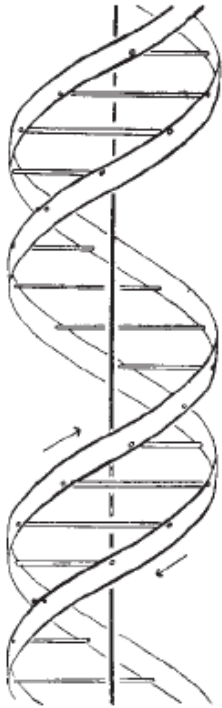
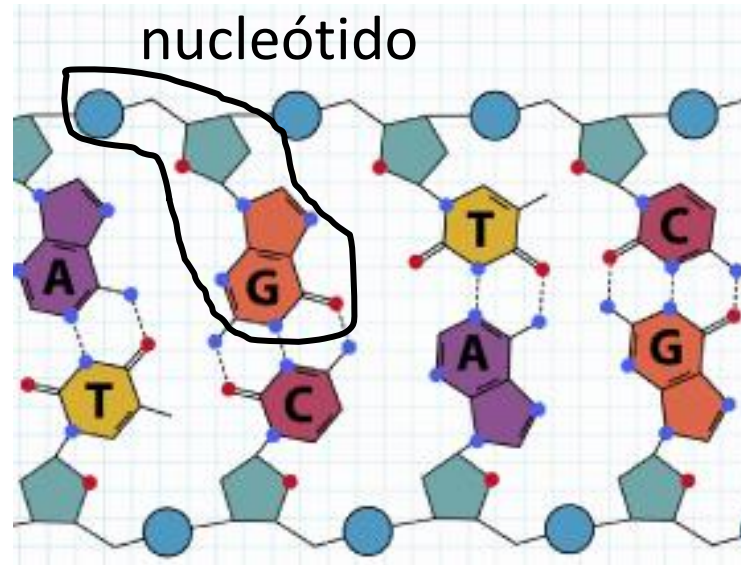


Estudio de la dinámica de transmisión y de la  
variabilidad genética de SARS-CoV-2 circulantes en  
Paraguay a través del análisis de secuencias del  
genoma viral (PINV20-239)

# ¿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?

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 abstract text is partially visible, describing the use of a membrane channel for detecting single molecules of DNA or RNA.

**PNAS** Proceedings of the National Academy of Sciences of the United States of America Log in

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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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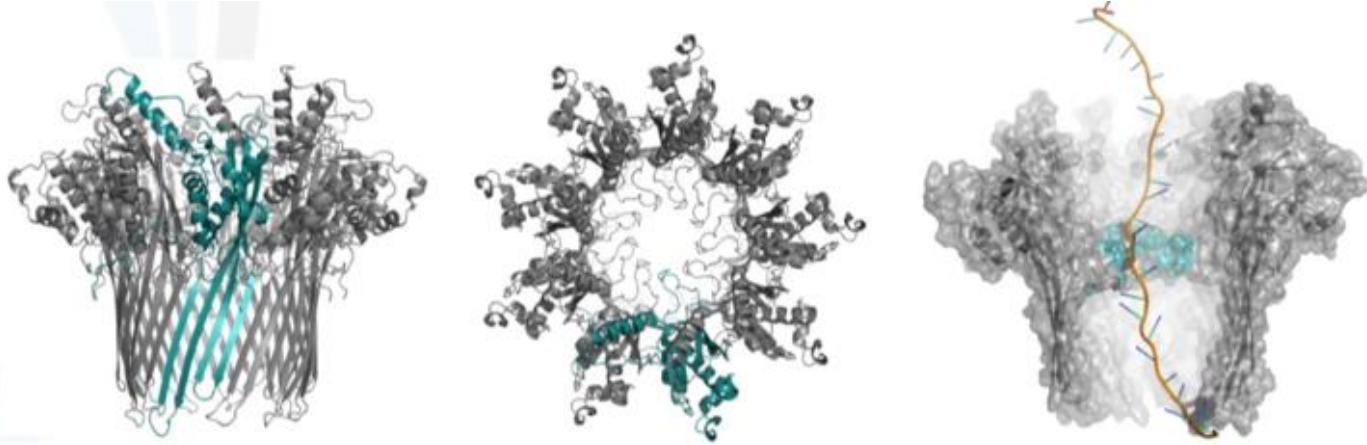
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<https://youtu.be/RcP85JHLmnl>

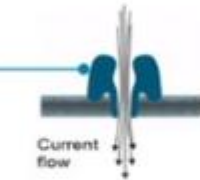
# Cómo funciona la tecnología Nanopore?



# Cómo funciona la tecnología Nanopore?

## NANOPORE SENSING

1 Nanopore creates hole in membrane  
Current passes through nanopore

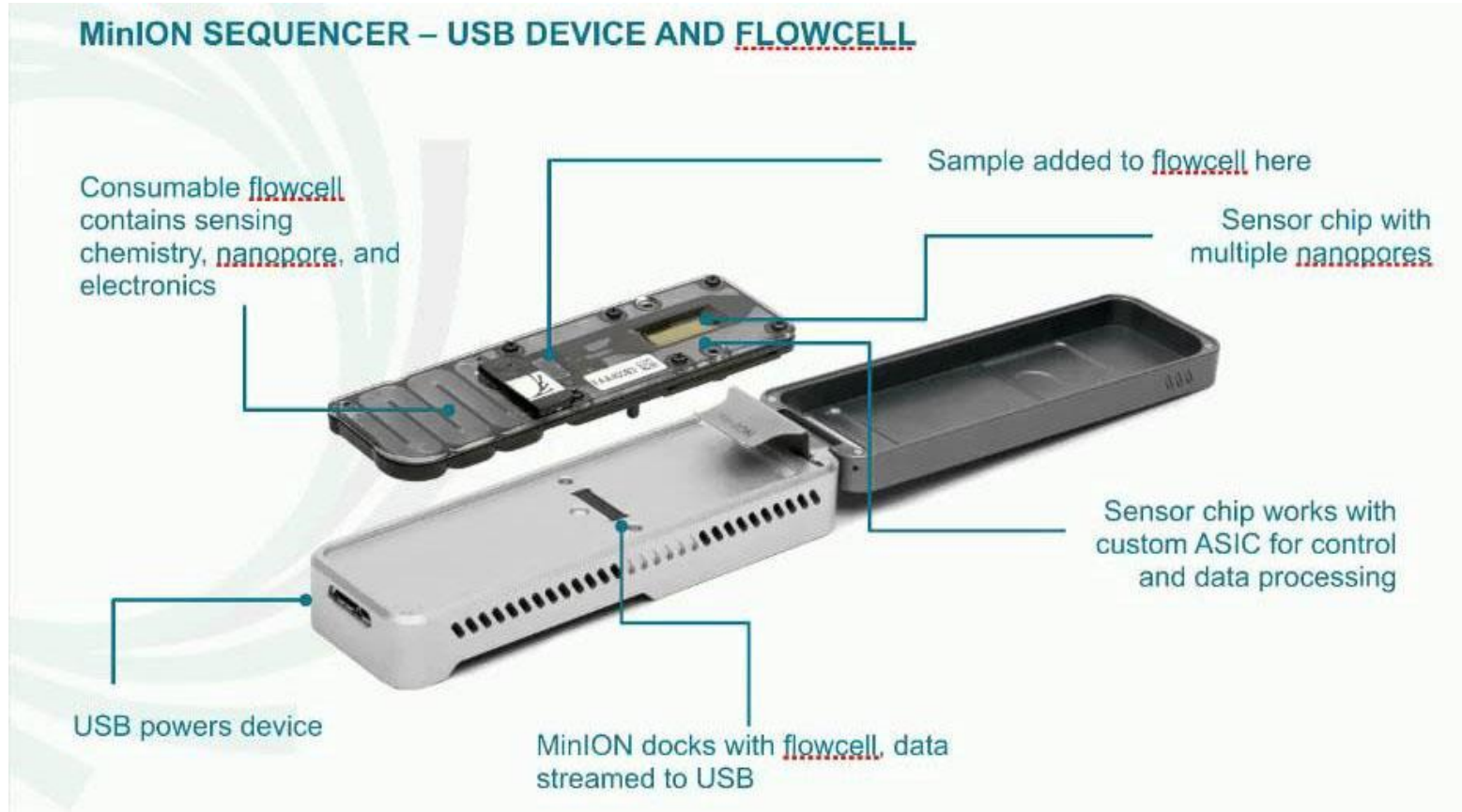


2 As analyte passes through or near the nanopore,  
this creates characteristic disruptions in the current



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

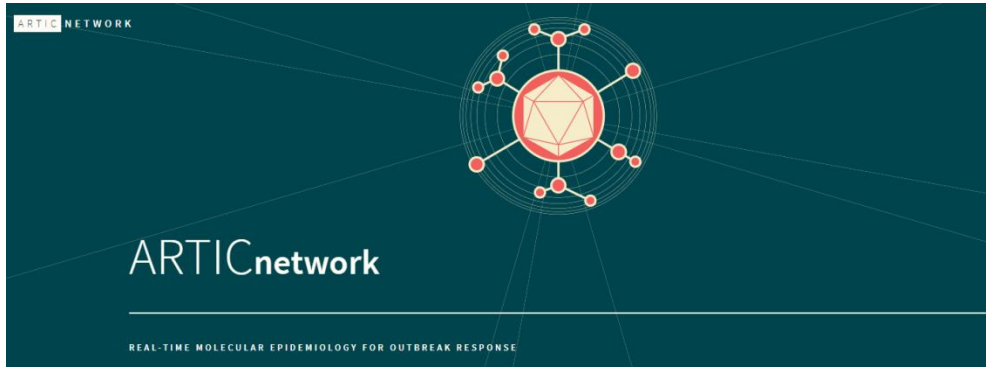
Metagenómica

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

Nanopore Community x Get started with your MinION x Introducción a Nanopore x 02 - How to sequence CC x WhatsApp x nCoV-2019 sequencing proto x

protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye

Addgene - Analyze... Importado de Inter... Artic Network International Com... Nextstrain / narrati... Virus Pathogen Dat... SARS-CoV-2 protei... Community - Gettin...

Josh Quick / Publications / nCoV-2019 sequencing protocol v3 (LoCost)

**nCoV-2019 sequencing protocol v3 (LoCost) V.3**

Version 1 is forked from Ebola virus sequencing protocol

In 1 collection

Josh Quick<sup>1</sup>

<sup>1</sup>University of Birmingham

Aug 25, 2020

22 Works for me This protocol is published without a DOI.

ARTIC Coronavirus Method Development Community 1 more workspace

Run

Bookmark

Copy / Fork

Josh Quick

View all 166 comments

Search in comments

**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

Steps Materials Forks Metadata Metrics

ABSTRACT

Amplicon sequencing protocol for SARS-CoV-2 v3 (LoCost)

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

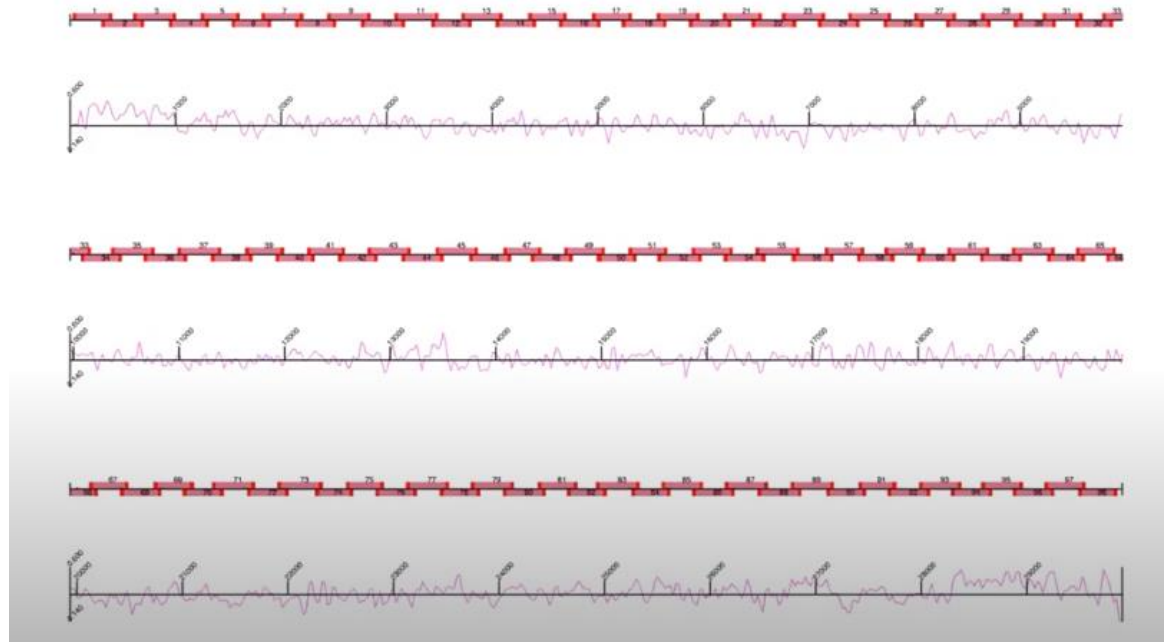
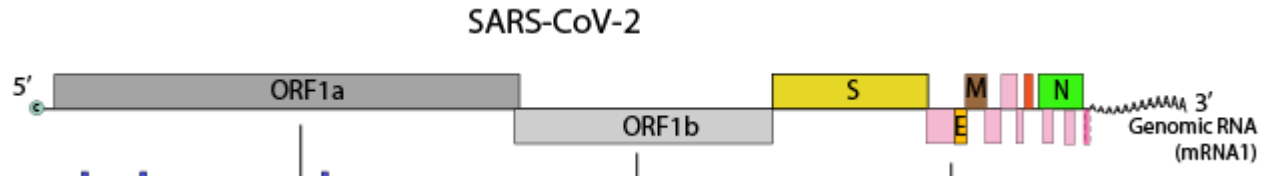
Prepare between 11 and 95 RNA samples plus 1 negative control using this protocol.

Activar Windows  
Ve a Configuración para activar Windows.

Escribe aquí para buscar

16:33 30/4/2021

# El método ARTIC usa multiplex PCR y barcoding



Amplicones  
400bp

C:\Users\user\Documents\Coronavirus\_files\  
minilON\artic\_network\artic-ncov2019-  
master\artic-ncov2019-master



Eddie Lusamaki

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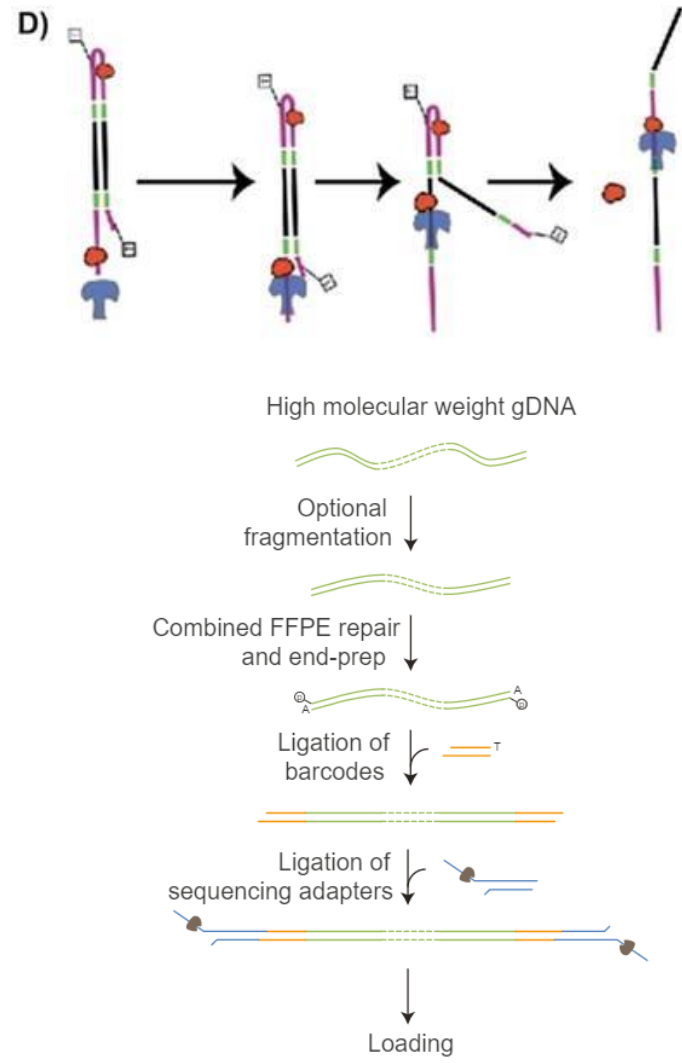
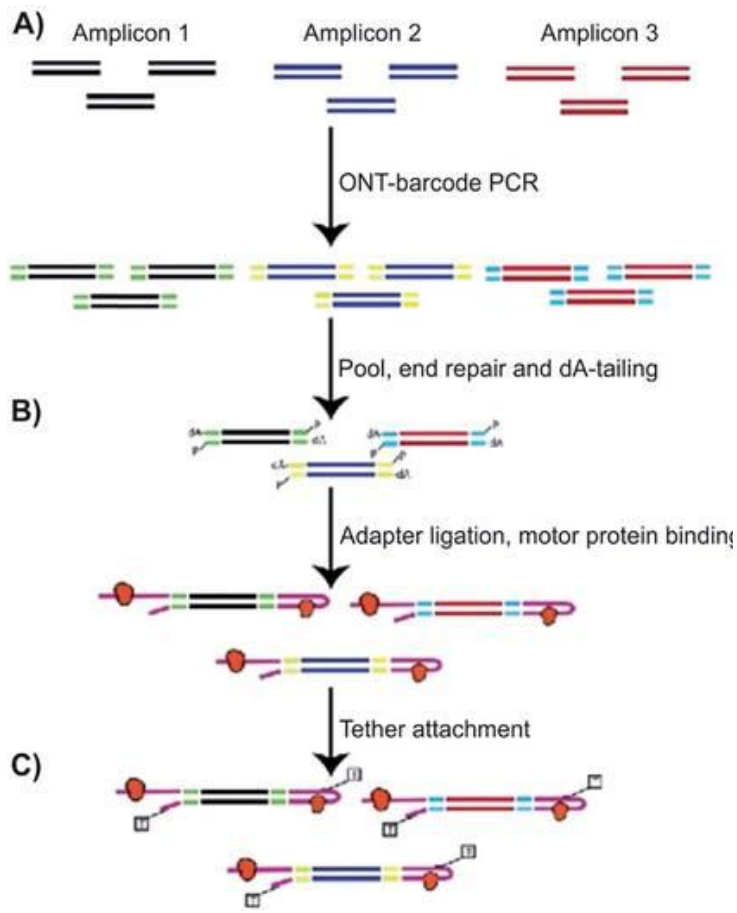
UK World Business Coronavirus Football More

## Coronavirus

### Antibody tests / Healthcare staff to get kits from next week

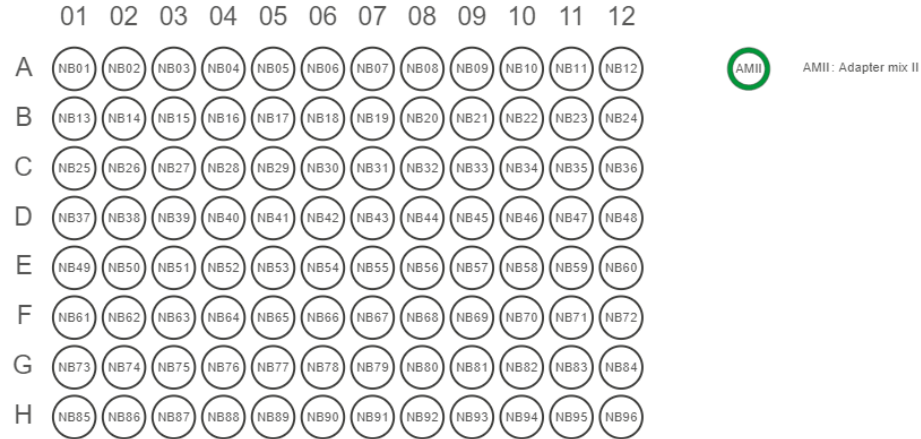
**NHS**  
Repayment scheme extended to low-paid workers

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



# 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
<b>Price per sample</b>	<b>\$599</b>	<b>\$104</b>	<b>\$54</b>	<b>\$7.3</b>

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

	01	02	03	04	05	06	07	08	09	10	11	12
A	NB01	NB02	NB03	NB04	NB05	NB06	NB07	NB08	NB09	NB10	NB11	NB12
B	NB13	NB14	NB15	NB16	NB17	NB18	NB19	NB20	NB21	NB22	NB23	NB24
C	NB25	NB26	NB27	NB28	NB29	NB30	NB31	NB32	NB33	NB34	NB35	NB36
D	NB37	NB38	NB39	NB40	NB41	NB42	NB43	NB44	NB45	NB46	NB47	NB48
E	NB49	NB50	NB51	NB52	NB53	NB54	NB55	NB56	NB57	NB58	NB59	NB60
F	NB61	NB62	NB63	NB64	NB65	NB66	NB67	NB68	NB69	NB70	NB71	NB72
G	NB73	NB74	NB75	NB76	NB77	NB78	NB79	NB80	NB81	NB82	NB83	NB84
H	NB85	NB86	NB87	NB88	NB89	NB90	NB91	NB92	NB93	NB94	NB95	NB96



AMII : Adapter mix II

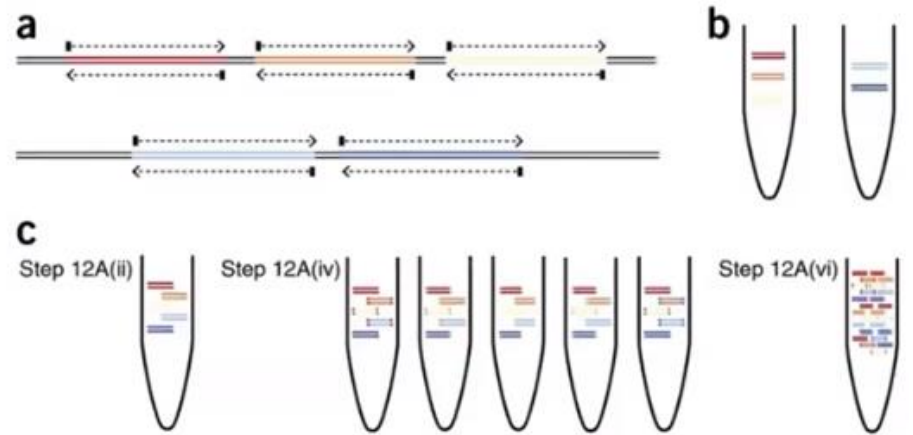
# Why it's needed

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



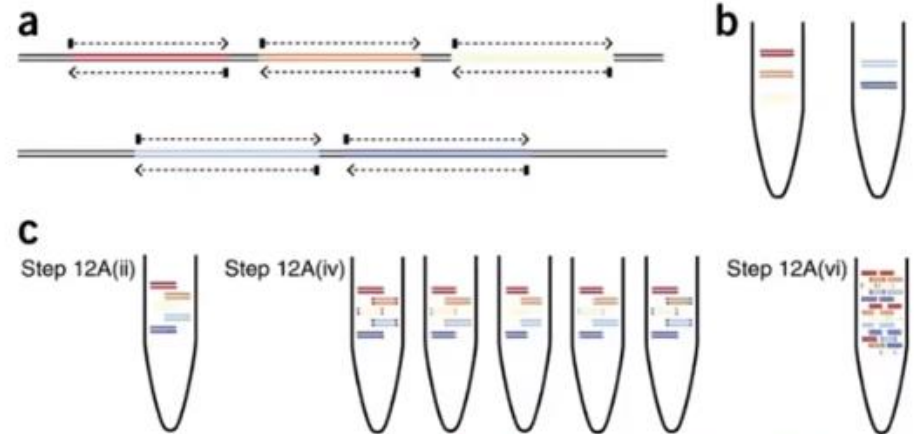
Quick et al., Nat. Prot. 2017  
Multiplex PCR method for nanopore sequencing of viral genomes.



# 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

prim

file c

# 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



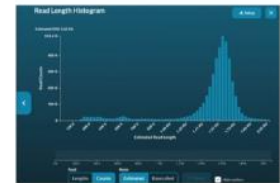
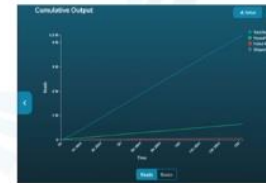
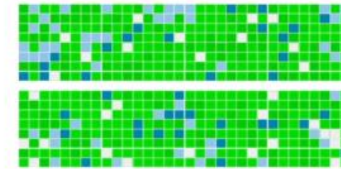
MinION Release 20.06.5



## Real-time data assessment

### Channels Panel

Live status of each channel's state during sequencing



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
		<b>12</b>	Price per sample	\$87.32
			Price per sample (including 3rd party reagents)	\$107.32

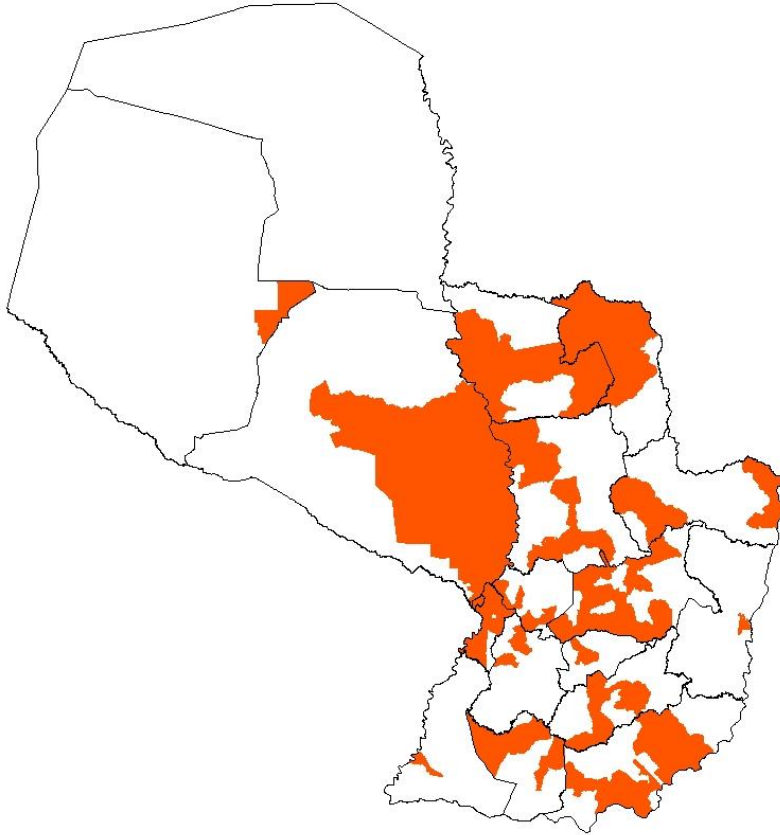
Third party reagents
\$240.00

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
		<b>24</b>	Price per sample	\$45.66
			Price per sample (including 3rd party reagents)	\$64.41

Third party reagents
\$450.00

# 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](#)

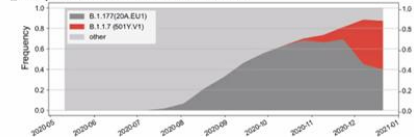
\* Apparent cumulative prevalence is the ratio of the sequences containing B.1.1.7 to all sequences collected since the identification of B.1.1.7 in that location. \*\* Dates are based on the sample collection date

[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)

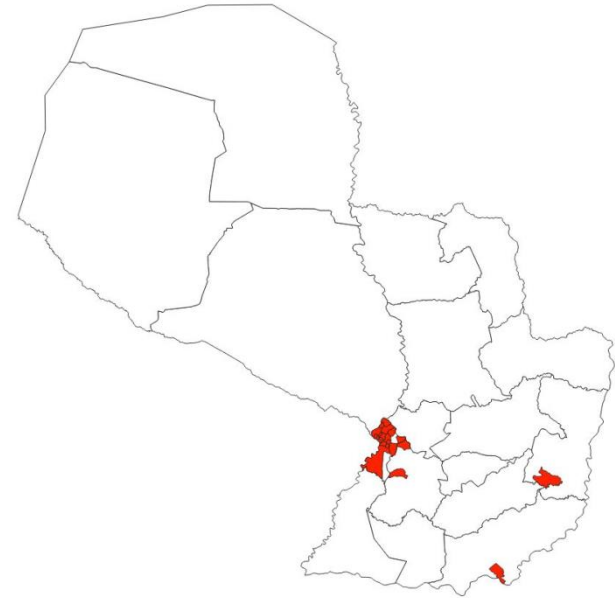
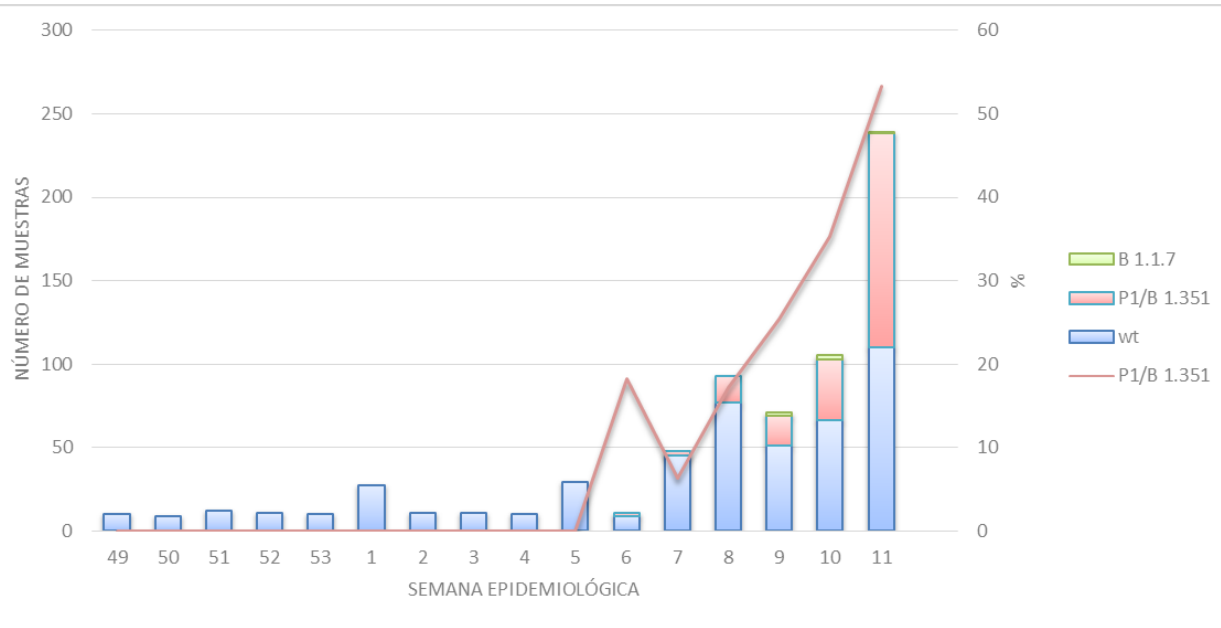
**B** Rapid rise of the B.1.1.7 variant in the UK



**C** Global surveillance of B.1.1.7 variants



# Muestras Diciembre 2020-Marzo 2021



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