CONSEJO NACIONAL DE CIENCIA Y TECNOLOGÍA

PROCIENCIA

PROGRAMA PARAGUAYO PARA EL DESARROLLO DE LA CIENCIA Y TECNOLOGÍA

ALTER VIDA

CENTRO DE ESTUDIOS Y FORMACIÓN PARA EL ECODESARROLLO

Con el apoyo de:

FEEI

Fondo para la Excelencia de la Educación y la Investigación

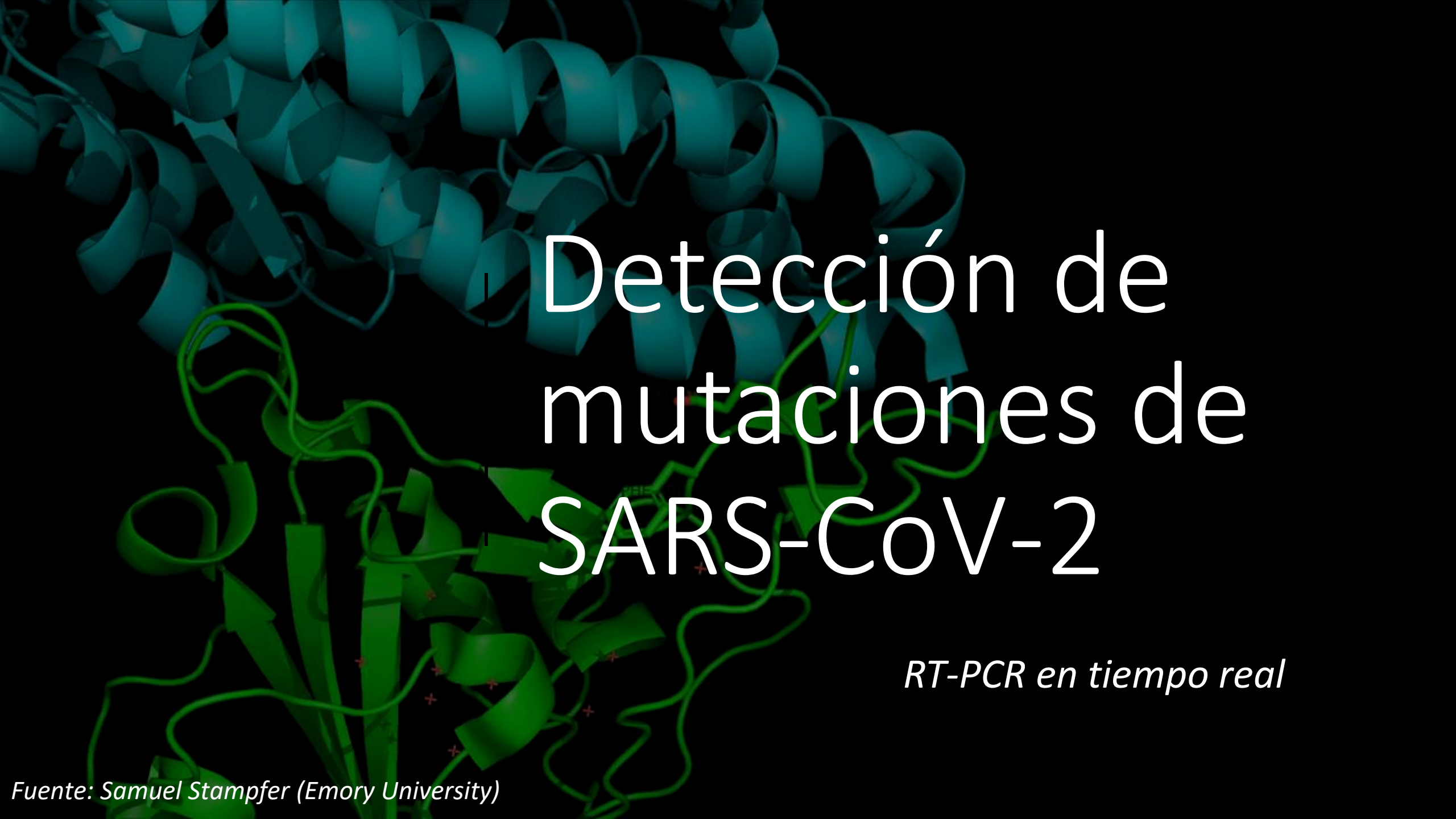
PROYECTO PINV20-239: "Estudio de la dinámica de transmisión y de la variabilidad genética de SARS-CoV 2 circulantes en Paraguay a través del análisis de secuencias del genoma viral"

"Curso de Secuenciación del genoma de SARS-CoV 2 con la plataforma MinION".

Fecha: 21 al 23 de julio de 2021.

"Este proyecto es cofinanciado por el Consejo Nacional de Ciencia y Tecnología - CONACYT con recursos del FEEI"

Para más informaciones: biomol@iics.una.py, imartinez@iics.una.py



Detección de mutaciones de SARS-CoV-2

RT-PCR en tiempo real

Fuente: Samuel Stampfer (Emory University)

VoCs

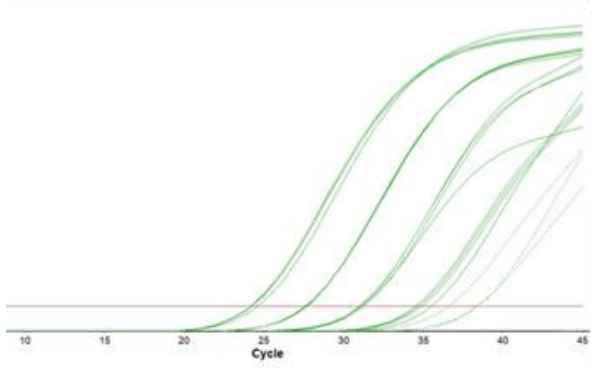
- CDC has 3 categories of SARS-CoV-2 variants
  - Variants of interest
  - Variants of concern
  - Variants of high consequence

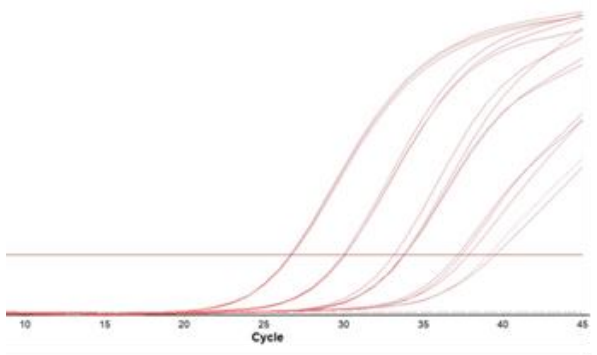
- Defined by potential significance of these strains on
  - Transmissibility
  - Clinical outcome
  - Detection
  - Treatment

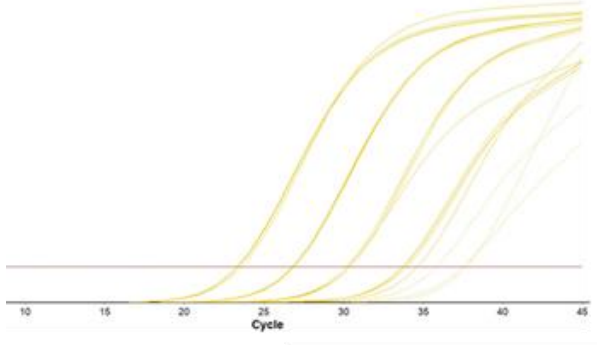
Name (Pango lineage)	Spike Protein Substitutions	Name (Nextstrain[a])	First Detected	BEI Reference Isolate[b]	Known Attributes
B.1.1.7	Δ69/70 Δ144Y (E484K*) (S494P*) N501Y A570D D614G P681H	20I/501Y.V1	United Kingdom	NR-54000	• ~50% increased transmission [5] • Likely increased severity based on hospitalizations and case fatality rates [6] • Minimal impact on neutralization by EUA monoclonal antibody therapeutics [7, 14] • Minimal impact on neutralization by convalescent and post-vaccination sera [8,9,10,11,12,13,19]
P.1	K417N/T E484K N501Y D614G	20J/501Y.V3	Japan/ Brazil	NR-54982	• Moderate impact on neutralization by EUA monoclonal antibody therapeutics [7,14] • Reduced neutralization by convalescent and post-vaccination sera [15]
B.1.351	K417N E484K N501Y D614G	20H/501.V2	South Africa	NR-54009	• ~50% increased transmission[16] • Moderate impact on neutralization by EUA monoclonal antibody therapeutics [7,14] • Moderate reduction on neutralization by convalescent and post-vaccination sera [8,12,18,19,20]
B.1.427	L452R D614G	20C/S:452R	US-California		• ~20% increased transmissibility [21] • Significant impact on neutralization by some, but not all, EUA therapeutics • Moderate reduction in neutralization using convalescent and post-vaccination sera [21]
B.1.429	S13I W152C L452R D614G	20C/S:452R	US-California		• ~20% increased transmissibility [21] • Significant impact on neutralization by some, but not all, EUA therapeutics • Moderate reduction in neutralization using convalescent and post-vaccination sera [21]

https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance

- Ensayo de Yale
- Ensayo de Emory
- Otros







Ensayo de Yale

Ensayo de Yale

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

Chantal B.F. Vogels[1†*], Mallery I. Breban[1†], Tara Alpert[1], Mary E. Petrone[1], Anne E. Watkins[1], Isabel M. Ott[1], Jaqueline Goes de Jesus[2], Ingra Morales Claro[2], Giulia Magalhães Ferreira[2,3], Myuki A.E. Crispim[4], Brazil-UK CADDE Genomic Network, Lavanya Singh[5], Houriiyah Tegally[5], Ugochukwu J. Anyaneji[5], NGS-SA, Emma B. Hodcroft[6], Christopher E. Mason[7], Gaurav Khullar[7], Jessica Metti[7], Joel T. Dudley[7], Matthew J. MacKay[7], Megan Nash[7], Jianhui Wang[8], Chen Liu[8], Pei Hui[8], Steven Murphy[9], Caleb Neal[9], Eva Laszlo[9], Marie L. Landry[10], Anthony Muyombwe[11], Randy Downing[11], Jafar Razeq[11], Tulio de Oliveira[5], Nuno R. Faria[2,12,13], Ester C. Sabino[2], Richard A. Neher[14,15], Joseph R. Fauver[1#*], Nathan D. Grubaugh[1,16#*]

- N2 (control)
- **S: Δ69/70** – associated with B.1.1.7
- **ORF1a: Δ3675/3677** – found in many VoCs (B.1.1.7, B.1.351, P.1) as well as some variants of interest (B.1.526)

"Dropout"

[1]Department of Epidemiology of Microbial Diseases,
[16]Department of Ecology and Evolutionary Biology
Yale School of Public Health, New Haven, CT 06510, USA.

Spike SNP rRT-PCR for COVID Variant Testing

Waggoner Lab

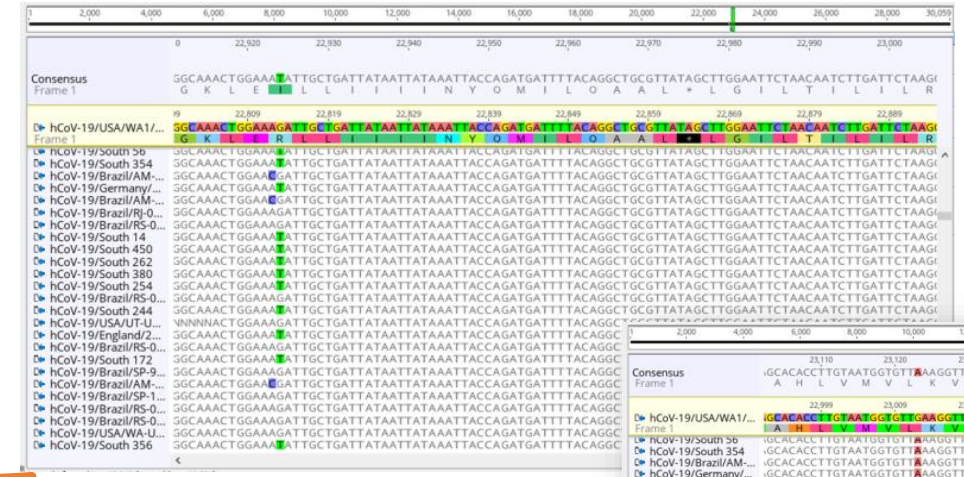
Emory University Department of Medicine

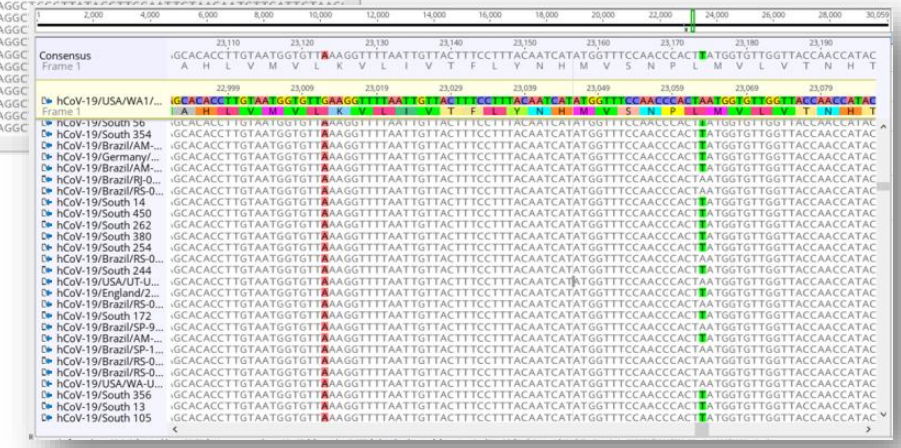
Jesse Waggoner

Ensayo de Emory -Waggoner Lab

- Spike SNP assay
  - Amplification of a specific region of the spike gene
  - Tiled probes to identify specific mutations

SpikeSNP Assay





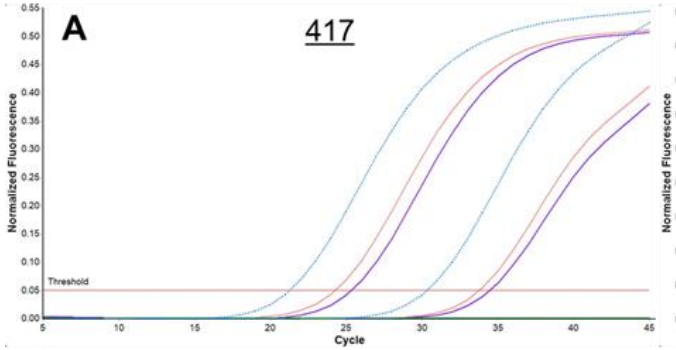
- Current design amplifies a **348bp** region of the spike gene
  - Includes the region encoding AA 413 – 514 (between the primers)
  - Original mix shown at right
- Probes have been designed to selectively identify
  - 417K (wt) – top sequences
  - 501Y – lower sequences (green)
  - 484K – lower sequences (red)
  - **452R**

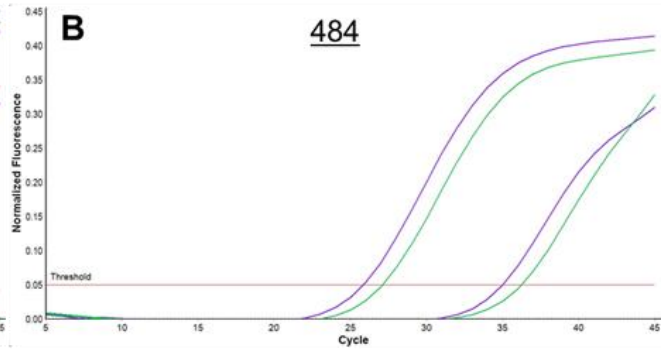
Nombre del Primer
SpikeSNP Ext
SpikeSNP R1
Nombre de la Sonda
417K Pr2
N501Y Pr1.S2
E484K Probe

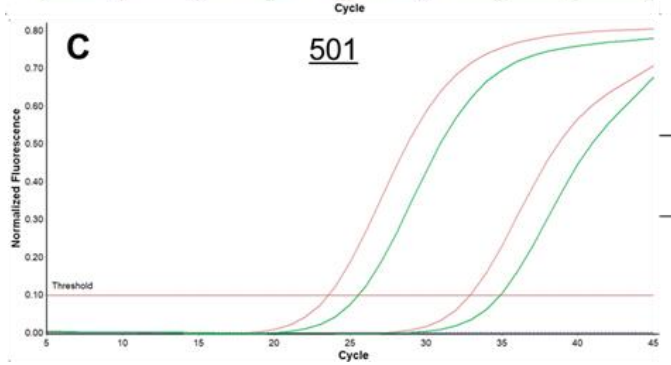
Original mix

Original Version

- Appearance as shown for 417, 484 and 501 targets







D

Spike SNP Result Interpretation

Variant (curve color)	Target (Pos/Neg)			Spike Amino Acid Call		
	417	484	501	417	484	501
BEI 52286 (blue)	Pos	Neg	Neg	K	E	N
B.1.1.7 (pink)	Pos	Neg	Pos	K	E	Y
B.1.525 (puple)	Pos	Pos	Neg	K	K	N
P.1 (green)	Neg	Pos	Pos	variant	K	Y

Ensayo Spike SNP “actual”

Primers
SpikeSNP Ext
SpikeSNP R1
Sondas
417K Pr2
N501Y Pr1.S2
E484K Probe
452R_unmod

Addition of probe for 452R did not affect performance for other targets

Ensayo Spike SNP “actual”

Primers
SpikeSNP Ext
SpikeSNP R1
Sondas
417K Pr2
N501Y Pr1.S2
E484K Probe
452R_unmod

Sondas LNA

Locked nucleic acids (LNAs)

THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Kinetics of DNA Strand Displacement Systems with Locked Nucleic Acids

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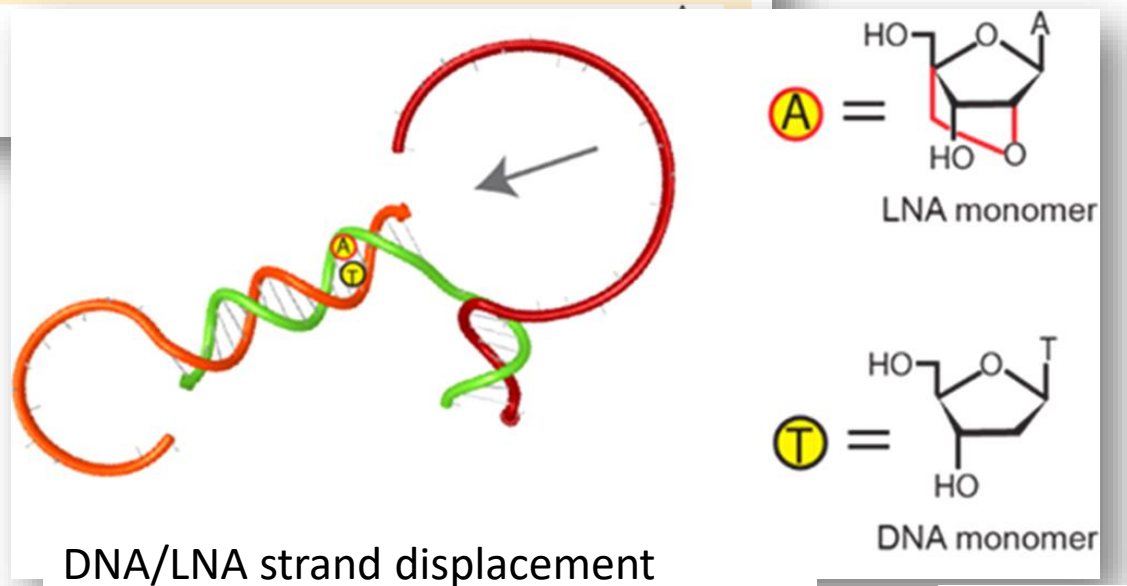
[†]Micron School of Materials Science & Engineering and [‡]Department of Electrical & Computer Engineering, Boise State University, 1910 University Drive, Boise, Idaho 83725, United States

Supporting Information

ABSTRACT: Locked nucleic acids (LNAs) are conformationally restricted RNA nucleotides. Their increased thermal stability and selectivity toward their complements make them well-suited for diagnostic and therapeutic applications.

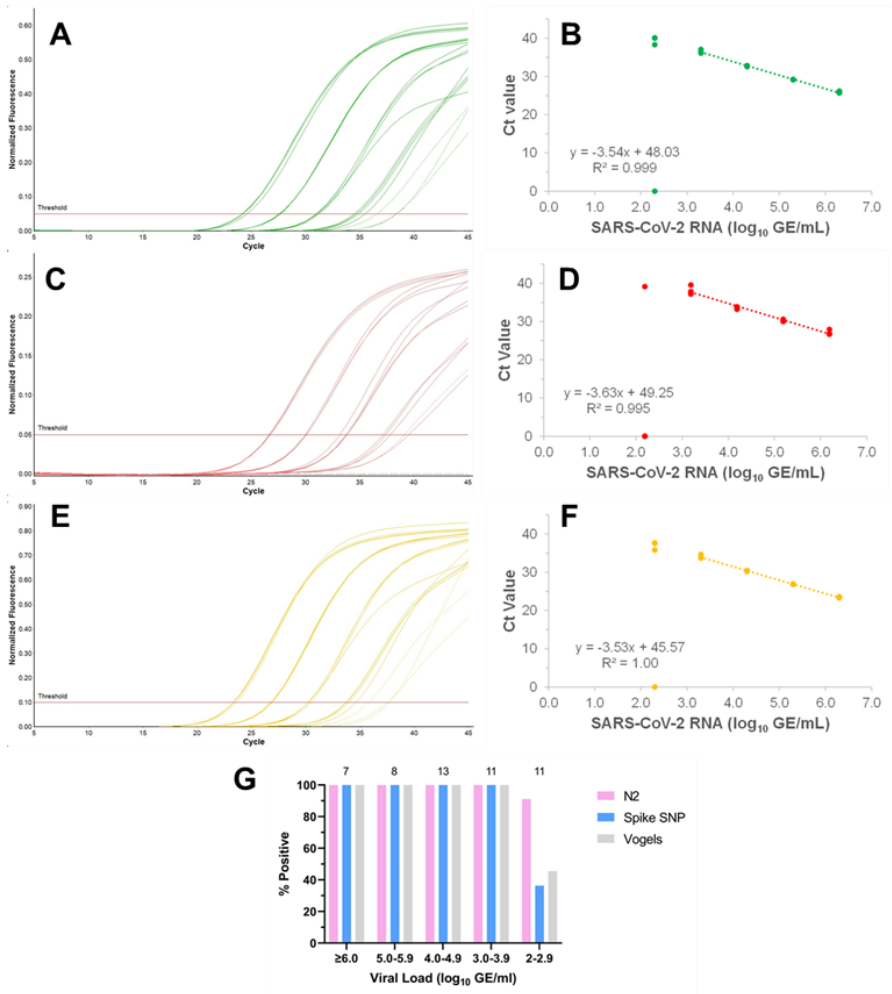


DNA/LNA strand displacement

DOI: 10.1021/acs.jpcb.7b01198
J. Phys. Chem. B 2017, 121, 2594−2602

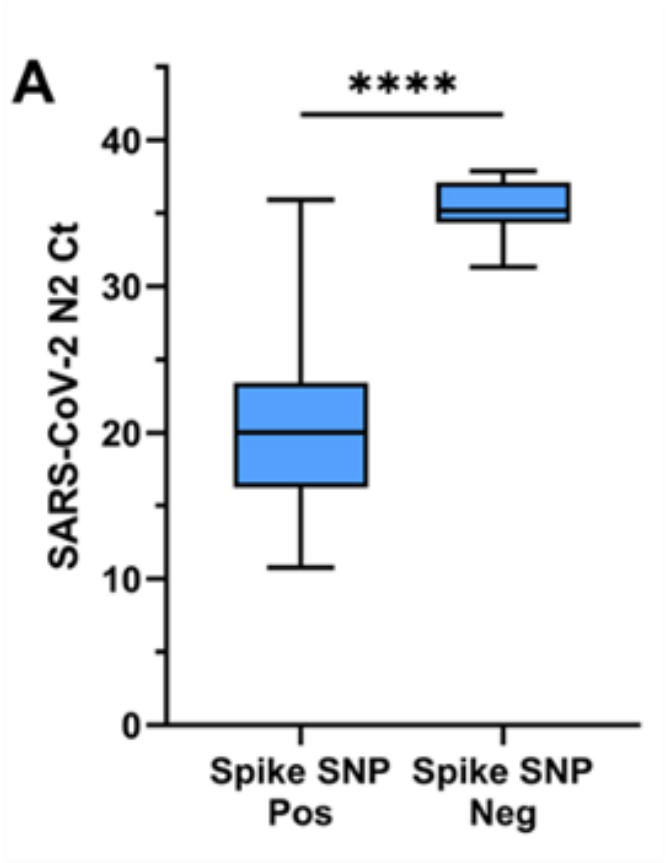
Linear Range and Variant Pools

- Lower limits of 95% detection in log_{10} GE/mL were calculated by probit analysis
  - 2.46-2.54 for the 417K, 484K, and 501Y targets, respectively
  - Correlate to ~1-2 GE/reaction.



Clinical Evaluation

- Spike SNP assay detected 238/253 (94.1%) N2-positive samples
- N2 Ct values were significantly higher in samples that tested negative in the Spike SNP assay
- All samples with N2 Ct values < 30 (220/220) were detected
- 18/33 samples with N2 Ct values ≥ 30 were detected



B

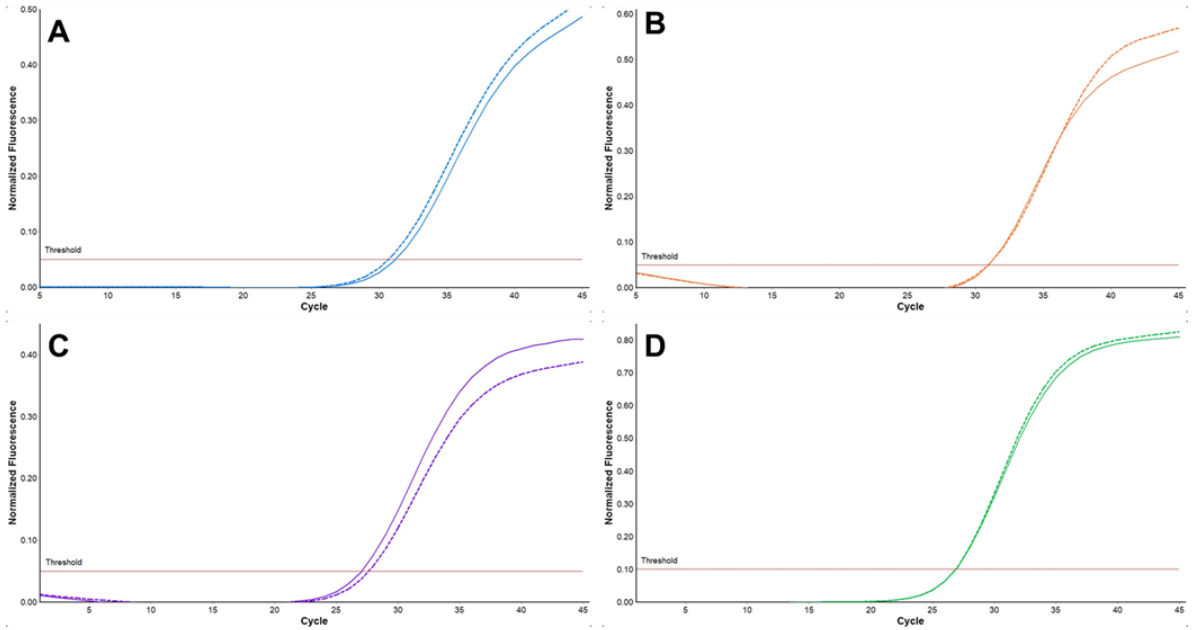
SARS-CoV-2 N2 Ct	N	Median (range)
< 30	220	19.39 (10.77-29.69)
≥ 30	33	33.57 (30.32-37.88)

Easily modified in target region

- Currently have probes for additional mutations
  - 452Q ---- Lambda (C.37)
  - 484Q ---- Kappa (B.1.617.1) y **B.1.617.3**
  - 490S ---- Lambda (C.37)

  - **478K ---- Delta**

- LNAs ----- unmodified probes



Other genotyping rRT-PCRs available

- Stanford Assay: multiplex rRT-PCR with two amplicons
- Commercial rRT-PCRs for specific mutations

Multiplex SARS-CoV-2 Genotyping RT-PCR for Population-Level Variant Screening and Epidemiologic Surveillance

Hannah Wang, MD[a*]; Jacob A. Miller, MD[b*]; Michelle Verghese, BA[a]; Mamdouh Sibai, BS[c]; Daniel Solis, BS[a]; Kenji O. Mfuh, PhD[c]; Becky Jiang, CLS[c]; Naomi Iwai, CLS[c]; Marilyn Mar, CLS[c]; ChunHong Huang, MD[a]; Fumiko Yamamoto, MS[a]; Malaya K. Sahoo, PhD[a]; James Zehnder, MD[a]; Benjamin A. Pinsky, MD, PhD[a,c,d]#

137 **Supplemental Table 1.** Primer and Probe Oligonucleotide Sequences and Characteristics

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Oligonucleotide	Sequence (5' → 3')	5' Mod	3' Mod	Tm[a] Match (°C)	Tm[a] Mismatch (°C)	Sequence Conservation			
						NCBI Pre-12/2020 (n=31,027)	GISAID B.1.427/B.1.429 (n=622)	GISAID B.1.1.7 (n=7,864)	GISAID B.1.351 (n=341)
L452R_FWD	CTCTCTCAAAAGGTTTGAGATTAGACT	-	-	62.7	-	99.6% (n=30,903)	100.0% (n=622)	99.9% (n=7,856)	99.7% (n=340)
L452R_REV	CTTGATTCTAAGGTTGGTGGTAA	-	-	60.5	-	99.0% (n=30,695)	99.5% (n=619)	99.8% (n=7,850)	98.8% (n=337)
L452R_MT_HEX	CCTAAACAATCTATACCGGTAATT	HEX	BHQ	58.7	51.6	<0.1% (n=19)	100.0% (n=622)	0.0% (n=0)	0.0% (n=0)
E484K_FWD	CTGAAATCTATCAGGCCGGTA	-	-	61.2	-	99.4% (n=30,823)	99.7% (n=620)	99.7% (n=7,843)	99.7% (n=340)
E484K_REV	GAAAGTACTACTACTCTGTATGG	-	-	57.4	-	98.6% (n=30,584)	99.7% (n=620)	99.8% (n=7,850)	98.5% (n=336)
E484K_MT_CY5	CTTGTAATGGTGTTAAAGGTTT	CY5	BHQ	57.6	53.7	<0.1% (n=13)	0.0% (n=0)	<0.1% (n=1)	99.7% (n=340)
N501Y_MT_FAM[b]	TTTCCAACCCACTTATGGT	FAM	BHQ	59.0	54.8	0.1% (n=38)	0.0% (n=0)	100.0% (n=7,861)	99.7% (n=340)
N501_WT_CY3.5[b]	TTTCCAACCCACTAATGGT	CY3.5	BHQ	59.0	56.8	99.2% (n=30,773)	100.0% (n=622)	0.0% (n=0)	0.0% (n=0)

Mod, modification; Tm, melt temperature; FWD, forward; REV, reverse; WT, wild-type; MT, mutant. A hyphen (-) indicates this cell is not applicable for this row (e.g., no 5' modification for the L452R_FWD primer).

[a] Calculated using IDT OligoAnalyzer (https://www.idtdna.com/pages/tools/oligoanalyzer) using qPCR conditions (DNA, 0.2μM [oligonucleotide], 50mM [Na^+], 3mM [Mg^{2+}],0.8mM [dNTPs]. Mismatch Tm is for wild-type → mutant or mutant → wild-type nucleotide annealing.

[b] Anneals to E484K_FWD/REV amplicon downstream of E484K_MT_CY5.

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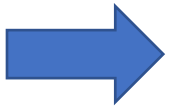
- Leda Bassit
- Greg Martin
- Wilbur Lam

Flujo IICS-UNA

rRT-PCR POSITIVO



Panel 1

- 417K
- Δ69-70
- Δ3675/3677

Panel 2

- N501Y
- E484K
- 452R

ANÁLISIS DE RESULTADOS

https://covariants.org/

Nextstrain Clade	Pango Lineage	WHO Label	Other Names	Old CoVariants Names
20I (Alpha, V1)	B.1.1.7	α Alpha	VOC 202012/01	20I/501Y.V1
20H (Beta, V2)	B.1.351	β Beta	501Y.V2	20H/501Y.V2
20J (Gamma, V3)	P.1	γ Gamma		20J/501Y.V3
21A (Delta)	B.1.617.2	δ Delta		21A/S:478K
21B (Kappa)	B.1.617.1	κ Kappa		21A/S:154K
21C (Epsilon)	B.1.427, B.1.429	ε Epsilon	CAL.20C	20C/S:452R
21D (Eta)	B.1.525	η Eta		20A/S:484K
21F (Iota)	B.1.526	ι Iota	(Part of Pango lineage)	20C/S:484K
21G (Lambda)	C.37	λ Lambda		
20E (EU1)	B.1.177		EU1	20A.EU1
21H	B.1.621			
20B/S:732A	B.1.1.519			
20A/S:126A	B.1.620			
20A.EU2	B.1.160			
20A/S:439K	B.1.258			
20A/S:98F	B.1.221			
20C/S:80Y	B.1.367			
20B/S:626S	B.1.1.277			
20B/S:1122L	B.1.1.302			

https://covariants.org/

CoVariants Home FAQ Variants Per country Per variant Shared Mutations Acknowledgements

Enabled by data from GISAID

Last updated: 2021-07-

Variant: S:E484

Variant

- 20I (Alpha, V1)
- 20H (Beta, V2)
- 20J (Gamma, V3)
- 21A (Delta)
- 21B (Kappa)
- 21F (Iota)

Propose changes to this section

Dedicated S:E484 Nextstrain build

Mutation Information

S:E484 has appeared multiple times independently around the world: each can be associated with different accompanying mutations

S:E484

This mutation is in the receptor binding domain (RBD), important to ACE2 binding and antibody recognition.

- Mutations at S:E484 may significantly reduce convalescent serum neutralization (Greaney et al., Cell Host & Microbe)
- There has been a case of reinfection associated with S:E484K: a woman previously infected with a non-S:E484K variant of SARS-CoV-2 was later reinfected with a virus carrying the S:E484K mutation (Nonaka et al., EID)
- In one study co-incubating SARS-CoV-2 with convalescent plasma, neutralization was completely escaped at day 73 due to an S:E484K mutation (Andreano et al., bioRxiv)
- In another study co-incubating pseudotyped virus with SARS-CoV-2 spike proteins and monocolonal antibodies, neutralization both by monoclonal antibodies and to convalescent sera was significantly reduced in viruses with S:E484 mutations (Liu et al., Cell Host &

https://covariants.org/

Mutaciones compartidas

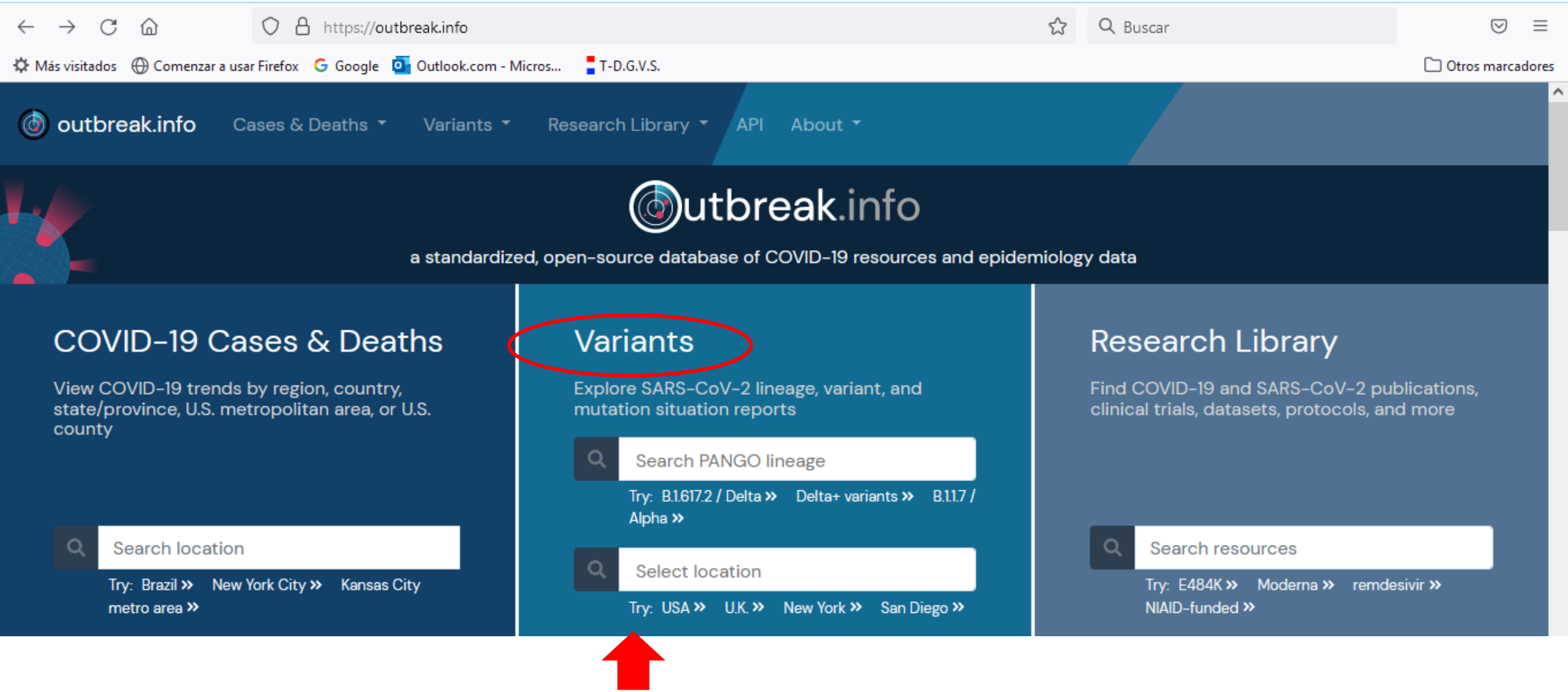
20I (Alpha, V1) (B.1.1.7)	20H (Beta, V2) (B.1.351)	20J (Gamma, V3) (P.1)	21A (Delta) (B.1.617.2)	21B (Kappa) (B.1.617.1)	20B/S.484K (P.2)	21C (Epsilon) (B.1.427/9)	21D (Eta) (B.1.525)	21F (Iota) (B.1.526)	21G (Lambda) (C.37)	21H (B.1.621)	20A/S:126A (B.1.620)
Shared mutations Sort by: Commonness / Position											
S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G
	S: E 484 K	S: E 484 K		S: E 484 Q	S: E 484 K		S: E 484 K	S: E 484 K		S: E 484 K	S: E 484 K
S: P 681 H			S: P 681 R	S: P 681 R						S: P 681 H	S: P 681 H
S: N 501 Y	S: N 501 Y	S: N 501 Y								S: N 501 Y	
S: Y 144 -							S: Y 144 -			S: Y 144 S	S: Y 144 -
			S: L 452 R	S: L 452 R		S: L 452 R			S: L 452 Q		
S: H 69 -							S: H 69 -				S: H 69 -
S: V 70 -							S: V 70 -				S: V 70 -
	S: L 18 F	S: L 18 F									
S: D 1118 H											S: D 1118 H
		S: T 1027 I									S: T 1027 I
			S: D 950 N							S: D 950 N	
	S: A 701 V							S: A 701 V			
	S: K 417 N	S: K 417 T									
		S: P 26 S									S: P 26 S
								S: D 253 G	S: D 253 N		

Mutaciones compartidas

https://covariants.org/

20I (Alpha, V1) (B.1.1.7)	20H (Beta, V2) (B.1.351)	20J (Gamma, V3) (P.1)	21A (Delta) (B.1.617.2)	21B (Kappa) (B.1.617.1)	20B/S.484K (P.2)	21C (Epsilon) (B.1.427/9)	21D (Eta) (B.1.525)	21F (Iota) (B.1.526)	21G (Lambda) (C.37)	21H (B.1.621)	20A/S:126A (B.1.620)
Shared mutations Sort by: Commonness / Position											
S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G	S: D 614 G
	S: E 484 K	S: E 484 K		S: E 484 Q	S: E 484 K		S: E 484 K	S: E 484 K		S: E 484 K	S: E 484 K
S: P 681 H			S: P 681 R	S: P 681 R						S: P 681 H	S: P 681 H
S: N 501 Y	S: N 501 Y	S: N 501 Y								S: N 501 Y	
S: Y 144 -							S: Y 144 -			S: Y 144 S	S: Y 144 -
			S: L 452 R	S: L 452 R		S: L 452 R			S: L 452 Q		
S: H 69 -							S: H 69 -				S: H 69 -
S: V 70 -							S: V 70 -				S: V 70 -
	S: L 18 F	S: L 18 F									
S: D 1118 H											S: D 1118 H
		S: T 1027 I									S: T 1027 I
			S: D 950 N							S: D 950 N	
	S: A 701 V							S: A 701 V			
	S: K 417 N	S: K 417 T									
		S: P 26 S									S: P 26 S
								S: D 253 G	S: D 253 N		

https://outbreak.info/



https://outbreak.info/

Variant of Concern Reports

Variants with increased transmissibility, virulence, and/or decreased diagnostic, therapeutic, or vaccine efficacy Read more

How to interpret these reports

include VOCs classified by: outbreak.info CDC ECDC Public Health England WHO CLEAR

VARIANT	CLASSIFICATIONS	FIRST IDENTIFIED	TOTAL FOUND	S-GENE MUTATIONS*
AY.1 WHO: **Delta** PHE: **VOC-21APR-02** related: **B.1.617.2 • AY.2 • AY.3**	VOC: outbreak.info 11 Jun 2021; CDC 6 Jul 2021; ECDC report; Public Health England 11 Jun 2021; WHO report VOI: VUM:		334	T19R W258L K417N L452R T478K D614G P681R D950N Explore all genes
AY.2 WHO: **Delta** PHE: **VOC-21APR-02** related: **B.1.617.2 • AY.1 • AY.3**	VOC: outbreak.info 18 Jun 2021; CDC 6 Jul 2021; ECDC report; Public Health England 18 Jun 2021; WHO report VOI: VUM:		546	T19R V70F DEL157/158 A222V K417N L452R T478K D614G P681R D950N Explore all genes
AY.3 related: **B.1.617.2 • AY.1 • AY.2**	VOC: outbreak.info 21 Jul 2021; CDC 20 Jul 2021; ECDC; Public Health England; WHO VOI: VUM:		1,640	T19R DEL157/158 L452R T478K D614G P681R D950N Explore all genes

https://outbreak.info/

SARS-CoV-2 (hCoV-19) Mutation Reports

Lineage Comparison

CHANGE LINEAGES

Updated 23h ago

2.375.554 sequences

Enabled by data from GISAID

How to interpret these reports

Mutation prevalence across lineages

CHANGE LINEAGES

Mutations with > 75% prevalence in at least one lineage

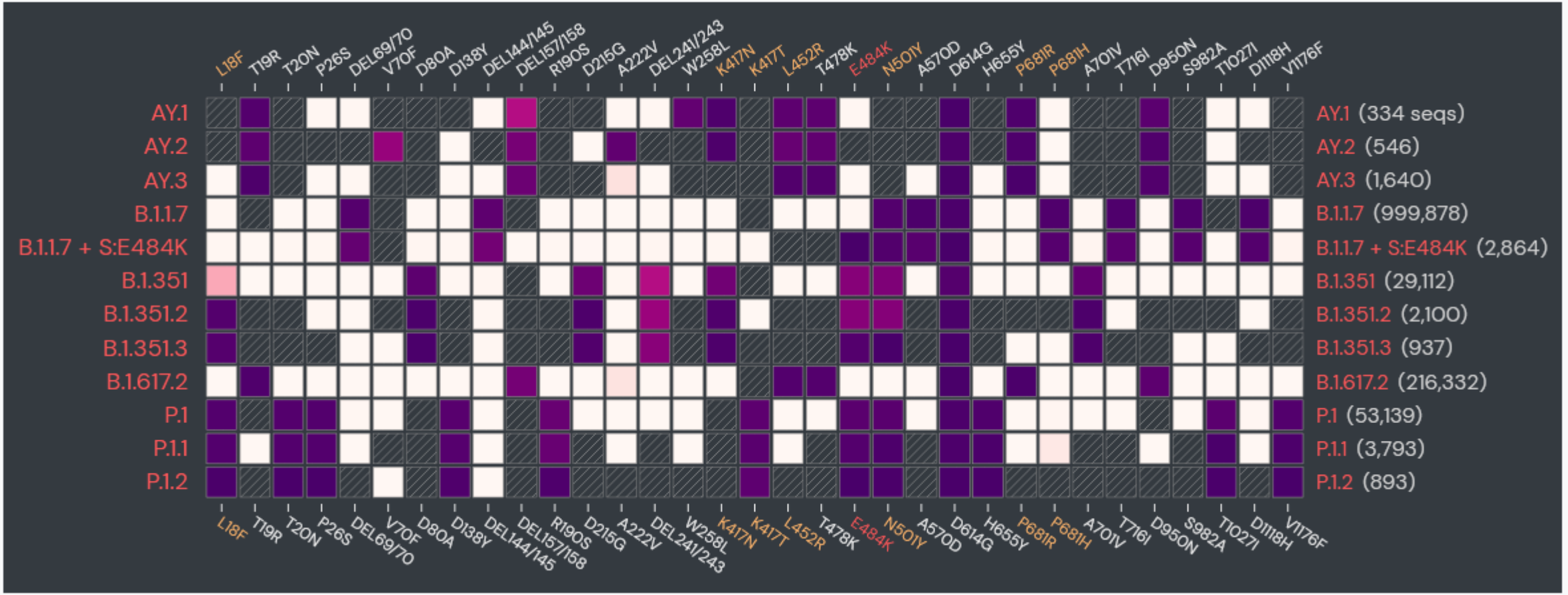
include genes: ORF1a, ORF1b, S, ORF3a, E, M, ORF6, ORF7a, ORF7b, ORF8, N, ORF10

Min. mutation prevalence: 75 %

dark mode

Mutation prevalence in lineage: 0 – 100%; not detected; Variant / Mutation of Concern; Variant / Mutation of Interest

S



Variantes y mutaciones

	Al fa (B.1.1.7)/Alfa + ★	**Beta** (B.1.351) ★	**Gamma** (P.1) ★	**Delta/Delta +** (B.1.617.2 / AY.1 / AY.2) ★	**Kappa** (B.1.617.1)	**Epsilon** (B.1.427 /429)	**Zeta** (P.2)	**Lambda** (C.37)		
417K										
Δ69-70										
Δ3675/3677										
N501Y										
E484K										
L452R										

★ VOC: Variantes de preocupación

	Al fa (B.1.1.7)/Alfa +	**Beta** (B.1.351)	**Gamma** (P.1)	**Delta/Delta +** (B.1.617.2)	**Kappa** (B.1.617.1)	**Epsil on** (B.1.427 /429)	**Zeta** (P.2)	**Lambda** (C.37)		
417K FAM	+	-	-	+	-	+	+	+		
Δ69-70 Cal Orange	-	+	+	+	+	+	+	+		
Δ3675/3677 Cal Red	-	-	-	+	+	+	+	-		
N501Y HEX	+	+	+	-	-	-	-	-		
E484K Cy5	-	+	+	+	-	-	-	+	-	
L452R Cal Orange	-	-	-	+	+	+	-	+		