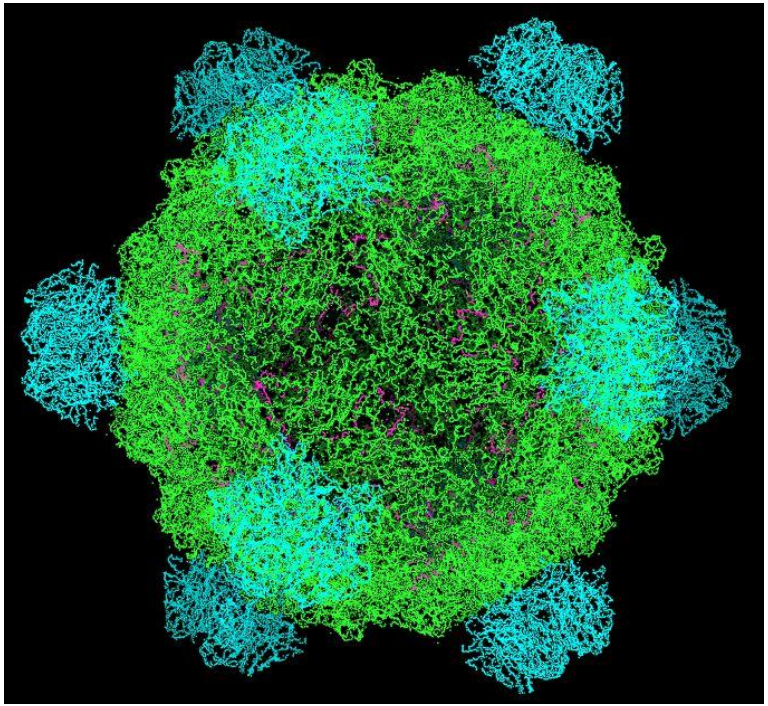
GLST 287

The Science of COVID-19 Part II: Testing and Vaccine Development

Dr. Mary Ellard-Ivey

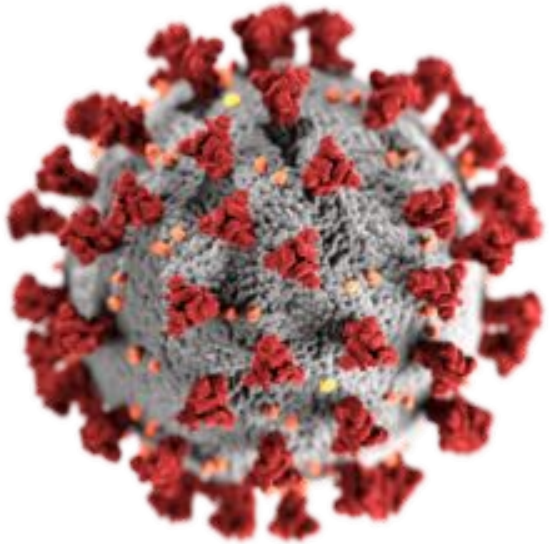
Department of Biology

1977 Sequencing of ΦX174 - Sanger



5,386 nucleotide
long sequence

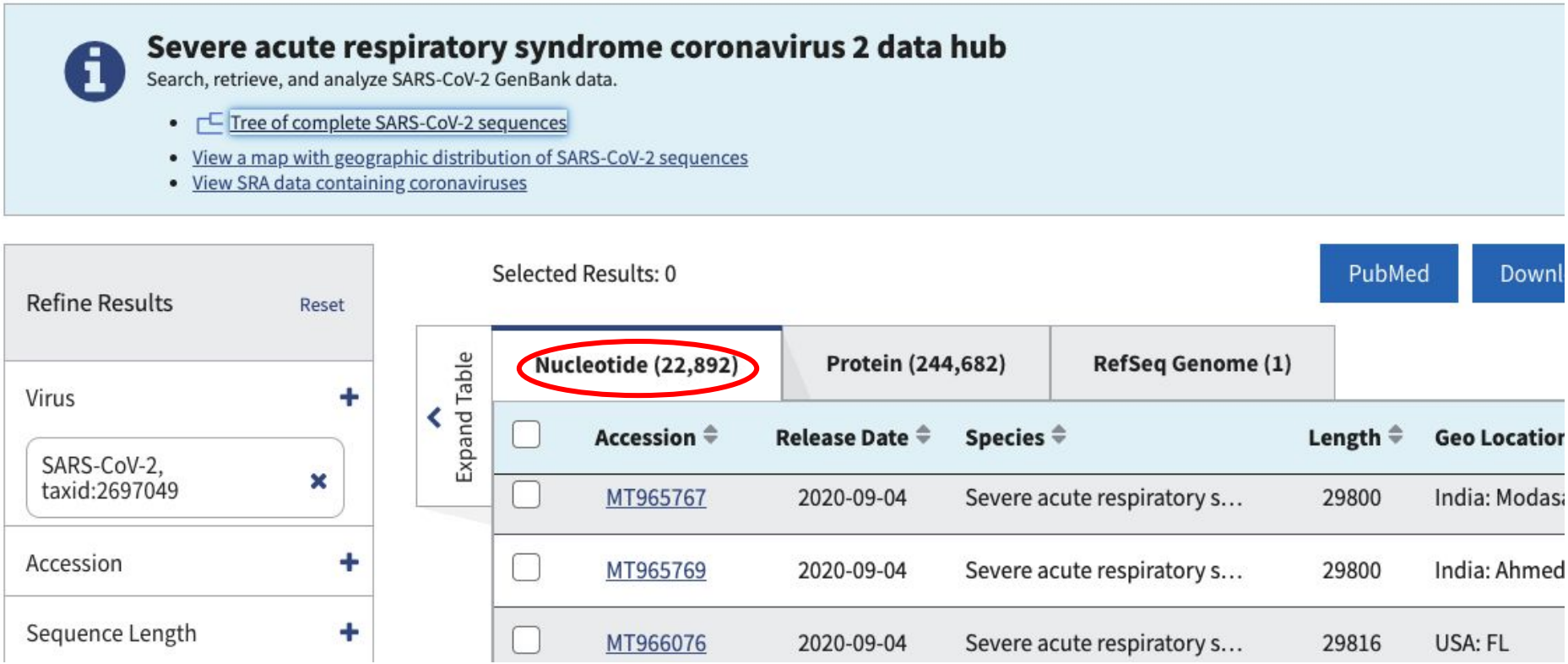
Took four years!



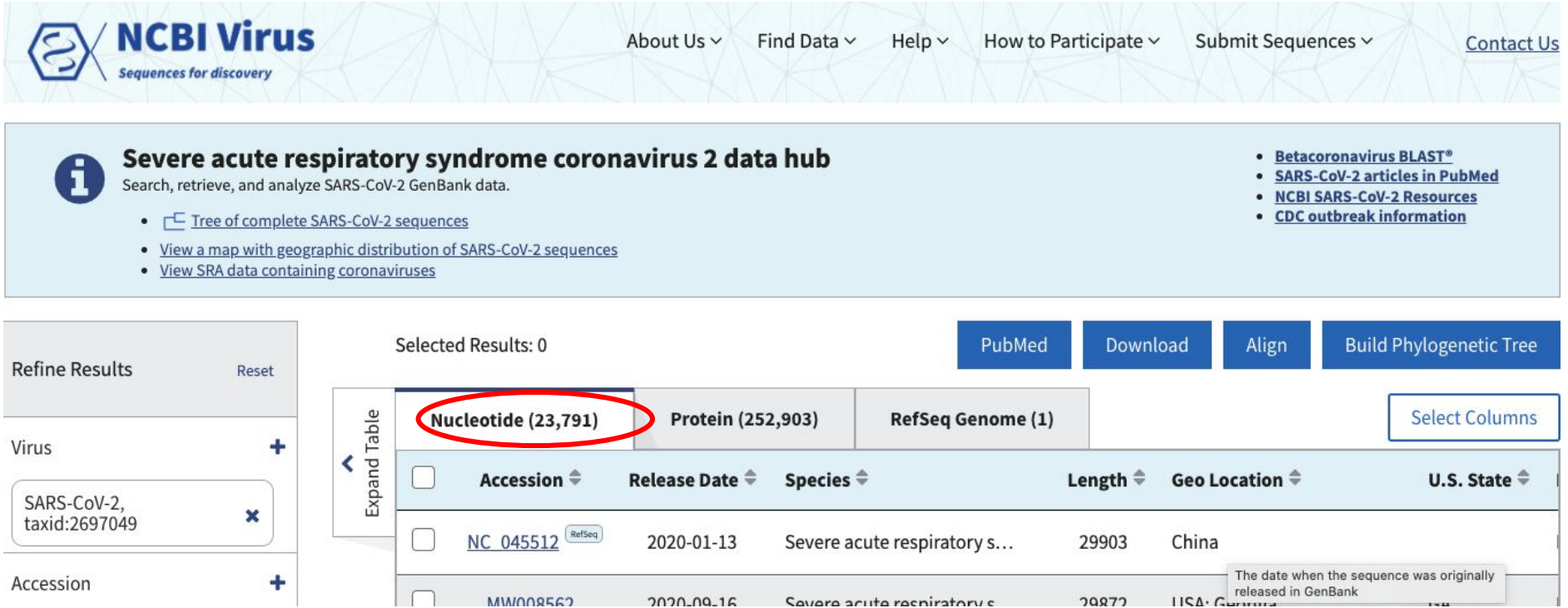
On 31 December 2019- WHO China Country Office was informed of cases of pneumonia of unknown etiology in Wuhan.

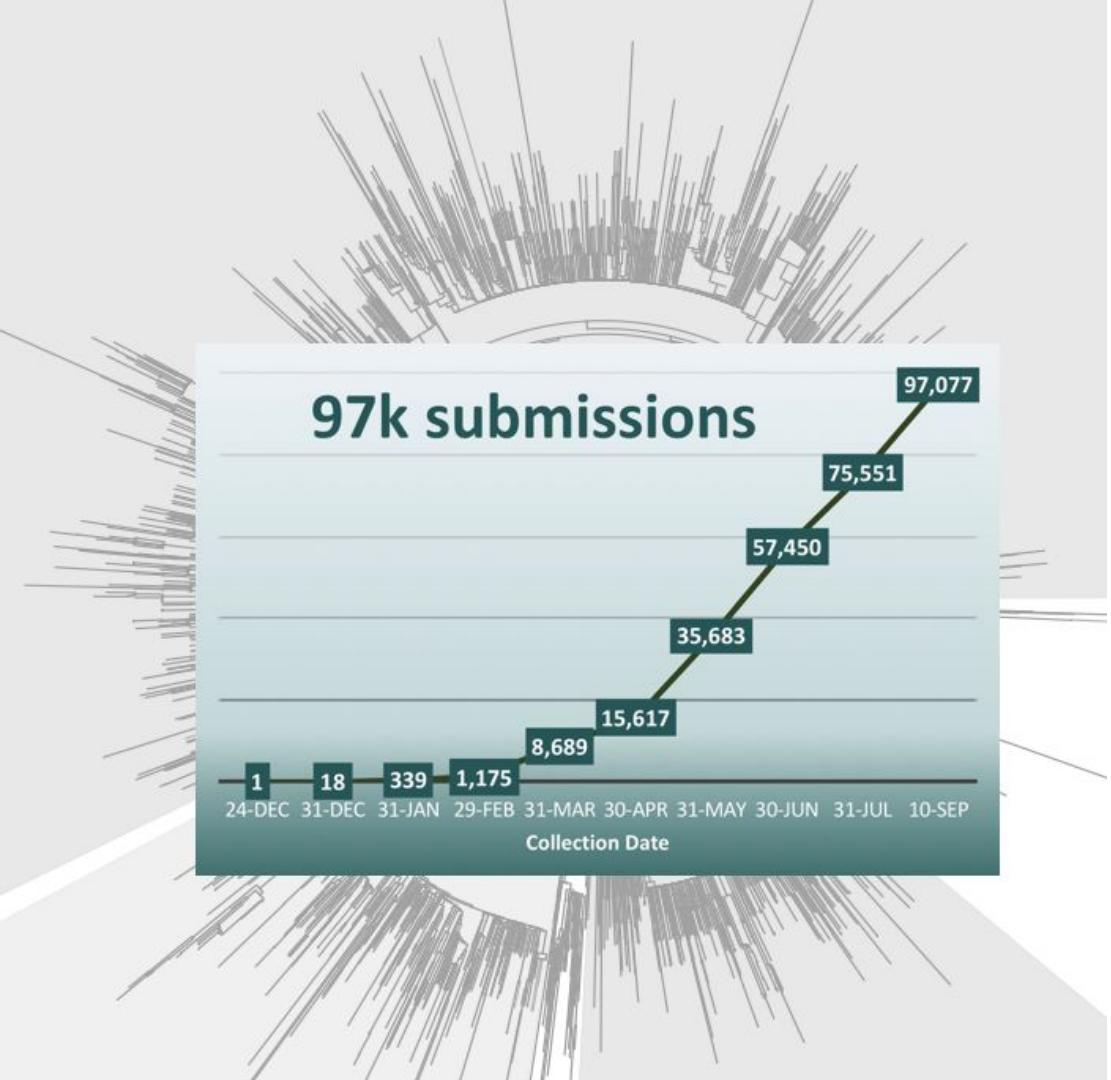
10th January 2020 - The first novel coronavirus genome sequence (~29,000 nt) was made publicly available

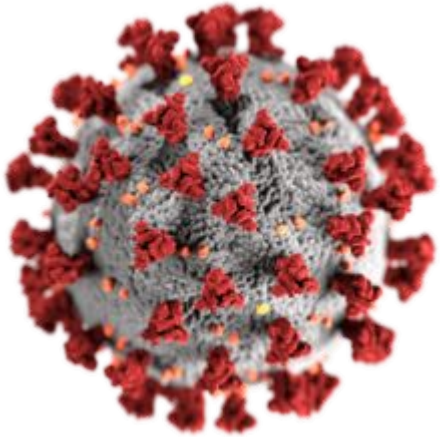
Monday 09/07


Severe acute respiratory syndrome coronavirus 2 data hub
Search, retrieve, and analyze SARS-CoV-2 GenBank data.
Tree of complete SARS-CoV-2 sequences
View a map with geographic distribution of SARS-CoV-2 sequences
View SRA data containing coronaviruses
Refine Results
Reset
Virus
SARS-CoV-2, taxid:2697049
Accession
Sequence Length
Selected Results: 0
PubMed
Downl
Expand Table
Nucleotide (22,892)
Protein (244,682)
RefSeq Genome (1)
Accession
Release Date
Species
Length
Geo Location
MT965767 2020-09-04 Severe acute respiratory s… 29800 India: Modas
MT965769 2020-09-04 Severe acute respiratory s… 29800 India: Ahmed
MT966076 2020-09-04 Severe acute respiratory s… 29816 USA: FL

Wednesday 09/16

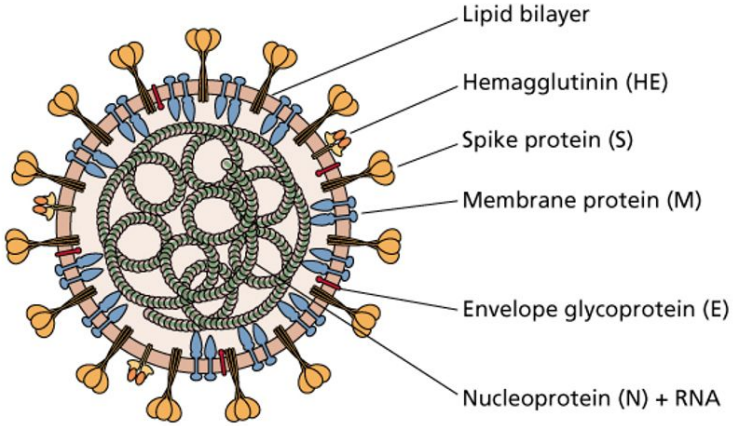



97k submissions
1
18
339
1,175
8,689
15,617
35,683
57,450
75,551
97,077
24-DEC
31-DEC
31-JAN
29-FEB
31-MAR
30-APR
31-MAY
30-JUN
31-JUL
10-SEP
Collection Date

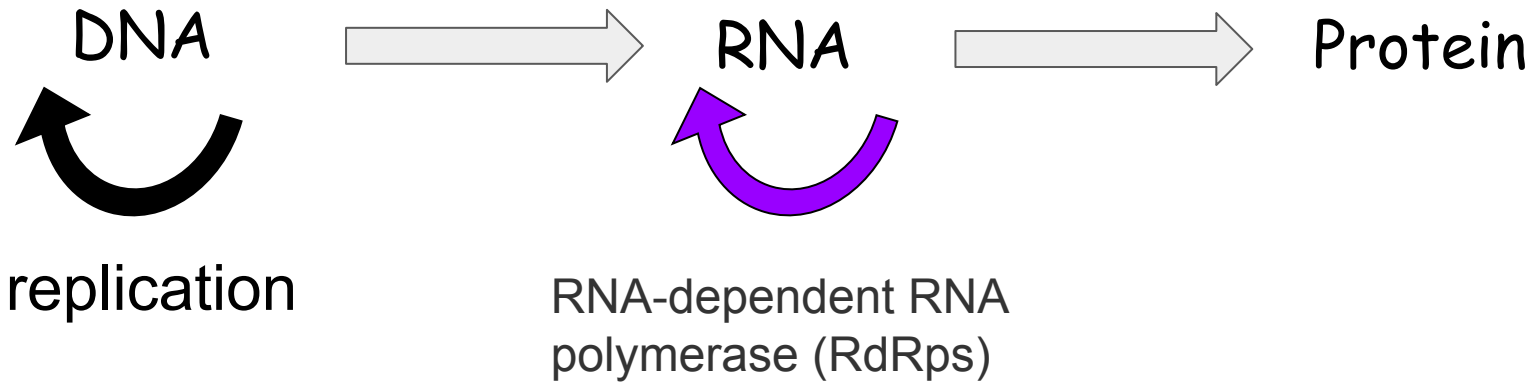


What type of genome does SARS CoV-2 have?

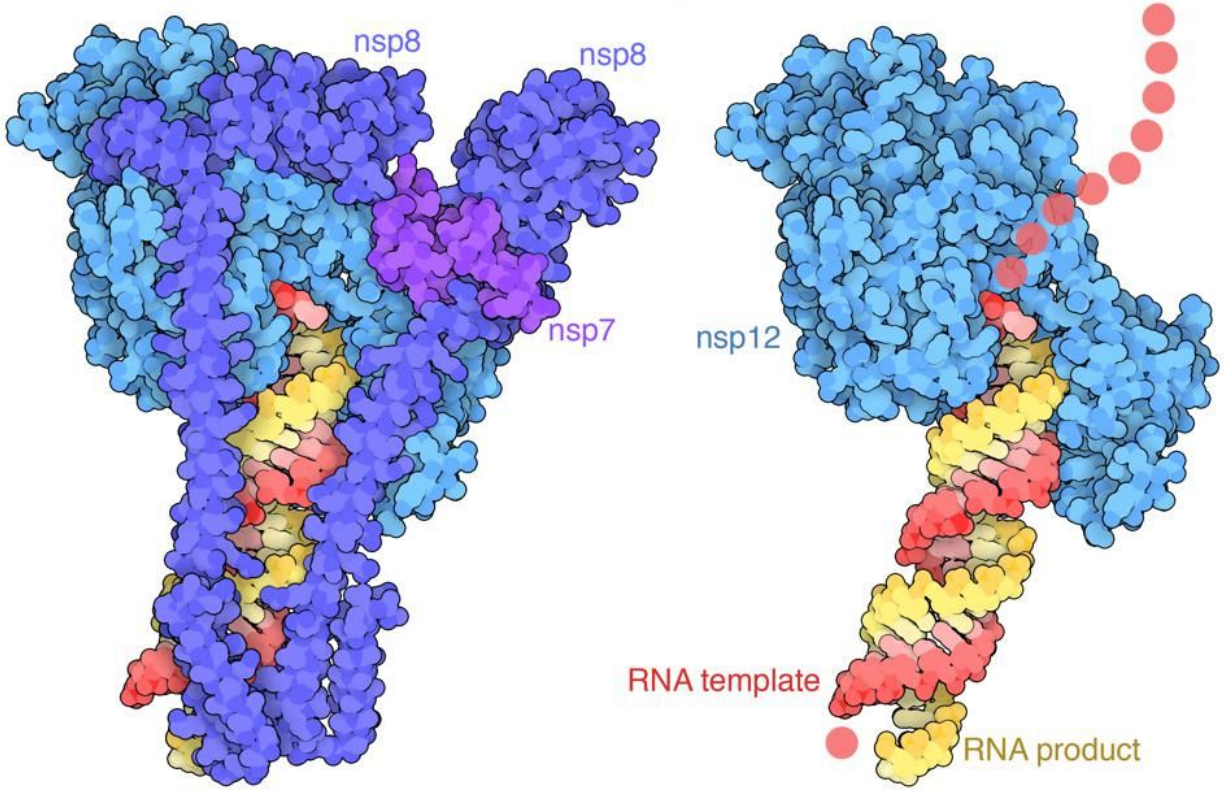
Single Stranded RNA genome



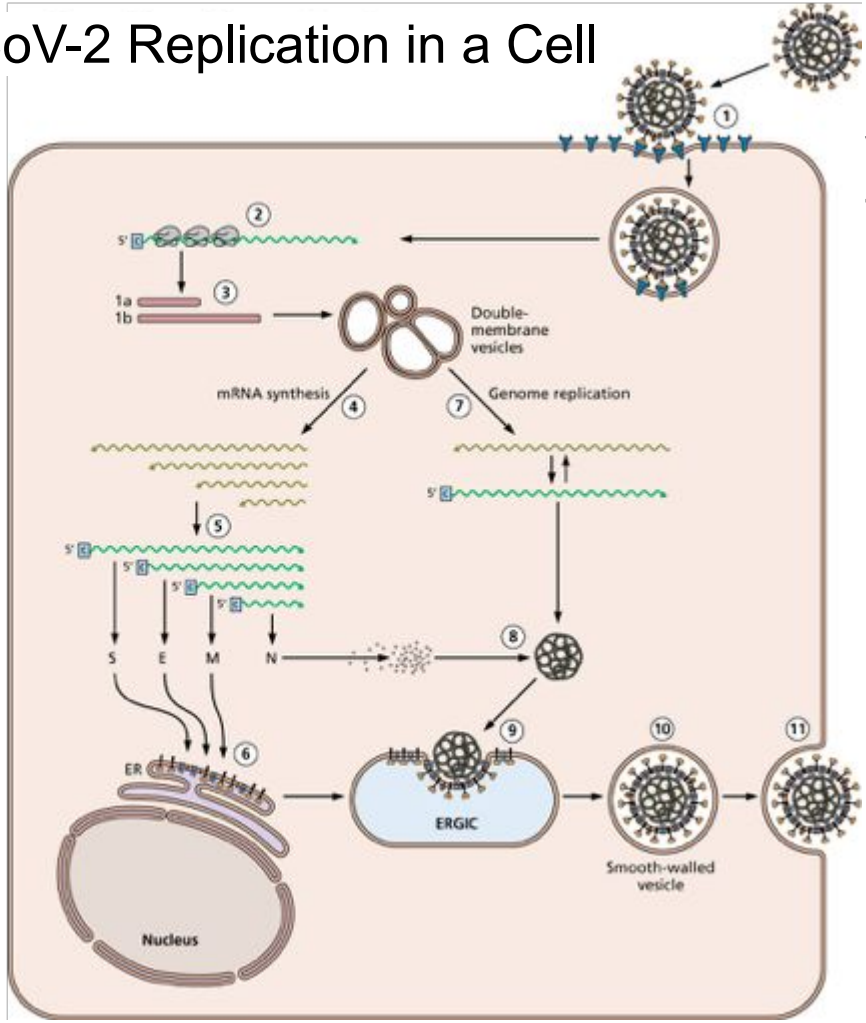
The Central Dogma of Molecular Biology



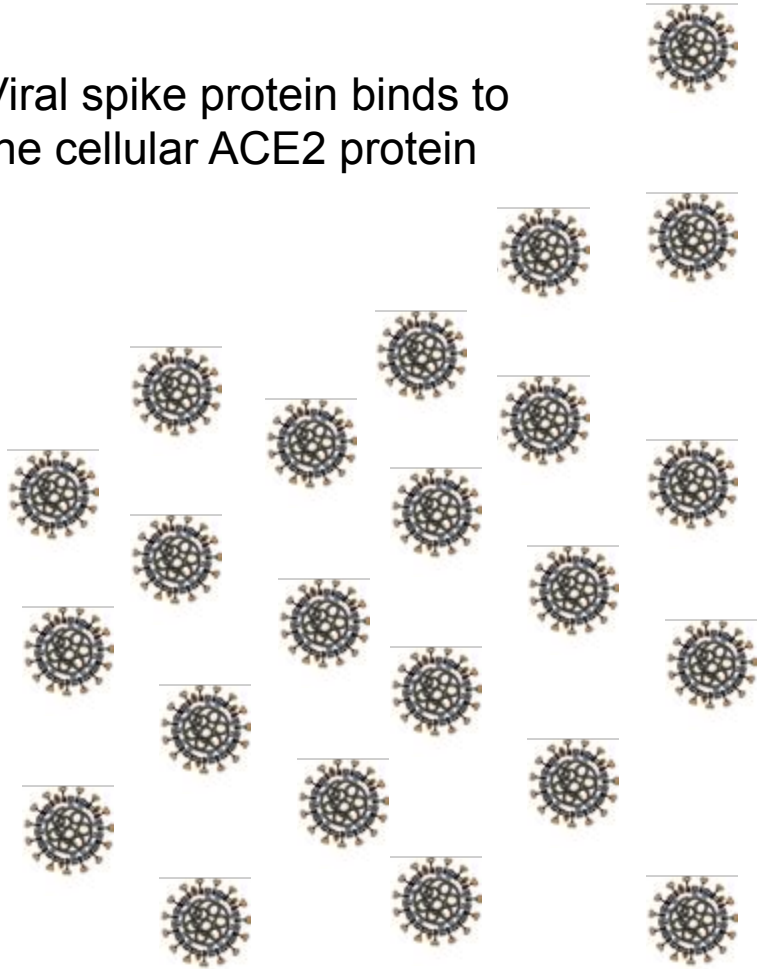
RdRp - RNA dependent RNA Polymerase



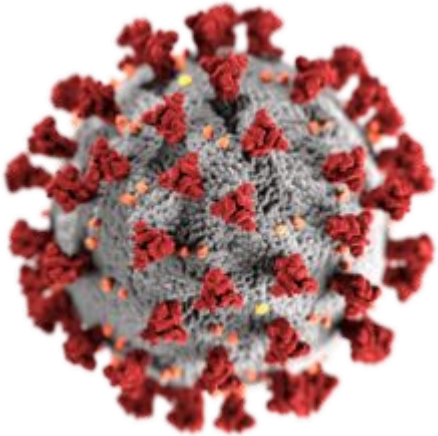
SARS CoV-2 Replication in a Cell



Viral spike protein binds to the cellular ACE2 protein



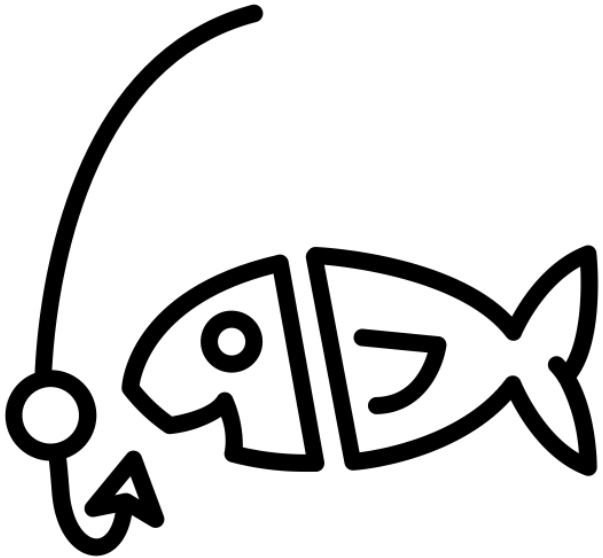
If you wanted to see if this virus is in a patient, what might you look for?



Strategies for testing for infection

Look for	Specific target	Tool	Comments
RNA	ORF1ab includes RdRp E gene - Envelope protein	Real Time PCR	Gold standard. High sensitivity/specificity Requires specialized equipment and expertise
Proteins/Antigens	Two targets on the Spike protein	ELISA/Antigen Tests	Lower sensitivity More false negatives
RNA	Nucleoprotein and E-protein	CRISPR	Requires specialized equipment and expertise Highly sensitive and specific

Molecular biologist and fishing



Created by Nicole Steffen
from Noun Project

CATGCATCGACACTATGC

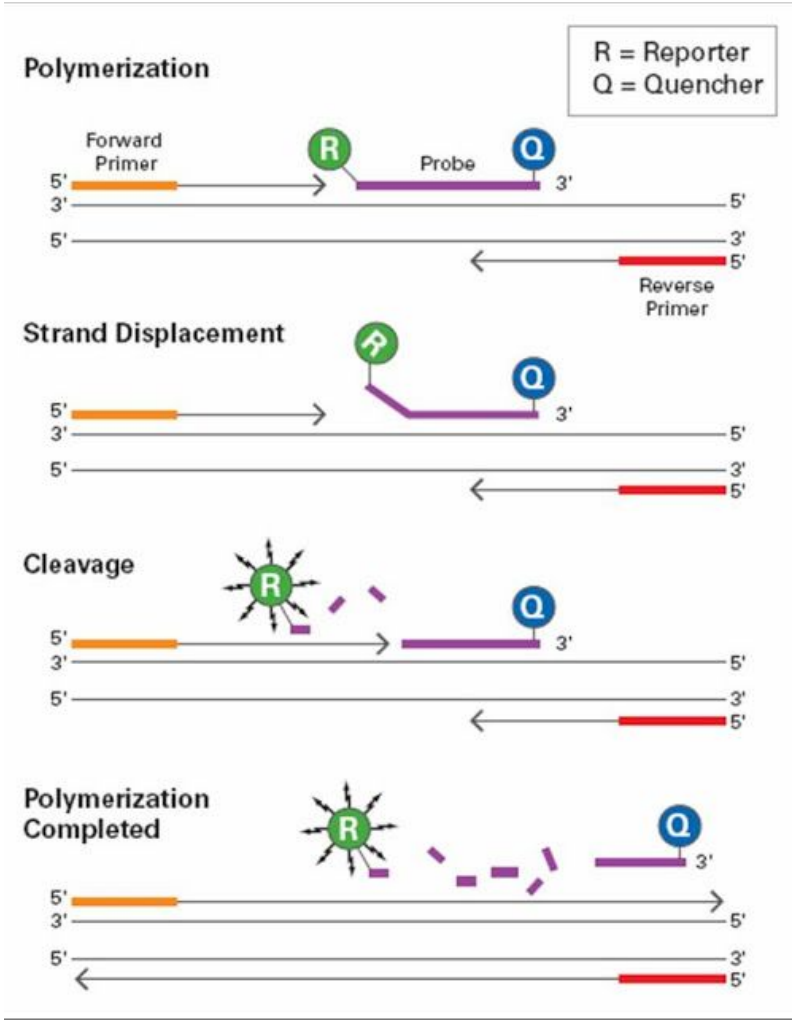
GTACGTAGCTGTGATACG

PCR identifies and copies a DNA molecule using a bait.

RNA

↓

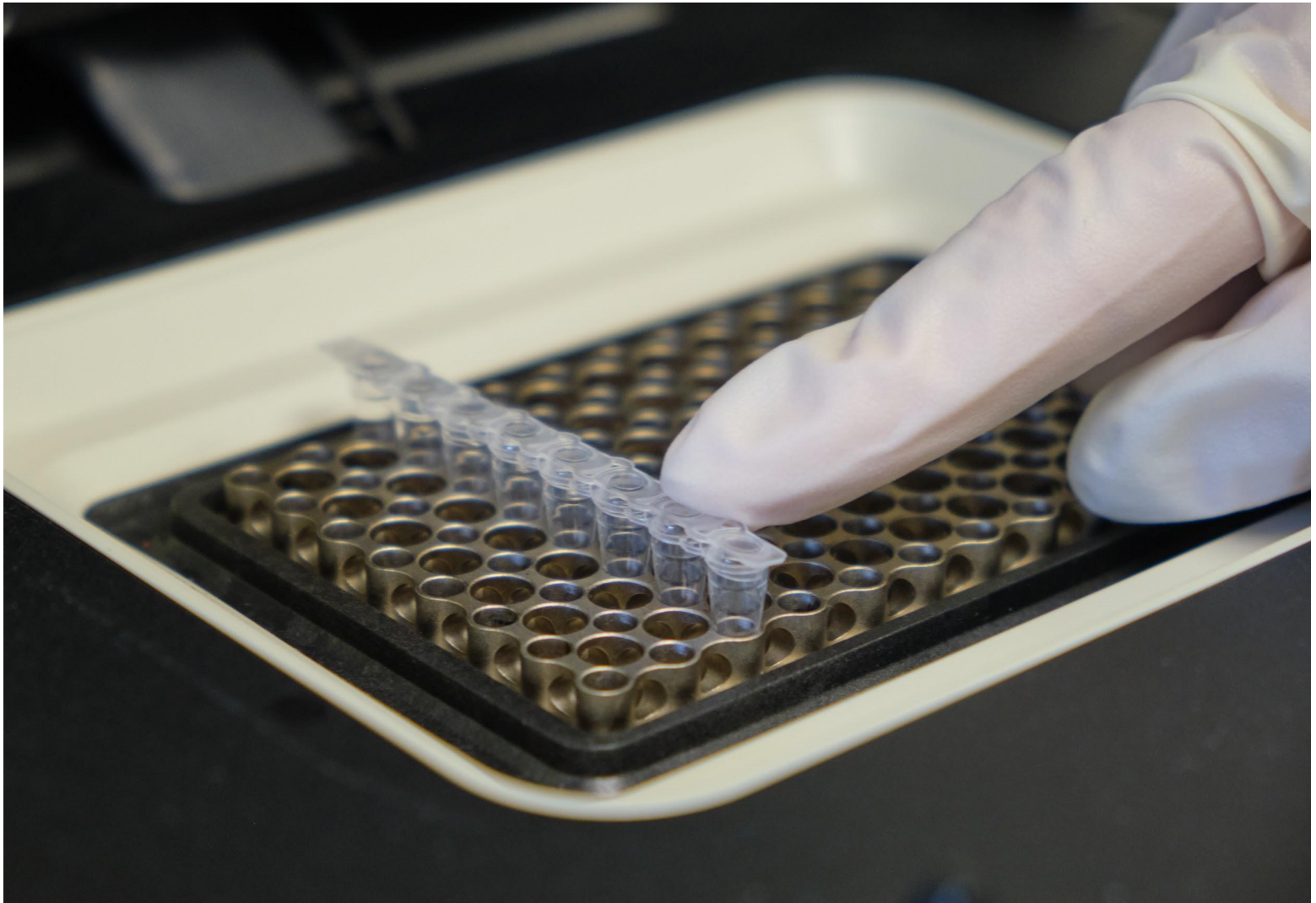
DNA

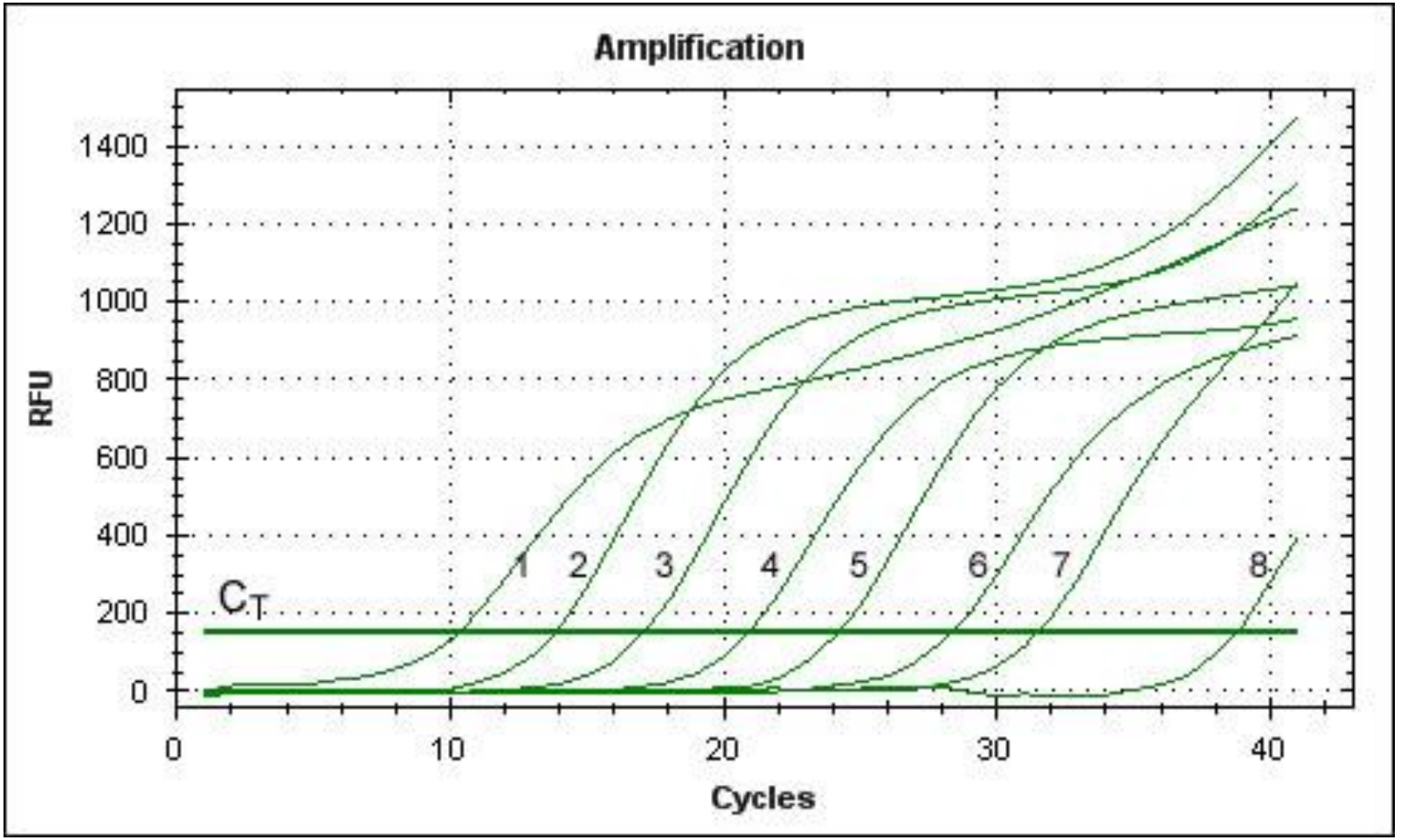


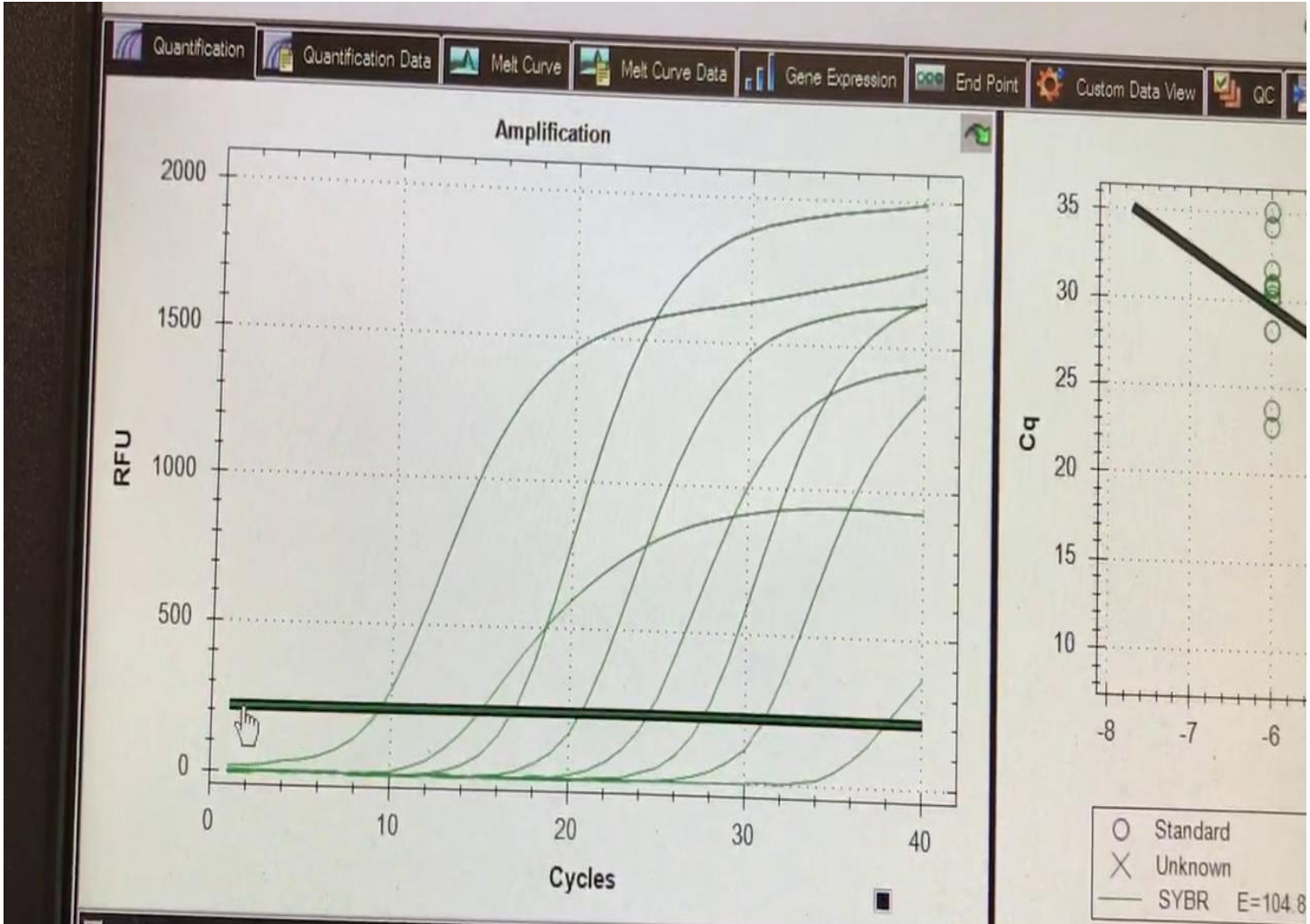
- Real Time PCR track the exponential accumulation of product

- Quantitative and sensitive


FHCRC
M6



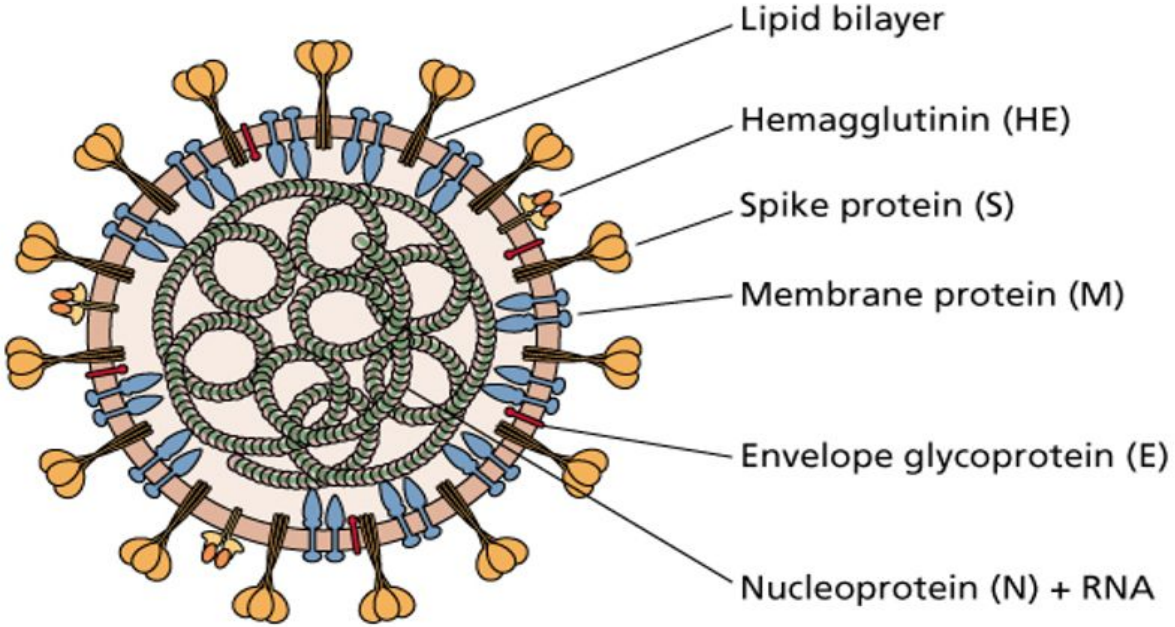

Amplification
RFU
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
Cycles
C_T
1
2
3
4
5
6
7
8

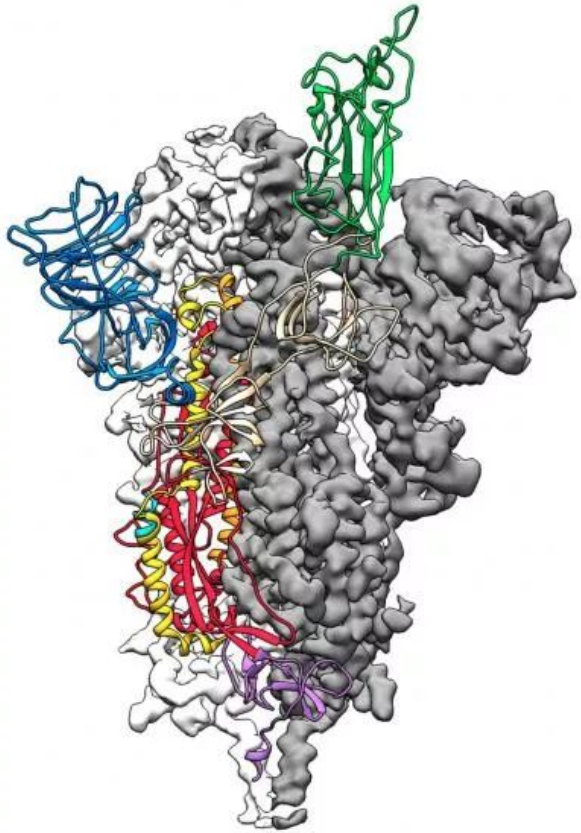

Quantification
Quantification Data
Melt Curve
Melt Curve Data
Gene Expression
End Point
Custom Data View
QC
Amplification
RFU
2000
1500
1000
500
0
0
10
20
30
40
Cycles
Cq
35
30
25
20
15
10
-8
-7
-6
Standard
Unknown
SYBR
E=104.8

Strategies for testing for infection

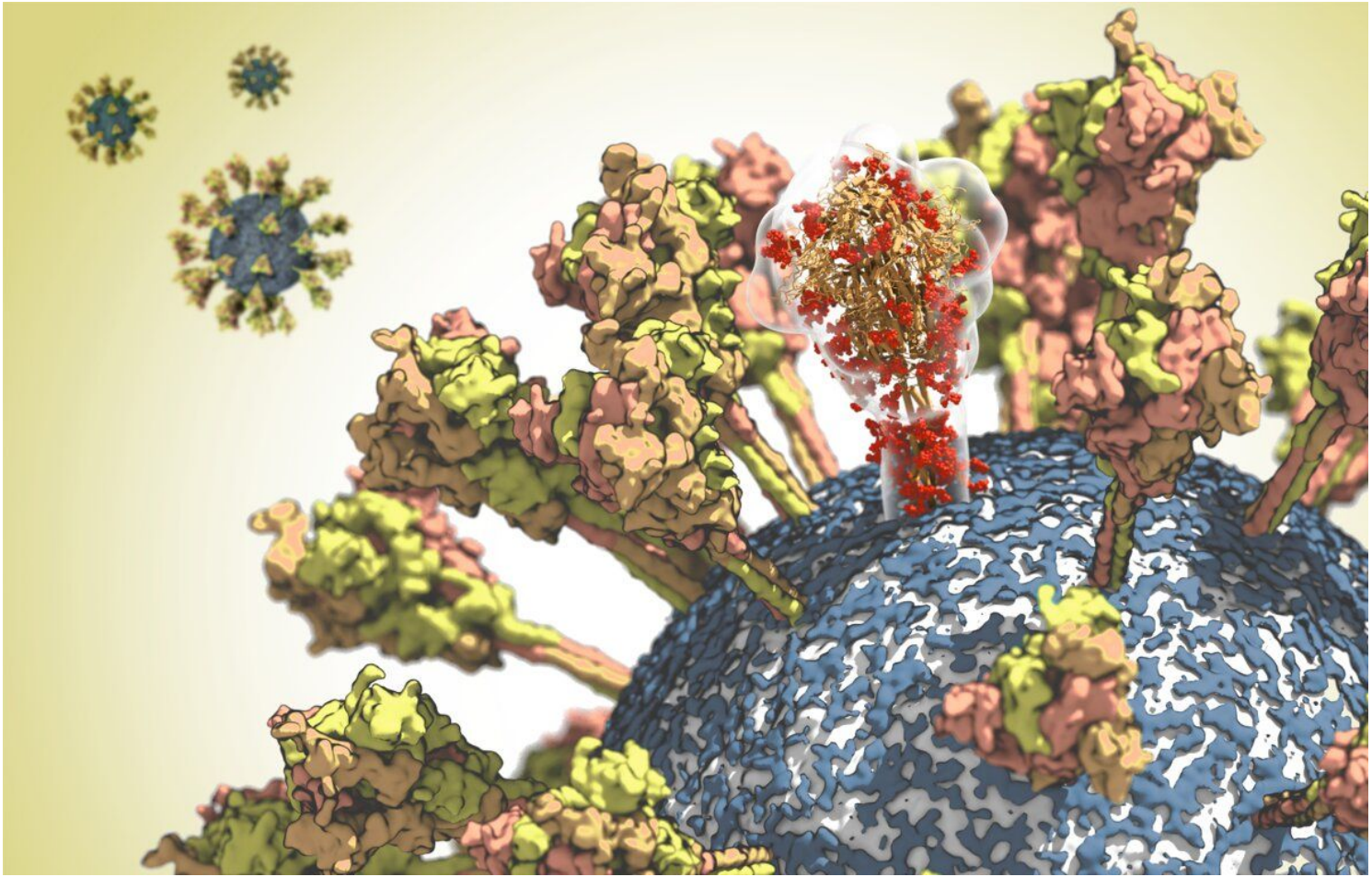
Look for	Specific target	Tool	Comments
RNA	ORF1ab includes RdRp E gene - Envelope protein	Real Time PCR	Gold standard. High sensitivity/specificity Requires specialized equipment and expertise
Proteins/Antigenic protein	Two targets on the Spike protein	ELISA/Antigen Tests	Lower sensitivity More false negatives Can be adapted to home use
RNA	Nucleoprotein and E-protein	CRISPR	Requires specialized equipment and expertise Highly sensitive and specific

Protein/Antigen tests



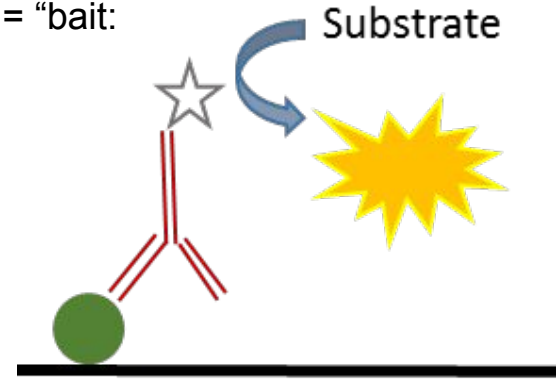


Surface of the CoV-2 virus. A molecule of the spike protein is shown translucently to emphasize its complex spatial structure.
Credit: MPI f. Biophysics



Protein/Antigen Test

Antibody = “bait:



Direct ELISA

no amplification step!

Rapid Antigen Detection (RAD)

- Quick Strep Tests
- Ovulation Predictor tests
- Pregnancy Tests


COVID-19
C
T

J Clin Virol. 2020 Aug; 129: 104500.
Published online 2020 Jun 8. doi: 10.1016/j.jcv.2020.104500
PMCID: PMC7278630
PMID: 32585619

Evaluation of rapid antigen test for detection of SARS-CoV-2 virus

Gannon CK Mak,* Peter KC Cheng, Stephen SY Lau, Kitty KY Wong, CS Lau, Edman TK Lam, Rickjason CW Chan, and Dominic NC Tsang

Table 1

Comparison of RT-PCR, viral culture and rapid antigen detection (RAD) test for the limit of detection of SARS-CoV-2 virus.

Dilution[b]	Test results[a]			
	RT-PCR[c]	Viral culture	RAD test, sample processing method[d] for:	
			less viscous samples	viscous samples
10^{-1}	ND	ND	POS	NEG
10^{-2}	ND	POS	POS	NEG
10^{-3}	ND	POS	NEG	NEG
10^{-4}	25.17	POS	NEG	NEG
10^{-5}	28.47	POS	NEG	NEG
10^{-6}	31.08	NEG	NEG	ND
10^{-7}	36.41	NEG	ND	ND

Strategies for testing for infection

Look for	Specific target	Tool	Comments
RNA	ORF1ab includes RdRp E gene - Envelope protein	Real Time PCR	Gold standard. High sensitivity/specificity Requires specialized equipment and expertise
Proteins/Antigenic protein	Two targets on the S protein	ELISA/Antigen Tests	Lower sensitivity More false negatives Can be adapted to home use
RNA	Nucleoprotein and E-protein	CRISPR	Requires specialized equipment and expertise Highly sensitive and specific

Testing needs to be:

- Rapid
- Accurate
- Sensitive
- Accessible
- Commensurate with risk

SHARE

1K

30

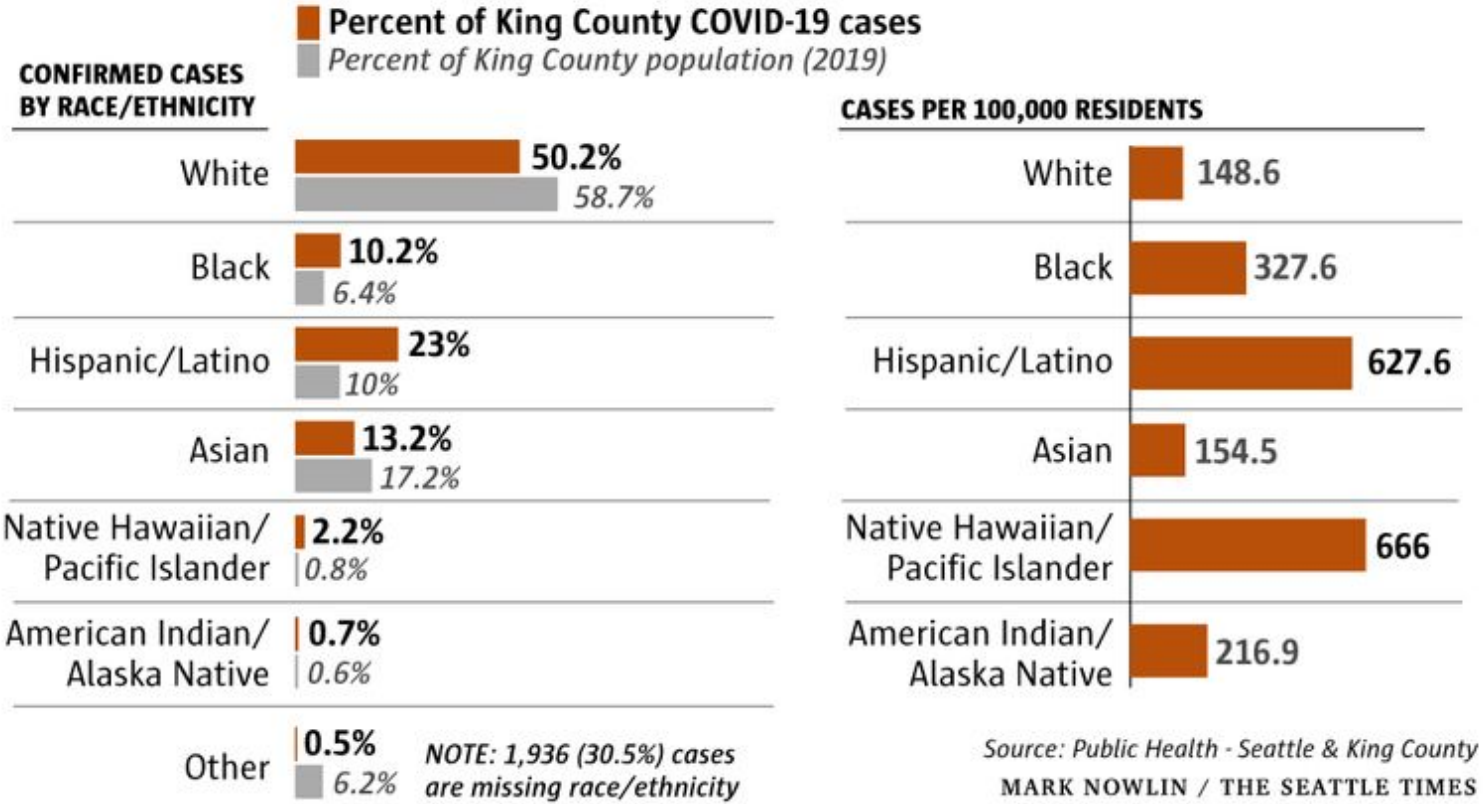
Complete data from COVID-19 testing sites in low-income areas, such as this one at Interbay Village in Seattle, are crucial to fighting the pandemic. DAVID RYDER/REUTERS

‘Huge hole’ in COVID-19 testing data makes it harder to study racial disparities

By Kelly Servick | Jul. 10, 2020 , 6:25 PM

COVID-19 not affecting all races equally in King County

In King County, some communities of color have been infected with the novel coronavirus at higher rates than white people, according to a new analysis of public health data.



Source: Public Health - Seattle & King County

MARK NOWLIN / THE SEATTLE TIMES

The race for a vaccine

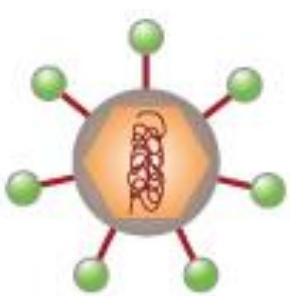
Created by Takao Umehara
from Noun Project

Operation Warp Speed

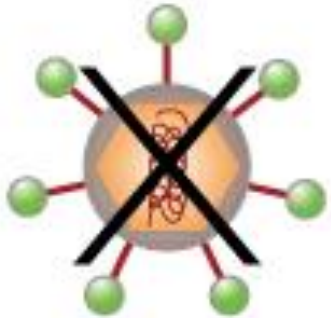
What features are desirable in a vaccine?

General Strategies for Vaccination

Created by Takao Umehara
from Noun Project



Live attenuated vaccine



Inactivated vaccine

Subunit vaccine

Nucleic acid-based vaccine

Candidate Vaccines for SARS CoV-2

Created by Takao Umehara
from Noun Project

These three vaccines have support of Operation Warp Speed and are in phase three clinical trials

- The University of Oxford and AstraZeneca's AZD1222
- Moderna's mRNA-1273
- Pfizer and BioNTech's BNT162b2

Oxford Vaccine

Replication deficient chimpanzee virus (adenovirus)

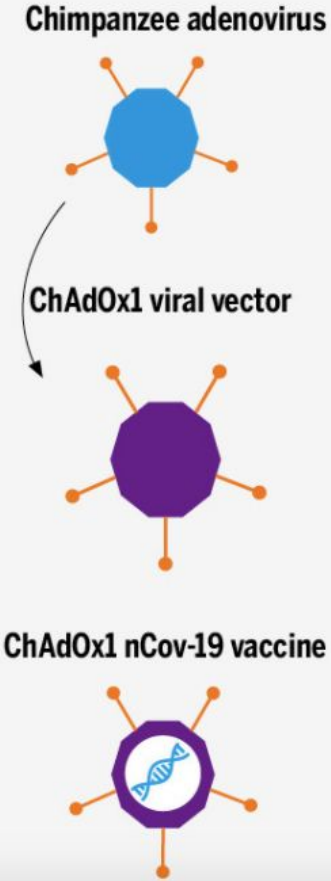
Causes common cold

STEP 1

Genetic code of the coronavirus spike protein is identified

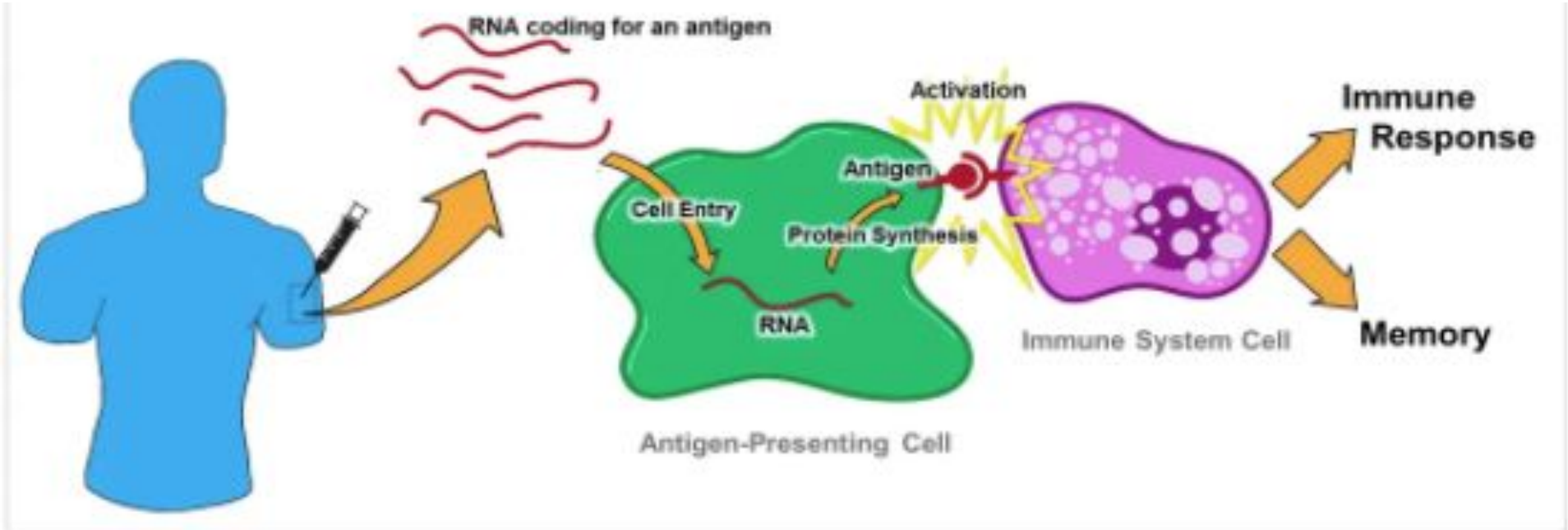
STEP 2

The vaccine contains the chimpanzee adenovirus in a weakened and genetically modified form so it doesn't infect humans and produces spike proteins

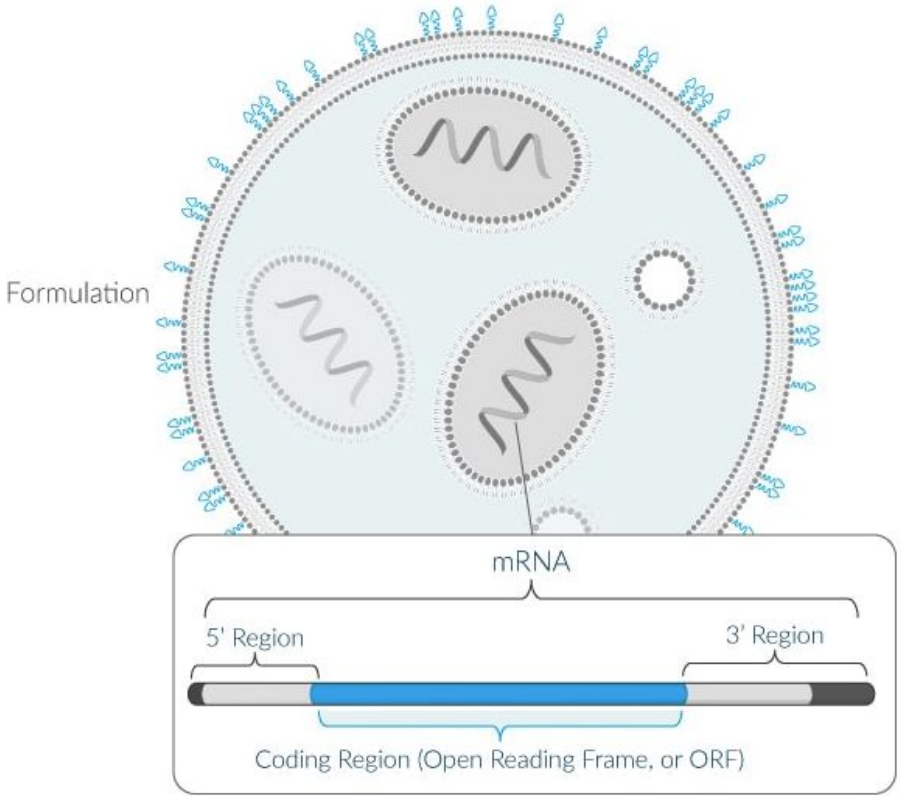


Moderna vaccine

About mRNA-1273, Moderna's Vaccine Candidate Against COVID-19



Delivered in a lipid based nanoparticle



RNA Vaccines

A completely novel and unproven technology

No RNA vaccines currently approved

Pfizer and BioNTech's BNT162b2

Another mRNA vaccine against the full length spike protein.

Do these vaccines meet the criteria for a successful vaccine?