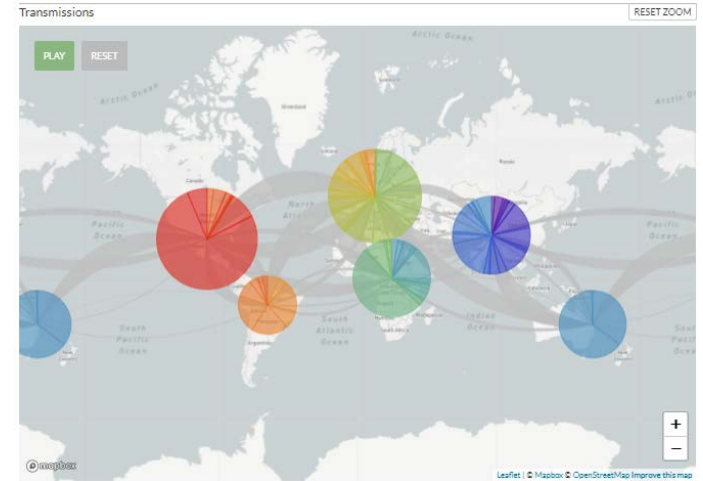


Variantes del SARS-CoV-2



Conversatorio

«Vacunas, alternativas terapéuticas, variantes del SARS-CoV-2 y el rol del laboratorio de Bioseguridad Nivel 3»

Facultad de Ciencias de la Salud

UCA-Campus Itapua

Magaly Martínez

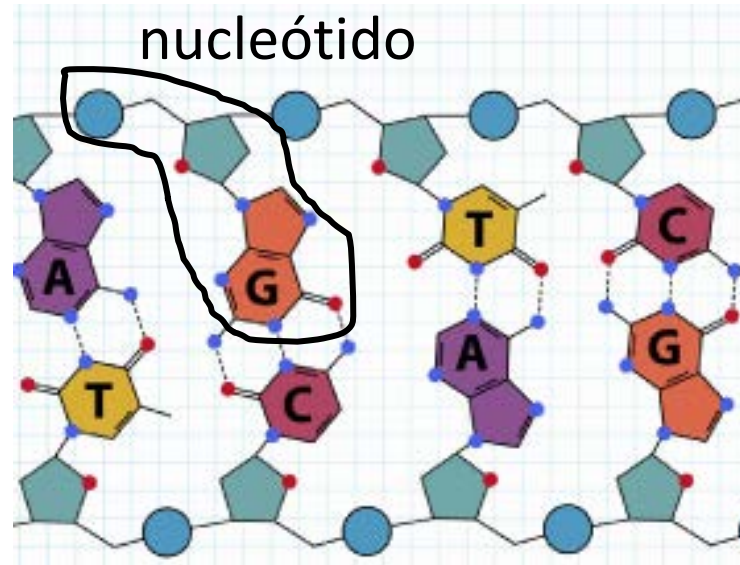
IICS-UNA

Abril 2021

¿Qué hacemos al secuenciar?



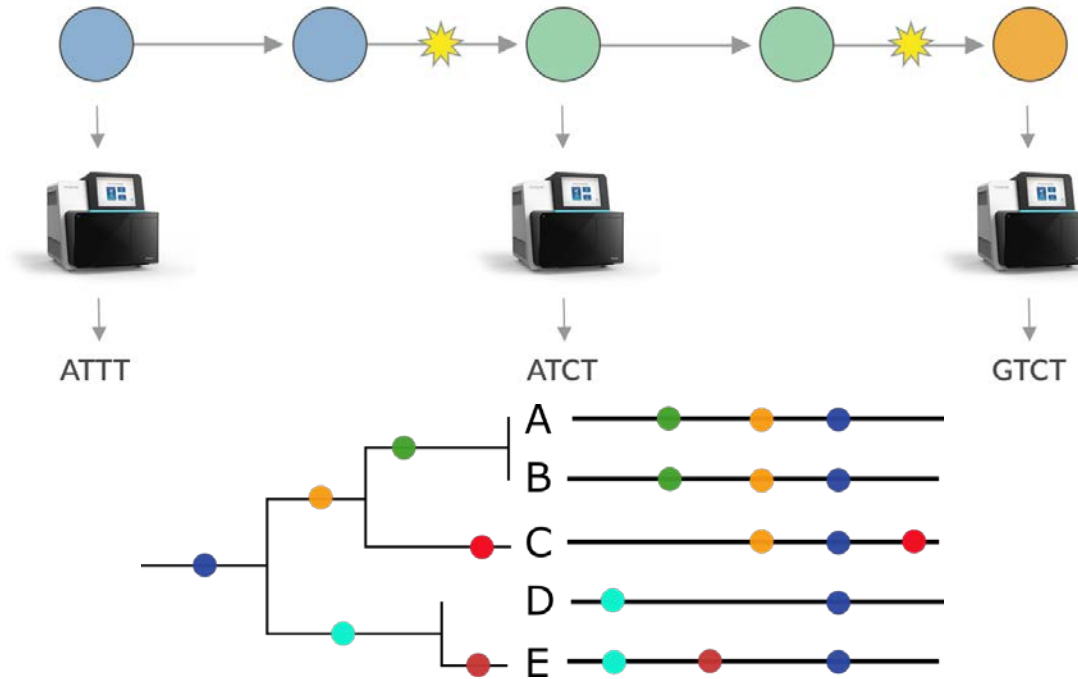
This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis



nucleótido

¿Qué hacemos al analizar la secuencia del genoma de un virus?

Los virus mutan rápidamente y acumulan cambios a medida que se transmiten de un hospedero a otro



La primera respuesta que dio la genómica al inicio de la pandemia:

¿Ante que patógeno estamos?



Novel 2019 coronavirus genome

SARS-CoV-2 coronavirus



edward_holmes

6 Jan '20

10th January 2020

This posting is communicated by Edward C. Holmes, University of Sydney on behalf of the consortium led by Professor Yong-Zhen Zhang, Fudan University, Shanghai

The Shanghai Public Health Clinical Center & School of Public Health, in collaboration with the Central Hospital of Wuhan, Huazhong University of Science and Technology, the Wuhan Center for Disease Control and Prevention, the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control, and the University of Sydney, Sydney, Australia is releasing a coronavirus genome from a case of a respiratory disease from the Wuhan outbreak. The sequence has also been deposited on GenBank ([accession MN908947](#) **29.5k**) and will be released as soon as possible.

Update: [This genome is now available on GenBank and an updated version has been posted](#) **29.5k**.

Disclaimer:

Please feel free to download, share, use, and analyze this data. We ask that you communicate with us if you wish to publish results that use these data in a journal. If you have any other questions –then please also contact us directly.

Professor Yong-Zhen Zhang,
Shanghai Public Health Clinical Center & School of Public Health,
Fudan University,
Shanghai, China.

email: zhangyongzhen@shphc.org.cn

<https://virological.org/t/novel-2019-coronavirus-genome/319>

GenBank Send to

Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome

GenBank: MN908947.3

[FASTA](#) [Graphics](#)

Go to:

LOCUS MN908947 29903 bp ss-RNA linear VRL 18-MAR-2020

DEFINITION Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome.

ACCESSION MN908947

VERSION MN908947.3

KEYWORDS -

SOURCE Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

ORGANISM *Severe acute respiratory syndrome coronavirus 2*
Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
Nidovirales; Coronavirineae; Coronaviridae; Orthocoronavirinae;
Betacoronavirus; Sarbecovirus.

REFERENCE 1 (bases 1 to 29903)

AUTHORS Wu, P., Zhao, S., Yu, B., Chen, Y. H., Wang, W., Song, Z. G., Wu, Y.,
Tao, Z. W., Tian, J. H., Pei, Y. V., Yuan, H. L., Zhang, Y. L., Dai, F. H.,
Liu, Y., Wang, Q. H., Zheng, J. J., Xu, L., Holmes, E. C. and Zhang, Y. Z.

TITLE A new coronavirus associated with human respiratory disease in China

TOUCHSTAMP 170 / 73281 365,360 (3836)



Five new genomes have been deposited in the GISAID platform:

<https://gisaid.org/CoV2020> 3.6k

Newly discovered betacoronavirus, Wuhan 2019-2020

A previously unknown betacoronavirus was detected in patients during an outbreak of respiratory illnesses, including atypical pneumonia, that started mid-December 2019 in the city of Wuhan, the capital of Central China's Hubei Province.

The newly discovered coronavirus is similar to some of the betacoronaviruses detected in bats, but it is distinct from SARS-CoV and MERS-CoV.

The genome of the newly discovered CoV consists of a single, positive-stranded RNA that is approximately 30k nucleotides long. The overall genome organization of the newly discovered CoV is similar to that of other coronaviruses. The newly sequenced virus genome encodes the open reading frames (ORFs) common to all betacoronaviruses, including ORF1ab that encodes many enzymatic proteins, the spike-surface glycoprotein (S), the small envelope protein (E), the matrix protein (M), and the nucleocapsid protein (N), as well as several nonstructural proteins.

Virus name	Accession ID	Passage det	Collection date	Host	Originating lab
BetaCoV/Wuhan/IVDC-HB-01/2019	EPI_ISL_402119	Virus Isolate	2019-12-30	Human	National Institute for Viral Disease Control and Prevention, China CDC
BetaCoV/Wuhan/IVDC-HB-04/2020	EPI_ISL_402120	Original	2020-01-01	Human	National Institute for Viral Disease Control and Prevention, China CDC
BetaCoV/Wuhan/IVDC-HB-05/2019	EPI_ISL_402121	Original	2019-12-30	Human	National Institute for Viral Disease Control and Prevention, China CDC
BetaCoV/Wuhan/IPBCAMS-WH-01/2019	EPI_ISL_402123	Original	2019-12-24	Human	Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Pel
BetaCoV/Wuhan/WIV04/2019	EPI_ISL_402124	Original	2019-12-30	Human	Wuhan Jinyintan Hospital

NEWS RELEASE 31-JAN-2020

Institut Pasteur sequences the whole genome of the Wuhan coronavirus, 2019-nCoV

INSTITUT PASTEUR



PRINT E-MAIL

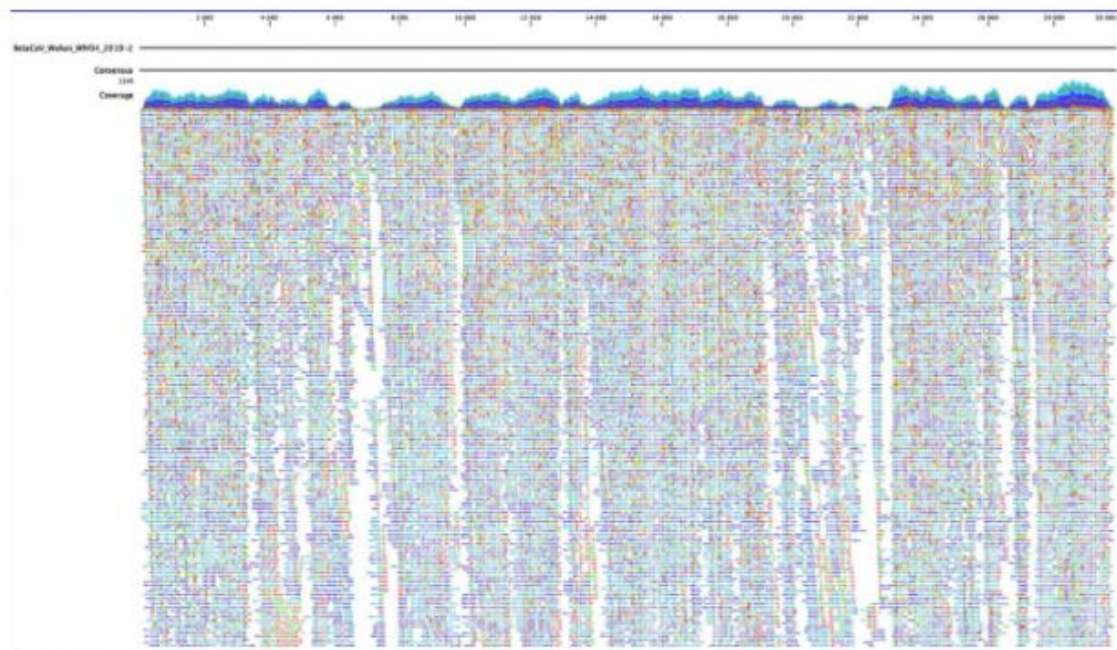
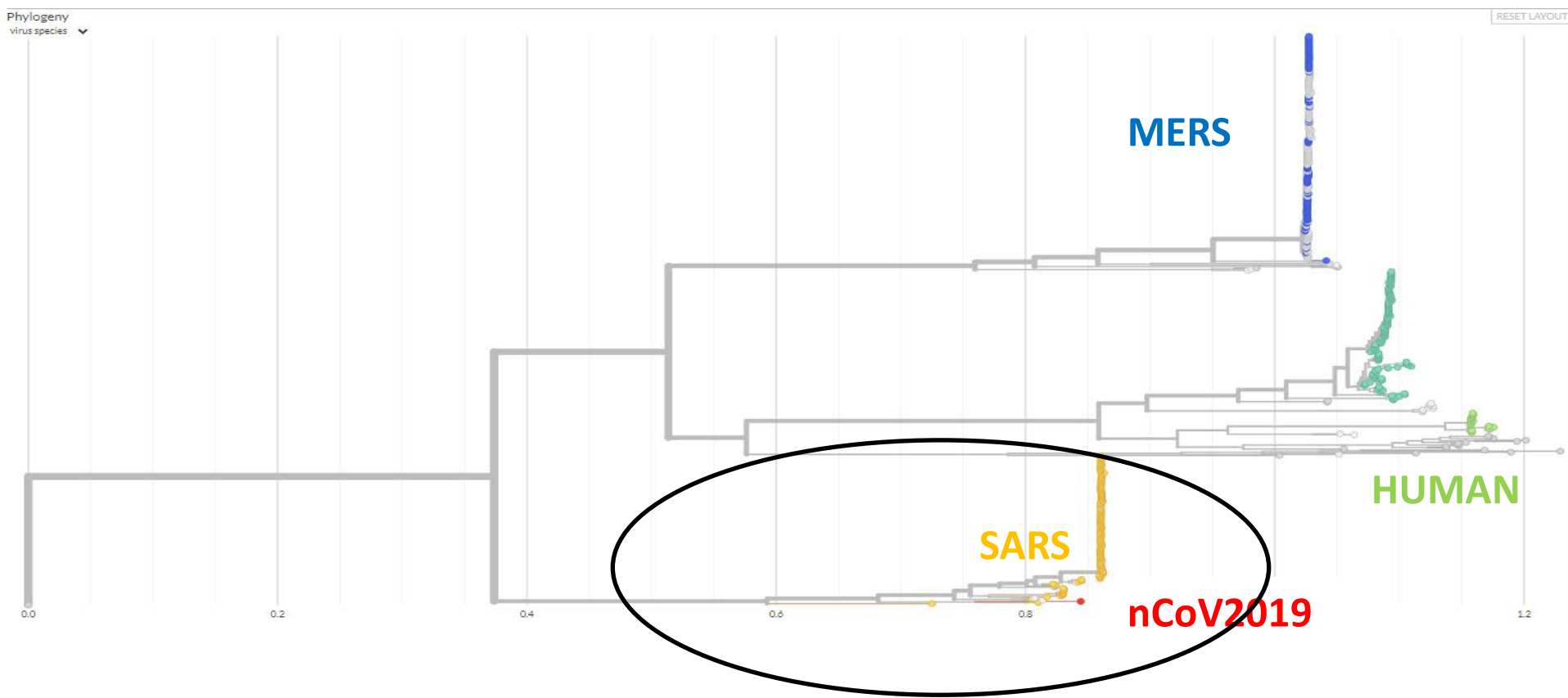


IMAGE: WHOLE GENOME SEQUENCE OF THE 2019-NCOV CORONAVIRUS, IN ONE OF THE FIRST FRENCH CASES, MADE AT THE INSTITUT PASTEUR (PARIS), USING A UNIQUE PLATFORM (P2M), OPEN TO ALL FRENCH NATIONAL... [view more >](#)

Genetic diversity of betacoronaviruses including novel coronavirus (nCoV)

Built with github.com/blab/beta-cov. Maintained by Trevor Bedford.



Que nos puede responder la epidemiología:

a Detection



b Snapshot



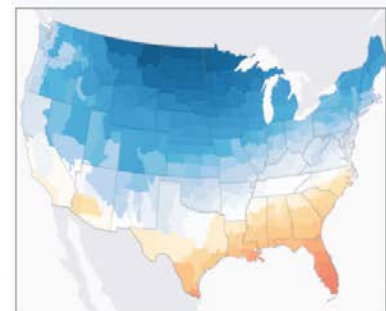
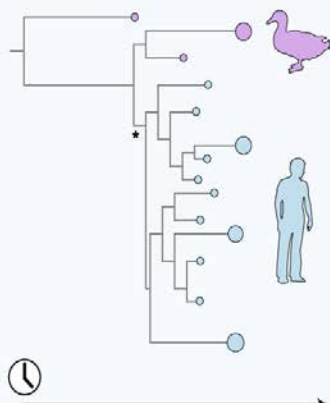
c Transmission chain



d Spread

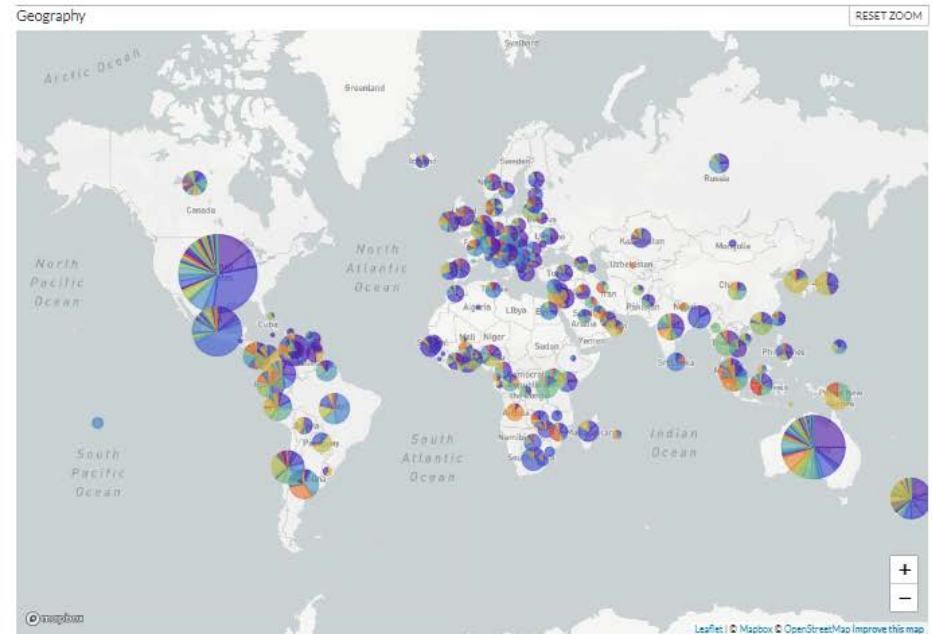
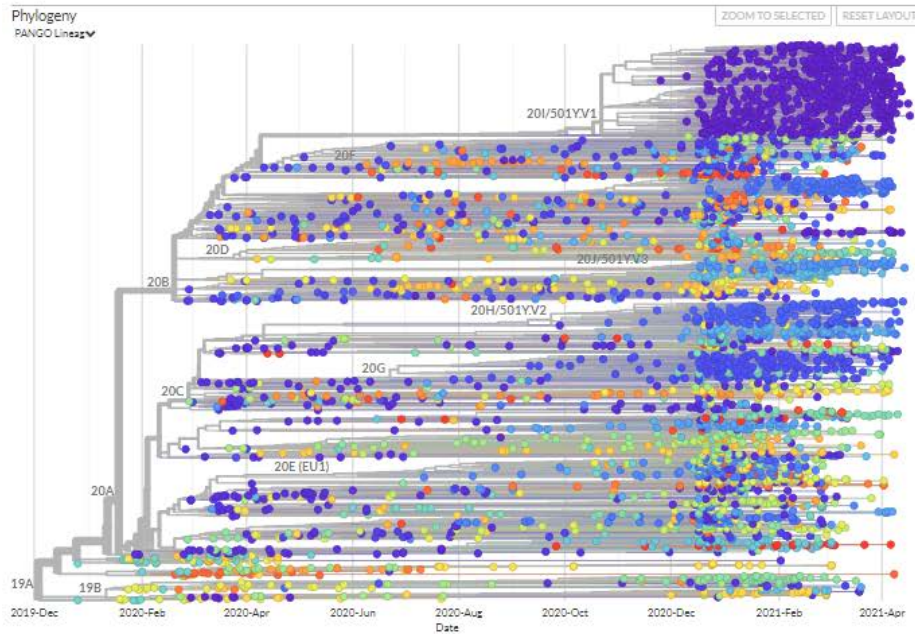


TTGCCGTATAAGCAT
CGATGATTGCCGCATT
AATTAGCCG
GATGATTGCCGCATT
CGATGATTGCCGCATT
AATTAGCCG
TTATTAAGAT
ATTAGATGCCGAT
AATTAGCCG
TAGGAAGSAGCCGAC
CGATGATATACAGCAGAC
GATATACAGCAGACTGC
CGATAAAGGCCATGATTG



Que nos puede responder la epidemiología genómica ahora:

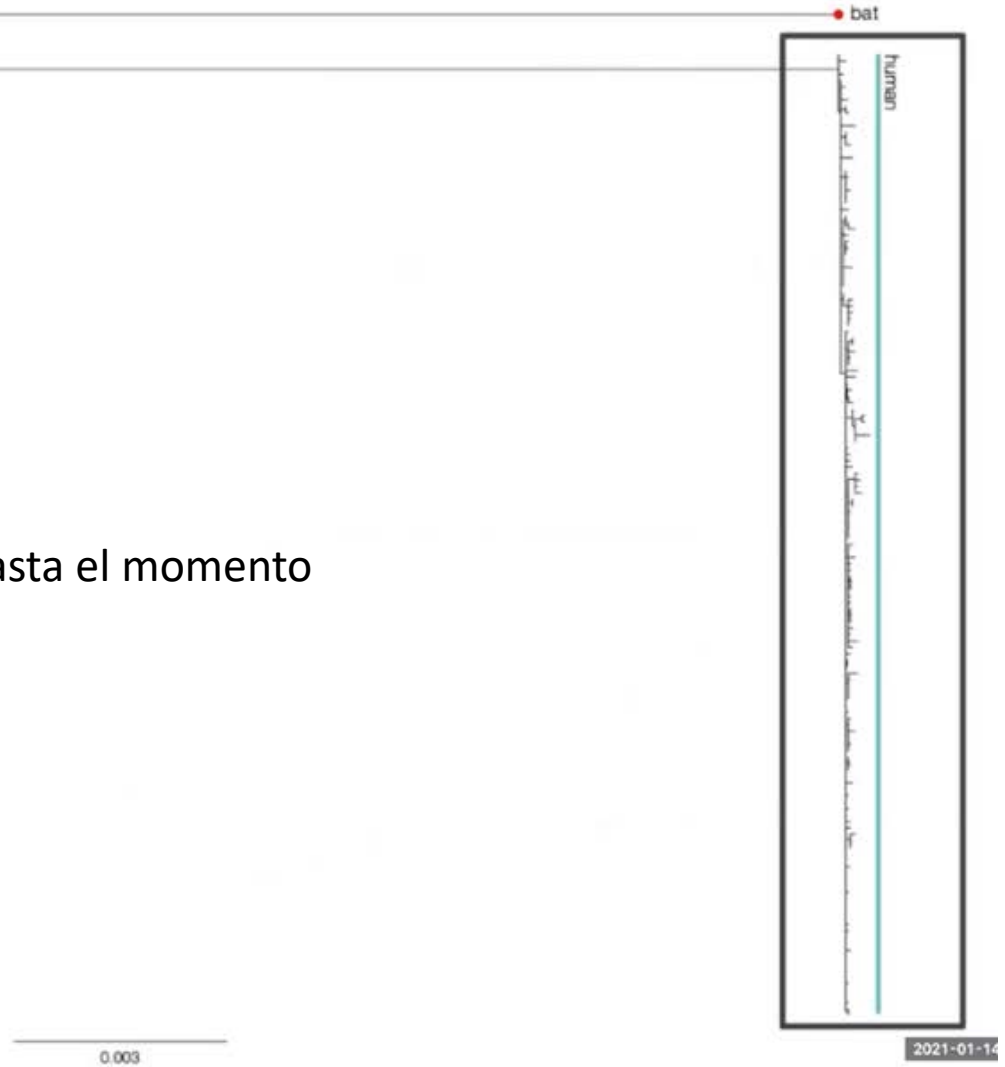
Cómo evoluciona y cuál es la diversidad viral a medida que pasa el tiempo



SARS-CoV-2 & bat/Yunnan/RaTG13

Sólo hay UNA «cepa» de SARS-CoV-2 hasta el momento

Figure by Andrew Rambaut

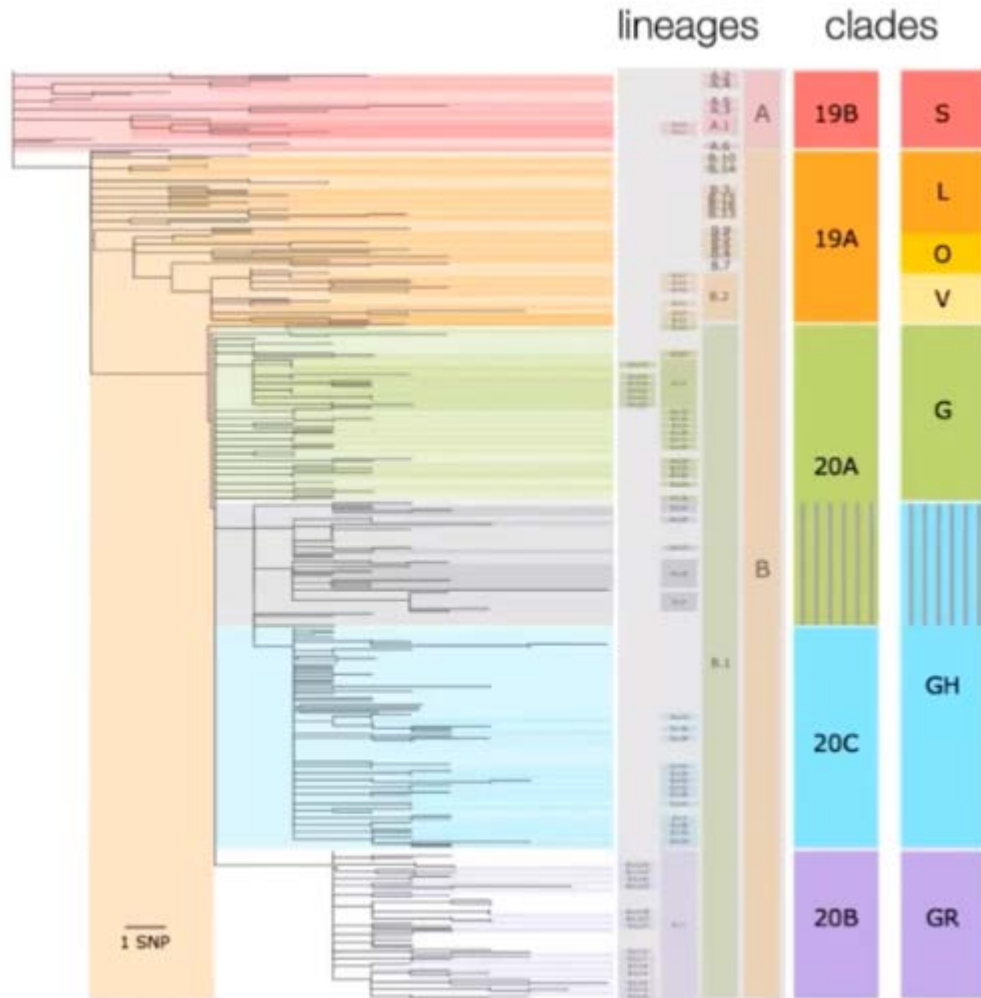


Clados

- Diversidad a una escala mayor, se basa en SNPs a lo largo de la filogenia
- Esta agrupación no varía en el tiempo y se puede observar su comportamiento por años

Linajes

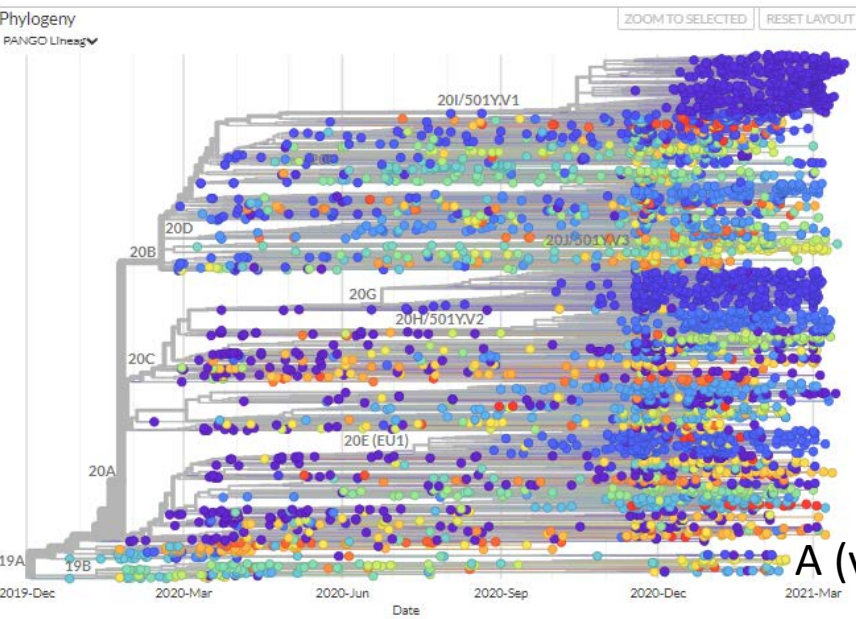
- Utiliza información epidemiológica: ej. geográfica además de la genómica
- Pueden extenderse mucho
- Al ser una clasificación jerárquica y más fina, puede ser menos estable y cambiar



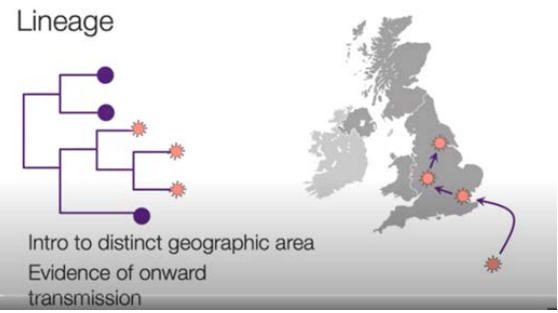
Genomic epidemiology of novel coronavirus - Global subsampling

Maintained by the Nextstrain team. Enabled by data from **GISAID**

Showing 3885 of 3885 genomes sampled between Dec 2019 and Mar 2021.

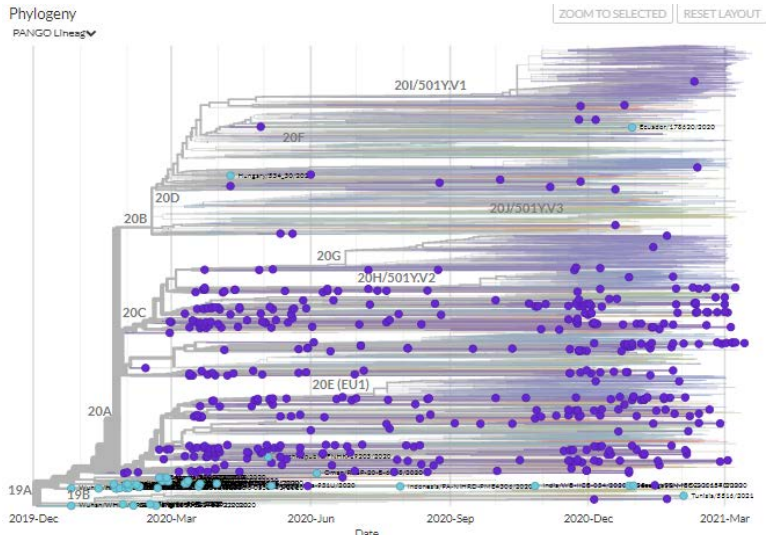
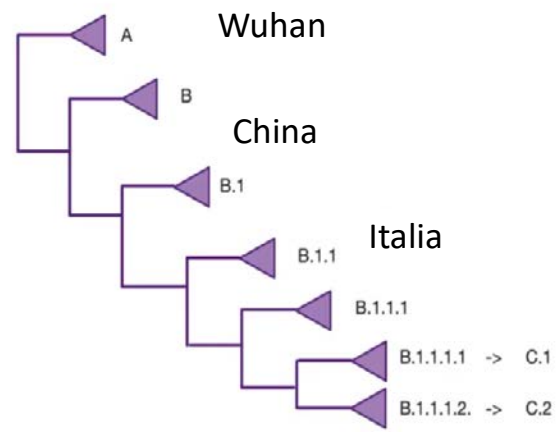


A (verde)



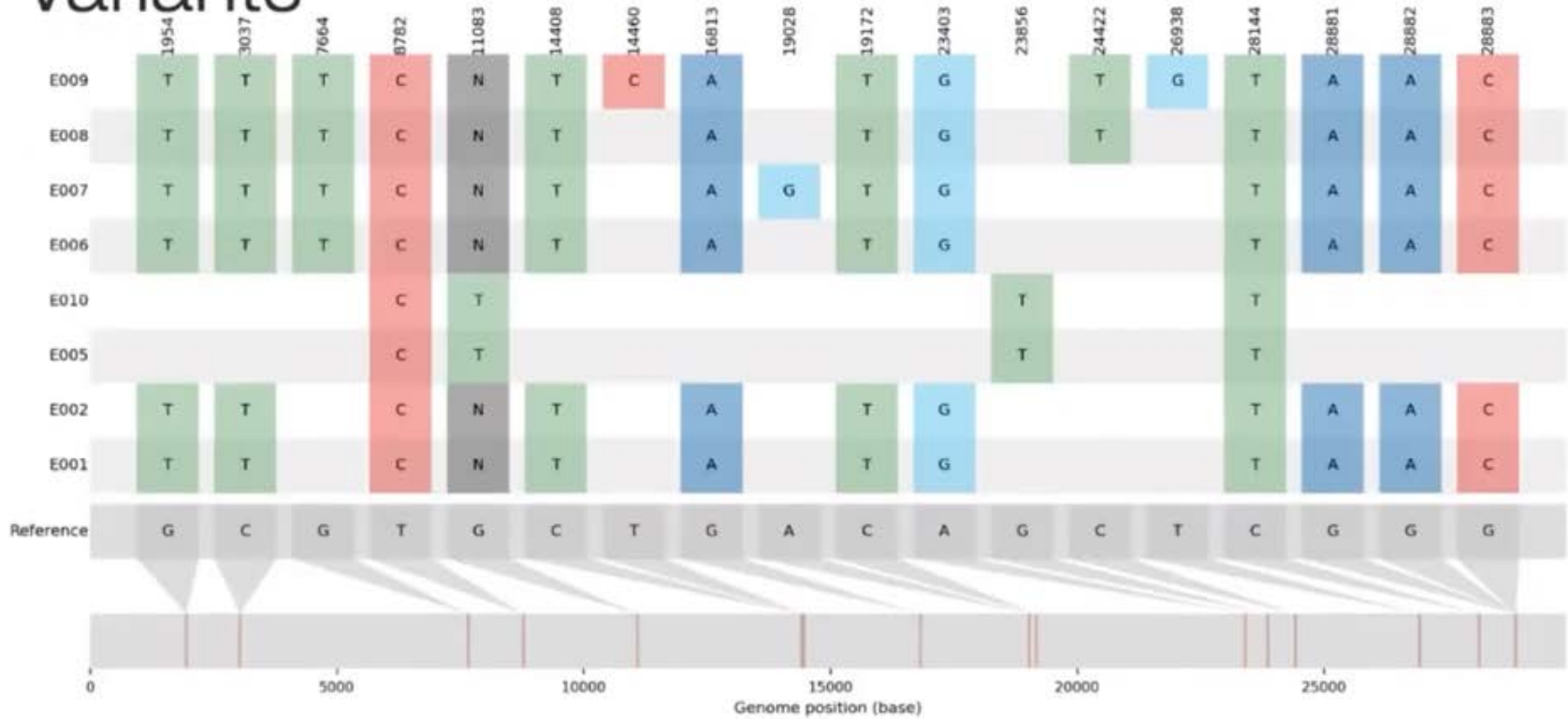
B verde agua
B1 púrpura

Lineage hierarchy



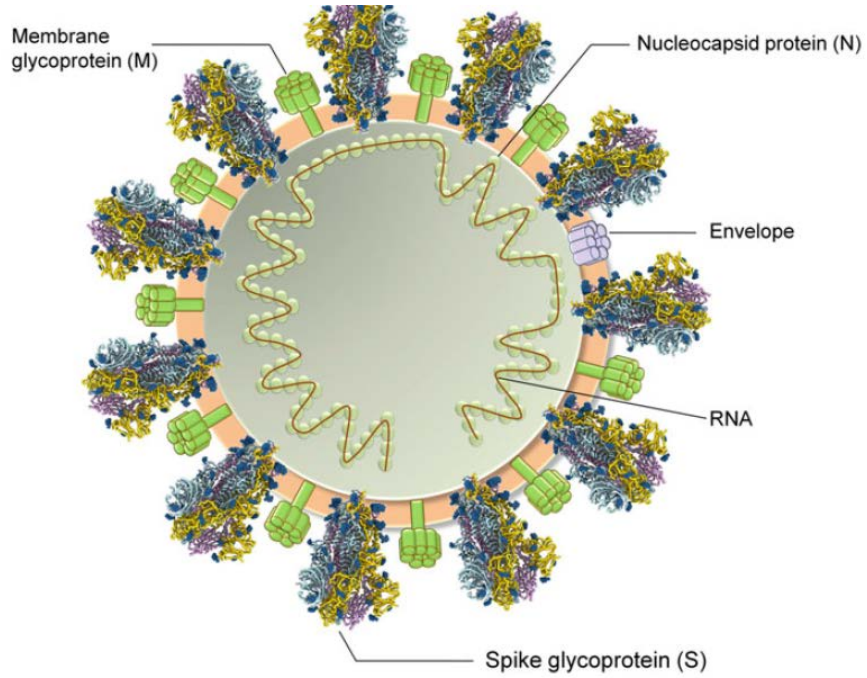
Distinguimos un virus de otro mediante mutaciones

Variants

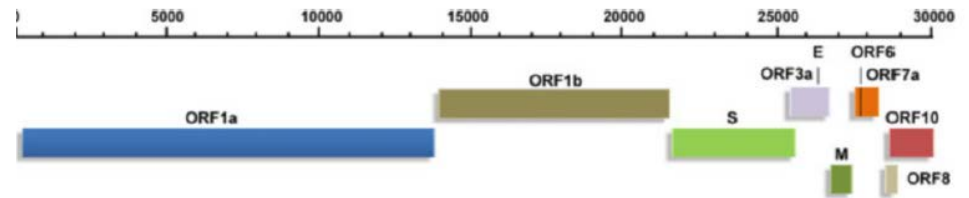


¿Porqué podrían preocuparnos estas variaciones?

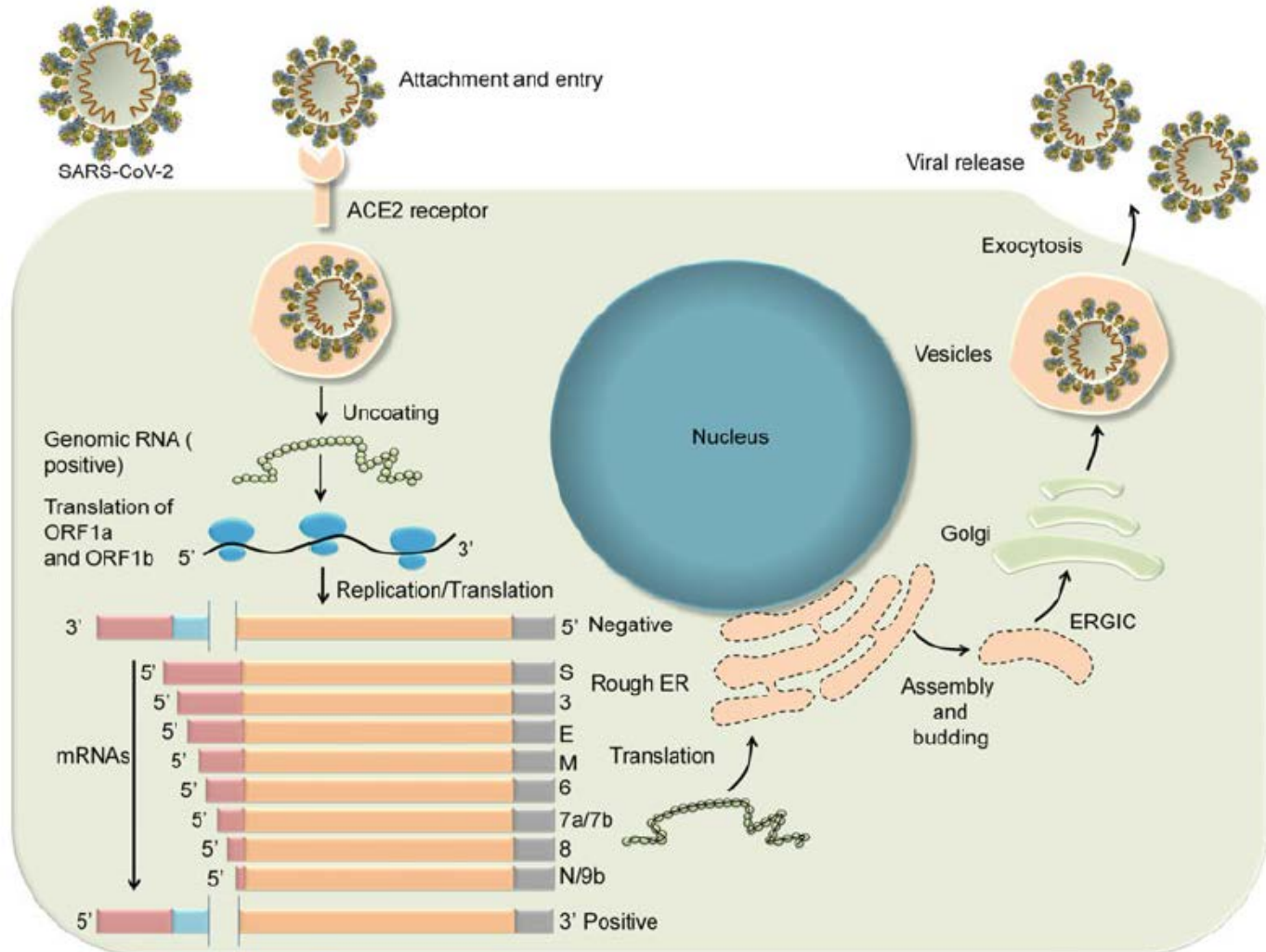
Partícula viral

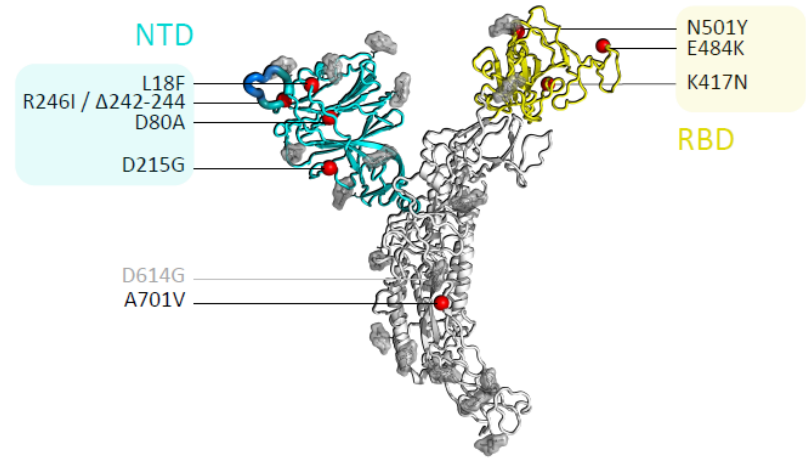
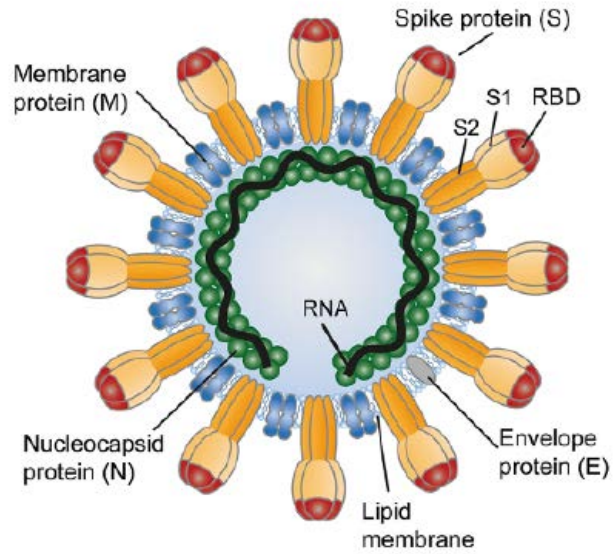


Genoma



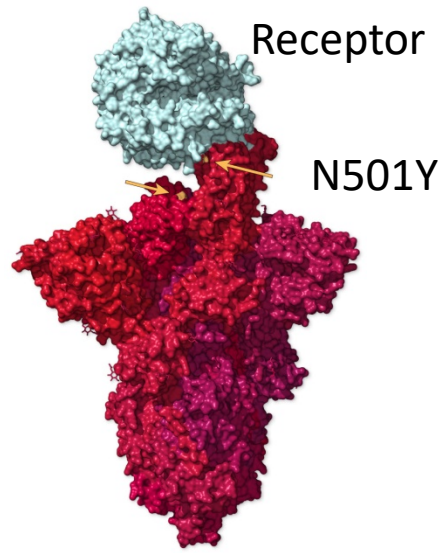
Ciclo replicativo



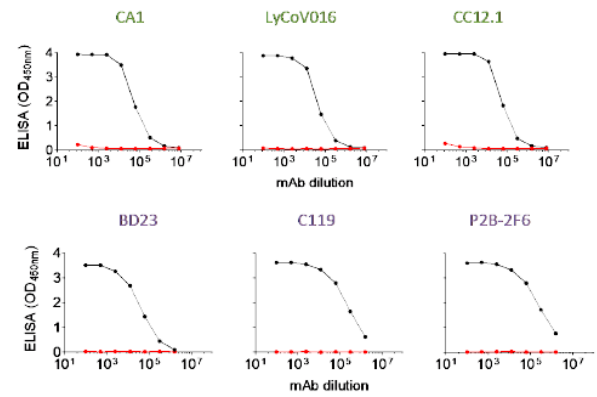
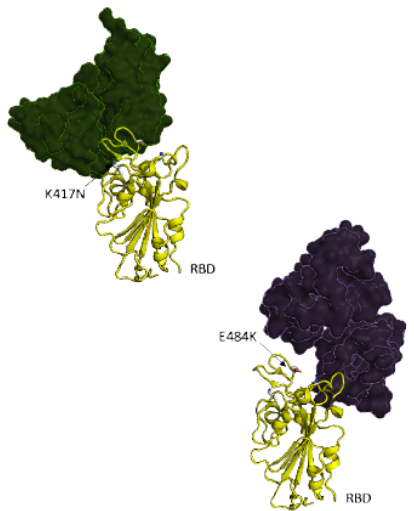


Kurt Wibmer, NIGD

B.1.351



Facilita la entrada a la célula



Escape a anticuerpos neutralizantes

Key ● Original ● RBD mutant ● 501Y.V2

Distinguiamos un virus de otro mediante mutaciones

Lineage Reports

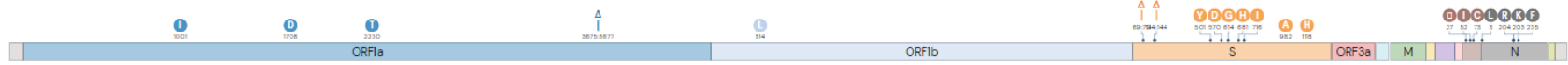
sequences classified as a particular PANGO lineage

How to interpret these reports

B.1.1.7

first identified in United Kingdom
a.k.a. Variant of Concern 202012/01, VOC-202012/01, 20B/501Y.V1, 20J/501Y.V1

VARIANT OF CONCERN



B.1.351

first identified in South Africa
a.k.a. 20H/501Y.V2

VARIANT OF CONCERN



B.1.427

first identified in California
a.k.a. CA VUII, CAL.20C

VARIANT OF CONCERN



B.1.429

first identified in California (United States)
a.k.a. CA VUII, CAL.20C

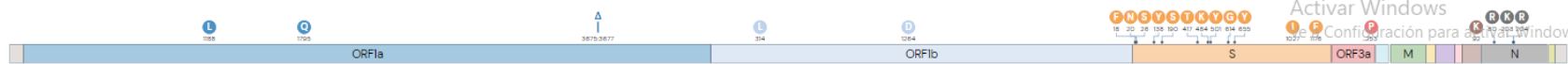
VARIANT OF CONCERN



P.1

first identified in Brazil
a.k.a. B.1.1.281, 20J/501Y.V3

VARIANT OF CONCERN

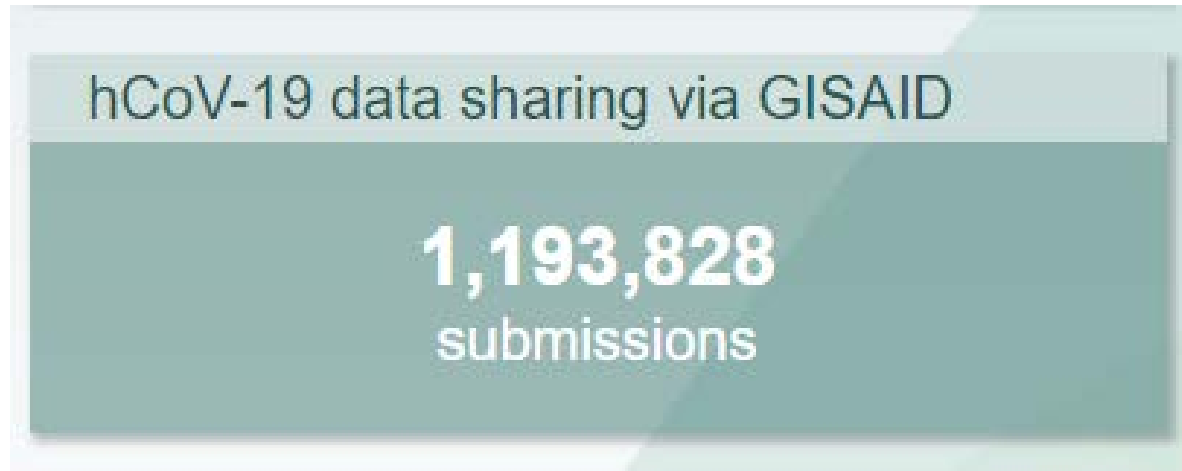


Activar Windows

Configuración para activar Windows.

	B.1.1.7	B.1.351	P.1	B.1.526/5	B.1.429/7	B.1.617
Alternate name	501Y.V1	501Y.V2	501Y.V3	-----	-----	-----
Country identified	United Kingdom	South Africa	Brazil	US, New York	US, California	India
Spike mutations, N	9	5	11	6	4	5
Key mutations In Spike (others are important)	N501Y, E69/70 del, P681H, 144Y del	N501Y, E484K, K417N, P71L	N501Y, E484K, K417T	E484K, S477N, H69-V70 del	L452R, W152C, S131	L452R, E484Q, P681R
Transmissibility Δ (vs ancestral)	Increased ~50-70%	Not established	Increased, % unresolved	Not established	Increased ~20%	Not established
Lethality Δ	Increased ? %	?	?	?	?	?
Immune evasion	Minimal	Yes	Yes, less than B.1.351	Minimal	Yes, less than B.1.351	Very likely
Vaccine efficacy Clinical Trials and Lab Studies	Minimal reduction <10% point decline in 2 trials (Novavax, AZ)	60+% Efficacy Novavax, J&J No AZ effect vs mild/mod infections	No clinical trials (only P.2); Sera from vaccinees for mRNA and AZ effective vs P.1	No trials; Sera from vaccinees for mRNA efficacy preserved	No trials; Sera from vaccinees for mRNA efficacy preserved	No data

Epidemiología genómica: Cantidad sin precedentes de datos generados



22/04/21

Bases de datos y herramientas on-line disponibles

Pangolin COVID-19 Lineage Assigner
Phylogenetic Assignment of Named Global Outbreak LINEages

Drag and drop fasta file
Select fasta file to upload

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 [Aine O'Toole](#), [Yusuf Hill](#), [JT McCrone](#) and [Andrew Rambaut](#). The source code can be found [here](#)

Recommended browsers: or

Centre for Genomic Pathogen Surveillance

es Events Collaborations References Registration Help

Login

Enabled by hCoV-19 data shared via GISAID

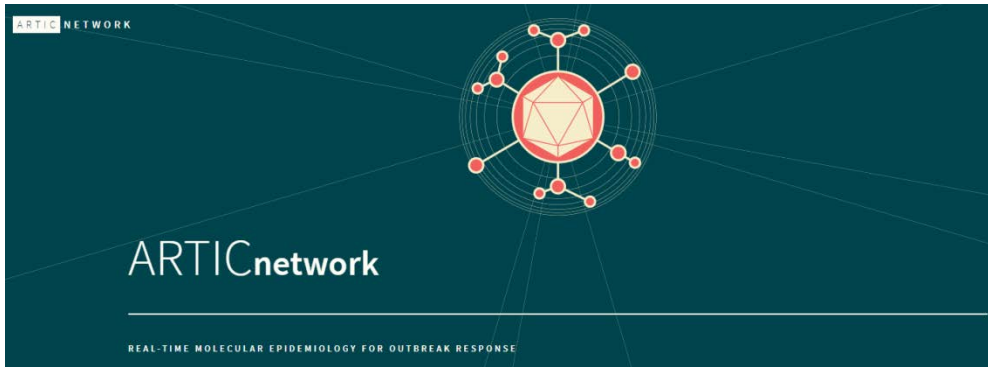
- Mutation Situation Reports**
The Andersen Lab at Scripps Research (USA)
Tracking the prevalence of several lineages or sets of mutations within the hCoV-19 genome
- CoVariants**
University of Bern (Switzerland)
SARS-CoV-2 variants and mutations of interest
- Covid-Miner**
Regina Elena National Cancer Institute (Italy)
Detect the emergence and geographical distribution of hCoV-19 Spike protein genetic variants
- CoVizu**
Western University (Canada)
Near real-time visualization of hCoV-19 genomic variation
- COVID-19 CoV Genetics Browser**
Broad Institute of MIT and Harvard (USA)
Track transmission, evolution, emergence, immune interactions, diagnostics, therapeutics & vaccines
- NAAT Amplicons**
European Commission (Belgium)
Performance database for diagnostic medical devices related test methods for COVID-19
- Sequence Analysis Pipeline**
Los Alamos National Lab (USA)
Analyses of accruing mutations geographically and over time, with an emphasis on Spike protein
- CoV-GLUE**
University of Glasgow (United Kingdom)
Amino acid variation database of amino acid replacements, insertions and deletions
- Genomic Signature Analysis**
CSIRO (Australia)
Representation of the similarity of viral genomes in 2-dimensional space
- Geographic Mutation Tracker**
KAUST (Saudi Arabia)
COVID-19 virus geographic mutation tracker and visualization tool
- Interactive Real-Time Mutation Tracker**
Michigan State University (USA)
Explore and animate mutations over time
- Global Testing and Genomic Variability**
Lebanese American University (Lebanon)
Identification and tracking of mutations and variations across time and geographies
- Spike Protein Mutations Monitoring**
KAUST (Saudi Arabia)
Visualization of frequency of point mutations in the S protein amino acid sequences
- GE \$\$**
Indiana University (USA)
Genome position / Region / Area / Concurrence search and SNV birth query

Estudio de la dinámica de transmisión y de la
variabilidad genética de SARS-CoV-2 circulantes en
Paraguay a través del análisis de secuencias del
genoma viral (PINV20-239)

PINV20-239-Epidemiología genómica de SARS-CoV-2 en Paraguay

Protocolo accesible

Sostenible en el tiempo



Partners

- University of Edinburgh
- University of Birmingham
- University of Cambridge
- KU Leuven
- University of Oxford
- Fred Hutchinson Cancer Research Center
- University of California Los Angeles

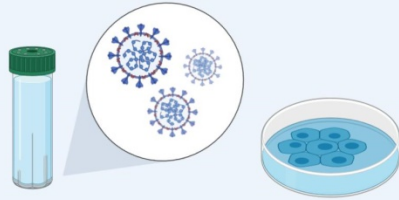


MinION-Oxford Nanopore Technology

SARS-CoV-2 sequencing workflow

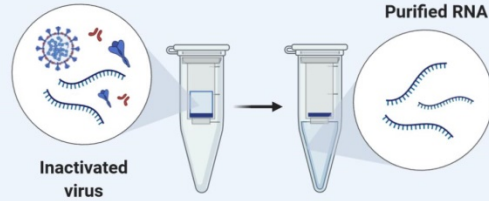
1 Specimens collected

Clinical samples and isolates



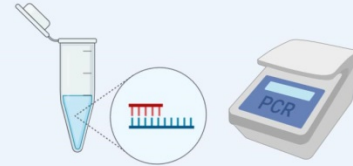
2 Viral RNA extraction

NucleoMag Virus kit (Macherey-Nagel) ~45 min

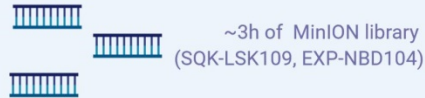


3 cDNA synthesis and multiplex PCR

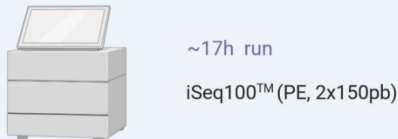
ProtoScript II First Strand cDNA Synthesis Kit (NEB) ~25 min
Q5 Hot Start High-Fidelity DNA Polymerase (NEB) ~4h per primer set



4 MinION (ONT) and iSeq100™ (Illumina) library preparation



5 MinION and iSeq100™ sequencing

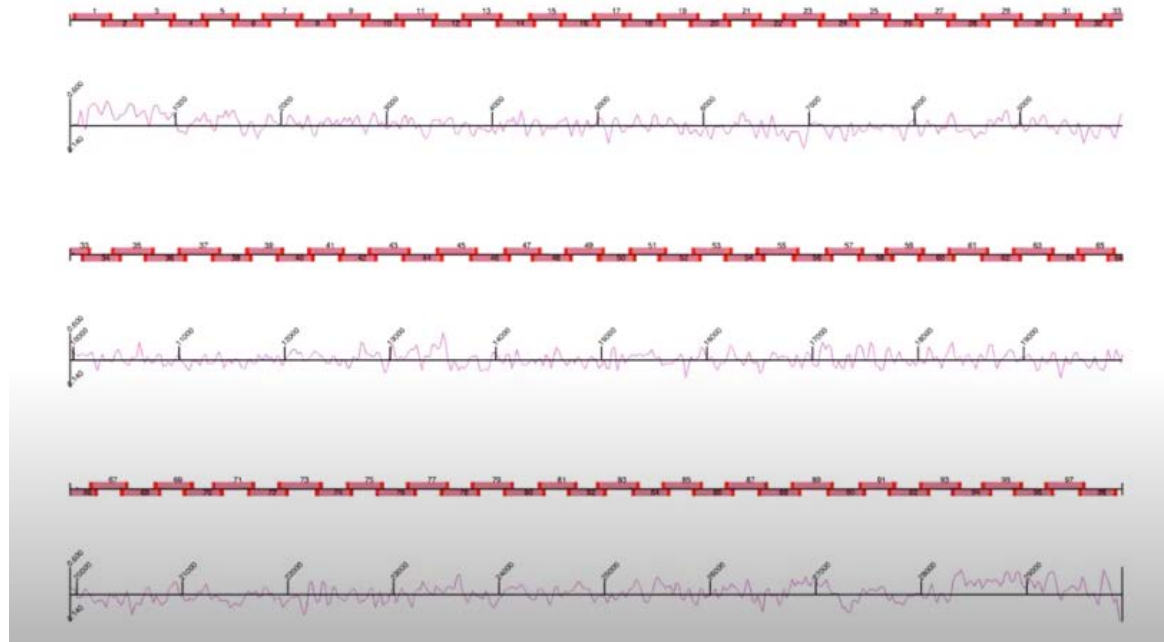
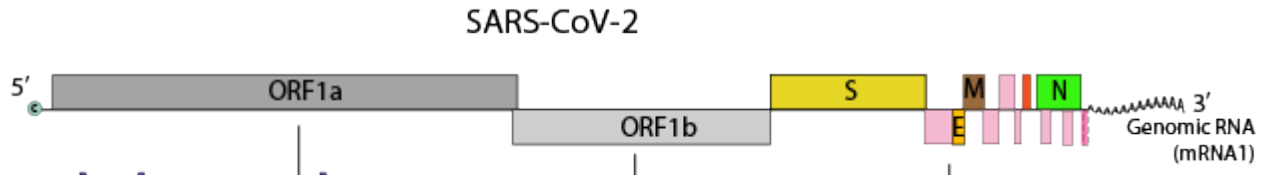


6 Data analysis

MinION/Illumina bioinformatics specific pipelines ~1h



Secuenciación mediante síntesis

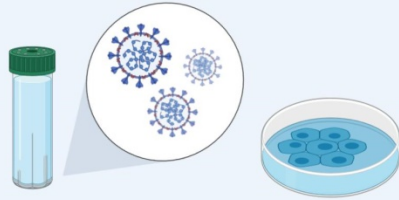


Amplicones
400bp

SARS-CoV-2 sequencing workflow

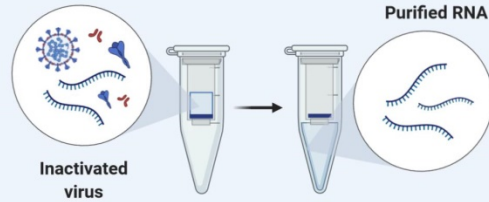
1 Specimens collected

Clinical samples and isolates



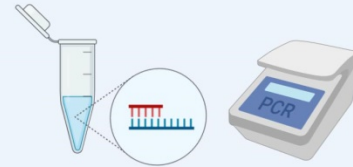
2 Viral RNA extraction

NucleoMag Virus kit (Macherey-Nagel) ~45 min

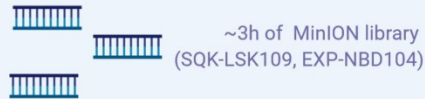


3 cDNA synthesis and multiplex PCR

ProtoScript II First Strand cDNA Synthesis Kit (NEB) ~25 min
Q5 Hot Start High-Fidelity DNA Polymerase (NEB) ~4h per primer set



4 MinION (ONT) and iSeq100™ (Illumina) library preparation



5 MinION and iSeq100™ sequencing



6 Data analysis

MinION/Illumina bioinformatics specific pipelines ~1h



Muestras

Mayo – Diciembre 2020



Summary

As of 24 March 2021, **192,927** sequences in the B.1.1.7 lineage have been detected since the lineage was identified:

location 📍	B.1.1.7 found		when found**	
	total	cumulative prevalence*	first	last
United Kingdom	137,775	57%	20 Sep 2020	17 Mar 2021
Worldwide	192,927	23%	14 Feb 2020	21 Mar 2021
United States	6,586	4%	22 May 2020	21 Mar 2021
California, United States	337	2%	17 Dec 2020	7 Mar 2021

[view change over time](#)

[change locations](#)

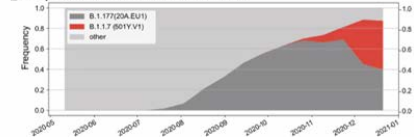
* Apparent cumulative prevalence is the ratio of the sequences containing B.1.1.7 to all sequences collected since the identification of B.1.1.7 in that location. ** Dates are based on the sample collection date

[Read about biases](#)

Rapid global rise of B.1.1.7

- ~50% more transmissible (Davies et al., 2020)
- 0.4-0.7 higher reproductive rate (Volz et al., 2021)
- 30%–50% higher secondary attack rate (PHE, 2020)

B Rapid rise of the B.1.1.7 variant in the UK

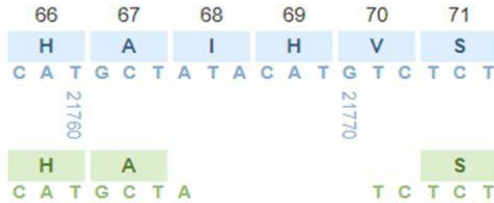


C Global surveillance of B.1.1.7 variants



Se podría hacer vigilancia de variantes con otros métodos aparte de la secuenciación?

A



The S Gene Advantage: TaqPath COVID-19 Tests May Help Early Identification of B.1.1.7

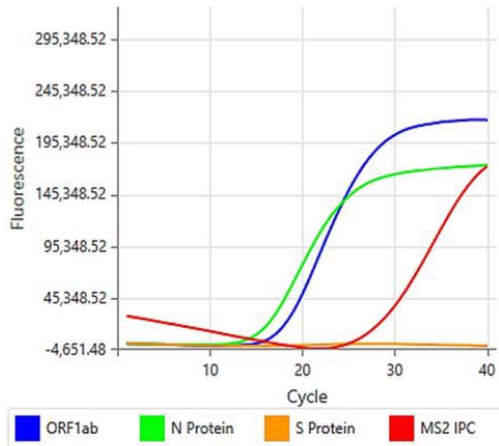
By Ask A Scientist Staff
01.14.2021

This blog represents the most up-to-date information about the B.1.1.7 SARS-CoV-2 variant as of February 1, 2021.

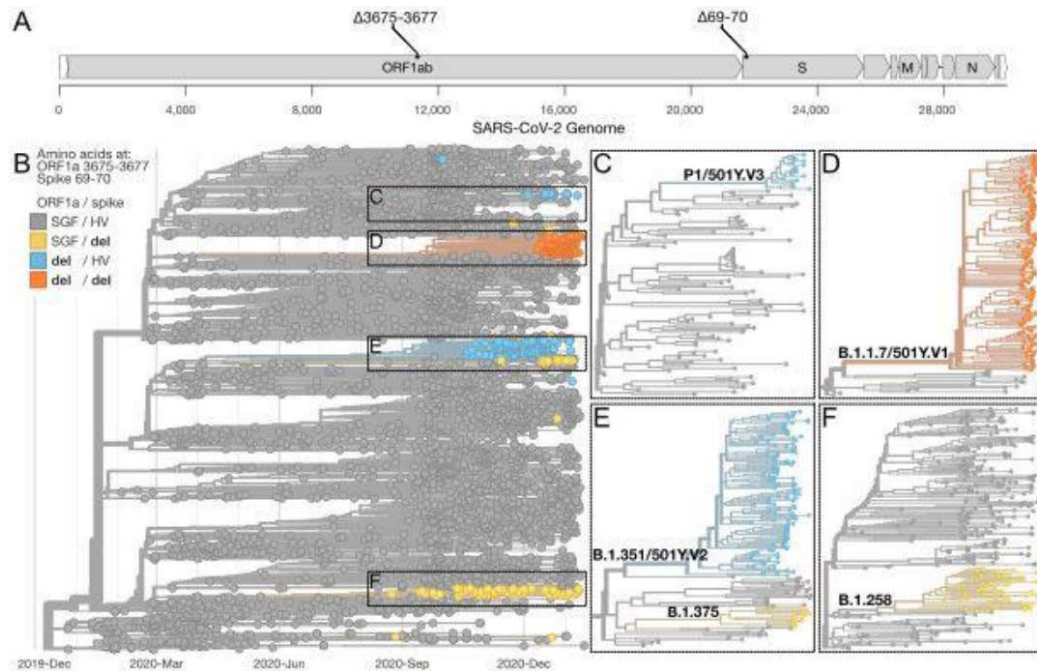
A new, highly transmissible variant of SARS-CoV-2 discovered in the United Kingdom, B.1.1.7 (or VOC202012/01 or 501Y.V1) has complicated the COVID-19 situation. B.1.1.7's markedly higher transmissibility creates challenges for COVID-19 wards, assisted-living facilities, and other situations in which close contact between potentially infected people is unavoidable, making the detection and tracking of this variant and others like it even more important to the long-term containment of SARS-CoV-2. While B.1.1.7 shows increased transmissibility, [preliminary evidence](#) suggested there was no indication it was associated with increased disease severity. However, [more recent studies](#) do indicate that there is a realistic possibility of increased risk of death in patients infected with B.1.1.7.

B.1.1.7 has a specific mutation in the S-gene which results in a deletion of two amino acids at sites 69 (histidine) and 70 (valine), commonly referred to as *69-70del*. This has raised concerns about whether the B.1.1.7 variant, or other lineages and variants which include the same S-gene mutation, can be detected.

B



Bal 2020



PCR assay to enhance global surveillance for SARS-CoV-2 variants of concern

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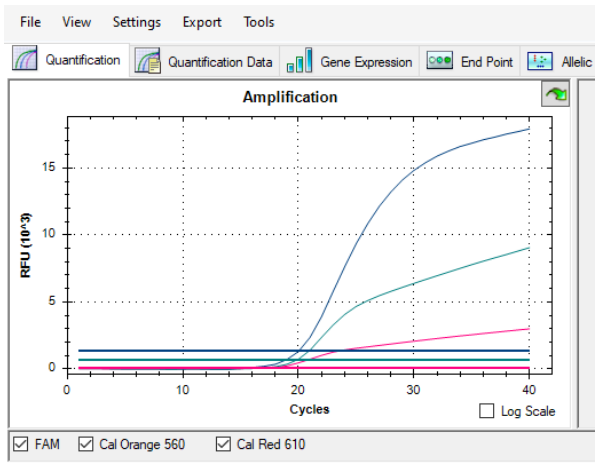
[†] These authors contributed equally

* Senior authors

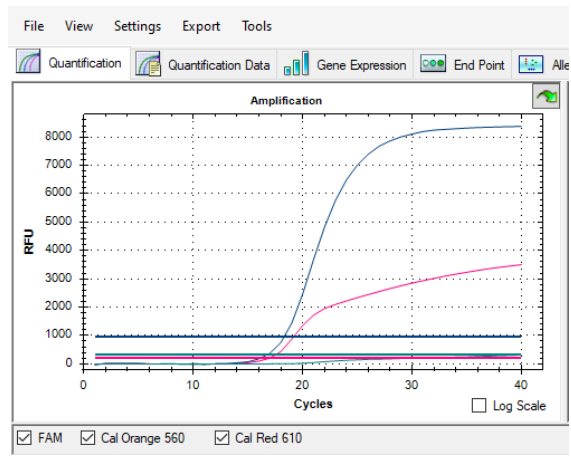
[†] Correspondence: Chantal B.F. Vogels (chantal.vogels@yale.edu), Joseph R. Fauver (joseph.fauver@yale.edu), Nathan D. Grubaugh (nathan.grubaugh@yale.edu)

Supplementary Table 3: Interpretation of results from the multiplexed RT-qPCR variant screening assay.

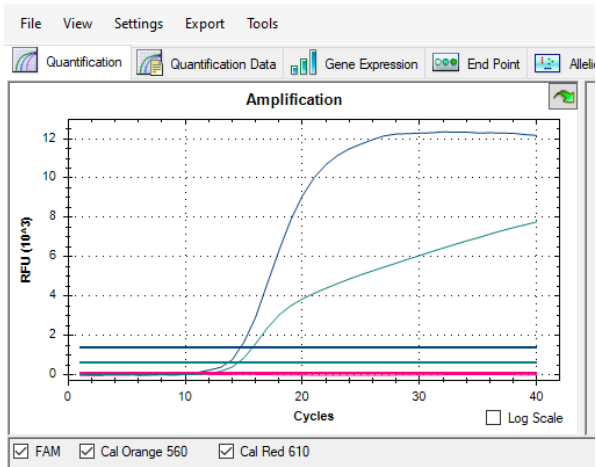
Result	N1-FAM	Orf1a-Cy5	Spike-HEX
Potentially B.1.1.7	CT ≤ 35	Undetected	Undetected
Potentially B.1.351 or P.1	CT ≤ 35	Undetected	CT ≤ 35
Potentially B.1.375	CT ≤ 35	CT ≤ 35	Undetected
Other lineages	CT ≤ 35	CT ≤ 35	CT ≤ 35
Inconclusive	CT > 35 or undetected	Any value	Any value



«wt»

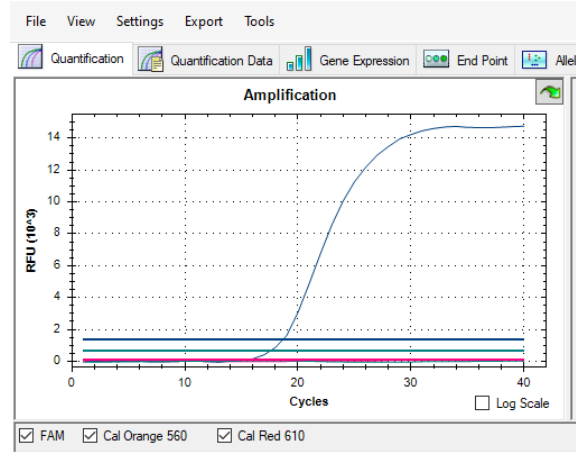


Dropout S (no voc, 14/03/2021)



Dropout Orf1a

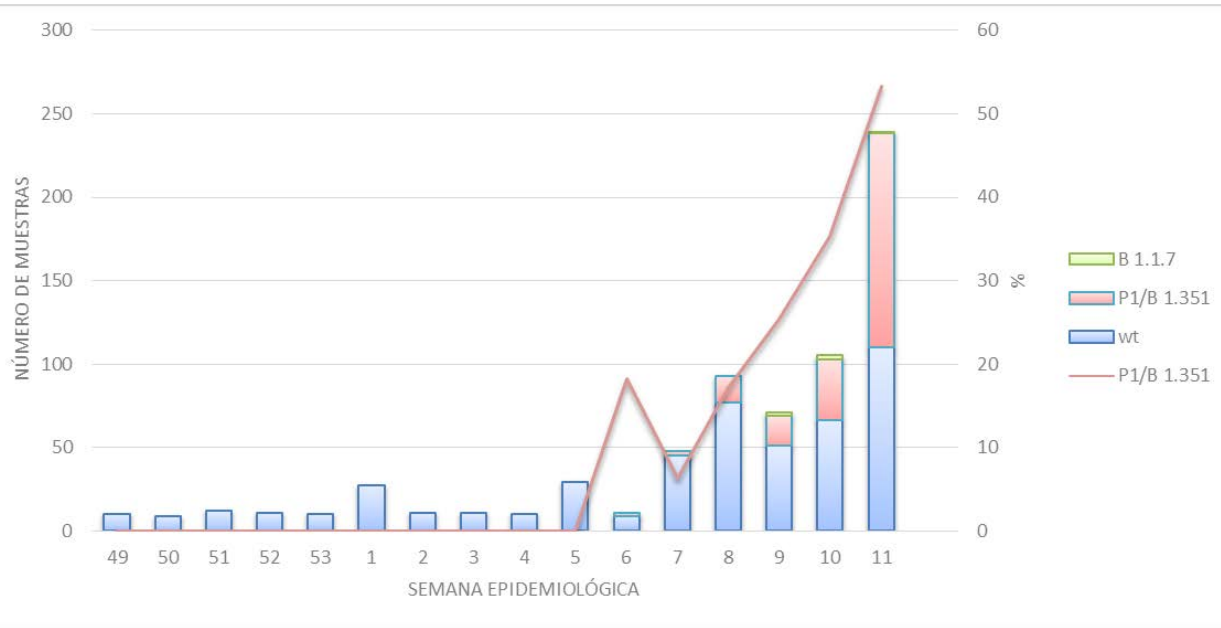
13/02/2021



Dropout Orf1a/S

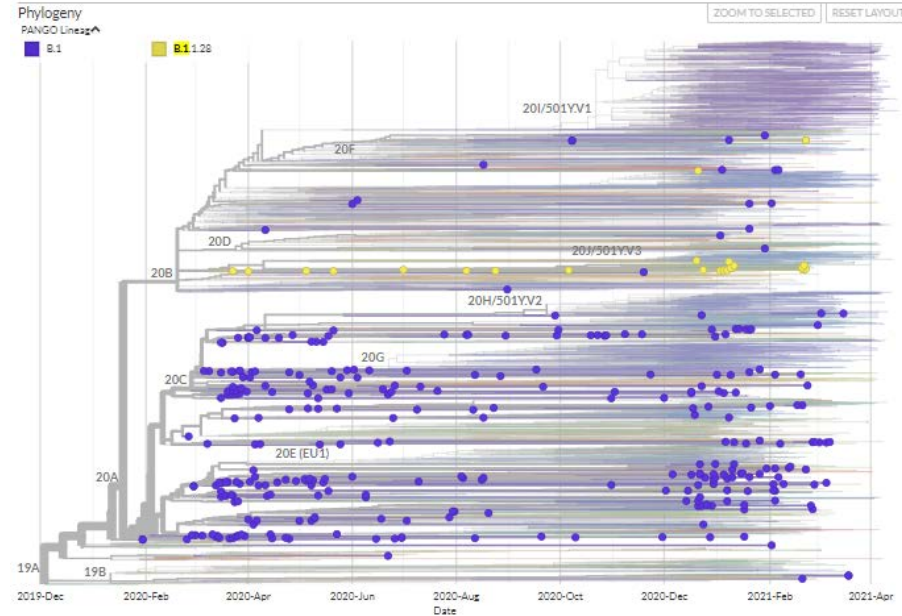
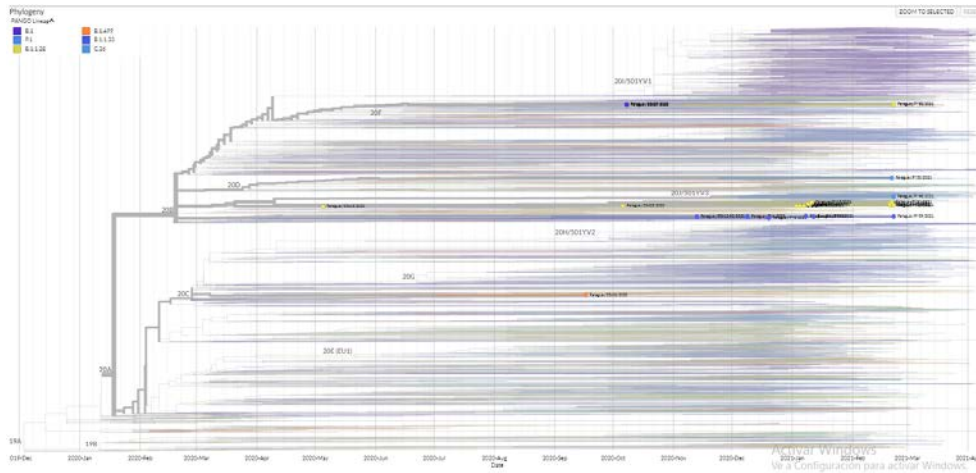
28/02/2021

Muestras Diciembre 2020-Marzo 2021

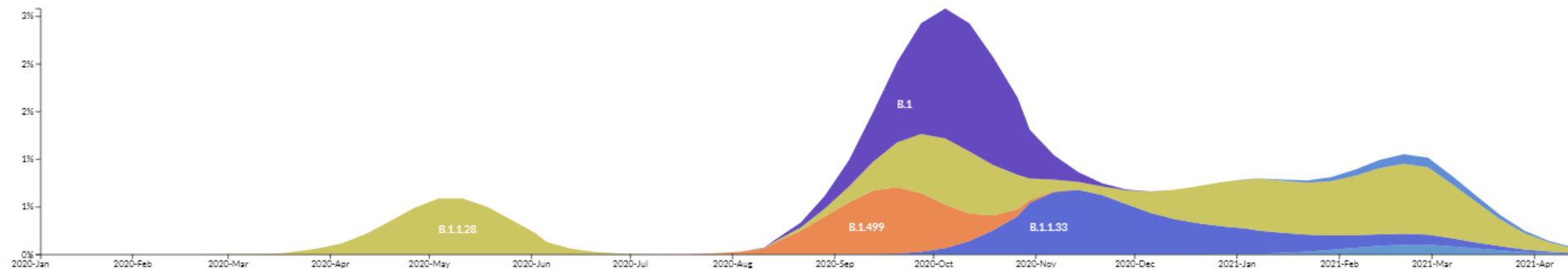


Abril: P1/B.1.351: 81%
B.1.1.7: 3,2%

Datos de Paraguay en Nextstrain- MSPyBS

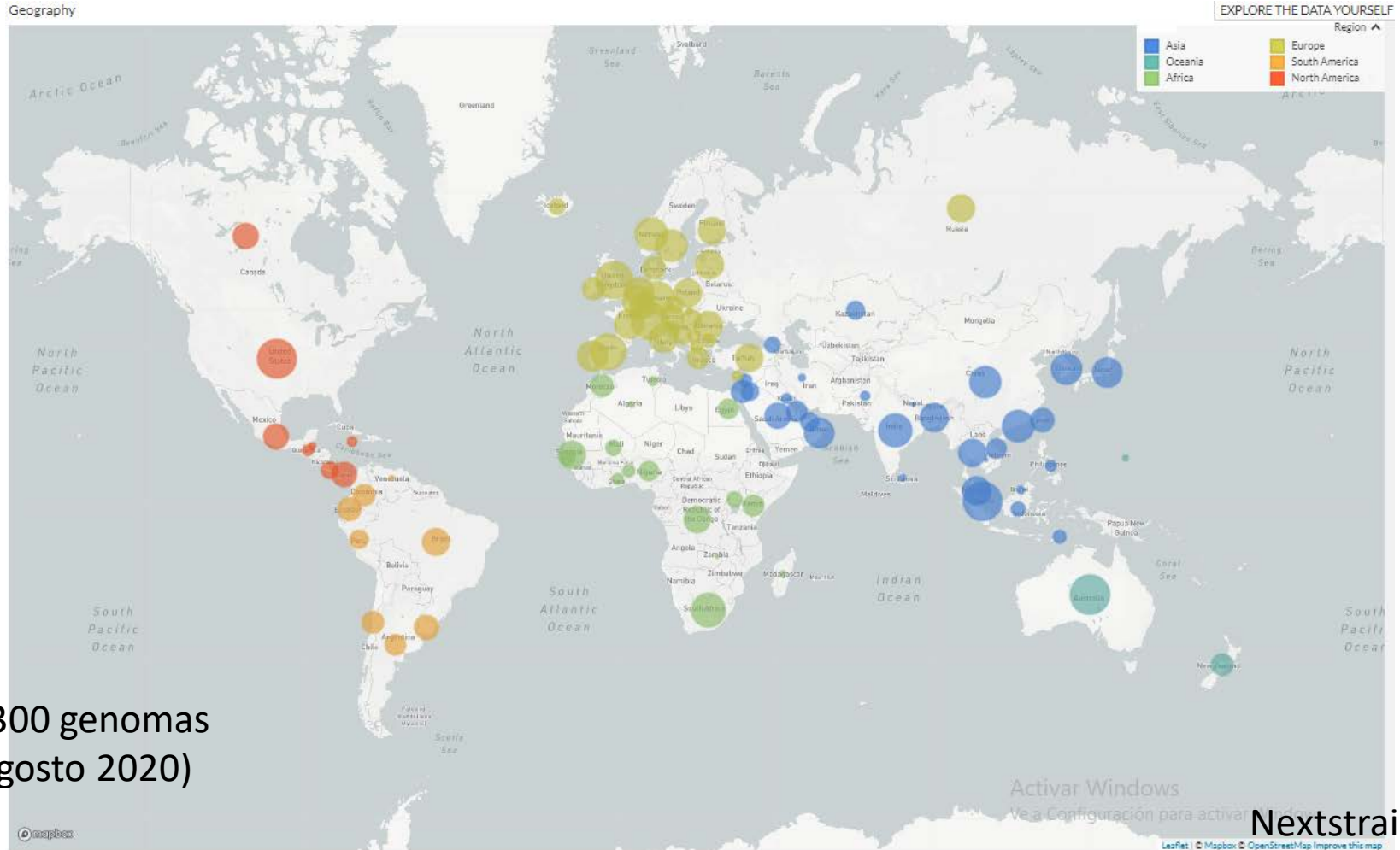


Frequencies (colored by PANGO Lineage as a fraction of all sequences at each time point showing 26 out of a total of 3861 tips)



Open data

Geography



GRACIAS

Eva Nara

Maru Galeano

Laura Franco

Leticia Rojas

Chyntia Díaz

Florencia del Puerto

Fátima Cardozo

Adriana Valenzuela

Laura Mendoza

Alejandra Rojas

Ana Ayala

Vicente Martínez

Nancy Riquelme

Magdalena Molinas

Marta Diarte

José Pereira

Letizia Carpinelli

Gladys Estigarribia

Sandra Caballero

Graciela Riera

Roque Morel

Analía Ortíz

Fátima Ovando

Lizza Ramírez

Fátima Macchi

Alexandra Mercado

Diego González

Mario Martínez

Joel Ortíz

Jonás Barreto

Marta Giovanetti

Jesse Waggoner