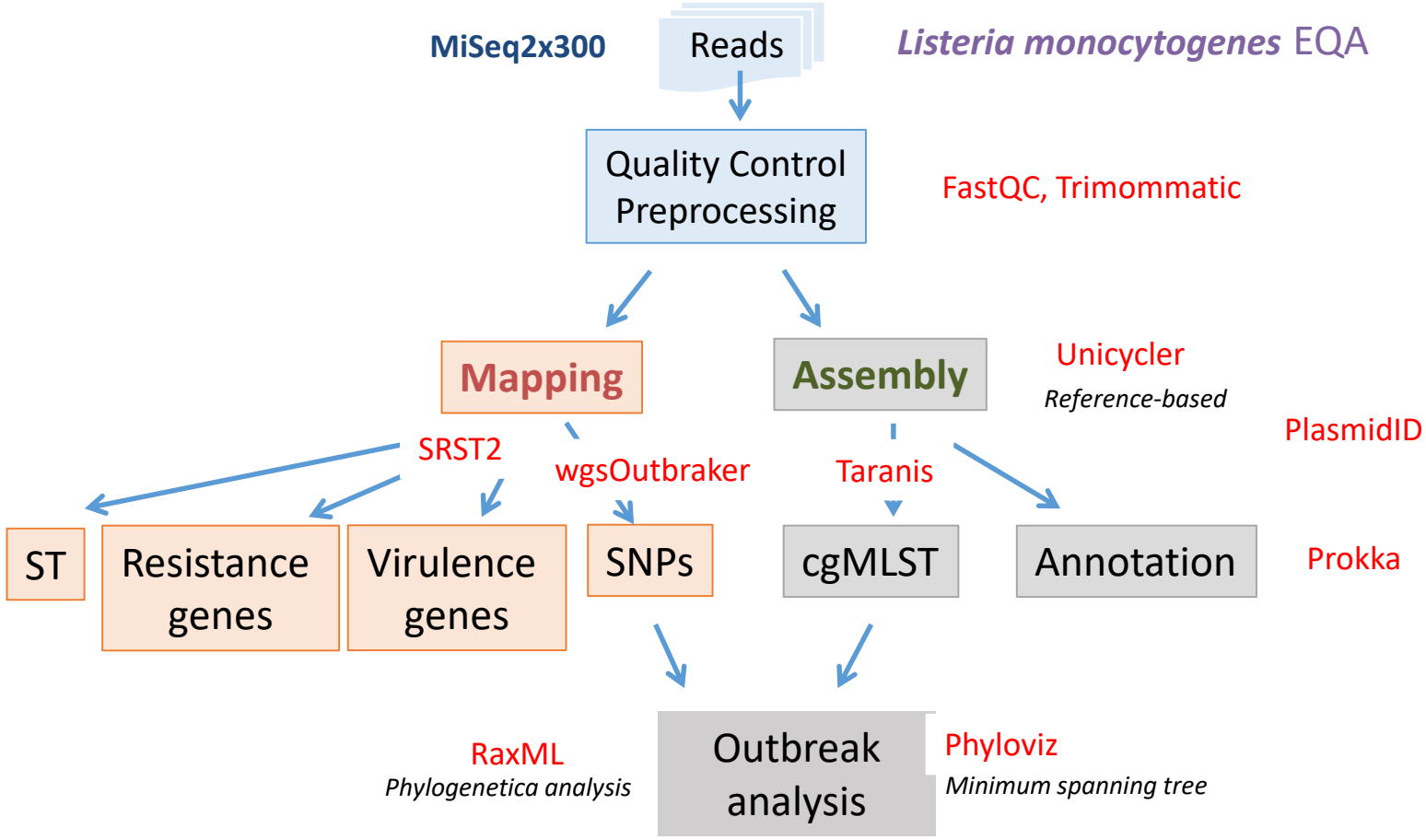


https://galaxyproject.github.io/training-material/topics/genome-annotation/tutorials/genome-annotation/tutorial.html

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
  - HAPLOYD GENOME
  - LOW FREQUENCY VARIANT – QUASISPECIES
  - SNPs MATRIX – PHYLOGENETIC ANALYSIS
- STRUCTURAL AND FUNCTIONAL ANNOTATION
  - RESISTOME, VIRULOME, SEQUENCE-TYPE

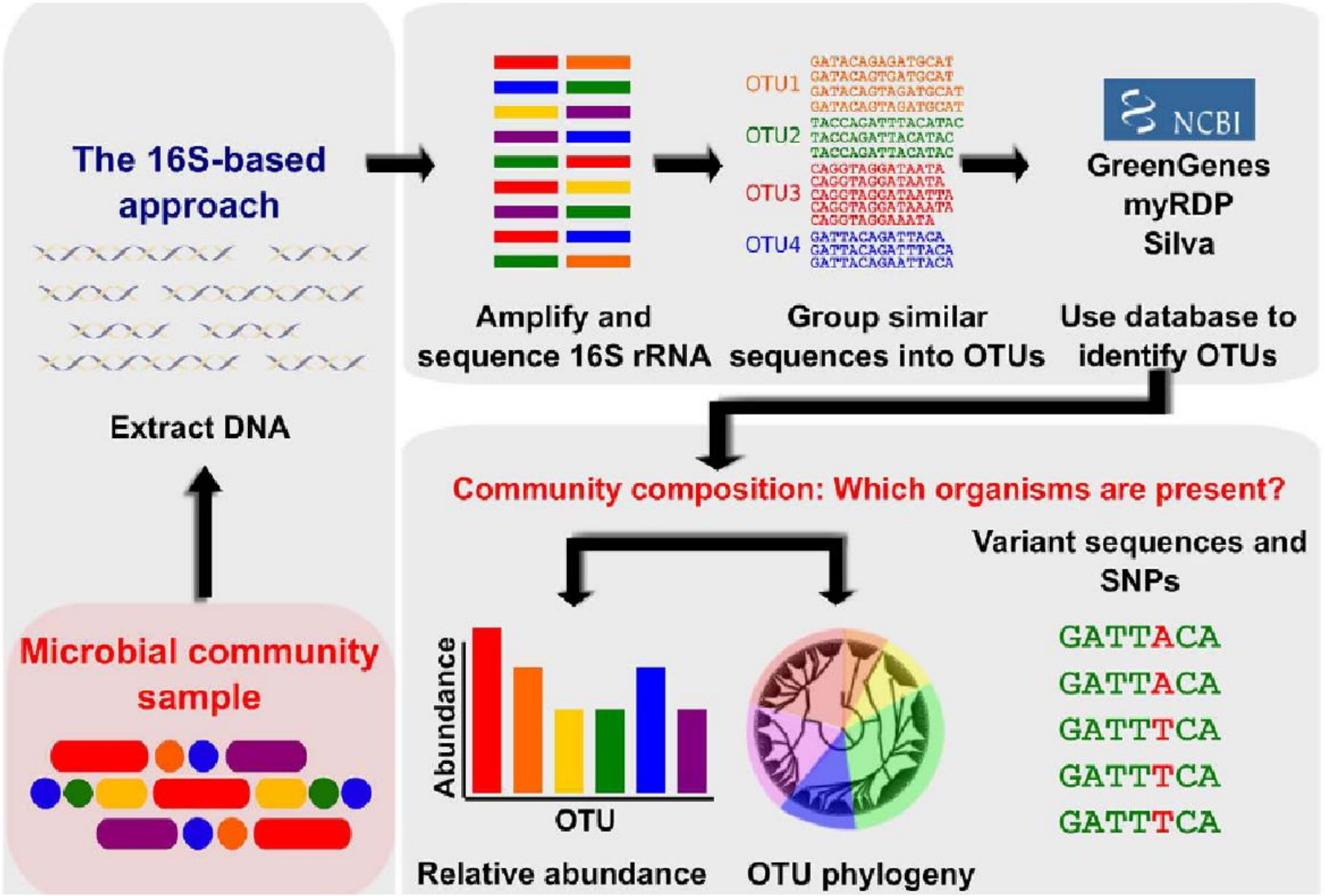
Workflow example



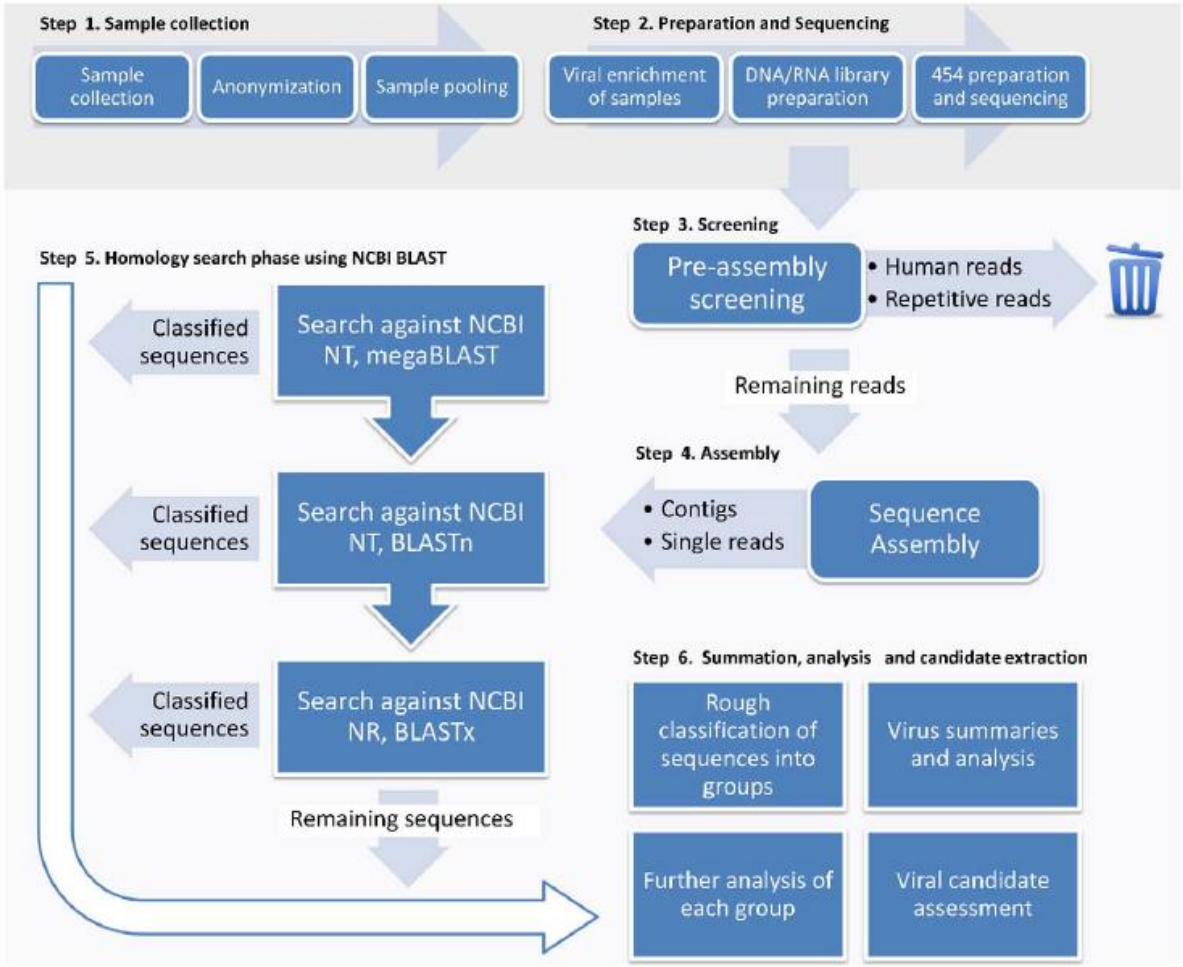
Metataxonomics vs Metagenomics (16S vs Shotgun)

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

Metataxonomics

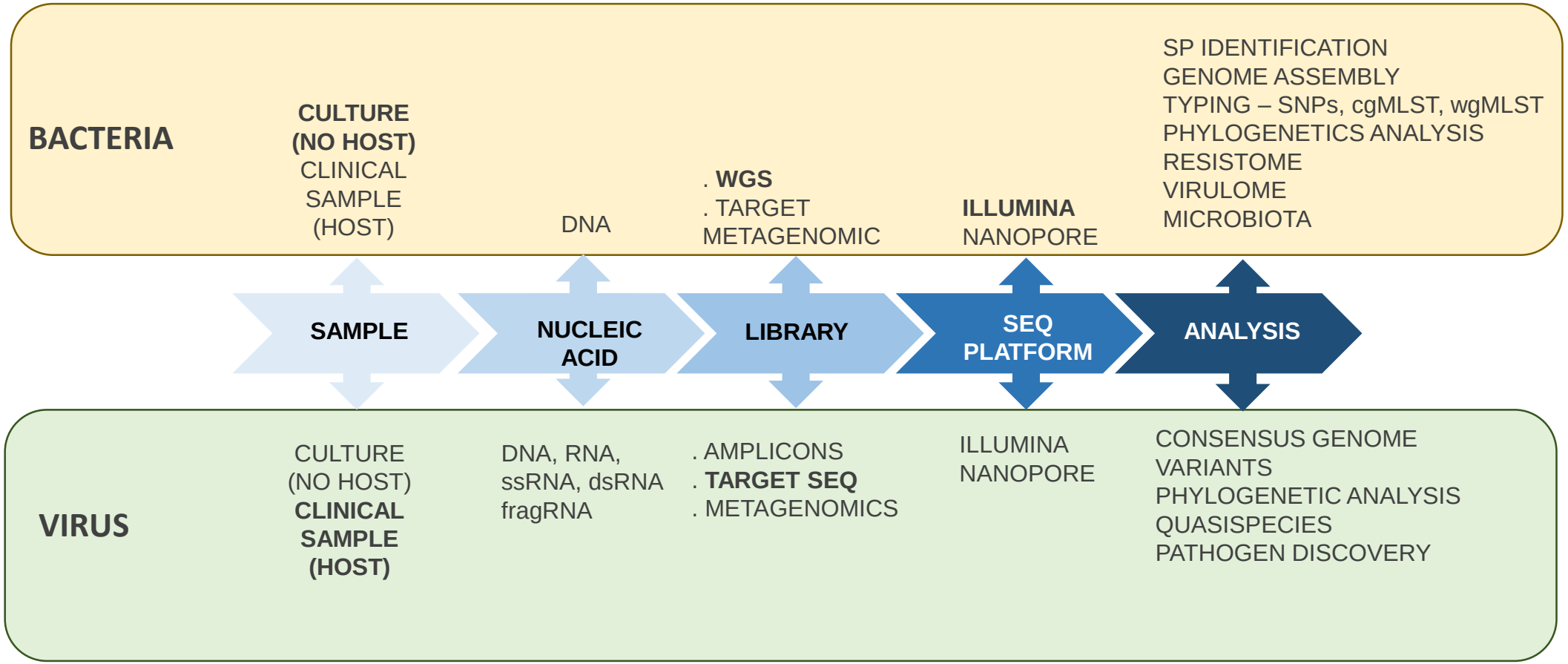


Metagenómica, pipeline de análisis



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

Bacterial and Viral Genome Sequencing



Retos de la Bioinformatica en High-Throughput Sequencing

- Tecnología que evoluciona muy rápido
  - nuevos formatos de ficheros
  - nuevas aplicaciones
  - nuevos análisis

- Coste de la secuenciación disminuye el embudo es el análisis de datos

- Adquisición de secuenciador debe ir ligado a la compra de computo y contratación de bioinformático

Retos de la Bioinformatica en High-Throughput Sequencing

- Necesidades de computo
  - ficheros de gran volumen (10Gb)
  - elevado uso de CPU y/o memoria
  - software no comercial en SO Unix

- Necesidades son dependientes de proyecto
  - No es lo mismo secuenciar un genoma 500Gb que 50 genomas 25Tb

- Si el proyecto es la aplicación en clínica
  - Las necesidades de almacenamiento aumentan por número de pacientes y por tiempo

Retos de la Bioinformatica en High-Throughput Sequencing

- Desarrollo de BD curadas (confianza = reference)
- Algoritmos que resuelvan el problema biológico planteado.
- Necesidades de Bioinformáticos
  Análisis de los datos

Retos de la Bioinformatica en High-Throughput Sequencing

- Tecnología que evoluciona muy rápido
  - nuevos formatos de ficheros
  - nuevas aplicaciones
  - nuevos análisis
  - nuevos algoritmos

- Software en continuo desarrollo (Unix)

Thanks for your attention!

Questions???