



SARS-COV-2 SEQUENCING

WEBINAR GIHSN

Antonin Bal, Gregory Destras, Hadrien Regue, Quentin Semanas, Gwendolyne Burfin, Bruno Simon, Bruno Lina, Laurence Josset

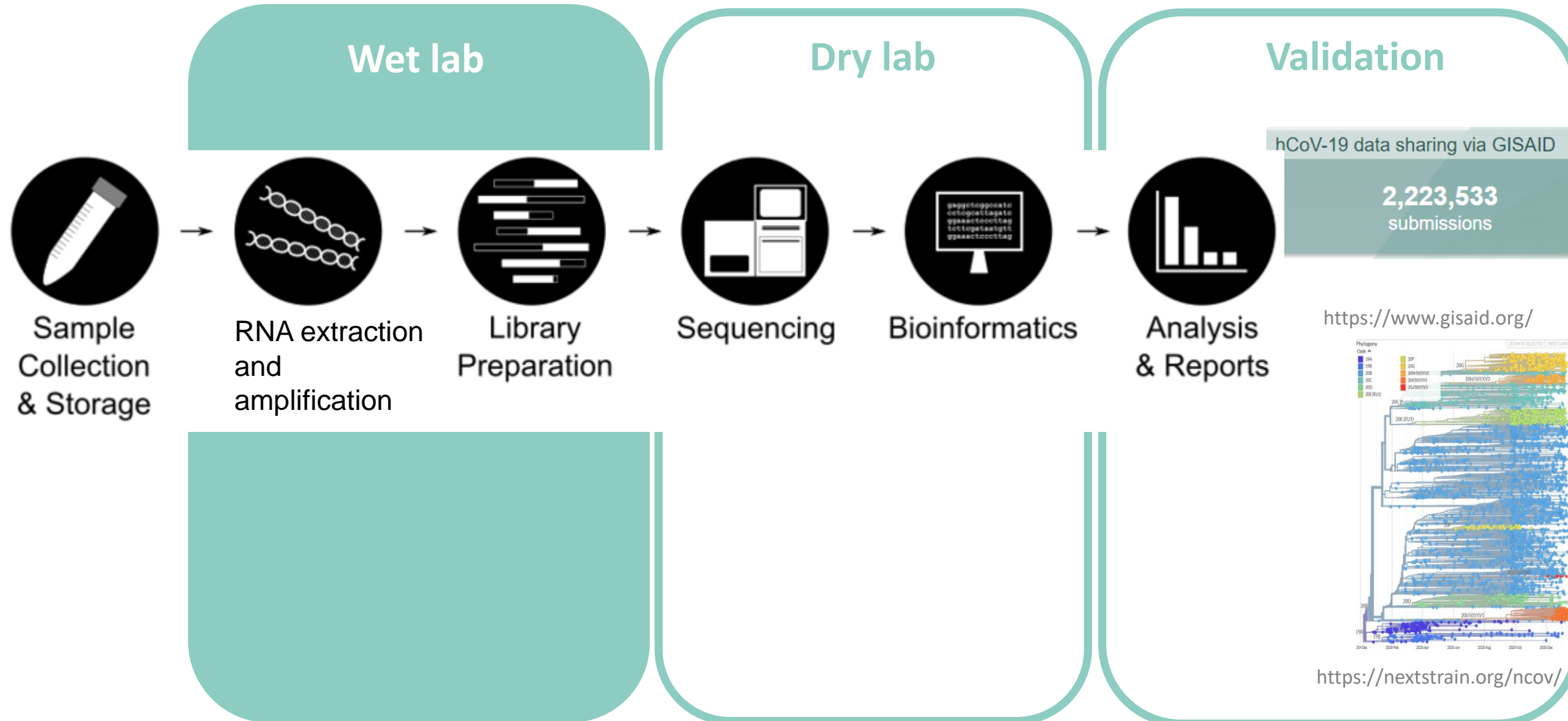
28/01/2021 NGS TEAM- CNR VIRUS RESPIRATOIRES FRANCE SUD

HCL
HOSPICES CIVILS
DE LYON

www.chu-lyon.fr

SARS-COV-2 SEQUENCING

3 STEPS

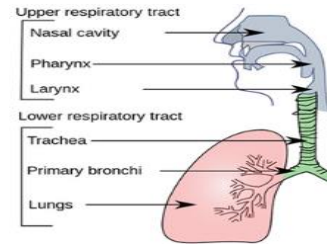


METAGENOMICS-NGS

METAGENOMICS-NGS

Detection of **known** and **unknown** viruses

Universal method for viral detection and whole genome sequencing



Influenza A & B, AdV, CMV, HHV-6, EBV, HBoV, HRV, RSV, PIV, MPV, CoV, Enterovirus, Measles, SARS-CoV-2

Bal et al., BMC Inf Dis, 2018

Extraction

DNase

cDNA
Amplification
(WTA)

Lib prep
(Nextera XT /
DNA prep)

Illumina
Sequencing

February 2020

Molecular characterization of SARS-CoV-2 in the first COVID-19 cluster in France reveals an amino acid deletion in nsp2 (Asp268del)

A. Bal ^{1,2,3,4,*}, G. Destras ^{1,2,3,*}, A. Gaymard ^{1,2,3}, M. Bouscambert-Duchamp ^{1,2}, M. Valette ^{1,2}, V. Escuret ^{1,2,3}, E. Frobert ^{1,2,3}, G. Billaud ^{1,2}, S. Trouillet-Assant ^{3,4}, V. Cheynet ⁴, K. Brengel-Pesce ⁴, F. Morfin ^{1,2,3}, B. Lina ^{1,2,3}, L. Josset ^{1,2,3,*}

PROS AND CONS FOR mNGS

REFERENCE METHOD

- Non-targeted method adapted for new emerging variant detection
- Low sensitivity: $Ct < 20$
- Expensive
- Low throughput
- Time-consuming process

Charre, *Virus Evol*, 2020

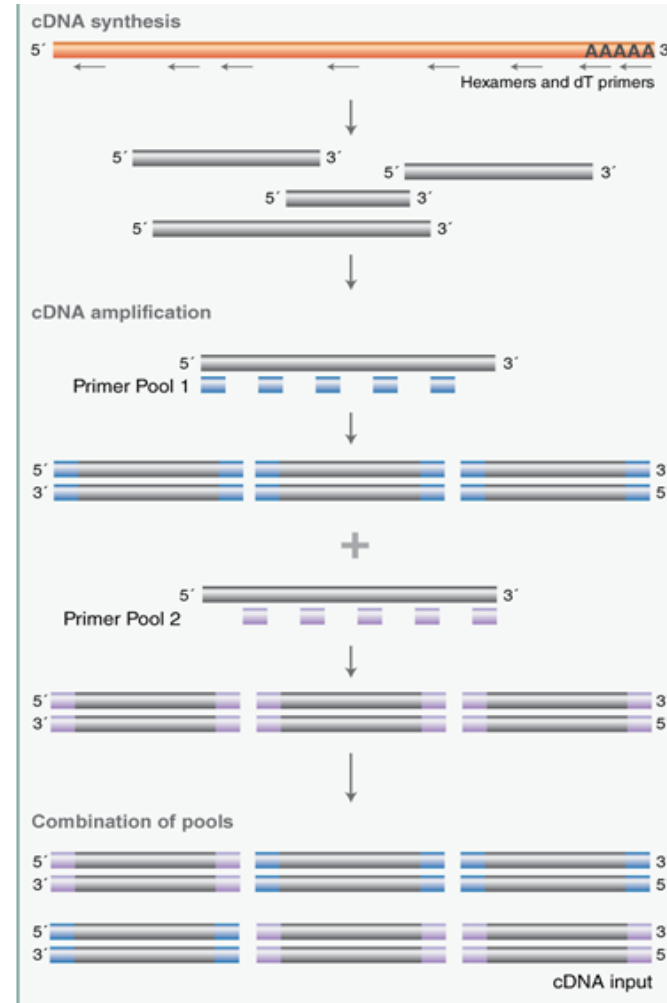
AMPLICON-BASED APPROACHE

AMPLICON-BASED APPROACHE

ARTIC V3, 98 AMPLICONS ~400 BP

1) RNA
extraction
96-Well
plate

2) cDNA
synthesis and
amplification



3) Lib preparation

- Illumina : DNA Prep/ Nextera XT ,
Tagmentation of reads
- ONT: sequencing of 400nt amplicons

4) Illumina / ONT Sequencing

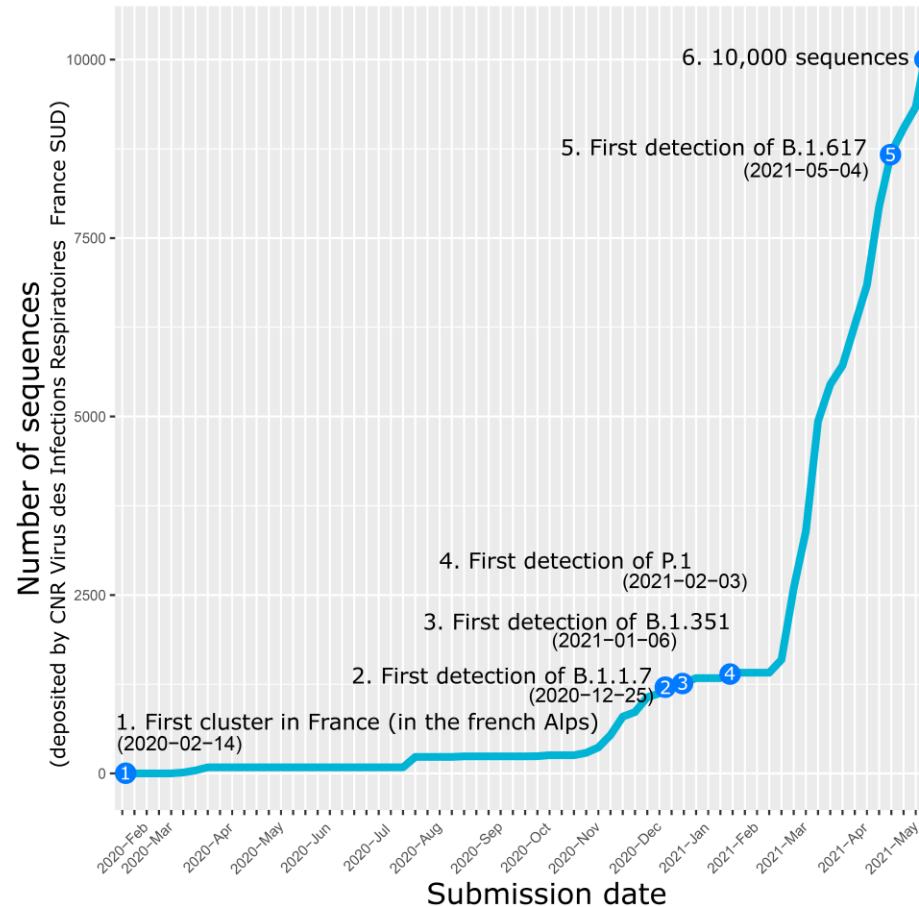
AMPLICON PROTOCOLS DERIVED FROM ARTIC

COMMERCIAL OR IN-HOUSE PROTOCOLS

- COVID-Seq (Illumina)
- QIAseq SARS-CoV2 + FX DNA Lib Prep (Qiagen)
- NEBNext[®] ARTIC SARS-CoV-2 Companion Kit
- Wellcome Sanger Institute

PROS AND CONS FOR AMPLICONS APPROACHES

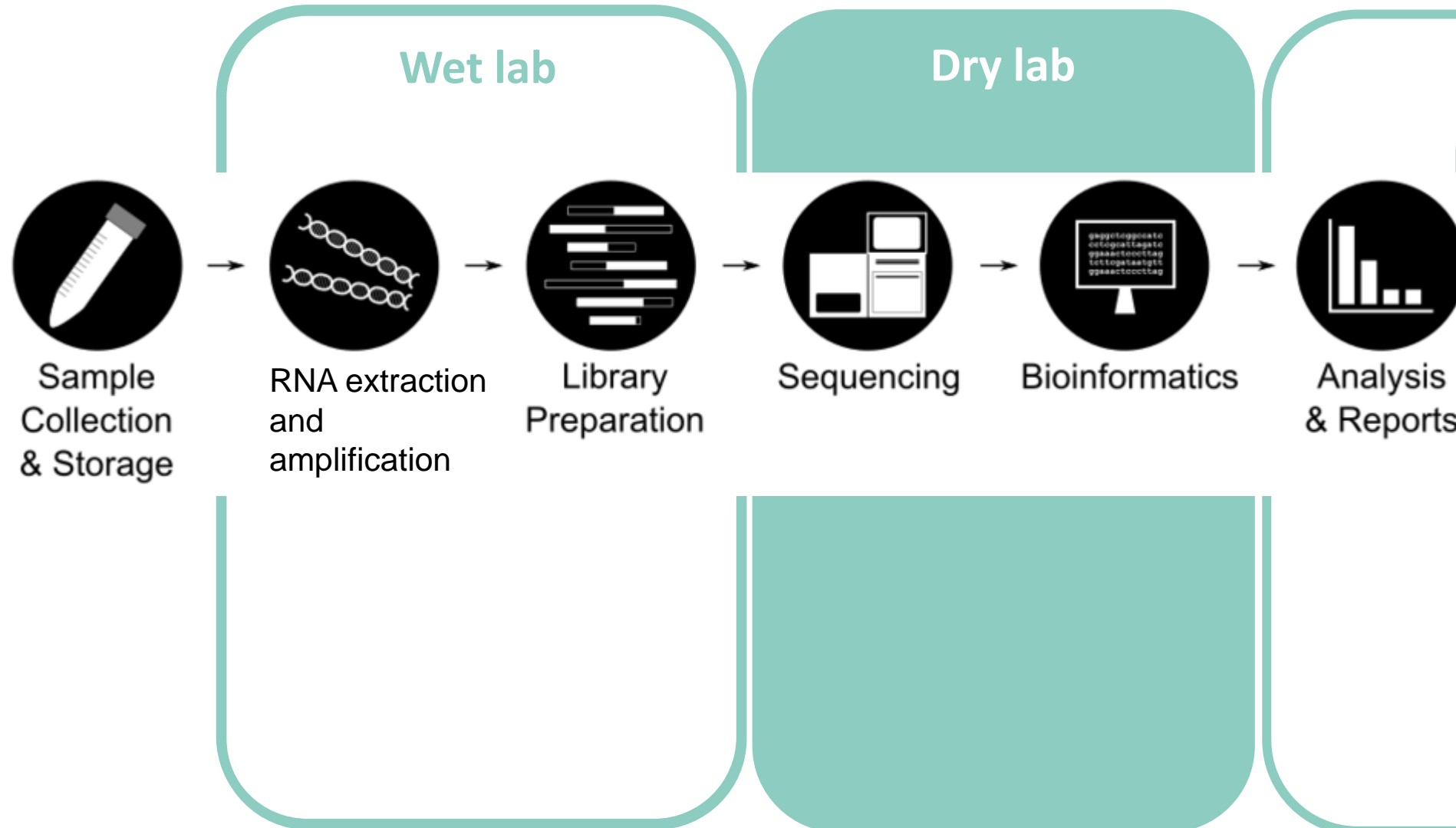
- Sensitivity
- Cost-effective
- High throughput
- Short-time process



- Impacted by little genome variations (SNPs or indels)

SARS-COV-2 SEQUENCING

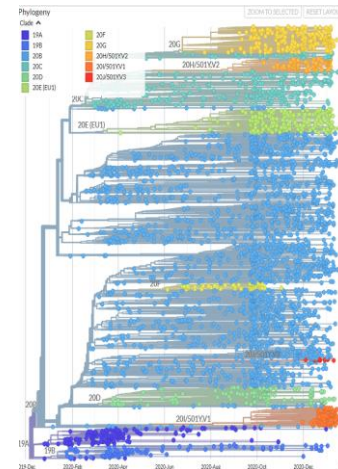
3 STEPS



hCoV-19 data sharing via GISAID

2,223,533
submissions

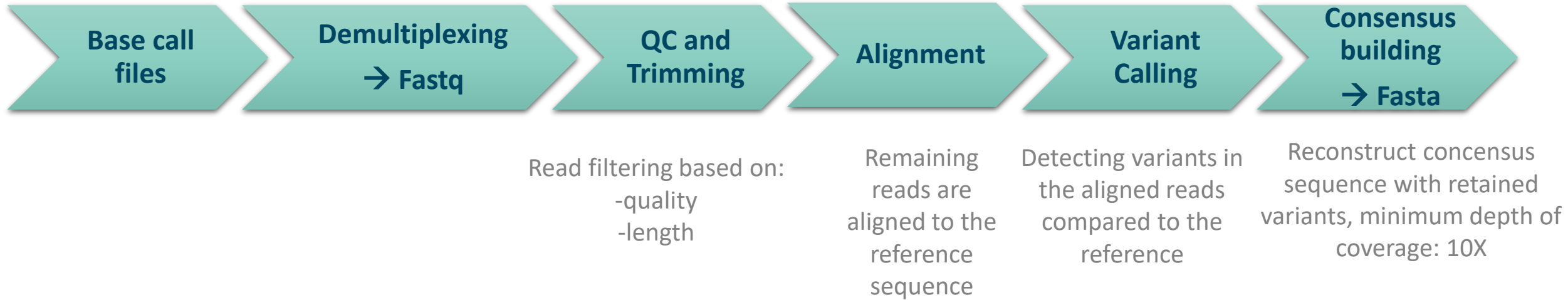
<https://www.gisaid.org/>



<https://nextstrain.org/ncov/>

BIOINFORMATICS

FROM RAW DATA TO THE CONSENSUS SEQUENCE: ILLUMINA SEQUENCING



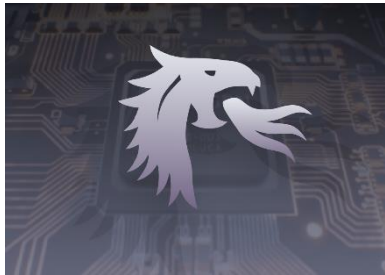
BIOINFORMATICS

OPEN SOURCE AND COMMERCIAL SOLUTIONS

For Illumina users...



« SEQMET », our « in house » pipeline freely available here:
<https://github.com/jossetlab/seqmet>



But see also the the Illumina's cloud solution « DRAGEN »
Illumina can provide online data analysis without bioinformatic skills
<https://basespace.illumina.com/>

... and ONT adepts

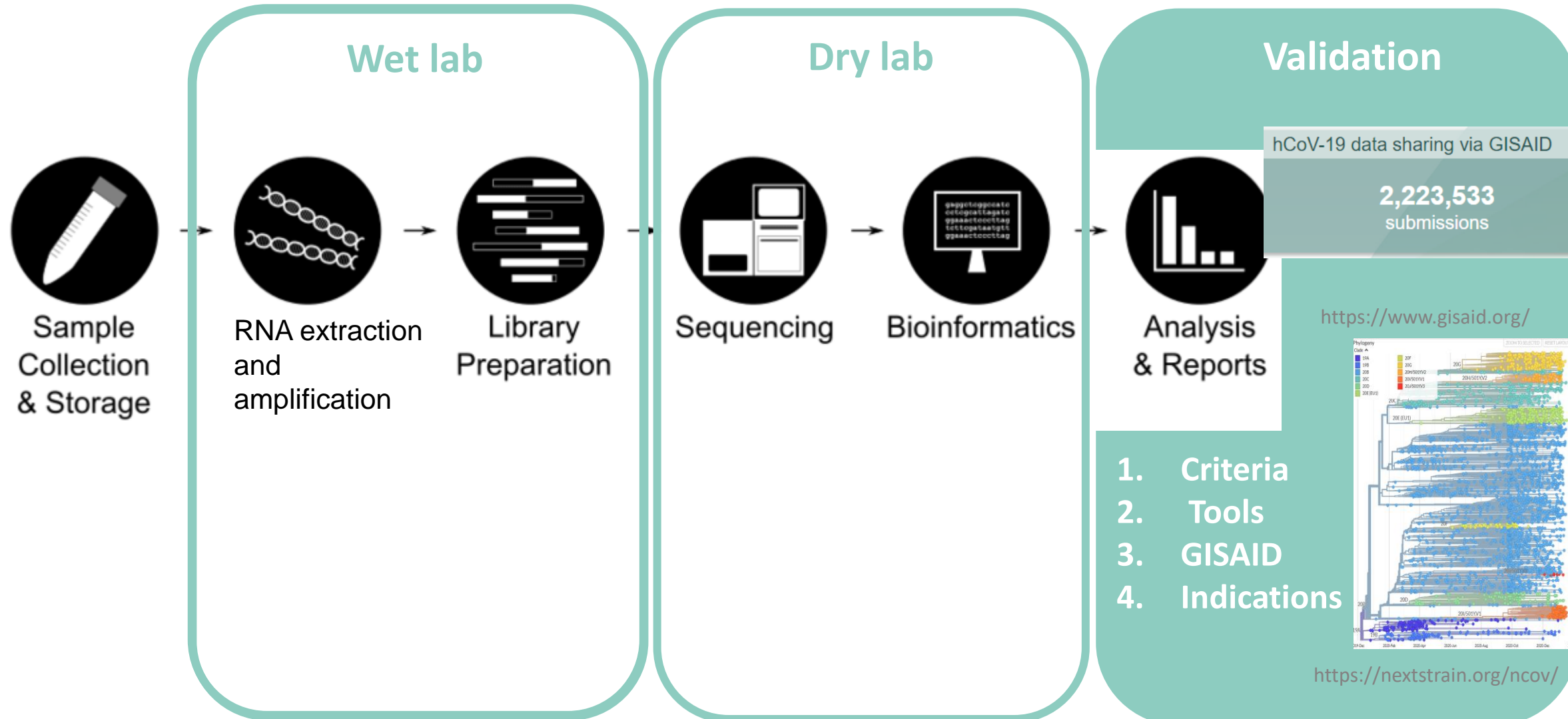
We haven't pipeline dedicated to ONT data analysis
Several analysis workflows are proposed by ONT



<https://labs.epi2me.io/> provide a large analysis panel
(and tutorials) to process online your data

SARS-COV-2 SEQUENCING

3 STEPS



VALIDATION CRITERIA

VALIDATION CRITERIA

NEGATIVE CONTROLS - CONTAMINATION

- **3 No-Template-Controls (NTC)** per 96-well plate
- Nuclease free-water **processed through the whole** workflow (including extraction step)

SARS-CoV-2 genome coverage for NTCs < 5%

VALIDATION CRITERIA

POSITIVE CONTROL

- Viral isolate belonging to **20A clade**
- **Known mutations**
- **One positive control / 96-well plate**

Viral genome coverage for positive control > 90%

VALIDATION CRITERIA

COVERAGE

- Minimal depth of coverage / base for consensus sequence generation: 10X for Illumina sequencing
- Otherwise : **N**

The sequence is validated if the genome coverage is > 90 % with a mean depth of coverage > 200X

VALIDATION CRITERIA

NUMBER AND TYPE OF MUTATIONS

- Divergence (number of mutations): QC nextclade
- Frameshift mutation: CoV-GLUE / QC nextclade
- New mutations: CoV-GLUE
- Atypical set of mutation
- Molecular epidemiology (Clade not circulating in a given area)

Repeat
extraction
+/- mNGS

TOOLS FOR QUALITY CONTROL

NEXTCLADE

[HTTPS://CLADES.NEXTSTRAIN.ORG/](https://clades.nextstrain.org/)

The screenshot shows the Nextclade web application interface. At the top, the browser address bar displays `clades.nextstrain.org`. The Nextclade logo, featuring a colorful circular icon and the text "Nextclade beta", is in the top left. Navigation links include "What's new", a language selector set to "English", and social media icons for Twitter, GitHub, and Docker. The main heading "Nextclade beta" is followed by "v0.12.0" and the tagline "Clade assignment, mutation calling, and sequence quality checks".

Below the heading, there are two main sections. On the left, a grid of four cards describes the application's features:

- Simple**: No installation or setup - drop a file and see the results.
- Private**: No remote processing - sequence data never leaves your computer.
- Mutation Calling**: Find differences of your sequences relative to the reference in standard numbering.
- Clade Assignment**: Find out which Nextstrain clades your samples are from.
- Phylogenetic Placement**: See where on the SARS-CoV-2 tree your sequences fall.
- Quality Control**: Check your data against multiple QC metrics.

On the right, a large interactive panel is shown. It has a dropdown menu set to "SARS-CoV-2" and a toggle switch for "Simple mode" (which is currently turned on). Below this, there are three buttons: "Sequences" (marked as "required"), "From file", "From URL", and "Paste". A large dashed box contains the text "Drag & Drop a file here" and a "Select a file" button. A small icon of a document with "<>" and "FASTA" is also present. At the bottom of this panel is a link "Show me an Example".

At the very bottom, a yellow warning banner states: "Nextclade is currently under active development. Implementation details and data formats are subjects to change. The app may contain bugs. Please report any issues and leave feedback at github.com/nextstrain/nextclade".



Done. Total sequences: 384. Succeeded: 276. Failed: 108



COV-GLUE

[HTTP://COV-GLUE.CVR.GLA.AC.UK/](http://cov-glue.cvr.gla.ac.uk/)

- Listing of known substitutions, insertions and deletions

Sequence	Classification			Primer/probe analysis			Differences from reference
	hCoV-19?	Lineage	Total LWR	Diagnostics issues	Sequencing issues	Full report	
SC719_S95	Yes	B.1.1	96.70%	4	1	View	SNPs: C913T, C3037T, C3267T, C5388A, C5986T, T6954C, C14408T, C14676T, C15279T, T16176C, A23063T, C23271A, A23403G, C23604A, C23709T, T24506G, G24914C, C27972T, G28048T, A28111G, G28280C, A28281T, T28282A, G28881A, G28882A, G28883C, C28977T Known amino acid replacement in nsp3: T183I (Tree) Known amino acid replacement in nsp3: A890D (Tree) Known amino acid replacement in nsp3: I1412T (Tree) Known amino acid replacement in nsp12: P323L (Tree) Known amino acid replacement in S: N501Y (Tree) Known amino acid replacement in S: A570D (Tree) Known amino acid replacement in S: D614G (Tree) Known amino acid replacement in S: P681H (Tree) Known amino acid replacement in S: T716I (Tree) Known amino acid replacement in S: S982A (Tree) Known amino acid replacement in S: D1118H (Tree) Known amino acid replacement in ORF 8: Q27* (Tree) Known amino acid replacement in ORF 8: R52I (Tree) Known amino acid replacement in ORF 8: Y73C (Tree) Known amino acid replacement in N: D3L (Tree) Known amino acid replacement in N: R203K (Tree) Known amino acid replacement in N: G204R (Tree) Known amino acid replacement in N: S235F (Tree) Known codon-aligned deletion in nsp6: codons 106-108 (Tree) Known non-codon-aligned deletion in S: nucleotides 21765-21770 (Tree) Known non-codon-aligned deletion in S: nucleotides 21991-21993 (Tree)

COV-GLUE

[HTTP://COV-GLUE.CVR.GLA.AC.UK/](http://cov-glue.cvr.gla.ac.uk/)

- Genome visualisation tool

Analysis of sequence file 'tours.txt'

Summary

Genome visualisation

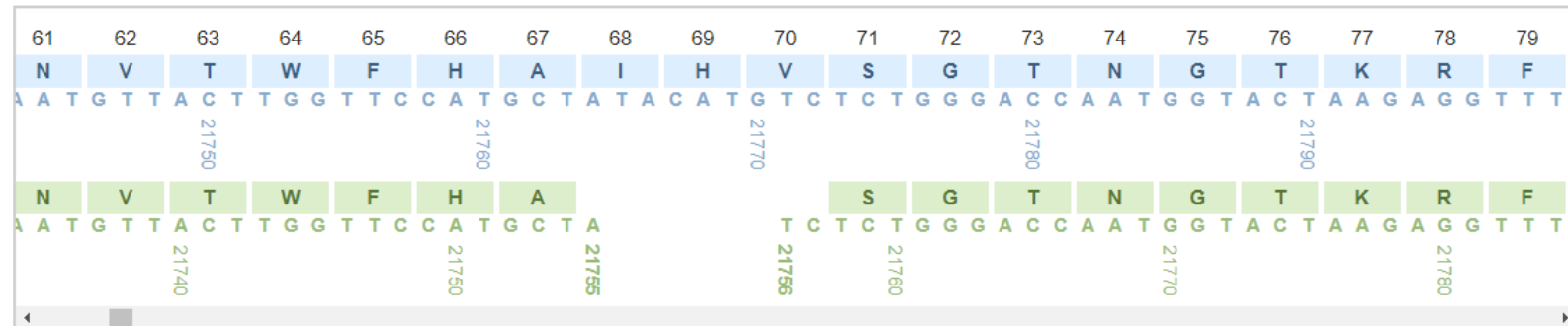
Phylogenetic placement

Download summary ▾

Download details ▾

Visualise coding region: of sequence: (green), highlighting differences with [Wuhan-Hu-1](#) (blue)

Update



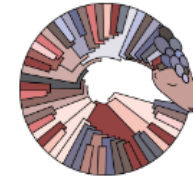
PANGOLIN

[HTTPS://PANGOLIN.CO-UK.IO/](https://pangolin.cog-uk.io/)

Drag and drop fasta file

Select fasta file to upload

Pangolin COVID-19 Lineage Assigner






Phylogenetic Assignment of Named
Global Outbreak LINEages

You can upload one or more sequences by dragging and dropping a (multi)fasta file or clicking "Select fasta file to upload" and selecting a (multi)fasta file.

This Web Application assigns lineages to COVID-19 sequences based on the methodology described in this [article](#)

The software to assign lineages based on the algorithm that was developed by [Áine O'Toole](#), [Verity Hill](#), [JT McCrone](#), [Emily Scher](#) and [Andrew Rambaut](#).

The source code can be found [here](#)

Recommended browsers   or 

GISAID

GISAID

CREATING AN ACCOUNT

The screenshot displays the GISAID website interface. At the top, there is a navigation bar with links: About us, Database Features, Events, Collaborations, References, Registration, and Help. A search bar and a 'Login' button are also present. The main content area is divided into several sections:

- In Focus:** A section titled 'Comment on recent spike protein changes' with a detailed paragraph about viral mutations and their effects. It includes a small 3D molecular model of a spike protein. Below the text are five small circular icons.
- Genomic epidemiology of hCoV-19:** A section featuring a world map and a bar chart showing the distribution of hCoV-19 sequences across different regions.
- hCoV-19 Tracking of Variants:** A section with a world map showing the global distribution of hCoV-19 variants.
- Enabled by hCoV-19 data from GISAID:** A section highlighting the GESS (Genome position / Region / Area / Concurrence search and SNV birth query) tool and the Status of Detection Systems (University of Turin (Italy) Diagnostic detection of 2019-nCoV by realTime).
- hCoV-19 Data Sharing via GISAID:** A section showing a bar chart of '420k submissions' and a table of recent submissions.
- Recent hCoV-19 Data Submissions:** A list of recent submissions, including hCoV-19/Hebei/IVDC-10-01/2021, hCoV-19/Australia/NSW3878/2021, and hCoV-19/Romania/GR-93715/2021.
- Recent Influenza Data Submissions:** A list of recent influenza submissions, including A/turkey/Poland/464/2020(H5N8), A/Guangdong/SF16348/2020, and A/chicken/Netherlands/20019879-001005/2020.

The bottom of the page shows a Windows taskbar with various application icons and a system clock indicating 14:28 on 26/01/2021.

SUBMISSION OF SEQUENCES: BATCH OR PER SEQUENCE

The screenshot displays the GISAID website interface. At the top, the GISAID logo is on the left, and the copyright notice "© 2008 - 2021 | Terms of Use | Privacy Notice | Contact" is on the right. Below the logo, a navigation bar includes "Registered Users", "EpiFlu™", "EpiCoV™", and "My profile". A secondary bar contains icons for "EpiCoV™", "Search", "Downloads", "Upload", and "My Unreleased". The main content area features a headline "Pandemic coronavirus causing COVID-19" with a detailed paragraph about the virus's origin and detection. To the right of the text is a circular phylogenetic tree. Below the text, a section titled "Analysis Update" includes a small image of a virus particle and a link to "Full genome derived from outbreak sequence". Overlaid on the page are two white boxes: the left one, labeled "Single upload", shows a form for uploading a single sequence; the right one, labeled "Batch upload", shows a table for uploading multiple sequences. At the bottom, a row of five cards provides additional analysis tools: "Temporal and regional distribution of clades in the first year", "Spike comparison between pangolin, bat, human", "Spike comparison to SARS and bat precursor", "Highly conserved drug targets between hCoV-19 and SARS", and a button labeled "analysis update.pdf" with a downward arrow icon.

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Registered Users EpiFlu™ EpiCoV™ My profile

EpiCoV™ Search Downloads Upload My Unreleased

Pandemic coronavirus causing COVID-19

A previously unknown human coronavirus (hCoV-19) was first detected in late 2019 in patients in the City of Wuhan, who suffered from respiratory illnesses including atypical pneumonia, an illness that has become known as coronavirus disease (COVID-19). The coronavirus originated from an animal host and is closely related to the virus responsible for the Severe Acute Respiratory Syndrome coronavirus (SARS).

On 10. January 2020, the first virus genomes and associated data were publicly shared via GISAID. The World Health Organization announced on 11. March 2020 the first coronavirus pandemic to the public.

Full genome derived from outbreak sequence

Analysis Update

Temporal and regional distribution of clades in the first year

Spike comparison between pangolin, bat, human

Spike comparison to SARS and bat precursor

Highly conserved drug targets between hCoV-19 and SARS

analysis update.pdf

Single upload

Batch upload

GISAID

FASTA FILE (SEQ CONSENSUS) + EXCEL FILE (METADA)

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You are logged in as **Antonin Bal** - [logout](#)

[Registered Users](#) | [EpiFlu™](#) | **[EpiCoV™](#)** | [My profile](#)

[EpiCoV™](#) | [Search](#) | [Downloads](#) | [Upload](#) | [My Unreleased](#)

GISAID hCoV-19 Batch Upload

Upload genetic sequence as single FASTA-File and metadata, available clinical and epidemiological data, geographical as well as species-specific data as XLS or CSV. Data will be reviewed by a curator prior to release. An email confirmation will be issued upon release.

Metadata as Excel or CSV*

max size: 5M

Choisir un fichier

Aucun fichier choisi

Sequences as FASTA*

max size: 32M

Choisir un fichier

Aucun fichier choisi

Report

Upload XLS/CSV and FASTA.

Download Instructions and Template

Contact Curator

Check and Submit

GISAID

EXCEL FILE (METADA) = MANDATORY

Column information		
Submitter	mandatory	enter your GISAID-Username
FASTA filename	mandatory	the filename that contains the sequence without path (e.g. all_sequences.fasta <u>not</u> c:\users\meier\docs\all_sequences.fasta)
Virus name	mandatory	e.g. hCoV-19/Netherlands/Gelderland-01/2020 (Must be FASTA-Header from the FASTA file all_sequences.fasta)
Type	mandatory	default must remain "betacoronavirus"
Passage details/history	mandatory	e.g. Original, Vero
Collection date	mandatory	Date in the format YYYY or YYYY-MM or YYYY-MM-DD
Location	mandatory	e.g. Europe / Germany / Bavaria / Munich
Additional location information		e.g. Cruise Ship, Convention, Live animal market
Host	mandatory	e.g. Human, Environment, Canine, Manis javanica, Rhinolophus affinis, etc
Additional host information		e.g. Patient infected while traveling in
Sampling Strategy		e.g. Sentinel surveillance (ILI), Sentinel surveillance (ARI), Sentinel surveillance (SARI), Non-sentinel-surveillance (hospital), Non-sentinel-surveillance (GP network), Longitudinal sampling on same patient(s), S gene dropout
Gender	mandatory	Male, Female, or <i>unknown</i>
Patient age	mandatory	e.g. 65 or 7 months, or <i>unknown</i>
Patient status	mandatory	e.g. Hospitalized, Released, Live, Deceased, or <i>unknown</i>
Specimen source		e.g. Sputum, Alveolar lavage fluid, Oro-pharyngeal swab, Blood, Tracheal swab, Urine, Stool, Cloakal swab, Organ, Feces, Other
Outbreak		Date, Location e.g. type of gathering, Family cluster, etc.
Last vaccinated		provide details if applicable
Treatment		Include drug name, dosage
Sequencing technology	mandatory	e.g. Illumina Miseq, Sanger, Nanopore MinION, Ion Torrent, etc.
Assembly method		e.g. CLC Genomics Workbench 12, Geneious 10.2.4, SPAdes/MEGAHIT v1.2.9, UGENE v. 33, etc.
Coverage		e.g. 70x, 1,000x, 10,000x (average)
Originating lab	mandatory	Where the clinical specimen or virus isolate was first obtained
Address	mandatory	
Sample ID given by the originating laboratory		
Submitting lab	mandatory	Where sequence data have been generated and submitted to GISAID
Address	mandatory	
Sample ID given by the submitting laboratory		
Authors	mandatory	a comma separated list of Authors with complete First followed by Last Name
Comment	leave empty	do not use this column
Comment text	leave empty	do not use this column

GISAID: VIRUS NAME FOR GIHSN SEQUENCES

Example

hCoV-19/Ukraine/GIHSN-021073674801/2021

How can I find my sequence and the accession number?

GISAID

You are logged in as **Antonin Bal** - [logout](#)

Registered Users | EpiFlu™ | **EpiCoV™** | My profile

EpiCoV™ Search Downloads Upload

Search

Accession ID Virus name ☐ complete ☐ high coverage

Location Host ☐ low coverage excl ☐ w/Patient status





Collection to Submission to ☐ collection date compl

Clade Lineage Substitutions Variants












GISAID: VIRUS NAME FOR GIHSN SEQUENCES

How can I find my sequence and the accession number?

[Registered Users](#) [EpiFlu™](#) [EpiCoV™](#) [My profile](#)




 [EpiCoV™](#)  [Search](#)  [Downloads](#)  [Upload](#)

Search

<input checked="" type="checkbox"/>	Virus name	Passage de	Accession ID	Collection da	Submission D		Length	Host	Location	Originating
<input checked="" type="checkbox"/>	hCoV-19/France/ARA-HCL990000346767/2	Original	EPI_ISL_2676804	2021-06-17	2021-06-25		29 763	Human	Europe / France	CNR Vir
<input checked="" type="checkbox"/>	hCoV-19/France/ARA-HCL990000346728/2	Original	EPI_ISL_2676803	2021-06-16	2021-06-25		29 763	Human	Europe / France	CNR Vir
<input checked="" type="checkbox"/>	hCoV-19/France/ARA-HCL990000346540/2	Original	EPI_ISL_2676802	2021-06-17	2021-06-25		29 769	Human	Europe / France	CNR Vir
<input checked="" type="checkbox"/>	hCoV-19/France/ARA-HCL990000343952/2	Original	EPI_ISL_2676801	2021-06-12	2021-06-25		29 769	Human	Europe / France	CNR Vir
<input checked="" type="checkbox"/>	hCoV-19/France/ARA-HCL990000341939/2	Original	EPI_ISL_2676800	2021-06-17	2021-06-25		29 769	Human	Europe / France	CNR Vir
<input checked="" type="checkbox"/>	hCoV-19/France/ARA-HCL990000341874/2	Original	EPI_ISL_2676799	2021-06-17	2021-06-25		29 769	Human	Europe / France	CNR Vir
<input checked="" type="checkbox"/>	hCoV-19/France/ARA-HCL990000340768/2	Original	EPI_ISL_2676798	2021-06-15	2021-06-25		29 763	Human	Europe / France	CNR Vir
<input checked="" type="checkbox"/>	hCoV-19/France/ARA-HCL990000295086/2	Original	EPI_ISL_2676797	2021-06-14	2021-06-25		29 763	Human	Europe / France	CNR Vir
<input checked="" type="checkbox"/>	hCoV-19/France/ARA-HCL990000281949/2	Original	EPI_ISL_2676796	2021-06-05	2021-06-25		29 763	Human	Europe / France	CNR Vir
<input checked="" type="checkbox"/>	hCoV-19/France/ARA-HCL121013879201/2	Original	EPI_ISL_2676795	2021-06-16	2021-06-25		29 763	Human	Europe / France	CNR Vir

Total: 11,632 viruses

<< < 1 2 3 4 5 > >>

 [Select](#)  [Analysis](#)  [Download](#)

GISAID: VIRUS NAME FOR GIHSN SEQUENCES

How can I find my sequence and the accession number?

The screenshot shows the GISAID EpiCoV interface. At the top, there are navigation tabs: 'Registered Users', 'EpiFlu™', 'EpiCoV™', and 'My profile'. Below these are icons for 'EpiCoV™', 'Search', 'Downloads', and 'Upload'. A search bar contains the text 'CNR Virus des Infections res'. A 'Download' modal window is open, showing options for the download format:

- ☒ Sequences (FASTA)
- ☐ Patient status metadata
- ☐ Sequencing technology metadata
- ☐ Dates and Location
- ☐ Input for the Augur pipeline
- ☐ Acknowledgement (Supplemental table)
- ☒ Replace spaces with underscores in FASTA header

At the bottom of the modal are 'Back' and 'Download' buttons. The background shows a table of search results with columns: Virus name, Accession number, Date, and Location. The table lists several hCoV-19 sequences from France, all originating from CNR Virus. The bottom of the interface shows a pagination bar with 'Total: 11,632 viruses' and a 'Select' button.

STORAGE / SHIPMENT

STORAGE / SHIPMENT TO OUR LAB

- RNA stored at -80°C until shipment
- Ct<28, 50 µl min
- Metadata excel file for GISAID Submission and recording : mandatory


Column information		
Submitter	mandatory	enter your GISAID-Username
FASTA filename	mandatory	the filename that contains the sequence without path (e.g. all_sequences.fasta not c:\users\meier\docs\all_sequences.fasta)
Virus name	mandatory	e.g. hCoV-19/Netherlands/Gelderland-01/2020 (Must be FASTA-Header from the FASTA file all_sequences.fasta)
Type	mandatory	default must remain "betacoronavirus"
Passage details/history	mandatory	e.g. Original, Vero
Collection date	mandatory	Date in the format YYYY or YYYY-MM or YYYY-MM-DD
Location	mandatory	e.g. Europe / Germany / Bavaria / Munich
Additional location information		e.g. Cruise Ship, Convention, Live animal market
Host	mandatory	e.g. Human, Environment, Canine, Manis javanica, Rhinolophus affinis, etc
Additional host information		e.g. Patient infected while traveling in
Sampling Strategy		e.g. Sentinel surveillance (ILI), Sentinel surveillance (ARI), Sentinel surveillance (SARI), Non-sentinel-surveillance (hospital), Non-sentinel-surveillance (GP network), Longitudinal sampling on same patient(s), S gene dropout
Gender	mandatory	Male, Female, or unknown
Patient age	mandatory	e.g. 65 or 7 months, or unknown
Patient status	mandatory	e.g. Hospitalized, Released, Live, Deceased, or unknown
Specimen source		e.g. Sputum, Alveolar lavage fluid, Oro-pharyngeal swab, Blood, Tracheal swab, Urine, Stool, Cloacal swab, Organ, Feces, Other
Outbreak		Date, Location e.g. type of gathering, Family cluster, etc.
Last vaccinated		provide details if applicable
Treatment		Include drug name, dosage
Sequencing technology	mandatory	e.g. Illumina Miseq, Sanger, Nanopore MinION, Ion Torrent, etc.
Assembly method		e.g. CLC Genomics Workbench 12, Geneious 10.2.4, SPAdes/MEGAHIT v1.2.9, UGENE v. 33, etc.
Coverage		e.g. 70x, 1,000x, 10,000x (average)
Originating lab	mandatory	Where the clinical specimen or virus isolate was first obtained
Address	mandatory	
Sample ID given by the originating laboratory		
Submitting lab	mandatory	Where sequence data have been generated and submitted to GISAID
Address	mandatory	
Sample ID given by the submitting laboratory		
Authors	mandatory	a comma separated list of Authors with complete First followed by Last Name
Comment	leave empty	do not use this column
Comment 2	leave empty	do not use this column

STORAGE / SHIPMENT

- Shipment organized by world courier
 - File need to be completed and send to World courier
 - They will take in charge the shipment :
 - Provide the boxes
 - Provide the dry ice

HCL SAMPLES PROCESSED :

- LEBANON: RNA – 200 SAMPLES SENT – SEQUENCING OK
- UKRAINE : RNA – 43 SAMPLES SENT – SEQUENCING IN PROGRESS

 **World Courier®**
AmerisourceBergen

Customer Order Form

Account#: _____ Account Name: _____
Billing Reference Number (if applicable): _____

Your Name: _____ Your Phone#: _____
Your E-mail: _____
Would you like to receive automatic email alerts? ☐ Order Entry: ☐ Pick-up: ☐ Delivery: ☐

Pick-up From:	Company Name	Deliver To:	Company Name
	Contact Name		Contact Name
	Street Address		Street Address
	Street Address		Street Address
	City, State/Province, Zip Country		City, State/Province, Zip Country
	Contact Phone Number		Contact Phone Number
	Contact E-mail		Contact Email

Auto Alerts? ☐ Order Entry: ☐ Pick-up: ☐ Delivery: ☐ Auto Alerts? ☐ Order Entry: ☐ Pick-up: ☐ Delivery: ☐

Pick-up Date: _____ Time: _____ (leave blank to just pre-advise paperwork)

Product Description: _____
Hazardous? ☐ YES ☐ NO If Yes: UN# _____ Class: _____
Number of Pieces: _____ * Total Weight: _____ Dimensions: _____ in _____ cm
Value for Customs: _____ Incoterms: _____

Shipments over \$2500 or shipments subject to export license require EEI/SED.
If you would like WC to file the SED for you, please fill out the Shipper's Letter of Instructions section below.
If you will file your own SED, please advise the ITN#: _____

SHIPPER'S LETTER OF INSTRUCTIONS:
This section should only be completed if you would like WC to file the SED on your behalf.

Type of transaction: ☐ Routed ☐ Non-routed
Parties to transaction: ☐ Related ☐ Non-related
Ultimate Consignee Type: ☐ Direct Consumer ☐ Government entity ☐ Reseller ☐ Other/Unknown
License Value: _____
U.S. Principal Party of Interest (USPPI): _____ Foreign Principal Party of Interest: _____

SARS-COV-2 SEQUENCING INDICATIONS

SARS-COV-2 SEQUENCING INDICATIONS

- Genomic surveillance: detect and monitor new variant of concern
- Cluster investigation
- Targeted sequencing of SARS-CoV-2 cases from special settings or populations
 - Vaccine breakthrough infections (COVID-IVAC Study) and reinfections
 - Immunocompromised patients : detecting new variant and treatment monitoring

CONCLUSION :

- Long +/- manual process = potential errors at all steps
- Validation criteria must be respected
- Need to be critical: re-sequence + possible control by mNGS
- Sharing of sequences on GISAID (date + location)
- Random genomic surveillance / targeted sequencing
- Quality of sequencing (wet, dry, validation) = crucial for analysis and interpretation

MERCI

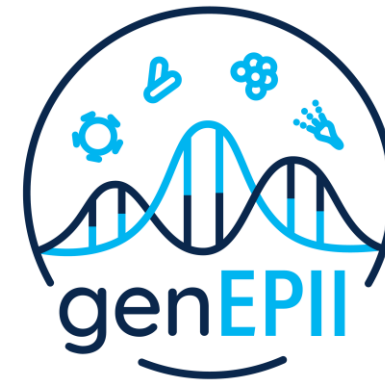
Respiratory virus National Reference Center (NRC) :

NGS team

- Dr Laurence Josset
- Dr Antonin Bal
- Dr Grégory Destras
- Dr Bruno Simon
- Grégory Quéromès (PhD Student)
- Hadrien Regue (Bioinformatician)
- Gwendolyne Burfin (technician)
- Quentin Semanas (technician)

Pr Florence Morfin
Dr Alexandre Gaymard
Dr Emilie Frobert
Dr Martine Valette
Pr Bruno Lina

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**GENomique
EPIdémiologique
des maladies
Infectieuses**

Public resources for SARS-CoV-2 genome analysis :

- **GISAID**
- **NextStrain**
- **COV-GLUE**

HCL
**HOSPICES CIVILS
DE LYON**