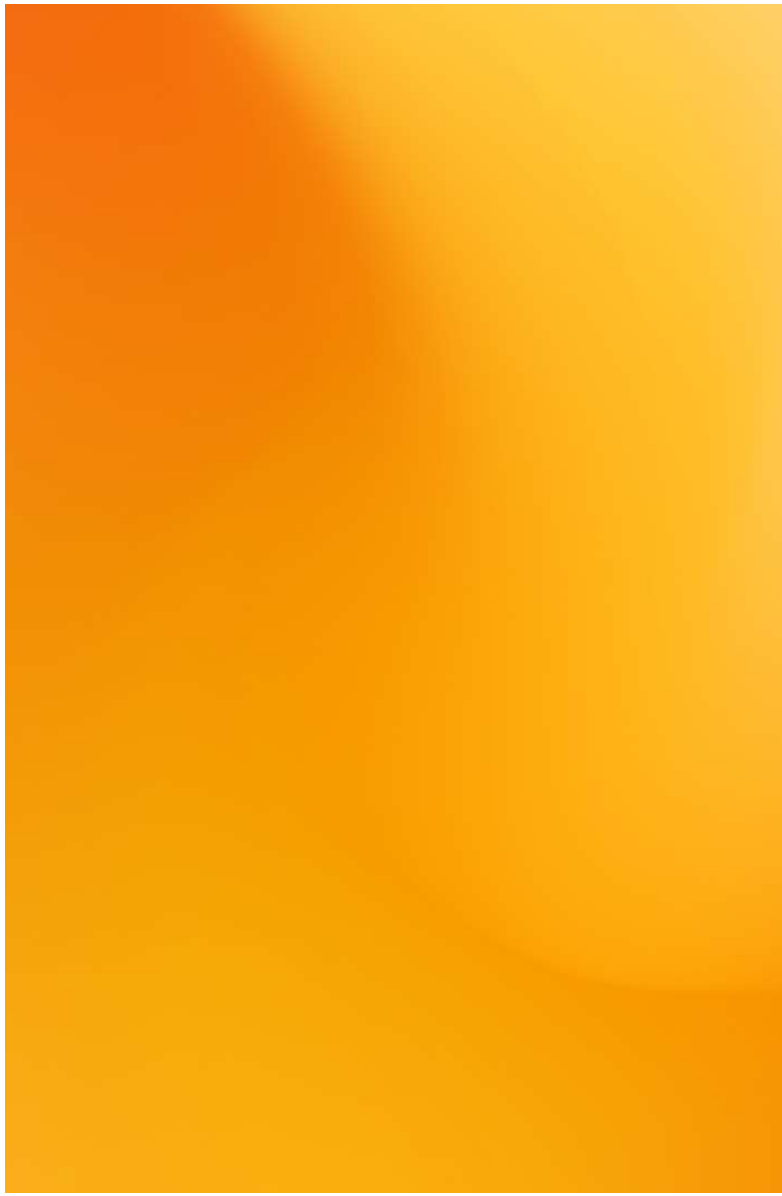
Implementación de nuevas tecnologías en seguridad alimentaria

Lourdes Fernández Calvino

Technical Sales Specialist

24th November 2022



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Agenda

1. Introducción Illumina
2. Secuenciación: Como funciona
3. Ventajas de NGS en seguridad alimentaria
4. Aplicaciones de NGS en microbiología
5. Análisis de datos

ACCELERATING ACCESS TO GENOMICS

Illumina-Introducción

illumina®

Where We Are

United States
- San Diego (Headquarters)
- Foster City
- Hayward
- Baltimore
- Madison

Brazil
- São Paulo

United Kingdom
- Cambridge

Belgium
- Mechelen

France
- Évry
- Rennes

Germany
- Berlin

Italy
- Milan

Israel
- Tel Aviv

Netherlands
- Eindhoven

Russia
- Moscow

Turkey
- Istanbul

United Arab Emirates
- Dubai

China
- Beijing
- Shanghai
- Guangzhou
- Hangzhou
- Taipei City

Japan
- Tokyo
- Osaka

Singapore

Australia
- Melbourne

South Korea
- Seoul

Who We Are

Illumina is a global leader in DNA sequencing and array-based technologies, serving customers in the research, clinical and applied markets. Our products are used for applications in the life sciences, oncology, reproductive health, agriculture and other emerging segments.

$4.5 billion (2021)
Annual revenue

~9,100
Number of employees

Francis deSouza
President & CEO

San Diego, CA, USA
Headquarters

1998
Year founded

>25 employees based in Spain: 7 TAM, 2 ISAM, 2 Technical specialist, 1 Genotyping specialist, 1 Infectious diseases specialist, 1 Oncology specialist, 12 Field Support team

Whom We Serve

MARKETS

Oncology

Reproductive Health

Genetic Disease

Microbiology

Agriculture

Molecular and Cell Biology

CUSTOMERS

Universities and Academic Research Centers

Pharmaceutical Companies

Genome Centers

Biotechnology Companies

Hospitals

Consumer Genetics Companies

Government Agencies

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Accelerating Access to Genomics

We are driven by our conviction that genomics should be available to the many, not the few.

We believe we have an obligation to make our technology affordable and accessible to as many patients as possible, while setting the highest standard for the ethical use and security of data.

Cost of sequencing, per human whole genome

Since 2001, with innovation the cost of DNA sequencing has dropped more than 100,000x, from $100 million USD per human genome to $200 USD today.

More than 20,000 Sequencing Systems Installed Around the World

Our sequencing instruments

Low-throughput

MiSeq™

MiniSeq

iSeq 100

Mid-throughput

NextSeq 500

NextSeq 550

NextSeq 1000/2000

IVD Instruments

MiSeq™Dx

NextSeq™ 550Dx

High-throughput

NovaSeq X
Novaseq X Plus

NovaSeq™ 6000 Dx

NovaSeq™ 6000

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Sequencing Systems and Key Applications Overview

		Larges WGS (human, plant, animal)	Small WGS (microbe, virus)	Exome Sequencing	Targeted Gene Sequencing	Whole-Transcriptome Sequencing	Gene Expression Profiling with mRNA-Seq	Targeted Gene Expression Profiling
High-throughput	**NovaSeq 6000** • Our broadest range of application • Enables lowest price per sample	■		■		■		
	Novaseq X Plus • Enabled the $200 Genome	■		■		■		
	NovaSeq X • Production-scale sequencing	■		■		■		
Mid-throughput	**NextSeq 1000/2000** • Incorporate blue / green SBS and super resolution optics		■	■	■	■	■	
	NextSeq 500/550 • Flexible output options (mid and high)		■	■	■	■	■	
Low-throughput	**MiSeq** • Our first benchtop sequencer		■		■			
	MiniSeq • <1 day turnaround time		■		■			■
	iSeq 100 • Our most affordable sequencer		■		■			

Note: Only key applications highlighted, which does not reflect each system's entire set of capabilities.

Secuenciación: Como funciona

2

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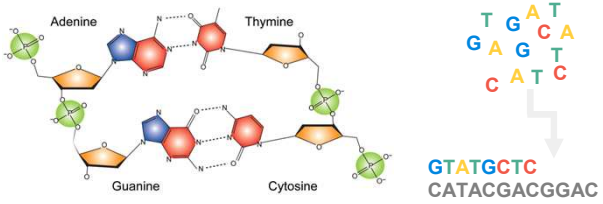
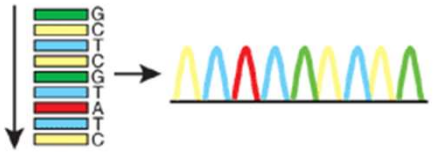
The Ability to Read the Genetic Code



Basics of DNA sequencing

- To be able to read the sequence of DNA (or letters) requires the ability to distinguish between the different nucleotides
- Various methods are available:
  - Some recognize size and shape
  - The majority use pre-labelled fluorescent nucleotides that only bind to their complementary partner



Sanger-based electrophoresis (1977)

- 1 read / capillary
- 96 capillaries / run
- 1.2Mb / day

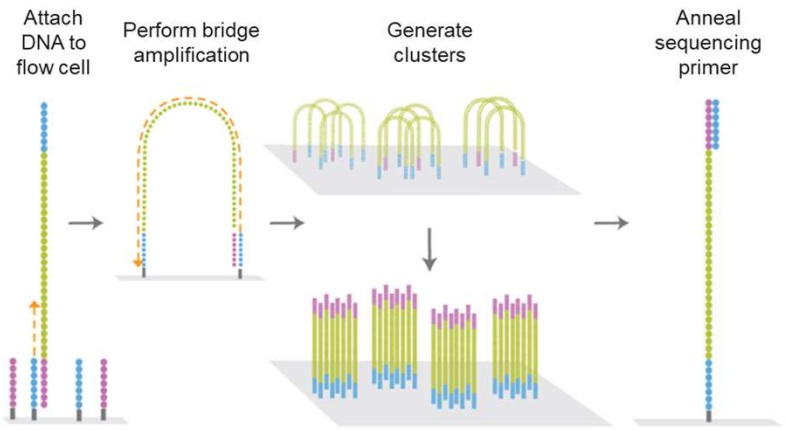
NGS Cyclic reads (2007)

- 4M-20B reads / flow cell
- ~1500Gb / day

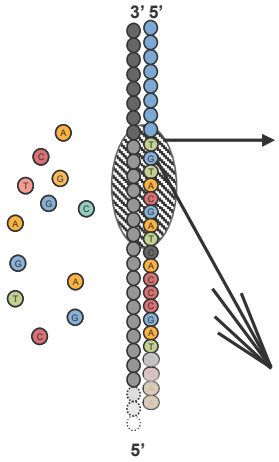
Nucleic acids research 36, 3978–92 (2008)
Nature Biotechnology 26, 1135–1145 (2008), Published online: 9 October 2008

Sequencing

Cluster generation



Sequencing by Synthesis



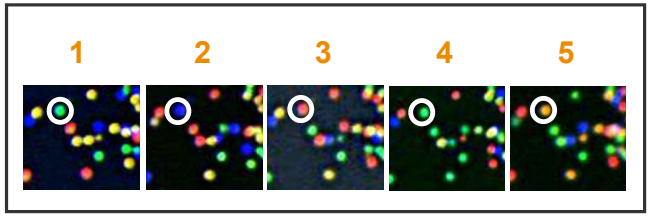


Image Acquisition

Common Sequencing Workflow for All Applications

Sample preparation

The sample is extracted and purified for the next stage in the process

Library preparation

Prepares the sample for sequencing by adding special adapters that are recognized by the sequencer

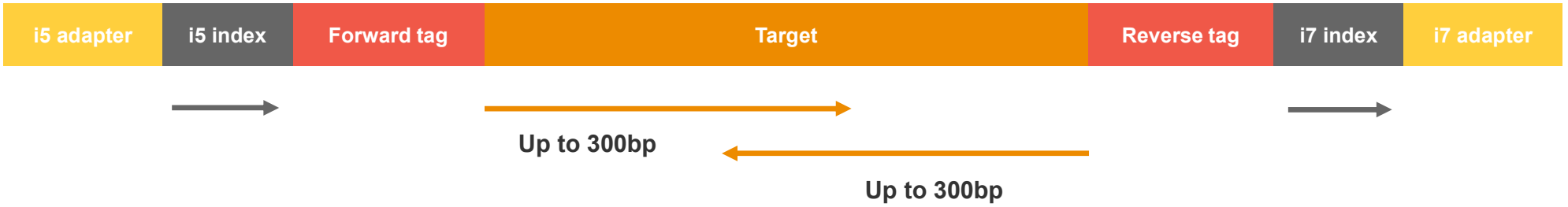
Sequencing

Reads the sequence of the sample and creates the raw data

Analysis

The analysis of the sequencing data has three stages: primary and secondary analysis, and interpretation

Library Preparation

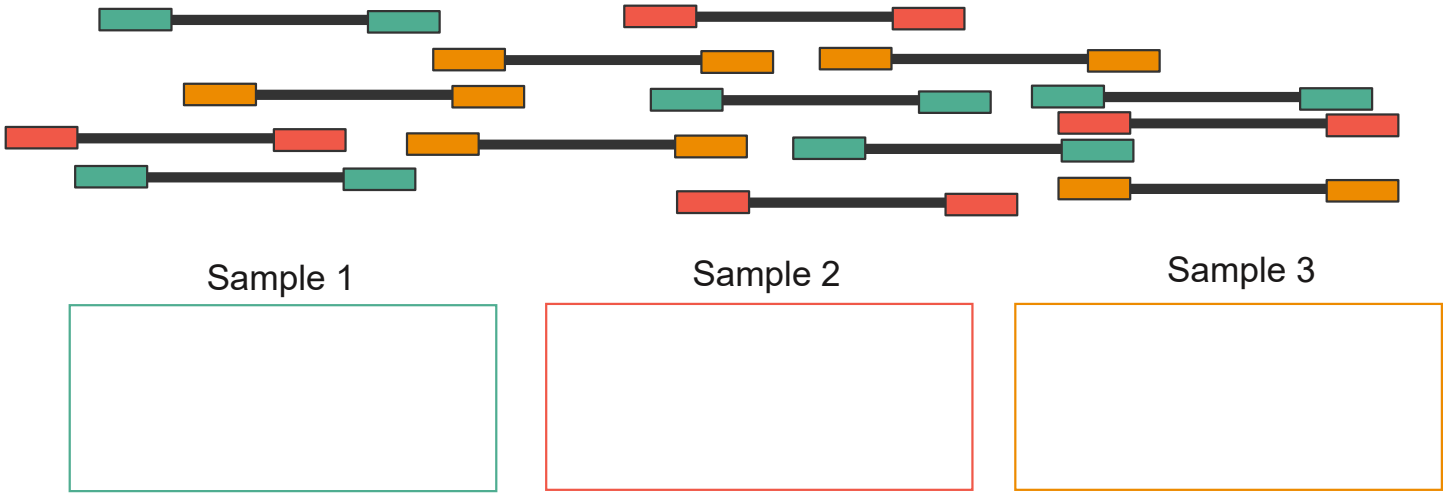


Depending on application, read length and target, the sequencing reads may overlap

The index regions of the library allow multiple samples to be sequenced all at the same time and individual sample data to be segregated

Different types of data analysis

- Demultiplexing
  - Determine which sequence for which sample



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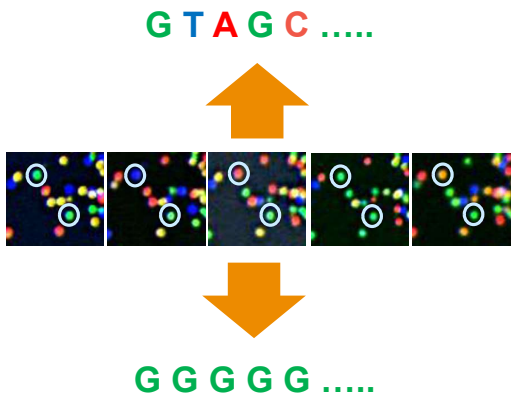
Analysis

Primary analysis

- Runs in parallel to the sequencing
- Uses the images to provide base calls of the reads

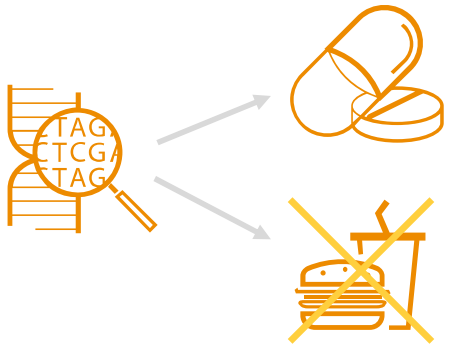


Secondary analysis

- Maps the reads to the reference genome (alignment)
- Analyses the mapped reads for any differences compared to the reference genome (variant calling)

Interpretation

- Leads to knowledge and insights into basic biology
- Potential causes of genetic disease
- Possible treatment or prevention



https://emea.illumina.com/informatics/sequencing-data-analysis.html

Different types of data analysis

- Quality score calculation
  - For each base call, a number of quality predictor values are computed. These are observable properties of clusters from which the base calls are extracted.

ASCII Quality Score	Probability of Incorrect Based Call	Base Call Accuracy	Q-score
+	1 in 10	90%	Q10
5	1 in 100	99%	Q20
?	1 in 1000	99.9%	Q30
I	1 in 10000	99.99%	Q40

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Ventajas del NGS en seguridad alimentaria

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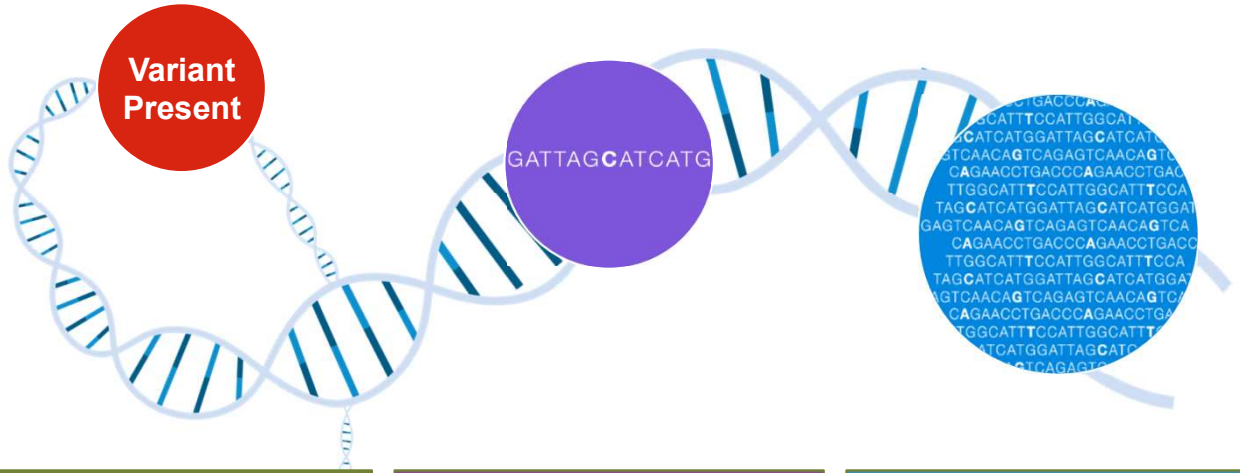
Comparing technologies



q/RT-PCR	Sanger/CE	Next-gen Sequencing
+ High sensitivity Capital equipment already found in most labs	+ Cost effective for small stretches of DNA Well known technique	+ High resolution and sensitivity More data from smaller amounts of DNA and RNA Higher throughput with sample multiplexing
– Low discovery power Low variant resolution Low scalability	– Low sensitivity (down to 20%) Low discovery power Low scalability	– Less cost effective when interrogating a low number of samples

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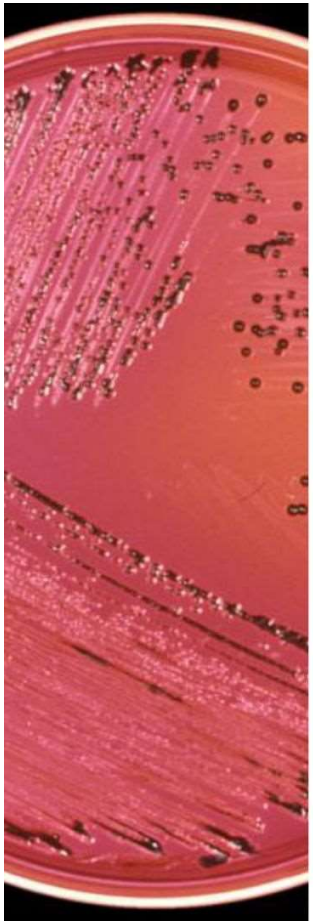

Why NGS in Microbiology?

- Characterization of un-culturable organisms
- Increase power of discovery of new organisms
- Higher sensitivity to detect low abundant species
- Better strategies in outbreak management
- Monitor host-pathogen interactions

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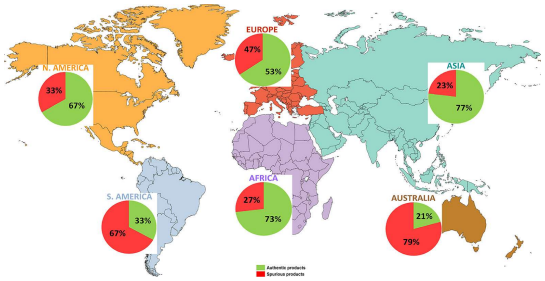
Why NGS in Food authenticity?

- Characterization of multiple species at once
- Untargeted detection of thousands of organisms with no requirement for previous knowledge
- Most relieable method for species identification
- Allows better dealing with damaged DNA



REVIEW ARTICLE
Front. Pharmacol., 24 October 2019 | https://doi.org/10.3389/fphar.2019.01227

The DNA-Based Authentication of Commercial Herbal Products Reveals Their Globally Widespread Adulteration

Mihael Cristin Ichim*
*"Stejarul" Research Centre for Biological Sciences, National Institute of Research and Development for Biological Sciences, Piatra Neamt, Romania

Aplicaciones de NGS en microbiología

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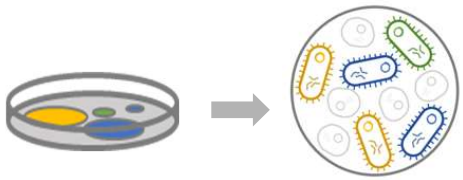
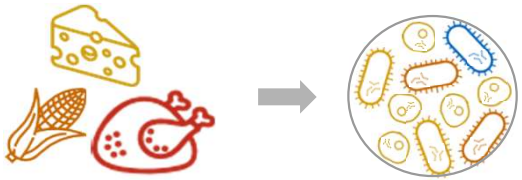
Applications for Culture vs. Culture-Free Sequencing



Whole-Genome Sequencing (WGS)

Sequencing a **cultured isolate** for taxonomy, evolution, and function

- Food outbreaks, identify source
- Monitor for pathogens in product and facilities
- Differentiate resident, persistent, and transient pathogens
- Virulence factors, AR genes, and other genetic markers of interest
- Maintain or enhance food quality (starter cultures)



Amplicon Sequencing

Sequencing **specific genes** for taxonomy and relative abundance

Shotgun metagenomics

Sequencing **"everything"** in a sample for taxonomy, abundance, and **function**

- Community profiling to predict pathogen, spoilage risk, and allergen
- Detect genetic modifications (GMOs)
- Authenticate high value foods (olive oil)
- Verify speciation (kosher, halal)
- Procure correct ingredients for final product (gluten free)

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Choose the Right NGS Method for Microbial Identification

Classification example for *E. coli*	
Domain	Bacteria
Phylum	Protobacteria
Class	Gammaproteobacteria
Order	Enterobacterales
Family	Escherichia
Genus	Coli

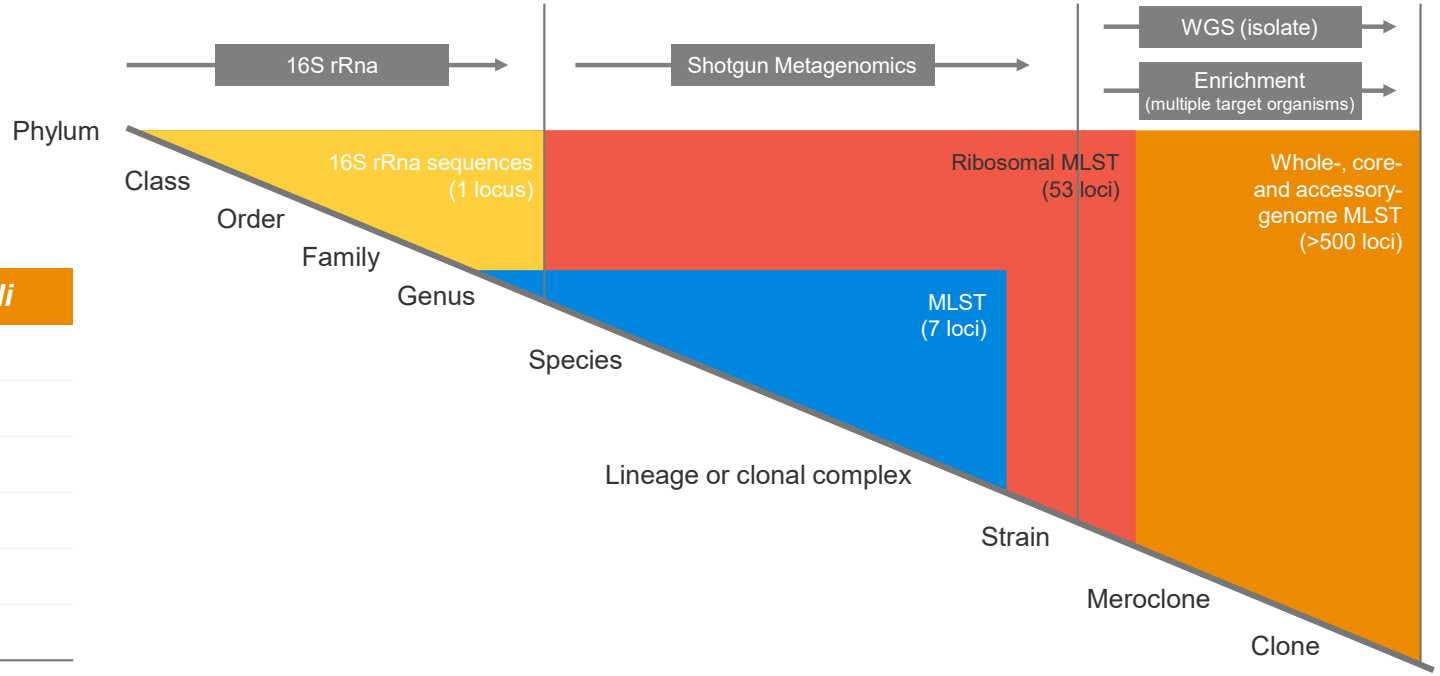

Figure 2. Relating sequence data to nomenclature schemes

Maiden et al. (2013) MLST revisited: The gene-by-gene approach to bacterial genomics. Nat Rev Microbiol. 2013 October

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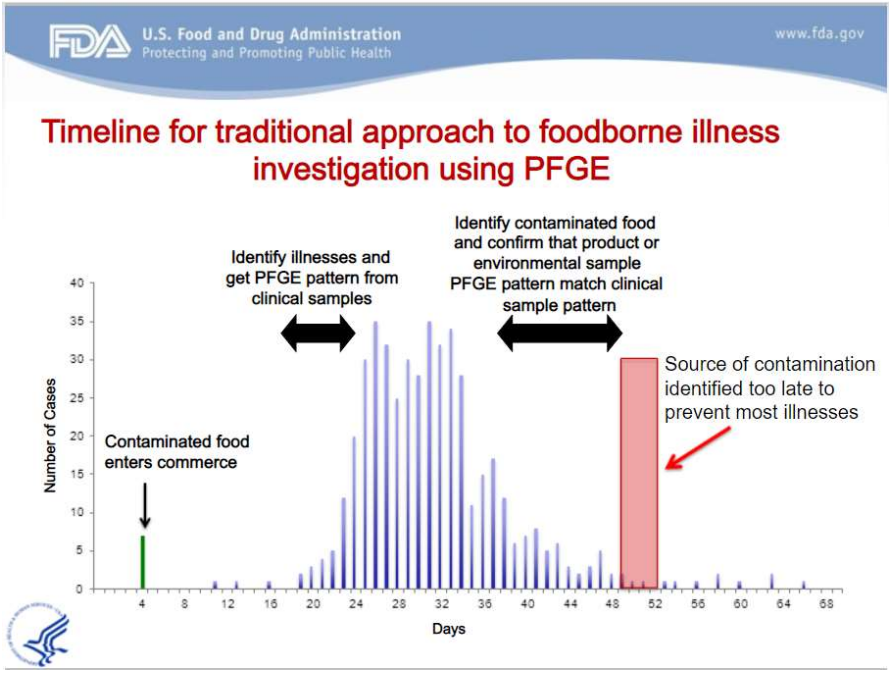
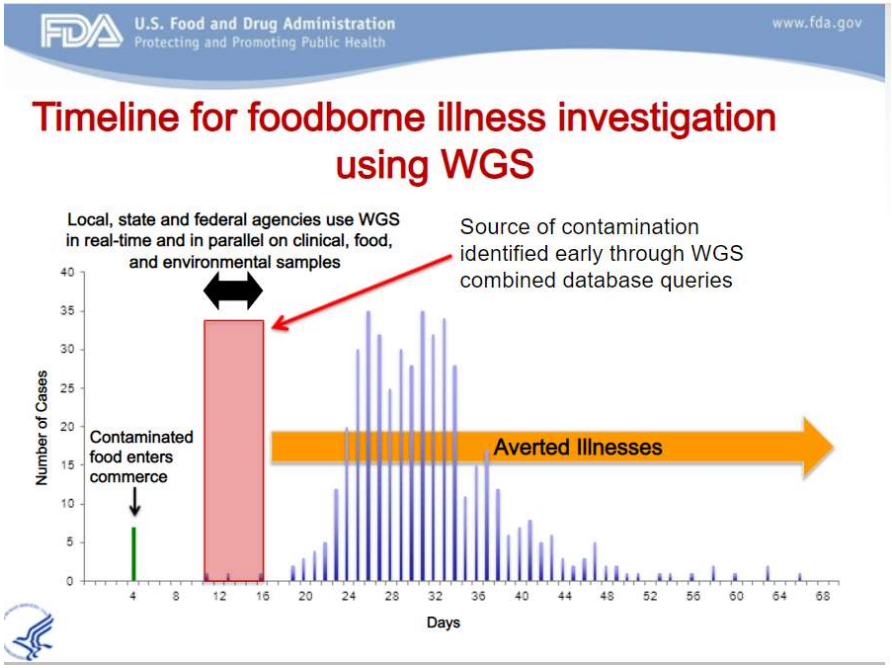
NGS speeding up foodborne illness outbreak investigations





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Using NGS to Assess Food Pathogen Outbreak

20 minutos | Actualidad | Nacional | Internacional | Deportes | Cultura | Opinión | Más

Una misma cepa de listeria procedente de la carne La Mechá ha contagiado a 144 pacientes

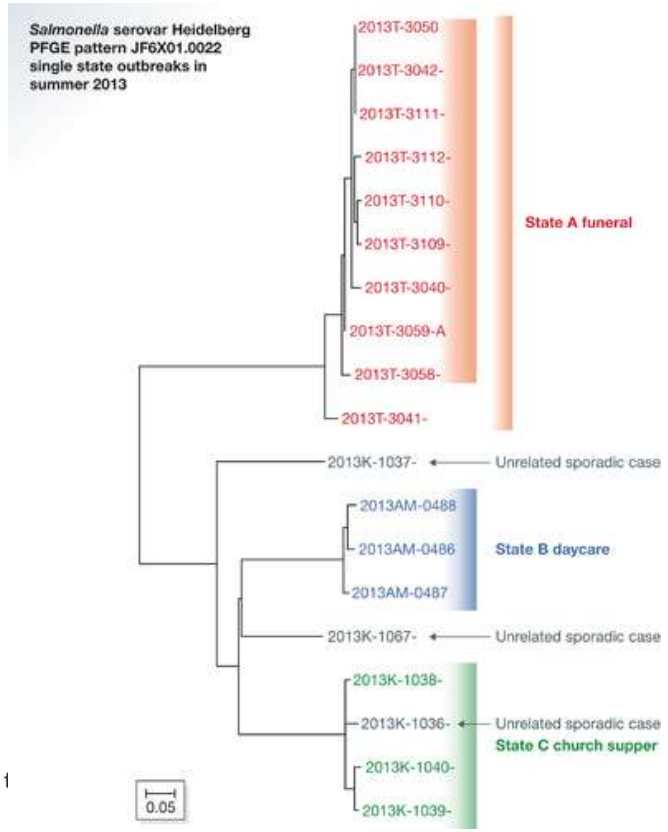
EFE 12.09.2019 - 15:21H

GOBIERNO DE ESPAÑA · MINISTERIO DE CIENCIA E INNOVACIÓN · Instituto de Salud Carlos III

French officials confirm some E. coli cases linked to Nestlé pizza

- 75 pacientes, 41 con Síndrome Urémico Hemolítico.
- 2 niños fallecidos.
- Posible origen en harina.
- Detección foco en Marzo 2022.
- Primeros enfermos en Enero

CDC discovers Listeria outbreak linked t
enoki mushrooms



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WGS as a reactive and proactive approach

WGS more a tool to determine the scope of outbreaks and speeding traceback investigation

- **Industry tool for monitoring ingredient supplies**
- **Allow to evaluate effectiveness of preventive and sanitary controls**
- **Used to determine the persistence of pathogens in the environment**
- **Possible indicator of antimicrobial resistance**

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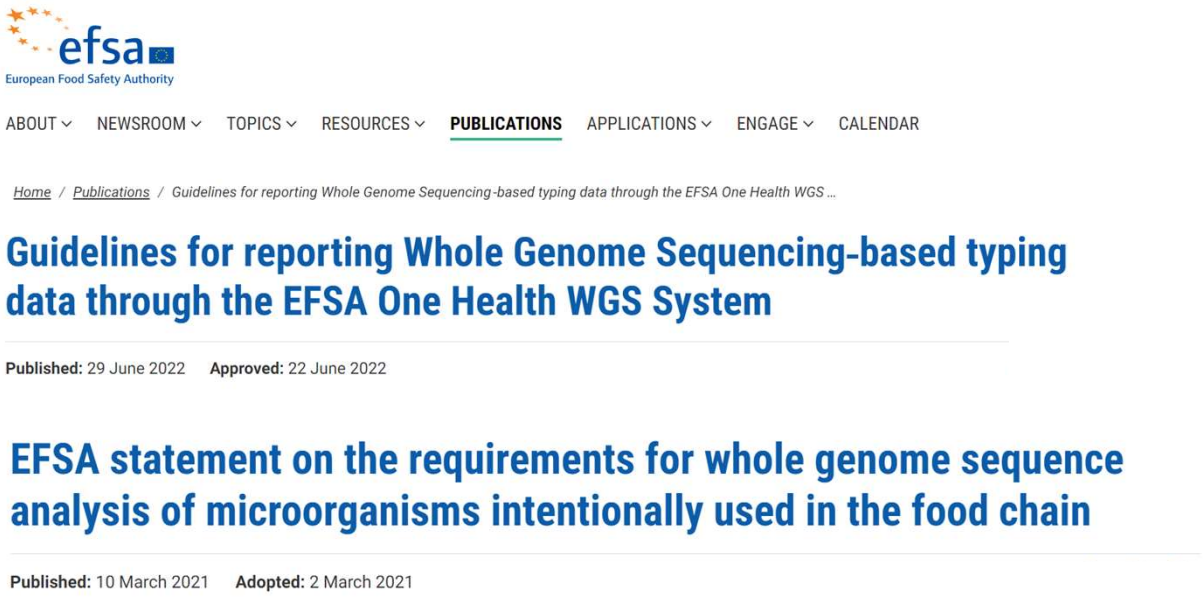
European Agency Guidelines for WGS



efsa
European Food Safety Authority

ABOUT NEWSROOM TOPICS RESOURCES **PUBLICATIONS** APPLICATIONS ENGAGE CALENDAR

Home / Publications / Guidelines for reporting Whole Genome Sequencing-based typing data through the EFSA One Health WGS ...

Guidelines for reporting Whole Genome Sequencing-based typing data through the EFSA One Health WGS System

Published: 29 June 2022 **Approved:** 22 June 2022

EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain

Published: 10 March 2021 **Adopted:** 2 March 2021

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Análisis de datos

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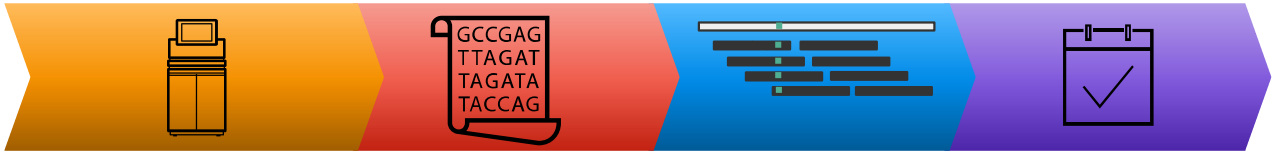
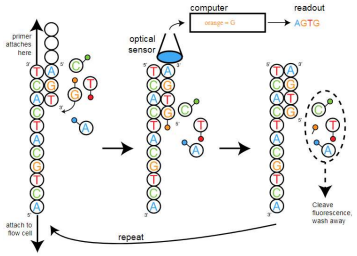
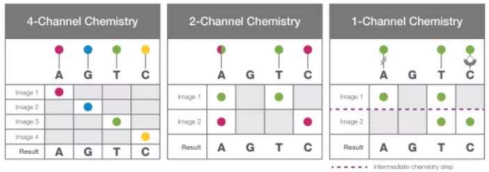
Different types of data analysis



Sequencing | Primary analysis | Secondary analysis | Tertiary analysis

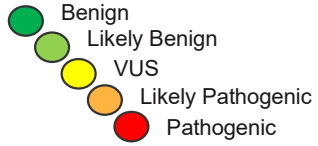


Sequencing By Synthesis



MiSeq™ NovaSeq6000™
HiSeq4000™ NextSeq™ iSeq100™
HiSeqX™ MiniSeq™

Fastq BAM VCF



- On Instrument
- On Instrument
- On or Off Instrument
- Off Instrument

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BaseSpace™ Sequence Hub

Enables anyone to analyze their NGS data

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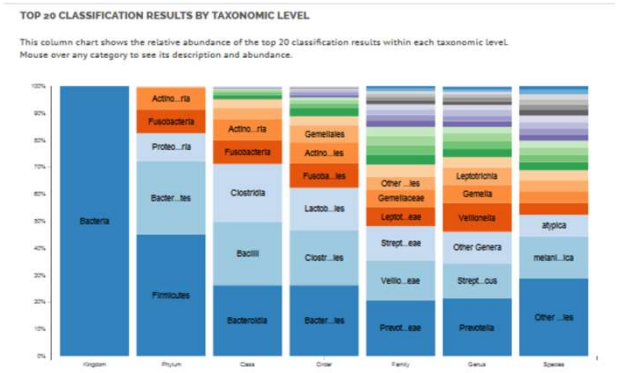
Metagenomics: analysis tools

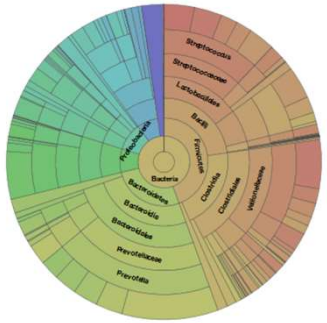
Secondary

16S Metagenomics

Illumina, Inc.

- Aligns amplicon reads against GreenGenes or Custom databases.
- Performs taxonomic classification
- Provides interactive visualization and raw classification output for per-sample and aggregate analysis.





SAMPLE INFORMATION

Total Reads	Reads PF	% Total Reads PF
5,373,983	4,876,386	90.74%

CLASSIFICATION STATISTICS

Taxonomic Level	Reads PF Classified to Taxonomic Level	% Reads PF Classified to Taxonomic Level
Kingdom	4,870,933	99.89%
Phylum	4,804,887	98.53%
Class	4,712,567	96.64%
Order	4,679,070	95.95%
Family	4,574,172	93.80%
Genus	4,496,803	92.22%
Species	3,104,027	63.65%

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Amplicon Sequencing: FOOD FRAUD analysis

Secondary

16S Metagenomics Database Creator

BaseSpace Labs

- Creates custom databases in FASTA and TSV input files.
- Allows to use Custom Databases for FOOD AUTHENTICITY analysis
  - Meat Food Authenticity
  - Fish Food Authenticity
  - Spices and herbs Food Authenticity

16S Metagenomics

Illumina, Inc.

Targeted Sequencing, Metageno...

- Aligns amplicon reads against Custom databases of species (COI, 18s, Cytochrome B…).
- Performs taxonomic classification
- Provides interactive visualization and raw classification output for per-sample and aggregate analysis.

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Whole-Genome Analysis and Shotgun metagenomics

Secondary

De novo assembly and identification

BaseSpace™ Apps

SPAdes Genome Assembler

- Assembly of small genomes (bacterial, both Multiple Displacement Amplified single-cell and standard isolates, fungal and others).
- Optimized utilization of singe read, mate pair and Paired end libraries
- Contains optional kmer based error correction before assembly
- v3.9.0 Significantly improved SPAdes family of tools now includes metaSPAdes, rnaSPAdes and metaSPAdes pipelines

SRST2

- Reports the presence of STs (sequence types) from pubMLST database and virulence genes, resistance genes, and plasmid replicons form ResFinder, ARG-Annot, PlasmidFinder and more
- Does not require prior assembly

Velvet *de novo* Assembly

- Multi-kmer de Bruin graph de novo assembly pipeline for bacterial samples using the Velvet assembler
- Optimized for data from Nextera™ Mate-pair library kit

Dragen Metagenomics

- Performs taxonomic classification of reads using the Kraken2 algorithm and a corresponding taxonomic database. The app provides interactive visualizations and raw classification output for per-sample and aggregate analyses.

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Do you want to try an NGS instrument for 6 months in your lab?

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Easy access to the power of NGS

Our new flexible reagent rental option makes the iSeq™ 100 sequencing system more accessible than ever.

Introduce Illumina's innovative next-generation sequencing technology into your lab with no upfront capital investment

New to NGS?

Harness the power of Illumina NGS technology with an easy-to-use, cost-effective system.

Want more control over your experiments?

Get results at your own pace in your own lab. This offer lets you deliver on your projects on time.

Need assistance?

Come up to speed by attending a training course online, at the Illumina Solution Centres, or in your own lab.

Find answers efficiently across a range of applications

Microbiology

Sequence the entire genome of bacteria, viruses, and other microbes. Perform metagenomic studies by sequencing 16S rRNA or ITS.

Amplicon sequencing

Enable a diverse set of research applications for the discovery, validation, or screening of genetic variants.

Gene panels

Analyze key genes, or regions of interest, to high depth using pre-designed, analytically validated panels.

How does it work?

- Get in-house access to an iSeq 100 instrument for 6 months[1] for a minimum commitment of € 9000[2]
- Place an upfront purchase order for the whole amount of consumables together with a shipment schedule
- Your 6-month agreement will commence upon delivery of the instrument and first batch of consumables

If you would like to make use of this limited offer*, please contact your Illumina Sales Representative to learn more about this opportunity.

1. A 12-month option is also available with different terms and conditions.
2. Local VAT is not included in this amount. Local VAT will be added to payments.

Click here to learn more about the iSeq 100

*PROMOTION TERMS AND CONDITIONS:
Promotion Terms and Conditions: Illumina is pleased to offer Customer, on a limited time only, access to an iSeq 100 instrument for 6 months for a minimum commitment of € 9,000 which must be spent on iSeq 100 sequencing kits and/or Illumina library prep kits, contingent on the Customer meeting all of the conditions listed below.

SPECIAL CONDITIONS OF SALE:
- Customer agrees to provide to Illumina an upfront purchase order for iSeq 100 sequencing kits and/or Illumina library prep kits before December 17, 2021.
- The contract must be signed by December 31, 2021.
- This Promotion is limited to Customers located in and/or receiving shipments in Belgium, France, Luxembourg, the Netherlands, Italy & Spain.
- Only applicable to new Illumina customers.

M-EMEA-00243
For Research Use Only. Not for use in diagnostic procedures.

Thank You

Lourdes Fernández
lfernandez1@illumina.com

Lourdes Fernández Calvino
lfernandez1@illumina.com

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