

ONE KIT ONE PRODUCT

WORKSHOP

Primer & Probe Design

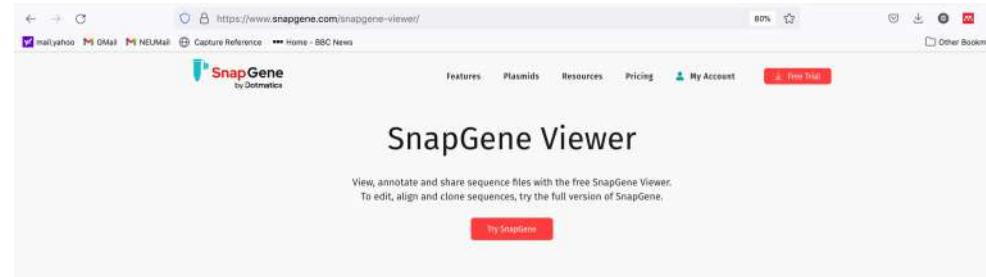
Dr. Gülten Tuncel, *PhD*

Polymerase Chain Reaction (PCR)

Workshop: Primer Design

- 1988YDU1988 wifi password
- Please download **SnapGene Viewer**

<https://www.snapgene.com/snapgene-viewer/>



Gain Unparalleled Visibility of Your Plasmids

- Identify pathogen of interest
 - Understand its genomic structure
 - Isolate genomic material
- Design kit for;
Detection of viral genome in the biological samples.

- 1- Download data from databases
- 2- Design primer and probes for PCR
- 3- Kit optimization
- 4- Use in routine medical laboratories

- Find your target reference sequence from reliable databases

GISAID (Global Initiative on Sharing Avian Influenza Data) – needs registration with an institutional account

NCBI database (The National Center for Biotechnology Information) – publicly available

The GISAID website features a large phylogenetic tree centered on the COVID-19 virus. Below the tree are several links to analytical tools: Audacity, Audacity Instant, BLAST, Covizid, Emerging Variants, Official GISAID reference sequence, PrimerChecker, Spike glycoprotein mutation surveillance, and Wastewater. At the bottom, there is an "Analysis Update (2022-07-19)" section and a legal note about the Database Access Agreement.

The NCBI homepage includes a "Welcome to NCBI" section, a sidebar with "Popular Resources" like PubMed and BLAST, and a "COVID-19 Information" section. The main area features sections for "Submit", "Download", "Learn", "Develop", "Analyze", and "Research". Each section has a brief description and an icon. On the right, there are news items and a "More..." link.

Search NCBI

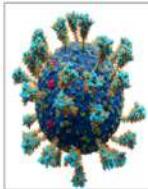
severe acute respiratory syndrome coronavirus 2



Search

Results found in 27 databases

TAXONOMY



Severe acute respiratory syndrome coronavirus 2

Severe acute respiratory syndrome coronavirus 2 is a below-species classification of Severe acute respiratory syndrome-related coronavirus

Taxonomy ID: 2697049

Was this helpful?



LitCovid

A curated literature hub for the latest scientific information on COVID-19

In NCBI homepage

Search for;

' severe acute respiratory syndrome coronavirus 2'



NCBI Virus

Browse and download

Literature

Bookshelf

1,146

MeSH

26

NLM Catalog

157

PubMed

173,294

Genes

Gene

37

GEO DataSets

1,159

GEO Profiles

0

HomoloGene

0

Proteins

Conserved Domains

8

Identical Protein Groups

3,449,480

Protein

32,124,147

Protein Family Models

5

Search NCBI

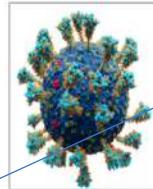
severe acute respiratory syndrome coronavirus 2



Search

Results found in 27 databases

TAXONOMY



[Severe acute respiratory syndrome coronavirus 2](#)

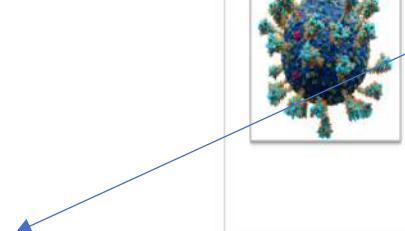
Severe acute respiratory syndrome coronavirus 2 is a below-species classification of Severe acute respiratory syndrome-related coronavirus

Taxonomy ID: 2697049

Was this helpful?



Click



[NCBI SARS-CoV-2 resources](#)



NCBI Virus

Browse and download

Literature

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NLM Catalog

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GEO DataSets

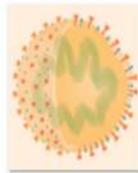
1,159

GEO Profiles

0

HomoloGene

0



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A curated literature hub for the latest scientific information on COVID-19

Proteins

Conserved Domains

8

Identical Protein Groups

3,449,480

Protein

32,124,147

Protein Family Models

5

Severe acute respiratory syndrome coronavirus 2 ★

Severe acute respiratory syndrome coronavirus 2 is a below-species classification of *Severe acute respiratory syndrome-related coronavirus*

[Browse taxonomy](#)

Current scientific name *Severe acute respiratory syndrome coronavirus 2*

Acronym SARS-CoV-2

Genome type ssRNA(+)

NCBI Taxonomy ID 2697049

For more details see [NCBI Taxonomy](#)

Genome

[Browse all genomes in NCBI Virus](#)

Reference genome ASM985889v3

Jan 13, 2020

RefSeq GCF_00985889.2

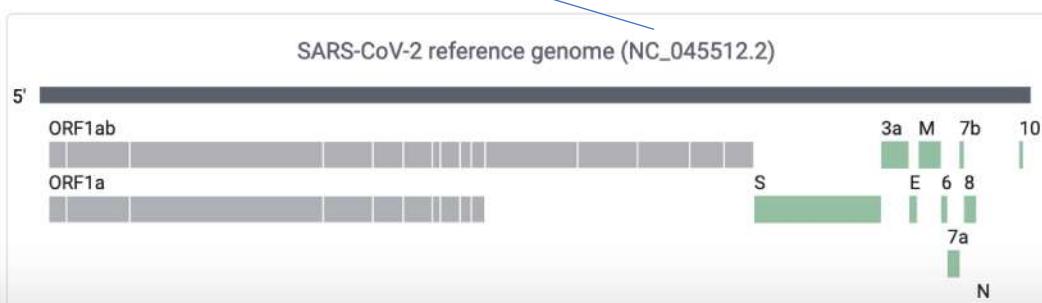
[Download](#)

Genome size 29.9 kb

Viral segments 1

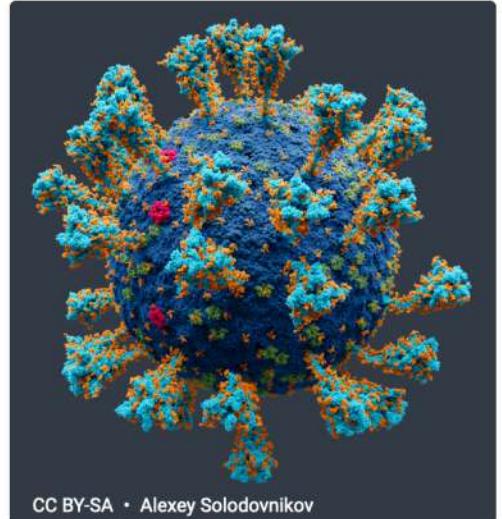
Genes 11

Annotation from NCBI RefSeq



We can obtain several information about the virus from the database.

We are interested in the reference genome sequence.



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Image may not have been verified for accuracy by NCBI Taxonomy.

External links

[Encyclopedia of Life](#)

[Wikipedia](#)

- NCBI Reference Sequence of SARS-CoV-2 Wuhan Isolate: **NC_045512.2**



Accession numbers:

NC_ : complete genomic molecule

NG_ : genomic sequence

NM_ : mRNA sequence

NP_ : protein sequence

An official website of the United States government [Here's how you know](#)

National Library of Medicine

National Center for Biotechnology Information

All Databases

Welcome to NCBI

The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.

[About the NCBI](#) | [Mission](#) | [Organization](#) | [NCBI News & Blog](#)

Submit
Deposit data or manuscripts into NCBI databases


Download
Transfer NCBI data to your computer


Learn
Find help documents, attend a class or watch a tutorial


Develop
Use NCBI APIs and code libraries to build applications


Analyze
Identify an NCBI tool for your data analysis task


Research
Explore NCBI research and collaborative projects


Popular Resources

- PubMed
- Bookshelf
- PubMed Central
- BLAST
- Nucleotide
- Genome
- SNP
- Gene
- Protein
- PubChem

NCBI News & Blog

RefSeq Release 213 18 Jul 2022
RefSeq Release 213 is now available online, from the FTP site and through

Try out the latest BLAST ClusteredNR database results. Now with in-cluster analyses! 14 Jul 2022

NLM's all-new NCBI Datasets genome table is now available 13 Jul 2022
We are excited to introduce new and

[More...](#)

COVID-19 Information

[Public health information \(CDC\)](#) | [Research information \(NIH\)](#) | [SARS-CoV-2 data \(NCBI\)](#) | [Prevention and treatment information \(HHS\)](#) | [Español](#)

Paste the accession number here
(SARS-CoV-2: NC_045512.2)

https://www.ncbi.nlm.nih.gov/nuccore/NC_045512.2/

An official website of the United States government [Here's how you know](#)

National Library of Medicine
National Center for Biotechnology Information

Log in

Nucleotide Nucleotide Advanced Search Help

GenBank Send to: Change region shown

Customize view

Severe acute respiratory syndrome coronavirus 2 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 18-JUL-2020

DEFINITION Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome.

ACCESSION NC_045512

VERSION NC_045512.2

DBLINK BioProject: [PRJNA485481](#)

KEYWORDS RefSeq.

SOURCE Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

ORGANISM [Severe acute respiratory syndrome coronavirus 2](#)
Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes; Nidovirales; Cornidovirinae; Coronaviridae; Orthocoronavirinae; Betacoronaviruses; Sarbecovirus.

REFERENCE 1 (bases 1 to 29903)

AUTHORS Wu,F., Zhao,S., Yu,B., Chen,Y.M., Wang,W., Song,Z.G., Hu,Y., 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 new coronavirus associated with human respiratory disease in China

JOURNAL Nature 579 (7798), 265–269 (2020)

PUBMED [32015508](#)

REMARK Erratum: [Nature. 2020 Apr;580(7803):E7. PMID: 32296181]

REFERENCE 2 (bases 13476 to 13503)

AUTHORS Baranov,P.V., Henderson,C.M., Anderson,C.B., Gesteland,R.F., Atkins,J.F. and Howard,M.T.

TITLE Programmed ribosomal frameshifting in decoding the SARS-CoV genome

JOURNAL Virology 332 (2), 498–510 (2005)

PUBMED [15680415](#)

Analyze this sequence
Run BLAST
Pick Primers
Highlight Sequence Features
Find in this Sequence

NCBI Virus
Retrieve, view, and download SARS-CoV-2 coronavirus genomic and protein sequences.

Related information
Assembly
BioProject
PubMed
Taxonomy
Full text in PMC
Gene
Genome
Identical GenBank Sequence
Mature Peptides

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NIH National Library of Medicine
National Center for Biotechnology Information

Log in

Nucleotide Nucleotide Search Advanced Help

GenBank Send to: Change region shown

Severe acute respiratory syndrome coronavirus 2 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 18-JUL-2020
DEFINITION	Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome.			
ACCESSION	NC_045512			
VERSION	NC_045512.2			
DBLINK	BioProject: PRJNA485481			
KEYWORDS	RefSeq.			
SOURCE	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)			
ORGANISM	Severe acute respiratory syndrome coronavirus 2 Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes; Nidovirales; Cornidovirinae; Coronaviridae; Orthocoronavirinae; Betacoronavirus; Sarbecovirus.			
REFERENCE	1 (bases 1 to 29903)			
AUTHORS	Wu,F., Zhao,S., Yu,B., Chen,Y.M., Wang,W., Song,Z.G., Hu,Y., 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 new coronavirus associated with human respiratory disease in China			
JOURNAL	Nature 579 (7798), 265-269 (2020)			
PUBMED	32015508			
REMARK	Erratum: [Nature. 2020 Apr;580(7803):E7. PMID: 32296181]			
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AUTHORS	Baranov,P.V., Henderson,C.M., Anderson,C.B., Gesteland,R.F., Atkins,J.F. and Howard,M.T.			
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PUBMED	15680415			

Send to: Change region shown

Analyze this sequence Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

NCBI Virus Retrieve, view, and download SARS-CoV-2 coronavirus genomic and protein sequences.

Related information Assembly

BioProject

PubMed Taxonomy

Full text in PMC

Gene

Genome

Identical GenBank Sequence

Mature Peptides

★ Designing primers to detect presence of viral genome:

Nucleocapsid (N)

Envelope (E)

Membrane (M)

Orf1ab

gene LSTDGTGVEHVTFFIYNKIVDEPEEHVQIHTIDGSSGVNPVMEPIYDEPTTTTSVPL"

26245..26472

/gene="E"

/locus_tag="GU280_gp04"

/db_xref="GeneID:43740570"

26245..26472

/gene="E"

/locus_tag="GU280_gp04"

/note="ORF4; structural protein; E protein"

/codon_start=1

/product="envelope protein"

/protein_id="YP_009724392.1"

/db_xref="GeneID:43740570"

/translation="MYSFVSEETGLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCC
NINVSLVKPSFYYSRVRKVNLSRVPDLLV"

26523..27191

/gene=""

/locus_tag="GU280_gp05"

/db_xref="GeneID:43740571"

26523..27191

/gene="M"

/locus_tag="GU280_gp05"

/note="ORF5; structural protein"

/codon_start=1

/product="membrane glycoprotein"

/protein_id="YP_009724393.1"

/db_xref="GeneID:43740571"

/translation="MADSNGTITVEELKKLQQNLVIGFLFTWLCLLQFAYANRNR
FLYIIKLFLWLPVTLACFVLAAVYRINWITGGIAIAMACLVGLMWLSFYIASFRL
FARTRSMSFNPEPNETNILLNVPLHGTILTRPLLESELVIGAVILRGLRIAGHHGLRC
IKDLPKKEITVATSRSLSYKLGAQRVAGDSGFAASRYRIGNYKLNTDHSSSDNIA
LIVQ"

gene

CDS

gene

CDS

- **Primer selection/design tools**

NCBI Primer BLAST : <https://www.ncbi.nlm.nih.gov/tools/primer-blast/>

SnapGene

- NCBI Primer BLAST : <https://www.ncbi.nlm.nih.gov/tools/primer-blast/>

National Library of Medicine
National Center for Biotechnology Information

Primer-BLAST

A tool for finding specific primers

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

Primers for target on one template Primers common for a group of sequences

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Range
From To
Or, upload FASTA file Dosya seçilmedi.

Retrieve recent results Publication Tips for finding specific primers

Primer Parameters

Use my own forward primer (5'->3' on plus strand)
Use my own reverse primer (5'->3' on minus strand)

PCR product size
Min Max
of primers to return

Primer melting temperatures (T_m)
Min Opt Max Max T_m difference

Exon/intron selection
A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span

Exon junction match
Min 5' match Min 3' match Max 3' match

Select
'Genomes for
selected
organisms'

Write 'SARS-
CoV-2' to the
organism
region

Click!

Primer Pair Specificity Checking Parameters

Specificity check Enable search for primer pairs specific to the intended PCR template [?](#)

Search mode [?](#)

Database Refseq mRNA
 Refseq representative genomes
 Genomes for selected organisms (primary reference assembly only) **Selected**
 Uncultured/environmental sample sequences [?](#)
 [?](#)

Exclusion nr
Refseq RNA (refseq_rna)
Custom

Organism (Enter taxonomy id or select from the suggestion list as you type. [?](#))

Entrez query (optional)

Primer specificity stringency
Primer must have at least total mismatches to unintended targets, including
at least mismatches within the last bps at the 3' end. [?](#)
Ignore targets that have or more mismatches to the primer. [?](#)

Max target amplicon size [?](#)

Allow splice variants Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input) [?](#)

Show results in a new window Use new graphic view [?](#)

Primer-BLAST » Job ID:t71o45r1112wY41mgAapVPoduGbxDqN71g

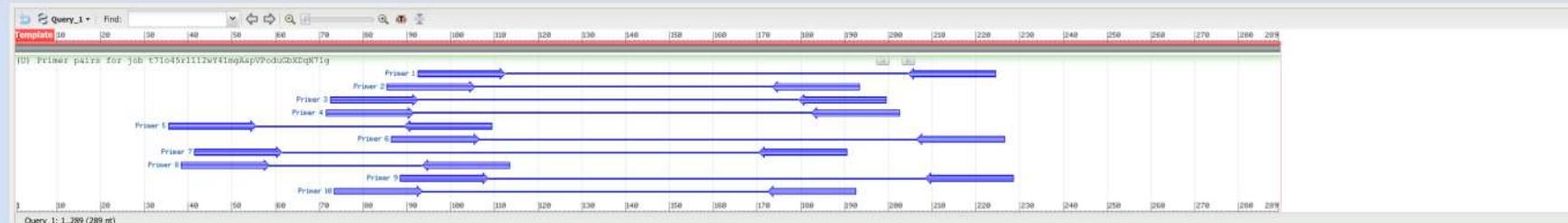
Primer-BLAST Results 

Input PCR template: Query_1
Range: 1 - 289

Specificity of primers: Primers may not be specific to the input PCR template as targets were found in selected database: RefSeq Representative Genome Database (Organism limited to Severe acute respiratory syndrome coronavirus 2)...help on specific primers

Other reports > Search Summary

Graphical view of primer pairs



Detailed primer reports

Primer pair 1

	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGTGACATCAAGGACCTGCC	Plus	20	93	112	59.96	55.00	5.00	1.00
Reverse primer	GCCAATCCCTGTAGCGACTGT	Minus	20	224	205	60.11	55.00	4.00	3.00
Product length	132								

Products on potentially unintended templates

>NC_045512.2 Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome

product length = 132
Forward primer 1 TGTGACATCAAGGACCTGCC 20
Template 26997 27016

Reverse primer 1 GCCAATCCCTGTAGCGACTGT 20
Template 27128 27109

Primer pair 2

	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGGACCGCTGTGACATCAAGG	Plus	20	86	105	60.04	55.00	5.00	2.00
Reverse primer	CCTGAGTCACCTGCTACACG	Minus	20	193	174	60.11	60.00	5.00	2.00
Product length	108								

Products on potentially unintended templates

>NC_045512.2 Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome

Select the most suitable primer set.

- Check product size
- Check non-specific products
- Check self complementarity

⭐ Checking the specificity of the selected primers:

Leave blank

Paste your selected primer sequences here

NIH National Library of Medicine
National Center for Biotechnology Information

Primer-BLAST A tool for finding specific primers

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

Primers for target on one template Primers common for a group of sequences

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Range Forward primer From To Reverse primer

Or, upload FASTA file Dosya seçilmedi.

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Use my own reverse primer (5'->3' on minus strand)

PCR product size: Min 70 Max 1000

of primers to return: 10

Primer melting temperatures (T_m): Min 57.0 Opt 60.0 Max 63.0 Max T_m difference 3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span: No preference

Exon junction match: Min 5' match 7, Min 3' match 4, Max 3' match 8

Select
'Genomes for
selected
organisms'

List the
organisms that
you want to
control

Click!

Primer Pair Specificity Checking Parameters

Specificity check Enable search for primer pairs specific to the intended PCR template [?](#)

Search mode [?](#)

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 Refseq representative genomes
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+ Advanced parameters