

Document TG-TER-03, Version 4Date:Work5/1/2025P

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 NumberDocument NameTG-TER-04Linking BaseSpace and Importing<br/>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! Reference 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 *	
n-training-workspaces	Enter a name	
	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 ad	d 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					
Upload the files to associate with this collection by dragging them into the table below, or clicking the Upload button.					
You may upload as many files as you wish, but each filename must be unique.           200230517_GhanaTraining/         Image: Delete					
O Name	Size	Last modified			
Q 13 513 1001 01 001 forte er	41 MB	Vesterday			

#### Figure 5



III WORKSPACE	theiagen-training-workspaces APHL_OM_training_ELD					Change
	SC2_training_data					Change
DATA FILES	Includes 40 files					Change
			5			
reating a new Table: illumin this table looks right to you, click t	a_pe_specimen he button on the right to create th	UPLOAD YOUR METADATA FILE e table in your workspace.	S		CANCEL	CREATE TABLE
this table looks right to you, click t entity: Illumina_pe_specimen	a_pe_specimen he button on the right to create th read1	UPLOAD YOUR METADATA FILE     table in your workspace.     read2	s run_ld	ī	CANCEL	CREATE TABLE
this table looks right to you, click t entity: Illumina_pe_specimen Sample_01	he_pe_specimen he button on the right to create th read1 13_513_L001_R1_001.faster.	UPLOAD YOUR METADATA FILE e table in your workspace.  read2 13_S13_LOO1_R2_001_fastq	s run_id training_data	Ĩ	CANCEL	CREATE TABLE
reating a new Table: illumin this table looks right to you, click t <i>ently:</i> illumina_pe_specimen [] Sample_01 Sample_02	a_pe_specimen he button on the right to create th read1 13.513.L001.R1.001fasto 15.515.L001.R1.001fasto	UPLOAD YOUR METADATA FILE table in your workspace.  read2  13.513_L001_R2_001/fasta  15.515_L001_R2_001/fasta	s run_ld training_data training_data	1	CANCEL	CREATE TABLE
reating a new Table: Illumin this table looks right to you, click t <i>entity</i> : Illumina_pe_specimen Sample_01 Sample_02 Sample_03	Be_specimen     He button on the right to create th     read1     13_515_L001_P1_001_fasta,-     15_515_L001_R1_001_fasta.     7_517_L001_R1_001_fasta.	UPLOAD YOUR METADATA FILE     table in your workspace.     read2     13.513.L001.R2_001fastq     15.515.L001.R2_001fastq     17.517_L001.R2_001fastq	s run_ld training_data training_data training_data	100	CANCEL	



Getting Started in Terra: Im Metadata, Workflows,	porting Reads, , and More
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DASHBOARD DATA	ANAL	YSES WORKFLOWS JOB	HISTORY		
ThelaCoV_IIIumL (5)  C EDIT X OPEN WITH B+ EXPORT  SETTINGS O rows selected					
illumina pe sp. (20)	•	illumina_pe_specimen_id 🕴 🕕	readl	read2	run_id
		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.oz	15_S15_L001_R2_001.fastq.oz	training_data
■ KIIITLH3N2 (39) ()	0	Sample_03	17_S17_L001_R1_001_fastq.gz	<u>17_S17_L001_R2_001.fastq.oz</u>	training_data
■ kilifi_H3N2_set (5) ①		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 1)
  - 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 1)





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	A	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
- Set the first input setting to this.sra\_accession, where sra\_accession is the TSV file column name containing SRA numbers (Figure 1)
- 10. In the outputs tab, click *use defaults* (Figure 1) 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

COTPOIS RON ANALISIS		
Variable	Туре	Attribute
sra_accession	String	this.sra_accession
cpus	Int	Optional
disk_size	Int	Optional
	Variable sra_accession cpus disk_size	Variable     Type       sra_accession     String       cpus     Int       disk_size     Int

Figure 74

		Download json   Drag or click to upload json   Clear outputs SEARCH OUTPUTS	
τy	уре	Input value   Use defaults	
Fi	ile	this.abricate_abaum_plasmid_tsv []	
St	tring	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

Getting Started in Terr Metadata, Workfl	a: Importing Reads, ows, and More	
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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	В	С	D	E	F
1	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)

O Upload D New folder		🗹 Analyze in Seqr	C Refresh
Name	Size	Last modified	
D00734_R1.fastq.gz	35 MB	Dec 1, 2024	:
00734_R2.fastq.gz	42 MB	Dec 1, 2024	()

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 (Figure 22)
  - *a.* To find a Theiagen workflow
    - *i.* click *organizations* (Figure 2)
    - *ii.* In the search bar type *Theiagen*
    - 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)

	CES	Worl WO	kspaces > theiagen- Irkflows	training-worksp
ASHBOARD DATA	ANAL	/SES	WORKFLOWS	JOB HISTOR
WORKFLOWS				
Find a Workflow		Augu	ur_PHB	
0		V. ma Sourc	in :e: Dockstore	(!)
Freyja_Dashboard_PHB		Freyj	a_FASTQ	

Figure 20

Dockstore.org		Terra Workflow Repository
A community repository of best pra that offers integration with GitHub.	clice workflows	A repository of WDL workflows that offers private workflows hosted in the platform.
Curated collections from our commun	ity:	d Dinelines 17
Curated collections from our commun GATK Best Practices ශ් VDL Analysis Research Pipelines ශ්	i <b>ity:</b> Long Rea Viral Gen	id Pipelines (? omics (?
turated collections from our commun .ATK Best Practices ගී VDL Analysis Research Pipelines ග් fsit our documentation to loarn how to	ity: Long Rea Viral Gen import and configu	d Pipelines 67 omics 67 ure your workflow, as well as how to save time and money.

Figure 21



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Lockstore Q Search 📩 Organizations 🐲 About 🛛 🕤 Docs 🔍 Forum Q Explore Workflows 🐉 Workflows - Tools Expand All Collapse All Copy search link Search: the Language is WDL 🗘 Reset Search Notice: Your search has returned greater than 200 results, however only 2 Enter search term... TheiaCov\_Illumina\_PE × your search to find more relevant results. pen Advanced Searc Category A Workflow can use multiple containers and executes multiple actions Search for category outlined by one or more descriptors tậ ↓ª SingleCellAnalysis 17 Name and Description Verified Author COVID-19 15 MicrobialGenomics 9 DataBiosphere/topmed-RNASeq 9

1. Organizations		
rgenizations	Sort by •	h Digentratices
Alaxy     The Galaxy Intergalactic Workflow Comission     The Galaxy Intergalactic workflow Comission	Theiagen Genomics	* 23
nna grung o chain to chain an a bhine a region y o chai pheasac annina ny Philippi de chain to chain an ann a bhine a region y o chai pheasac annina ny Philippi de chain anni anni anni anni anni anni anni	Contraction accommission on portragen an extension     Contraction accom     Contraction accommission ac	

Figure 23

Dockstore Q Search 🖧 Organizations 🖝 About 👔 f	Docs 퇸j Forum Login Regist
ally Organizations / Theisgen Benemics / Public Health Bioinformatics (PHB)	
Collection: Public Healt	h Bioinformatics (PHB) gen genomics
Vorkflows and Tools	
	About the Collection
github.com/thelager/public_health_bloinformatics/ThelaCoV_Clear     Labs_PHBv1.0.0     Los updatel Jul 21,322     With     Wate	About the Collection Public Health Bioinformatics (PHB)
Other Convirteiagen/public_health_bioinformatics/TheiaCoV_Clear Labs_PH5v1.0.0      Introduct Al 27.2022     WR      Other Convirteiagen/public_health_bioinformatics/TheiaCoV_ONT_ PH5v100	About the Collection Public Health Bioinformatics (PHB) Bioinformatics workflows for characterization, epidemiology and sharing of pathoge genomes.

Figure 24



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<b>)</b>	github.com/theiag	gen/public_health	n_bioinformatics/Tl	heiaCoV_ClearLat	os_PHB:v1.0.0	☆
ig creat	ted: 38 days ago					
ist upda	ate to source repository:	4 hours ago				
bels th	nelagen-phb					
<	Info	Launch	Versions	Files	Tools >	Launch with
Wo Sour TRS Topi	rkflow Informatio	n <u>thelagen/public_health</u> wws for genomic charact	bioinformatics/TheiaCoV_ terization, submission prep	ClearLabs_PHB	demiology of	Terra Vel.wation AnVIL
path	ogens of public health o	oncern.				NHLBI BioData Catalyst®
Chee	cker Workflow:				n/a	

Figure 25

Importing from Dockstore	Workflow Name
github.com/theiagen/public_health_bioinformatics/TheiaCoV_ClearLabs_P HB V.V1.0.0	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_nchi_scrub_wdl" as pois 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 8)
  - 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 🛓 D	ownload TSV 0 rows selected		
in (5) ( Add variable				
	Upload TSV	•		
in (1) (-	Delete selected variables	token		

Figure 28



Key     Value     Description       pagolin_docker_image     us-docker,pkg.dev/general-theiagen/staphb/pagolin/4.3     updated by Curtis on 2025-02-13	🎤 Edit	L Download TSV 0 rows selected			Search
pangolin_docker_image us-docker.pkg.dev/general-theiagen/staphb/pangolin:4.3 updated by Curtis on 2025-02-13	•	Кеу	Value	Description	
	1.1	pangolin_docker_image	us-docker.pkg.dev/general-theiagen/staphb/pangolin:4.3	updated by Curt	tis on 2025-02-13
String v			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	
FreyjaLineageMetadata	<u>curated_lineages.json</u>	Taken from Freyja_Workflows Demo Data
FreyjaUsherBarcodes	usher_barcodes.csv	Updated 8/3/23; taken from Freyja_Workflows D
Freyja_ReferenceGenome	<u>nCoV-2019.reference.fasta</u>	MN908947.3
Midnight_primer_bed	Midnight_Primers_SARS-CoV-2.scheme.bed	
SWIFT_primer_bed	SWIFT_SARS-CoV-2.scheme.bed	Updated 2023-07-05