Analyzing Bacterial Da Theiagen's TheiaProl	C C	
Document TG-TP-ONT, Version 2		
Date:	Workflow Version	
5/1/2025	PHB v3.0.0	

1. PURPOSE/SCOPE

To standardize the process of analyzing bacterial next generation sequence (NGS) data using Theiagen's TheiaProk_ONT_PHB workflow in Terra to generate assemblies, quality control (QC) metrics, and determine predicted taxonomy, serotype/serogroup, sequence type (ST), AMR profile, and plasmid content. Additional analyses are optional in TheiaProk but are not addressed herein. Acceptable data types include ONT raw read file format. Lab-specific QC metrics and acceptance criteria should be established to ensure the integrity of the end-to-end NGS test system. Read the docs here.

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*.

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
- ONT raw sequencing read files uploaded to Terra workspace, see TG-TER-03
- Theaigen's TheiaCoV_ONT_PHB workflow in Terra, see TG-TER-03 appendix 9.2

3. RELATED DOCUMENTS

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

4. PROCEDURE

4.1 CREATE A SAMPLE METADATA FILE (TSV FILE) FOR RAW READS OR SRA FETCH

- 1. In Excel, <u>create a list</u> containing the following sample information:
 - a. For all analyses:
 - Column 1 header (Fig 1): <u>BacterialONT_id</u> where <u>BacterialONT</u> is the name of the data table/group of samples to be analyzed
 - ii. List all *sample IDs* in column 1

	А	В			
1	BacterialONT_id	reads			
2	Sample_01	gs://fc-a0493432-4788-42e6-8b			
3	Sample_02	gs://fc-a0493432-4788-42e6-8b			
4	Sample_03	gs://fc-a0493432-4788-42e6-8b			
E Comple 04					
Figure 1: Raw Read Metadata File. 2-4788-42e6-8					



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BacterialONT id

Sample_01

Sample 07

Sampla 11

sra accession

SRX17082331

SRX17082330

CDV17000200

Figure 2: SRA Accession Metadata File.

run id

SEQ137

SEQ137

SEQ137

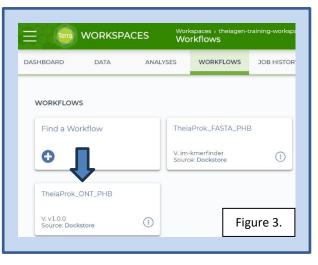
SEO137

b. For analysis from raw sequencing reads (Fig 1)

- i. Column 2 header: reads
- ii. List the *full file paths* to read1 files in the cloud
- c. For analysis using SRA fetch (Fig 2):
 - i. Column 2 header: sra_accession
- d. <u>Optional</u>: remaining columns may be used to add metadata like run_id, additional lab results, sample collection information, demographic data, etc
- e. Do not include spaces in the headers
- 2. Save as a txt or tsv file
- Upload to Terra workspace; see TG-TER-03 for details

4.2 RUNNING THE THEIAPROK WORKFLOW

- Open Terra and navigate to the workflows tab of the workspace containing bacterial data
- 2. Select the *TheiaProk_ONT_PHB* workflow (Fig 3)
- Choose the *latest version of the workflow* in the version dropdown field, or the workflow version that was used during internal assay validation (Fig 4, a)



- 4. Select the second bullet to *run workflow(s) with inputs defined by data table* (Fig 4, b)
- 5. Select the relevant data table under the select data table dropdown (Fig 4, c), e.g. BacterialONT
- 6. Click select data (Fig 4, d)

TheiaProk_ONT_PHB
Version: v1.2.1 v Caral a
Source: github.com/theiagen/public_health_bioinformatics/TheiaProk_ONT_PHB:v1.2.1
Synopsis:
No documentation provided
O Run workflow with inputs defined by file paths
Run workflow(s) with inputs defined by data table
RestorialONT A RestorialONT A RestorialONT_set named
BacterialONT SELECT DATA SELECT DATA SELECT DATA SELECT DATA
Use call caching 🕕 Delete intermediate outputs 🕕 Use reference disks 🕕 Retry with more memory 🕕 Ignore e Figure 4.



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- In the pop-up window select the checkbox for each sample to be included in the analysis (Fig 5)
 - a The checkbox at the top may be used to select all samples listed on the page
 - b Click the down arrow and select all to process all specimens
 - c Additionally, a subset of samples may be chosen using the search bar to filter before selecting the

Selec	t BacterialONTs to process	SETTINGS 5 rows selected	⊟ ADVANCED SEARCH	Search
		reads		
	Sample_01	F13916_Kpne.fastq.gz		
	Sample_02	<u>F16067_Paer.fastq.gz</u>		
	Sample_03	F16068_Kpne.fastq.gz		
	Sample_04	F16390_Abau.fastq.gz		
	Sample_05	F16398_Abau.fastq.gz		
Selecte	d BacterialONTs will be save	d as a new BacterialONT_set named:	1-5of5 « < 1	> > Items per page: 100 •
Thois	Prok_ONT_PHB_2023-12-19T17	-07-09		

checkbox at the top to only select samples matching the search criteria

- d The set of samples will be saved in the *BacterialONT_set* Terra data table and can be named for easier identification using the text field in the bottom left of this pop-up window (e.g. the group of samples could be saved as the run ID, HAI, etc)
- e Scroll to the bottom and click ok
- 8. Uncheck *Use call caching* (Fig 4)
- Set the first two input attributes in the table to *this.reads* and *this.BacterialONT_id*, respectively (Fig 6) where:
 - a *this.BacterialONT_id* is the unique name of your data table in Terra

SCRIPT •• INPUTS ()	OUTPUTS RUN ANALYSIS		CANCEL SAV
lide optional inputs		Download json	Drag or to upload json Clear inputs SEARCH INPUTS
Task name ↓	Variable	Туре	Attribute
theiaprok_ont	readl	File	this.reads
theiaprok_ont	samplename	String	this.BacterialONT_id []
Figure 6.	сри	Int	Optional {

- 10. Specify outputs by clicking on the *outputs* tab and *use defaults* (Fig 7)
- 11. Click *save*
- 12. Launch the workflow by clicking *run analysis* (Fig 7); enter desired comments and click *launch*

	Th	eiagen's	cterial Data in Terra using TheiaProk ONT Workflow	
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SCRIPT •• INPUTS Output files will be saved to Files / submission unique ID / theiapu References to outputs will be written to Tables / BacterialONT Fill in the attributes below to add or upd	rok_ont / workfi			CANCEL SAVE
Task name l	Variable	Time		SEARCH OUTPUTS
Task name ↓ theiaprok_ont	Variable abricate_abaum_plasmid_tsv	Type	Download json Drag or click to upload json Clear outputs Attribut Use defaults this.abricate_abaum_plasmid_tsv	SEARCH OUTPUTS
			Attribut Use defaults	

4.3 RAW READ AND ASSEMBLY QUALITY ASSESSMENT

- 1. Follow all quality assessment procedures specified by the instrument manufacturer, sequencing program (PulseNet, GenomeTrakr, etc), and those determined during internal validation procedures, as appropriate
- 2. Raw read data quality assessment may include looking at parameters such as average read quality scores; these should be determined during validation activities
- 3. Assembly-level quality assessment may include evaluating outputs such as average coverage, assembly length, contig number, etc; these should be determined during validation activities

4.4 VIEWING EXAMPLE QUALITY METRICS IN TERRA

- 1. In the data tab of the Terra workspace containing TheiaProk results, open the relevant data table
- 2. Click *Select Columns* above the data table, select *none* (Fig 8)
- 3. Select the appropriate columns:
 - a. assembly_length
 - b. est_coverage_clean
 - c. gambit_predicted_taxon
 - d. nanoplot_r1_mean_q_clean
 - e. number_contigs
 - f. quast_gc_percent
 - *g.* <u>Optional</u>: save this column group for future use by clicking the save this column selection field, naming it (e.g. QC), and clicking save (Fig 8)
- 4. Click done
- 5. Compare all metrics to relevant QC requirements and determine pass/fail calls for all samples



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- 6. For samples not passing QC metrics, re-sequence
 - a. Failed QC samples may proceed to analysis at the discretion of the laboratory
- 7. For samples passing QC metrics, continue to analysis section 4.5

DASHBOARD DATA	ANAL	YSES WORKFLOWS	SUBMISSION HISTORY	Show: II none	Sort: alphabetical	Search	Q
amr_phoenixSD_set (1) ①	P Edit	🗙 Open With 🕒 Export	: 🏚 Select Columns 🛛 0	agrvate_summary agrvate_version		Save This Column Selection	
🛙 amr_theiaprokS (225) 🕕	•	bacterialONT_id 10	abricateplasmid_ts	amrfinderplus_all_report		Your saved column selections:	•
🖪 amr_theiaprokSD (3) ①		Sample_01		amrfinderplus_amr_classes amrfinderplus_amr_core_genes		GenomeCharacteristics () QC ()	
🛙 augur (25) 🕕		Sample_02		amrfinderplus amr core genes		-	
🗈 augur_set (3) 🕕		Sample_03		amrfinderplus_amr_subclasses			
🗉 bacterialONT (5) 🕕		Sample_04		amrfinderplus_db_version amrfinderplus_stress_genes			
🔳 bacterialONT_set (1) 🕕		Sample_05	Sample_05_abricate_hits.tsv	amrfinderplus_stress_report			
🖲 bacterialphylog (108) 🕕				amrfinderplus_version amrfinderplus_virulence_genes			
🖪 bacterialphylogen (7) 🕕							Cancel Do
🛙 cdphKmer (87) 🕕							
🖪 cdphKmer_set (1) 🕕							
Figure 8.							

4.5 DETERMINING GENOME CHARACTERISTICS

- 1. Navigate to the *data* tab of the Terra workspace containing bacterial data
- 2. Open the data table by clicking on the name of the data table in the left sidebar
- 3. Click Select Columns above the data table, select none (Fig 8)
- 4. Select the following columns:
 - a. amrfinderplus_amr_core_genes
 - b. gambit_predicted_taxon
 - c. plasmidfinder_results
 - d. For serotype and serogroup results:
 - *i. ectyper_predicted_serotype*: serotype predicted by ECTyper for *Escherichia coli*
 - ii. *kaptive_k_type*: Kaptive predicted K type for *Acinetobacter baumannii*
 - iii. kleborate_ktype: Kleborate predicted K type (capsule) for Klebsiella spp. serotyping
 - iv. kleborate_otype: Kleborate predicted O type (LPS) for Klebsiella spp. serotyping
 - v. *lissero_serotype*: serotype predicted by LisSero for *Listeria monocytogenes*
 - vi. *meningotype_serogroup*: serogroup predicted by meningotype for *Neisseria meningitidis*
 - vii. *pasty_serogroup*: serogroup predicted by Pasty for *Pseudomonas aeruginosa*



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viii. *seqsero2_predicted_serotype*: serotype predicted by SeqSero2 for *Salmonella spp*.

ix. *serotypefinder_serotype*: serotype predicted by SerotypeFinder for *E. coli* and *Shigella spp*.

x. *shigatyper_predicted_serotype*: serotype predicted by ShigaTyper for *Shigella sonnei*

xi. *shigeifinder_serotype*: serotype predicted by ShigEiFinder for *Shigella sonnei*

xii. *sistr_predicted_serotype*: serotype predicted by SISTR for *Salmonella spp*.

xiii. *sistr_serogroup*: serogroup predicted by SISTR for *Salmonella spp*.

xiv. *srst2_vibrio_serogroup*: O1 and O139 serotype prediction by SRST2 for *Vibrio spp*.

e. For sequence type (ST) results:

- i. *kleborate_mlst_sequence_type*: Kleborate predicted ST for *Klebsiella spp*.
- ii. *legsta_predicted_sbt*: Legsta predicted sequence-based typing for *Legionella pneumophila*

iii. ngmaster_ngmast_sequence_type: Ngmast predicted ST for Neisseria gonorrohoeae

- iv. *ngmaster_ngstar_sequence_type*: Ngstar predicted ST for *Neisseria gonorrohoeae*
- v. *sistr_serotype_cgmlst*: SISTR predicted ST for *Salmonella spp*.
- vi. *ts_mlst_predicted_st*: Torsten Seemann predicted ST for bacterial 7-gene MLST
- f. <u>Optional</u>: save this column group for future use by clicking the save this column selection field, naming it (e.g. Pseudo), and clicking save (Fig 8)
- 5. Click done
- 6. Determine the predicted results for each sample by viewing the respective columns, as desired
- 7. Follow lab-specific resulting and reporting procedures, as applicable

5. QUALITY RECORDS

- Raw read files
- Sample read, assembly, and result-specific QC metrics
- All workflow outputs relevant to results including tool and database versions

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

- 1. The TheiaProk_ONT_PHB workflow only runs on bacterial, single-end ONT data
- 2. Poor base quality, short read length, and nonuniform sequencing depth can impact the ability to adequately perform de novo assembly and affect downstream tool predictions
- 3. Sequencing of mixed/contaminated cultures will affect the accuracy of result predictions
- 4. GAMBIT may misclassify certain *E. coli* samples as *Shigella* species; therefore, interpret results in combination with additional tests or tools to ensure confident *E. coli* identification.



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8. **REFERENCES**

 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>

2. Theiagen Genomics Public Health Bioinformatics Workflow Documentation. https://theiagen.github.io/public_health_bioinformatics

9. **REVISION HISTORY**

Revision	Version	Release Date
Document creation	1	12/2023
Minor edits to align with Terra interface, add new outputs for species typing, improve formatting	2	5/2025

10. APPENDICES

None