

Concatenation of ONT Reads in Terra		
Document TG-ONT-01, Version 1		
Date:	Workflow Version:	
09/19/2025	PHB v3.1.1	

1. PURPOSE/SCOPE

This procedure describes how to run the ONT_Barcode_Concatenation_PHB workflow using the Terra platform. This is intended for use with unconcatenated Oxford Nanopore Technology (ONT) reads, where there are multiple reads per barcode that need to be concatenated into a single file for each barcode. The output of this workflow is per-barcode concatenated ONT reads that can be used as the input for a workflow that requires one read file per sample.

2. REQUIRED RESOURCES

- Computer
- Internet connection: at least 10 and 5Mbps for download and upload speed, respectively
- Internet browser
 - o Google Chrome, Firefox, or Edge
- Google account
- Terra account, linked to Google account
- ONT raw sequencing read files uploaded to Terra workspace
- Theiagen's ONT Barcode Concatenation PHB workflow in Terra

3. RELATED DOCUMENTS

Document Number	Document Name
TG-TER-03	Getting Started in Terra: Importing Reads, Metadata, Workflows, and More
<u>TG-TP-ONT</u>	Analyzing Bacterial Data in Terra using Theiagen's TheiaProk ONT Workflow

4. PROCEDURE

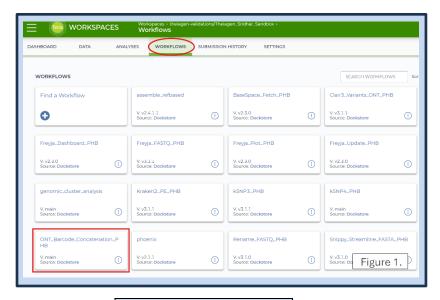
Prior to running the ONT_Barcode_Concatenation_PHB workflow, the workflow must be imported into the Terra workspace. For details see **Appendix 10.1**.

4.1 Configure and run the ONT_Barcode_Concatenation_PHB Workflow

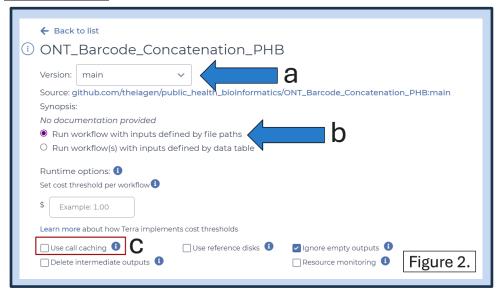
- 1. Open Terra, navigate to the workflows tab, and select the ONT_Barcode_Concatenation_PHB workflow (Fig 1).
 - a. To import the workflow for the first time, refer to Appendix 10.1.



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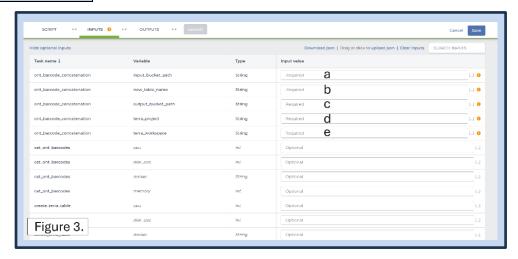
- 2. In the version dropdown field, *select the workflow version* that was internally validated, or the latest version of the workflow (Fig 2, a).
- 3. Select the first bullet to run workflow with inputs defined by file paths (Fig 2, b).
- 4. Uncheck *Use call caching* (Fig 2, c).





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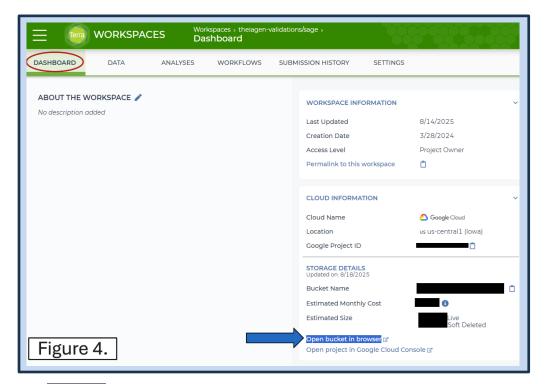
- 5. There are five required input fields (Fig 3). Skip to **step 12** if you already know the input and output bucket paths and have uploaded your raw ONT data to the input bucket.
 - a. *input_bucket_path* (Fig 3, a)
 - b. *new_table_name* (Fig 3, b)
 - c. output_bucket_path (Fig 3, c)
 - d. terra_project (Fig 3, d)
 - e. terra_workspace (Fig 3, e)



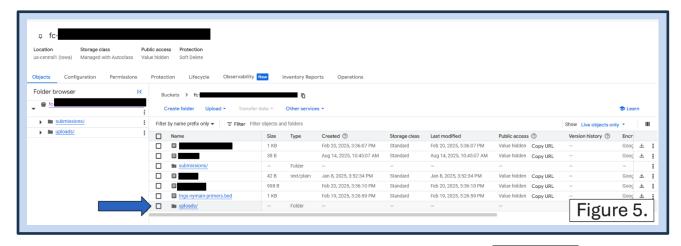
- 6. There are additional optional input fields you may wish to change. See section 4.2 for instructions on these inputs. These inputs are:
 - a. barcode_renaming_file
 - b. file extension
- 7. To determine and select the appropriate <u>input_bucket_path</u>, navigate to the Terra workspace Dashboard tab and click on "Open bucket in browser" under the "Cloud Information" toggle on the right-hand side (Fig 4).



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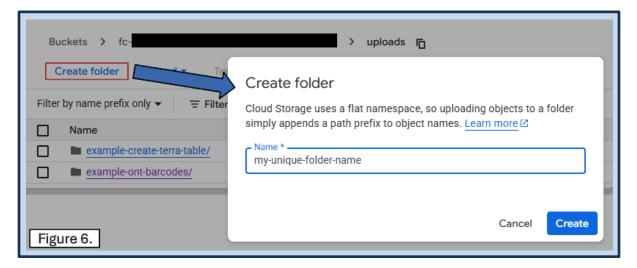
8. Click on the uploads/ folder (Fig 5).



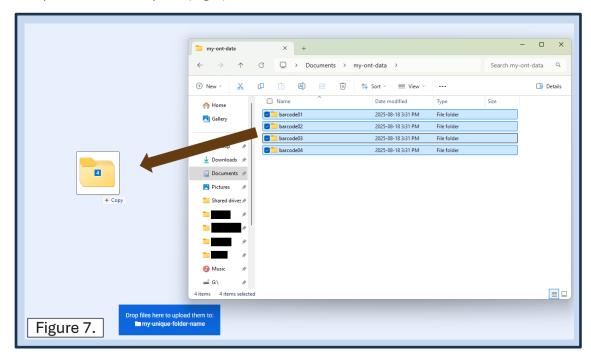
- 9. If you need to create a new folder for your data, click on the blue text <u>Create folder</u> on the top left (Fig 6). Give the folder a unique name to identify the run or group of data. Click on <u>Create</u> once you have typed the new folder name.
 - a. If you have an existing folder that you want to add data to, click on the existing folder.



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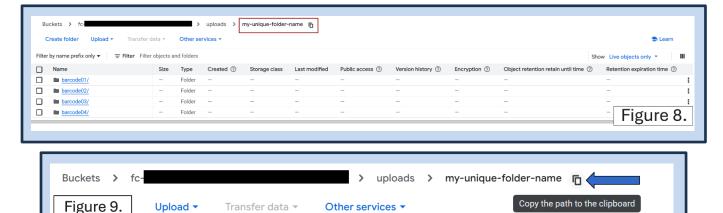
10. Click on the newly created or existing folder to navigate into it. You can now drag and drop entire barcode directories from your computer into this folder (Google bucket). This directly copies the data to your Terra workspace (Fig 7).



- 11. Check that your files have been uploaded correctly (Fig 8).
- 12. Click on the icon to the right of the folder name to copy the file path to your clipboard (Fig 9).



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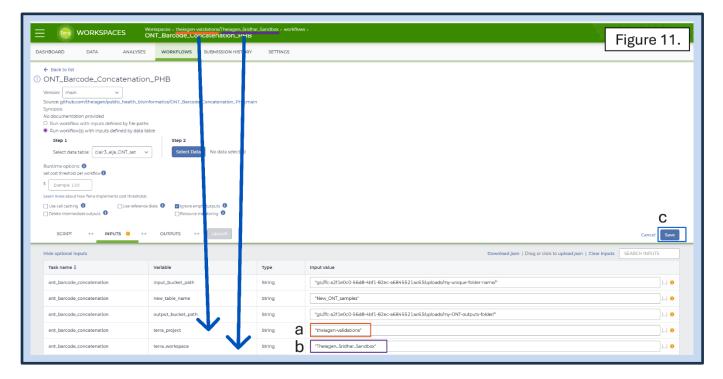
- 13. Paste this input bucket path into the required input <code>input_bucket_path</code> in the ONT_Barcode_Concatenation workflow (Fig 3a), making sure that the file path begins with "gs://" and that the full path is in quotes ("") (Fig 10, a).
- 14. The <u>new_table_name</u> input can be a string of your choosing. If you are adding new reads to an existing data table, use the same table name. Otherwise, create a new table name and enclose it in quotes (Fig 10, b).
- 15. The <u>output_bucket_path</u> is the path to where you would like the concatenated files to go. This output bucket can be created in the same way as the input bucket above (see steps 6-8, 11). It is recommended that a new output bucket is created each time the workflow is run if you wish to avoid overwriting the <u>upload_date</u> column in the workflow output (Fig 10, c).



- 16. The input <u>terra_project</u> is the name of the Terra billing project your workspace is in. It can be determined by looking at the top bar in green following "Workspaces >" (Fig 11, a). This string should be inputted in quotes.
- 17. The input <u>terra_workspace</u> is the name of your specific workspace. This can be found following the Terra project name in the green bar at the top (Fig 11, b). This string should be inputted in quotes.



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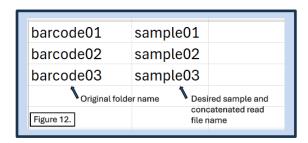
- 18. Once all required inputs have been filled in, press the *Save* button above the inputs section (Fig 11, c).
- 19. If running with default options, press "Launch" to begin the workflow.

4.2 Running optional parameters

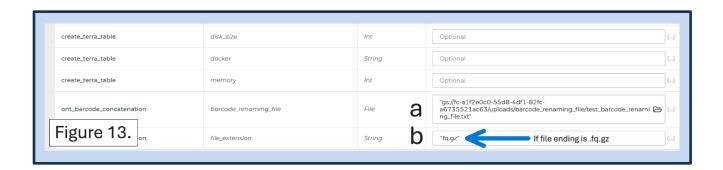
- 1. By default, each concatenated file and sample name will take the name of the parent folder that contains the unconcatenated files. (E.g. if the barcode folder was called barcode01, the concatenated file and sample name will be named barcode01.) Thus, if you wish to change the read file name for the concatenated file and the sample's name in the new data table, it is recommended to provide a file to the input barcode_renaming_file. The file format is a tabdelimited (.txt or .tsv) file with no header. The first column is the original folder name (e.g. barcode01), and the second column is the desired sample and read file name (e.g. sample01) (Fig 12).
- 2. Upload this file to your Terra bucket using either the Data Uploader or by clicking on the file icon on the right sidebar. Refer to *Appendix 10.2* for how to upload a file to a Terra bucket.



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- 3. Copy the file path into the barcode renaming file variable (Fig 13, a).
- 4. The input <u>file_extension</u> should be used if the reads files are not in the conventional format. In the case that files are unzipped fastq files (.fastq instead of .fastq.gz) or have a different naming scheme (.fq.gz instead of .fastq.gz), you can supply the ending of your files. Input the file ending in quotes (Fig 13, b).



5. QUALITY RECORDS

- Raw read files
- Workflow version and input and output parameters
- Any required sample ID nomenclature for concatenation

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 support@theiagen.com



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7. LIMITATIONS

- The workflow will not be able to successfully concatenate files in an incorrect file structure. Files to be concatenated together must be provided within a discrete folder.
- If the same output file bucket path is provided for multiple runs, and it contains existing files from prior runs, the workflow will overwrite the date of upload in the data table for existing files in the output bucket. It will not, however, overwrite any files as long as each file has a unique name.

8. REFERENCES

¹"ONT_Barcode_Concatenation_PHB." Theiagen Public Health Bioinformatics, Theiagen Genomics,

https://theiagen.github.io/public health bioinformatics/main/workflows/data import/ont b arcode concatenation/#data-upload. Accessed 29 September 2025.

9. REVISION HISTORY

Revision	Version	Release Date
Document creation	1	10/2025

10. APPENDICES

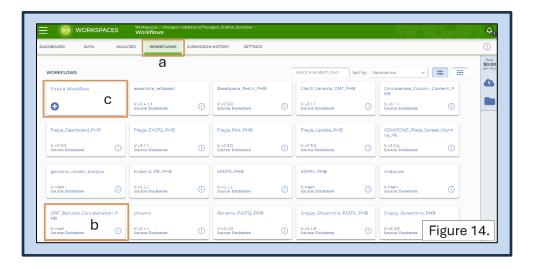
10.1 Find and Import the ONT_Barcode_Concatenation_PHB Workflow

- 1. Navigate to the workflows tab of the workspace (Fig 14, a).
- Users who already have the ONT_Barcode_Concatenation_PHB workflow in their workspace can select the workflow (Fig 14, b) and proceed to the Configure and Run the ONT_Barcode_Concatenation_PHB Workflow section of this SOP.

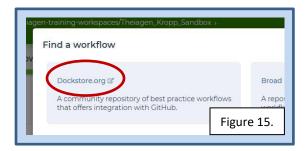


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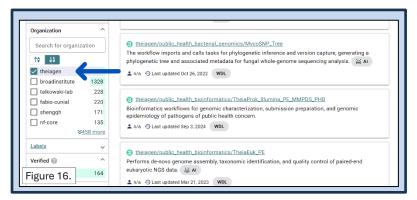
a. To import the workflow, click find a workflow (Fig 14, c).



b. In the pop-up window, click *Dockstore.org* in the bottom left (Fig 15).



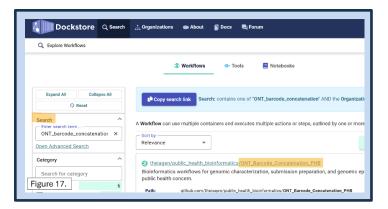
c. Look at the left bar and scroll down until you reach the *Organization* heading. Check the box for *theiagen* to filter for only Theiagen workflows (Fig 16).





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d. Scroll back to the top of the page and in the *Search* bar on the left, type *ONT_barcode_concatenation* (Fig 17). Click on the workflow.





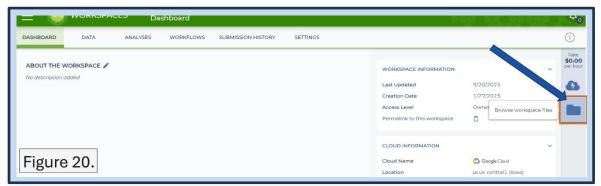
- e. Click on the Terra icon (Fig 18) to import the workflow into a Terra workspace.
- f. Select the workspace in the destination workspace dropdown field and click Import (Fig 19).



10.2 Uploading a file to a Terra bucket

One can add a file to the Uploads folder in Terra the same way a new folder was created above to upload reads to the input bucket (steps 6-9 of section 4.1). However, one can alternatively navigate to the Uploads folder and upload a file as follows:

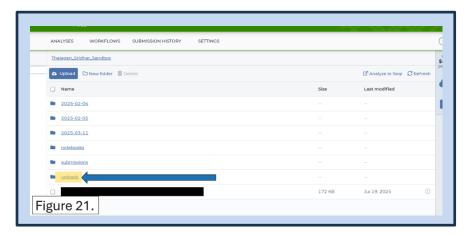
1. From any tab in Terra, click on the Folder icon on the right hand side of the page (Fig 20).





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2. Click on the Uploads folder within the Workspace files (Fig 21).



3. If you wish to create a new folder, click on <u>New Folder</u>. When you are ready to upload your barcode renaming file, click on the <u>Upload</u> button. This will open a file explorer on your computer to find the file locally and upload it to your Terra folder (Fig 22).



4. Click on the clipboard icon directly to the right of the newly uploaded file to copy the path for the ONT_barcode_concatenation workflow (Fig 23). Refer to step 3 and Figure 13 in section 4.2 for further detail.

