

# Genomics and Bioinformatics Unit

## Department of Infectious Diseases

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**MCBBi | NOVA**  
UNIVERSIDADE NOVA  
DE LISBOA

# Instituto Nacional de Saúde Dr. Ricardo Jorge

## State Laboratory (NIH)

- Under the Ministry of Health
- Broad mission towards the improvement of public health



## Divided in several departments

- Department of Food and Nutrition
- Department of Infectious Diseases
- Department of Epidemiology
- Department of Human Genetics
- Department of Health Promotion and Prevention of Non-Transmissible Diseases
- Department of Environmental Health

## Housing the National Reference Laboratories

### Laboratory surveillance of infectious diseases

#### Priorities

- Detection of infectious agents
- Discrimination and classification (typing techniques)



#### Benefits for public health:

- Tracking the emergence and spread of pathogenic agents
- Detect and solve outbreaks
- Identify factors that contribute to the persistence of endemic clones
- Complement/Support diagnosis
- Guide treatment by providing antibiotic resistance profile of the infectious agents and helping in the evaluation of the reasons behind treatment failure

etc

# Departament of Infectious Diseases (DDI)

- DDI collaborates in the epidemiological surveillance of infectious diseases, through the activity of several 'Reference Laboratories'
  - Each 'Reference Laboratory' is specialized in a topic, including sexually transmissible infections, respiratory infections, gastrointestinal and food-borne infections, vector-based infections, parasites, fungi, antimicrobial resistance, etc...
- All samples from the national health service suspected of containing an infectious agent are sent to INSa for analysis
  - Samples are analysed by an appropriate 'Reference Laboratory', with some cases being analysed by several different laboratories
- DDI communicates and collaborates with european and world entities towards the evaluation of biological hazard in the context of public health emergencies
  - Eg. SARS-CoV-2 and Mpox surveillance, among several others



## Laboratory surveillance of infectious diseases

### Whole-genome sequencing (WGS) as the gold standard typing method

#### Traditional typing methods

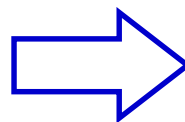
PFGE  
MLVA  
MLST  
PCR assays for detection of virulence genes

Antibiotic susceptibility tests

Serotyping

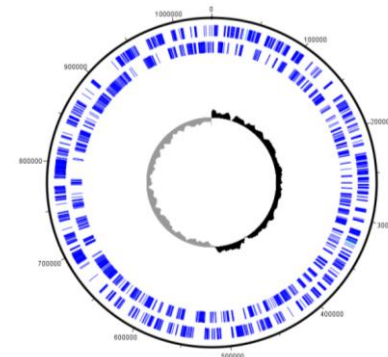
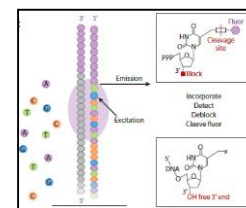
(...)

- *Difficult standardization*
- *Time consuming*
- *Low discriminatory power*
- (...)



#### Next-generation sequencing (NGS)

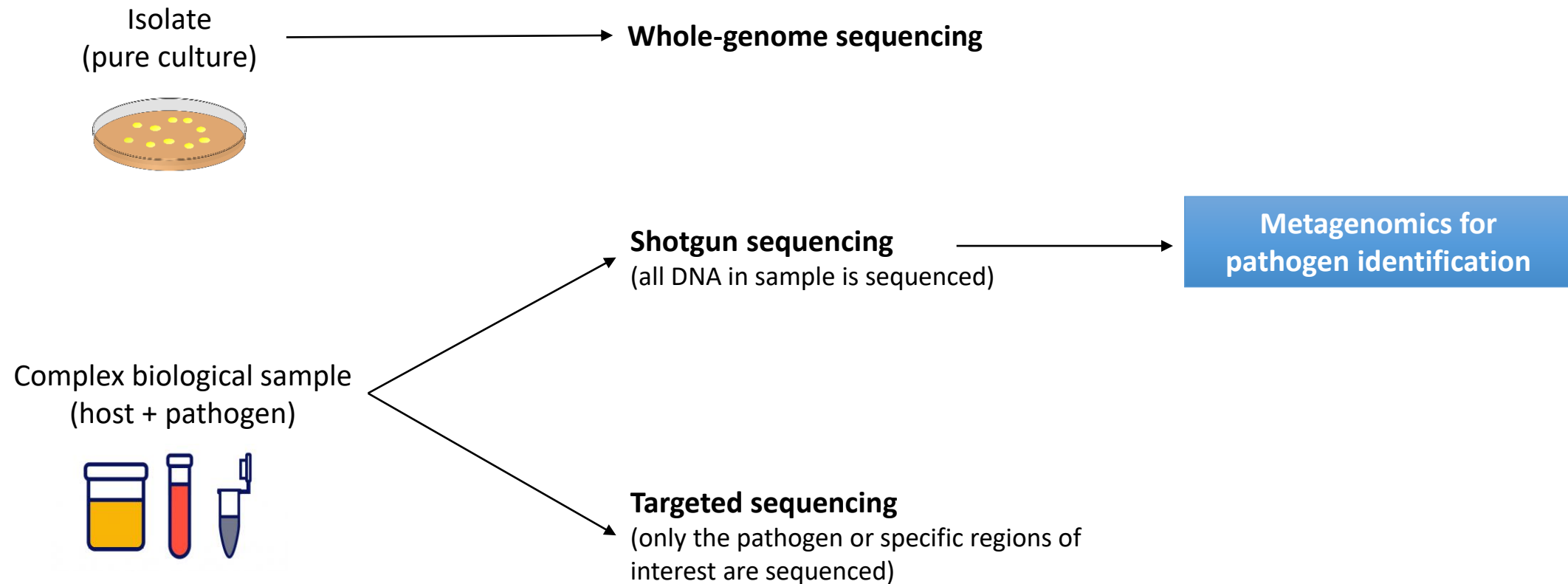
*“massive parallel sequencing”*



- *Capture of the “whole” genome*
- *High discriminatory power*
- *Applicable to “all” organisms*
- *Multiple tests in silico from a single assay*
- *Cost and time - already competitive*
- (...)

## Laboratory surveillance of infectious diseases

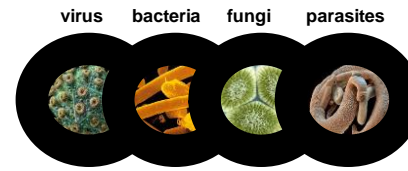
### Next-generation sequencing



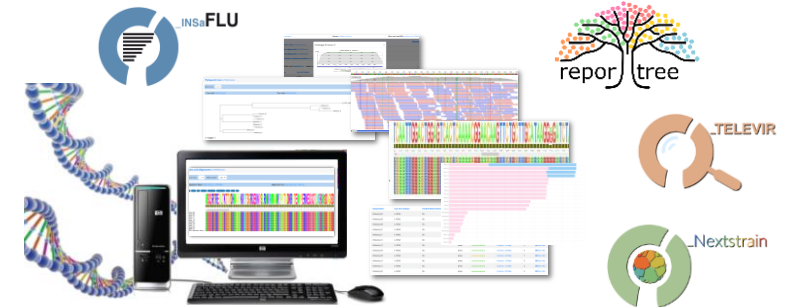
illumina



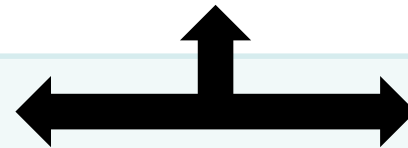
OXFORD  
NANOPORE  
technologies



NATIONAL REFERENCE  
LABORATORIES



NEXT-GENERATION SEQUENCING (NGS)



BIOINFORMATICS / (META)GENOMICS



**Top from left to right:** Sílvia Duarte, Sara Rangel, Camila Fernandes, Luís Vieira, José Ferrão, Catarina Silva. **Bottom from left to right:** Joana Mendonça, Dina Carpinteiro, Daniela Santos.



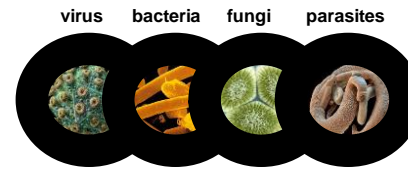
**Top from left to right:** Vítor Borges, Rita Ferreira, Verónica Mixão, João Paulo Gomes, Joana Isidro, João Dourado, Alexandra Nunes. **Bottom from left to right:** Daniel Sobral, Miguel Pinto, Luís Coelho.



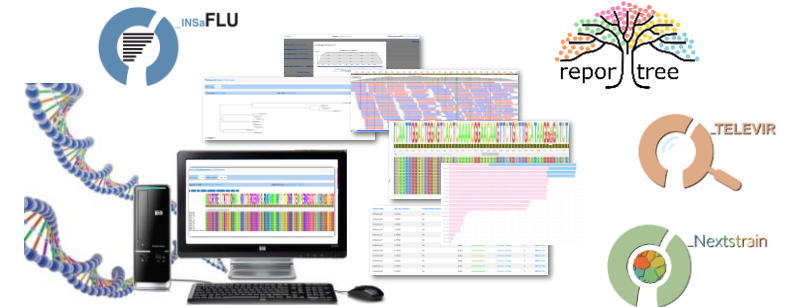
illumina



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NEXT-GENERATION SEQUENCING (NGS)

BIOINFORMATICS / (META)GENOMICS

## Pathogen's detection

- Response to emerging threats

Monitoring vaccine  
antigens, antibiotic  
resistance, etc

## Technology transfer

- WGS directly from clinical samples  
(uncultivable or fastidious pathogens)  
- Transition from traditional typing  
methods to WGS for multiple  
pathogens

# Routine Surveillance / Outbreak Investigation

## R&D activities

- Evolutionary adaptation
- Genotype-phenotype associations
- Prediction of antibiotic resistance  
profiles
- Transcriptomic studies  
(...)

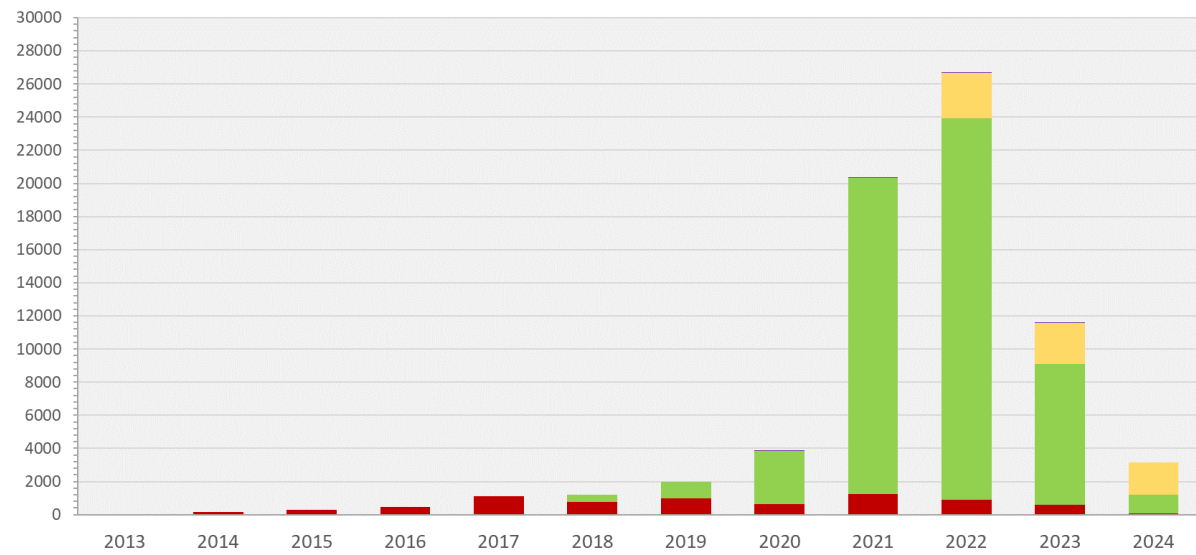
## Diagnosis support

## Software Development

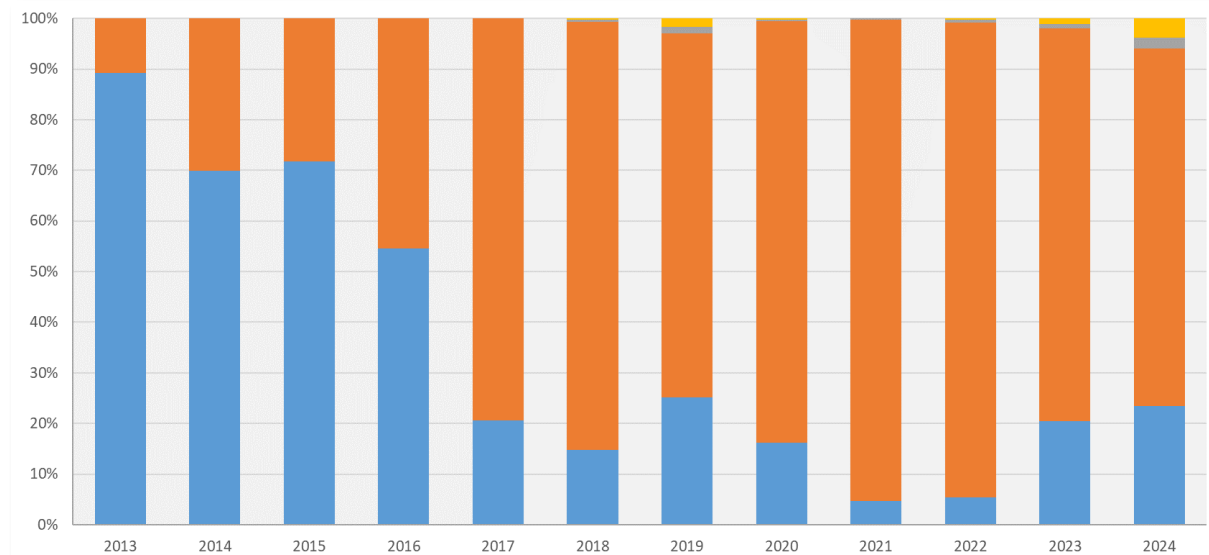
- Support/Facilitate Laboratory  
surveillance activities



Total number of samples sequenced over time at INSA



Total number of samples sequenced over time at INSA by purpose



Illumina MiSeq



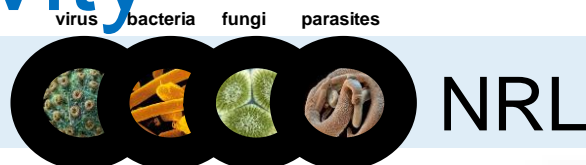
Illumina NextSeq 550



Illumina NextSeq 2000

# Examples of GBU activity

“NEXT GENERATION SEQUENCING” (NGS)



Genomic surveillance of influenza, SARS-CoV-2, and Mpox virus, using the INSaFLU bioinformatics platform

Borges V, Pinheiro M, et al, 2018. *Genome Medicine*, 10:46.  
Borges V, Isidro J, Trovão N et al, 2022, *Commun Medicine* (...)

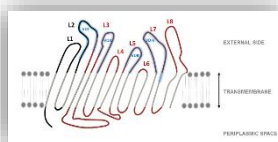
Assisting Diagnosis of Complex Clinical Cases using Metagenomics



Ferreira et al. (in preparation).

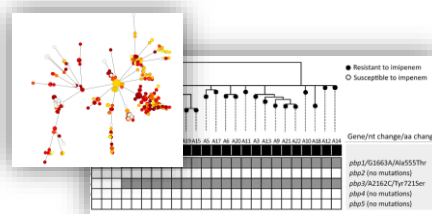
Detection and investigation of outbreaks of:

- Sexually transmitted infections:



Pinto et al., 2016, *Nat Microbiol*  
Borges et al, 2019, *M Genomics*, 5  
Pinto et al, 2021, *M Genomics*, 7  
Borges et al, 2021, *CID*, 73

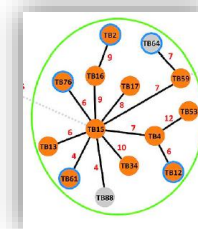
- Food-bourne infections



Llarena et al, 2018, *EFSA*  
Isidro et al, 2018; *EID*, 24:741-745  
Silveira et al, 2019, *IJG*  
Silveira L, Nunes A et al, 20  
Alves F, Nunes A et al., 2022; *Front. Microbiol.* 13:858310

Monitoring dissemination of multi-resistant *Mycobacterium tuberculosis*

Macedo R, Nunes A et al, 2018, *Tuberculosis*, 110:44-51  
Macedo et al, 2019, *Tuberculosis*, 115, 81–88.



First sequencing of *Treponema pallidum* (Syphilis) genome directly from clinical samples

Pinto M, Borges V et al. 2016. *Nature Microbiol.* 2:16190

Investigation of the large outbreak of *Legionella pneumophila*

Borges V, Nunes A et al. 2016. *Sci Reports*; 6:26261  
Correia AM et al. 2016. *N Engl J Med*; 374: 497-8.

(...)

# INSaFLU-TELEVIR platform (<https://insaflu.insa.pt>)

## SOFTWARE

Open Access



INSaFLU: an automated open web-based bioinformatics suite “from-reads” for influenza whole-genome-sequencing-based surveillance

Vitor Borges<sup>1†</sup>, Miguel Pinheiro<sup>2†</sup>, Pedro Pechirra<sup>3</sup>, Raquel Guiomar<sup>3</sup> and João Paulo Gomes<sup>1</sup>

## SOFTWARE

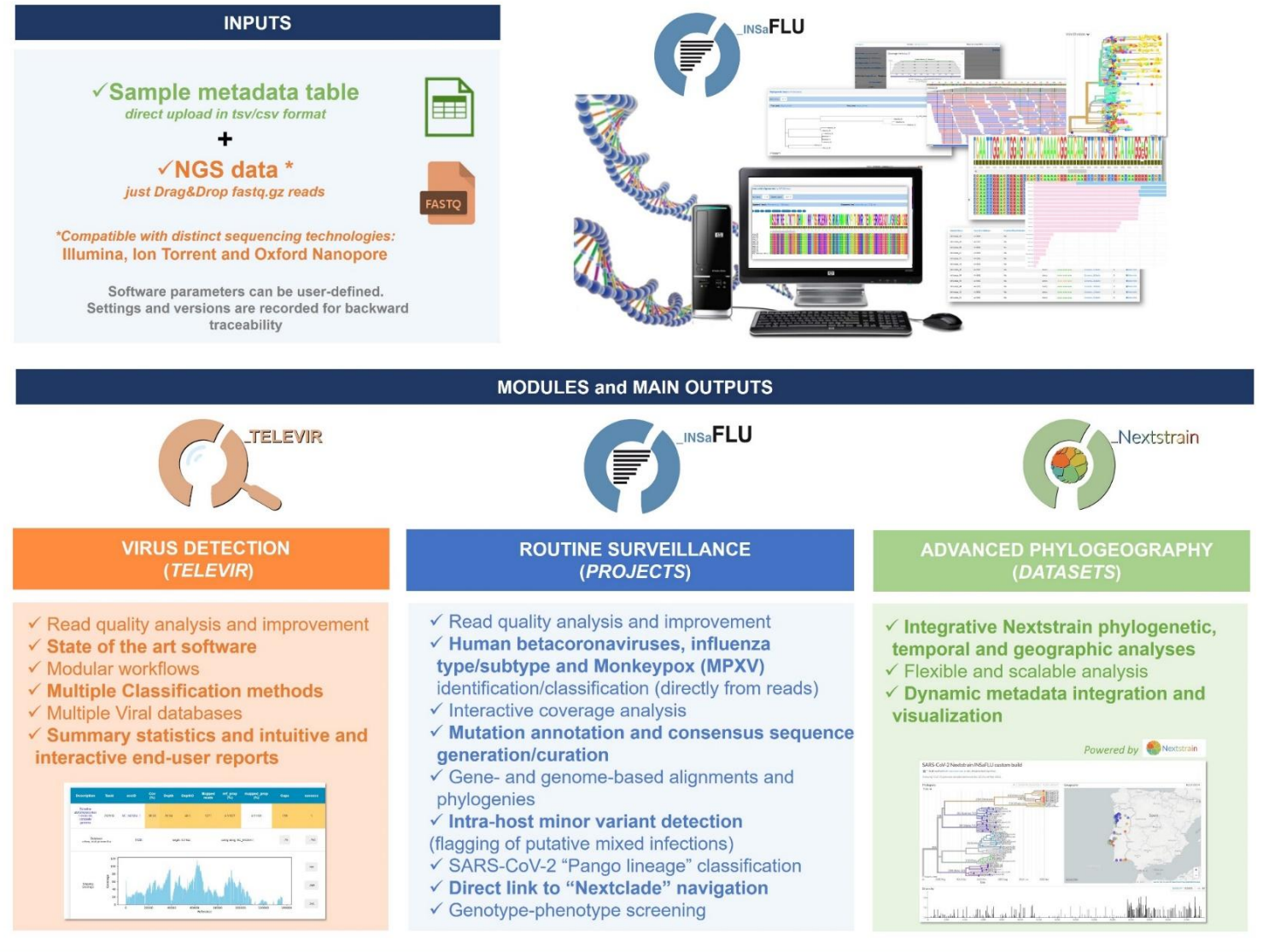
Open Access



INSaFLU-TELEVIR: an open web-based bioinformatics suite for viral metagenomic detection and routine genomic surveillance

João Dourado Santos<sup>1</sup>, Daniel Sobral<sup>1</sup>, Miguel Pinheiro<sup>2</sup>, Joana Isidro<sup>1</sup>, Carlijn Bogaardt<sup>3</sup>, Miguel Pinto<sup>1</sup>, Rodrigo Eusébio<sup>1</sup>, André Santos<sup>1</sup>, Rafael Mamede<sup>4</sup>, Daniel L. Horton<sup>3</sup>, João Paulo Gomes<sup>1,5</sup>, TELEVIR Consortium and Vitor Borges<sup>1\*</sup>

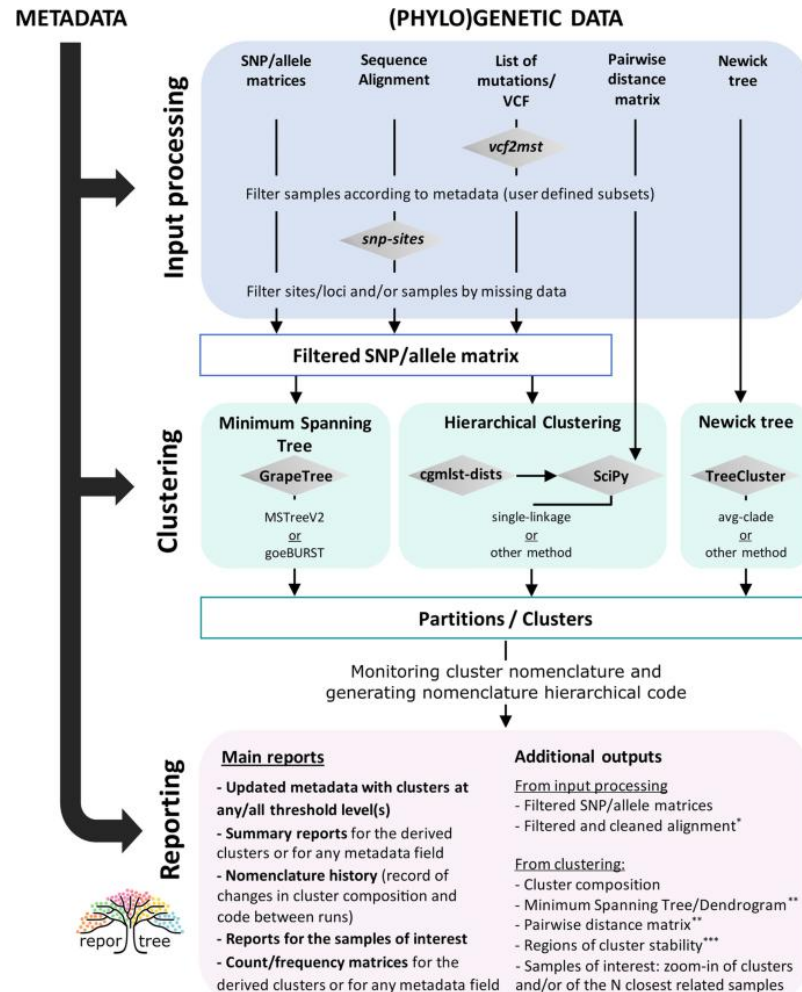
- **Online** (also installable locally)
- **Free** (confidential accounts)
- **User- and surveillance-oriented**
- **Compatible with main sequencing technologies**
- **Multiple features and workflows**







# ReporTree: a surveillance-oriented tool to strengthen the linkage between genetic clusters and epidemiological data



- Identification of genetic clusters at all resolution levels (multiple input formats and clustering methods)
- Surveillance-oriented reports linking to epi data
- Flexible and routine-oriented cluster nomenclature

## Outcomes

- ✓ Key-role in *routine surveillance* and outbreak investigation of bacterial and viral pathogens in Portugal
- ✓ Under implementation in WGS systems dispersed across the world



Mixão et al. (2023)  
Genome Medicine 15:43

Contributes to a **sustainable and efficient** public health genomics-informed pathogen surveillance