

# Basics concepts of molecular virology

**Dr Liã Bárbara Arruda**

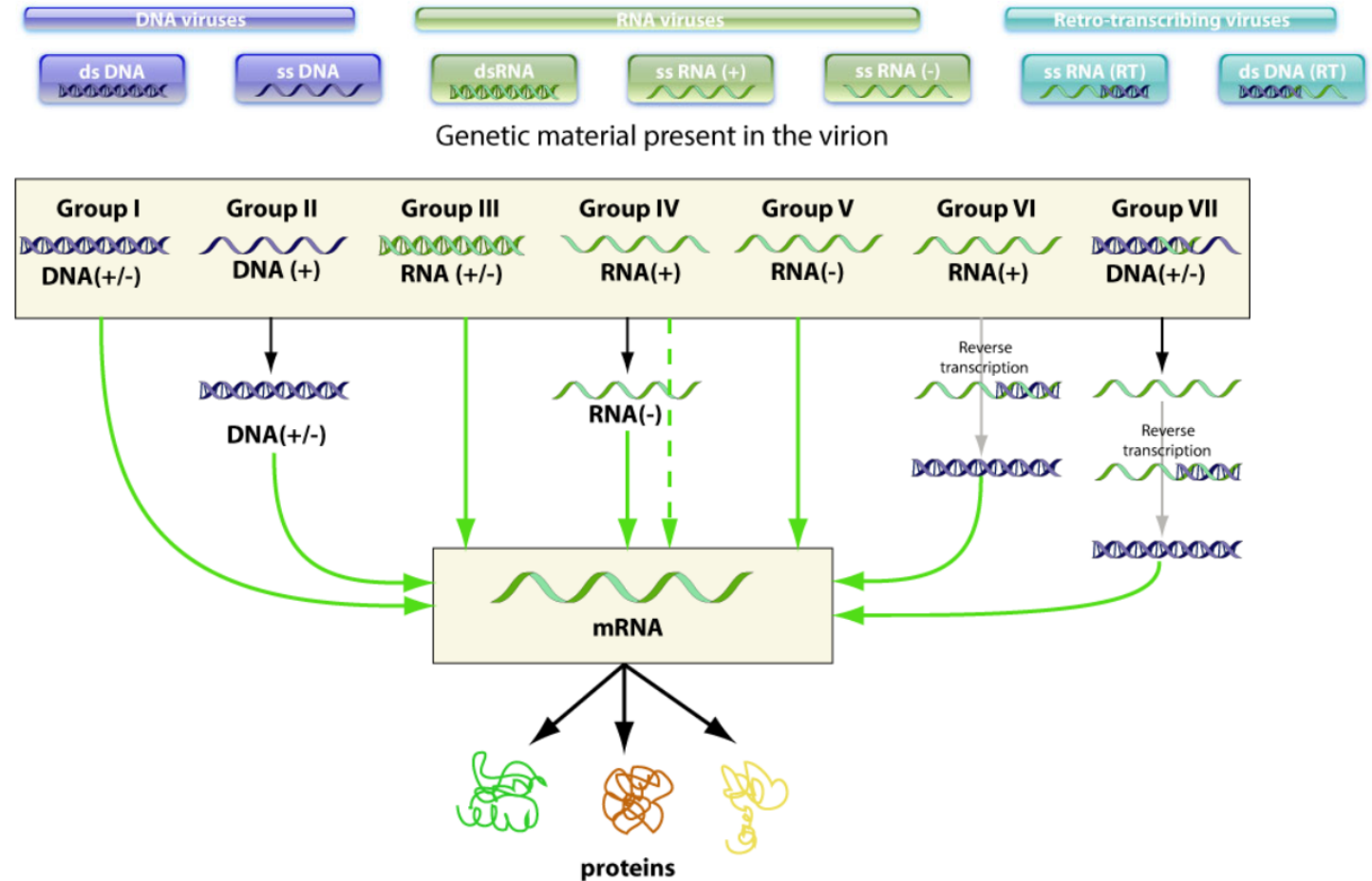
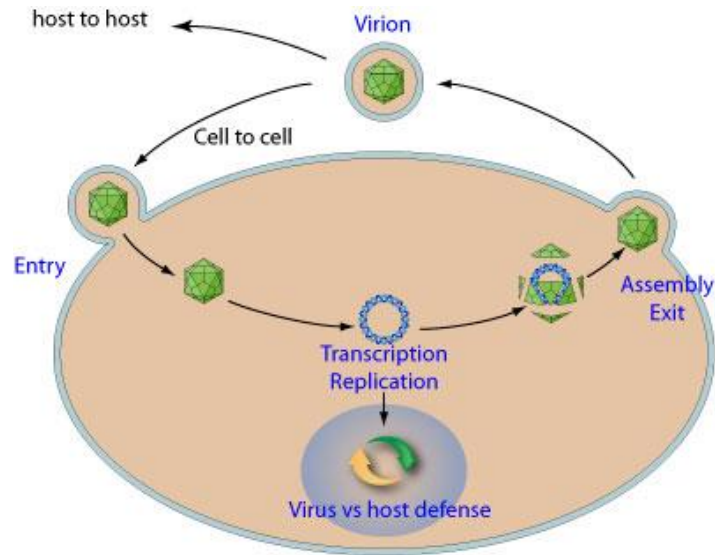
Postdoctoral Research Associate

Centre for Clinical Microbiology

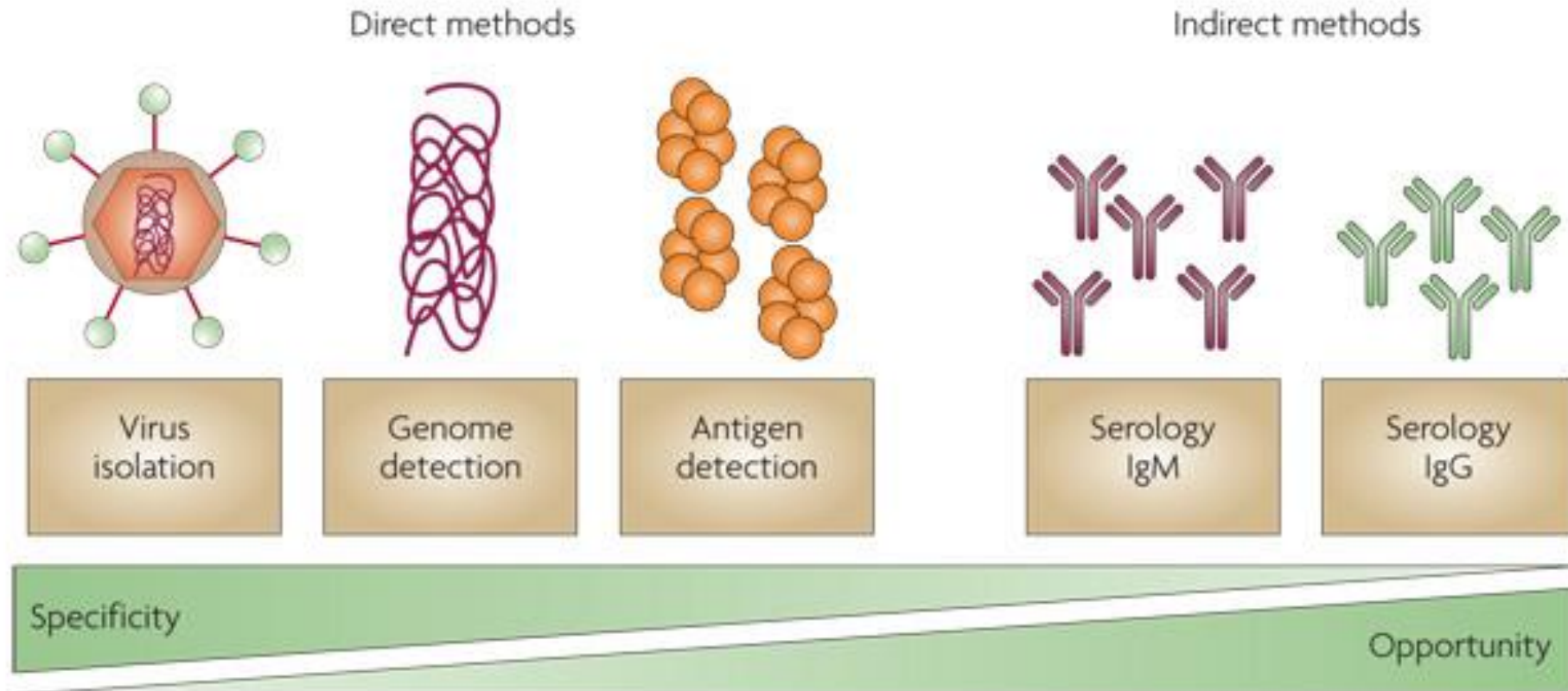
PANDORA-ID-NET Consortium

14 Feb 2020

# Viruses are just the coolest



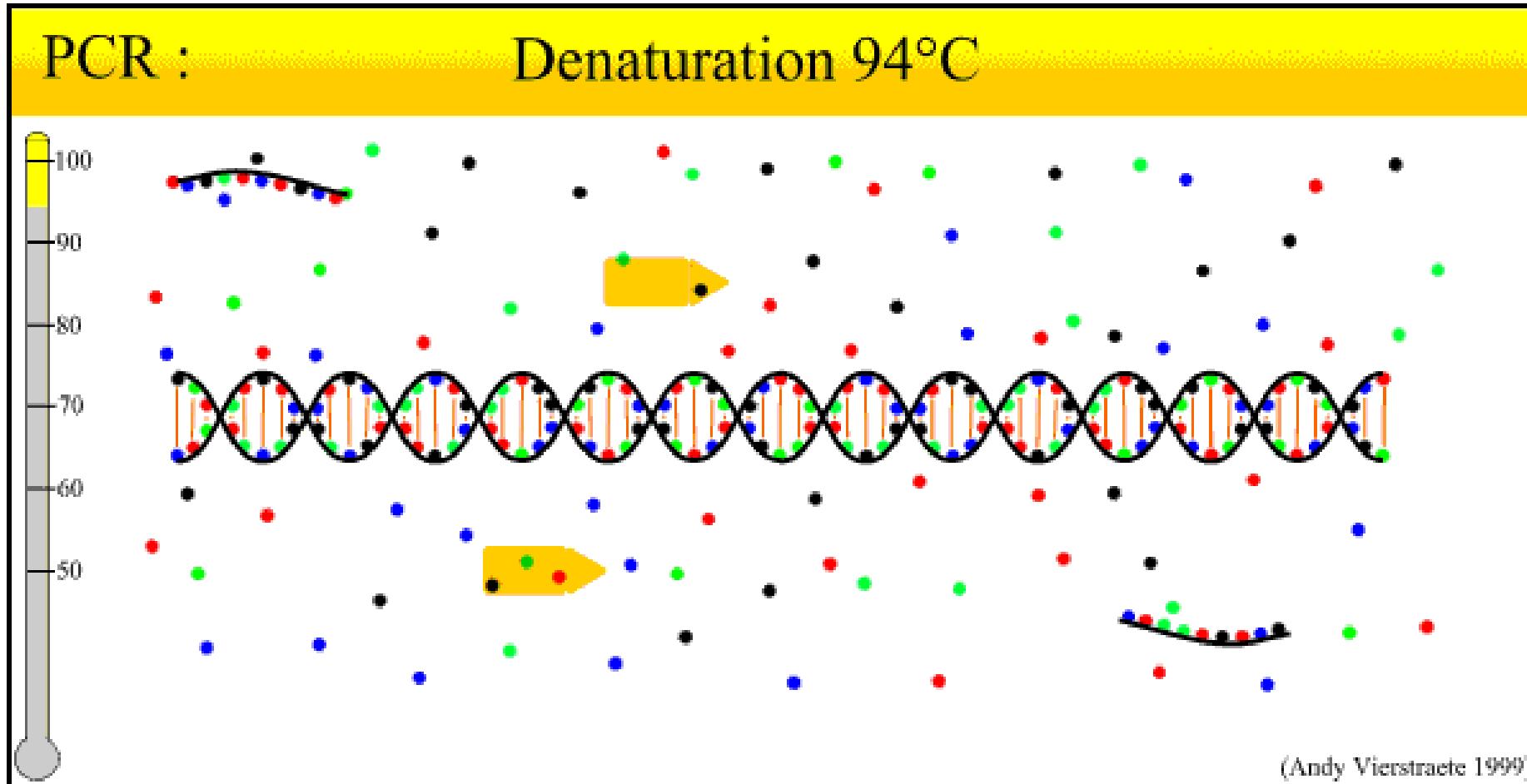
# Detection of viruses



<https://www.who.int/in-vitro-diagnostic/en/>

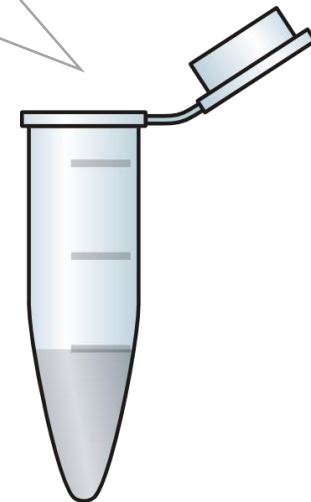
Peeling et al. Nat Rev Microbiol. 2010 Dec;8(12 Suppl):S30-8. doi: 10.1038/nrmicro2459

# Polymerase chain reaction (PCR)

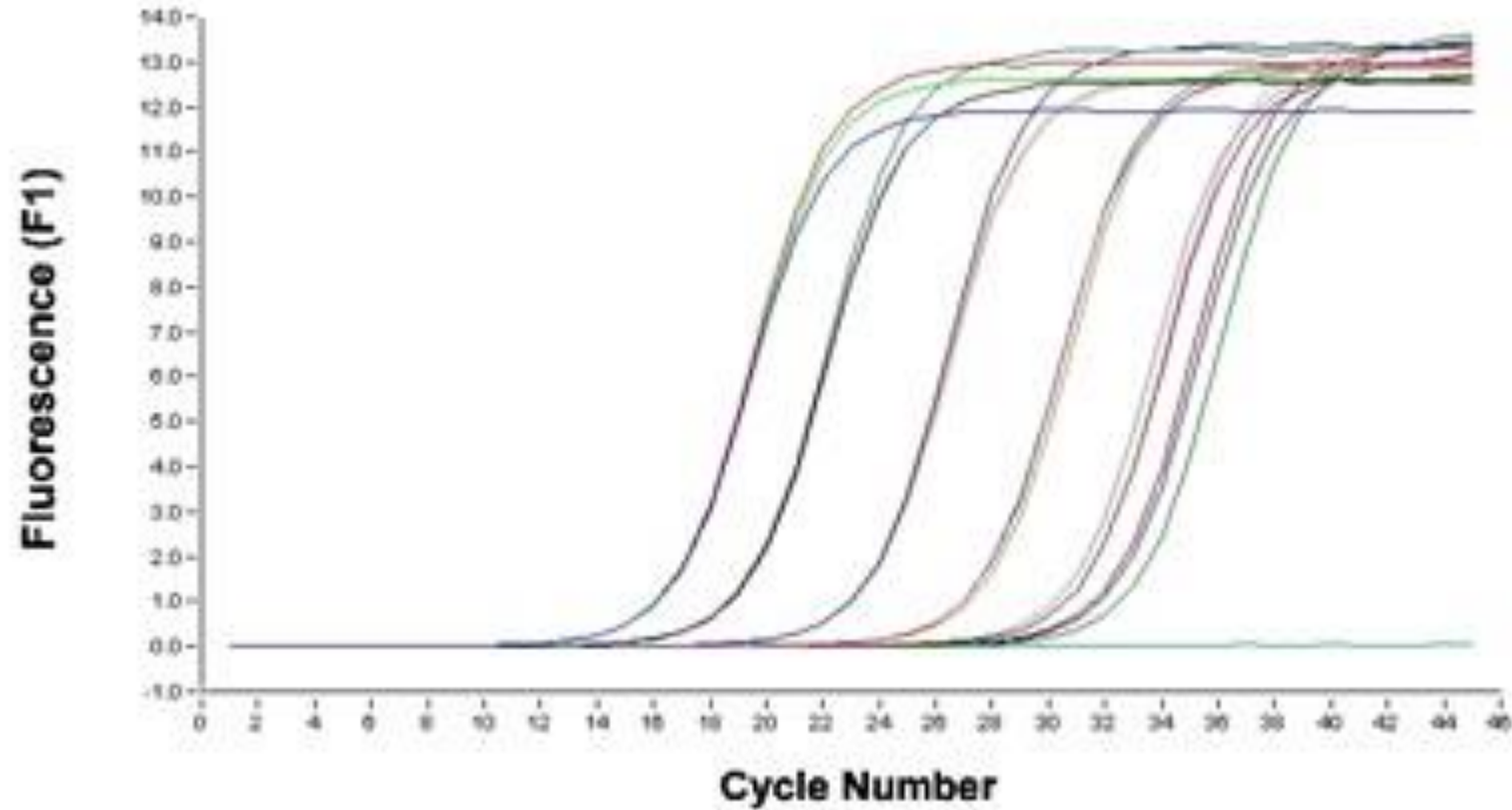


# PCR components

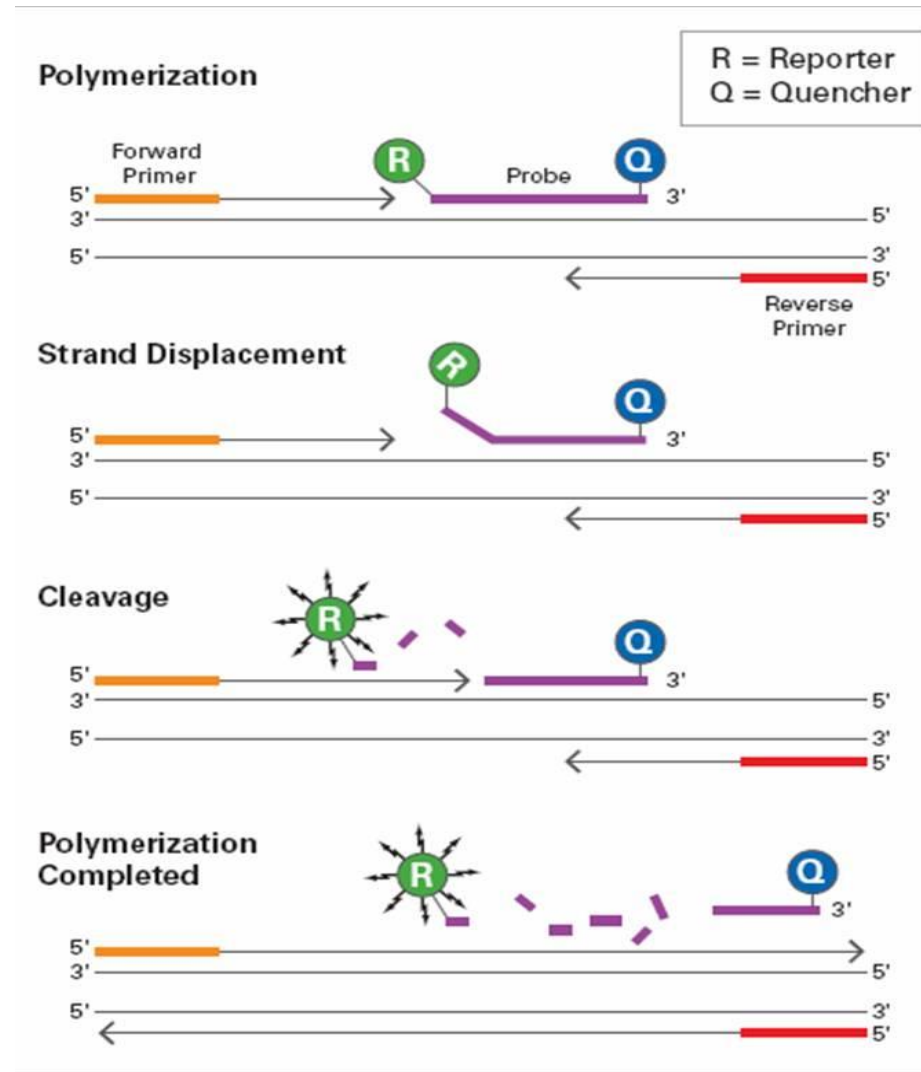
Nuclease-free water  
Buffer  
*Taq* DNA Polymerase\*  
*Taq* DNA Polymerase co-factor (Mg++)  
dNTPs  
**Oligonucleotides**  
**Template**



# Real-time PCR (qPCR)



# TaqMan system



# TaqMan labels

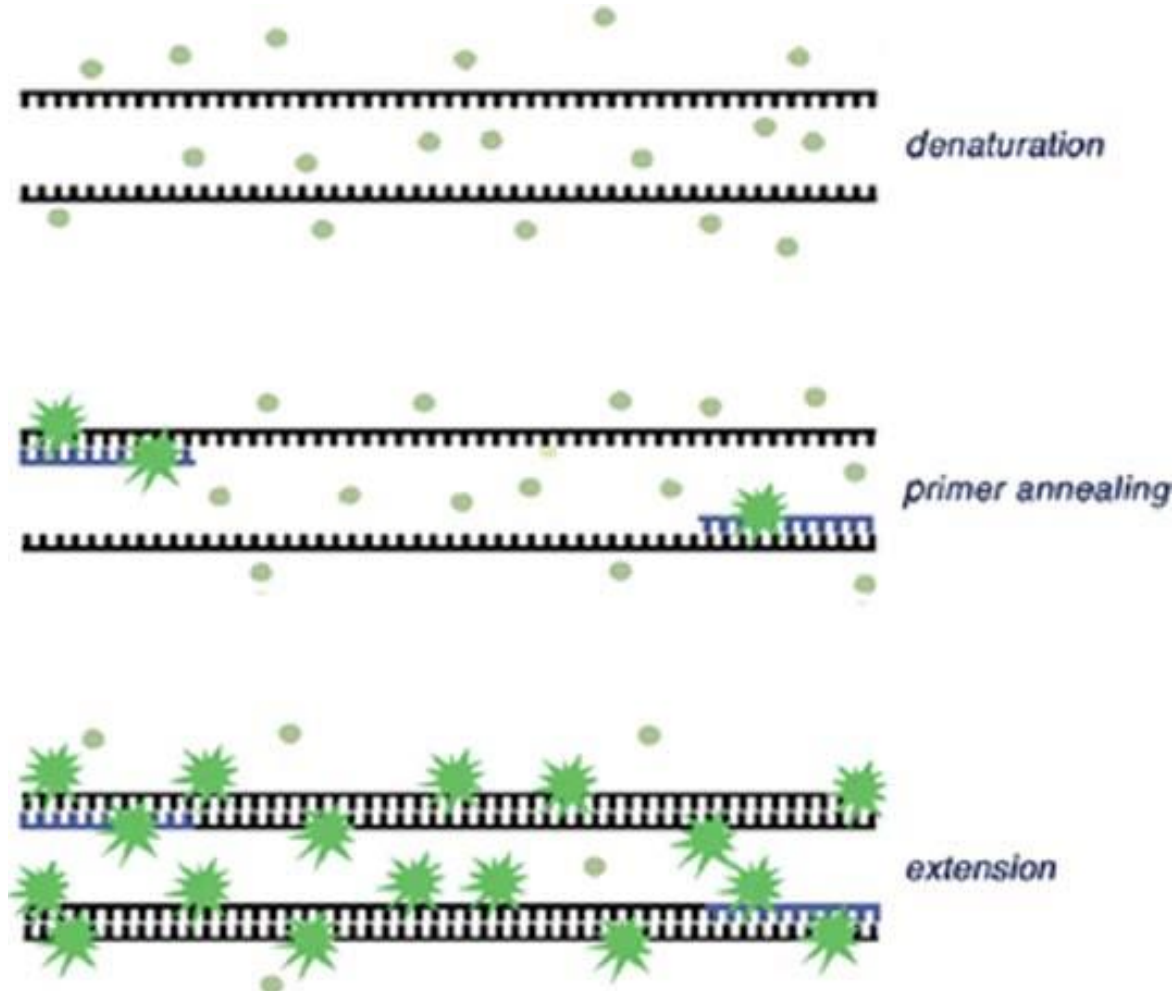
5' FLUOROPHORE	Abs [nm]	Em [nm]	3' QUENCHER
FAM [FAM]	495	520	TAM, BHQ1, DAB, Eclip
TET [TET]	521	536	TAM, BHQ1
JOE [JOE]	520	548	TAM, BHQ1, BHQ2
Yakima Yellow [YAKYE]	530	549	BHQ1, Eclip
HEX [HEX]	535	556	TAM, BHQ1, BHQ2, Eclip
Cyanine3 [CY3]	552	570	BHQ1, BHQ2, BBQ650
ATTO 550 [ATTO550]	554	576	TAM, BHQ2
TAMRA [TAM]	544	576	BHQ2
ROX [ROX]	575	602	TAM, BHQ2, BBQ650
Texas Red [TxRed]	583	603	BHQ2, BBQ650
Cyanine3.5 [CY35]	588	604	BHQ2
LightCycler 610 [LC610]	590	610	BHQ2
LightCycler 640 [LC640]	625	640	BHQ2, BBQ650
ATTO 647N [ATTO647N]	644	669	BHQ2, BBQ650
Cyanine5 [CY5]	649	670	BHQ2, BBQ650
Cyanine5.5 [CY55]	675	694	BHQ2, BBQ650
ATTO 680 [ATTO680]	680	700	BBQ650

Table of available dye-quencher combinations.

Rotor Gene 5 plex HRM		
Coulour	Source (nm)	Detector (nm)
Green	470	510
Yellow	530	555
Orange	585	610
370/510	530	510
370/555	530	555
Red	625	660
Crimson	680	710
HRM	460	510

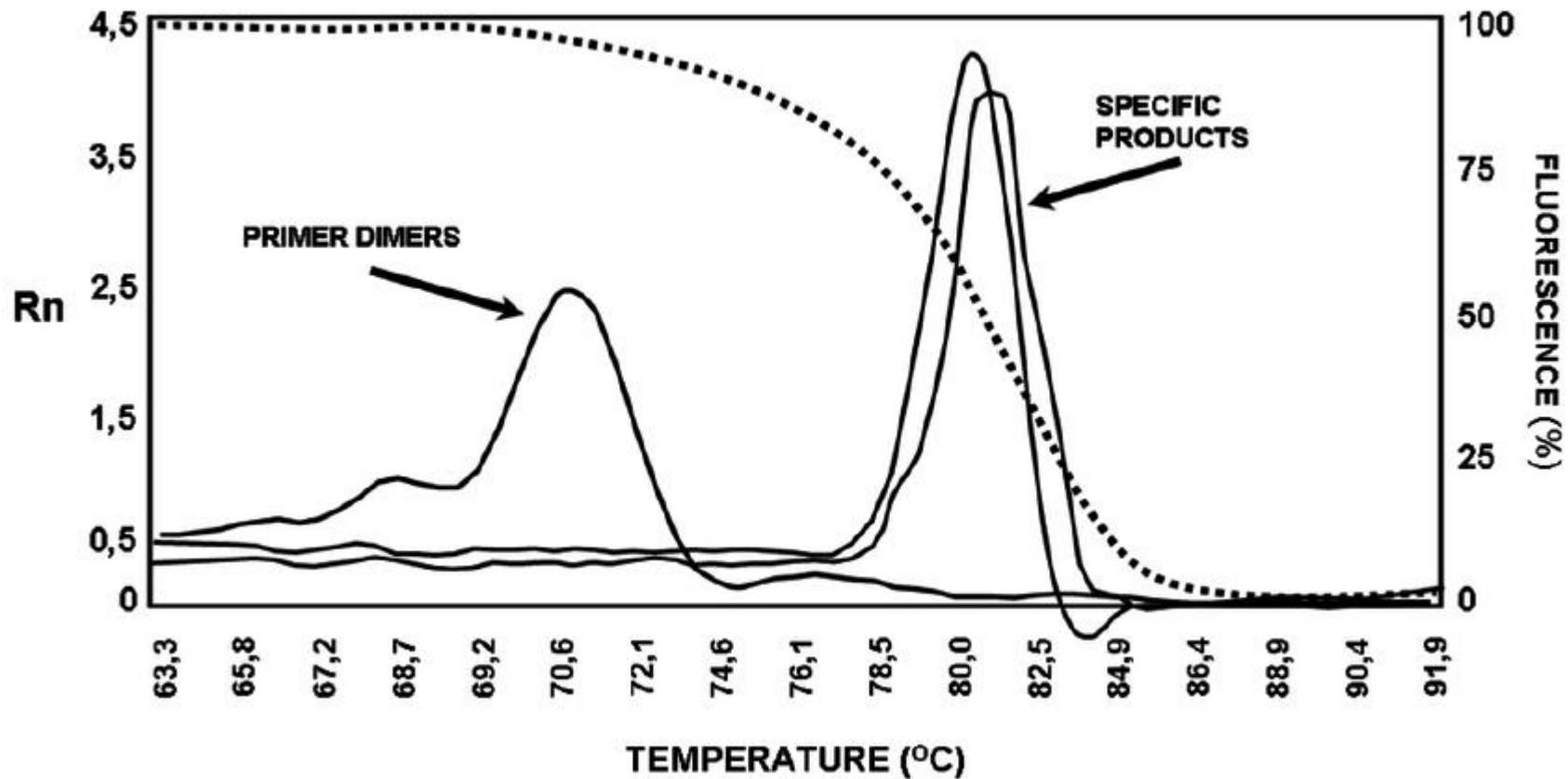


# SYBR Green system



# Melting curve

Temperature at which 50% of the DNA is denaturated



- Ensure high quality of nucleic acid
  - Please prevent inhibitors: excess of phenol, proteinase K, chelating agents, haemoglobin, SDS, salt
  - Please prevent contamination: amplicons and nucleases
- RNA – cannot be used as template for PCR
  - Synthesis of complementary DNA (cDNA)
  - RT-PCR = reverse transcriptase PCR

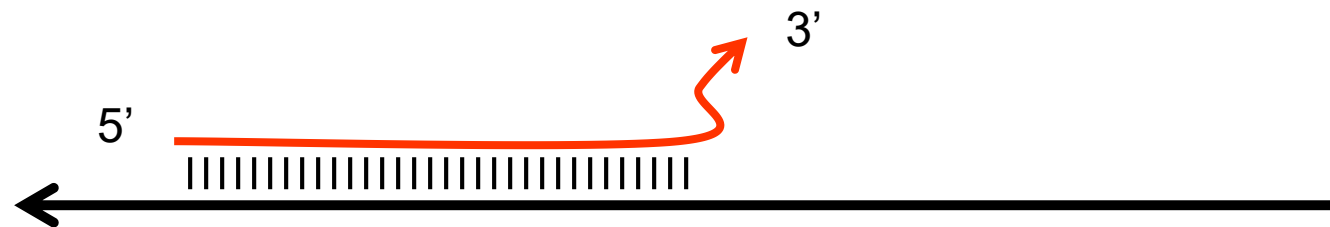
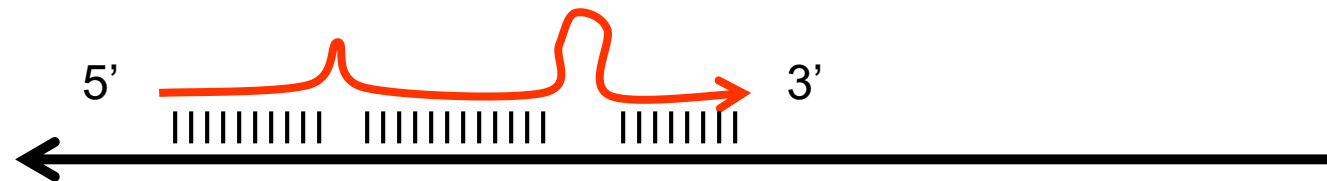
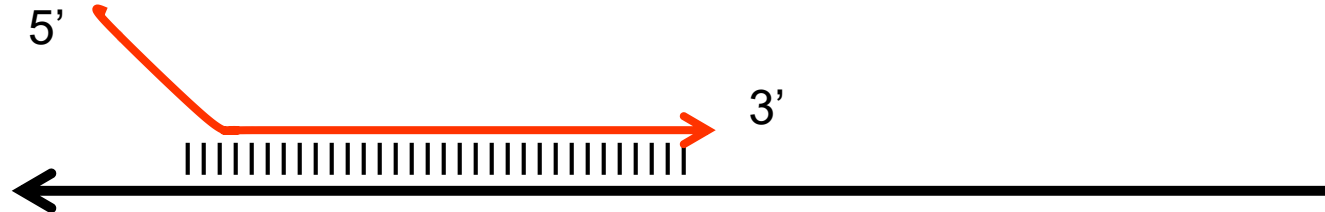
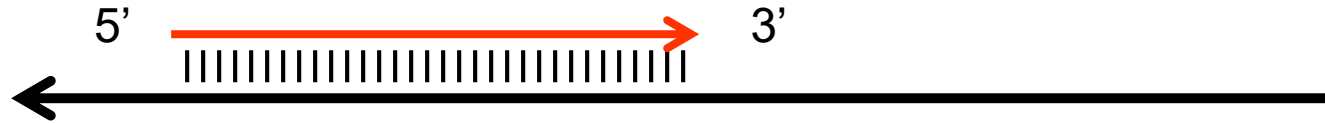
- Positive = sample known to have the targeted region
- Negative = sample known to NOT have the target region (Nuclease free water/blank)
- Spike-in = exogenous nucleic acid added in known amount into the tested samples and controls before extraction
- Housekeeping = constitutive gene that is always expressed in the tested organism

- Standards = serial diluted positive control
- Reverse transcriptase negative (RT-) = control of DNA contamination in RNA samples
- No template control (NTC)/blank = water nuclease free instead of the sample. MANDATORY!

# What about the oligos?

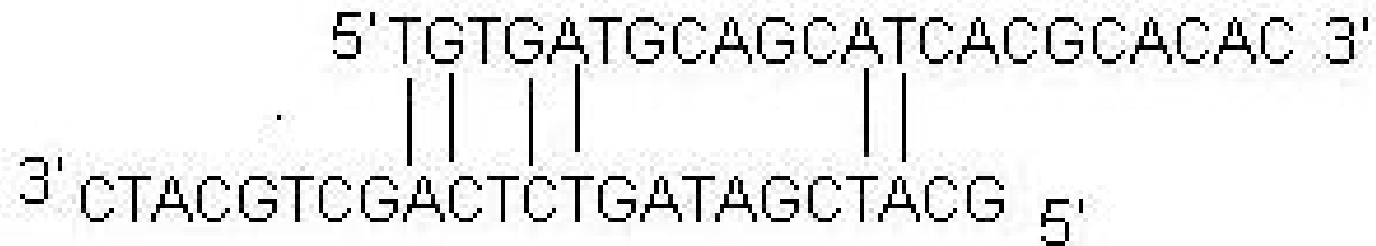
- Size ~ 20 base pairs (bp)
- C+G content ~ 50%
- 3' end should contain CG
- Similar annealing temperature ( $T_a$ )  
 $T_a = \text{Melting temperature } (T_m) - 5\text{ }^{\circ}\text{C}$   
 $T_m = [2 \times (T+A)] + [4 \times (C+G)]$

# What you want

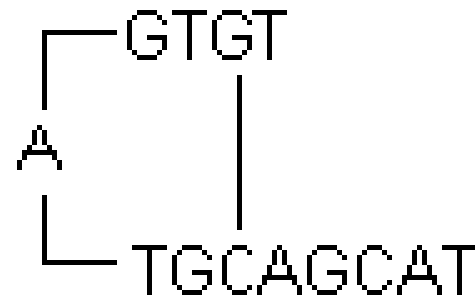


# What you want to avoid

- Primer dimer



- Primer hairpin





# Reverse complement

5' -GGATGGAACACTGGGGGGAGCCGATACCCAGGACAGGGCAGTCCTGGAGGCAACCGTTATCCACCTCAGG-3'  
3' -CCTCCGTTGGCAATAGGTGGAGT-5'  
5' -ATGGAACACTGGGGGGAGCC-3'  
3' -CCTACCTTGTGACCCCCCTCGGCTATGGGTCCTGTCCCGTCAGGACCTCCGTTGGCAATAGGTGGAGTCC-5'

*Primers (5' > 3')*

*Forward:* **ATGGAACACTGGGGGGAGCC**

*Reverse:* **TGAGGTGGATAACGGTTGCCTCC**

# How can I trust in the literature?

## Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China



Chaolin Huang\*, Yeming Wang\*, Xingwang Li\*, Lili Ren\*, Jianping Zhao\*, Yi Hu\*, Li Zhang, Guohui Fan, Jiuyang Xu, Xiaoying Gu, Zhenshun Cheng, Ting Yu, Jiaan Xia, Yuan Wei, Wenjuan Wu, Xuelei Xie, Wen Yin, Hui Li, Min Liu, Yan Xiao, Hong Gao, Li Guo, Jungang Xie, Guangfa Wang, Rongmeng Jiang, Zhancheng Gao, Qi Jin, Jianwei Wang†, Bin Cao†

### Procedures

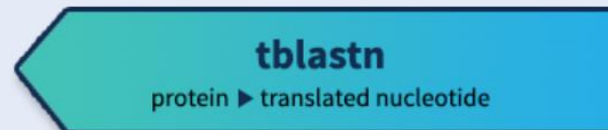
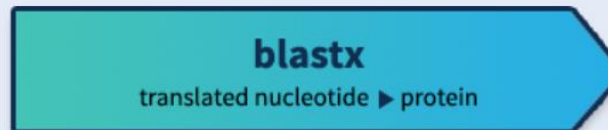
Local centres for disease control and prevention collected respiratory, blood, and faeces specimens, then shipped them to designated authoritative laboratories to detect the pathogen (NHC Key Laboratory of Systems Biology of Pathogens and Christophe Mérieux Laboratory, Beijing, China). A novel coronavirus, which was named 2019-nCoV, was isolated then from lower respiratory tract specimen and a diagnostic test for this virus was developed soon after that.<sup>14</sup> Of 59 suspected cases, 41 patients were confirmed to be infected with 2019-nCoV. The presence of 2019-nCoV in respiratory specimens was detected by next-generation sequencing or real-time RT-PCR methods. The primers and probe target to envelope gene of CoV were used and the sequences were as follows: forward primer 5'-TCAGAAATGCCAATCTCCCAAC-3'; reverse primer 5'-AAAGGTCCACCCGATACATTGA-3'; and the probe 5'-CY5-CTAGTTACACTAGCCATCCTTACTGC-3'-BHQ1. Conditions for the amplifications were 50°C for 15 min, 95°C for 3 min, followed by 45 cycles of 95°C for 15 s and 60°C for 30 s.

.....



- Basic Local Alignment Search Tool
  - Finds regions of similarity between biological samples

## Web BLAST



- **The higher the percent identity is, the more significant the match.**
- The percent identity is a number that describes how similar the query sequence is to the target sequence (how many characters in each sequence are identical).

- **The BLAST E-value is the number of expected hits of similar quality (score) that could be found just by chance.**
- E-value of 10 means that up to 10 hits can be expected to be found just by chance, given the same size of a random database.
- E-value can be used as a first quality filter for the BLAST search result

- **The higher the bit-score, the better the sequence similarity**
- The bit-score is the requires size of a sequence database in which the current match could be found just by chance.
- Bit-score does not depend on database size. The bit-score gives the same value for hits in databases of different sizes and hence can be used for searching in an constantly increasing database.



# Testing published primers/probe

## Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China



Chaolin Huang\*, Yeming Wang\*, Xingwang Li\*, Lili Ren\*, Jianping Zhao\*, Yi Hu\*, Li Zhang, Guohui Fan, Jiuyang Xu, Xiaoying Gu, Zhenshun Cheng, Ting Yu, Jiaan Xia, Yuan Wei, Wenjuan Wu, Xuelei Xie, Wen Yin, Hui Li, Min Liu, Yan Xiao, Hong Gao, Li Guo, Jungang Xie, Guangfa Wang, Rongmeng Jiang, Zhancheng Gao, Qi Jin, Jianwei Wang†, Bin Cao†

### Procedures

Local centres for disease control and prevention collected respiratory, blood, and faeces specimens, then shipped them to designated authoritative laboratories to detect the pathogen (NHC Key Laboratory of Systems Biology of Pathogens and Christophe Mérieux Laboratory, Beijing, China). A novel coronavirus, which was named 2019-nCoV, was isolated then from lower respiratory tract specimen and a diagnostic test for this virus was developed soon after that.<sup>14</sup> Of 59 suspected cases, 41 patients were confirmed to be infected with 2019-nCoV. The presence of 2019-nCoV in respiratory specimens was detected by next-generation sequencing or real-time RT-PCR methods. The primers and probe target to envelope gene of CoV were used and the sequences were as follows: forward primer

5'-TCAGAATGCCAATCTCCCAAC-3'; reverse primer 5'-AAAGGTCCACCCGATACATTGA-3'; and the probe 5'-CY5-CTAGTTACACTAGCCATCCTTACTGC-3'-BHQ1.

Conditions for the amplifications were 50°C for 15 min, 95°C for 3 min, followed by 45 cycles of 95°C for 15 s and 60°C for 30 s.

- Copy only the nucleotides
- Forward primer and probe are in sense direction
- Reverse primer is in reverse complementary direction



# Paste in query

← → ↻ 🔒 blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE\_TYPE=BlastSearch&LINK\_LOC=blasthome

NIH U.S. National Library of Medicine NCBI National Center for Biotechnology Information libarbara My NCBI Sign Out

**BLAST®** >> blastn suite **Make sure you are using blastn** Home Recent Results Saved Strategies Help

### Standard Nucleotide BLAST

blastn blastp blastx tblastn tblastx

BLASTN programs search nucleotide databases using a nucleotide query. [more...](#) [Reset page](#) [Bookmark](#)

**Enter Query Sequence**

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#) Query subrange [?](#)

TCAGAAATGCCAATCTCCCAAC

**Paste forward primer on query**

From

To

Or, upload file  No file chosen [?](#)

Job Title

Enter a descriptive title for your BLAST search [?](#)

☐ Align two or more sequences [?](#)

**Choose Search Set**

Database ☒ Standard databases (nr etc.): ☐ rRNA/ITS databases ☐ Genomic + transcript databases ☐ Betacoronavirus


Nucleotide collection (nr/nt) [?](#)

Organism [Optional](#)  ☐ exclude [+](#)

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown [?](#)

Exclude [Optional](#) ☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to [Optional](#) ☐ Sequences from type material

**BLAST results will be displayed in a new format by default**  
You can always switch back to the Traditional Results page. 

**Make sure you selected nucleotide collection**

**Make sure you selected the correct organism collection**

# Bottom of the query page

← → ↻ [blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) ★ 🌐 🏠 🗨️ 👤 ⋮

Or, upload file  No file chosen ⓘ Traditional Results page.

Job Title   
Enter a descriptive title for your BLAST search ⓘ

☐ Align two or more sequences ⓘ

**Choose Search Set**

**Database** ☒ Standard databases (nr etc.): ☐ rRNA/ITS databases ☐ Genomic + transcript databases ☐ Betacoronavirus  
 ⓘ

**Organism** Optional  
 ☐ exclude   
Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown ⓘ

**Exclude** Optional  
☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

**Limit to** Optional  
☐ Sequences from type material

**Entrez Query** Optional  
  
Enter an Entrez query to limit search ⓘ [YouTube](#) [Create custom database](#)

**Program Selection**

**Optimize for** ☒ Highly similar sequences (megablast)  
☐ More dissimilar sequences (discontiguous megablast)  
☐ Somewhat similar sequences (blastn)  
Choose a BLAST algorithm ⓘ

**BLAST** Search **database Nucleotide collection (nr/nt)** using **Megablast (Optimize for highly similar sequences)**  
☒ Show results in a new window

You may choose the default  
“Megablast” for this analysis

# Additional parameters

← → ↻ [blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) ★ G A | 👤 ⋮

**Algorithm parameters** [Restore default search parameters](#)

**General Parameters**

Max target sequences: 100 Select the maximum number of aligned sequences to display ?

Short queries: ☒ Automatically adjust parameters for short input sequences ?

Expect threshold: 10 ?

Word size: 28 ?

Max matches in a query range: 0 ?

**Scoring Parameters**

Match/Mismatch Scores: 1,-2 ?

Gap Costs: Linear ?

**Filters and Masking**


Filter: ☒ Low complexity regions ?  
☐ Species-specific repeats for: Homo sapiens (Human) ?

Mask: ☒ Mask for lookup table only ?  
☐ Mask lower case letters ?

**Blast it**

**BLAST** Search **database Nucleotide collection (nr/nt)** using **Megablast (Optimize for highly similar sequences)**  
☒ Show results in a new window

# Output - top page (1 of 3)

 U.S. National Library of Medicine  
National Center for Biotechnology Information


libarara

BLAST® » [blastn suite](#) » results for RID-4D27GKA6014

Home Recent Results Saved Strategies Help

[← Edit Search](#) [Save Search](#) [Search Summary ▾](#)

[? How to read this report?](#) [▶ BLAST Help Videos](#) [↶ Back to Traditional Results Page](#)

 Your search parameters were adjusted to search for a short input sequence.  
Your search is limited to records that include: Coronaviridae (taxid:11118)

Job Title	Nucleotide Sequence
RID	<a href="#">4D27GKA6014</a> Search expires on 02-15 18:48 pm <a href="#">Download All ▾</a>
Program	BLASTN <a href="#">?</a> <a href="#">Citation ▾</a>
Database	nt <a href="#">See details ▾</a>
Query ID	lcl Query_3059
Description	None
Molecule type	nucleic acid
Query Length	22
Other reports	<a href="#">Distance tree of results</a> <a href="#">MSA viewer</a> <a href="#">?</a>

Descriptions

Graphic Summary

Alignments

Taxonomy

Filter Results

Organism only top 20 will appear ☐ exclude

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity

to

E value


to

Query Coverage

to

Filter

Reset

 Feedback

# Descriptions tab (2 of 3)

blast.ncbi.nlm.nih.gov/Blast.cgi

Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments Download Manage Columns Show 100 ?

☐ select all 0 sequences selected

GenBank Graphics Distance tree of results

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input type="checkbox"/>	Porcine epidemic diarrhea virus strain AJ1102(F12), complete genome	26.3	50.5	68%	9.5	100.00%	<a href="#">MK584552.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate GDS09, complete genome	26.3	70.9	68%	9.5	100.00%	<a href="#">MH726408.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate GDS15, complete genome	26.3	50.5	68%	9.5	100.00%	<a href="#">MH726391.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate GDS40, complete genome	26.3	50.5	68%	9.5	100.00%	<a href="#">MH726390.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate GDS39, complete genome	26.3	50.5	68%	9.5	100.00%	<a href="#">MH726389.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate GDS36, complete genome	26.3	50.5	68%	9.5	100.00%	<a href="#">MH726388.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate GDS35, complete genome	26.3	50.5	68%	9.5	100.00%	<a href="#">MH726387.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate GDS34, complete genome	26.3	50.5	68%	9.5	100.00%	<a href="#">MH726386.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate GDS33, complete genome	26.3	50.5	68%	9.5	100.00%	<a href="#">MH726385.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate GDS32, complete genome	26.3	50.5	68%	9.5	100.00%	<a href="#">MH726384.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate GDS31, complete genome	26.3	70.9	68%	9.5	100.00%	<a href="#">MK796238.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate GDS30, complete genome	26.3	50.5	68%	9.5	100.00%	<a href="#">MK392335.1</a>
<input type="checkbox"/>	Porcine epidemic diarrhea virus isolate F.I2011, complete genome	26.3	50.5	68%	9.5	100.00%	<a href="#">MH708895.1</a>

Sort by description

**Observe:**

- Do the organisms belong to the same taxonomical clade as your query?
- Ideal identity should be ~ 100%
- Ideal e-value should be < 1

# Alignments tab (3 of 3)

blast.ncbi.nlm.nih.gov/Blast.cgi

Descriptions Graphic Summary **Alignments** Taxonomy

Alignment view Pairwise ☐ CDS feature ?

100 sequences selected ?

New Designing or Testing PCR Primers? Try your search in Primer-BLAST

Download v GenBank Graphics Sort by: E value

**Porcine epidemic diarrhea virus strain AJ1102(F12), complete genome**  
Sequence ID: [MK584552.1](#) Length: 28044 Number of Matches: 2

Range 1: 1227 to 1239 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
26.3 bits(13)	9.5	13/13(100%)	0/13(0%)	Plus/Minus
Query 2	CAGAATGCCAATC	14		
Sbjct 1239	CAGAATGCCAATC	1227		

Range 2: 20907 to 20918 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
24.3 bits(12)	37	12/12(100%)	0/12(0%)	Plus/Minus
Query 5	AATGCCAATCTC	16		
Sbjct 20918	AATGCCAATCTC	20907		

## Observe:

- Although the identity is 100%, not the entire primer is aligned to the output sequences
- E-value is too high

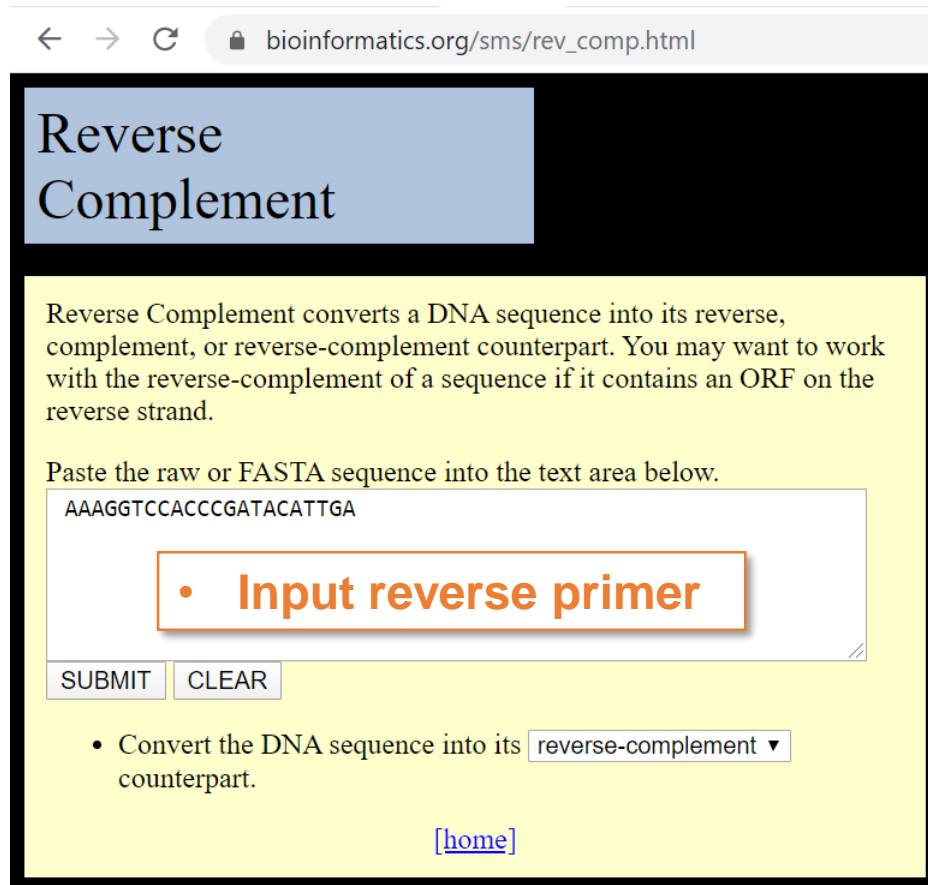
## Conclusions:

- This primer is poor because it is not specific to the species analysed (COVID-19), although it aligns to other coronavirus (Pan-coronavirus primer)
- It is specifically poor to be able to align to as many species from the clade as possible
- It can generate unespecific PCR products



# Reverse primer

- Must be converted to “reverse complementary” before searching it on the query.



Reverse Complement

Reverse Complement converts a DNA sequence into its reverse, complement, or reverse-complement counterpart. You may want to work with the reverse-complement of a sequence if it contains an ORF on the reverse strand.

Paste the raw or FASTA sequence into the text area below.

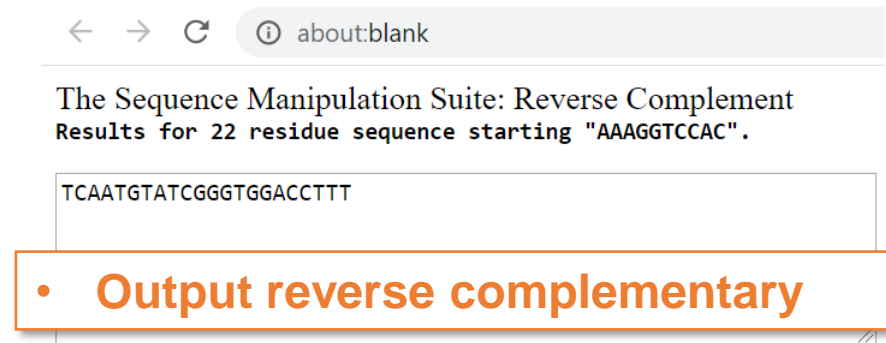
AAAGGTCCACCCGATACATTGA

- **Input reverse primer**

SUBMIT CLEAR

• Convert the DNA sequence into its reverse-complement ▼ counterpart.

[\[home\]](#)



← → ↻ ⓘ about:blank

The Sequence Manipulation Suite: Reverse Complement  
Results for 22 residue sequence starting "AAAGGTCCAC".

TCAATGTATCGGGTGGACCTTT

- **Output reverse complementary**

- **Copy/paste the reverse primer and “submit” it**
- **Copy the output and paste on the blastn query like for the forward primer**
- **Repeat the analyses**

# Reverse primer output

blast.ncbi.nlm.nih.gov/Blast.cgi

Descriptions Graphic Summary **Alignments** Taxonomy

Alignment view Pairwise ☐ CDS feature [?](#) Download [v](#)

100 sequences selected [?](#)

**New** Designing or Testing PCR Primers? Try your search in **Primer-BLAST**. [Go](#)

---

[Download](#) [v](#) [GenBank](#) [Graphics](#) [v](#) Next [Previous](#) [Descriptions](#)

**Infectious bronchitis virus partial S1 gene for spike 1 protein, genomic RNA, strain NGA/324/2006**

Sequence ID: [FN182277.1](#) Length: 1614 Number of Matches: 1

Range 1: 576 to 588 [GenBank](#) [Graphics](#) [v](#) Next Match [Previous](#)

Score	Expect	Identities	Gaps	Strand
26.3 bits(13)	9.5	13/13(100%)	0/13(0%)	Plus/Plus

Query 8 ATCGGGTGGACCT 20  
Sbjct 576 ATCGGGTGGACCT 588

---

[Download](#) [v](#) [GenBank](#) [Graphics](#) [v](#) Next [Previous](#) [Descriptions](#)

**Canine coronavirus genomic RNA, 3' end side sequence**

Sequence ID: [D13096.1](#) Length: 9580 Number of Matches: 1

## Observe:

- Similar “poor” results as for the forward primer

## Conclusions:

- Pan-coronavirus primer



# Repeat the process using the probe

blast.ncbi.nlm.nih.gov/Blast.cgi

Descriptions Graphic Summary **Alignments** Taxonomy

Alignment view Pairwise ☐ CDS feature ? Download

100 sequences selected ?

**New** Designing or Testing PCR Primers? Try your search in Primer3

Download GenBank Graphics Sort by: E value

**Wuhan seafood market pneumonia virus isolate 2019-nCoV/USA-AZ1/2020, complete genome**  
Sequence ID: [MN997409.1](#) Length: 29882 Number of Matches: 2

Range 1: 26326 to 26351 [GenBank](#) [Graphics](#) [Next Match](#)

Score	Expect	Identities	Gaps	Strand
52.0 bits(26)	3e-07	26/26(100%)	0/26(0%)	Plus/Plus
Query 1	CTAGTTACACTAGCCATCCTTACTGC	26		
Sbjct 26326	CTAGTTACACTAGCCATCCTTACTGC	26351		

Range 2: 25875 to 25884 [GenBank](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
20.3 bits(10)	918	10/10(100%)	0/10(0%)	Plus/Minus
Query 3	AGTTACACTA	12		
Sbjct 25884	AGTTACACTA	25875		

[Feedback](#)

## Observe:

- The whole probe is aligned to the COVID-19 sequence, which identity is 100%
- E-value is quite low

## Conclusions:

- The probe is specific to COVID-19
- The set of primers detect coronavirus in general, while the probe confers specificity to detect COVID-19

# Designing your own primers/probes

- Use primer-BLAST or any other designing tool
- Always use a Reference Sequence (RefSeq) as template to desing your oligos
- Use GenBank tools to get RefSeqs
- Double-check your designed oligos testing them using the same procedures as described previously

# Search for sequences on the nucleotide database

← → ↺ ncbi.nlm.nih.gov/nuccore

NCBI Resources How To libarbara My NCBI Sign Out

Nucleotide Nucleotide

Wuhan seafood market pneumonia virus

Wuhan seafood market pneumonia virus orf1ab

Wuhan seafood market pneumonia virus S

Wuhan seafood market pneumonia virus 2'-O-ribose methyltransferase

Wuhan seafood market pneumonia virus 3'-to-5' exonuclease

Wuhan seafood market pneumonia virus 3C-like proteinase

Wuhan seafood market pneumonia virus RNA-dependent RNA polymerase

Wuhan seafood market pneumonia virus endoRNase

Wuhan seafood market pneumonia virus helicase

Wuhan seafood market pneumonia virus leader protein

Wuhan seafood market pneumonia virus nsp10

Wuhan seafood market pneumonia virus nsp11

Wuhan seafood market pneumonia virus nsp2

Wuhan seafood market pneumonia virus nsp3

Wuhan seafood market pneumonia virus nsp4

Wuhan seafood market pneumonia virus nsp6

Wuhan seafood market pneumonia virus nsp7

Wuhan seafood market pneumonia virus nsp8

Wuhan seafood market pneumonia virus nsp9

Wuhan seafood market pneumonia virus orf1a polyprotein;orf1ab polyprotein

Wuhan seafood market pneumonia virus genome

Search

Help

GenBank, RefSeq, TPA and research and discovery.

Using Nucleotide

[Quick Start Guide](#)

[FAQ](#)

[Help](#)

[GenBank FTP](#)

[RefSeq FTP](#)

You are here: NCBI > DNA & RNA > Nucleotide Database

Support Center

# Select preferably RefSeq sequences



ncbi.nlm.nih.gov/nuccore/?term=Wuhan+seafood+market+pneumonia+virus

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Nucleotide Nucleotide Wuhan seafood market pneumonia virus Search

Create alert Advanced Help

Species Viruses (79) Customize ...

Molecule types genomic DNA/RNA (79) Customize ...

Source databases INSDC (GenBank) (78) RefSeq (1) Customize ...

Sequence Type Nucleotide (79)

Sequence length Custom range...

Release date Custom range...

Revision date Custom range...

Clear all Show additional filters

Summary 20 per page Sort by Default order

Send to: Filter your results:

All (79)

Bacteria (0)

INSDC (GenBank) (78)

mRNA (0)

ncRNA (0)

RefSeq (1) **CLICK**

rRNA (0)

Manage Filters

GENOME ASSEMBLY Was this helpful?

Severe acute respiratory syndrome coronavirus 2 genome

Severe acute respiratory syndrome coronavirus 2 (Host: human,vertebrates)

ssRNA(+)

RefSeq GCF\_009858895

RefSeq genomic segment

5'

polyprotein

polyprotein

S

E

ORF6

ORF10

M

ORF7b

ORF7a

ORF8

NCBI Virus BLAST Download

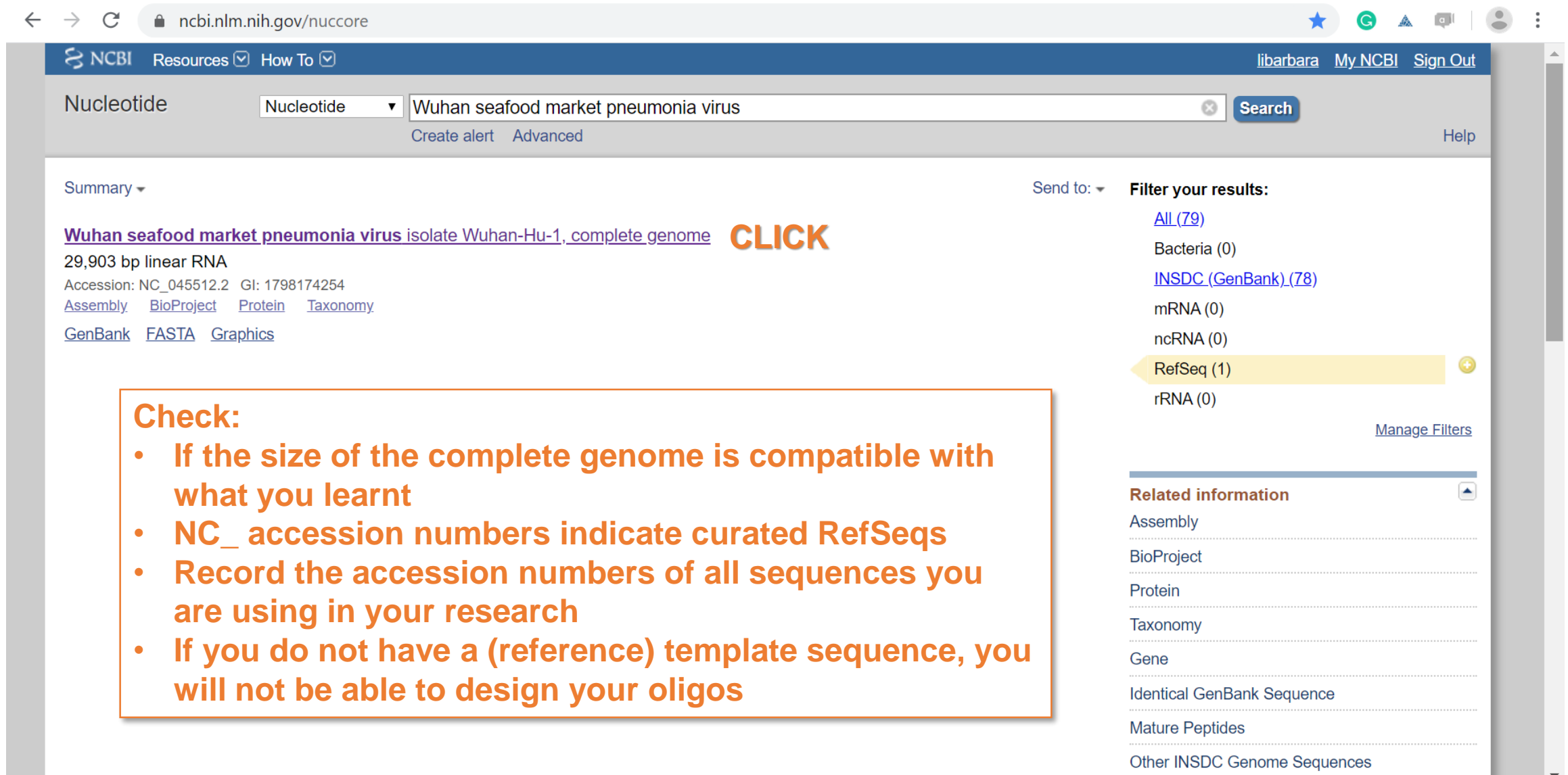
**RefSeqs are curated whole genome sequences and can be trusted.**

**In the absence of a RefSeq, chose a “complete genome sequence” from a reliable publication.**

Search details

"Wuhan seafood market pneumonia virus"  
[Organism] OR Wuhan seafood market pneumonia virus[All Fields]

# Check the information you know



The screenshot shows the NCBI Nucleotide search results for the query 'Wuhan seafood market pneumonia virus'. The search results page includes a summary section with the following information:

- Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1, complete genome** **CLICK**
- 29,903 bp linear RNA
- Accession: NC\_045512.2 GI: 1798174254
- Assembly BioProject Protein Taxonomy
- GenBank FASTA Graphics

On the right side of the page, there is a 'Filter your results:' section with the following options:

- All (79)
- Bacteria (0)
- INSDC (GenBank) (78)
- mRNA (0)
- ncRNA (0)
- RefSeq (1)
- rRNA (0)

Below the filters, there is a 'Related information' section with the following links:

- Assembly
- BioProject
- Protein
- Taxonomy
- Gene
- Identical GenBank Sequence
- Mature Peptides
- Other INSDC Genome Sequences

**Check:**

- If the size of the complete genome is compatible with what you learnt
- NC\_ accession numbers indicate curated RefSeqs
- Record the accession numbers of all sequences you are using in your research
- If you do not have a (reference) template sequence, you will not be able to design your oligos

# Learn more from the organism genome



← → ↻ [ncbi.nlm.nih.gov/nuccore/NC\\_045512.2](https://ncbi.nlm.nih.gov/nuccore/NC_045512.2) ★

NCBI Resources ▾ How To ▾ [libarbara](#) [My NCBI](#) PhishProtect Beta Has access to this site

Nucleotide   [Advanced](#) [Help](#)

GenBank ▾ [Send to: ▾](#) [Change region shown ▾](#)

## Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1, complete genome

NCBI Reference Sequence: NC\_045512.2  
[FASTA](#) [Graphics](#)

[Go to: ▾](#)

LOCUS	NC_045512	29903 bp ss-RNA	linear	VRL 28-JAN-2020
DEFINITION	Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1, complete genome.			
ACCESSION	NC_045512			
VERSION	NC_045512.2			
DBLINK	BioProject: <a href="#">PRJNA485481</a>			
KEYWORDS	RefSeq.			
SOURCE	Wuhan seafood market pneumonia virus			
ORGANISM	<a href="#">Wuhan seafood market pneumonia virus</a> Viruses; Riboviria; Nidovirales; Coronaviridae; Orthocoronavirinae; Betacoronavirus; unclassified Betacoronavirus.			
REFERENCE	1 (bases 1 to 29903)			
AUTHORS	Wu,F., Zhao,S., Yu,B., Chen,Y.-M., Wang,W., Hu,Y., Song,Z.-G., Tao,Z.-W., Tian,J.-H., Pei,Y.-Y., Yuan,M.L., Zhang,Y.-L., Dai,F.-H., Liu,Y., Wang,Q.-M., Zheng,J.-J., Xu,L., Holmes,E.C. and Zhang,Y.-Z.			
TITLE	A novel coronavirus associated with a respiratory disease in Wuhan of Hubei province, China			
JOURNAL	Unpublished			
REFERENCE	2 (bases 1 to 29903)			

**Customize view**

**Basic Features**

- ☒ All features
- ☐ Gene, RNA, and CDS features only

**Display options**

- ☒ Show sequence
- ☐ Show reverse complement

[Update View](#)

**SARS Coronavirus Resource**

Retrieve, view, and download SARS coronavirus genomic and protein sequences.

Make sure you selected “all features” to explore all annotated genes and other information from the RefSeq genome

# Primer-BLAST query (1 of 2)

ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK\_LOC=BlastHome

NIH U.S. National Library of Medicine NCBI National Center for Biotechnology Information libarbara My NCBI Sign Out

## Primer-BLAST

A tool for finding specific primers

Finding primers specific to your PCR template (using Primer3 and BLAST).

[Reset page](#) [Save search parameters](#) [Retrieve recent results](#) [Publication](#) [Tips for finding specific primers](#)

### PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) [Clear](#)

NC\_045512.2

Or, upload FASTA file  No file chosen

Range

Forward primer  From  To  [Clear](#)

Reverse primer

### Primer Parameters

Use my own forward primer (5'->3' on plus strand)  [Clear](#)

Use my own reverse primer (5'->3' on minus strand)  [Clear](#)

PCR product size

Min	Max
<input type="text" value="70"/>	<input type="text" value="250"/>

# of primers to return

Primer melting temperatures (T<sub>m</sub>)

Min	Opt	Max	Max T <sub>m</sub> difference
<input type="text" value="57.0"/>	<input type="text" value="60.0"/>	<input type="text" value="63.0"/>	<input type="text" value="3"/>

- Copy/paste the RefSeq accession number into the query on primer-blast page
- You can change the settings according to your choices
- Use “?” to learn more about the parameters

# Primer-BLAST query (2 of 2)

Note: Parameter values that differ from the default are highlighted in yellow

### Primer Pair Specificity Checking Parameters

**Specificity check** ☒ Enable search for primer pairs specific to the intended PCR template

**Search mode** Automatic

**Database** nr

**Exclusion** ☐ Exclude predicted Refseq transcripts (accession with XM, XR prefix) ☐ Exclude uncultured/environmental sample sequences

**Organism** Coronaviridae (taxid:11118)  
Enter an organism name (or organism group name such as enterobacteriaceae, rodents), taxonomy id or select from the suggestion list as you type.  
[Add more organisms](#)

**Entrez query (optional)**

**Primer specificity stringency** Primer must have at least 2 total mismatches to unintended targets, including at least 2 mismatches within the last 5 bps at the 3' end.  
Ignore targets that have 6 or more mismatches to the primer.

**Max target size** 4000

**Allow splice variants** ☐ Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)

- Make sure you selected the “nr” database and the correct organism taxid

### Internal hybridization oligo parameters

**Hybridization oligo** ☒ Pick internal hybridization oligo

	Min	Opt	Max
<b>Hyb Oligo Size</b>	18	20	27
<b>Hyb Oligo tm</b>	57.0	60.0	63.0
<b>Hyb Oligo GC%</b>	20.0	50	80.0

**Get Primers** ☒ Show results in a new window ☒ Use new graphic view

- To design probes, select “hybridization oligo” on advanced parameters at the bottom of the page



# Additional information

ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi

NIH U.S. National Library of Medicine NCBI National Center for Biotechnology Information libarbara My NCBI Sign Out

## Primer-BLAST

A tool for finding specific primers

Finding primers specific to your PCR template (using Primer3 and BLAST)

**Input PCR template** [NC\\_045512.2](#) Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1, complete genome  
**Range** 1 - 29903

Your PCR template is highly similar to the following sequence(s) from the search database. To increase the chance of finding specific primers, please review the list below and select all sequences (within the given sequence ranges) that are intended or allowed targets.

Select: [All](#) [None](#) Selected:2

Accession	Title	Identity	Alignment length	Seq. start	Seq. stop
<input checked="" type="checkbox"/> <a href="#">MN988669.1</a>	Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV WHU02, complete genome	100%	29881	1	29881
<input checked="" type="checkbox"/> <a href="#">MN988668.1</a>	Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV WHU01, complete genome	100%	29881	1	29881
<input type="checkbox"/> <a href="#">MN994468.1</a>	Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-CA2/2020, complete genome	99.99%	29883	1	29883
<input type="checkbox"/> <a href="#">MN975262.1</a>	Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV_HKU-SZ-005b_2020, complete genome	99.98%	29891	1	29891
<input type="checkbox"/> <a href="#">MN985325.1</a>	Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-WA1/2020, complete genome	99.99%	29882	1	29882
<input type="checkbox"/> <a href="#">MN997409.1</a>	Severe acute respiratory syndrome corona			1	29882
<input type="checkbox"/> <a href="#">MN988713.1</a>	Severe acute respiratory syndrome corona			1	29882
<input type="checkbox"/> <a href="#">MN994467.1</a>	Severe acute respiratory syndrome corona			1	29882
<input type="checkbox"/> <a href="#">MN938384.1</a>	Severe acute respiratory syndrome corona			1	29838

**In some cases primer-BLAST may ask you to confirm additional information to narrow down the analyses**

☒ Show results in a new window

# Predicted oligos output (1 of 2)

← → ↻ [ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi?ctg\\_time=1581693560&job\\_key=4ug945cj mou9sQq0B9Quhn3PP7RQ3CSpUQ](https://ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi?ctg_time=1581693560&job_key=4ug945cj mou9sQq0B9Quhn3PP7RQ3CSpUQ) ★ G ▲ 🗨 👤 ⋮

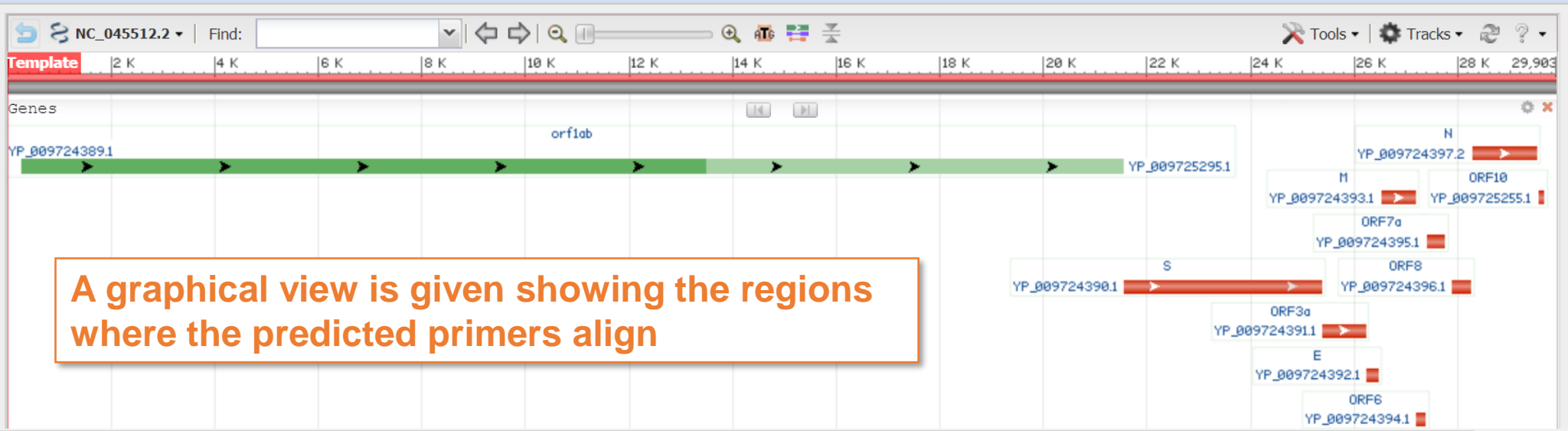
**NIH** U.S. National Library of Medicine **NCBI** National Center for Biotechnology Information libarbara My NCBI Sign Out

**Primer-BLAST** >> JOB ID:4ug945cj mou9sQq0B9Quhn3PP7RQ3CSpUQ

Primer-BLAST Results ⓘ

**Input PCR template** [NC\\_045512.2](#) Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1, complete genome  
**Range** 1 - 29903  
**Specificity of primers** Primers may **not** be specific to the input PCR template as targets were found in selected database:Nucleotide collection (nt) (Organism limited to Coronaviridae)...[help on specific primers](#)  
**Other reports** ▶ [Search Summary](#)

**Graphical view of primer pairs**



A graphical view is given showing the regions where the predicted primers align

# Predicted oligos output (2 of 2)

ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi?ctg\_time=1581693560&job\_key=4ug945cj mou9sQq0B9Quhn3PP7RQ3CSpUQ



## Detailed primer reports

You can re-search for specific primers by accepting some of the unintended targets, check the box(es) next to the ones you accept and try again to re-search for specific primers

### Primer pair 1

	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ACCTTCCCAGGTAACAAACCA	Plus	21	14	34	59.15	47.62	5.00	0.00
Reverse primer	ACTCGTGCCTGTCAACGAC	Minus	20	168	149	59.97	55.00	4.00	3.00
Internal oligo	GTGGCTGTCACTCGGCTGCA	Plus	20	88	107	59.63	65.00		
Product length	155								

### Products on intended targets

>[MN988669.1](#) Wuhan seafood market pneumonia virus isolate 2019-nCoV WHU019/2019

product length = 155

Forward primer 1 ACCTTCCCAGGTAACAAACCA 21  
Template 13 .....

Reverse primer 1 ACTCGTGCCTGTCAACGAC 20  
Template 167 .....

>[MN988668.1](#) Wuhan seafood market pneumonia virus isolate 2019-nCoV WHU019/2019

product length = 155

Forward primer 1 ACCTTCCCAGGTAACAAACCA 21  
Template 13 .....

Reverse primer 1 ACTCGTGCCTGTCAACGAC 20  
Template 167 .....

A few oligo set options will be given. Chose the best result.

A good oligo set should have:

- GC% (GC content) > 50%
- Similar Tm (melting temperature) for all oligos in the set
- Self complementarity score near 0.00

# Your new best friends

- ViralZone <https://viralzone.expasy.org/>
- International Committee on Taxonomy of Viruses <https://talk.ictvonline.org/taxonomy/>
- Pubmed <https://www.ncbi.nlm.nih.gov/pubmed/>
- GenBank <https://www.ncbi.nlm.nih.gov/genbank/>
- Blast <https://blast.ncbi.nlm.nih.gov/Blast.cgi>