

Document TG-TER-03, Version 4 Date: Wor 5/1/2025

Workflow Versions: PHB v3.0.0

IMPORTANT NOTES

Metadata column headers and workflow input text indicated in gray in this SOP are

customizable; **black** is required text.

1. PURPOSE/SCOPE

To standardize the process of uploading next generation sequencing (NGS) data from local storage or the Sequencing Reads Archive (SRA) and creating and uploading a results metadata table using the online Terra platform for downstream Theiagen workflow analysis. Additional instructions are provided for importing workflows and adding workspace data elements and files. Acceptable NGS data types include Illumina, Oxford Nanopore Technology (ONT), ClearLabs, and FASTA file formats. Read the comprehensive workflow 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.

3. RELATED DOCUMENTS

Document Number	Document Name
TG-TER-04	Linking BaseSpace and Importing
<u>10-12R-04</u>	BaseSpace Reads to Terra Workspace

4. PROCEDURE

4.1 IMPORTING LOCAL RAW READS

- 1. Sign in to <u>https://app.terra.bio/</u> using a Gmail account and Google Authentication (Figure 1)
- 2. Click on the *hamburger icon* in the top left and navigate to *Workspaces* (Figure 2)
- 3. *Open the workspace* designated for analysis
- 4. In the data tab, click *Import data* and select *Open data uploader* (Figure 3**Error! Reference source not found.**)
- 5. For new data sets, click *Create a New Collection* (Figure 4Error! R eference source not found.)
 - a. The following nomenclature may be useful: YYYYMMDD_# (do not include spaces)
- 6. Click Upload or drag and drop raw sequencing reads into the data Figure 1 table





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- 7. When upload is complete, files will populate on this page with *file names hyperlinked* to their corresponding Google Bucket location (Figure 5)
- 8. Continue to section 4.2 to upload sample metadata.





Figure 2

Figure 3

	Collection name *	
en-training-workspaces -practices-demo	Enter a name	
	COLLECTION NAME MAY NOT CONTAIN SPACES, FORWARD SLASHES, OR ANY OF THE FOLLOWING CHARACTERS: # ? [] Cancel Create Collection	
es with a single metadata	a file describing the table structure. You can create a new collection, or add	files to an e
	+ Create a new collection	

Figure 4

4.2 IMPORTING SAMPLE METADATA (TSV FILE) AFTER CREATING COLLECTION OF READ FILES

- 1. Once files have successfully uploaded, click *Next* (Figure 5)
- 2. Import the associated sample metadata file or drag and drop from file explorer (Figure)
 - a. See appendix 10.1 to create a sample metadata file
 - b. Alternatively, use the auto-generation option and carefully review

AUTOGENERATE TABLE FOR SINGLE OR PAIRED END SEQUENCING

- *i.* When using this function, the collection name should be the desired table name
- 3. Click *create table* (Figure)

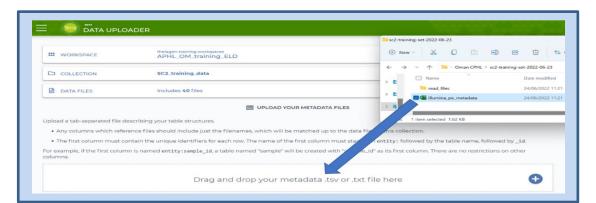


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- 4. View the uploaded read data and metadata in the Terra data table by clicking *View the...table in the workspace* at the bottom of the screen
- 5. In the data tab, all read files and metadata are now populated for each sample in the associated table (e.g. *illumina_pe_specimen* table)
 - a. Open the data table by clicking on the table name in the sidebar (Figure 8)

UPLOAD YOUR DATA	FILES		о т >
Upload the files to associate with this collection by dragging them into the tabl		d button.	
You may upload as many files as you wish, but each filename must be unique. 200230517_GhanaTraining/			ete
Name	Size	Last modified	
13_S13_L001_R1_001.fastq.gz	41 MB	Yesterday	*

Figure 5



WORKSPACE	theiagen-training-workspaces					Change
	SC2_training_data					Change
DATA FILES	Includes 40 files					Change
		UPLOAD YOUR METADATA FILI	ES			
			ES		CANCEL	CREATE TABL
Treating a new Table: illumin this table looks right to you, click t entity: illumina_pe_specimen			run_id	i i	CANCEL	CREATE TABL
this table looks right to you, click t	he button on the right to create th	e table in your workspace.		ĩ	CANCEL	CREATE TABLE
this table looks right to you, click t	he button on the right to create th	read2	run_id	1	CANCEL	CREATE TABL
this table looks right to you, click t ent/ty: illumina_pe_specimen Sample_01	he button on the right to create th read1 13_S13_L001_R1_001.fastq	read2	run_id training_data	1	CANCEL	CREATE TABLE



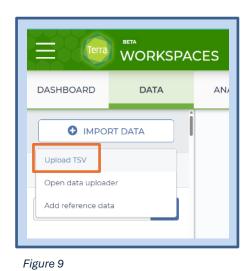
Getting Started in Terra: Metadata, Workflov				
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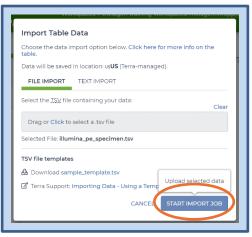
DASHBOARD DATA	ANAL	YSES WORKFLOWS JOB	HISTORY		
TheiaCoV_IIIumi (5) (i)		T 🔀 OPEN WITH 🕒 EXPORT	SETTINGS 0 rows selected		
TheiaCoV_Illumi (1) (1) Illumina_pe_sp (20) (1)	••	illumina_pe_specimen_id	readl	read2	run_id
illumina_pe_sp (14) (1		Sample_01	13_S13_L001_R1_001.fastq.gz	13_S13_L001_R2_001.fastq.gz	training_data
		Sample_02	15_S15_L001_R1_001.fastq.gz	15_S15_L001_R2_001.fastq.gz	training_data
kilifi_H3N2 (39)	0	Sample_03	17_S17_L001_R1_001.fastq.gz	17_S17_L001_R2_001.fastq.gz	training_data
kilifi_H3N2_set (5)		Sample_04	18_S18_L001_R1_001.fastq.gz	18_S18_L001_R2_001.fastq.gz	training_data

Figure 8

4.3 IMPORTING SAMPLE METADATA (TSV FILE) WITHOUT CREATING A READ COLLECTION

- 1. To upload the metadata file to Terra, return to the *Terra workspace* containing data of interest
- 2. In the *data* tab, click *import data*, and *upload TSV* (Figure)
- 3. In the pop-up window, *drag and drop* the file in the gray box or *click to select* the metadata file, then click *start import job* (Figure 10)
 - a. See appendix 10.1 to create a sample metadata file
 - b. If adding metadata to samples in an existing data table, ensure the data table name indicated in the TSV file in cell A1 contains the same data table name listed in Terra (e.g. [DATATABLE_NAME]_id)
- 4. All samples and metadata should now be populated in the corresponding Terra data table (Figure 8)



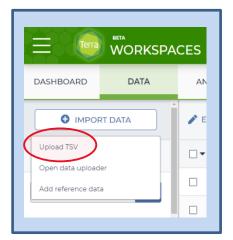




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4.4 IMPORTING RAW READS USING SRA_FETCH

- 1. Navigate to the Terra workspace that will be used to import reads
- 2. In the data tab, click on import data and upload TSV (Figure)
- 3. Import a TSV file containing the table of SRA accession numbers for desired samples: *select* or *drag and drop* the file, then click *start import job* (Figure 4)
 - a. A template TSV file can be downloaded from this pop-up; follow appendix 10.1 to create a metadata/TSV file (for SRA Fetch, the TSV file does not need read1 or read2 columns, only sra_accession) (Figure 5)



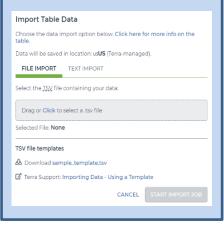


Figure 11

Figure 42

kleb_training_id	sra_accession	acquisition	hospital	month
INF004	ERR1023740	HA	Α	4
INF026	ERR1023759	HA	Α	4
INF029	ERR1023762	Nosocomial	Α	4
INF055	ERR1023788	Nosocomial	С	5
INF064	ERR1023715	HA	Α	5
INF074	ERR1008633	Nosocomial	Α	5

- 4. In the workflows tab, click SRA_Fetch_PHB
 - a. Refer to appendix 10.2 for how to import a workflow into a workspace
- 5. Set the workflow *version* to the latest version, or the workflow version used for internal validations
- 6. Choose the second bullet to run workflow(s) with inputs defined by data table
- 7. Select the sample table to use under *select data table*
 - a. Do not choose the "set" table version



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- 8. Click select data and mark the checkboxes to specify which sample reads to import
- 9. Set the first input setting to *this.sra_accession*, where *sra_accession* is the TSV file column name containing SRA numbers (Figure 7)
- 10. In the outputs tab, click *use defaults* (Figure 6) and *save* the workflow
- 11. Then click run analysis, enter any comments if desired, and click launch
- 12. Fetched reads will be populated in the *read1* and *read2* columns of the data table

SCRIPT •• INPUTS	•• OUTPUTS 🔒 •• RUN	ANALYSIS	
de optional inputs			
Task name ↓	Variable	Туре	Attribute
etch_sra_to_fastq	sra_accession	String	this.sra_accession
etch_sra_to_fastq	cpus	Int	Optional
etch_sra_to_fastq	disk size	Int	Optional

Figure 74

	Download json Drag or click to upload json Clear outputs SEARCH OUTPUTS
Туре	Input value Use defaults
File	this.abricate_abaum_plasmid_tsv []
String	this.abricate_abaum_plasmid_type_genes {}

Figure 65

4.5 IMPORTING RAW READS FROM BASESPACE

- 1. Command line steps are required for the initial setup between BaseSpace and Terra. Refer to the following sites for details on initial setup and the import process:
 - a. Theiagen's BaseSpace Fetch Documentation
 - b. Document TG-TER-04, Version 3

5. QUALITY RECORDS

- Raw read files
- Metadata results table
- Workspace elements and files



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6. TROUBLESHOOTING

- 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
- For workspace data and files, search for them in workflow input dropdowns using the prefix *workspace*.
- If the first cell in the metadata table does not end with <u>_id</u>, an error message will prevent file import; adjust the metadata text in cell A1 and re-upload
- If any workflow, input, or output settings are entered incorrectly, the analysis will not run as expected; verify all settings are correct and re-launch analysis
- If analysis fails, navigate to the job history in the workspace and click on the job submission for details; for help resolving run failures, email <u>support@theiagen.com</u>

7. LIMITATIONS

N/A

8. REFERENCES

- 1. 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). https://doi.org/10.1099/mgen.0.001051
- 2. Theiagen Genomics Public Health Bioinformatics Workflow Documentation
- 3. Theiagen's <u>BaseSpace Fetch</u> and <u>SRA Fetch</u> workflow resource pages
- 4. Theiagen's Docker Image and Reference Materials for SARS-CoV-2
- 5. Theiagen's Public Health Resources

9. REVISION HISTORY

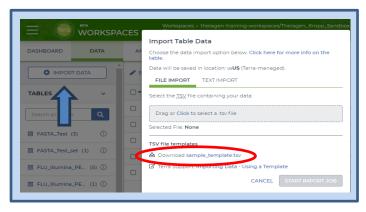
Revision	Version	Release Date
Document Creation	1	7/2023
Added internet speeds recommended for up/download, important notes, section 4.5, SRA metadata info in appendix 9.1, and appendix 9.3	2	9/2023
Formatting (Figure references and cross-references check), section 4.1 - 4.4 updates, inclusion of figure 15, appendix 10.1 updates	3	4/2024
Edits for version release, aligning with Terra interface, and formatting	4	5/2025

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10. APPENDICES

10.1 CREATING A METADATA FILE (TSV FILE)

1. Open the downloadable TSV template located in the Import Data/Upload TSV pop-up window (Figure 6)





- 2. Cell A1 must contain the following text: [name]_id (Figure 17)
 - a. Do not include spaces or characters other than hyphens (-) or underscores (_)
 - *b.* The data table name should contain text to indicate the project name (e.g. Cell A1 may read *HAI_id* to create a table for all HAI organisms)
- 3. Enter all *sample IDs* into column 1 below cell A1
- 4. Label column headers for desired metadata (do not include spaces or special characters)
 - *a.* <u>Optional</u>: enter run_id as a column header and the run ID for each sample in the column
 - b. Optional: add additional metadata columns and sample information, as needed

HAI <mark>_id</mark>	<mark>run_id</mark>	sample_matrix	<mark>county</mark>
2168435186	SEQ217	NP swab	Adams
2168435187	SEQ217	Buccal swab	Alameda



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- 5. **For manually-uploaded reads** via the *Data Uploader*, include read columns (Figure 18)
 - a. For paired-end (PE) sequencing: read1 and read2

	A	B	C	D	E	F
l	HAI_id	read1	read2	run_id	sample_matrix	county
2	2168435186	100734_R1.fastq.gz	100734_R2.fastq.gz	SEQ217	NP swab	Adams
3	2168435187	100735_R1.fastq.gz	100735_R2.fastq.gz	SEQ217	Buccal swab	Alameda
4	2168435188	100735_R1.fastq.gz	100735_R2.fastq.gz	SEQ217	Buccal swab	Tulare
5	2168435189	100736_R1.fastq.gz	100736_R2.fastq.gz	SEQ217	NP swab	Gilpin

Figure 18

- b. For single-end (SE) sequencing: only read1
- c. Use the *file name* as the value for this column (e.g. 100734_R1.fastq.gz) (Figure 19)

Opload □ New folder		🖾 Analyze in Seqr 🖌	🕽 Refresh
Name	Size	Last modified	
00734_R1.fastq.gz	35 MB	Dec 1, 2024	1
00734_R2.fastq.gz	42 MB	Dec 1, 2024	0

Figure 19

6. For SRA uploads:

- a. *Label the column header* for column 2 as *sra_accession*, or similar
- b. Enter SRA accessions as the values for this column (e.g. SRR# or ERR#)

HAI <mark>_id</mark>	sra_accession	hospital	month	year	age	run_id
2168435186	ERR1023740	D	4	2013	30-39	SEQ217
4831845358	SRR11445892	А	2	2014	80-89	SEQ217
8415835241	ERR4087740	С	6	2013	60-69	SEQ217
6846813545	SRR11842392	А	3	2013	80-89	SEQ217

- 7. In Excel, click *save as* and change the file type to *Text (Tab delimited)*.
- 8. Refer to section 4.2 to import TSV files at the same time as creating new read collections (importing files locally) and section 4.3 to import TSV files without creating read collections



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10.2 IMPORTING A WORKFLOW FROM DOCKSTORE

- 1. In the *Terra workspace* of interest, navigate to the *workflows* tab and click *find a workflow* (Figure 20)
- 2. In the pop-up window, click *Dockstore.org* (Figure 21)
- 3. Workflows may be found through the search bar or by navigating through the organization if it is known
 - *a.* To find a Theiagen workflow
 - *i.* click *organizations* (Figure 22)
 - *ii.* In the search bar type *Theiagen* (Figure 22)
 - iii. Click on the *logo*, *view*, or *# collections*. (Figure)
 - *iv.* Click on the collection to see available workflows: *Public Health Bioinformatics (PHB)*
- 4. Find and *open the workflow* (workflow name listed at the end of the file path) (Figure 24)
- 5. Click Terra to launch the workflow in Terra (Figure)
- 6. Choose the *destination workspace* in the dropdown
- 7. Click *import* or *create a new workspace* (Figure 26)

			rkflows	_
SHBOARD DATA	ANAL	YSES	WORKFLOWS	JOB HISTO
WORKFLOWS				
Find a Workflow		Augu	ur_PHB	
0		V. ma	in :e: Dockstore	(1)
		Source	e: Dockstore	
Freyja_Dashboard_PHB		Freyj	a_FASTQ	
		V. ma	in	(;)

Figure 20

Dockstore.org 2"		Terra Workflow Repository
A community repository of best pract that offers integration with GitHub.	ice workflows	A repository of WDL workflows that offers private workflows hosted in the platform.
Curated collections from our communit		ad Disalinas (2
ATK Best Practices 🛙		ad Pipelines (? omics G*
ATK Best Practices II VDL Analysis Research Pipelines II	Long Rea Viral Gen	

Figure 21

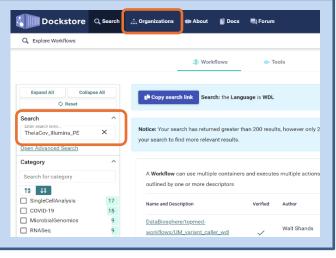


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1. Organizations		
orgenizations	Sort by •	ganitations
20 Conception Conceptio	Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration	* 23
7 Collections View	4 Collections	View

Figure 23

Dockstore Q Search 🚓 Organizations 📾 About 🕤	Docs 뤈 Forum Login Regist
ally Organizations / Theisgen Generaliss / Public Health Bioinformatics (PHB)	
Collection: Public Heal	th Bioinformatics (PHB)
Vorkflows and Tools	About the Collection
Ofthub conv/theiagen/public_health_bioinformatics/TheiaCoV_Cleas Ofthub conv/theiagen/public_health_bioinformatics/TheiaCoV_Cleas Instrupterel Al 21.3023 Ont Vime	
github.com/theiager/public_health_bioinformatics/TheiaCoV_Clear Labs_PHByt1.0.0	Public Health Bioinformatics (PHB) Bioinformatics workflows for characterization, epidemiology and sharing of pathoge

Figure 24



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github.com/theiagen/public_health_bioinformatics/TheiaCoV_ClearLabs_PHB:v1.0.0					
ag created: 38 days ago					
Last update to source repository: 4 hours ago					
abels thelagen-phb					
< Info	Launch	Versions	Files	Tools >	Launch with
Workflow Information Source Code TRS: #workflow/github.com/theisgen/gublic.health.bioinformatics/TheisCoV.ClearLabs.PHE Topic: Bioinformatics workflows for genomic characterization, submission preparation, and genomic epidemiology of pathogens of public health concern. Checker Workflow: n/g					

Figure 25

Importing from Dockstore	Workflow Name
github.com/theiagen/public_health_bioinformatics/TheiaCoV_ClearLabs_P HB V. V10.0	TheiaCoV_ClearLabs_PHB
Please note: Dockstore cannot guarantee that the WDL and Docker image referenced by this Workflow will not change. We advise you to review the WDL before future runs.	Destination Workspace
<pre>1 version 1.0 2 3 import "//tasks/assembly/task_artic_consensus.wdl" as artic_consensus 4 import "//tasks/quality_control/task_assembly_metrics.wdl" as assembly_metr 5 import "//tasks/quality control/task ncbi scrub.wdl" as ncbi scrub</pre>	TheiaCoV_Training_Demos



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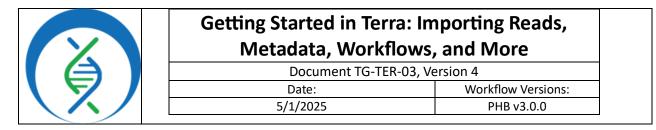
10.3 ADDING AND UPDATING WORKSPACE DATA ELEMENTS AND FILES

- 1. Navigate to the *Terra workspace* where analyses will be run
- 2. To upload local files:
 - a. Open the *workspace files* tab in the right-side panel of the workspace (Figure 7).
 - b. Click upload
 - c. Following upload, click the *clipboard* or *right click* on the file name to *copy link*
- 3. To add workspace variables:
 - a. Click the *workspace data* tab at the bottom of the left-side panel (Figure 27)
 - b. Click *Edit* in the top menu (Figure 28)
 - c. Click Add Variable (Figure 28)
 - d. Click in the *key field* and *name the element* being added (Figure 9)
 - e. In the value field, choose *string* as the value type
 - f. Paste the file path copied above in step 2, or enter the desired file path (see below)
 - i. E.g. to add the Artic V4-1 primer bed file, the key Artic_V4-1_primer_bed may be used with the file path as the value (Figure 30)
- 4. Docker images and tags for TheiaCoV workflows should be included in workspace variables.
 - a. Ensure the docker images and dataset tags are aligned with versions used for internal validation procedures or are re-verified before use.
 - b. Available in the Docker Image and Reference Materials for SARS-CoV-2
 - c. All resources can be found on the <u>Theiagen Public Health Resources</u> page



DATA	ANALYSES	WORKFLOWS	SUBMISSION HISTORY	
I Edit Download TSV 0 rows selected				
in (5) (i Add variable Upload TSV Delete selected variables				

Figure 28



	Edit	La Download TSV 0 rows selected		Search
	Ŧ	Key	Value	Description
		pangolin_docker_image	us-docker.pkg.dev/general-theiagen/staphb/pangolin:4.3	updated by Curtis on 2025-02-13
L			String v	
_				

Figure 29

Key		Value	Description
Artic_V3	_primer_bed	V3_nCoV-2019,primer.bed	
Artic_V4	-1_primer_bed	V4-1_nCoV-2021.primer.bed	
Artic_V4	_primer_bed	V4_nCoV-2021.primer.bed	
FreyjaLin	eageMetadata	curated_lineages.json	Taken from Freyja_Workflows Demo Data
FreyjaUsl	herBarcodes	usher_barcodes.csv	Updated 8/3/23; taken from Freyja_Workflows D
Freyja_Re	eferenceGenome	<u>nCoV-2019.reference.fasta</u>	MN908947.3
Midnight	t_primer_bed	Midnight_Primers_SARS-CoV-2.scheme.bed	
SWIFT_p	rimer_bed	SWIFT_SARS-CoV-2.scheme.bed	Updated 2023-07-05