

Analyzing SARS-CoV-2 Da	ata in Terra using Theiagen's
TheiaCoV Clea	arLabs Workflow
Document TG-	SC2-CL, Version 4
Date:	Workflow Versions
5/1/2025	PHB v3.0.0

## 1. PURPOSE/SCOPE

To standardize the process of analyzing SARS-COV-2 (SC2) next generation sequencing (NGS) data using Theiagen's TheiaCoV\_ClearLabs\_PHB workflow in Terra to generate assemblies, quality control (QC) metrics, and determine Nextclade clade and Pangolin lineage assignments. Acceptable data types include ClearLabs raw read file format. Read the documentation <u>here</u>.

## 2. REQUIRED RESOURCES

- Computer
- Internet connection: at least 10 and 5Mbps for download and upload speeds, respectively
- Internet browser
  - Google Chrome, Firefox, or Edge
- Google account
- Terra account, linked to Google account
- ClearLabs raw sequencing read files uploaded to Terra workspace, see TG-TER-03
- Theiagen's TheiaCoV\_ClearLabs\_PHB workflow in Terra, see appendix 10.1

# 3. RELATED DOCUMENTS

# IMPORTANT NOTES

- Metadata column headers and workflow input text indicated in gray in this SOP are customizable; black is required text
- Terra data table column headers become available as workflow inputs when running workflows, search for them in workflow input dropdowns using the prefix this. to filter
- Filter for workspace data and files in workflow input dropdowns using the prefix workspace.

Document Number	Document Name
TG-TER-03	Getting Started in Terra: Importing Reads, Metadata, Workflows, and More

## 4. PROCEDURE

## 4.1 RUNNING THE THEIACOV WORKFLOW

 Open Terra and navigate to the workflows tab within the workspace containing SC2 data (Fig 1)

2.	Select the
	TheiaCoV_ClearLabs_PHB
	(Fig 1)

DASHBOARD	DATA	ANALYSES	WORKFLOWS	JOB HISTO	NRY	
WORKFLOW	s		labs	× S	ort By: Alphabe	-
Find a Wo	rkflow	Thei	aCoV_ClearLabs		TheiaCoV_ClearLabs_P	нв
Eiguro 1		V. v2. Source	3.2 e: Dockstore	(i)	V. v1.0.0 Source: Dockstore	(i)

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TheiaCoV_ClearLabs_PHB	
Source: github.com/theirgen/public health hisinformatics/	TheipCoV Clearlinhs BHB1/110
Synopsis:	
No documentation provided	
O Run workflow with inputs defined by file paths	h
Run workflow(s) with inputs defined by data table	
S	
s 🕻 territoria KK_ClearLabs_Test 🗸	
🗌 Use call caching 🜖 🔄 Delete intermediate outputs 🜖	Use reference disks 🕚 🗌 Retry with mo Figure 2.

- 3. Uncheck call caching (Fig 2)
- 4. Choose the *latest version* of the workflow, or the version used for internal validation (Fig 2, a)
- 5. Select the second bullet to *run workflow(s) with inputs defined by data table* (Fig 2, b)
- 6. Select the relevant data table under the select data table dropdown (Fig 2, c)
- 7. Click select data (Fig 2, d)
- 8. In the pop-up window *select the checkbox* for each sample to be included in the analysis (Fig 3)



- b. Additionally, a subset of samples may be chosen using the search bar to filter before selecting the checkbox at the top to only select samples matching the search criteria
- c. Scroll to the bottom and click *ok*

Choose	e learLabs_Tests	s to process os_Tests		
ect K	s_Tests	SETTINGS     2 rows     selected	ADVANCED SEARCH     Search	વ
-	KK_ClearLabs_Test_id	1 O QC_Call	() assembly_length_unambiguous	() as
Page	-00003868	FAIL	1061	1.
All (2)	-00003870	FAIL	715	1.
All [2] None	-00003870	FAIL	715 of 2 « c 3 > 0 Iter pe:	100
Selected I	KK_ClearLabs_Tests will	be saved as a new KK_ClearL	.abs_Test_set named:	
		71717 64 41		

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- 9. Specify the desired *dataset tags* and *docker image* inputs
  - a. To run TheiaCoV\_ClearLabs\_PHB for the first time or configure with the newest dataset tags and docker images, upload the TheiaCov input json file on the inputs tab by navigating to the Key Resources Notion page titled Docker Image and Reference Materials for SARS-CoV-2 Genomic Characterization
    - i. **NOTE**: TheiaCoV PHB v2.0.0+ workflows are not backwards compatible with older versions of Nextclade; <u>use Nextclade Dataset Tag</u> 2024-04-15—15-08-222 or <u>newer</u>
    - ii. Expand the TheiaCoV in PHB (v2.0.0 or higher) section, followed by the Terra.Bio Input
       JSONs for PHB v2.0.0 or higher; click on the json file associated with the ClearLabs platform, TheiaCoV\_ClearLabs\_PHB\_2025-04-02.json, or newer
    - iii. Right click and save the file (text does not have to be selected to save properly)
    - *iv.* Return to the workflow in Terra, click *upload json* (Fig 4, red circle), *select* the saved json file, and click *open*
  - b. To run the workflow with previously saved dataset tags and docker images, no changes are needed
  - c. To add docker images and dataset tags as workspace files for availability in input dropdowns, refer to appendix 10.2

Use call caching	e outputs 0 Use reference disks 0 F	Retry with more memor	y 0 Ignore empty outputs 0	
SCRIPT ** INPUTS 0 **	OUTPUTS ** RUN ANALYSIS		П	CANCEL
Hide optional inputs			Download json   Drag or	dick to upload json   diear inputs SEARCH INPUT
Task name 🌡	Variable	Туре	Attribute	$\smile$
theiacov_clearlabs	clear_lab_fastq	File	thisreads	B
theiacov_clearlabs	primer_bed	File	workspace.Artic_V4-1_primer_bed	Þ
theiacov_clearlabs	samplename	String	Required	
	cpu		this.KK_ClearLabs_Test_id	
Figure 4.	disk_size	Int	thisreads	

10. Set the first and third attributes in the table to *this.reads* and *this.KK\_ClearLabs\_Test\_id*, respectively (Fig 4)

- a. Where *this.KK\_ClearLabs\_Test\_id* is the unique name of your data table in Terra
- 11. Manually choose the *primer\_bed* file for the primer set used to sequence samples
  - Labs using the Artic V4-1 will choose workspace.Artic\_V4-1\_primer\_bed; for other primer bed files, see <u>Docker Image and Reference Materials for SARS-CoV-2 Genomic Characterization</u> for available primer bed files

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b. To add workspace files for availability in input dropdowns, refer to appendix 10.2

SCRIPT ** INPUTS * Output files will be saved to ☐ Files / submission unique ID / theiacov_cleart	OUTPUTS     RUN ANALYSIS abs / work/ wunique ID			
References to outputs will be written to Tables / KK_ClearLabs, Test Fill in the attributes below to add or update colu	imns in you a table		revented jun   Drag or cick to upload jun   Clear outputs SEACCH OUTPUT	s
Task name ↓	Variable	Туре	Attribute   Use defaults	
theiacov_clearlabs	aligned_bai	File	thisaligned_bar	
Figure 5	aligned_bam	File	thisaligned_bam	
	antia dantan	Chiles.	Akis satis studen	

12. Specify outputs by clicking on the *outputs* tab and *use defaults* (Fig 5)

# 13. Click save

14. Launch the workflow by clicking run analysis (Fig 5); enter desired comments and click launch

# 4.2 QUALITY ASSESSMENT OF THEIACOV OUTPUTS

- 1. Navigate to the *data* tab of the workspace containing SC2 data and open the pertinent data table
- 2. Click <u>Select Columns</u> (Fig 6, green rectangle) and select <u>none</u> to deselect all output columns (Fig 6, yellow highlight)
- 3. To simplify the table, select the three following outputs that will be used to make a QC assessment: *assembly\_length\_unambiguous*, *Number\_N*, and *percent\_reference\_coverage* 
  - a. <u>Optional</u>: save this selection by clicking in the save this column selection field and naming it (e.g. QC\_assessment); do not include any spaces in the name (Fig 6, red rectangle)
  - b. Click done

	CES	Workspaces + the Data	ingen-training-workspaces/APHL_OF
DASHBOARD DATA	ANA	LYSES WORKF	LOWS SUBMISSION HISTORY
O import Data	/ Edi	C 🗙 Open With.	Bi Erpor 🏟 Select Columns
rables ~	0.	illumina_ 10	QC_assignment
Search all tables		Sample_01	PASS
B acinetobacter_test (3)		Sample_02	PASS
Baspergillus_fumigatus (2) ()		Sample_03	FAIL
🛙 aspergillus_furnigatu (1) 🛈		Sample_04	FAIL.
aps validation (500)		Sample_05	PASS
Illumina na snael /201 ()		Sample_06	FAIL
n monimul projection (100 C)	D	Sample_07	FAIL
Figure 6		Sample_08	FAIL
inguic 0.		Sample,09	FAIL

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# 4. Optional: add a column to record QC PASS/FAIL by clicking edit, add a column (Fig 7) a. Name the new

spaces

column (e.g. QC\_Call); do

TABLE Q (5) (6) not include any b. Set the value Figure 7. type as string

- c. Click save 5. Use table 1 to assess the quality of each sample's genome assembly (see next page) &/or labspecific quality metrics
- 6. Optional: notate in the QC\_assessment field for each sample PASS or FAIL by clicking the pencil icon in the corresponding field (Fig 7, red circle)
- 7. For samples that pass the guidance thresholds, proceed to section 4.3
  - a. For samples that do not pass guidance thresholds, resequence
    - i. Samples not meeting guidance thresholds indicated here may proceed to analysis at the discretion of the laboratory

## Table 1. Guidance thresholds for genome assembly QC

QC Metric	Data Table Column	Guidance Threshold* <sup>1</sup>
Number N	number_N	<5kbp
Assembly length unambiguous	assembly_length_unambiguous	>24kbp
Percent reference coverage	percent_reference_coverage	>83%

<sup>&</sup>lt;sup>1</sup> Metrics and thresholds presented for guidance only as there are currently no standard assembly metric requirements; internal validation procedures will ultimately define acceptable assembly QC parameters

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	•	

## 4.3 DETERMINING SARS-CoV-2 CLADES, LINEAGES, AND WHO VARIANTS OF CONCERN (VoC)

- 1. Navigate to the *data* tab of the Terra workspace containing SC2 data of interest
- 2. *Open the data table* by clicking on the name of the data table in the left sidebar
- 3. View settings above the data table (Fig 6), select none (Fig 7)
- 4. Select the following columns: *nextclade\_clade* and *pango\_lineage* 
  - a. <u>Optional</u>: save this column group for future use by clicking the save this column selection field, naming it (e.g. SC2\_Results), and clicking save
- 5. Click done
- 6. Determine the Nextclade clade for each sample
  - a. In the data table, find the column titled <u>nextclade\_clade</u>; result formats will use the following nomenclature: <u>21L (Omicron)</u> where:
    - i. 211 indicates the sample clade and
    - ii. In parentheses, (Omicron), contains the WHO variant of concern classification
       1. Not every sample will belong to a WHO classification
  - b. Samples indicated as recombinant may indicate a case where multiple strains have combined during viral replication producing a new lineage
  - c. More information on SARS-CoV-2 recombinants can be found at the following Github site: <u>pipeline-resources/docs/sc2-recombinants.md at main · pha4ge/pipeline-resources · GitHub</u>
- 7. Identify the Pangolin lineage for each sample
  - a. In the data table, find the column titled *pango\_lineage*; nomenclature will be similar to the following: B.1.167
  - b. For more information on each of the lineages, visit <u>https://cov-lineages.org/lineage\_list.html</u>
- 8. Follow lab-specific QC, resulting, and reporting procedures, as applicable

# 5. QUALITY RECORDS

- Raw read files
- Workflow version and input parameters
- Reference sequence, if applicable
  - SC2: Wu, F., et al. (2020). Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome. NC\_045512.2. [FASTA Genome Assembly]. NCBI. https://www.ncbi.nlm.nih.gov/nuccore/1798174254.
  - Sample read, assembly, and result-specific QC metrics
- Sample read, assembly, and result-specific QC metrics
   Allowed flow as tests as lower to use the including test and allowed to use test and allowed test and a
- All workflow outputs relevant to results, including tool and database versions

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## 6. TROUBLESHOOTING

- Consult with internal staff familiar with this procedure or contact <u>support@theiagen.com</u> for troubleshooting inquiries
- For document edit requests, contact <u>support@theiagen.com</u>

## 7. LIMITATIONS

- This SOP is written for the analysis of SC2 data; v2+ of the TheiaCoV\_ClearLabs\_PHB workflow is also compatible with the following pathogens: monkeypox virus (MPXV), human immunodeficiency virus (HIV), west nile virus (WNV), influenza virus, and respiratory syncytial viruses A and B (RSV). Refer to <u>Theiagen Public Health Resources documentation</u> for organismspecific parameters and details.
- TheiaCoV PHB v2.0.0+ workflows are not backwards compatible with older versions of Nextclade; use Nextclade Dataset Tag 2024-04-15-15-08-222 or newer

# 8. REFERENCES

- Smith, E., Wright, S., & Libuit, K. (2022, June 28). *Identifying SARS-CoV-2 Recombinants*. Github. Retrieved June 16, 2023, from <u>https://github.com/pha4ge/pipeline-</u> resources/blob/main/docs/sc2-recombinants.md#identifying-sars-cov-2-recombinants
- O'Toole, Áine et al. "Tracking the international spread of SARS-CoV-2 lineages B.1.1.7 and B.1.351/501Y-V2 with grinch." Wellcome open research vol. 6 121. 17 Sep. 2021, doi:10.12688/wellcomeopenres.16661.2
- Libuit, Kevin G., Emma L. Doughty, James R. Otieno, Frank Ambrosio, Curtis J. Kapsak, Emily A. Smith, Sage M. Wright, et al. 2023. "Accelerating Bioinformatics Implementation in Public Health." Microbial Genomics 9 (7). <u>https://doi.org/10.1099/mgen.0.001051</u>
- 4. Theiagen Genomics Public Health Bioinformatics Workflow Documentation

#### 9. REVISION HISTORY

Revision	Version	Release Date
Document creation	1	7/2023
Added TG-TER-04 reference, uncheck call caching, updated input json, figures, and formatting	2	9/2023
Removed section 4.1 for creating a metadata tsv file (refer to TG- TER-03 and TG-TER-04 for details); updated quality records and limitations sections; added primer bed file upload instructions; added appendices 10.1 and 10.2	3	5/2024
Updates for version release, aligning with Terra interface, formatting	4	5/2025

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# **10. APPENDICES**

## 10.1 IMPORTING THE THEIACOV\_CLEARLABS\_PHB WORKFLOW FROM DOCKSTORE

	Workspaces > thelagen-training-workspa Workflows	Suggested Workflows	Figure 9
ASHBOARD DATA ANA	LYSES WORKFLOWS JOB HISTORY	hapletypecaller-guef-gatk4 Runs maxiobpecaller from GATK4 in CVCF mode on a single sample	mutect2-gatk4 Implements GATK4 Mutect 2 on a single tumor normal pair
Find a Workflow	Augur_PHB V. main Service Declarates (1)	processing for variant discovery-gatk4 implements data pre-processing according to the CATK Best Practices	Validate-barn This WDL performs format validation on : SAM/bank files in a list.
Freyja_Dashboard_PHB	Freyja_FASTQ	paired fastq to unmapped barn	generate-sample-map
Figure 8.	V. main Source: Dockstore	Find Additional Workflows Dackstore Envose WDL warkflows in Dackstore, an open platform used by the CA4CH for sharing Docker bated workflows	Bread Methods Repository Use Broad workflows in Terra. Share your own choose from > 700 public workflows

- 1. In the *Terra workspace* of interest, open the *workflows* tab and click *find a workflow* (Fig 8)
- 2. In the pop-up window, click *dockstore* (Fig 9)
- 3. In the top banner click *Organizations*; then click *Theiagen Genomics* (Fig 10)
- 4. Open the Public Health Bioinformatics (PHB) collection (Fig 11)



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Theiagen	Theiagen Genomics	
GENOMICS	Public health bioinformatics for pathogen surve	illance
Collections 4	Members 2 C Updates 10	About the Organization
🍨 Public Health Bioint	formatics (PHB)	This organization does not have a description
Public Health Bioint	formatics (PHB)	This organization does not have a descrip

 To find the TheiaCoV\_ClearLabs\_PHB workflow in Windows environments, hold <u>Ctrl + F</u> and <u>search TheiaCoV\_ClearLabs</u>, then click on the link (Fig 12)



6. Click Terra to launch the workflow in Terra (Fig 13)

Last update to source repository: 3 hours ago Labels Theiagen-phb Info Launch Versions Files Tools DAG Metrics Launch with Workflow Information Source Code: TRS: #workflow/github.com/theiagen/gublic.health_bioinformatics/TheiaCoV_ClearLabs_PHB  Source Code:	ray created. 529 day	is ago						
Labels Thetagen-phb Info Launch Versions Files Tools DAG Metrics Launch with Workflow Information Source Code TRB: @workflow/qithub.com/theiagen/qublic.health.bioinformatics/TheiaCoV.Cleart.abs.PHB	Last update to sourc	e repository: 3 ho	ours ago					
Info Launch Versions Files Tools DAG Metrics Launch with Workflow Information Source Code: TR8: #workflow/qithub.com/theiagen/qublic_health_bioinformatics/TheiaCoV_ClearLabs_PHB }	Labels theiagen-phb							
Workflow Information       DNAnexus:         Source Code       Trra         TRS: #workflow/github.com/theiagen/gublic_health_bioinformatics/TheiaCoV_ClearLabs_PHB       Source Code	Info	Launch	Versions	Files	Tools	DAG	Metrics	Launch with
Workflow Information     Image: Terra       Source Code:     Source Code:       TRS: #workflow/github.com/theiagen/gublic.health_bioinformatics/TheiaCoV_ClearLabs_PHB								DNAnexus
Source Code: TRS: #workflow/github.com/theiagen/public.health.bioinformatics/TheiaCoV_ClearLabs.PHB								
TRS: #workflow/github.com/theiagen/public_health_bioinformatics/TheiaCoV_ClearLabs_PHB	Workflow In	formation						Terra
	Workflow Ir	formation						Terra
Topic: Bioinformatics workflows for genomic characterization, submission preparation, and genomic epidemiology of	Workflow Ir Source Code: TRS: <u>#workflow</u>	formation	agen/public_health_b	ioinformatics/The	iaCoV_ClearLabs_F	<u>2HB</u>		Terra
	Workflow Ir Source Code: TRS: <u>#workflow</u> Topic: Bioinform	formation	agen/public_health_b for genomic characte	ioinformatics/The	iaCoV_ClearLabs_F on preparation, and	PHB <b>S</b> genomic epidemic	ology of	Terra

7. Choose the *destination workspace* in the dropdown and click *import* (Fig 14)

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# **10.2 ADDING WORKSPACE DATA ELEMENTS**

- 1. Navigate to the *Terra workspace* where analysis will be run
- To upload local files, open the *Files* tab in the right-side panel of the workspace (Fig 15, box)
   a. Click *upload* (Fig 16)
  - b. Once the upload is complete, *right click* on the file name and click *copy link*

DASHBOARD DATA	ANALYSES WORKFLOWS	SUBMERSION HISTORY Select a data type from the navgation panel on the left	() Sata
TABLES V			\$0.00 par hour
Billumine_PE_v2-1-2 (25)		-	
No references have been added. Add reference data			
Workspace Data			Figure 15.
= 向 w	ORKSPACES	Workspaces > theiagen-training-workspaces/Theiagen_Kropp_Sandbox >	

= 🤎	WORKSP	ACES Data
DASHBOARD	DATA	ANALYSES WORKFLOWS JOB HISTORY
sd_theiaprok	(103) ①	Elles a UPLO
sd_theiaprok.	_set (2) ①	Name
🗉 sra_fetch (28	I) ①	Assemblies/
sra_fetch_set	(4) ③	Candida_albicans_ATCC_10231.fasta
Figure 16.	(5) ①	B EASTAG

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- 3. Open the *workspace data* tab (Fig 15, circle)
- 4. Click Edit and Add variable in the top tool bar (Fig 17)



- 5. Click in the key field and name the element being added (Fig 18)
  a. E.g. to add the Artic v4-1 primer bed file, the key Artic\_v4-1\_primer\_bed may be used
- 6. In the value field, choose *string* as the value type
  - a. Paste the file path; the value should start with gs://
  - b. NOTE: For other string elements like dataset tags and docker images paste the ID value i.E.g. for the nextclade docker image, add nextstrain/nextclade:2.14.0
     ii.Always ensure the docker images and dataset tags are aligned with versions used for internal validation procedures
- 7. <u>Optional</u>: A description may be added to denote the date updated with staff initials
- 8. Click the blue check mark on the right-hand side of the variable to save it
  - a. The variable will now be available as a workflow input which can be found by typing the prefix *workspace*. plus the key name *artic\_v4-1\_primer\_bed* 
    - i.e.g. *workspace.*artic\_v4-1\_primer\_bed

Key	Value	Description Figure 18
Artic_V4-1_primer_bed	V4-1_nCoV-2021,primer.bed	
Artic_V4_primer_bed	V4_nCoV-2021,primer.bed	
FreyjaLineageMetadata	surated_lineages.json	Taken from Freyja_Workflows Demo Data
FreyjaUsherBarcodes	usher_barcodes.csv	Updated 8/3/23; taken from Freyja_Workflows Demo Data
Freyja_ReferenceGenome	nCoV-2019.reference.fasta	MN908947.3
Midnight_primer_bed	Midnight_Primers_SARS-CoV-2.scheme.bed	
SWIFT_primer_bed	gs://theiagen-public-files/terra/theia	Updated 2023-07-05 kk