

SARS-CoV-2 GENOMICS: BIOINFORMATICS WORKFLOWS (PANGOLin and COVDB online tools)

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Division of the National Health Laboratory Service



Overview

- PANGOLin (<u>https://pangolin.cog-uk.io/</u>)
- Covdb tool (<u>https://covdb.stanford.edu/sierra/sars2/by-sequences/</u>)

GISAID (<u>https://www.gisaid.org/</u>)

PANGOLin (PANGO lineages)

- > PHYLOGENETIC ASSIGNMENT OF NAMED GLOBAL OUTBREAK LINEAGES
- Lineages are sequences of biological entities connected by ancestry-descent relationships.
- Pangolin was developed to implement the dynamic nomenclature of SARS-CoV-2 lineages, known as the Pango nomenclature.
- It allows a user to assign a SARS-CoV-2 genome sequence the most likely lineage (Pango lineage) to SARS-CoV-2 query sequences.
- Web-based and command line tool.



Overview

PANGO lineages		
НОМЕ		
PANGO LINEAGES		
MAJOR LINEAGES	~	
SUGGEST A NEW LINEAGE		
PANGOLIN		
PANGOLIN DOCS	~	
INTERNATIONAL LINEAGE REPORT	~	
LLAMA		
FREQUENTLY ASKED QUESTIONS		
CONTRIBUTORS		
HOW TO CITE		
tps://cov-lineages.org/pangolin.html		

PANGO lineages

pangolin

PHYLOGENETIC ASSIGNMENT OF NAMED GLOBAL OUTBREAK LINEAGES

pangolin was developed to implement the dynamic nomenclature of SARS-CoV-2 lineages, known as the Pango nomenclature. It allows a user to assign a SARS-CoV-2 genome sequence the most likely lineage (Pango lineage) to SARS-CoV-2 query sequences.







Overview

INTERNATIONAL LINEAGE REPORT	
LLAMA	
FREQUENTLY ASKED QUESTIONS	
CONTRIBUTORS	
HOW TO CITE	





Command-line tool

GNU General Public License v3.0

QUICK LINKS

- How it works
 Dependencies
 Usage
- Model training
 Installation
- pangolin tutorial
 Updating
 Citing pangolin

Output



Web application

Developed by the <u>Centre for Genomic Pathogen</u> Surveillance.

Web-Interface (<u>https://pangolin.cog-uk.io/</u>)

Drag and drop fasta file

Select fasta file to upload

Pangolin COVID-19 Lineage Assigner

Phylogenetic Assignment of Named Global Outbreak LINeages



You can upload one or more sequences by dragging and dropping a (multi)fasta file or clicking "Select fasta file to upload" and selecting a (multi)fasta file.

This Web Application assigns lineages to COVID-19 sequenes based on the methodology described in this article

The software to assign lineages based on the algorithm that was developed by Áine O'Toole, Verity Hill, JT McCrone, Emily Scher and Andrew Rambaut. The source code can be found here

Recommended browsers 📀 ڬ or 🖉



Centre for Genomic Pathogen Surveillance Pangolin (version v.2.4.2, lineages version 2021-04-28) is built by Áine, JT, Verity, Emily and Andrew. Web Application by

Uploading files and start analysis

	Start analysis Reset entries U	pload another file		Help
	File name	Sequence name	Lineage	Assignment Conflict
— REA	DY FOR ANALYSIS 7 sequences			
Β	Multi-Fasta.fasta	EPI_ISL_2003169		
Ζ	Multi-Fasta.fasta	hCoV-19/SouthAfrica/819503/2021		
Ζ	Multi-Fasta.fasta	hCoV-19/SouthAfrica/819931/2021		
Ζ	Multi-Fasta.fasta	hCoV-19/SouthAfrica/400919678/2021		
Ζ	Multi-Fasta.fasta	2019-nCoV_MN908947 S03911-20		
Χ	Multi-Fasta.fasta	N2618		
Ζ	Multi-Fasta.fasta	N2625		

Analysis and Interpretation

	Retry Failed Sequences	Reset entries Upload another file		Help
	File name	Sequence name	Lineage	Assignment Conflict
- FAII	LED (Click warning icon for more	info) 2 sequences		
()	Multi-Fasta.fasta	N2618		
!	Multi-Fasta.fasta	N2625		
- AN/	ALYSED (Click tick icon for more i Multi-Fasta.fasta	nfo) 5 sequences EPI_ISL_2003169	R1 💧 🚱 🛈	0
~	Multi-Fasta.fasta	hCoV-19/SouthAfrica/819503/2021	B.1.351 S ()	0
~	Multi-Fasta.fasta	hCoV-19/SouthAfrica/819931/2021	B.1.1.7 🏂 🚱 🛈	0
\checkmark	Multi-Fasta.fasta	hCoV-19/SouthAfrica/400919678/2021	B.1.617.2 🚯 🛈	0.0
\checkmark	Multi-Fasta.fasta	2019-nCoV_MN908947 S03911-20	B.1.1.57 🏂 🚱 🛈	0.0

Pangolin (version v.2.4.2, lineages version 2021-04-28) is built by Áine, JT, Verity, Emily and Andrew. Web Application by Sector for Genomic Pangolin (version v.2.4.2, lineages version 2021-04-28) is built by Áine, JT, Verity, Emily and Andrew. Web Application by

Downloading Results and Quality Calling

	ᡖᠳ᠂᠅᠂᠇				Pangolin Results	- Excel		西 —	
	File Home Insert Page Layout	Formulas	Data	Review View Acrobat	♀ Tell me what yo	ou want to do		Daniel Amoako	0
Pa	Clipboard r₂ Format Painter	11 - A		E = ≫ → E Wrap Text E = Merge & Cente Alignment	General r - \$ - % *	Conditional Format as Formatting ▼ Table ▼ Styles		 ➤ AutoSum * Area ▼ Fill * Sort & Find & Filter * Select * Editing 	
D	17 • : $\times \checkmark f_x$								
	А	В	с	D	E	F	G	н	
1	Sequence name	Lineage	Conflict	Most common countries	Number of taxa	Date range	Days since last sampling		_
2	EPI_ISL_2003169	P.1	0	Brazil, Switzerland, Colombia	134	December-04, February-09	92		
З	hCoV-19/SouthAfrica/819503/2021	B.1.351	0	South_Africa, UK, Belgium	1236	September-28, February-12	87		
4	hCoV-19/SouthAfrica/819931/2021	B.1.1.7	0	UK, Denmark, France	65955	September-20, February-21	78		
5	hCoV-19/SouthAfrica/400919678/2021	B.1.617.2	0	UK, India, Australia	109	February-27, April-16	24		
6	2019-nCoV_MN908947 \$03911-20	B.1.1.57	0	South_Africa, India, Zimbabwe	58	March-31, November-13	178		
7									

Quality Calling:

- Passed_qc
- ➢ Failed_qc

Coverage vs Quality Calling

- N_content
- Sequence length

Downloading Results and Quality Calling

	А	В	С	D	E	F	G	Н	
55	N5193_S77_L001	B.1.351	0	2.4.2	5/12/2	2021 1.2.2	passed_qc	9/9 B.1.351 SNPs (0 ref and 0 other)	
56	N5206_S90_L001	B.1.351	0	2.4.2	5/12/2	2021 1.2.2	passed_qc	6/9 B.1.351 SNPs (0 ref and 3 other)	
57	N5208_S92_L001	B.1.351	0	2.4.2	5/12/2	2021 1.2.2	passed_qc	9/9 B.1.351 SNPs (0 ref and 0 other)	
58	Negative-2_S192_L001	B.1.351	0	2.4.2	5/12/2	2021 1.2.2	passed_qc	7/9 B.1.351 SNPs (0 ref and 2 other)	
59	Positive-1_S95_L001	B.1	0	2.4.2	5/12/2	2021 1.2.2	passed_qc		
0	Positive-3_S287_L001	B.1	0	2.4.2	5/12/2	2021 1.2.2	passed_qc		
71	N2618_S295_L001	None	0	2.4.2	5/12/2	2021 1.2.2	fail	seq_len:330	
2	N2625_S302_L001	None	0	2.4.2	5/12/2	2021 1.2.2	fail	N_content:0.67	
3	N2626_S303_L001	None	0	2.4.2	5/12/2	2021 1.2.2	fail	N_content:0.55	
4	N2629_S306_L001	None	0	2.4.2	5/12/2	2021 1.2.2	fail	N_content:0.72	
5	N2630_S307_L001	None	0	2.4.2	5/12/2	2021 1.2.2	fail	N_content:0.77	
6	N2631_S308_L001	None	0	2.4.2	5/12/2	2021 1.2.2	fail	N_content:0.67	
7	N2632_S309_L001	None	0	2.4.2	5/12/2	2021 1.2.2	fail	N_content:0.89	
8	N2634 S311 L001	None	0	2.4.2	5/12/2	2021 1.2.2	fail	seq_len:6299	

Quality Calling:

- Passed_qc
- ➤ Failed_qc

Coverage (threshold) vs Quality Calling

- > N_content
- Sequence length



Stanford tool (<u>https://covdb.stanford.edu/sierra/sars2/by-sequences/</u>)

Different options for inputs

	Stanford University CORONAVIRUS A A Stanford HIVDB team website. I	NTIVIRAL & RES	ISTANCE DATABASE	номе	SEARCH	ANALYSIS PROGRAM	DRUGS	TRIALS	CITATION	SUPPORT COVDB	
SARS-Co	oV-2 Sequen	ce Analysis									
			quence is entered. Sequences m uences containing ~30000 nucle			TA format if multiple sequ	ences are ente	ered. Seque	nces can be pa	sted in the text box or up	loaded
Input mutation	ns Input sequences	Input sequence read	s								
Header:				(optional)							
Upload text fil	e: Choose File	No file chosen	Load Examples								
Output opt	ions										
	O Printable HTML										
										Reset Analy.	ze

Stanford tool (https://covdb.stanford.edu/sierra/sars2/by-sequences/)

Outputs

- Sequence summary (Information on the specific genes coverage)
- Pangolin lineage assignment
- Outbreak information
- Sequence quality assessment (graphical visualization)
- Mutation list
- Mutation comments
- MAb susceptibility summary
- Convalescent plasma susceptibility summary
- Plasma from vaccinated persons susceptibility summary

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Outputs

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INPUT SEQUENCES AND ANALYSE

SARS-CoV-2 Sequence Analysis

SARS-CoV-2 Sequences can be entered as plain text if just one sequence is entered. Sequences must be entered using the FASTA format if multiple sequences are entered. Sequences can be pasted in the text box or uploaded using the File Upload option. The upper limit is currently 100 sequences containing ~30000 nucleotides per sequence.

Input mutations	Input sequences	Input sequence reads		
Header:			(optional)	
Upload text file:	Choose File	Multi-Fasta.fasta	Load Examples	
>EPI_ISL_2003169				
CCTTCCCAGGTAA	CAAACCAACCAACTT	CGATCTCTTGTAGATCTGTT	TTCTCTAAACGAACTTTAAAATCTGTGTGGCTG	
TCACTCGGCTGCA	TGCTTAGTGCACTCA	GCAGTATAATTAATAACTAAT	ATTACTGTCGTTGACAGGACACGAGTAACTCG	
TCTATCTTCTGCAG	GCTGCTTACGGTTTC	GTCCGTGTTGCAGCCGATC/	TCATCAGCACATCTAGGTTTTGTCCGGGTGTGAC	
CGAAAGGTAAGAT	GGAGAGCCTTGTCCC	TGGTTTCAACGAGAAAACAC	ACACGTCCAACTCAGTTTGCCTGTTTTACAGGTT	
CGCGACGTGCTC	GTACGTGGCTTTGGAG	ACTCCGTGGAGGAGGTCTT	TTATCAGAGGCACGTCAACATCTTAAAGATGGCAC	
TTGTGGCTTAGTA	GAAGTTGAAAAAGGCC	STTTTGCCTCAACTTGAACA(CAGCCCTATGTGTTCATCAAACGTTCGGATGCTC	4
Output options				
O HTML	Printable HTML			
			Re	eset Analyze

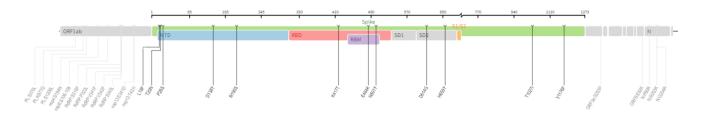


OUTPUTS

1. EP1_ISL_2003169 * 1 2 3 4 5 6 7 * This submission contains 7 sequences.
nsp1 · nsp2 · PLpro · nsp4 · 3CLpro · nsp6 · nsp7 · nsp8 · nsp9 · nsp10 · RdRP · nsp13 · nsp14 · nsp15 · nsp16 · Spike · ORF3a · E · M • ORF6 · ORF7a · ORF7b · ORF8 · N · ORF10
P.1 (Prob=1.0; pangolin: 2.4.2; pangoLEARN: 2021-05-12)
P.1 (n=16,994)

•

Sequence quality assessment





	1. EPI_ISL_2003169 * 1 2 3 4 5 6 7 * This submission contains 7 sequences.
Mutation list	
PLpro: S370L • K977Q • S1206L	Spike: L18F • T20N • P26S • D138Y • R190S • K417T • E484K • N501Y • D614G • H655Y • T1027I
nsp4: S184N	• V1176F
nsp6: Δ106-108	ORF3a: S253P
RdRP: S318F • P323L • V341F • V342F • S343L	ORF8: E92K
nsp13: E341D • T4311	N: P80R • R203K • G204R

Mutation comments	Last updated on May 1, 2021

nsp6:∆106

Each of the WHO-defined variants of concern, one of the two CDC-defined variants of concern, and a recently described novel E484K-containing U.S. lineage contains the same deletion of amino acids 104 to 106 in the non-structural protein 6 (nsp6) a component of the SARS-CoV-2 membrane-tethered replication complex that is also involved in antagonizes host-responsiveness to interferons^{[1][2][3][4]}. Although several additional non-spike mutations have been shared by more than one variant of concern, none have occurred as commonly as this mutation^[5].

L18F

L18F is an NTD mutation present in B.1.351, P.1, and a sub-lineage of B.1.1.7^[6]. It is associated with reduced susceptibility to several NTD-binding mAbs but by itself does not appear to reduce susceptibility to plasma from convalescent or vaccinated persons^{[7][8]}.

• K417T

K417N/T are ACE2-binding site RBM mutations present in the WHO-defined variants of concern B.1.351 (K417N) and P.1 (K417T). Both mutations reduce ACE2 binding^{[9][10]} and rarely occur in other virus variants. K417N confers >100-fold reduced susceptibility to ETE^[11] and ~30-fold reduced susceptibility to CAS^[7] but appears to retain susceptibility to the remaining mAbs in advanced clinical development^[7]. K417N/T retain full susceptibility to plasma samples from convalescent persons.

E484K

SARS-CoV-2-related Database- GISAID (<u>https://www.gisaid.org/</u>)



Pandemic coronavirus causing COVID-19

A previously unknown human coronavirus (hCov-19) was first detected in late 2019 in patients in the City of Wuhan, who suffered from respiratory illnesses including atypical pneumonia, an illness that has become known as coronavirus disease (COVID-19). The coronavirus originated from an animal host and is closely related to the virus responsible for the Severe Acute Respiratory Syndrome coronavirus (SARS).

On 10. January 2020, the first virus genomes and associated data were publicly shared via GISAID. The World Health Organization announced on 11. March 2020 the first coronavirus pandemic. As the pandemic progresses, scientists from around the globe are tracking the virus and its genome sequences to ensure optimal virus diagnostic tests, to track and trace the ongoing outbreak and to identify potential intervention options. Several analyses to assist with these efforts are offered here, including sequence alignments, diagnostic primer and probe coordinates, 3D protein models, drug targets, phylogenetic trees and many more.



Search



• EpiCoV

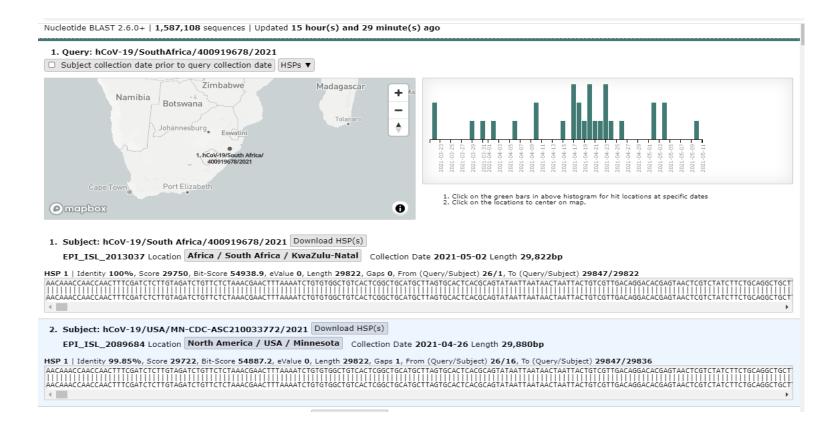
- Search
- Downloads
- Upload
- BLAST tool
- PrimerChecker

Using BLAST tool for Lineage confirmation

>hCoV-19/SouthAfrica/400919678/2021
NNNNNNNNNNNNNNNNNNNNAACAAACCAACCAACTTTCGATCTCTTGTAGATCT
GTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACT
CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATC
TTCTGCAGGCTGCTTACGGTTTCGTCCGTTTTGCAGCCGATCATCAGCACATCTAGGTTT
TGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAAC
ACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTTTGG

Submit hCoV-19 BLAST

Identifying similar sequences



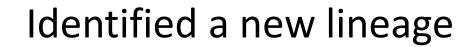
Searching for the similar sequences

GISAID	© 2008 - 2021 Terms of Use Privacy Notice Contact
	You are logged in as Daniel Gyamfi Amoako - logout
Registered Users EpiFlu™ EpiCoV™	My profile
EpiCoV™ 🌏 Search 🥫 Downloads	🅙 Upload 🛛 🎁 My Unreleased
Search	
Accession ID	Virus name hCoV-19/USA/MN-CDC-ASC2100337 🗸 🗌 complete 🔊 🗌 high coverage 🕤
Location	✔ Host
Collection 🧼 to	🥹 Submission 🛛 😨 to 🔤 collection date complo
Clade all V Lineage	✓ Substitutions ⑦ Reset Fulltext ▲
Virus name	Passage de Accession ID Collection da Submission E 🛈 Length Host Location Originating
hCoV-19/USA/MN-CDC-ASC210033772/20	Original EPI_ISL_2089684 2021-04-26 2021-05-13 ① 29,880 Human North America / U Aegis Scien
Total: 1 viruses	<< < 1 > >> Select Analysis 📑 Download
the Database contains data relating to non-influenza viru	is Agreement, you have accepted certain terms and conditions for viewing and using data regarding influenza viruses. To the extent uses, the viewing and use of these data is subject to the same terms and conditions, and by viewing or using such data you agree to Database Acress Agreement in censert of such data in the same manner as if they were data relation to influenza viruses.



Confirming the set of mutations in the specific lineage

virus uctari	
Virus name:	hCoV-19/USA/MN-CDC-ASC210033772/2021
Accession ID:	EPI_ISL_2089684
Type:	betacoronavirus
Clade	G
Pango Lineage	B.1.617.2 (version: 2021-05-12)
AA Substitutions	Spike D614G, Spike D950N, Spike E156G, Spike F157del, Spike L452R, Spike P681R, Spike R158del, Spike T19R, Spike T478K, M I82T, N D63G, N D377Y, N R203M, NS3 S26L, NS7a L116F, NS7a T120I, NS7a V82A, NSP2 P129L, NSP3 P822L, NSP4 A446V, NSP4 P29S, NSP6 V149A, NSP12 G671S, NSP12 P323L, NSP13 P77L, NSP14 P46L
Variant	G/452R.V3 (B.1.617+)
Passage details/history:	Original
Sample information	
Collection date:	2021-04-26
Location:	North America / USA / Minnesota
Host:	Human
Additional location information	on:
Gender:	Female
Patient age:	33
Patient status:	unknown
Specimen source:	Nasal - Anterior Nares
Additional host information:	
Sampling strategy:	
Outbreak:	
Last vaccinated:	
Treatment:	
Sequencing technology:	Illumina NovaSeq 6000
Assembly method:	Dragen COVID Lineage v3.5.1
Coverage:	2,059.93x
Comment:	③ Gap of 13 nucleotides when compared to the reference sequence.
Institute information	
Originating lab:	Aegis Sciences Corporation
Address:	501 Great Circle Road, Nashville, TN 37228
< Back	Contact Submitter 📑 Metadata 🧃 FAS



What next?

HOME		
PANGO LINEAGES		
MAJOR LINEAGES	~	
SUGGEST A NEW LINEAGE		
PANGOLIN		
PANGOLIN DOCS	~	How to: lineage
INTERNATIONAL LINEAGE REPORT	~	designation
LLAMA		So you think you've identified a new lineage? The following
FREQUENTLY ASKED QUESTIONS		is a step-by-step guide of how to add your new lineage to the growing list of lineages we maintain and can then be
CONTRIBUTORS		assigned using pangolin.
HOW TO CITE		

LLAMA

FREQUENTLY ASKED QUESTIONS

CONTRIBUTORS

HOW TO CITE



© SARS-CoV-2 lineages Design: HTML5 UP Andrew Banchich Áine O'Toole

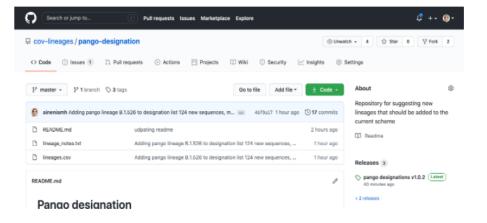
What to do

1. Does your cluster fulfil the definition of a lineage?

Refer to the Pango lineage guide to check if your cluster fits the new lineage guidelines.

2. Navigate to the new pango-designation repository

Go to the <u>github.com/cov-lineages/pango-designation</u> repository, shown below. Notice the lineage_notes.txt and lineages.csv files. These include the latest set of manually curated lineage designations.





THANK YOU