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ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE "G. CAPORALE"

Whole Genome Sequence for microbial pathogens: activities at the Italian Reference Centre GENPAT

Cesare Cammà

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WGS: a story started 30 years ago

Whole genome sequencing of pathogens: a new era in microbiology

E. Richard Moxon

Sequencing of DNA has brought about a revolution in biology by making available the immense fund of historical information contained in the genomes of cells. Unicellular organisms appeared some 2 million years before the first primitive algae and over a billion years before the first animals and higher plants. Thus, a sensible and obvious starting point in the sequencing of entire genomes might be to tackle the prokaryotes, the smallest chromosomes that contain all the information that is required for the free-living state. Fred Blattner (University of Wisconsin, Madison, WI, USA) emphasized at the recent Workshop on Bacterial Genome Sequencing (Wellcome Trust Frontiers in Biology Conference) that major funding agencies have been reluctant to support the sequencing of bacterial genomes.

Workshop on Bacterial Genome Sequencing, Wellcome Trust Frontiers in Biology Conference, Broadway, Worcestershire, UK, 23–26 April 1995.

E.R. Moxon is in the Institute of Molecular Medicine and Dept of Paediatrics, University of Oxford, UK. tel: +44 1865 221074, fax: +44 1865 220479, e-mail: richard.moxon@paediatrics.ox.ac.uk

led by Craig Venter and Rob Fleischmann at the Institute for Genomic Research (TIGR) in Gaithersburg, MD, USA, in collaboration with Ham Smith of Johns Hopkins University, Baltimore, MD, USA. The genome sequenced was that of *Haemophilus influenzae*, which was once mistakenly thought to cause epidemics of serious respiratory infections that are now

1.83 million nucleotides of circular double-stranded DNA in less than a year. A crucial factor in the success of the project was the application of a random sequencing approach and computational methods developed by TIGR for large-scale sequencing.

Predictably, most of the *H. influenzae* genome consists of sequences representing potentially functional genes; there are a total of 1749 open reading frames, of which about 1200 have similarity with database sequences. Among these are complete sets of ribosomal proteins with similarity to *Escherichia coli*, and amino-acyl-tRNA synthetases. The encoded proteins have been grouped into functional categories to provide a broad picture of the proportion of the genome that is involved in various cell processes. The *H.*

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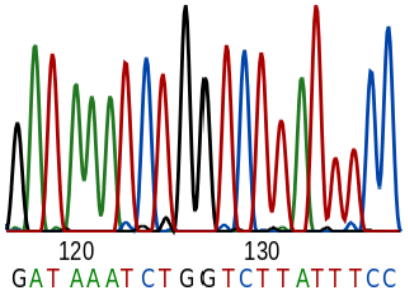
From the «old» to the «new» generation

AB3730xl Capillaries: 96
Throughput: 0.1 Mb/run
Lenght: 800 nt

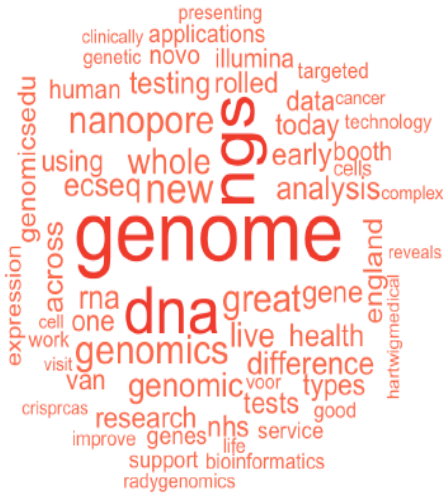


Sanger sequencing (1977)

Human Genome Project
1990 -2003

DNA Sequencing Technologies Key to the Human Genome Project
By: Heidi Chial, Ph.D. (*Write Science Right*) © 2008 Nature Education
Citation: Chial, H. (2008) DNA sequencing technologies key to the Human Genome Project. *Nature Education* 1(1):219

454 GS20 Roche
Length ~250
Throughput: 20 Mb/run



Next Generation Sequencing
mid 2000–present

= high-throughput sequencing

= massively parallel sequencing

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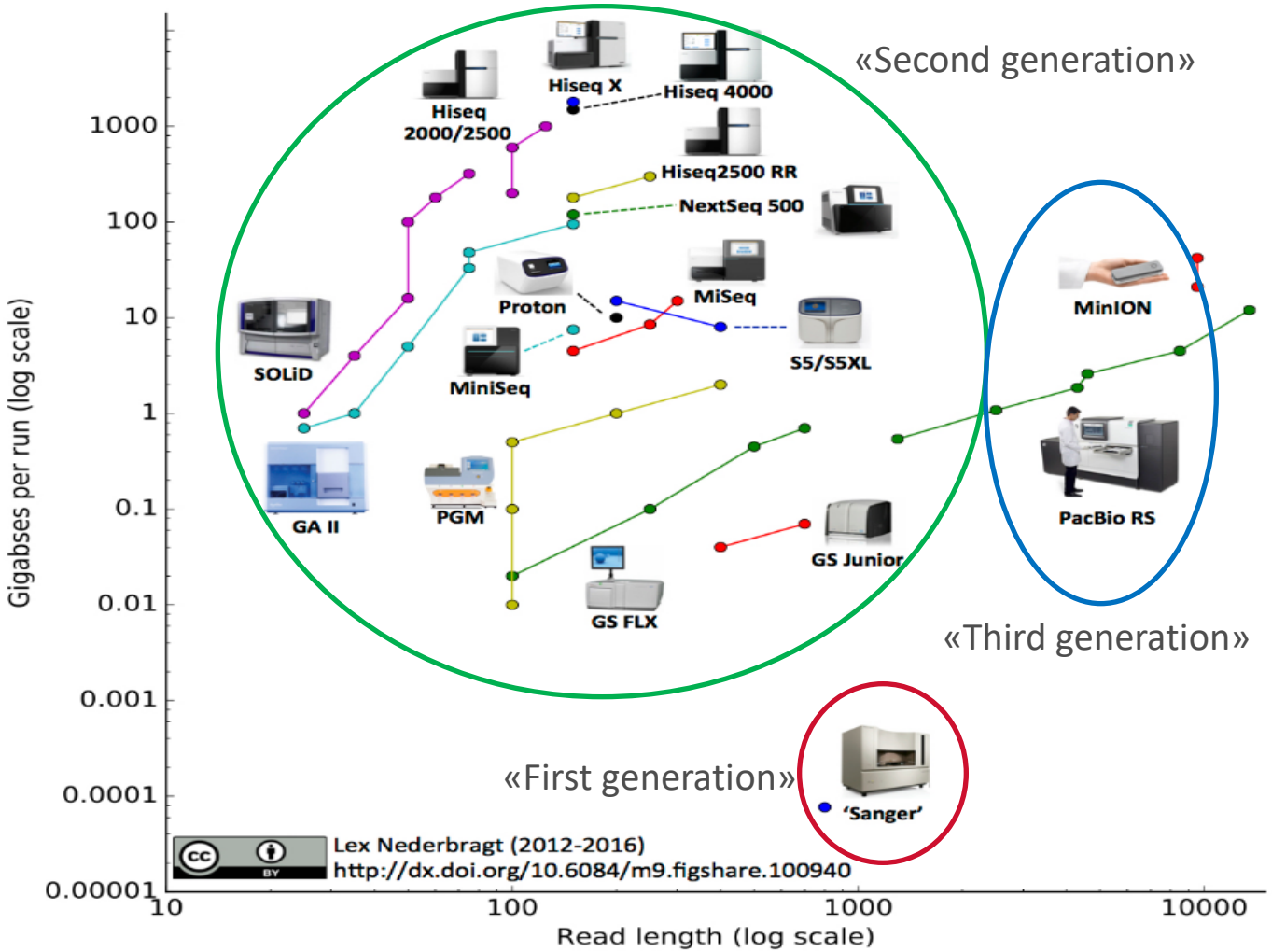
NGS battle



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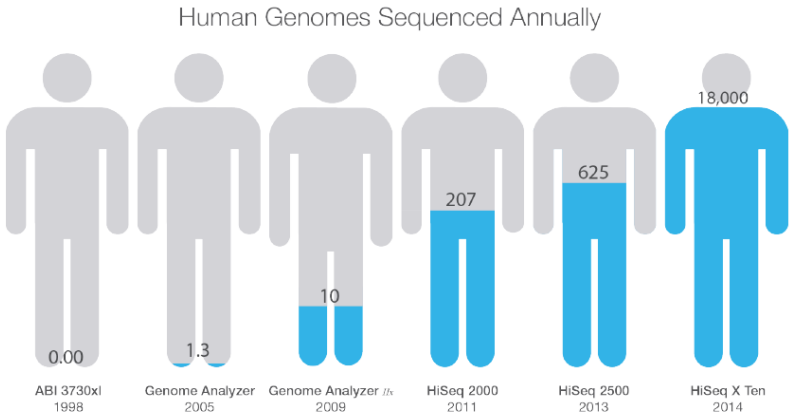
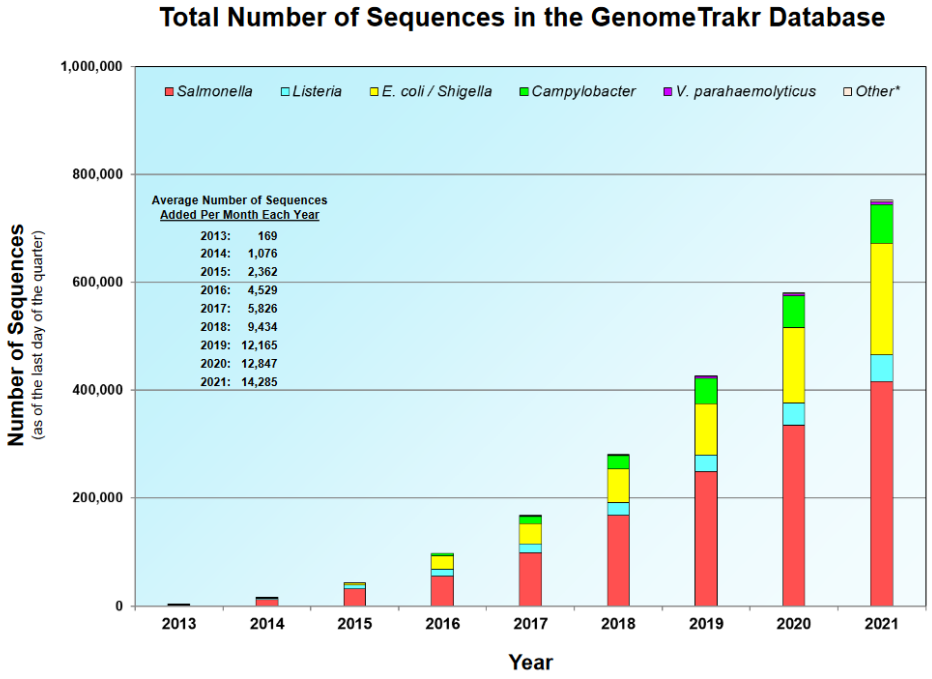
NGS
For human and microbial genomes



FDA Center for Food Safety and Applied Nutrition



First sequences uploaded in February 2013

* Other pathogens: *Cronobacter, V. vulnificus, C. botulinum, and C. perfringens*

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How it works (Illumina NGS technology)

- Microbial WGS has **three basic steps**: library preparation, sequencing, and analysis.
- During **library preparation**, DNA is fragmented; Illumina sequencing adapters linked to each fragment
- Each individual fragment is then **sequenced**
- The resulting nucleotide-level data is **analyzed** to generate an accurate reference genome

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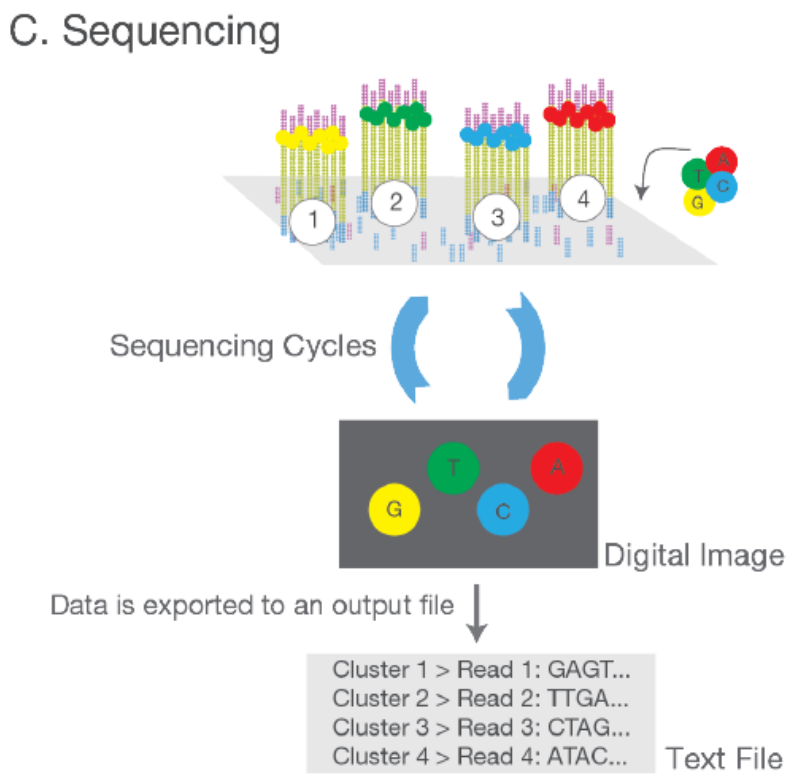
Sequencing by Synthesis (SBS)



DNA polymerase catalyzes the incorporation of **fluorescently labeled dNTPs** into a DNA template strand during **sequential cycles** of **DNA synthesis**. All four **reversible terminator–bound dNTPs** are present during each sequencing cycle

During **each cycle**, at the point of incorporation, **the nucleotides are identified by fluorophore excitation**.

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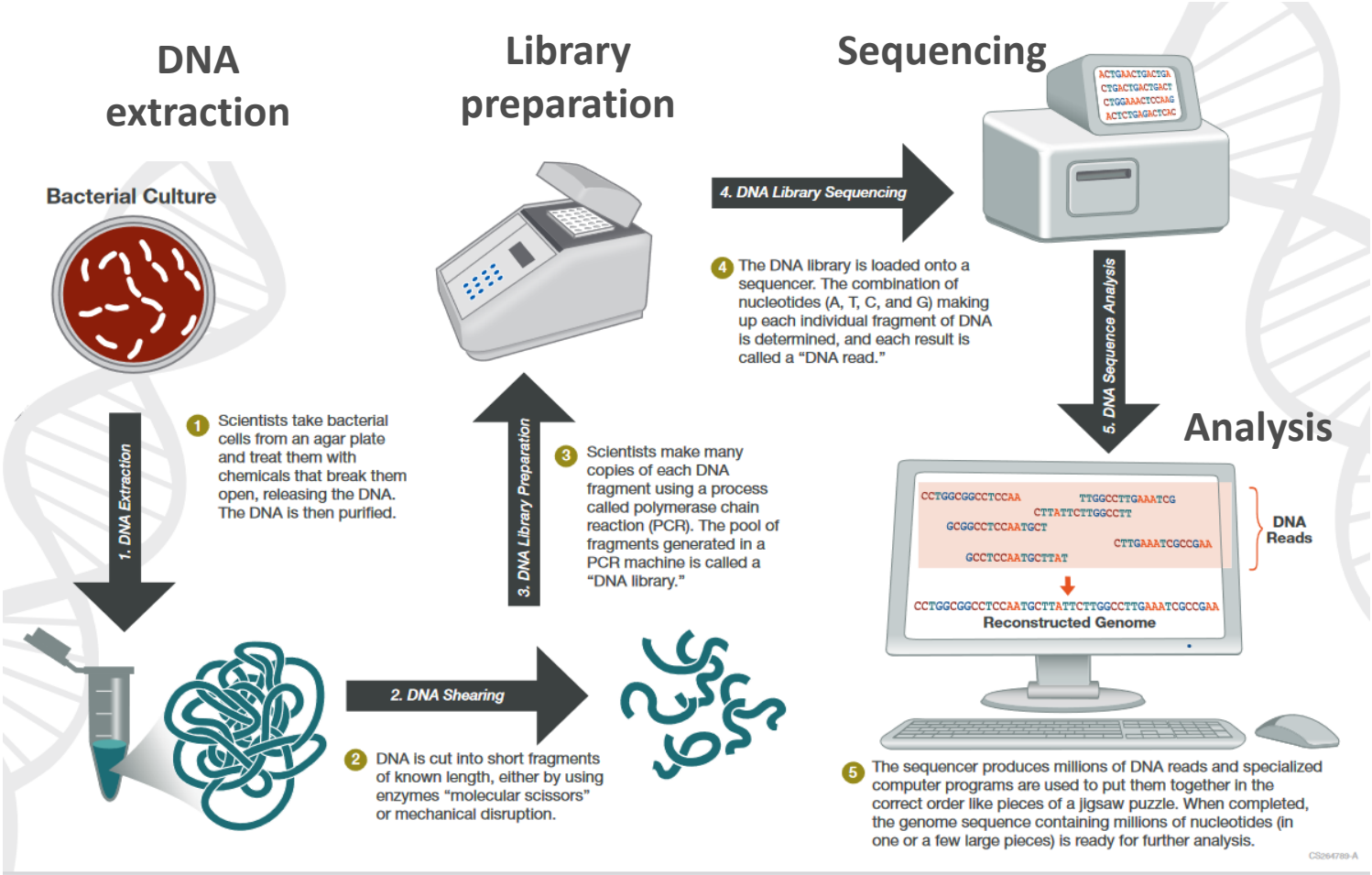
WGS workflow



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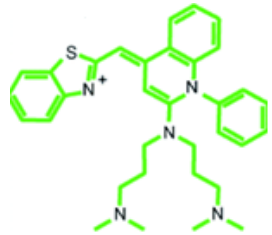
gDNA quantification and standardization

Nanodrop

>3,5 ng/µL

100-500 ng

Fluorimeter



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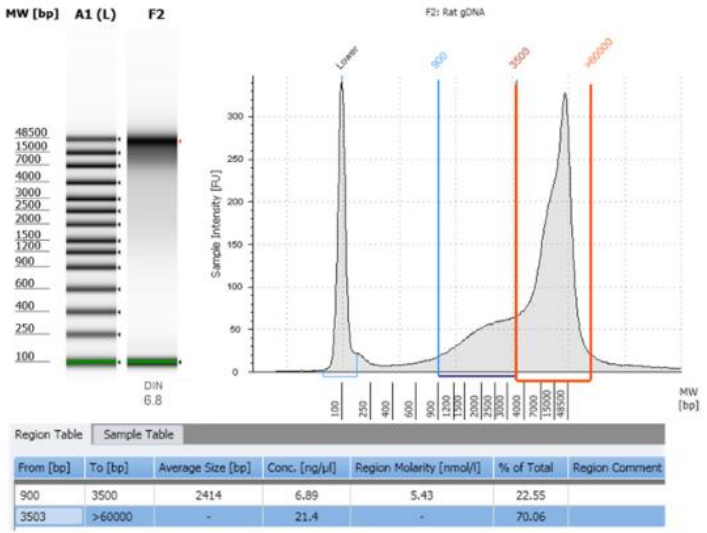
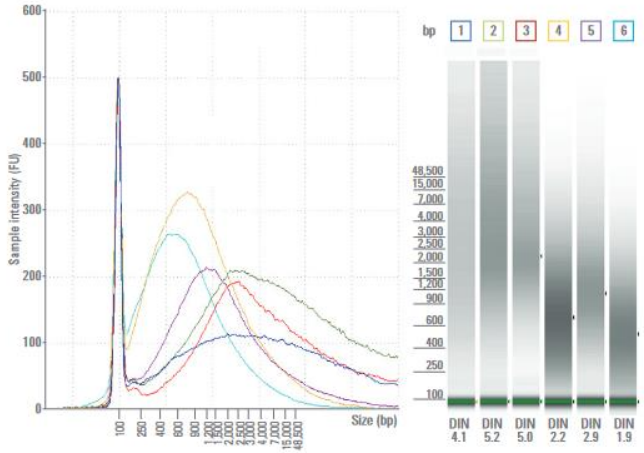
Integrity assessment of gDNA

Agilent Tapestation



Region Table | Sample Table

From [bp]	To [bp]	Average Size [bp]	Conc. [ng/µl]	Region Molarity [nmol/l]	% of Total	Region Comment
900	3500	2414	6.89	5.43	22.55	
3503	>60000	-	21.4	-	70.06	



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First step: library preparation

- The sequencing library is prepared by **tagmentation** which combines into a single step the random fragmentation of the DNA or cDNA sample and the adapter ligation reactions
- Adapter-ligated fragments are then **PCR amplified** and bead-based **purified**

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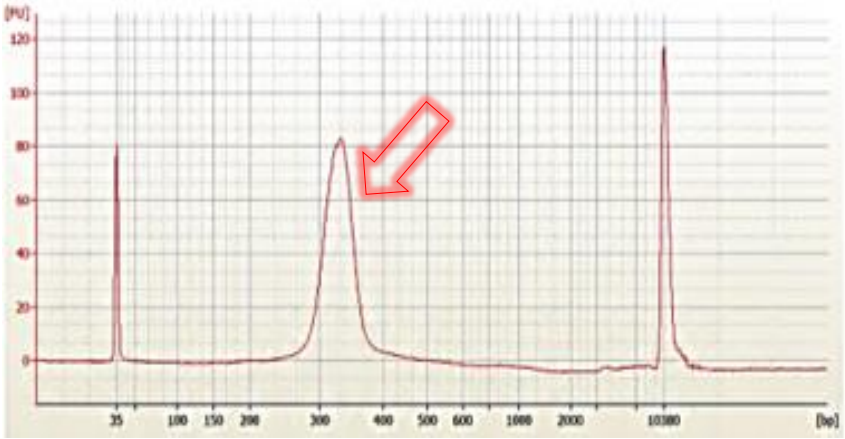
QC and pooling

Agilent Tapestation



20pM

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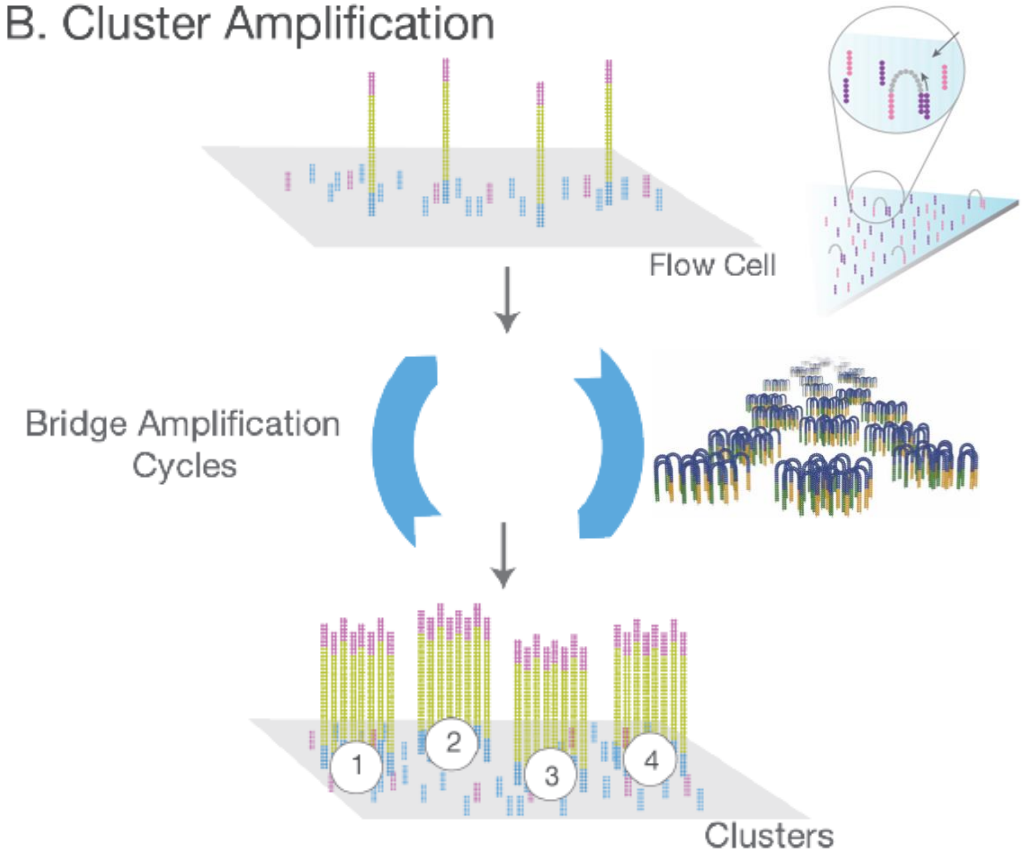
Cluster amplification and generation



Flow cell

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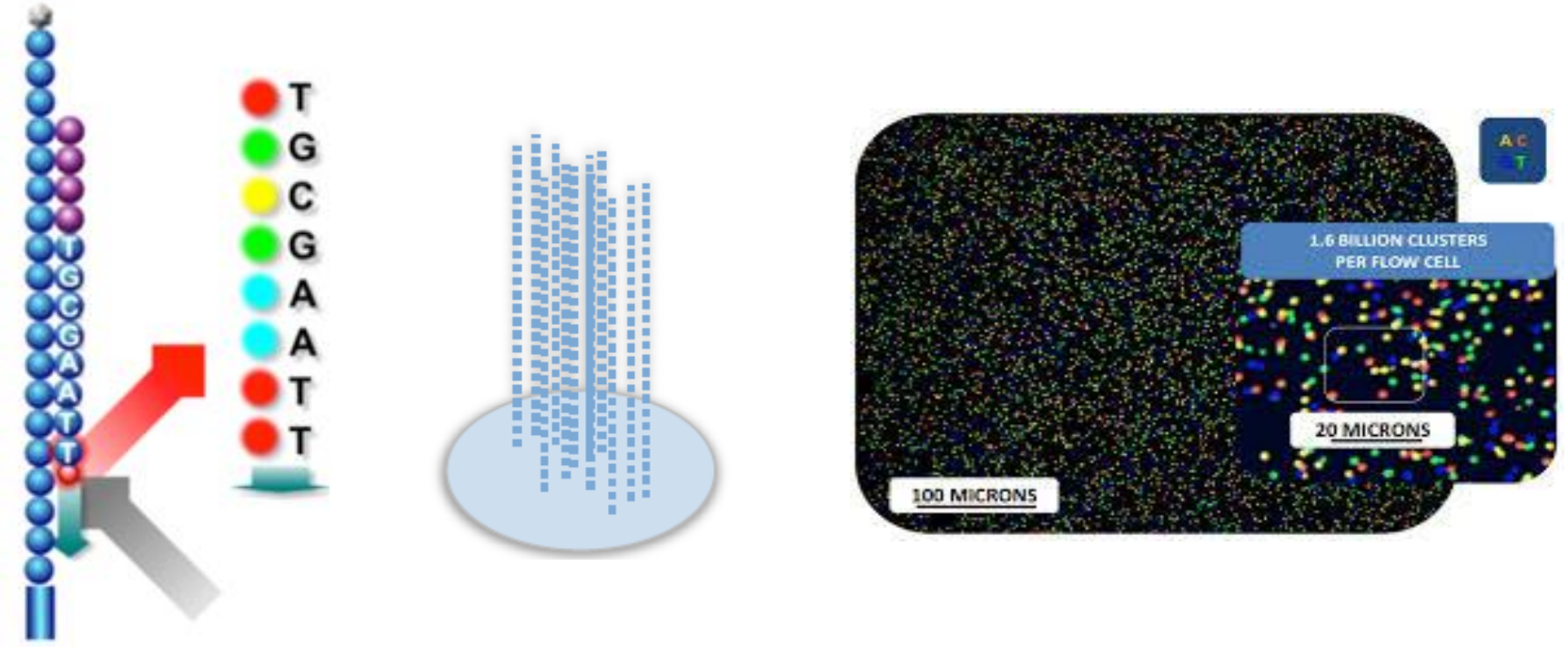
Sequencing by Synthesis (SBS)



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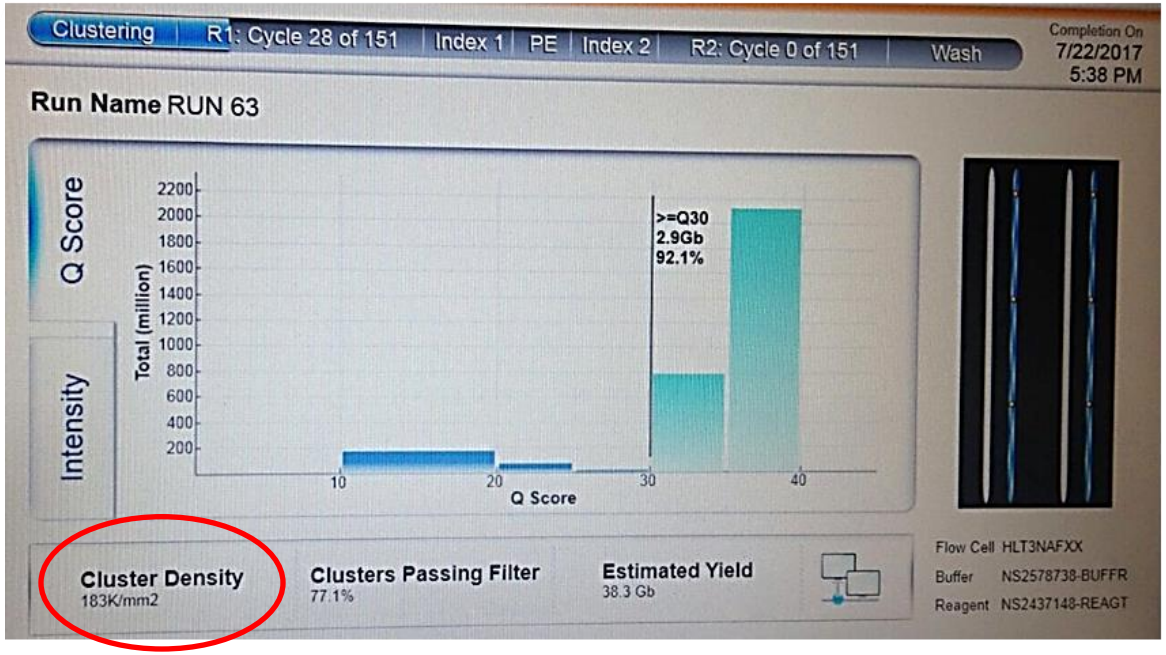
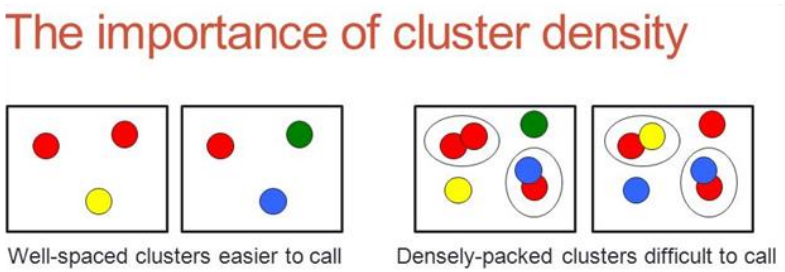
Sequencing run QC





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Illumina sequencing by synthesis (SBS)

- The critical **difference** with the **Sanger sequencing** is that, instead of sequencing **a single DNA fragment**, **NGS** extends this process across **millions of fragments in a massively parallel fashion**.
- The result is highly **accurate base-by-base sequencing** that virtually eliminates sequence specific errors, even within **repetitive sequence** regions and **homopolymers**.
- More than **90% of the world's sequencing** data are generated by **Illumina** SBS chemistry.

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Choosing a sequencing system

	iSeq 100 System	MiniSeq System	MiSeq System	NextSeq 550 System	NextSeq 2000 System	NovaSeq 6000 System
Bacterial genome samples processed/ flow cell[a]	1-4	1-18	1-24	1-384	1 - 384; limited by available indexes	1 - 1536; using all 4 lanes of S4 flow cell
Onboard informatics	●	●	●	–	●	–
Benchtop system	●	●	●	●	●	–
Production-scale capabilities	–	–	–	–	–	●
Flow cell options	Standard	Mid-output/ High-output	Standard v2, Micro v2, Nano v2, Standard v3	Mid-output/ High-output	P2, P3	SP, S1, S2, S4
Flow cells processed/run	1	1	1	1	1	1 or 2

a. Sample numbers may depend on genome size, read depth, and specific flow cell output. Calculations based on 5 Mb genome and 1M reads.

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Third generation sequencing: long reads

- Pacific Bioscience

Revio

Vega

- Oxford Nanopore **MinION**

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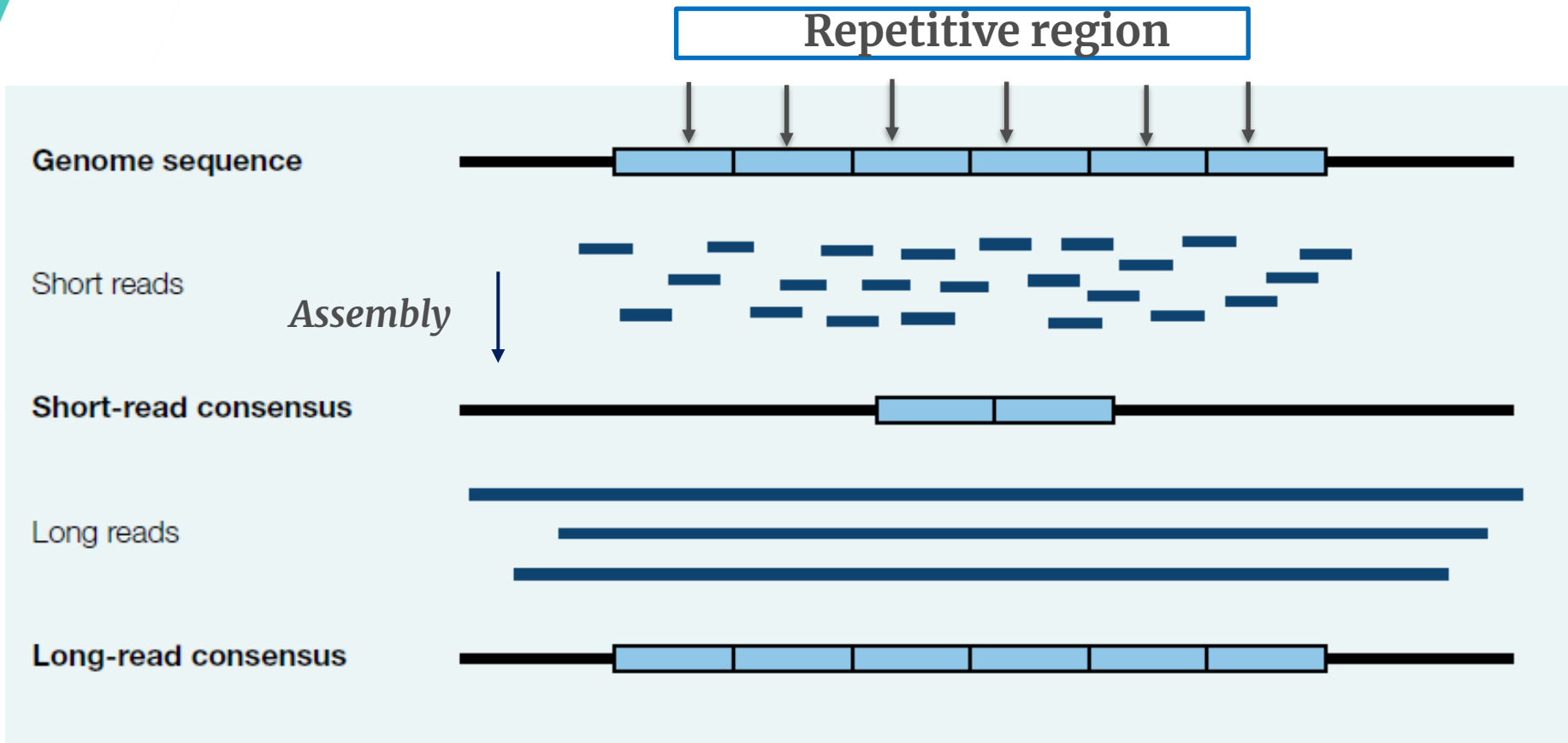
Why long reads?



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Advantage of Nanopore sequencing

- Using ultra-long nanopore reads it is possible to sequence entire **viral genomes in a single read**, bypassing the requirement for assembly completely
- A significant advantage of nanopore sequencing is that it streams data in **real time**, allowing real-time **data analysis**, which can drastically **reduce time to result**.
- Nanopore sequencing provides additional advantages as **very low capital investment**, and **shorter** (10 hours library preparation and sequencing) **turnaround time** compared to other NGS technologies

Oxford NANOPORE Technologies

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Why Whole Genome Sequencing (WGS)?

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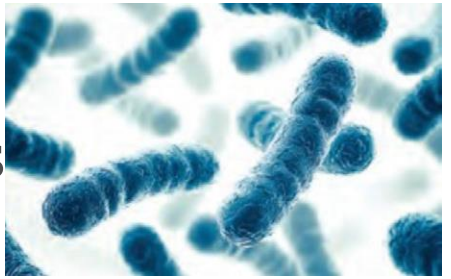
Whole Genome Sequencing WGS

Sequencing the entire bacterial, viral or protozoal genomes is important for:

- microbial identification
- comparative genomic studies
- generating accurate reference genomes



Use microbial WGS to:

- **analyze entire genomes and mobile genetic elements** from microbial isolates and cultures
- **discover new variants** in culture or clinical samples
- **investigate** and understand **outbreaks of** infectious disease **or antibiotic resistance**

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WGS for molecular typing

- Whole-genome sequencing (WGS) provides higher resolution and accuracy than traditional molecular typing methods (e.g. PFGE, MLVA) for foodborne bacterial pathogens.

- It can be considered a very useful tool in surveillance of FBD and **outbreak investigation**, facilitating the detection of potential clusters and increasing the chance of finding the outbreak source

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Comparative analysis between conventional microbiological methods and WGS

Aspect	Conventional Methods	Whole Genome Sequencing (WGS)
Principle	Based on phenotypic traits such as culture characteristics, serotyping, biochemical tests, or PCR-based detection (Oluwaseun et al., 2018; Foddai and Grant, 2020).	Sequencing the entire genome to identify pathogens and analyze genetic traits (Allard et al., 2019; Collineau et al., 2019).
Applications	Detection, identification, and enumeration of foodborne pathogens (Martinović et al., 2016; Oluwaseun et al., 2018).	Outbreak tracing, source attribution, evolutionary studies, and functional gene analysis (Baert et al., 2021).
Speed	Time-consuming (days to weeks, depending on the method) (Gill, 2017; Foddai and Grant, 2020).	Faster results once sequencing infrastructure is established (hours to days) (Scarano et al., 2024).
Sensitivity and Specificity	Varies; dependent on culture conditions and the detection method used (Oluwaseun et al., 2018; Foddai and Grant, 2020).	High sensitivity and specificity due to genome-wide analysis (Kovac et al., 2021).
Data Output	Qualitative or semi-quantitative results (e.g., presence/absence, counts) (Foddai and Grant, 2020; Saravanan et al., 2020).	Quantitative and comprehensive genetic data (e.g., SNPs, resistome, virulome) (Franz et al., 2016).

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Comparative analysis between conventional microbiological methods and WGS

Aspect	Conventional Methods	Whole Genome Sequencing (WGS)
Cost	Lower initial and operational costs (Gill, 2017; Foddai and Grant, 2020).	High initial cost for sequencing equipment; operational costs depend on scale and throughput; these elevated costs may limit some developing countries, or countries with fewer resources, from accessing this technology (World Health Organization, 2018).
Advantages	Cost-effective, well-established, and simple to implement in basic labs (Gill, 2017; Foddai and Grant, 2020).	Provides comprehensive genetic information, including antimicrobial resistance and virulence factors (Allard et al., 2019; Collineau et al., 2019).
Disadvantages	Limited accuracy in strain differentiation and inability to detect non-culturable organisms. Relies on viable pathogens; may not detect viable but non-culturable (VBNC) cells or unculturable pathogens (Gill, 2017; Foddai and Grant, 2020).	High initial cost requires advanced infrastructure, expertise, and bioinformatics capabilities; requires high-quality DNA and generates large datasets that need robust bioinformatics pipelines for analysis (World Health Organization, 2018; Brown et al., 2021).

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GENPAT

Italian National Reference Center for Whole Genome Sequencing of microbial pathogens: database and bioinformatic analysis

Italian Ministry of Health
Regulatory reference 30 May 2017
(G.U.R.I. n. 196 23 August 2017)

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Mission

- To develop a **national platform** for collection and storage of **genomic sequences** of pathogenic microorganisms, to perform **bioinformatic analyses**, to archive and to share the results
- To create a structured and permanent **network of reference people** from all veterinary public health institute (IIZZSS), National Health Institute (ISS) and other research institutes

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Why a National Platform

- The use of genomic data without a national repository would result in data loss and decrease of **efficiency of national surveillance plans**
- **Setting** of **quality standards** of data obtained by NGS would ensure armonization of bioinformatic analysis
- Capability to **integrate WGS data** with information present in **other national databases**

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NGS at IZASM

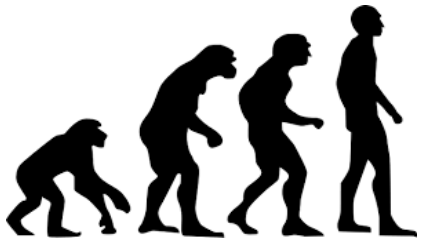
Illumina
- MiniSeq
- MySeq
- NS1000
- NS2000

Thermo Fisher Scientific
- Ion GeneStudio S5 System

ONT
- MinION
- GridION

PacBIO

..on going
- Vega



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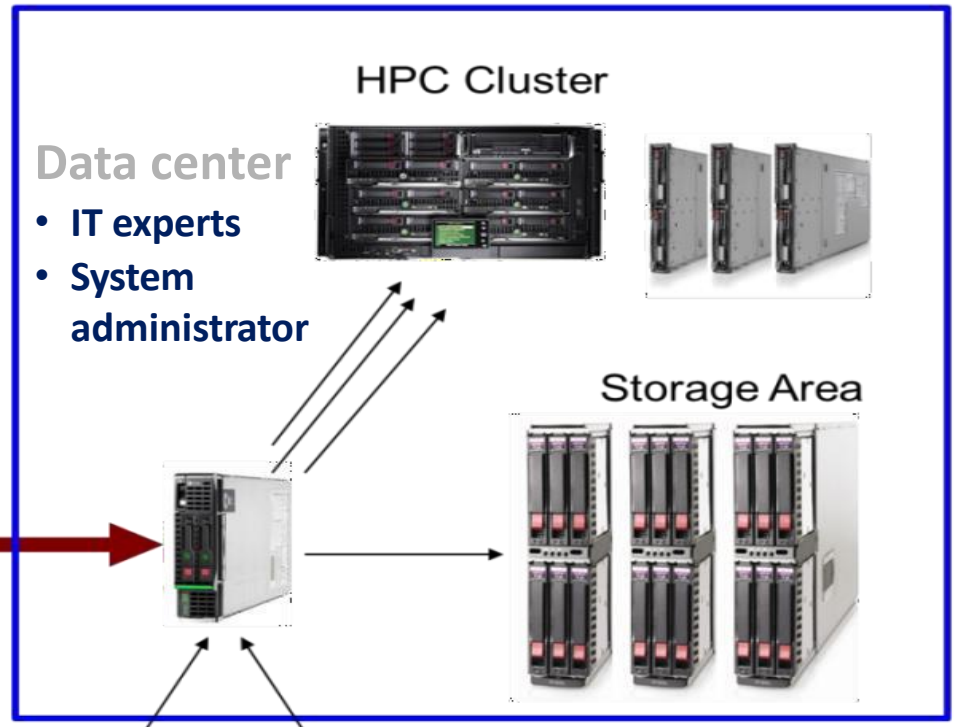
NGS Workflow at IZSAM



Data center
- IT experts
- System administrator

Genomic unit
- Molecular biologists
- Biotechnologists

Epidemiology and Risk analysis unit
- Epidemiologists

Bioinformatic unit
- Bioinformaticians

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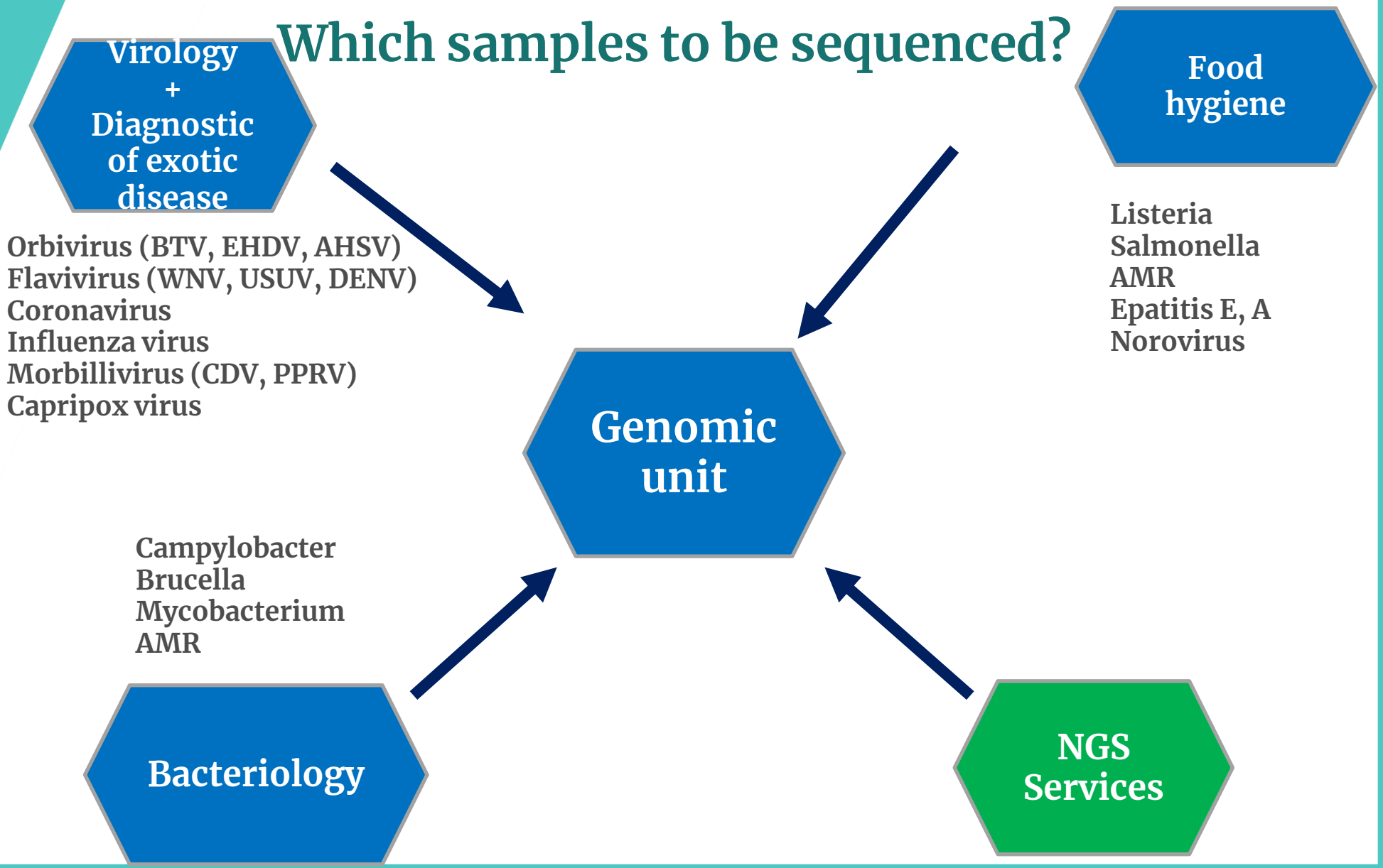
Which samples to be sequenced?



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GenPat for...

- National Reference lab for Listeria, Campylobacter and Brucella (IZSAM)
- National Reference lab for BTV, Flavivirus, Capripox virus, AHSV, PPRV (IZSAM)
- EURL for RVFV (IZSAM)
- FAO reference lab for zoonotic Coronavirus (IZSAM)
- National Reference lab for ASFV (IZSUM)
- National Reference lab for Salmonella
- EURL for Avian Influenza (IZSVe)
- National Reference lab for Staphylococcus (IZSPLV)

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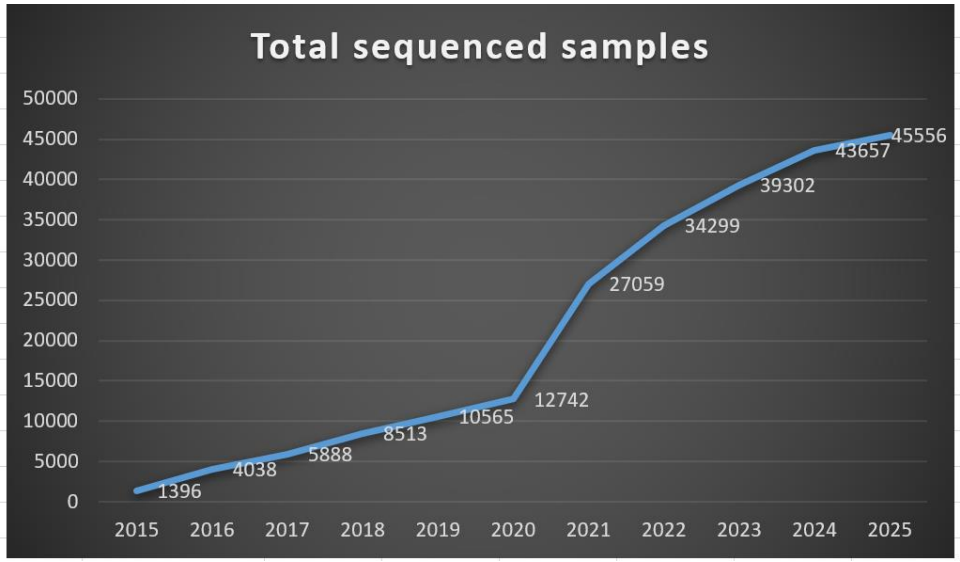
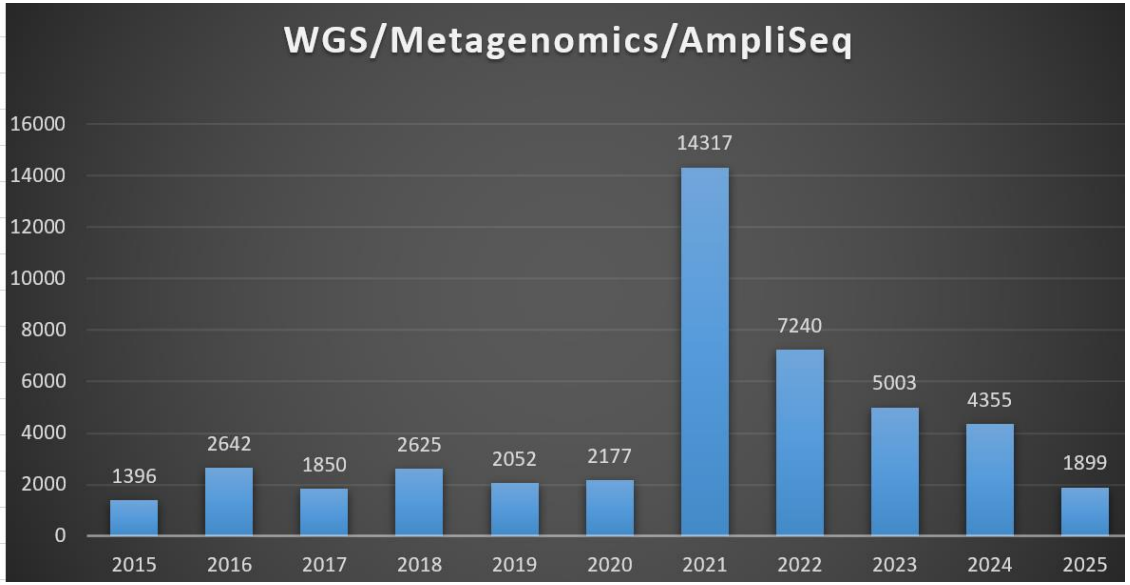
NGS at GenPat





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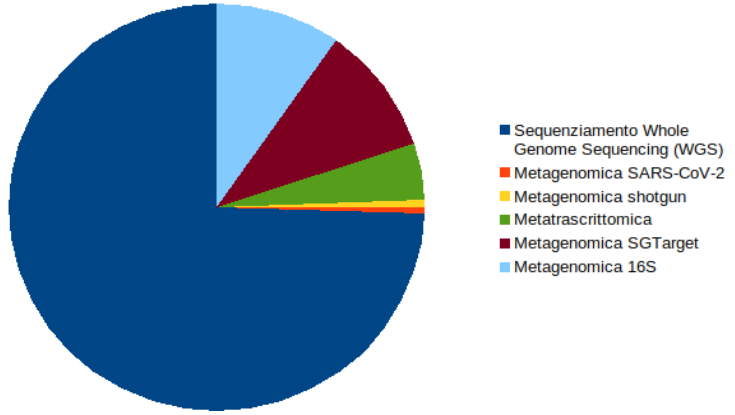
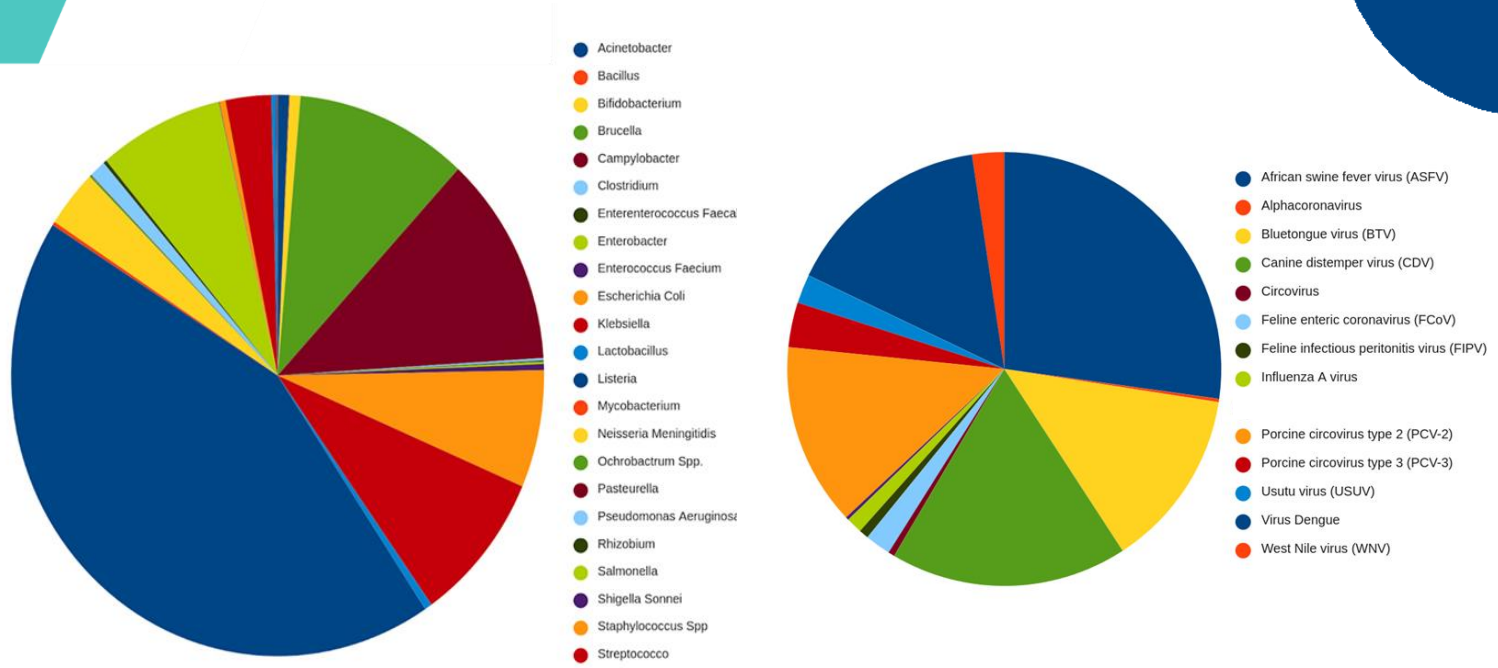
NGS activity 2024

Accertamento	Numero
Sequenziamento Whole Genome Sequencing (WGS)	2908
Metagenomica SARS-CoV-2	21
Metagenomica shotgun	22
Metatrascrittomica	183
Metagenomica SGTarget	385
Metagenomica 16S	397
Totale	**3916**





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ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE "G. CAPORALE"

WGS for foodborne outbreak investigation

JOURNAL OF MEDICAL MICROBIOLOGY Volume 70, Issue 3

Research Article

A large food-borne outbreak of campylobacteriosis in kindergartens and primary schools in Pescara, Italy, May–June 2018

Simona Sorgentone[1], Luca Busani[2], Paolo Calistri[3], Giorgio Robuffo[1], Stefania Bellino[2], Vicdalia Acciari[3], Maurizio Ferri[1], Caterina Graziani[2,†], Salvatore Antoci[3], Fabrizio Lodi[1], Valeria Alfonsi[2,‡], Cesare Cammà[3], Paolo Fazii[4], Xanthi Andrianou[2], Francesca Cito[3], Giuliano Lombardi[4], Gabriella Centorotola[3], Massimo D'Amario[1], Nicola D'Alterio[3], Vincenzo Savini[4], Fabrizio De Massis[3], Anna Pelatti[4], Marco Di Domenico[3], Guido Di Donato[3], Elisabetta Di Giannatale[3], Lisa Di Marcantonio[3], Violeta Di Marzio[3], Gabriella Di Serafino[3], Anna Janowicz[3], Cristina Marfoglia[3], Francesca Marotta[3], Daniela Morelli[3], Giacomo Migliorati[3], Diana Neri[3], Francesco Pomilio[3], Silvia Scattolini[3], Giovanni Rezza[2,§], Antonio Caponetti[1], Patrizio Pezzotti[2], Giuliano Garofolo[3]

View Affiliations

First Published: 21 January 2021 | https://doi.org/10.1099/jmm.0.001262

JOURNAL OF MEDICAL MICROBIOLOGY Volume 67, Issue 9

Research Article | Free

A severe outbreak of listeriosis in central Italy with a rare pulsotype associated with processed pork products

Anna Duranti[1,†], Michela Sabbatucci[2,3,†], Giuliana Blasi[1], Vicdalia Aniela Acciari[4], Massimo Ancora[4], Antonino Bella[2], Luca Busani[2], Patrizia Centorame[4], Cesare Cammà[4], Fabrizio Conti[5], Dario De Medici[2], Marco Di Domenico[4], Violeta Di Marzio[4], Giovanni Filippini[1], Alfonsina Fiore[2], Stefano Fisichella[1], Antonietta Gattuso[2], Monica Gianfranceschi[2], Caterina Graziani[2], Fabrizia Guidi[1], Maurilia Marcacci[4], Cristina Marfoglia[4], Diana Neri[4], Massimiliano Orsini[4], Donatella Ottaviani[1], Annalisa Petruzzelli[1], Patrizio Pezzotti[2], Caterina Rizzo[2], Anna Ruolo[4], Gaia Scavia[2], Stefania Scuota[1], Giuliano Tagliavento[6], Alberto Tibaldi[6], Francesco Tonucci[1], Marina Torresi[4], Giacomo Migliorati[4], Francesco Pomilio[4]

View Affiliations

First Published: 19 July 2018 | https://doi.org/10.1099/jmm.0.000785

Severe *Streptococcus equi* Subspecies *zooepidemicus* Outbreak from Unpasteurized Dairy Product Consumption, Italy

Serena Bosica, Alexandra Chiaverini, Maria Elisabetta De Angelis, Antonio Petrini, Daniela Averaimo, Michele Martino, Marco Rulli, Maria Antonietta Saletti, Maria Chiara Cantelmi, Franco Ruggeri, Fabrizio Lodi, Paolo Calistri, Francesca Cito, Cesare Cammà, Marco Di Domenico, Antonio Rinaldi, Paolo Fazii, Fabrizio Cedrone, Giuseppe Di Martino, Patrizia Accorsi, Daniela Morelli, Nicola De Luca, Francesco Pomilio, Giustino Parruti, Giovanni Savini

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 29, No. 5, May 2023

EILLANCE AND OUTBREAK REPORT

Outbreak of unusual *Salmonella enterica* serovar Typhimurium monophasic variant 1,4 [5],12:i:-, Italy, June 2013 to September 2014

F Cito [1], F Baldinelli [1], P Calistri [1], E Di Giannatale [1], G Scavia [2], M Orsini [1], S Iannetti [1], L Sacchini [1], I Mangone [1], L Candeloro [1], A Conte [1], C Ippoliti [1], D Morelli [1], G Migliorati [1], NB Barile [1], C Marfoglia [1], S Salucci [1], C Cammà [1], M Marcacci [1], M Ancora [1], AM Dionisi [2], S Owczartek [2], I Luzzi [2], on behalf of the outbreak investigation group [9]

1. Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy
2. Istituto Superiore di Sanità, Rome, Italy

microorganisms

MDI

Article

Whole Genome Sequencing for Studying *Bacillus anthracis* from an Outbreak in the Abruzzo Region of Italy

Alexandra Chiaverini [1,*], Mostafa Y. Abdel-Glil [2], Jörg Linde [2], Domenico Galante [3], Valeria Rondinone [3], Antonio Fasanella [3], Cesare Cammà [1], Nicola D'Alterio [1], Giuliano Garofolo [1] and Herbert Tomaso [2]

IZS TERAMO

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE "G. CAPORALE"

Using NGS directly from biological samples

- **Targeted resequencing** (amplicon o enrichment based): when the target microorganism is present or at least suspected
- **Metagenomics:** (metabarcoding) it allows many different species to be identified simultaneously in a single sample (e.g. microbial communities)
- **Shotgun metagenomics :** it allows all DNA molecules present in the sample to be sequenced

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Virus characterization at IZS-Te

Epidemics 39 (2022) 100578

Contents lists available at ScienceDirect

Epidemics

journal homepage: www.elsevier.com/locate/epidemics

Epidemiological and genomic findings of the first documented Italian outbreak of SARS-CoV-2 Alpha variant of concern

Laura Amato [a,*], Luca Candeloro [a], Arturo Di Girolamo [b], Lara Savini [a], Ilaria Puglia [a], Maurilia Marcacci [a], Marialuigia Caporale [a], Iolanda Mangone [a], Cesare Cammà [a], Annamaria Conte [a], Giuseppe Torzi [b], Adamo Mancinelli [b], Francesca Di Giallonardo [c], Alessio Lorusso [a], Giacomo Migliorati [a], Thomas Schael [b], Nicola D'Alterio [a], Paolo Calistri [a]

[a] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM), 64100 Teramo, Italy
[b] Lanciano-Vasto-Chieti Local Health Unit, 66100 Chieti, Italy
[c] The Kirby Institute, UNSW Sydney, Sydney, NSW 2052, Australia

Acta Tropica 260 (2024) 107416

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Identification and characterization of two atypical strains of bluetongue virus in sheep, Tunisia

Sara Thabet [a], Soufien Sghaier [b,c], Valentina Curini [d], Luana Fiorella Mincarelli [d], Dorsaf El Mansouri [a], Raja Ben Osmane [e], Sonia Ben Hassan [b,c], Ahmed Amara [f], Thameur Ben Hassine [g], Giovanni Savini [d], Simone Pulsoni [d], Ayda Sayadi [e], Ayda Krichene [b,c], Cesare Cammà [d], Massimo Spedicato [d], Alessio Lorusso [d], Maurilia Marcacci [d,*], Salah Hammami [a]

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome of African Swine Fever Virus Genotype II in Central Italy

Monica Giammarioli,[a] Maurilia Marcacci,[b] Maria Teresa Scicluna,[c] Antonella Cersini,[c] Claudia Torresi,[a] Valentina Curini,[b] Massimo Ancora,[b] Antonio Rinaldi,[b] Marcello Giovanni Sala,[c] Elisabetta Rossi,[a] Cristina Casciari,[a] Michela Pela,[a] Claudia Pellegrini,[a] Carmen Iscaro,[a] Cesare Cammà,[b] Francesco Feliziani[a]

nature communications

Article

https://doi.org/10.1038/s41467-023-42185-7

Spatial and temporal dynamics of West Nile virus between Africa and Europe

Received: 4 March 2023

Accepted: 29 September 2023

Published online: 13 October 2023

Check for updates

Giulia Mencattelli [1,2,3,7], Marie Henriette Dior Ndione [4,7], Andrea Silverj [2,3,5,7], Moussa Moise Diagne [4], Valentina Curini [1], Liana Teodori [1], Marco Di Domenico [1], Rassoul Mbaye [4], Alessandra Leone [1], Maurilia Marcacci [1], Alioune Gaye [6], ElHadji Ndiaye [6], Diawo Diallo [6], Massimo Ancora [1], Barbara Secondini [1], Valeria Di Lollo [1], Iolanda Mangone [1], Andrea Bucciacchio [1], Andrea Polci [1], Giovanni Marini [3], Roberto Rosà [2,3], Nicola Segata [5], Gamou Fall [4], Cesare Cammà [1], Federica Monaco [1], Mawlouth Diallo [6], Omar Rota-Stabelli [2,3,5], Oumar Faye [4,8], Annapaola Rizzoli [3,8] & Giovanni Savini [1,8]

pathogens

MDPI

Case Report

Full Genome Characterization of Respiratory Syncytial Virus Causing a Fatal Infection in an Immunocompromised Patient in Tunisia

Valentina Curini [1], Maurilia Marcacci [1,2,*], Salma Abid [3,4], Monia Ouederni [5], Awatef ElMoussi [3,4], Latifa Charaa [4], Wafa Achour [6,7], Ramzi Ouhichi [8], Latifa Maazaoui [9], Adriano Di Pasquale [1], Hakim ElGhord [9], Ahlem Gzara [9], Alessandro Ripani [1,10], Francesca Di Giallonardo [11], Cesare Cammà [1], Alessio Lorusso [1] and Ilhem Boutiba-Ben Boubaker [3,4]

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GenPat as Service

- Universities
- Research institutes
- ECDC
- International Atomic Energy Agency (IAEA)

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EFSA/ECDC service

12/2019
02/2024

European countries	Number of samples
Greece	258
Hungary	16
Netherlands	96
Poland	29
Estonia	105
Romania	192
Slovenia	71
Belgium	333
Portugal	50
Czech Republic	236
Bulgaria	12
Croatia	19
Lithuania	105
Italy	57
Total	**1579**

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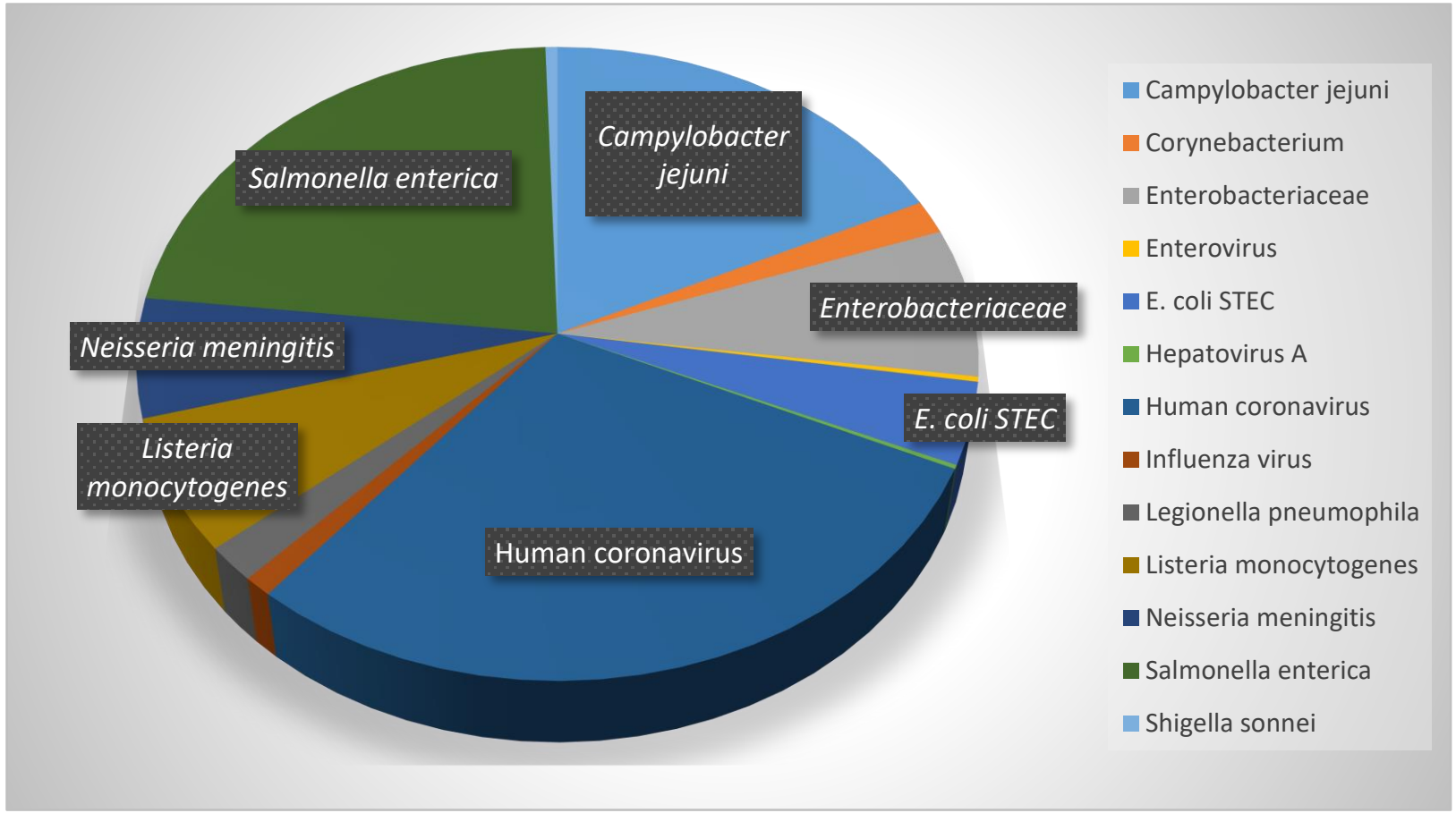
EFSA/ECDC service



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WGS services for Coordinated Research Project D32036

GenPat Platform

National Reference Centre for Whole Genome Sequencing of microbial pathogens: database and bioinformatic analysis

PURCHASE ORDER
No.: 202409684-AY
Date: 21-NOV-2024

Scope
Whole Genome Sequencing (WGS) services

The Contractor shall perform the WGS Services on the followings:

i) Whole genome PCR amplicons of the avian influenza viruses (AIVs);
ii) DNA extract from multiple capripox viruses;
iii) DNA extracts from pure cultures of brucella and salmonella

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ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE "G. CAPORALE"

Long term WGS service

Contract Purchase Agreement No. 202504962

between

the International Atomic Energy Agency

and

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale (IZSAM)

concerning

Provision of Whole Genome Sequencing Services for Member States

IAEA International Atomic Energy Agency

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01 August 2025 – 28 July 2028

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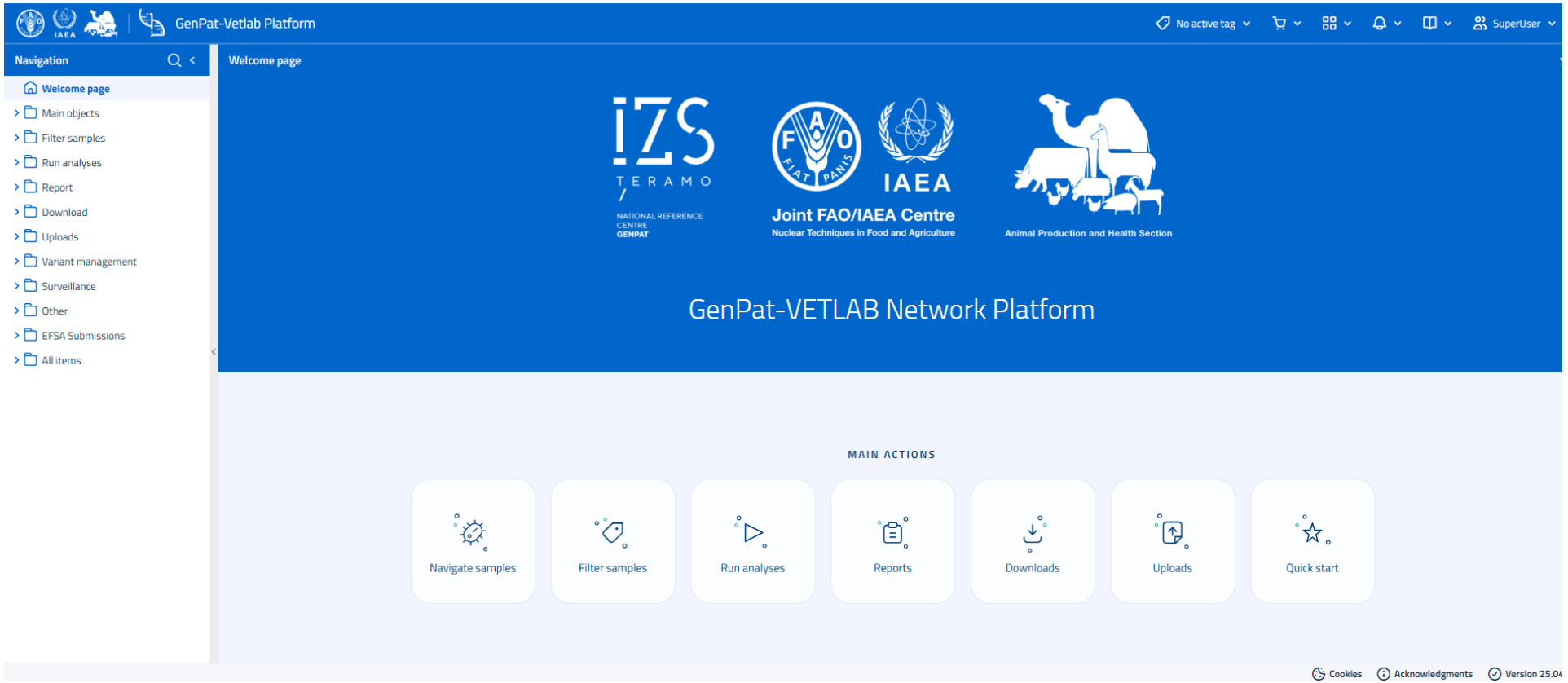
GENPAT-VETLAB platform



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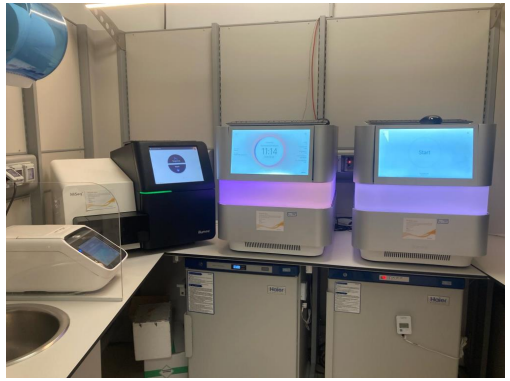
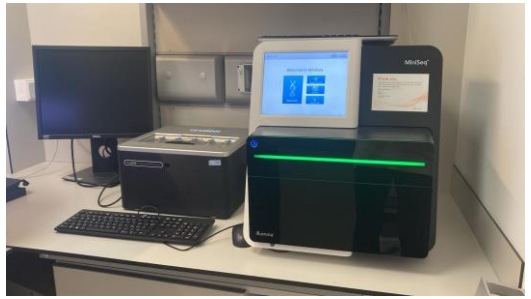
GenPat

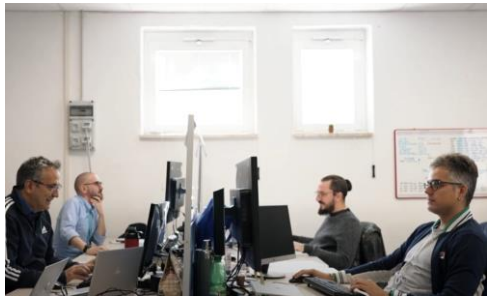
Genomic unit





Bioinformatic unit

GenPat Platform



https://genpat.izs.it/

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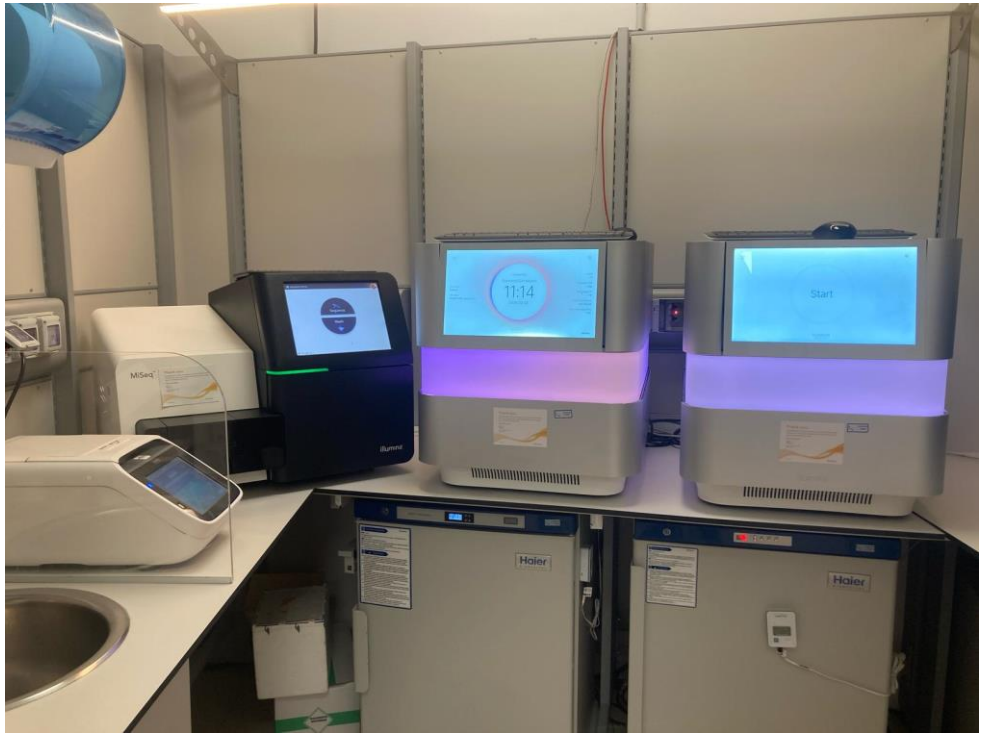
Illumina sequencing platforms



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E DEL MOLISE
"G. CAPORALE"

Nanopore Sequencing platforms

MinION Mk1B

MinION Mk1D

GridION

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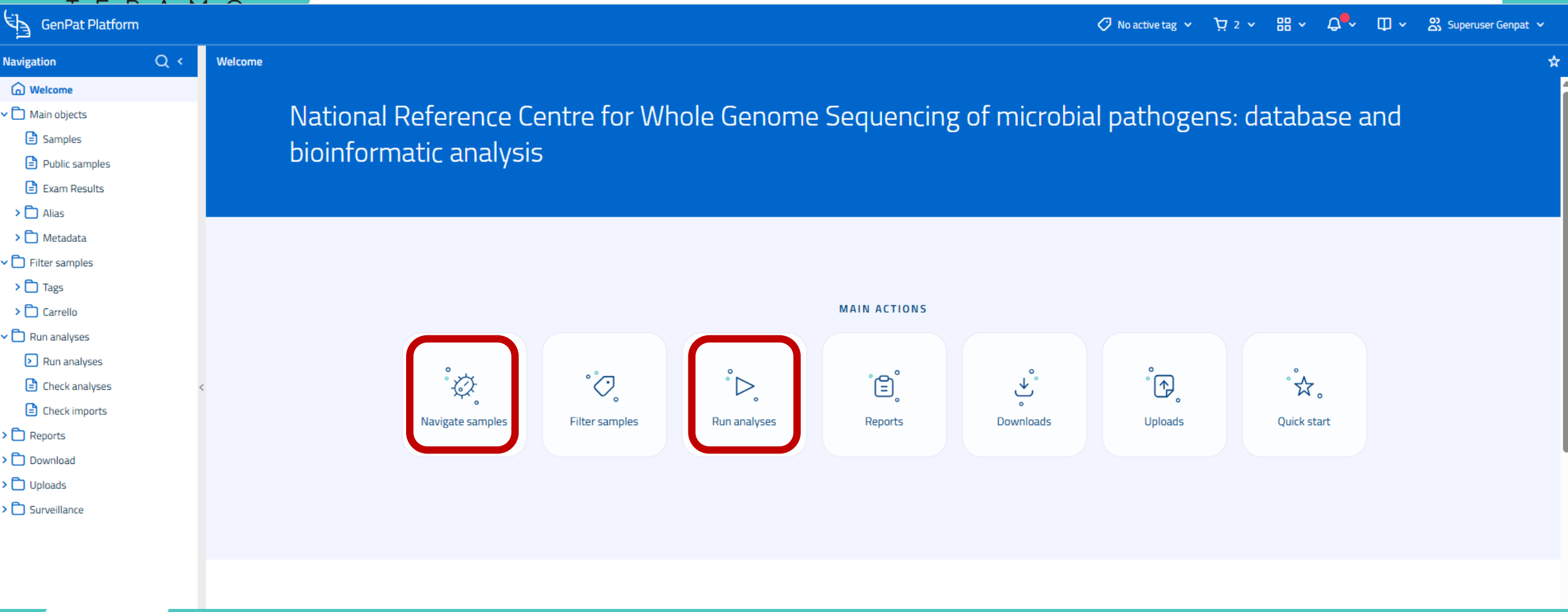
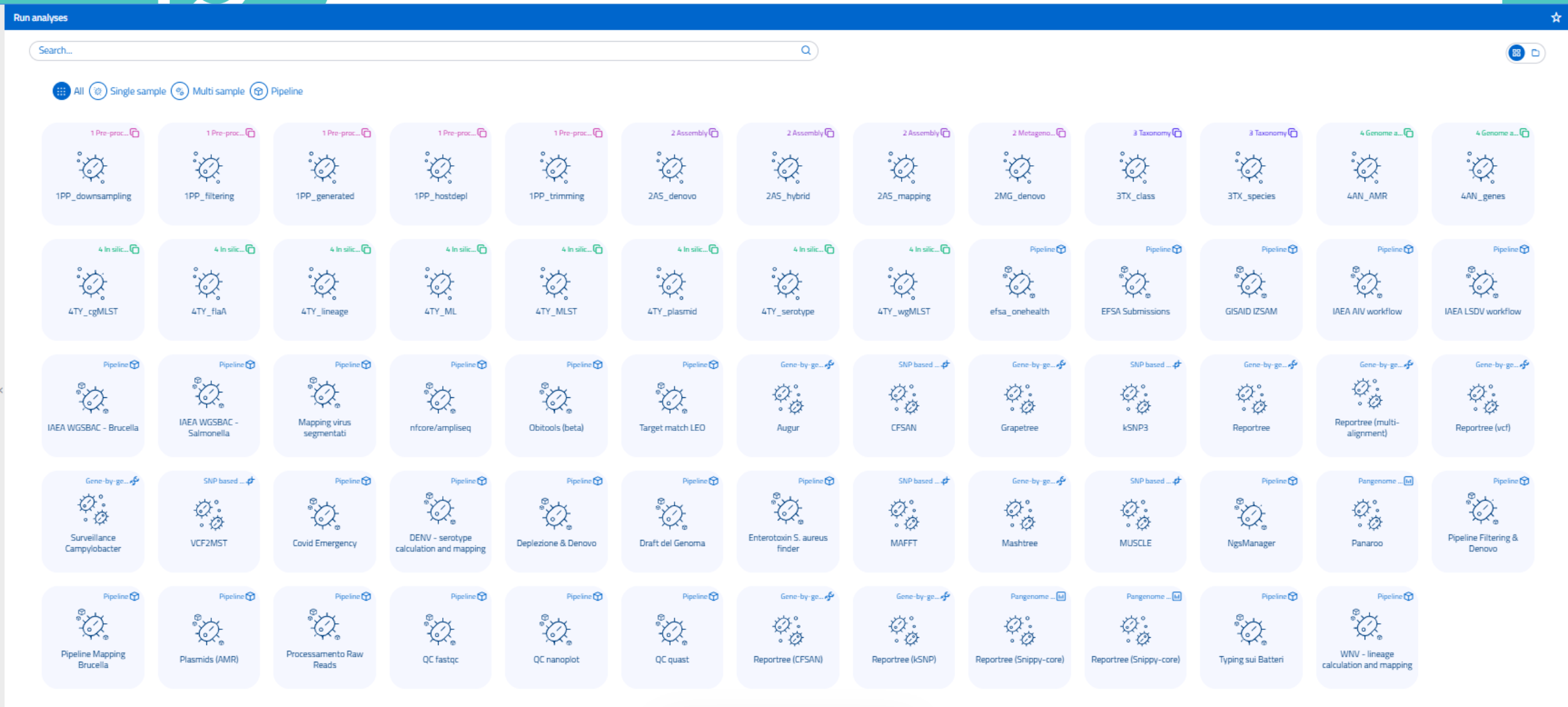
IonTorrent GeneStudio S5
IonChef System

GenPat Platform



Bioinformatic tools

GenPat Platform



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ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE "G. CAPORALE"

Contacts

NGSlab – Cesare Cammà
c.camma@izs.it
ngs@izs.it

Bioinfolab - Adriano Di Pasquale
a.dipasquale@izs.it
bioinformatica@izs.it

National Reference Center Genpat
genpat@izs.it