CONSEJO NACIONAL DE CIENCIA Y TECNOLOGÍA

CONACYT

PROCIENCIA

PROGRAMA PARAGUAYO PARA EL DESARROLLO DE LA CIENCIA Y TECNOLOGÍA

40 Años IICS 1980 - 2020

ALTER VIDA

CENTRO DE ESTUDIOS Y FORMACIÓN PARA EL ECODESARROLLO

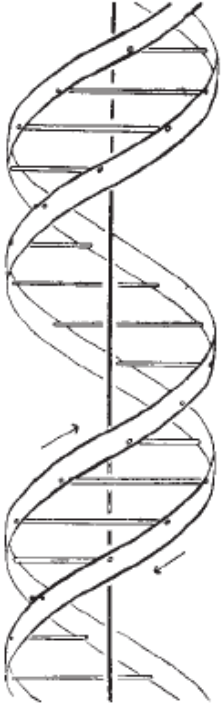
Con el apoyo de:

Feei

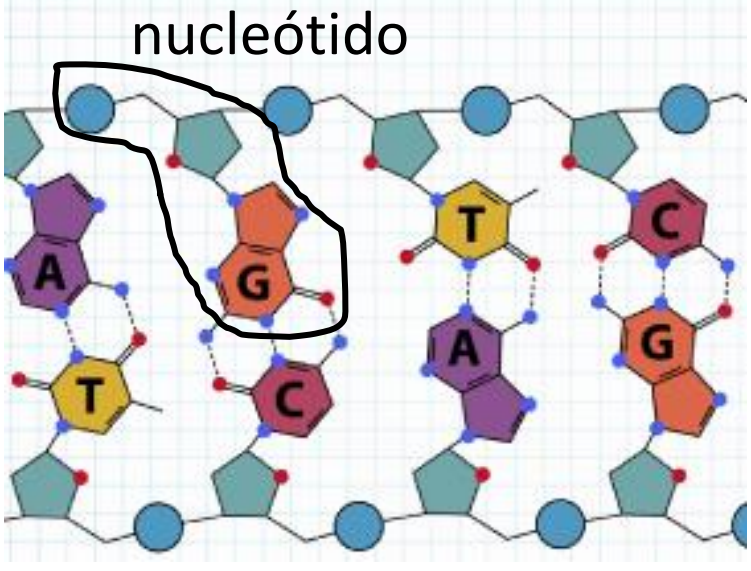
Fondo para la Excelencia de la Educación y la Investigación

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)

¿Qué hacemos al secuenciar?



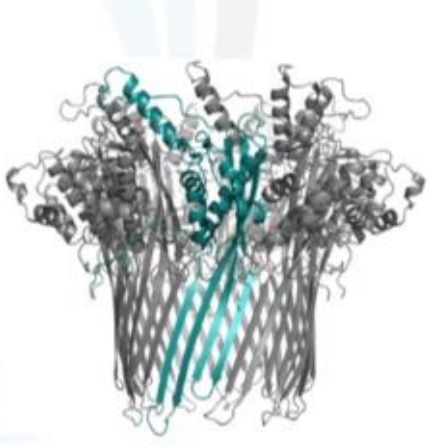
This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

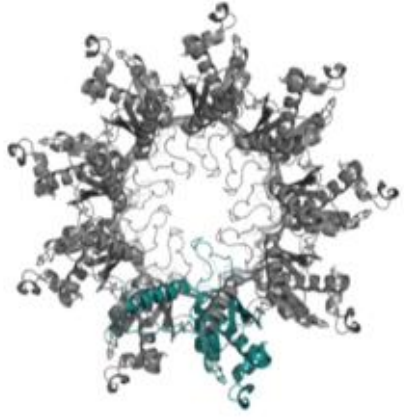


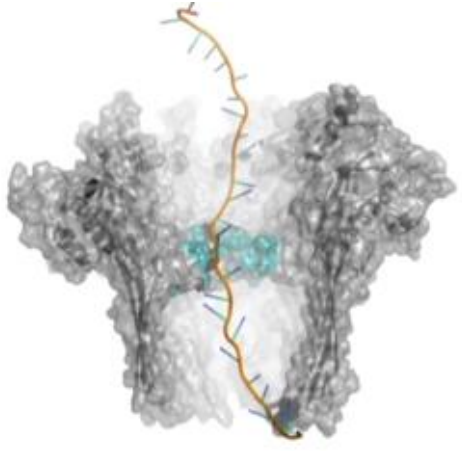
Cómo funciona la tecnología Nanopore?

https://youtu.be/RcP85JHLmnI

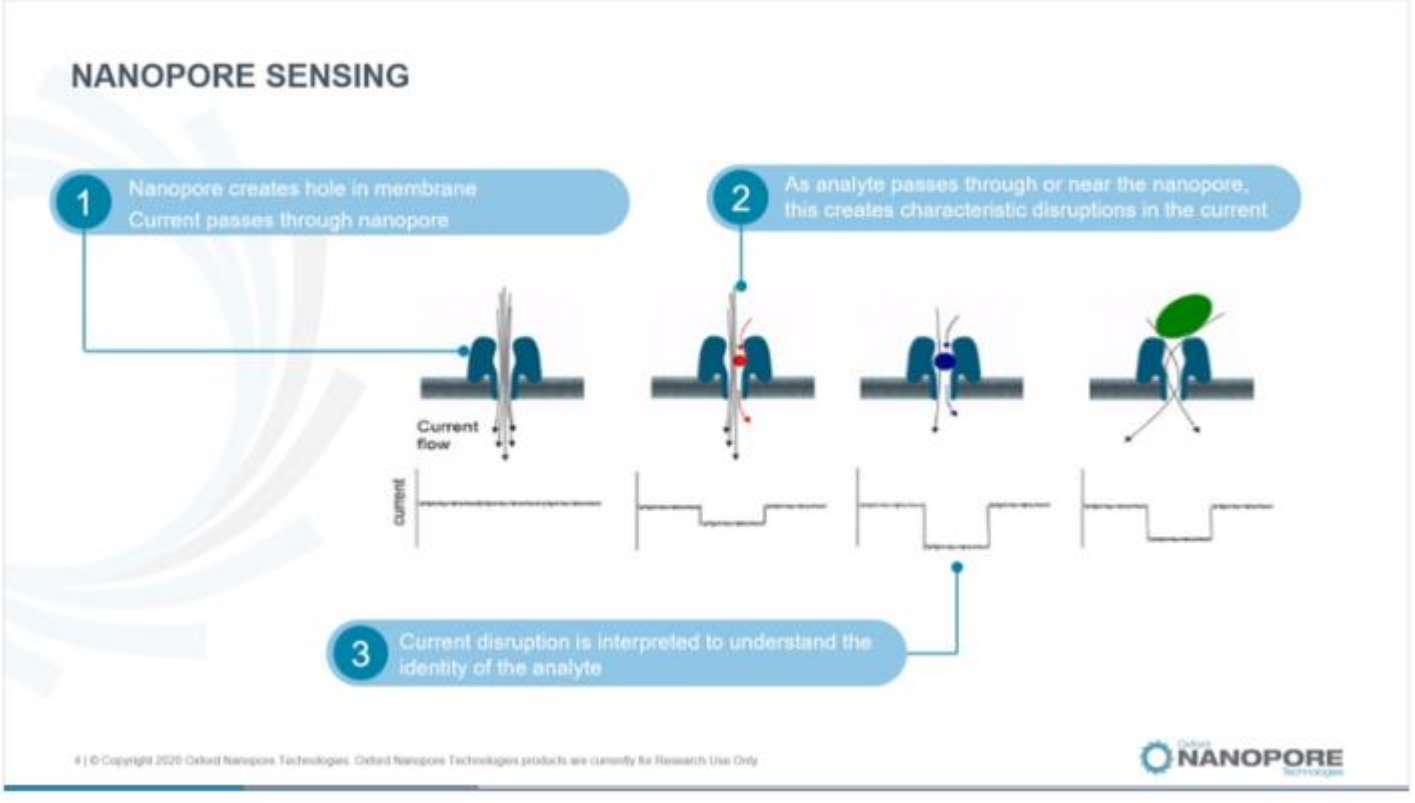
Cómo funciona la tecnología Nanopore?



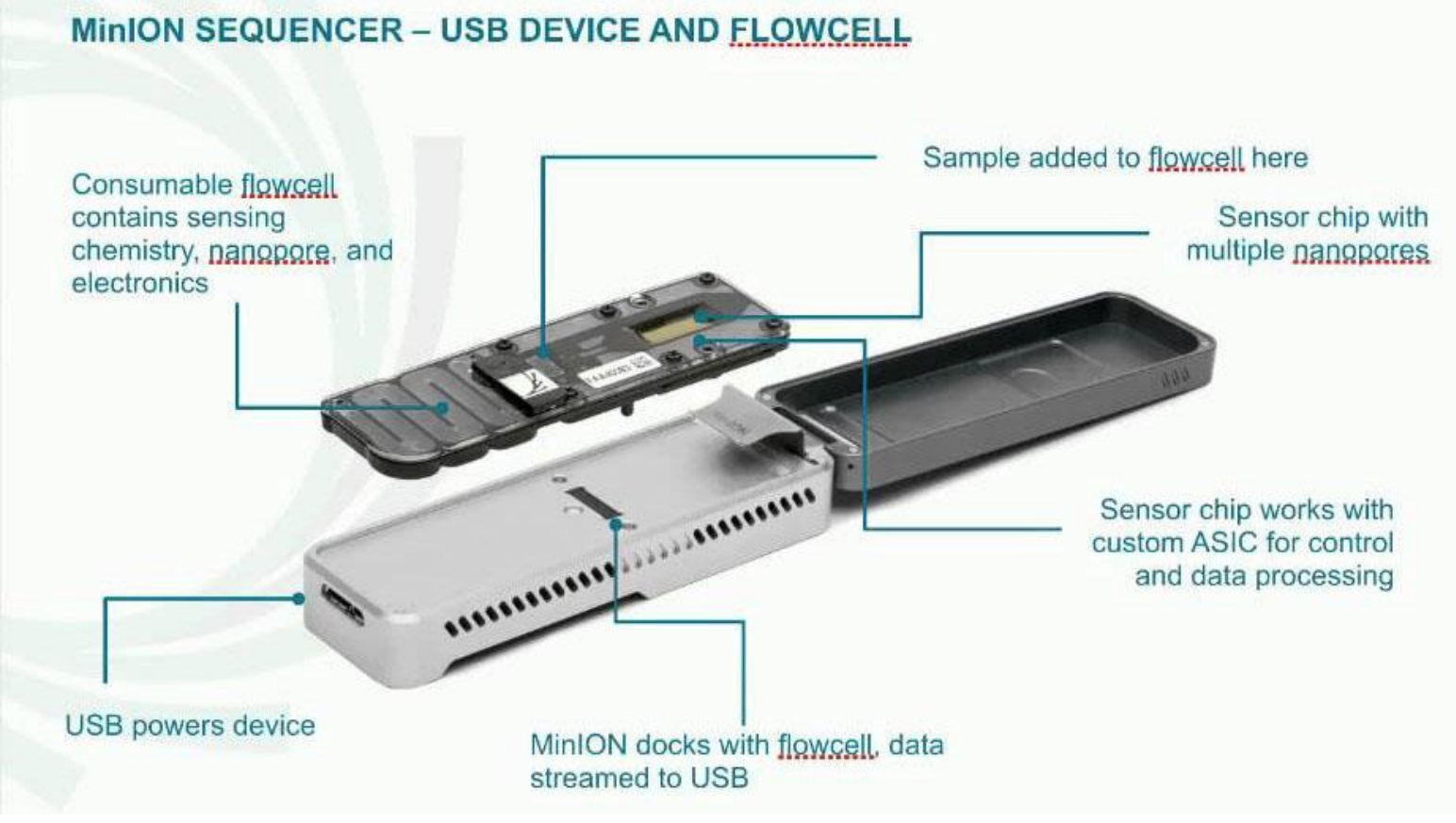




Cómo funciona la tecnología Nanopore?



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

ARTIC NETWORK

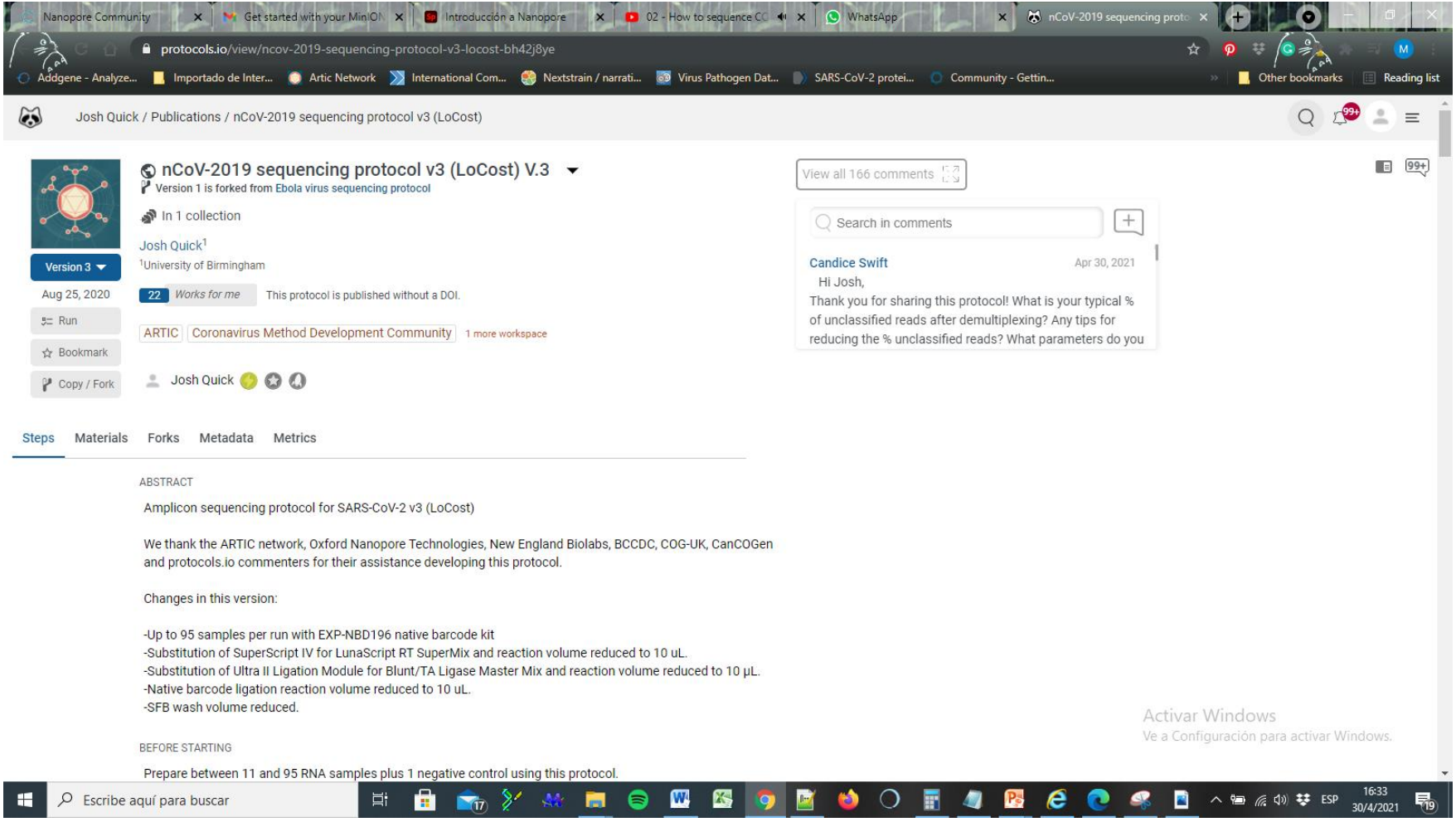
ARTICnetwork

REAL-TIME MOLECULAR EPIDEMIOLOGY FOR OUTBREAK RESPONSE

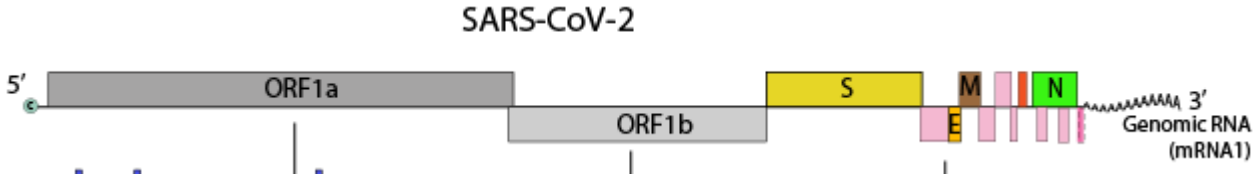
Partners

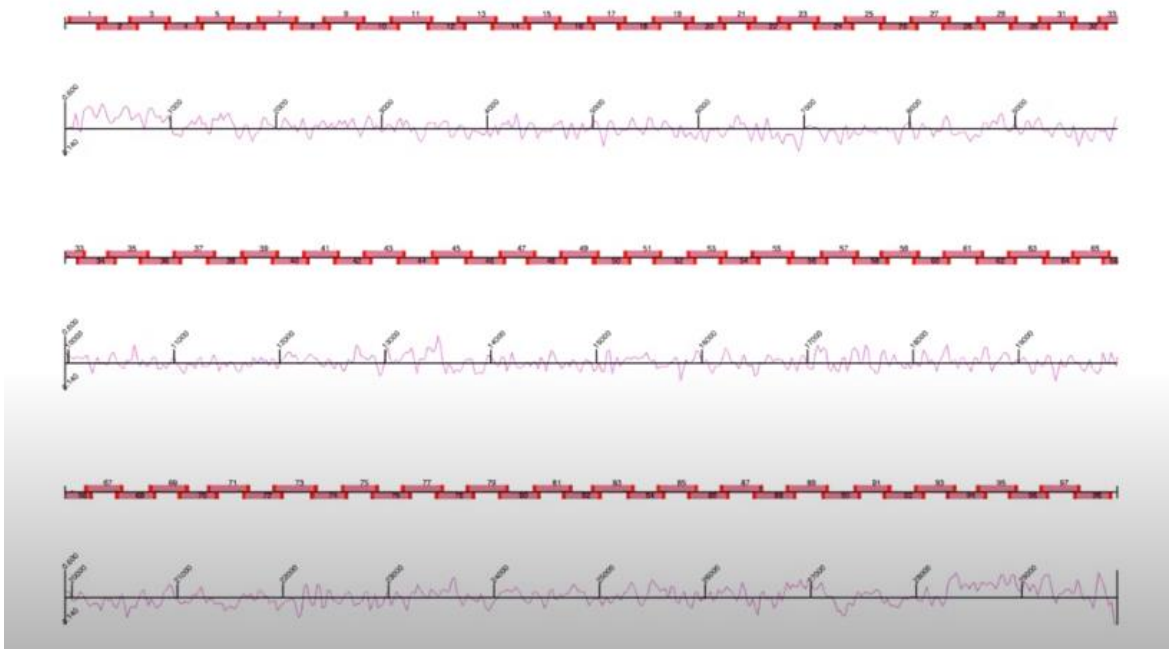
- University of Edinburgh
- University of Birmingham
- University of Cambridge
- KU Leuven
- University of Oxford
- Fred Hutchinson Cancer Research Center
- University of California Los Angeles

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



El método artic usa multiplex PCR y barcoding



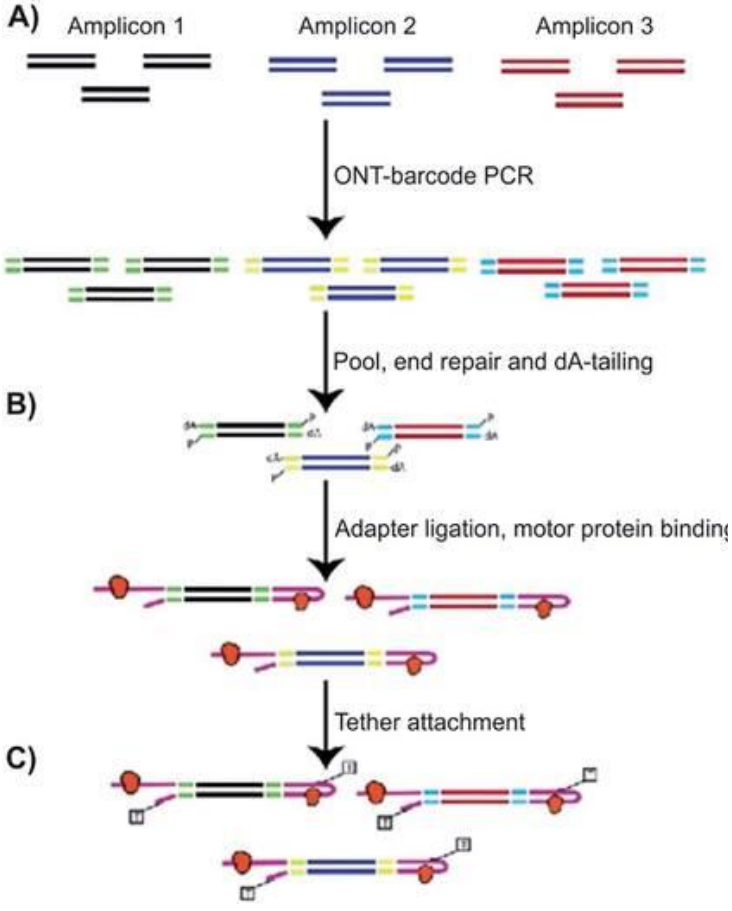


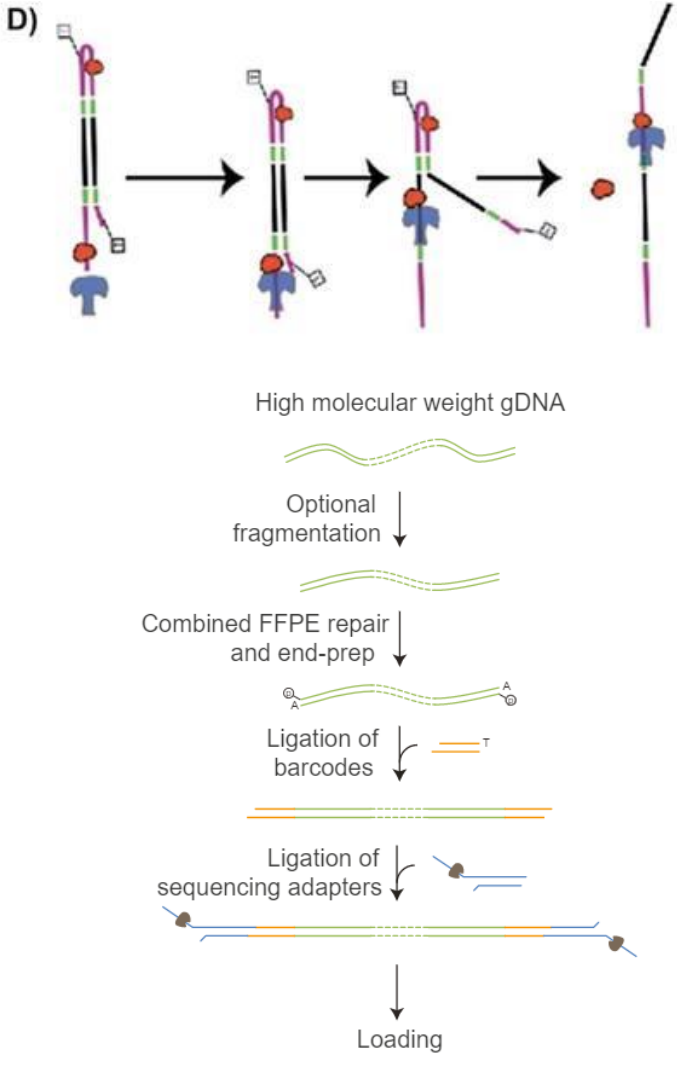
Amplicones
400bp

C:\Users\user\Documents\Coronavirus_files\miniION\artic_network\artic-ncov2019-master\artic-ncov2019-master

Eddie Lusamaki

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

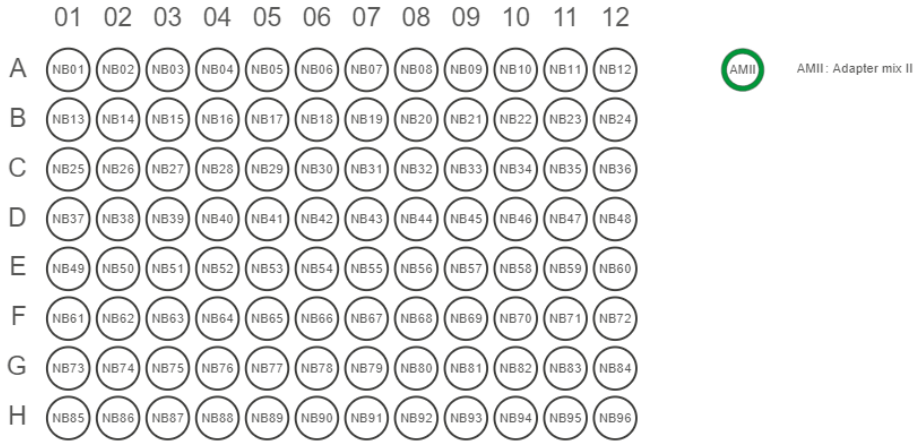

A)
Amplicon 1
Amplicon 2
Amplicon 3
ONT-barcode PCR
Pool, end repair and dA-tailing
B)
Adapter ligation, motor protein bindin
Tether attachment
C)


D)
High molecular weight gDNA
Optional fragmentation
Combined FFPE repair and end-prep
Ligation of barcodes
Ligation of sequencing adapters
Loading

Vu 2017

Barcoding

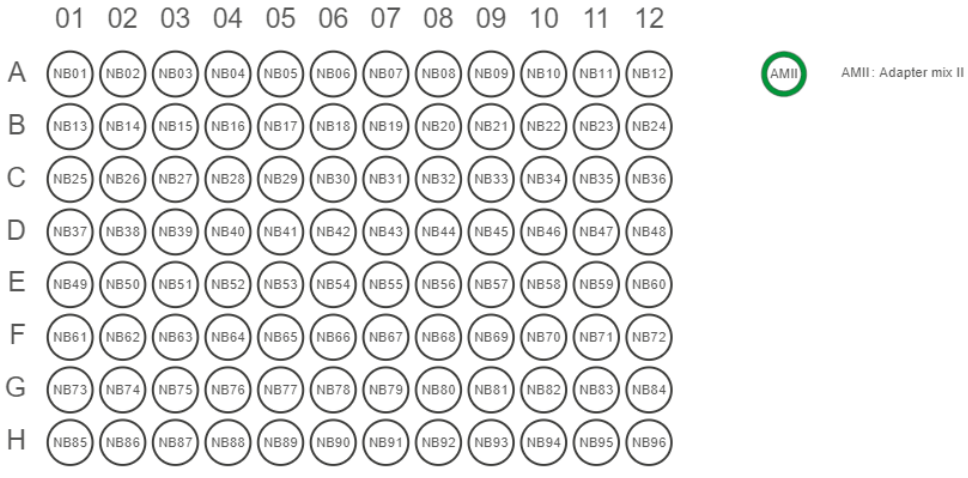
The Native Barcoding Expansion 96 contains 96 unique barcodes and sufficient reagents for 12 sequencing libraries.



	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**

https://store.nanoporetech.com/native-barcoding-expansion-96.html

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



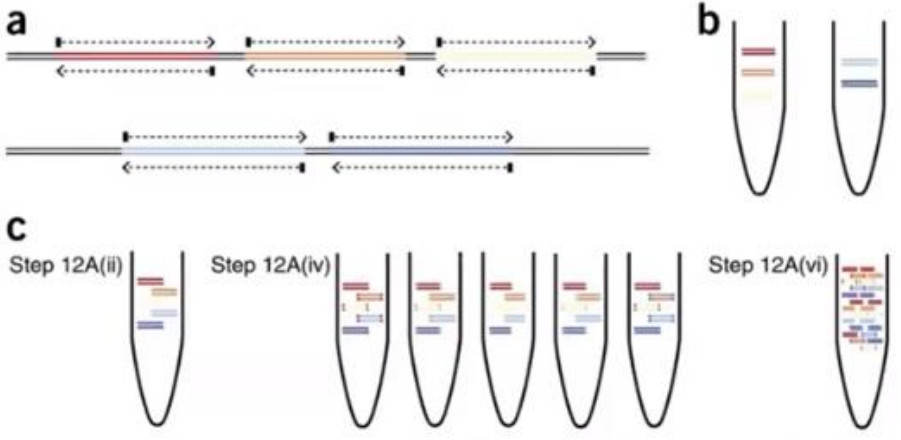
Why it's needed

Amplicon sequencing is good:

- Works on samples with low viral copy numbers
- Most reads are "on target" so cost is low

But has challenges:

- High sensitivity means contaminating template can be amplified
- Barcode cross over
- Reads contain synthetic material (primers)
- Nanopore data can be noisy

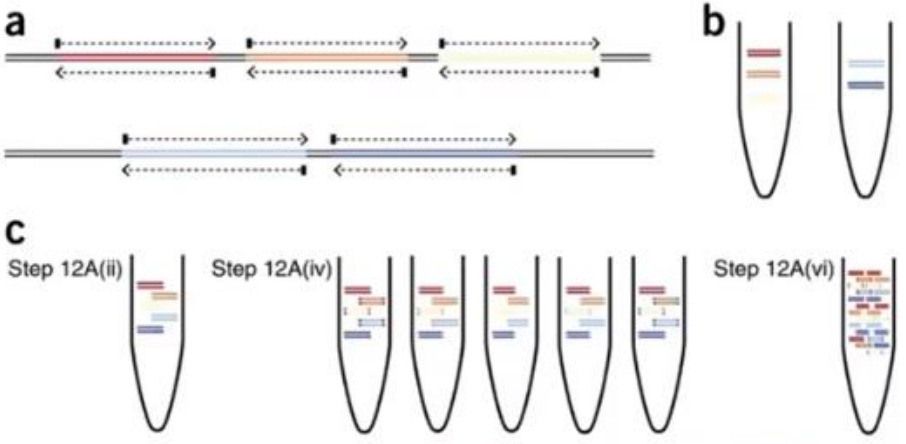


Quick et al., Nat. Prot. 2017
Multiplex PCR method for nanopore sequencing of viral genomes.

Why it's needed

Considerations:

- Appropriate sequencing controls (positive and negative)
- Stringent de-multiplexing to ensure same barcodes are present at both ends of a read
- Read filtering to expected amplicon lengths
- Removal of synthetic sequence
- Evaluation of variants against multiple primer pools to identify artifacts
- Appropriate parameterisation of Nanopore software (Nanopolish and Medaka)



Quick et al., Nat. Prot. 2017
Multiplex PCR method for nanopore sequencing of viral genomes.

2021-01-14 10:51:46

Amplicon handling

Reads are aligned to the reference genome and assigned to an amplicon if:

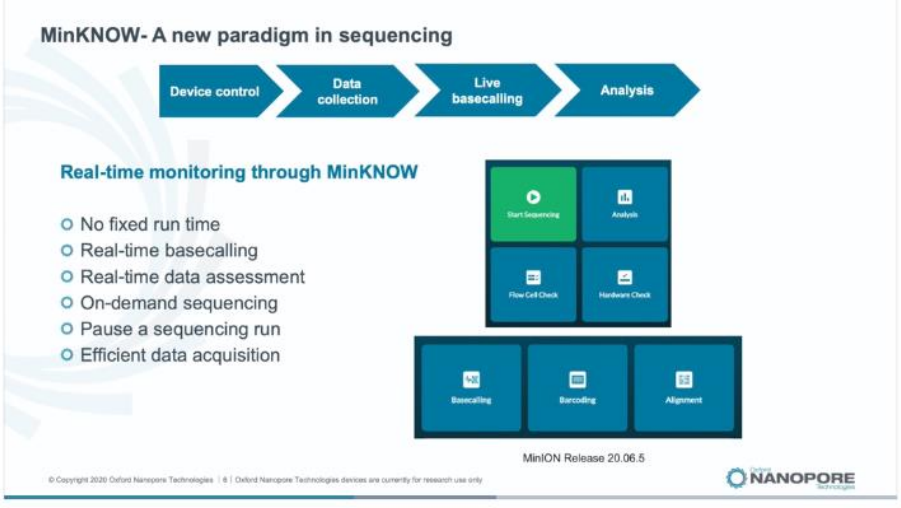
1. It has correctly paired primers
2. It covers entire amplicon
3. Amplicon read threshold not met

Amplicon assigned reads are then processed:

1. Trim sequence outside of amplicon boundary
2. Optionally trim primer sequence
3. Assign read group for primer pool

ARTIC Nanopore Bioinformatics Pipeline (Will Rowe)

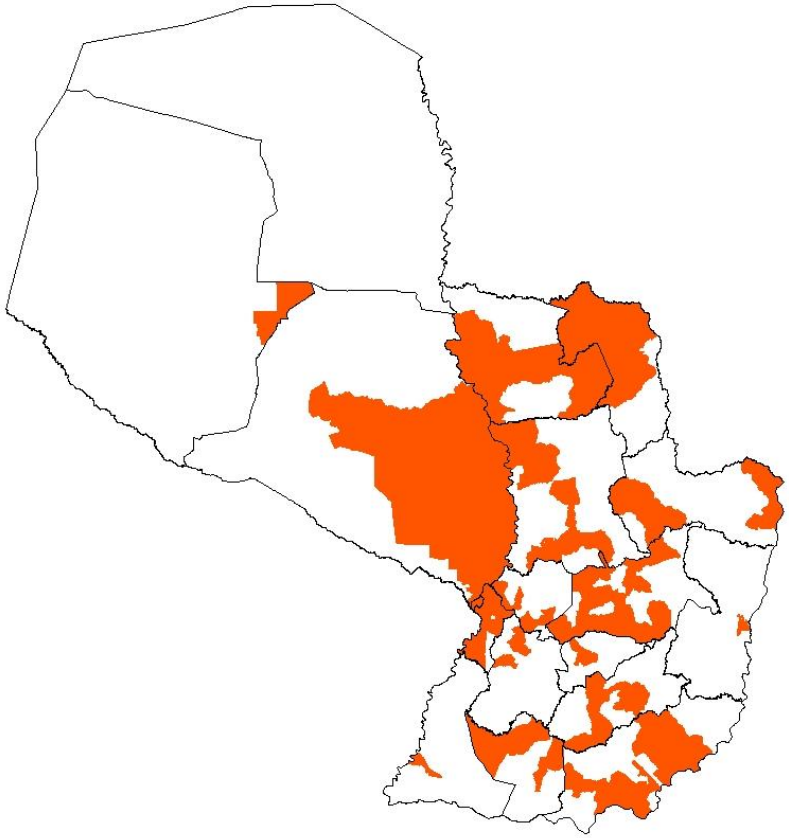
MinKnow: software de corrida



Item	Part Number	# reactions available	Price per item	Price per reaction	Third party reagents
Flow Cell	FLO-MIN106D	1	$900.00	$900.00	$240.00
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 (inluding 3rd party reagents)	$107.32	

Item	Part Number	# reactions available	Price per item	Price per reaction	Third party reagents
Flow Cell	FLO-MIN106D	1	$900.00	$900.00	$450.00
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 (inluding 3rd party reagents)	$64.41	

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

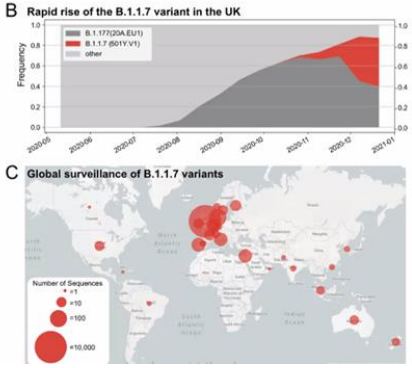
view change over time | change locations

** 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)



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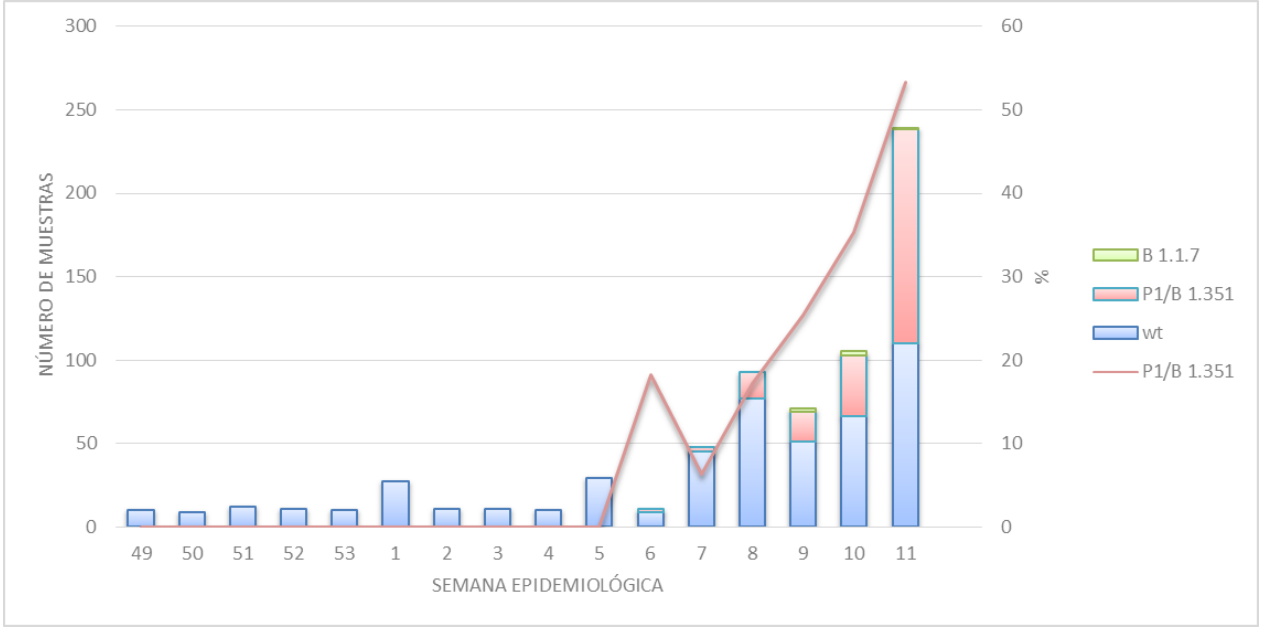
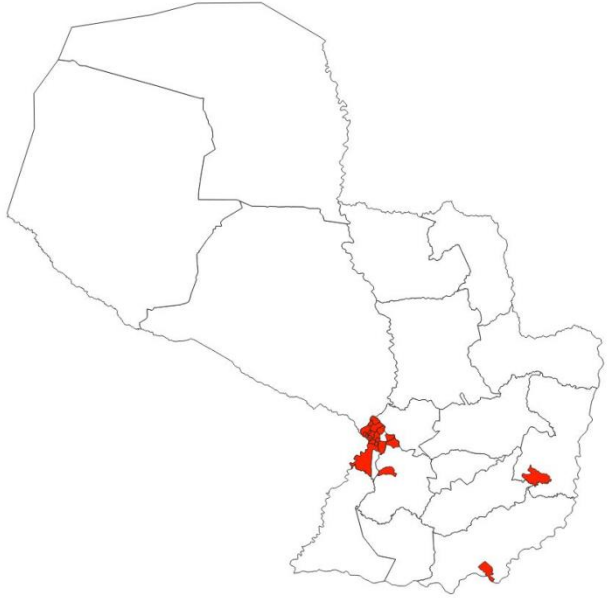
Con el apoyo de: Feei

Muestras Diciembre 2020-Marzo 2021





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