

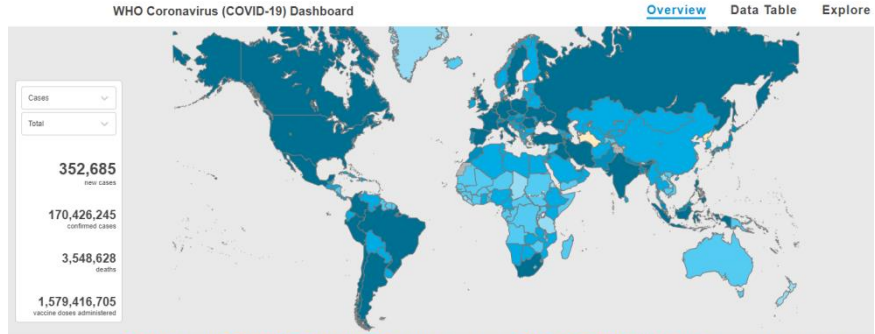
Estudio de la dinámica de transmisión y de la
variabilidad genética de SARS-CoV-2 circulantes en
Paraguay a través del análisis de secuencias del
genoma viral (PINV20-239)

Magaly Martinez

IICS-UNA

Mayo 2021

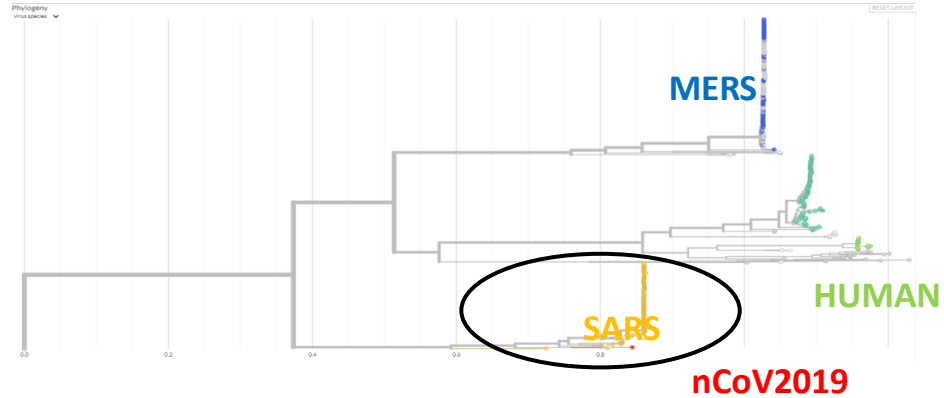
Pandemia SARS-CoV-2



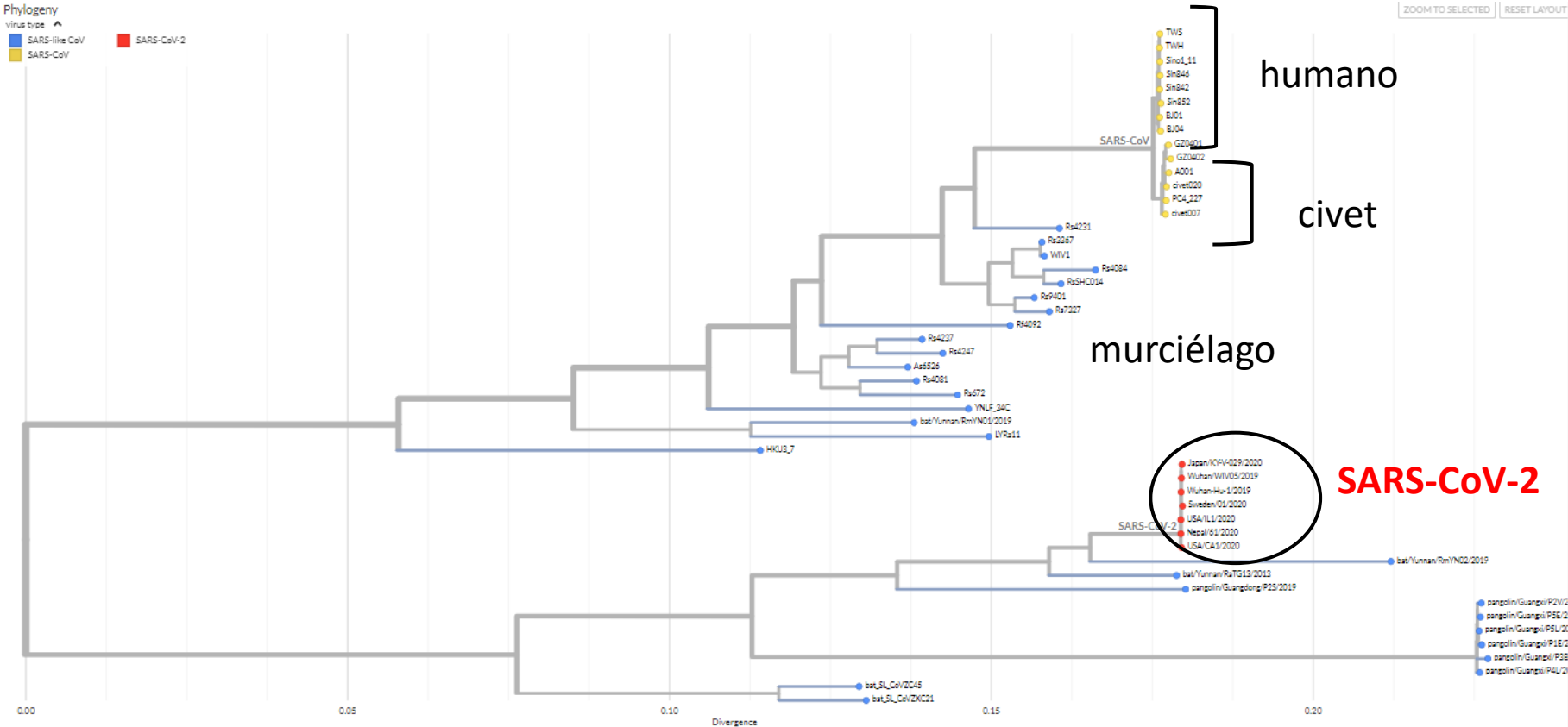
Globally, as of 5:06pm CEST, 1 June 2021, there have been 170,426,245 confirmed cases of COVID-19, including 3,548,628 deaths, reported to WHO. As of 31 May 2021, a total of 1,579,416,705 vaccine doses have been administered.

Genetic diversity of betacoronaviruses including novel coronavirus (nCoV)

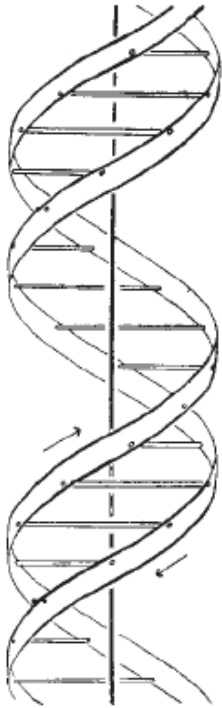
Built with github.com/blab/beta-cov. Maintained by Trevor Bedford.



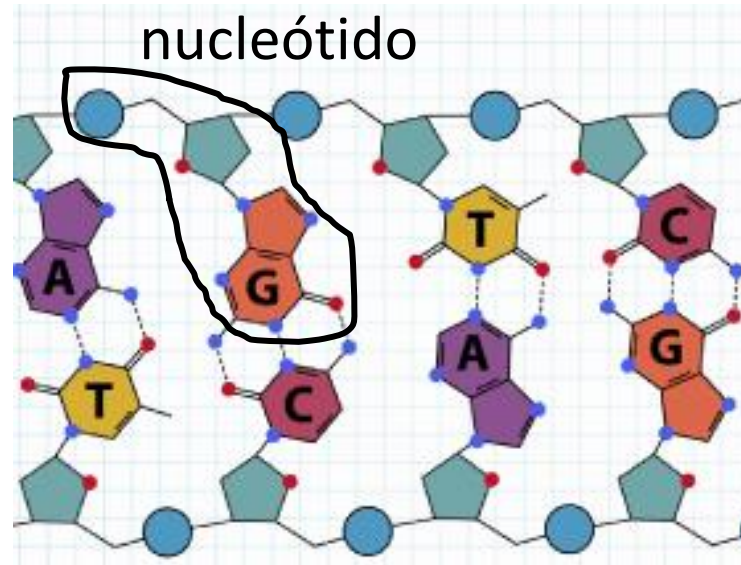
Cluster SARS-like



¿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



Cómo funciona la tecnología Nanopore?

The image shows a screenshot of a research article page from the Proceedings of the National Academy of Sciences (PNAS). The page features a blue header with the PNAS logo and navigation links. The main content area includes the article title, authors, and a brief abstract. On the right side, there are social media sharing options and a sign-up form for a newsletter. The article title is "Characterization of individual polynucleotide molecules using a membrane channel" by John J. Kasianowicz, Eric Brandin, Daniel Branton, and David W. Deamer. The abstract describes a method for measuring polynucleotide length using a membrane channel.

PNAS Proceedings of the National Academy of Sciences of the United States of America

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RESEARCH ARTICLE

Characterization of individual polynucleotide molecules using a membrane channel

John J. Kasianowicz, Eric Brandin, Daniel Branton, and David W. Deamer
+ See all authors and affiliations

PNAS November 26, 1996 93 (24) 13770-13773; <https://doi.org/10.1073/pnas.93.24.13770>

Article Figures & SI Info & Metrics PDF

We show that an electric field can drive single-stranded RNA and DNA molecules through a 2.6-nm diameter ion channel in a lipid bilayer membrane. Because the channel diameter can accommodate only a single strand of RNA or DNA, each polymer traverses the membrane as an extended chain that partially blocks the channel. The passage of each molecule is detected as a transient decrease of ionic current whose duration is proportional to polymer length. Channel blockades can therefore be used to measure polynucleotide length. With further improvements, the method could in principle provide direct, high-speed detection of the sequence of bases in single molecules of DNA or RNA.

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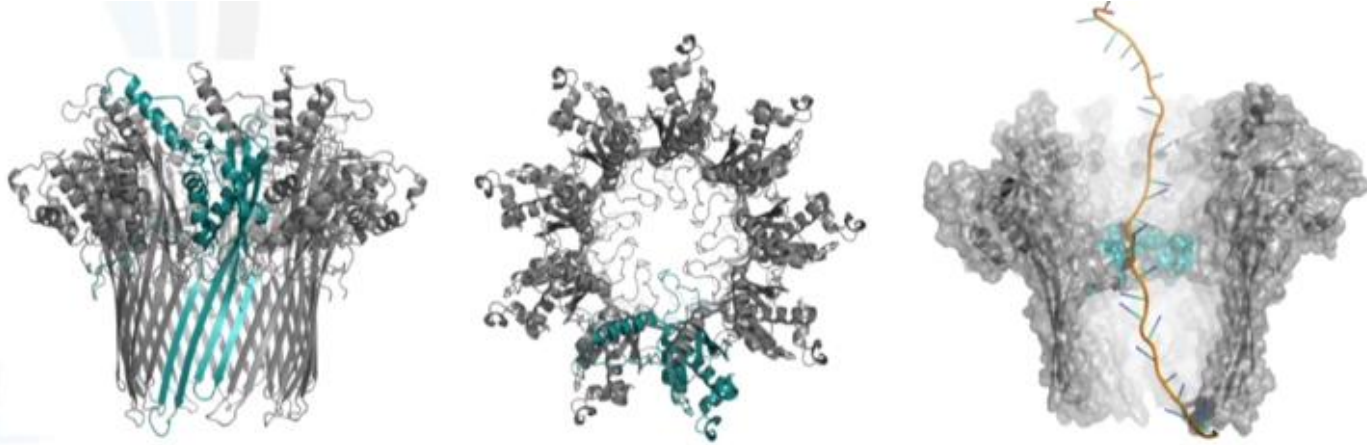
Table of Contents
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<https://youtu.be/RcP85JHLmnl>

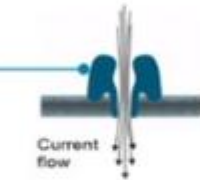
Cómo funciona la tecnología Nanopore?



Cómo funciona la tecnología Nanopore?

NANOPORE SENSING

1 Nanopore creates hole in membrane
Current passes through nanopore

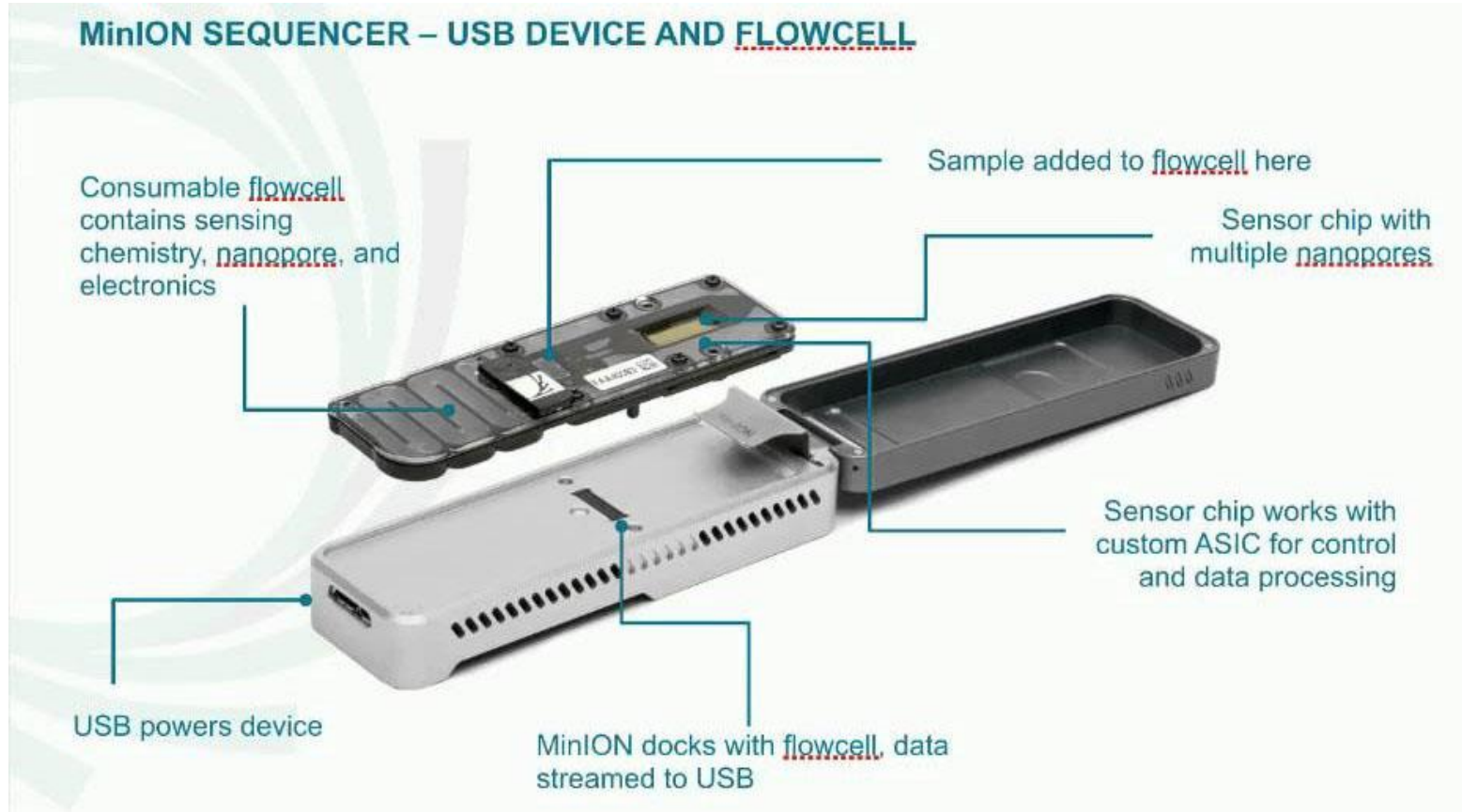


2 As analyte passes through or near the nanopore,
this creates characteristic disruptions in the current



3 Current disruption is interpreted to understand the
identity of the analyte

Cómo funciona la tecnología Nanopore?



VENTAJAS

Dispositivo portátil de bajo costo

Basecalling en tiempo real

Secuenciamiento directo de RNA ya que también detecta uracilo

Obtención de lecturas largas

Se puede escalar a cientos de genomas

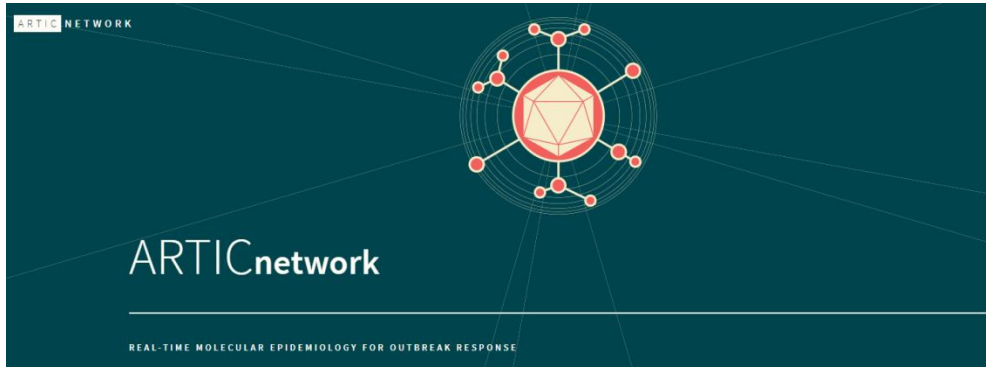
Metagenómica

Amplicones

DESVENTAJA

Igual que otras tecnologías de lectura larga, presenta una alta tasa de error depende de los métodos de preparación de la librería (inserciones y deleciones)

Protocolo ARTIC Network



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



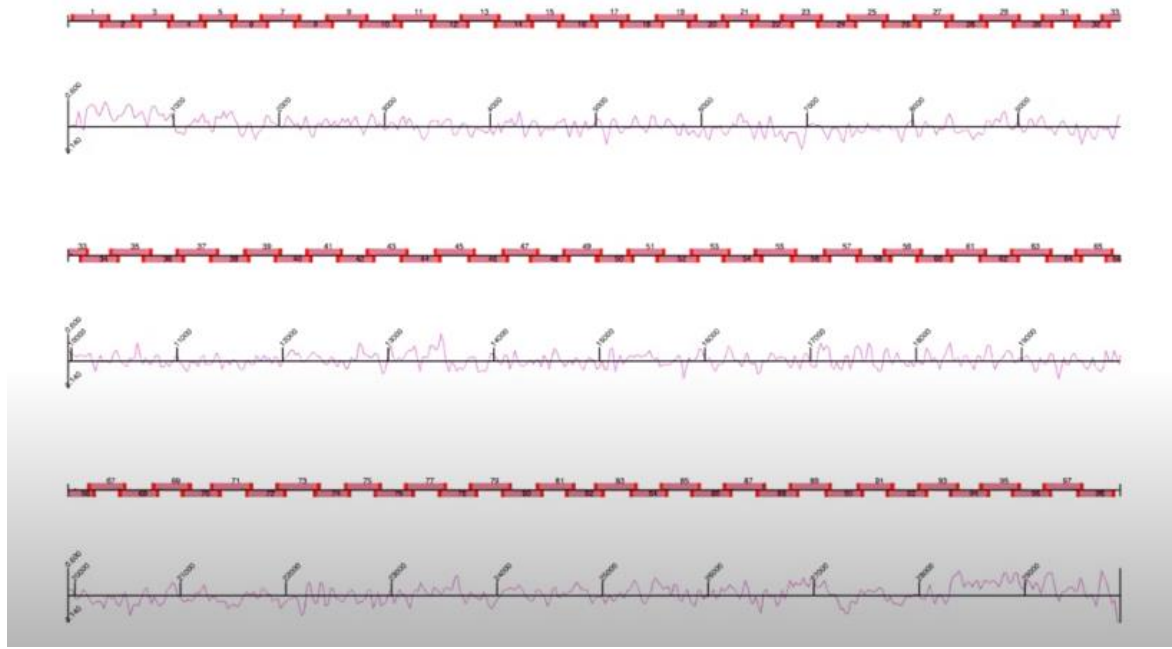
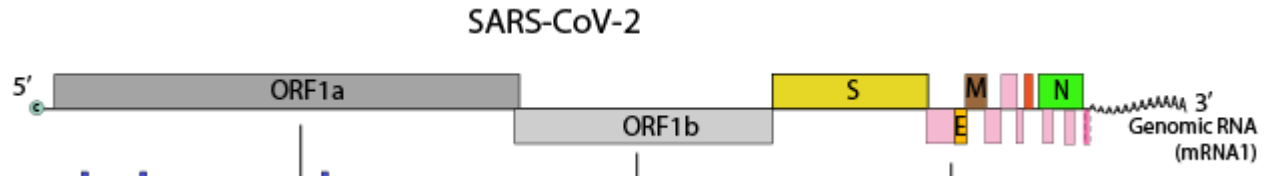
Protocolo ARTIC Network: protocolo detallado y una ventana de preguntas/respuestas están disponibles

The screenshot displays a web browser window with the following elements:

- Browser Tabs:** Nanopore Community, Get started with your MinION, Introducción a Nanopore, 02 - How to sequence CC, WhatsApp, nCoV-2019 sequencing proto.
- Address Bar:** protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye
- Page Header:** Josh Quick / Publications / nCoV-2019 sequencing protocol v3 (LoCost)
- Protocol Card:**
 - Title:** nCoV-2019 sequencing protocol v3 (LoCost) V.3
 - Version:** Version 3
 - Date:** Aug 25, 2020
 - Author:** Josh Quick¹, University of Birmingham
 - Tags:** ARTIC, Coronavirus Method Development Community
 - Actions:** Run, Bookmark, Copy / Fork
- Comments Section:**
 - View all 166 comments**
 - Search in comments**
 - Comment by Candice Swift (Apr 30, 2021):** Hi Josh, Thank you for sharing this protocol! What is your typical % of unclassified reads after demultiplexing? Any tips for reducing the % unclassified reads? What parameters do you
- Navigation:** Steps, Materials, Forks, Metadata, Metrics
- ABSTRACT:** Amplicon sequencing protocol for SARS-CoV-2 v3 (LoCost)
- Text:** We thank the ARTIC network, Oxford Nanopore Technologies, New England Biolabs, BCCDC, COG-UK, CanCOGen and protocols.io commenters for their assistance developing this protocol.
- Changes in this version:**
 - Up to 95 samples per run with EXP-NBD196 native barcode kit
 - Substitution of SuperScript IV for LunaScript RT SuperMix and reaction volume reduced to 10 uL.
 - Substitution of Ultra II Ligation Module for Blunt/TA Ligase Master Mix and reaction volume reduced to 10 uL.
 - Native barcode ligation reaction volume reduced to 10 uL.
 - SFB wash volume reduced.
- BEFORE STARTING:** Prepare between 11 and 95 RNA samples plus 1 negative control using this protocol.

The Windows taskbar at the bottom shows the search bar, taskbar icons for various applications, and the system clock indicating 16:33 on 30/4/2021.

El método ARTIC usa multiplex PCR y barcoding



Amplicones
400bp

	A	B	C	D	E	F	G	H	I
1	reference	name	pool	seq	position	length	%gc	tm (use 65)	
2	MN908947.3	nCoV-2019_1_LEFT	nCoV-2019_1	ACCAACCAACTTTCGATCTCTTGT	30	54	24	41.67	60.69
3	MN908947.3	nCoV-2019_1_RIGHT	nCoV-2019_1	CATCTTTAAGATGTTGACGTGCCCTC	385	410	25	44.00	60.45
4	MN908947.3	nCoV-2019_2_LEFT	nCoV-2019_2	CTGTTTTACAGGTTCCGCGACGT	320	342	22	50.00	61.67
5	MN908947.3	nCoV-2019_2_RIGHT	nCoV-2019_2	TAAGGATCAGTGCCAAGCTCGT	704	726	22	50.00	61.74
6	MN908947.3	nCoV-2019_3_LEFT	nCoV-2019_1	CGGTAATAAAGAGACTGGTGGC	642	664	22	54.55	61.32
7	MN908947.3	nCoV-2019_3_RIGHT	nCoV-2019_1	AAGGTGTCTGCAATTCATAGCTCT	1004	1028	24	41.67	60.32
8	MN908947.3	nCoV-2019_4_LEFT	nCoV-2019_2	GGTGTACTGCTGCCGTGAAC	943	965	22	54.55	61.56
9	MN908947.3	nCoV-2019_4_RIGHT	nCoV-2019_2	CACAAGTAGTGGCACCTTCTTAGT	1312	1337	25	44.00	60.97
10	MN908947.3	nCoV-2019_5_LEFT	nCoV-2019_1	TGGTGAAACTTCATGGCAGACG	1242	1264	22	50.00	61.39
11	MN908947.3	nCoV-2019_5_RIGHT	nCoV-2019_1	ATTGATGTTGACTTTCTCTTTTGGAGT	1623	1651	28	32.14	60.17
12	MN908947.3	nCoV-2019_6_LEFT	nCoV-2019_2	GGTGTGTTGGAGAAGGTTCCG	1573	1595	22	54.55	61.64
13	MN908947.3	nCoV-2019_6_RIGHT	nCoV-2019_2	TAGCGGCCTCTGTAAAACACG	1942	1964	22	50.00	61.18
14	MN908947.3	nCoV-2019_7_LEFT	nCoV-2019_1	ATCAGAGGCTGCTCGTGTGTA	1875	1897	22	50.00	61.73
15	MN908947.3	nCoV-2019_7_LEFT_alt0	nCoV-2019_1	CATTTGCATCAGAGGCTGCTCG	1868	1890	22	54.55	62.44
16	MN908947.3	nCoV-2019_7_RIGHT	nCoV-2019_1	TGCACAGGTGACAATTTGTCCA	2247	2269	22	45.45	60.95
17	MN908947.3	nCoV-2019_7_RIGHT_alt5	nCoV-2019_1	AGGTGACAATTTGTCCACCGAC	2242	2264	22	50.00	61.07
18	MN908947.3	nCoV-2019_8_LEFT	nCoV-2019_2	AGAGTTTCTTAGAGACGGTTGGGA	2181	2205	24	45.83	61.00
19	MN908947.3	nCoV-2019_8_RIGHT	nCoV-2019_2	GCTTCAACAGCTTCACTAGTAGGT	2568	2592	24	45.83	60.56
20	MN908947.3	nCoV-2019_9_LEFT	nCoV-2019_1	TCCCACAGAAGTGTTAACAGAGGA	2505	2529	24	45.83	61.18
21	MN908947.3	nCoV-2019_9_LEFT_alt4	nCoV-2019_1	TTCCCACAGAAGTGTTAACAGAGG	2504	2528	24	45.83	60.44
22	MN908947.3	nCoV-2019_9_RIGHT	nCoV-2019_1	ATGACAGCATCTGCCACAACAC	2882	2904	22	50.00	61.71
23	MN908947.3	nCoV-2019_9_RIGHT_alt2	nCoV-2019_1	GACAGCATCTGCCACAACACAG	2880	2902	22	54.55	62.26
24	MN908947.3	nCoV-2019_10_LEFT	nCoV-2019_2	TGAGAAGTGCTCTGCTTACAGT	2826	2850	24	45.83	61.12
25	MN908947.3	nCoV-2019_10_RIGHT	nCoV-2019_2	TCATCTAACCAATCTTCTTCTGCTCT	3183	3210	27	37.04	60.31
26	MN908947.3	nCoV-2019_11_LEFT	nCoV-2019_1	GGAATTTGGTGCCACTTCTGCT	3144	3166	22	50.00	61.66
27	MN908947.3	nCoV-2019_11_RIGHT	nCoV-2019_1	TCATCAGATTCAACTGCATGGCA	3507	3531	24	41.67	61.35
28	MN908947.3	nCoV-2019_12_LEFT	nCoV-2019_2	AAACATGGAGGAGGTGTGCGAG	3460	3482	22	50.00	61.08
29	MN908947.3	nCoV-2019_12_RIGHT	nCoV-2019_2	TTCACTCTTCATTTCCAAAAGCTTGA	3826	3853	27	33.33	60.36
30	MN908947.3	nCoV-2019_13_LEFT	nCoV-2019_1	TCGCACAAATGTCTACTTAGCTGT	3771	3795	24	41.67	60.56
31	MN908947.3	nCoV-2019_13_RIGHT	nCoV-2019_1	ACCACAGCAGTTAAACACCCT	4142	4164	22	45.45	60.36
32	MN908947.3	nCoV-2019_14_LEFT	nCoV-2019_2	CATCCAGATTCTGCCACTCTCTCT	4054	4077	22	47.82	60.62

218 primers



Eddie Lusamaki

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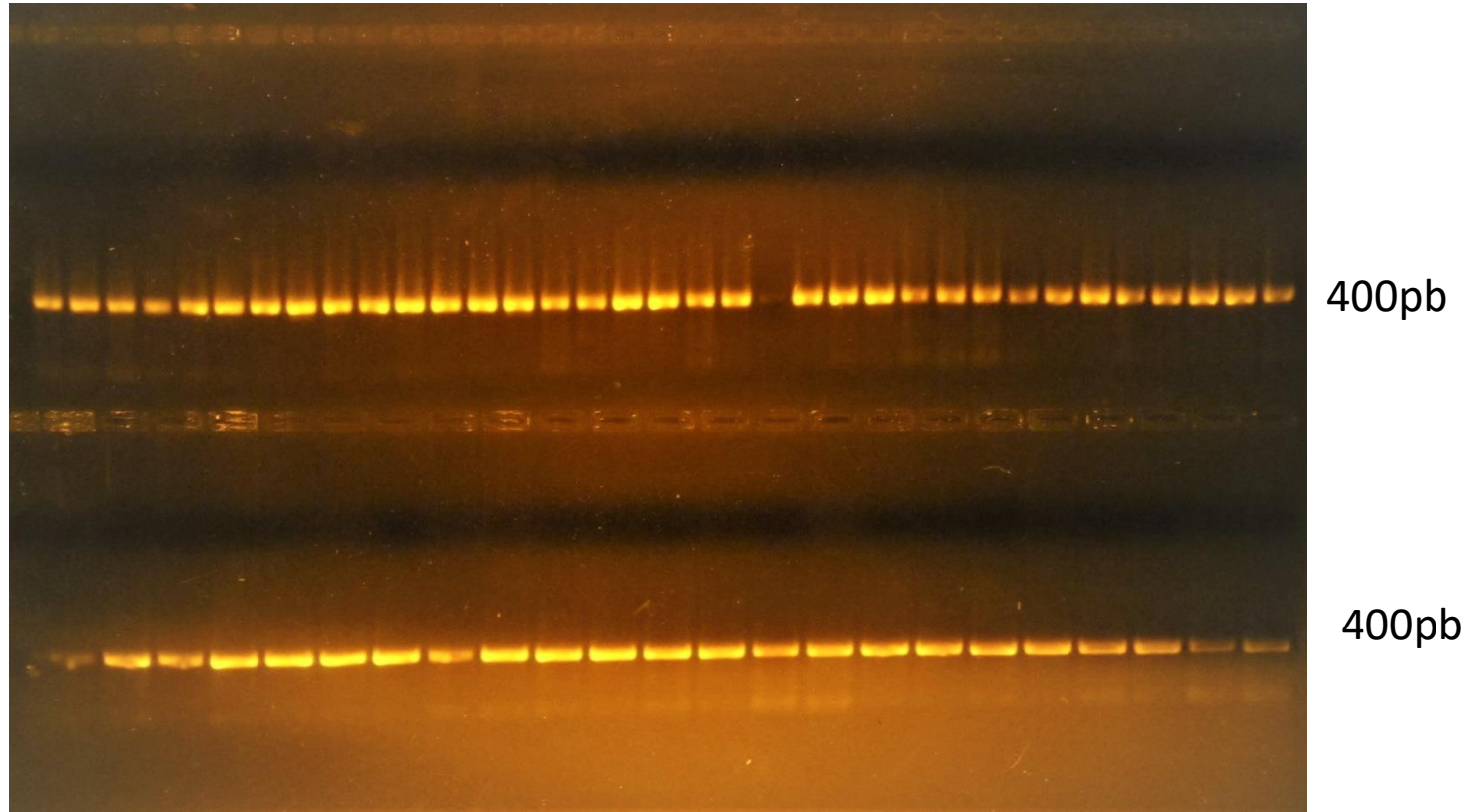
Coronavirus

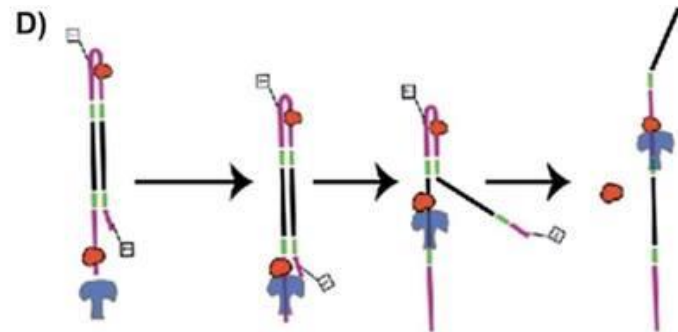
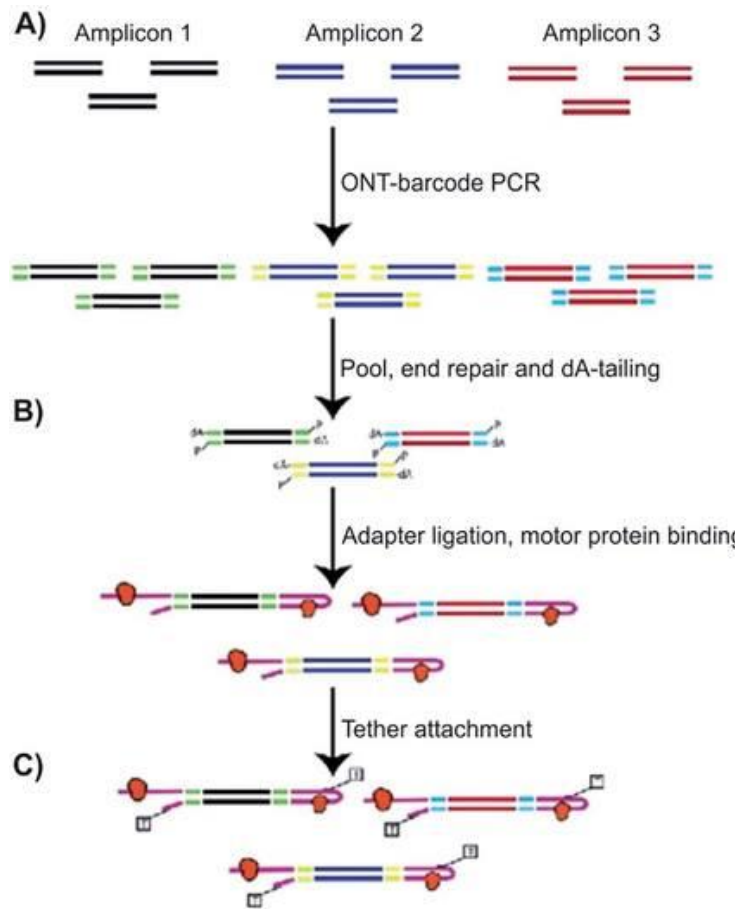
Antibody tests / Healthcare staff to get kits from next week

NHS
Repayment scheme extended to low-paid workers

<https://go.idtdna.com/NGS-Coronavirus-Research.html>

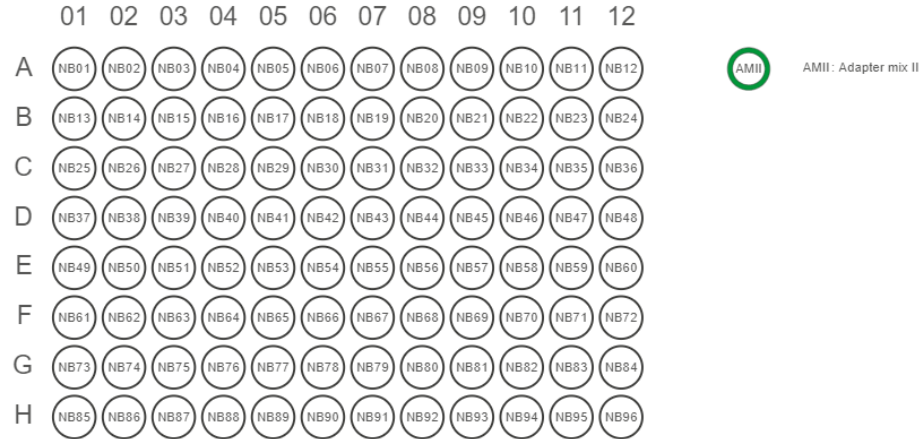
Amplicones pooles 1 y 2





Barcoding

The Native Barcoding Expansion 96 contains 96 unique barcodes and sufficient reagents for 12 sequencing libraries.



	No barcodes	6 barcodes	12 barcodes	96 barcodes
Flow cell price	\$500	\$500	\$500	\$500
Library price	\$99	\$99	\$99	\$99
Barcode price	-	\$25	\$50	\$100
Price per sample	\$599	\$104	\$54	\$7.3

4/5/2020

Gmail - Lo q mandó Nanopore ONT-Covid19 support

Item	Part Number	# reactions available	Price per item	Price per reaction
Flow Cell	FLO-MIN106D	1	\$900.00	\$900.00
Ligation Kit	SQK-LSK109	6	\$599.00	\$99.83
Native Barcoding kit I	EXP-NBD104	6	\$288.00	\$48.00
		# barcodes		\$1,047.83
		12	Price per sample	\$87.32
			Price per sample (including 3rd party reagents)	\$107.32

Third party reagents
\$240.00

Item	Part Number	# reactions available	Price per item	Price per reaction
Flow Cell	FLO-MIN106D	1	\$900.00	\$900.00
Ligation Kit	SQK-LSK109	6	\$599.00	\$99.83
Native Barcoding kit I	EXP-NBD104	6	\$288.00	\$48.00
Native Barcoding kit II	EXP-NBD114	6	\$288.00	\$48.00
		# barcodes		\$1,095.83
		24	Price per sample	\$45.66
			Price per sample (including 3rd party reagents)	\$64.41

Third party reagents
\$450.00

MinKNOW: software de corrida

MinKNOW- A new paradigm in sequencing



Real-time monitoring through MinKNOW

- No fixed run time
- Real-time basecalling
- Real-time data assessment
- On-demand sequencing
- Pause a sequencing run
- Efficient data acquisition



MinION Release 20.06.5

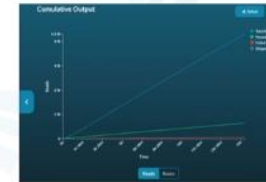
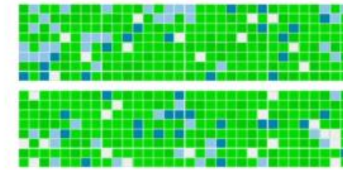
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Real-time data assessment

Channels Panel

Live status of each channel's state during sequencing

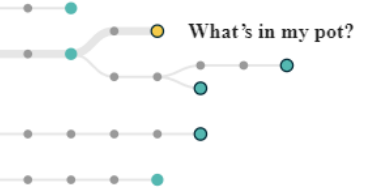
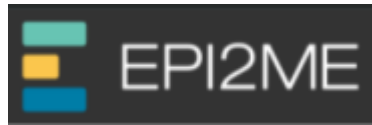


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>20 muestras
24 muestras
48 muestras


Rendimiento
Tiempo de análisis

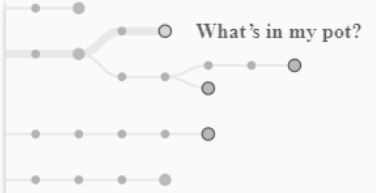


What's in my pot?

FASTQ WIMP 2020.05.19

Taxonomic assignment


20 



What's in my pot?


FASTQ WIMP (HUMAN + VIRAL) 2021.04.05

Centrifuge index created by <https://genexa.ch>. It is based on NCBI RefSeq and NCBI Virus genomes (VERSION 2, including 138 SARS-CoV-2 complete genomes from NCBI Virus in addition to RefSeq). Download (Data fetched March 27th, 2020 from NCBI)




FASTQ RNA CONTROL EXPERIMENT 2021.03.30

See how your experiment performed



FASTQ CONTROL EXPERIMENT 2021.03.30


See how your experiment performed



CUSTOM REFERENCE ALIGNMENT


FASTQ CUSTOM ALIGNMENT 2021.03.25

Custom Reference Alignment



FASTQ QC + ARTIC + NEXTCLADE 1.0.4


QC + ARTIC + NextClade for SARS-CoV2 / COVID19



> CONNECTION TEST

FASTQ CONNECTION TEST 3.10.4

Test your EPI2ME configuration

5 



HUMAN ALIGNMENT

FASTQ HUMAN ALIGNMENT 2020.03.11

GRCh38

Align against Human GRCh38 reference

Activar Windows 60 

Ve a Configuración para activar W

Muestras

Mayo – Diciembre 2020

Enero-Mayo 2021



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](#)

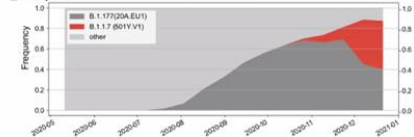
* Apparent cumulative prevalence is the ratio of the sequences containing B.1.1.7 to all sequences collected since the identification of B.1.1.7 in that location. ** Dates are based on the sample collection date

[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

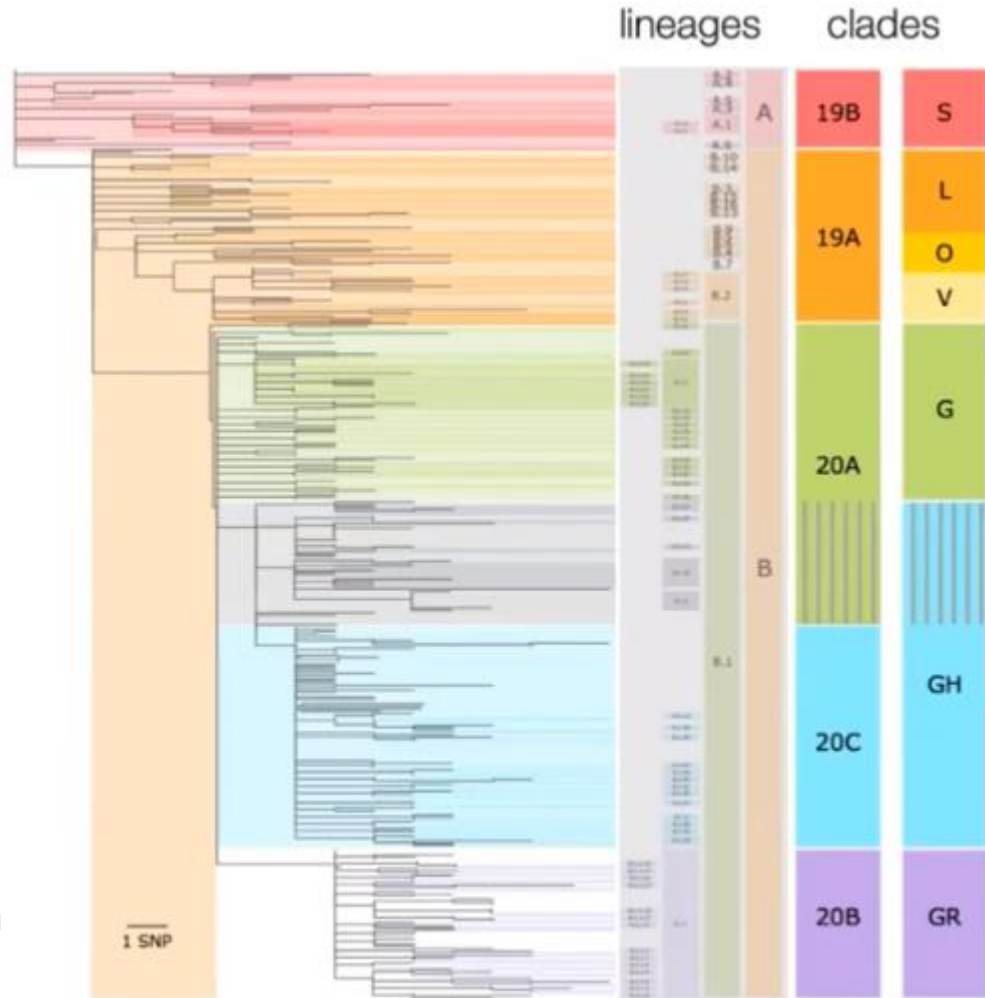


Clados

- 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



Nextclade

Analysing sequences: 49/108

What's new English

Back Run New Filter Export to CSV Show Tree Settings

Results filter

By sequence name
Ex: Wuhan_WH

By nucleotide mutations
Ex: C3037T_A

By aminoacid changes
Ex: ORF1b:P314L_S_384_ORF1b:P314-

By clades
Ex: 198_20

By quality
 Good quality
 Mediocre quality
 Bad quality
 Has errors

D	Sequence name	QC	Clade	Mut.	non-ACGTN	Ns	Gaps	Sequence view
0	hCoV-19/Paraguay/lics_9055/2021	N M P C	20J/501YV3	36	0	4	9	
1	hCoV-19/Paraguay/lics_9640/2021	N M P C	20J/501YV3	33	0	4	9	
2	hCoV-19/Paraguay/lics_11609/2021	N M P C	19B	33	0	279	9	
3	hCoV-19/Paraguay/lics_12516/2021	N M P C	20J/501YV3	31	0	226	9	
4	hCoV-19/Paraguay/lics_10354/2021	N M P C	20B	26	0	0	0	
5	hCoV-19/Paraguay/lics_9781/2021	N M P C	20B	28	0	0	0	
6	hCoV-19/Paraguay/lics_10776/2021	N M P C	20J/501YV3	33	0	2	9	
7	hCoV-19/Paraguay/lics_12384/2021	N M P C	20J/501YV3	31	0	780	9	
8	hCoV-19/Paraguay/lics_12523/2021	N M P C	20J/501YV3	35	0	3	9	
9	hCoV-19/Paraguay/lics_9105/2021	N M P C	20B	28	0	0	1	
10	hCoV-19/Paraguay/lics_9814/2021	N M P C	20J/501YV3	35	0	5	9	

Genome annotation

5000 10000 15000

Active Windows: Ve a 20000 duración para 25000 Windows.

MAFFT version 7

Multiple alignment program for amino acid or nucleotide sequences



Análisis filogenéticos preliminares

[Genomes](#)[Genome Browser](#)[Tools](#)[Mirrors](#)[Downloads](#)[My Data](#)[Projects](#)[Help](#)[About Us](#)

JShER: Ultrafast Sample placement on Existing tRee

Sequence name 'hCoV-19/Paraguay/iics_12209/2021' has already been used; ignoring subsequent usage (29903 bases, 2 N's, 0 ambiguous).

[view in Genome Browser](#)[view single subtree in Nextstrain](#)

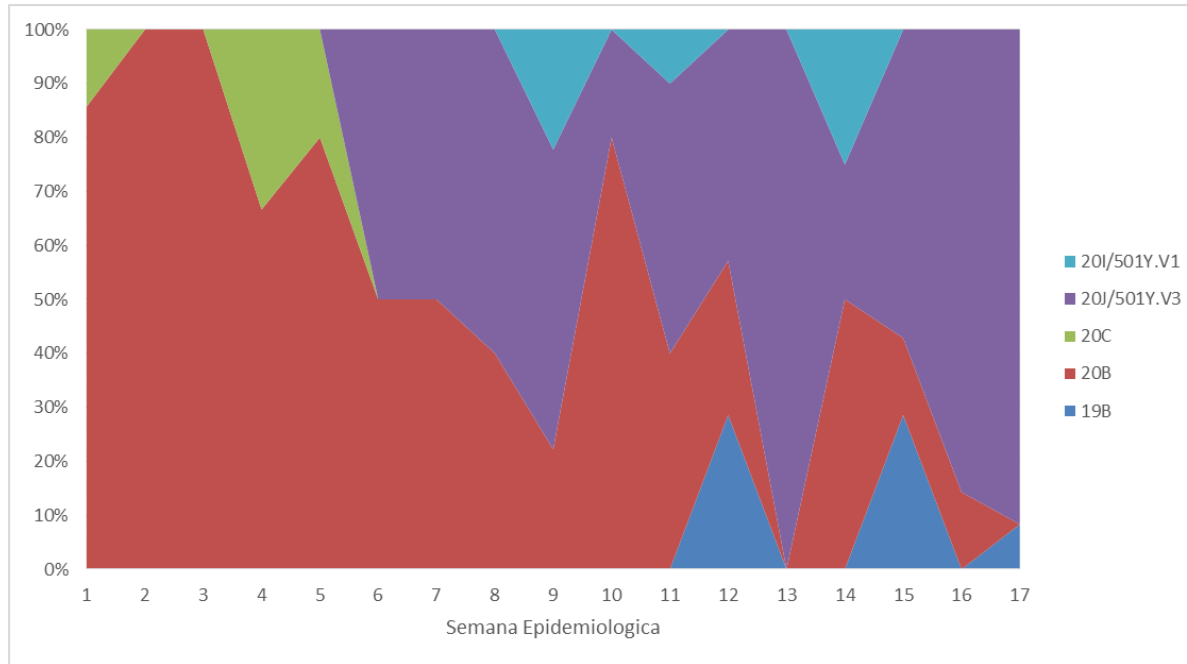
Nextstrain

Real-time tracking of pathogen evolution

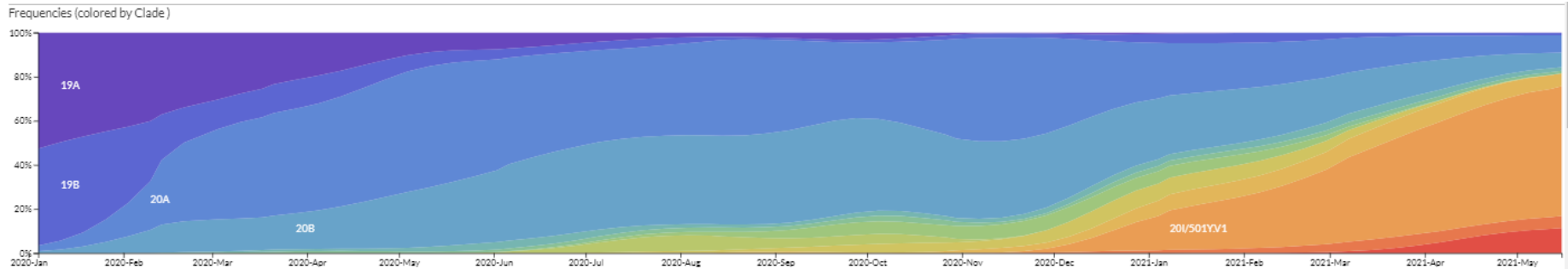
Nextstrain is an open-source project to harness the scientific and public health potential of pathogen genome data. We provide a continually-updated view of publicly available data alongside powerful analytic and visualization tools for use by the community. Our goal is to aid epidemiological understanding and improve outbreak response. If you have any questions, or simply want to say hi, please give us a shout at hello@nextstrain.org.

[READ MORE](#)

Clados detectados en el Dpto. Central Enero 2021-Mayo 2021



Frecuencia global de clados-Nexstrain



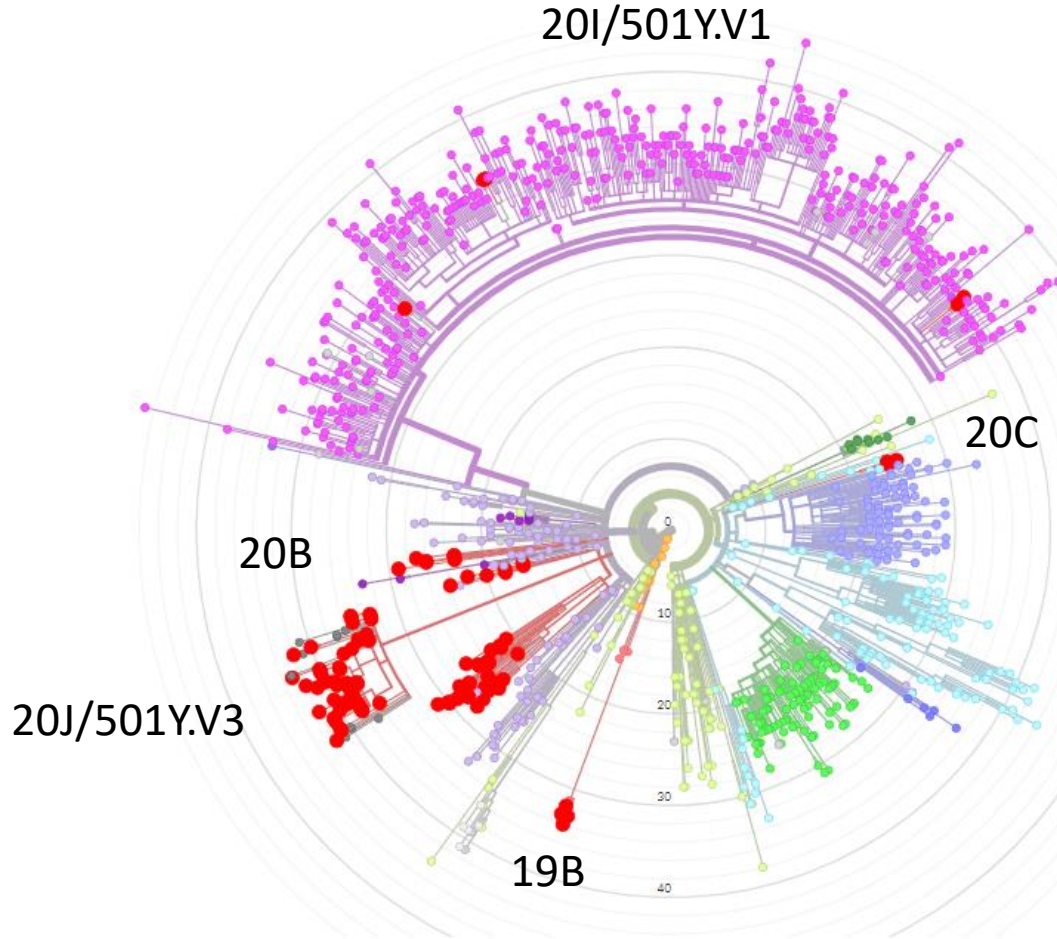
Subtree with hCoV-19/Paraguay/iics_9055/2021 and 106 other uploaded samples

Showing 1107 of 1107 genomes.

Nextstrain Clades

Phylogeny

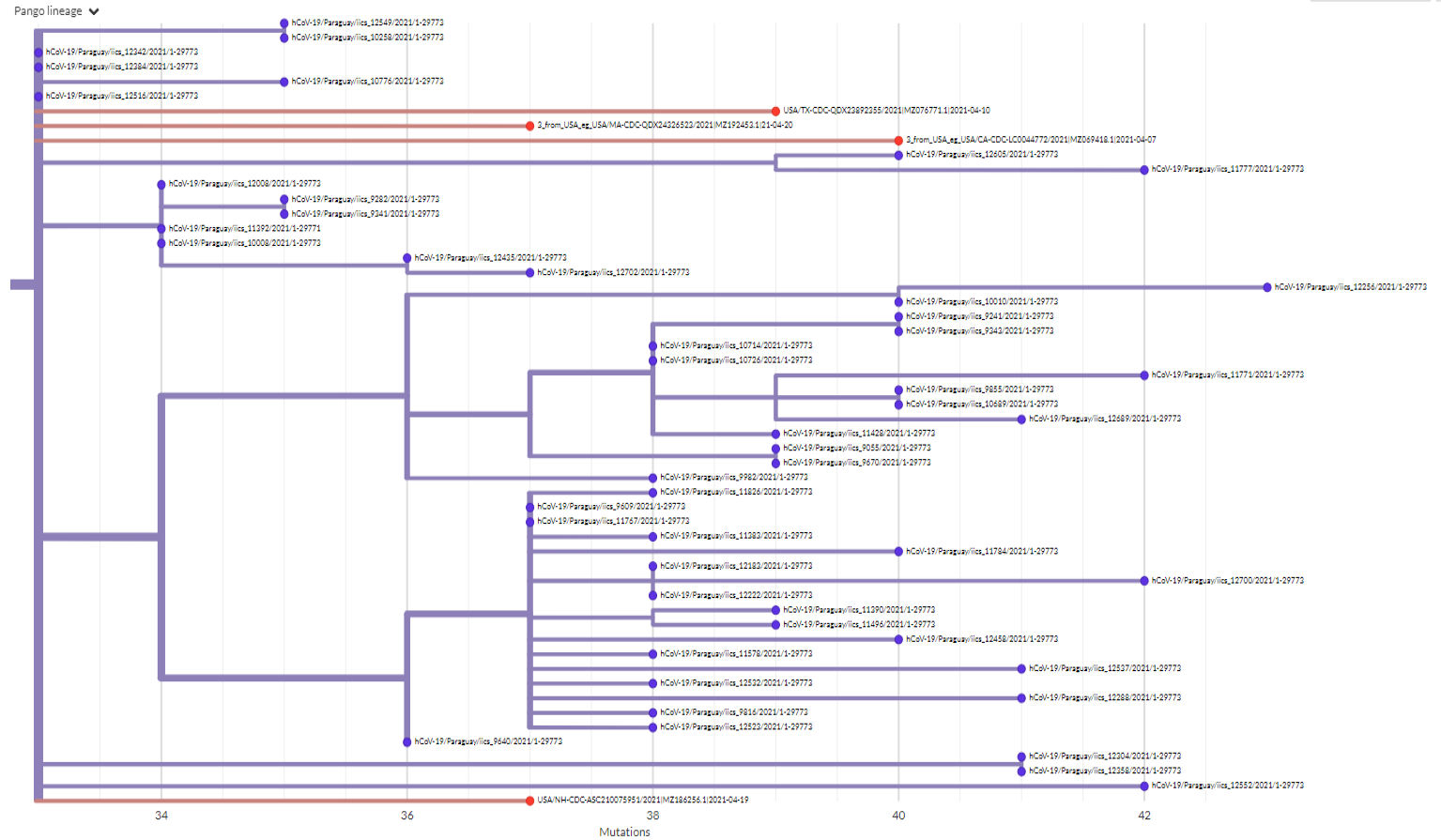
Nextstrain Clade ^



Linaje P1

Phylogeny

ZOOM TO SELECTED



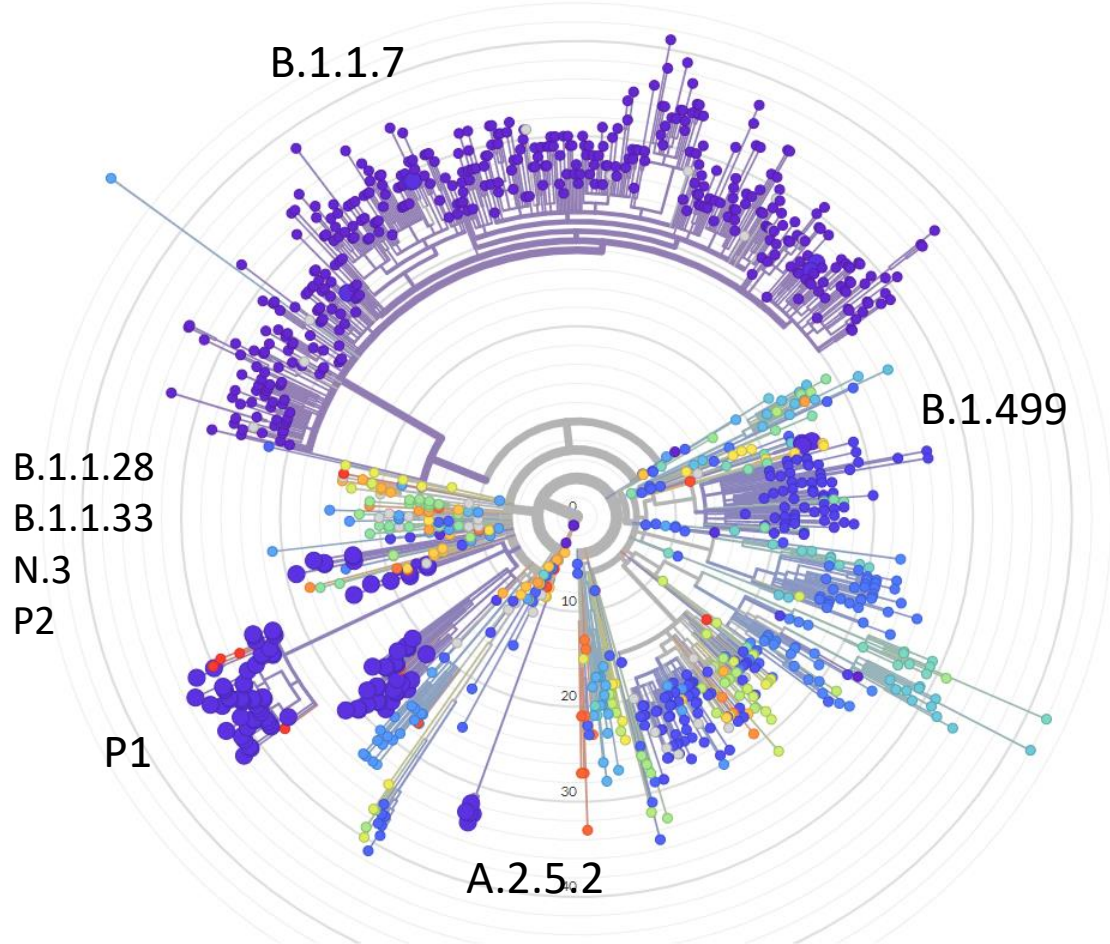
Showing 1107 of 1107 genomes.

Phylogeny

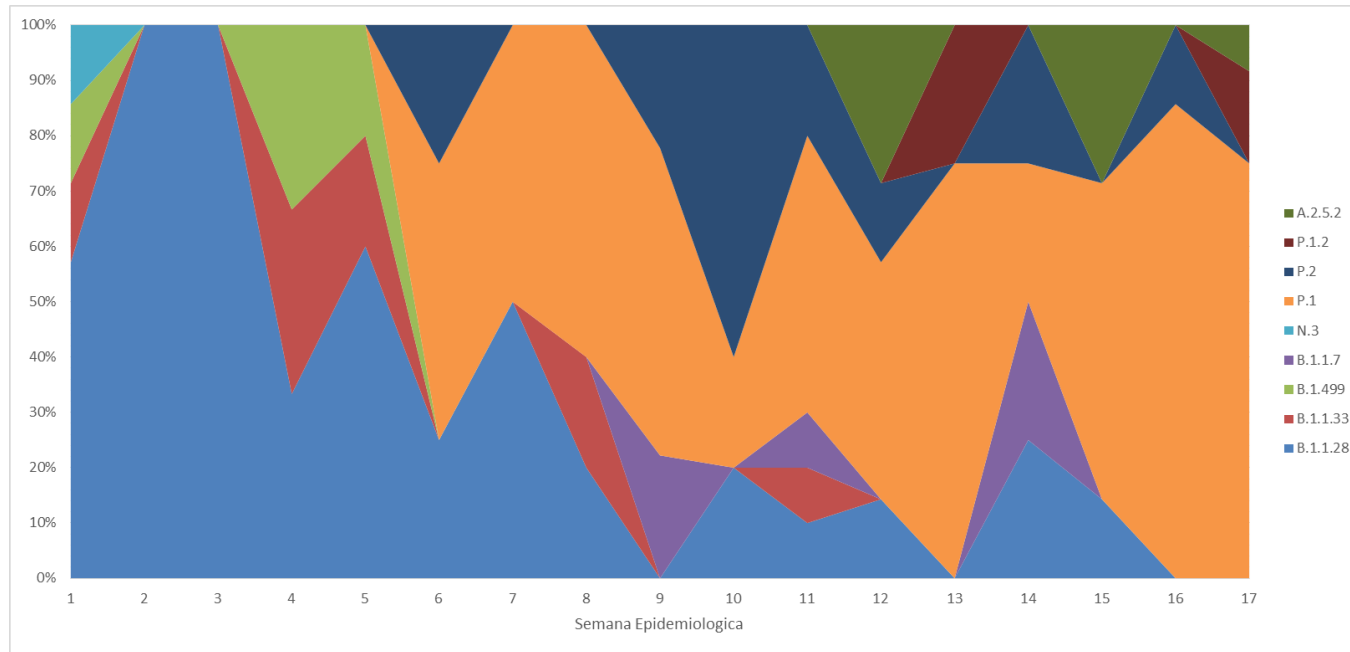
Pango lineage ^

B.1.1.7	B.1.1.361
uploaded sample	B.1.1.365
B.1.2	B.1.1.368
B.1.1.77	B.1.1.369
B.1	B.1.1.4
B.1.429	B.1.1.453
B.1.526	B.1.1.50
B.1.1.519	B.1.1.512
B.1.1	B.1.1.59
B.1.243	B.1.1.86
B.1.1.60	B.1.1.89
B.1.526.2	B.1.1.10.3
B.1.427	B.1.1.13
B.1.526.1	B.1.1.13
B.1.596	B.1.1.153
B.1.1.37	B.1.1.160.12
D.2	B.1.1.160.14
B.1.575	B.1.1.160.16
B.1.1.77.87	B.1.1.160.30
B.1.232	B.1.1.177.11
B.1.1.77.15	B.1.1.177.16
AD.2	B.1.1.177.19
B.1.1.39	B.1.1.177.23
B.1.234	B.1.1.177.43
B.1.369	B.1.1.177.53
B.1.577	B.1.1.177.54
A.1	B.1.1.177.57
B.1.1.1	B.1.1.177.7
B.1.1.119	B.1.1.177.82
B.1.1.315	B.1.1.177.84
B.1.1.70	B.1.1.195
B.1.1.77.4	B.1.222
B.1.240	B.1.258.14
B.1.258	B.1.258.17
B.40	B.1.3
P.1	B.1.311
B	B.1.313
B.1.1.253	B.1.324
B.1.1.279	B.1.337
B.1.1.289	B.1.349
B.1.1.311	B.1.351
B.1.1.77.18	B.1.36.22

Nextstrain Lineages

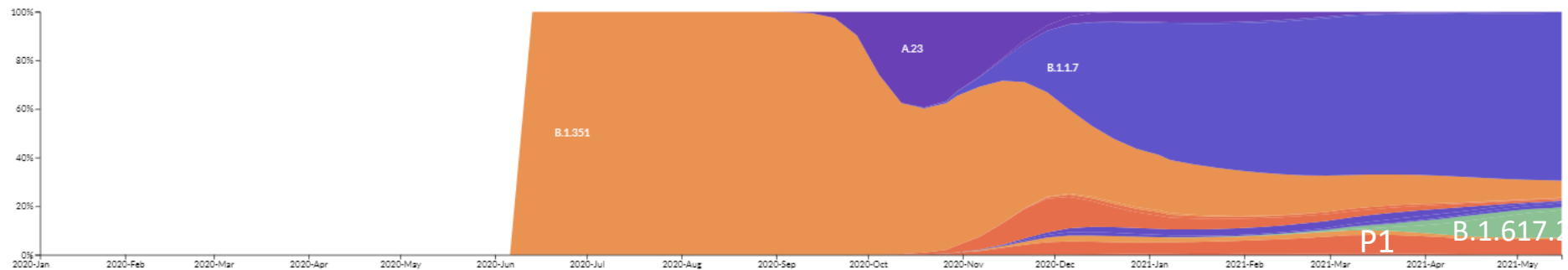


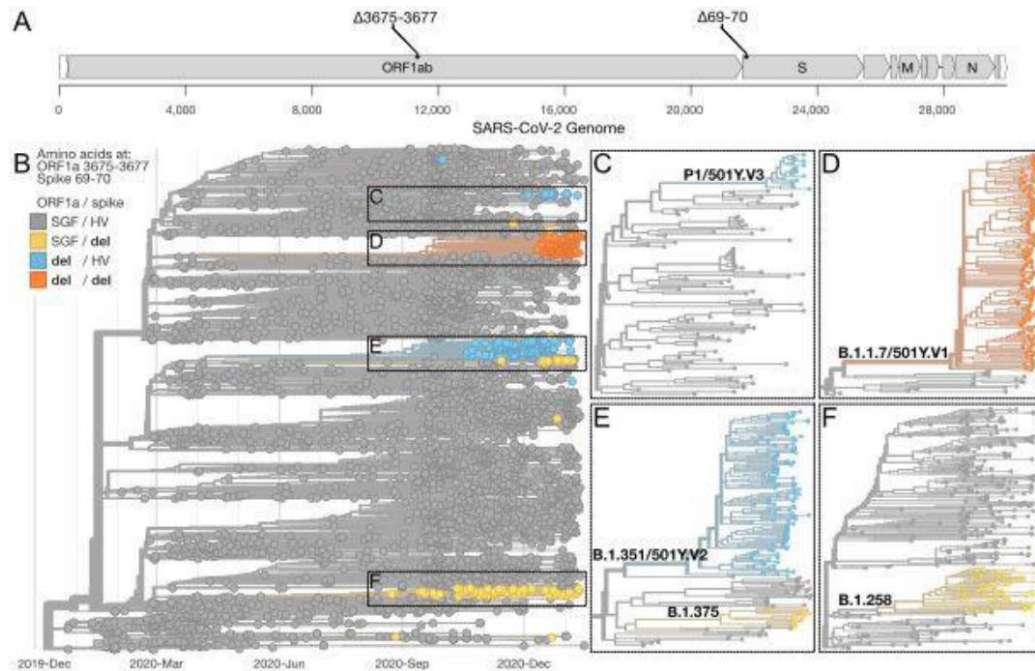
Linajes detectados en el Dpto. Central Enero 2021-Mayo 2021



Frecuencia global de linajes emergentes-Nextstrain

Frequencies (colored by PANGO Lineage and normalized to 100% at each time point for 1618 out of a total of 3883 tips)





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

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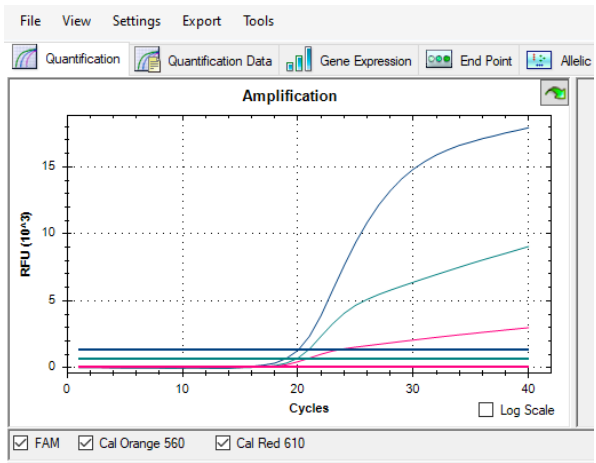
[†] These authors contributed equally

* Senior authors

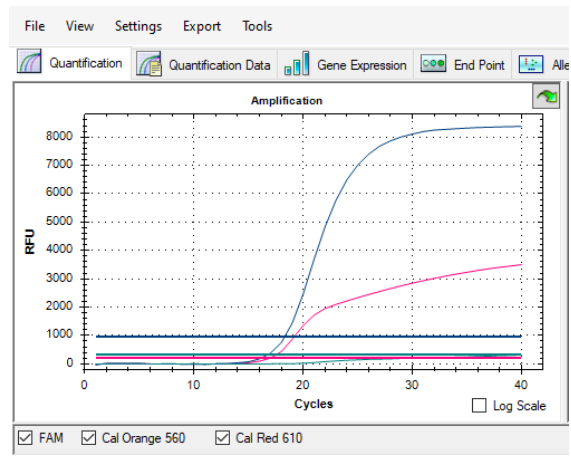
Correspondence: Chantal B.F. Vogels (chantal.vogels@yale.edu), Joseph R. Fauver (joseph.fauver@yale.edu), Nathan D. Grubaugh (nathan.grubaugh@yale.edu)

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

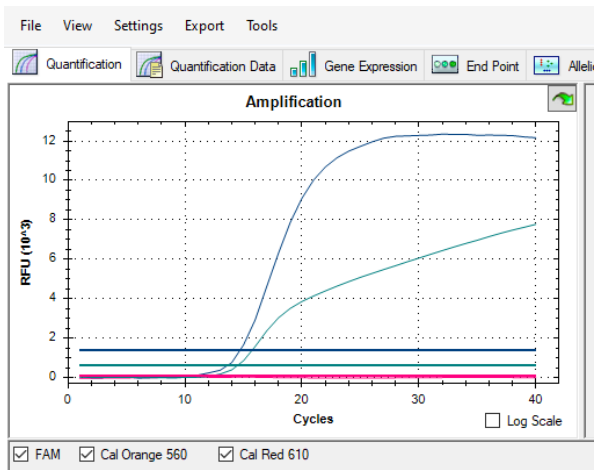
Result	N1-FAM	Orf1a-Cy5	Spike-HEX
Potentially B.1.1.7	CT ≤ 35	Undetected	Undetected
Potentially B.1.351 or P.1	CT ≤ 35	Undetected	CT ≤ 35
Potentially B.1.375	CT ≤ 35	CT ≤ 35	Undetected
Other lineages	CT ≤ 35	CT ≤ 35	CT ≤ 35
Inconclusive	CT > 35 or undetected	Any value	Any value



«wt»

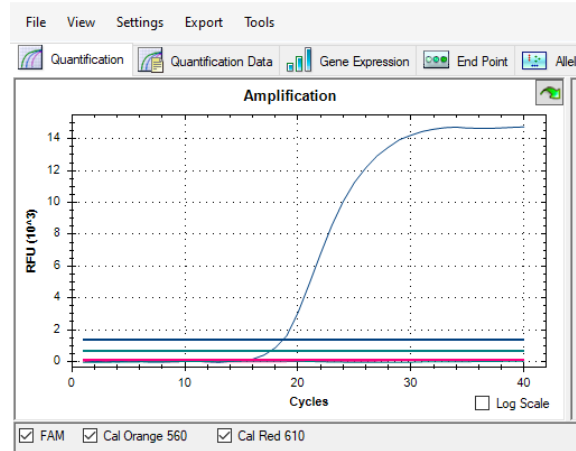


Dropout S (no voc, 14/03/2021)



Dropout Orf1a

13/02/2021

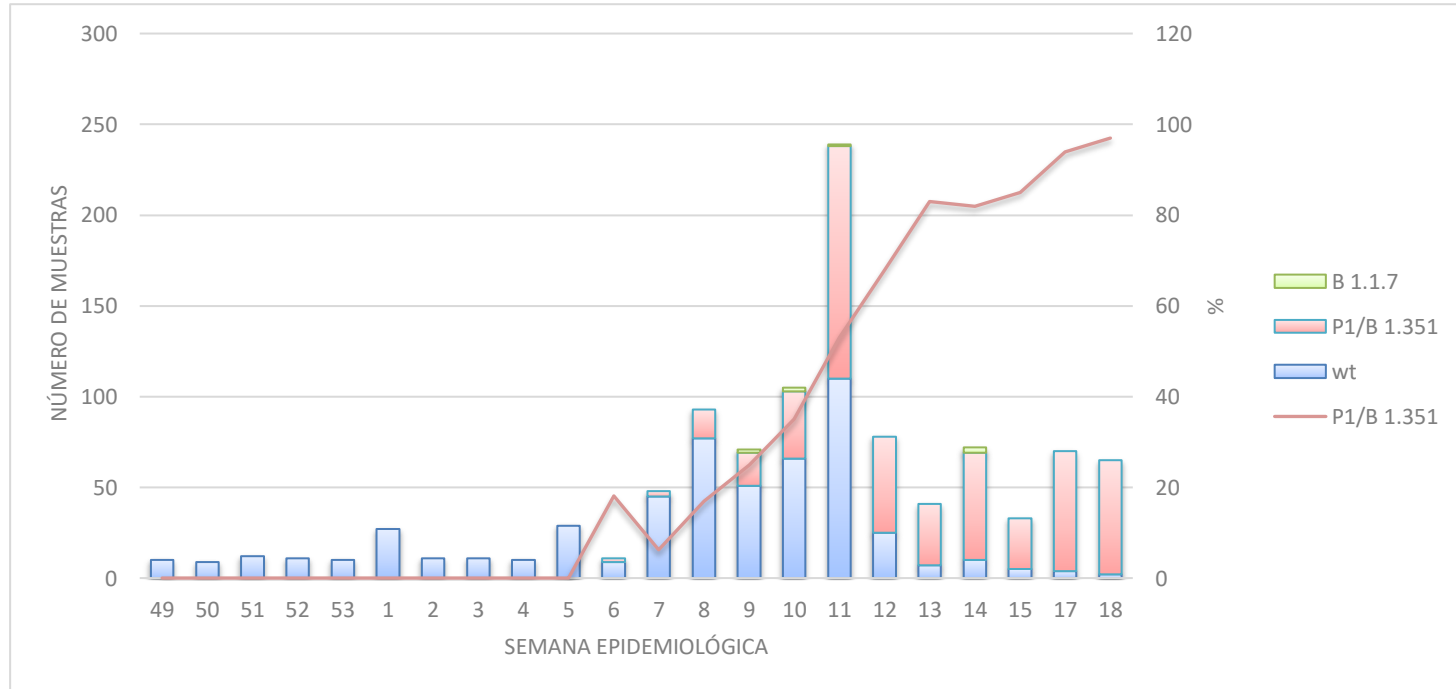


Dropout Orf1a/S

28/02/2021

Detección de variantes por RT-PCR-CENTRAL

Muestras Diciembre 2020-Marzo 2021

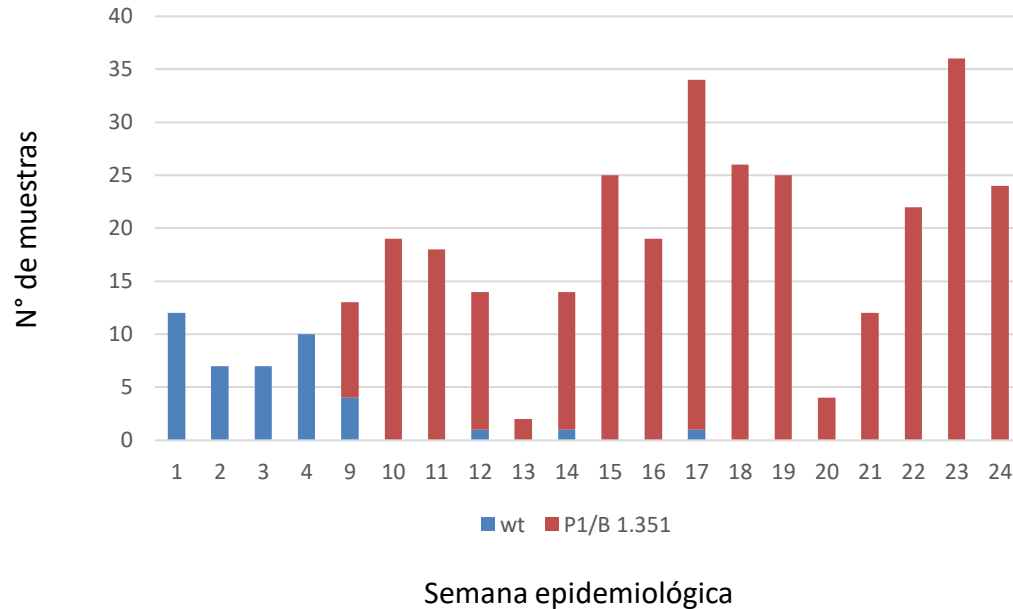


*Central junio: proporción P/B.1.351 sigue igual



ITAPUA

Enero hasta semana 2 de junio 2021

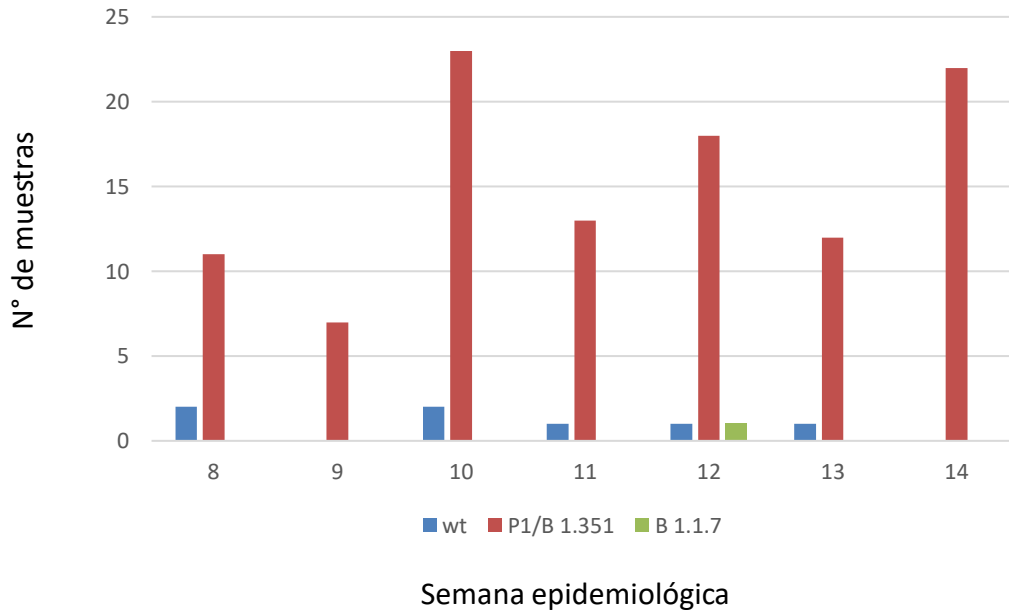


343 muestras analizadas RT-PCR variantes



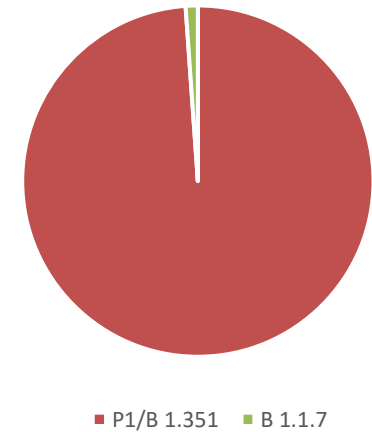
ALTO PARANA

Semana 4 de febrero hasta semana 1 de abril 2021



114 muestras analizadas RT-PCR variantes

Junio 2021



179 muestras analizadas RT-PCR variantes

En proceso (PINV-239):
Análisis filogenéticos (objetivos específicos)

Perspectivas:
Otras herramientas de análisis de datos crudos

GRACIAS

Eva Nara
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Laura Franco
Leticia Rojas
Chyntia Díaz
Florencia del Puerto
Fátima Cardozo
Adriana Valenzuela
Laura Mendoza
Alejandra Rojas
Ana Ayala
Vicente Martínez
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Graciela Riera
Roque Morel
Fátima Ovando

Fátima Macchi
Alexandra Mercado
Diego González
Mario Martínez
Joel Ortíz
Jonás Fernández
Marta Giovanetti
Jesse Waggoner

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