

Document TG-TER-03, Version 5

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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 in the appendices 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 here.

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3. REQUIRED RESOURCES

- Computer
- Internet connection: at least 10 and 5Mbps for download and upload speeds, respectively
- Internet browser
 - o Google Chrome, Firefox, or Edge
- Google account
- Terra account, linked to Google account

IMPORTANT NOTES

Metadata column headers and workflow input text indicated in gray in this SOP are customizable; **black** is required text.

4. RELATED DOCUMENTS

Document Number	Document Name
TG-TER-04	Linking BaseSpace and Importing
<u>1G-1ER-04</u>	BaseSpace Reads to Terra Workspace

5. PROCEDURE

5.1 IMPORTING LOCAL RAW READS

- 1. Sign in to https://app.terra.bio/ 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)
- 5. For new data sets, click Create a New Collection (Figure 4)
 - 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 table



Figure 1

- 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 5.2 to upload sample metadata.



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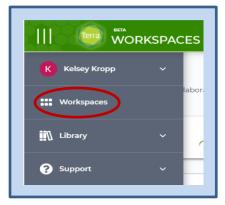




Figure 2

Figure 3

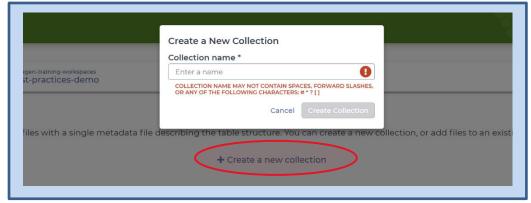


Figure 4

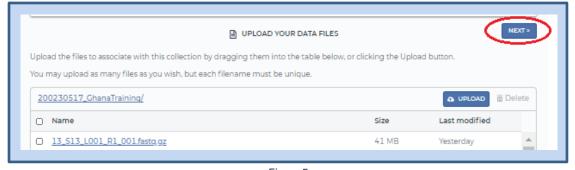


Figure 5

5.2 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 6)
 - a. See appendix 10.1 to create a sample metadata file



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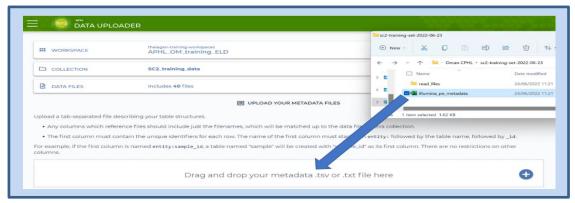


Figure 6

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 7)

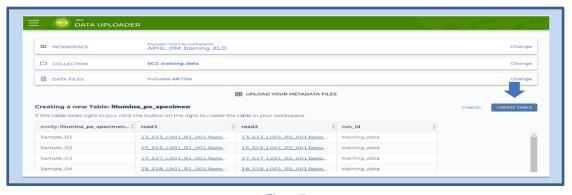


Figure 7

- 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)



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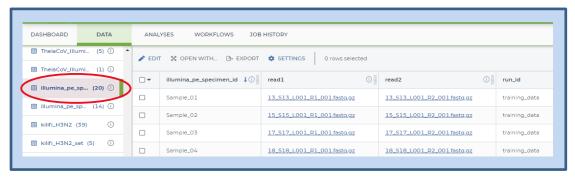
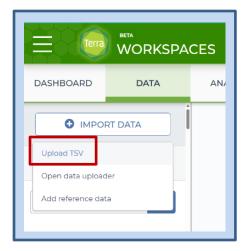


Figure 8

5.3 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)



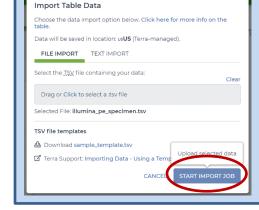


Figure 9

Figure 10

4. All samples and metadata should now be populated in the corresponding Terra data table (Figure 8)



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5.4 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)
 - 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 4)

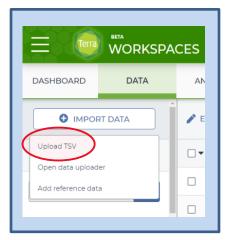




Figure 11

Figure 12

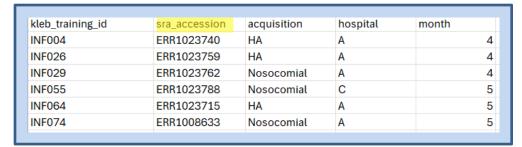


Figure 13

- 4. In the workflows tab, click SRA_Fetch_PHB
 - a. Refer to appendix 10.2 for how to import a workflow into a workspace



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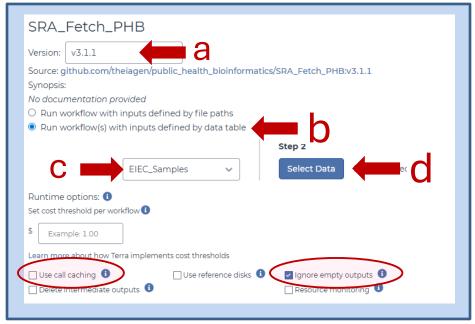


Figure 14

- 5. Set the workflow *version* to the latest version, or the workflow version used for internal validations (Fig 14, a)
- 6. Choose the second bullet to run workflow(s) with inputs defined by data table (Fig 14, b)
- 7. Select the sample table to use under *select data table* (Fig 14, c)
 - a. Do not choose the "set" table version
- 8. Click *select data* (Fig 14, d) and *mark the checkboxes* to specify which sample reads to import (Fig 15)

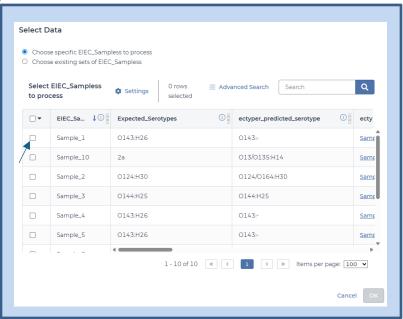


Figure 15



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- 9. Set the first input setting to *this.sra_accession*, where *sra_accession* is the TSV file column name containing SRA numbers (Figure)
- 10. In the outputs tab, click *use defaults* (Error! Reference source not found.) 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

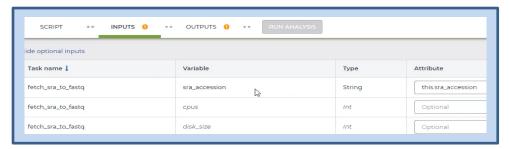


Figure 16

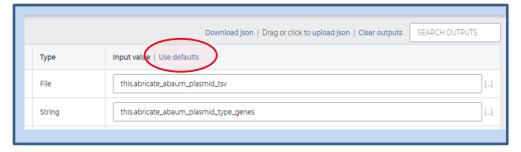


Figure 17

5.5 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 <u>BaseSpace Fetch Documentation</u>
 - b. Document TG-TER-04, Version 3

6. QUALITY RECORDS

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



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7. 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 support@theiagen.com

8. LIMITATIONS

N/A

9. 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 BaseSpace Fetch and SRA Fetch workflow resource pages
- 4. Theiagen's <u>Docker Image and Reference Materials for SARS-CoV-2</u>
- 5. Theiagen's Public Health Resources

10. 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	2	9/2023
9.3		
Formatting (Figure references and cross-references check), section	3	4/2024
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
Edits for clarity, including a table of contents, additional figures,		
appendix on referencing workspace elements in a workflow, and	5	10/2025
figure reference and caption corrections		



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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 18)

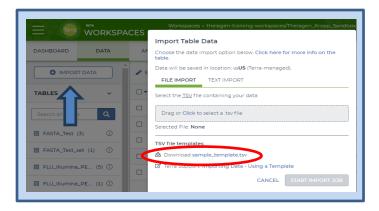


Figure 18

- 2. Cell A1 must contain the following text: [name]_id (Figure 19)
 - 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) (Table 1)
 - a. Optional: 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>	run_id	sample_matrix	county
2168435186	SEQ217	NP swab	Adams
2168435187	SEQ217	Buccal swab	Alameda

Table 1. Metadata setup with required and optional columns



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	A	В	C	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 19

- 5. **For manually-uploaded reads** via the *Data Uploader*, include read columns (Figure 19)
 - a. For paired-end (PE) sequencing: read1 and read2
 - 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 20)

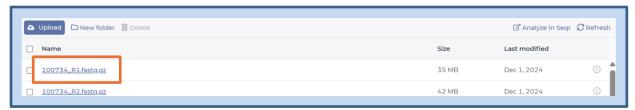


Figure 20

6. For SRA uploads:

- a. Label the column header for column 2 as sra_accession, or similar (Table 2)
- 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	Α	2	2014	80-89	SEQ217
8415835241	ERR4087740	С	6	2013	60-69	SEQ217
6846813545	SRR11842392	А	3	2013	80-89	SEQ217

Table 2. Metadata table setup for SRA uploads.

- 7. In Excel, click save as and change the file type to Text (Tab delimited).
- 8. Refer to section 5.2 to import TSV files at the same time as creating new read collections (importing files locally) and section 5.3 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 21)
- 2. In the pop-up window, click *Dockstore.org* (Figure 22)
- 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 23)
 - ii. In the search bar type *Theiagen* (Figure 23)
 - iii. Click on the logo, view, or # collections. (Figure 24)
 - 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 25)
- 5. Click *Terra* to launch the workflow in Terra (Figure 26)
- 6. Choose the destination workspace in the dropdown
- 7. Click import or create a new workspace (Figure 27)

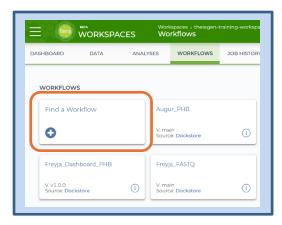


Figure 21

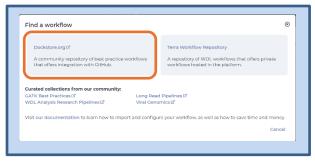


Figure 22



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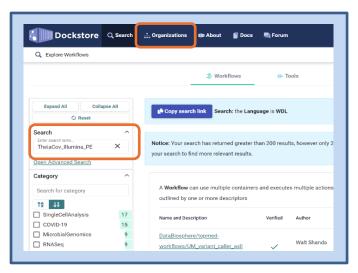


Figure 23

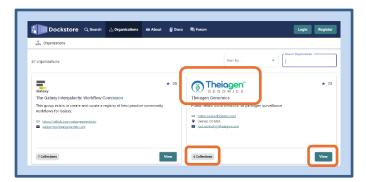


Figure 24



Figure 25



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Figure 26



Figure 27



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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 28).
 - b. Click upload
 - Following upload, click the *clipboard* or *right click* on the file name to *copy link* (Figure 29)
- 3. To add workspace variables:
 - a. Click the workspace data tab at the bottom of the left-side panel (Figure 28)
 - b. Click *Edit* in the top menu (Figure 30)
 - c. Click Add Variable (Figure 30)
 - d. Click in the key field and name the element being added (Figure 31)
 - 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 32)
- 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 Theiagen Public Health Resources page



Figure 28



Figure 29



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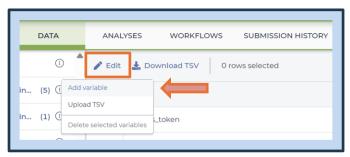


Figure 30



Figure 31

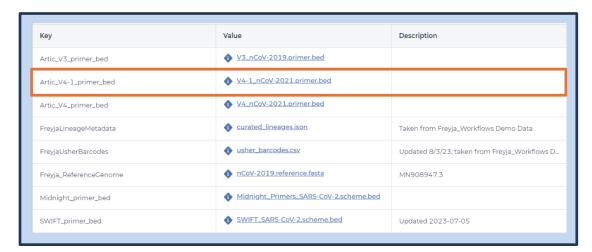


Figure 32



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10.4 REFERENCING A WORKSPACE FILE IN A WORKFLOW

When there is a workflow input that you may wish to reference regularly (e.g. a Kraken database or reference assembly file), you can indicate the file saved as a workspace element (see above section 10.3 for guidance on creating a workspace element).

1. Make sure the reference file you want to use is uploaded and stored as a workspace element by going to the Data tab of your workspace, scrolling down to the bottom to click where it says "Workspace data" (Figure 33).

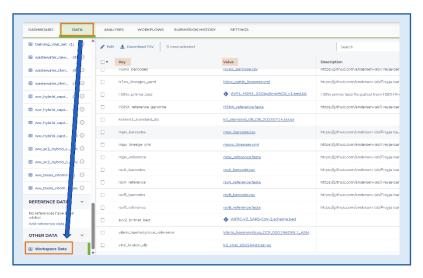


Figure 33

2. Return to the Workflows tab and go to the variable that would require a workspace data element. Type "workspace."--you will see all the workspace data elements you have saved appear in a dropdown. Continue typing or scroll to find the appropriate element for your workflow and select it (Figure 34).



Figure 34