1. **PURPOSE:** To describe the procedure for the performance of WGS proficiency testing within PulseNet. This is part of the PulseNet WGS QA/QC program.

2. **SCOPE:** This procedure applies to PulseNet certified personnel at participating laboratories who perform proficiency testing. Successful completion of the PulseNet proficiency test for WGS also satisfies the GenomeTrakr proficiency testing requirement.

3. **DEFINITIONS/TERMS:**

3.1 **7-gene MLST:** Multi-Locus Sequence Typing scheme based on seven conserved housekeeping genes
3.2 **Allele:** One of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome
3.3 **AMR:** Antimicrobial Resistance
3.4 **Analysis-certified:** An individual or laboratory that is certified for checking WGS data quality, performing analysis and uploading to the PulseNet National Database and NCBI using BioNumerics
3.5 **BaseSpace:** Illumina cloud-based computing environment for next generation sequencing data analysis and management, including data sharing
3.6 **BioNumerics:** Analysis software used by PulseNet, developed by Applied Maths (Sint-Martens-Latem, Belgium)
3.7 **Bundle file:** A packed BioNumerics file with a .bdl extension that contains a selection of database entries along with one or more experiments and information fields, as specified by the user
3.8 **CDC:** Centers for Disease Control and Prevention
3.9 **cgMLST:** Core Genome Multi-Locus Sequence Typing
3.10 **Critical Quality Metrics:** Average denovo coverage, average quality (Q score), assembly length, secondary species abundance (contamination), percent core present, allele difference compared to reference (wet lab PT only). Failing to meet the minimum thresholds/acceptable range for any one of these metrics will result in a failure of PT.
3.11 **Dry Lab Proficiency Test:** The annual challenge testing the participant's skills in analyzing sequence data using BioNumerics
3.12 **E-PT Survey Template:** an electronic Google Form that each participating laboratory is required to fill out and submit. This survey captures the submitting laboratory's RefID database QC results for the PT strains, results for the cluster detection challenge, general submission information and uploads of the library prep workbooks generated for the PT sequencing run(s). See Appendix PNQ09-2 for example screenshots.
3.13 **Fastq (wet lab)-certified:** An individual or a laboratory that is certified in laboratory methods for WGS
3.14 **Fastq file:** A text-based file format for storing both sequence and its corresponding quality scores
3.15 **FDA:** Food and Drug Administration
3.16 **FTP:** File Transfer Protocol; a standard network protocol used to transfer computer files from one host to another host via Internet
3.17 **"In-house fastq files":** sequencing results generated by the participating laboratory. This is a part of the proficiency testing program where laboratories produce fastq.gz files containing sequencing data.
3.18 **MB:** Mega Base (1 million base pairs)
3.19 **NCBI:** National Center for Biotechnology Information
3.20 **Organism-specific Database:** A BioNumerics database, v 7.6 or higher, used for comparing isolates for surveillance. Part of the standard PulseNet workflow.
3.21 **Proficiency Testing (PT):** A mandatory annual assessment of the quality of the work being performed in PulseNet participating laboratories

3.22 **Proficiency Test file evaluator (evaluator):** A PulseNet Central staff member who evaluates and signs off on the proficiency test reports submitted by PulseNet participants

3.23 **Proficiency Testing Packet:** An electronic PDF file including a cover letter and the proficiency testing evaluation that is emailed to the primary PulseNet contact for the laboratory and the participant's laboratory director

3.24 **Proficiency Testing Report:** A report that contains the evaluation and results of the participant's proficiency test. See templates in Appendix PNQ09-1a and PNQ09-1b.

3.25 **PulseNet Area Laboratory:** Laboratory, designated by CDC, which has agreed to assume responsibility for additional PulseNet duties for laboratories within their support zone. The current Area Laboratories include CO, MI, MN, NY, TN, VA and WA.

3.26 **PulseNet Central:** PulseNet team at CDC comprising of the PulseNet Response and Outbreak Management Team (PulseNet@cdc.gov) and the Next Generation Subtyping Methods Unit (PulseNetNGSlab@cdc.gov)

3.27 **QA/QC:** Quality Assurance/Quality Control

3.28 **RefID Database:** A BioNumerics database, v 7.6 or higher, used for quality control of raw sequence data, assembly of sequences, contamination detection, and species identification. Part of the standard PulseNet workflow.

3.29 **SOP:** Standard Operating Procedure

3.30 **Wet Lab Proficiency Test:** The annual challenge testing the participant's skills in the laboratory procedure to generate fastq files

3.31 **WGS:** Whole Genome Sequencing

4. RESPONSIBILITIES

4.1 Individuals performing PulseNet-related work (i.e., sequencing and/or analyzing data in BioNumerics) must be certified before being able to participate in the proficiency testing program. See PNQ08 (SOP for WGS Certification of PulseNet Personnel) for information on becoming certified.

4.2 Certified individuals in PulseNet participating laboratories must participate in PulseNet proficiency testing annually as part of continuing in-house QA/QC. It is recommended that all certified individuals in a laboratory perform wet lab and dry lab proficiency testing in-house, but only one set of fastq files and one set of analyzed data per pathogen per laboratory is selected and submitted to PulseNet Central for evaluation. ***GenomeTrakr participants must also share their BaseSpace projects/runs with FDA.*** **NOTE:** *Proficiency Testing is currently available for Listeria monocytogenes, Escherichia coli/Shigella* spp*., Salmonella enterica, Campylobacter* spp., and *Vibrio* spp.

- 4.2.1 Fastq (lab)-certified individuals will sequence 4 proficiency testing strains to generate in-house fastq files for submission and evaluation by PulseNet Central.
- 4.2.2 Analysis-certified individuals will analyze the 4 fastq files generated for the proficiency test strains and the bundle file containing the cluster detection challenge using the BioNumerics 7.6 PulseNet workflow. Results for the QC and the cluster detection and general submission information will be recorded on the E-PT Survey Template and submitted to PulseNet Central for evaluation together with organism database-specific BioNumerics bundle files.

5. PROCEDURE

5.1 Individuals at participating laboratories must perform the proficiency testing according to the current proficiency testing instructions that are emailed to primary PulseNet contacts and posted on SharePoint within the PulseNet Announcements forum.

5.2 Laboratories are required to include routine and/or historical *Salmonella* and/or *E. coli* isolates on the same cartridge as the proficiency testing isolates in order to fill up the run. Acceptable library preparation kits, sequencing platforms, sequencing chemistries and DNA loading capacities are listed in Appendix PNQ09-3.

5.3 At least one fastq-certified individual in a laboratory must perform wet lab proficiency testing using the complete set of 4 PT strains annually as part of in-house QA/QC. It is recommended that all fastq-certified individuals sequence at least one of the PT strains annually as part of in-house competency assessment. However, only one set of fastq files per pathogen per laboratory should be chosen for submission through the PulseNet/GenomeTrakr BaseSpace projects or the PulseNetQA FTP site.

- 5.3.1 BaseSpace sharing of **the fastq files AND the run** with PulseNet Central using the email: PulseNet@cdc.gov.
  - 5.3.1.1 Initially stream the files to a separate QC project on BaseSpace that is not shared with CDC. **NOTE:** ***do not share the fastq files until you know that the files pass critical quality metrics in the RefID database***.
  - 5.3.1.2 Files passing critical quality metrics can then be copied to a project shared with CDC. Use an existing organism-specific project already shared with the PulseNet inbox or alternatively set up a separate project for PT named "Lab ID_proficiency testing".
- 5.3.2 GenomeTrakr labs only: BaseSpace sharing of **the fastq files AND the run** with FDA using the email: GnomeTrakr@fda.hhs,gov.
- 5.3.3 Separate instructions will be provided for accessing the PulseNetQA FTP site. Email PulseNet@cdc.gov to request the FTP access credentials.
  - 5.3.3.1 If the PulseNetQA FTP site is used to share the data, also share the following files/folders so that run metrics can be evaluated: Interop folder, RunInfo, and RunParameters.

5.4 At least one analysis-certified individual in a laboratory must annually conduct dry lab proficiency testing by performing the BioNumerics analysis of the fastq files generated for the 4 wet lab PT strains and the bundle file containing the cluster detection challenge. It is recommended that all analysis-certified individuals in a laboratory perform the BioNumerics analysis of the PT materials annually as part of an in-house competency assessment. However, only one set of analysis results per pathogen per laboratory should be chosen for submission.

- 5.4.1 E-PT Survey Template is used to report general information about the PT sequencing runs, and results from the RefID database and the cluster detection challenge.
  - 5.4.1.1 Launch the survey either from the link provided in the PT announcement email or copy the link and paste into new browser screen.
  - 5.4.1.2 All questions marked with an asterisk are required.
- 5.4.2 Each of the 4 PT strains has a section for recording metrics from RefID. For each certified organism, complete the section "RefID Database Analysis Results" (Appendix PNQ09-2).
  - 5.4.2.1 In the event that the laboratory is not certified in analysis for a specific organism, respond "N/A" for the five questions associated with that organism.
- 5.4.3 For analysis of the 4 PT strains performed in the organism-specific databases, create database-specific bundle files for the analyzed results. Include all experiments and information fields that are listed in the PT instructions.
  - 5.4.3.1 Name the file following the PulseNet standard naming scheme for bundle files: *lab_id-year-sequential number for the file*. For example, the second bundle file created in 2021 by the GA lab would be named GA21002PN.bdl. **NOTE1:** Sequential numbering is not organism database-specific. **NOTE2:** when using the PulseNet bundle file tool  , the "PN" is automatically added.

5.4.4 For the analysis of the bundle file containing the cluster detection challenge in the organism-specific database, fill out the "Cluster Detection" section of the E-PT Survey Template (Appendix PNQ09-2) to record the results.

5.4.4.1 In the event the laboratory is not analysis certified for the organism included in the cluster detection challenge, select the column "N/A, does not group" for all ten isolates, allowing the required information to be processed when survey is submitted.

5.5 After filling out all the required fields and attaching the required files (cluster detection dendrogram and sequencing run workbook(s) in the E-PT Survey Template), submit the form by clicking "Submit". You will receive a confirmation email including a copy of your responses and a link facilitating editing of the responses after the survey submission.

5.6 Send an email to PulseNet@cdc.gov to notify PulseNet Central about the availability of the fastq files and the analyzed results. **NOTE:** *only send **one** email per laboratory*.

5.6.1 In the subject line of the email, include your laboratory ID and the words "WGS Proficiency Testing" (e.g., GA_WGS Proficiency Testing).

5.6.2 Attach the bundle files for the analysis PT that were created in the organism-specific databases.

5.7 The fastq files and the dry lab analysis results submitted for proficiency testing must be generated by certified individuals. Results submitted by non-certified individuals will automatically fail the proficiency testing round.

5.8 All results must be submitted correctly by the submission deadline to avoid penalty. Laboratories that cannot meet the deadline may submit a request for extension to PulseNet@cdc.gov before the submission deadline.

5.9 Laboratories must pass proficiency testing annually to maintain PulseNet certification.

5.9.1 In order to pass the wet lab PT:

5.9.1.1 The fastq files for the 4 PT strains must meet the critical quality thresholds outlined in the Appendix PNQ09-4.

5.9.1.2 A minimum of 85% of all points available must be accumulated.

5.9.2 In order to pass the dry lab PT:

5.9.2.1 The analyzed sequences for the 4 PT strains must meet the critical quality thresholds outlined in the Appendix PNQ09-4 for raw sequence data and allele calls. Correct genus & species, serotype (if applicable), virulence profile (if applicable), AMR profile, lineage (if applicable) and 7-gene MLST type must be indicated. Metadata fields must be filled out correctly.

5.9.2.2 The sequences included in the cluster detection challenge must be correctly designated either belonging to a cluster (i.e., being highly related to another sequence within the bundle file) or being sporadic (not highly related to another sequence in the bundle file). Cluster assignment must be based on the PulseNet guidelines for local cluster detection.

5.9.2.3 A minimum 85% of all points available must be accumulated.

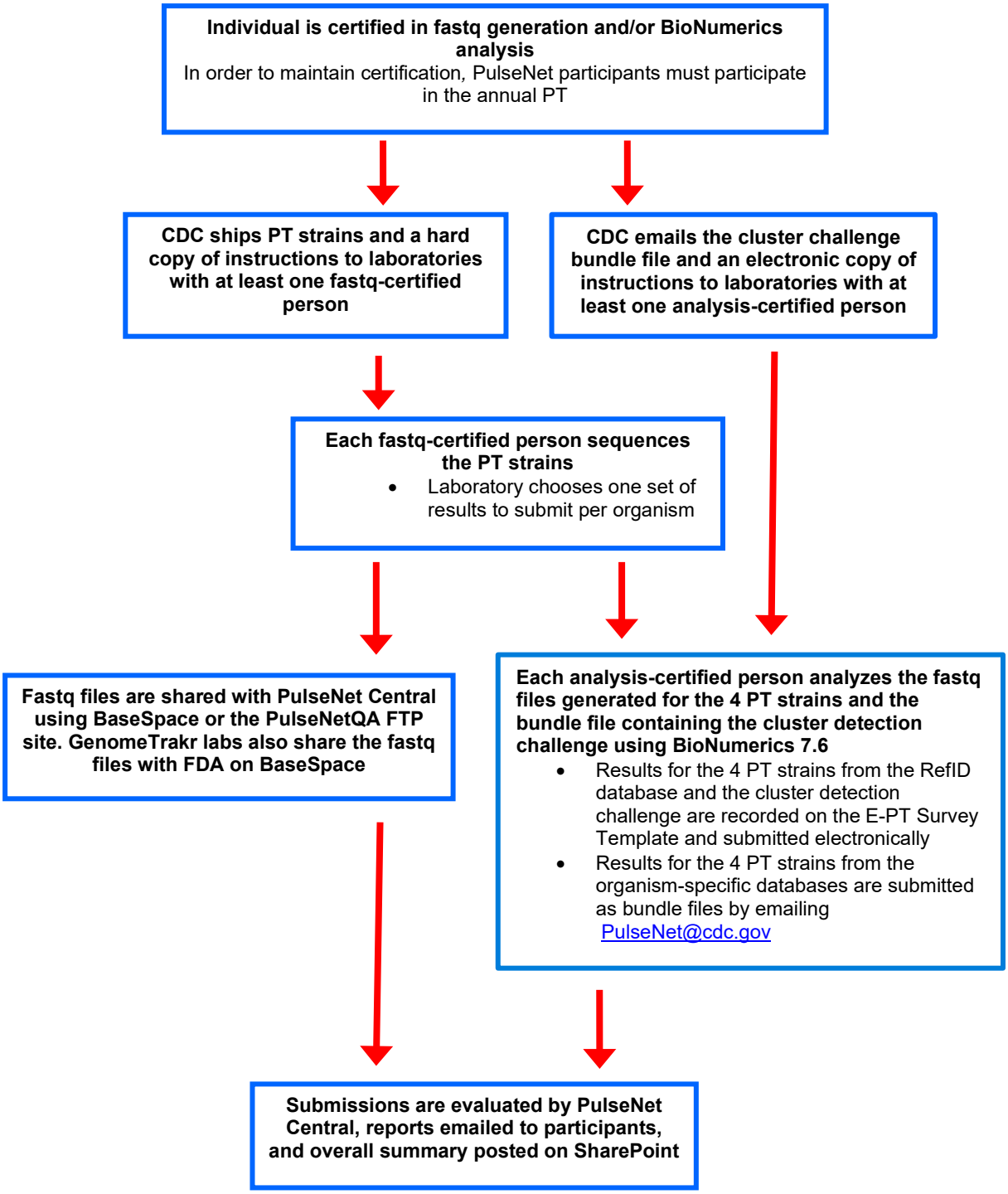
5.9.3 The evaluator will email the PT packet consisting of the cover letter (Appendix PNQ09-5) and the PT reports (Appendices PNQ09-1a and PNQ09-1b) to the participant(s), the main PulseNet contact, the laboratory director and other necessary personnel (e.g., a QA/QC manager) designated by the submitting lab. If the participating laboratory is also a GenomeTrakr lab, the PT reports are also emailed to the GenomeTrakr PT contact(s).

5.9.4 Laboratories that fail one proficiency testing round must resubmit the proficiency testing results for that pathogen again using the same strains. The evaluator will contact the submitter directly via email to ask for a resubmission. Resubmission due dates will be determined by the evaluator.

5.9.5 Laboratories that fail a round of PT for a particular organism three times in a row will lose their certification. The evaluator will email a failure notification letter (Appendix PNQ09-6) to the participant, the primary PulseNet contact, the laboratory director, the appropriate PulseNet Area Lab, and the GenomeTrakr PT contact, if the participating laboratory is also a GenomeTrakr laboratory. Individuals at those laboratories must submit new certification files and pass certification again before reinstatement of their certification. Please see PNQ08 for PulseNet WGS Certification information.

5.9.6 CDC has the right to revoke certifications at any time.

6. FLOW CHART:


Individual is certified in fastq generation and/or BioNumerics analysis
In order to maintain certification, PulseNet participants must participate in the annual PT
CDC ships PT strains and a hard copy of instructions to laboratories with at least one fastq-certified person
CDC emails the cluster challenge bundle file and an electronic copy of instructions to laboratories with at least one analysis-certified person
Each fastq-certified person sequences the PT strains
Laboratory chooses one set of results to submit per organism
Fastq files are shared with PulseNet Central using BaseSpace or the PulseNetQA FTP site. GenomeTrakr labs also share the fastq files with FDA on BaseSpace
Each analysis-certified person analyzes the fastq files generated for the 4 PT strains and the bundle file containing the cluster detection challenge using BioNumerics 7.6
Results for the 4 PT strains from the RefID database and the cluster detection challenge are recorded on the E-PT Survey Template and submitted electronically
Results for the 4 PT strains from the organism-specific databases are submitted as bundle files by emailing PulseNet@cdc.gov
Submissions are evaluated by PulseNet Central, reports emailed to participants, and overall summary posted on SharePoint

7. RELATED DOCUMENTS:

Document Number	Title
PNL33	**DNA Extraction and QC SOP**
PNL34	**Illumina Nextera XT Library Preparation SOP**
PNL35	**Illumina DNA Prep Library Preparation SOP**
PNL36	**Qiagen QIAseq FX Library Preparation SOP**
PNL37	**KAPA HyperPlus Library Preparation SOP**
PNL38	**Sequencing on the MiSeq SOP**
PNL39	**Sequencing on the MiniSeq SOP**
PNL40	**Sequencing on the iSeq SOP**
PNL42	**NEBNext Ultra II FS Library Preparation SOP**
PNL43	**Illumina DNA PCR-Free Library Preparation SOP**
PNQ07	**Illumina Sequence Data QC SOP**
PND20	**BioNumerics RefID Database Workflow SOP**
PND05	**BioNumerics Surveillance Database Workflow SOP**
PND06	**Cluster Detection SOP**
Provided via the email announcement	**PulseNet WGS PT Instructions/ Supplementary Instructions**
SharePoint: Library of PulseNet Documents/BioNumerics Data Management/Fields and Metadata	**Source Definition**
SharePoint: Library of PulseNet Documents/BioNumerics Data Management/Fields and Metadata	**BN Dictionary**
SharePoint: Library of PulseNet Documents/Quick Tips/Database_BioNumerics/WGS	**PulseNet WGS Cluster Coding Guidelines**

8. REFERENCES:

8.1 Timme, R.E., Lafon, P.C., Balkey, M., Adams, J.K., Wagner, D., Carleton, H., Strain, E., Hoffmann, M., Sabol, A., Rand, H., Lindsey, R., Sheehan, D., Baugher, J.D., Trees, E. (2020) Gen-FS coordinated proficiency test data for genomic foodborne pathogen surveillance, 2017 and 2018 exercises. Sci Data 7:402. doi: 10.1038/s41597-020-00740-7.

9. CONTACTS:

9.1 CDC PulseNet Response and Outbreak Management Team
(404) 639-4558
PulseNet@cdc.gov

9.2 CDC PulseNet Next Generations Subtyping Methods Unit
(404) 718-4185
PulseNetNGSlab@cdc.gov

9.3 GenomeTrakr Contacts
Maria Balkey

(240) 402-4946
Maria.Balkey@fda.hhs.gov

Tina S. L. Pfefer
(240) 402-2203
Tina.Pfefer@fda.hhs.gov

10. AMENDMENTS:

10.1 2017-02-15 changes:

- Updated Quality Metrics thresholds for Average Read Quality (≥30), Assembly-based calls: Multiple Alleles (now shown in red), and Percent Core Present (now shown in red) in Appendix 9-4 Table.

10.2 2017-12-15 changes:

- Updated SOP to include *Vibrio spp*
- Minor grammatical edits
- Updated Definitions for Proficiency Test evaluators, BaseSpace, WGS PT Reports, Category A and Category B infectious substances
- Updated Shipping requirements
- Updated duties outlined in Sections 5.2.3.4-5.2.3.6

10.3 2018-2-05 changes:

- Updated Definitions to include Category A, Category B infections substances, Proficiency Test Report, and wgMLST.
- Updated Proficiency Test Report Template in Appendix PNQ09-2.
- Added language to include testing of *Vibrio spp*.
- Revised Table in PNQ09-5 to include metrics for *Vibrio spp*.
- Removed section 4.5.2 regarding duties of database and laboratory reviewer, revised to include only one reviewer.
- Removed requirement to include labels identifying contents (i.e. 1x1.0 ml *Escherichia coli* O157:H7, 1 x 1.0 ml *Salmonella enterica* serotype Typhimurium). Replaced with requirement for Packing slips.
- Removed requirement to sequentially number participant addresses or packages for Scireson. Only the SET booking numbers are required.
- Added details to file submission in section 5.3.3.
- Updated file evaluation process in section 5.4.3.
- Updated Shipping contact information

10.4 2019-3-27 changes:

- Updated the SOP reference in section 5.2.2 from PNL32 to PNL38.
- Updated the Quality Metrics table in PNQ09-5
- Added CDC Technology Transfer Office to Contacts

10.5 2020-3-16 changes:

- Removed the CDC in-house procedure for planning and preparing PTs and evaluating submissions from PNQ09. This procedure is now outlined in an in-house SOP.
- Added analysis PT
- Added PulseNet Proficiency Testing Submission Template (Appendix PNQ09-2)
- Change in policy: a lab will now lose its certification status for a particular organism after failing three attempts of Proficiency Testing
- Added Appendix PNQ09-03 listing acceptable library prep kits and DNA loading capacities

- Updated the critical quality metrics in the Appendix PNQ09-4 to be aligned with the RefID database workflow. HqSNP difference compared to CDC sequenced reference was removed as a critical quality metric for the wet lab PT. Minimum average Q score requirement was increased from 28.0 to 30.0.

10.6 2021-2-23 changes:

- Added the cluster detection challenge to the Analysis PT

10.7 2022-3-25 changes:

- Clarified the data sharing instructions
- Clarified the bundle file naming instructions
- Added the related Documents section #7
- Updated the reports and the submission template in Appendices PNQ09-1 and PNQ09-2, respectively
- Updated the Appendix PNQ09-3 Acceptable Library Preparation Kits, Sequencing Platforms, Sequencing Chemistries and Validated DNA Loading Capacities

10.8. 2023-3-24 changes

- Proficiency Testing Submission Template in Excel was replaced by E-PT Survey Template utilizing Google Forms
- Overall pass/fail scoring of the wet lab and dry lab PTs was changed from organism-specific to a single wet lab (fastq) and a single dry lab (analysis) PT. A minimum of 85% of the total of 200 points available is required for passing each of the wet lab and the dry lab PT.

11. APPROVAL SIGNATURES:

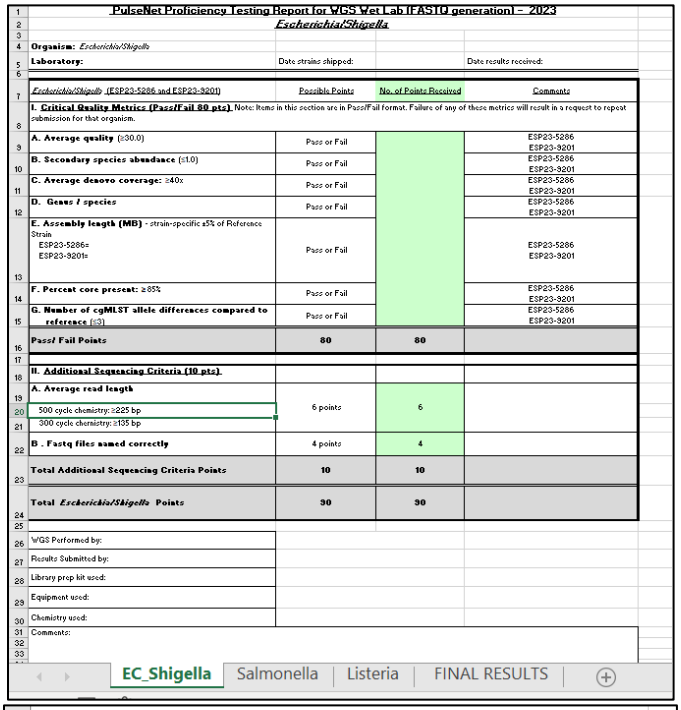
Approved By: Jennifer K. Adams Digitally signed by Jennifer K. Adams Date: 2023.03.31 13:38:12 -04'00' Date: 3/31/2023
QA/QC Personnel

Approved By: Kelley B Hise Digitally signed by Kelley B Hise Date: 2023.03.28 17:37:51 -04'00' Date: 3/28/2023
PulseNet Response and Outbreak Management Team Lead

Approved By: Rebecca L. Lindsey -S Digitally signed by Rebecca L. Lindsey -S Date: 2023.03.27 15:49:26 -04'00' Date: 3/27/2023
PulseNet Next Generation Subtyping Methods Unit Chief

Approved By: Efrain M. Ribot -S Digitally signed by Efrain M. Ribot -S Date: 2023.03.28 08:28:06 -04'00' Date: 3/28/2023
PulseNet Reference Outbreak Surveillance Team Lead

Appendix PNQ09-1a. WGS Proficiency Testing Example Report Template for the Wet Lab PT (Fastq file Generation)



PulseNet Proficiency Testing Report for WGS Wet Lab (FASTQ generation) – 2023

Escherichia/Shigella

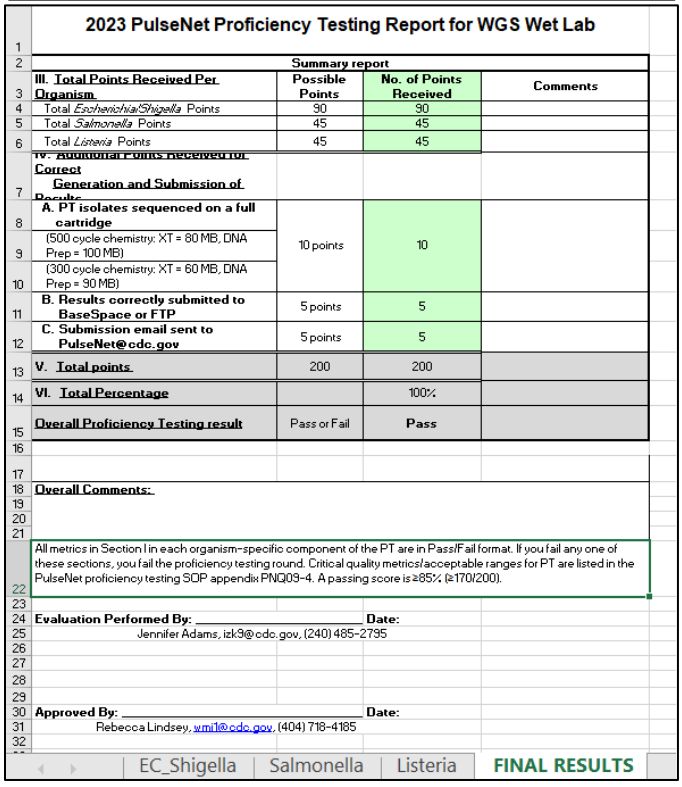
Organism: *Escherichia/Shigella*

Laboratory: | Date strains shipped: | Date results received:

Escherichia/Shigella (ESP23-5286 and ESP23-9201)	Possible Points	No. of Points Received	Comments
I. Critical Quality Metrics (Pass/Fail 80 pts) Note: Items in this section are in Pass/Fail format. Failure of any of these metrics will result in a request to repeat submission for that organism.			
A. Average quality (≥30.0)	Pass or Fail		ESP23-5286 ESP23-9201
B. Secondary species abundance (≤1.0)	Pass or Fail		ESP23-5286 ESP23-9201
C. Average genome coverage: ≥40x	Pass or Fail		ESP23-5286 ESP23-9201
D. Genus / species	Pass or Fail		ESP23-5286 ESP23-9201
E. Assembly length (MB) - strain-specific ±5% of Reference Strain ESP23-5286= ESP23-9201=	Pass or Fail		ESP23-5286 ESP23-9201
F. Percent core present: ≥85%	Pass or Fail		ESP23-5286 ESP23-9201
G. Number of cgMLST allele differences compared to reference (≤3)	Pass or Fail		ESP23-5286 ESP23-9201
Pass/ Fail Points	**80**	**80**	
II. Additional Sequencing Criteria (10 pts)			
A. Average read length			
500 cycle chemistry: ≥225 bp 300 cycle chemistry: ≥135 bp	6 points	6	
B . Fastq files named correctly	4 points	4	
Total Additional Sequencing Criteria Points	**10**	**10**	
Total *Escherichia/Shigella* Points	**90**	**90**	

WGS Performed by:	
Results Submitted by:	
Library prep kit used:	
Equipment used:	
Chemistry used:	
Comments:	

EC_Shigella | Salmonella | Listeria | FINAL RESULTS



2023 PulseNet Proficiency Testing Report for WGS Wet Lab

Summary report

III. Total Points Received Per Organism	Possible Points	No. of Points Received	Comments
Total *Escherichia/Shigella* Points	90	90	
Total *Salmonella* Points	45	45	
Total *Listeria* Points	45	45	
IV. Additional Points Received for Correct Generation and Submission of Results			
A. PT isolates sequenced on a full cartridge (500 cycle chemistry: XT = 80 MB, DNA Prep = 100 MB) (300 cycle chemistry: XT = 60 MB, DNA Prep = 90 MB)	10 points	10	
B. Results correctly submitted to BaseSpace or FTP	5 points	5	
C. Submission email sent to PulseNet@cdc.gov	5 points	5	
V. Total points	200	200	
VI. Total Percentage		100%	
Overall Proficiency Testing result	Pass or Fail	**Pass**	

Overall Comments:

All metrics in Section I in each organism-specific component of the PT are in Pass/Fail format. If you fail any one of these sections, you fail the proficiency testing round. Critical quality metrics/acceptable ranges for PT are listed in the PulseNet proficiency testing SOP appendix PNQ09-4. A passing score is ≥85% (≥170/200).

Evaluation Performed By: _____ **Date:**
Jennifer Adams, izk9@cdc.gov, (240) 485-2795

Approved By: _____ **Date:**
Rebecca Lindsey, wmi1@cdc.gov, (404) 718-4185

EC_Shigella | Salmonella | Listeria | FINAL RESULTS

Appendix PNQ09-1b. WGS Proficiency Testing Example Report Template for the Dry Lab PT (BioNumerics Analysis)

PulseNet Proficiency Testing Report for WGS Dry Lab (BioNumerics Analysis) - 2023

Escherichia/ Shigella

Organism: ***Escherichia/Shigella***

Laboratory: Date strains shipped: Date results received:

I. Analysis Critical Pass/ Fail Metrics (40 pts)	**Possible Points**	**No. of Points Received**	**Comments**
A. Genus/species: *Escherichia coli*	Pass or Fail		
B. Average quality: ≥30.0	Pass or Fail		
C. Secondary species abundance: ≤1.0	Pass or Fail		
D. Average denovo coverage: ≥40x	Pass or Fail		
E. Assembly length (MB) - Compared to Reference Strain (± 5%) ESP23-5286	Pass or Fail		
F. Percent core present: ≥85%	Pass or Fail		
G. Metadata - Source Type entered correctly	Pass or Fail		
Pass / Fail Points	**40**	**40**	

Note: Items in Section I are in Pass/Fail format. Failure of any of these metrics will result in a request to repeat submission for that organism. Sequences should not proceed to organism-specific database if any of these metrics fail.

II. Additional Analysis Criteria (20 pts)			
A. Genotyping (10)			
A1. Serotype_wgs ESP23-5286 ESP23-3201	2	2	
A2. Virulence Profile/Pathotype consistent with reference strain: ESP23-5286 ()	2	2	
A3. AMR Profile (Consistent with reference strain)	2	2	
(MLST_ST) ESP23-5286 () ESP23-3201 ()	2	2	
A5. Stx subtype (Toxin_WGS) ESP23-5286 () ESP23-3201 ()	2	2	
B. Metadata Fields (8 pts) Note: Provided metadata should be entered correctly in the organism-specific database			
B1. Source site entered correctly	2	2	
B2. Patient age entered correctly	2	2	
B3. Patient sex entered correctly	2	2	
B4. Isolation date entered correctly	2	2	
C. Bundle File Submission (2 pts)			
C1. Correct experiments and data fields included in the bundle file	2	2	
Total Additional Analysis Criteria Points	**20**	**20**	
Total *Escherichia/Shigella* Analysis Score	**60**	**60**	

Analysis Performed by:

EC_Shigella Analysis | Salmonella Analysis | Listeria Analysis | Cluster Detection | FINAL RESULTS

PulseNet Proficiency Testing Report for WGS Dry Lab (BioNumerics Analysis) - 2023

Cluster Detection

III. Cluster Detection - *Listeria* (35pts)			
A. Correct assignment of clusters	**Correct Assignment (Y/N)**	**Possible Points**	**No. of Points Received**
SRR6786636	Y		
SRR6829219	Y		
SRR7648453	Y		
SRR7692265	Y		
SRR7821164	Y		
SRR9961517	Y		
SRR9961515	Y		
SRR9114662	Y		
SRR9222635	Y		
SRR9600159	Y		
Passing Criteria ≥ 9 correct (30 Points)		35	35
B. Dendrogram (5 pts)		5	5
Total *Listeria* Cluster Detection Score		**40**	**40**

Note: Must have ≥9 correct assignment of clusters to receive full 35 points for assignment of clusters.

EC_Shigella Analysis | Salmonella Analysis | Listeria Analysis | **Cluster Detection** | FINAL RESULTS

2023 PulseNet Proficiency Testing Report for WGS Dry Lab (BioNumerics Analysis)

Summary Report

IV. Total Points Received Per Organism	Possible Points	No. of Points Received	Comments
A. *Escherichia/Shigella* Analysis Score	60	60	
B. *Salmonella* Analysis Score	45	45	
C. *Listeria* Analysis Score	45	45	
V. Total Cluster Detection Score	40	40	
VI. Submission of Results			
A. Results submitted by the deadline	5	5	
B. Submission email sent	5	5	
VII. Total points	**200**	**200**	
VIII. Total Percentage		**100%**	
Overall Proficiency Testing result	**Pass or Fail**	**Pass**	

Comments:

Critical quality metrics/acceptable ranges for PT are listed in the PulseNet Proficiency Testing SOP appendix PNQ09-4. A passing score is ≥85% (≥170/200) with no failures in the metrics with automatic Pass/Fail criteria.

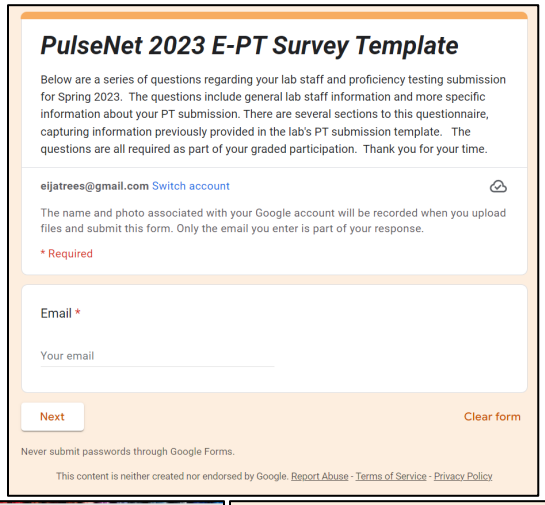
Proficiency Testing Evaluation and Approval:

Evaluation Performed By: _____ **Date:**
Jennifer Adams, izk3@cdc.gov, (240) 485-2795

Approved By: _____ **Date:**
Kelley Hise, kpb6@cdc.gov, (404) 639-0704

EC_Shigella Analysis | Salmonella Analysis | Listeria Analysis | Cluster Detection | **FINAL RESULTS**

Appendix PNQ09-2. Selected Screenshots of the PulseNet E-PT Survey Template



PulseNet 2023 E-PT Survey Template

Below are a series of questions regarding your lab staff and proficiency testing submission for Spring 2023. The questions include general lab staff information and more specific information about your PT submission. There are several sections to this questionnaire, capturing information previously provided in the lab's PT submission template. The questions are all required as part of your graded participation. Thank you for your time.

eijatrees@gmail.com Switch account

The name and photo associated with your Google account will be recorded when you upload files and submit this form. Only the email you enter is part of your response.

* Required

Email *

Your email

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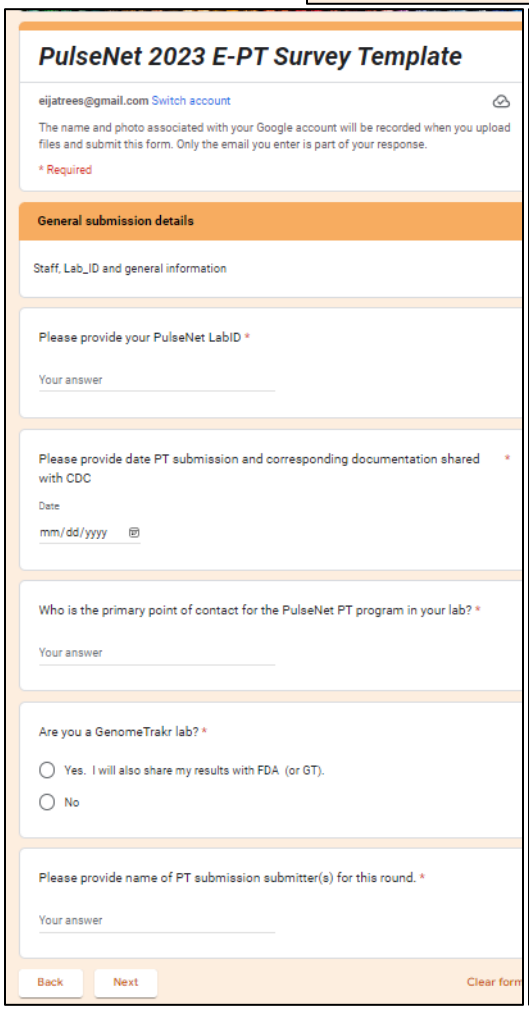

PulseNet 2023 E-PT Survey Template

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* Required

General submission details

Staff, Lab_ID and general information

Please provide your PulseNet LabID *

Your answer

Please provide date PT submission and corresponding documentation shared with CDC *

Date

mm/dd/yyyy

Who is the primary point of contact for the PulseNet PT program in your lab? *

Your answer

Are you a GenomeTrakr lab? *

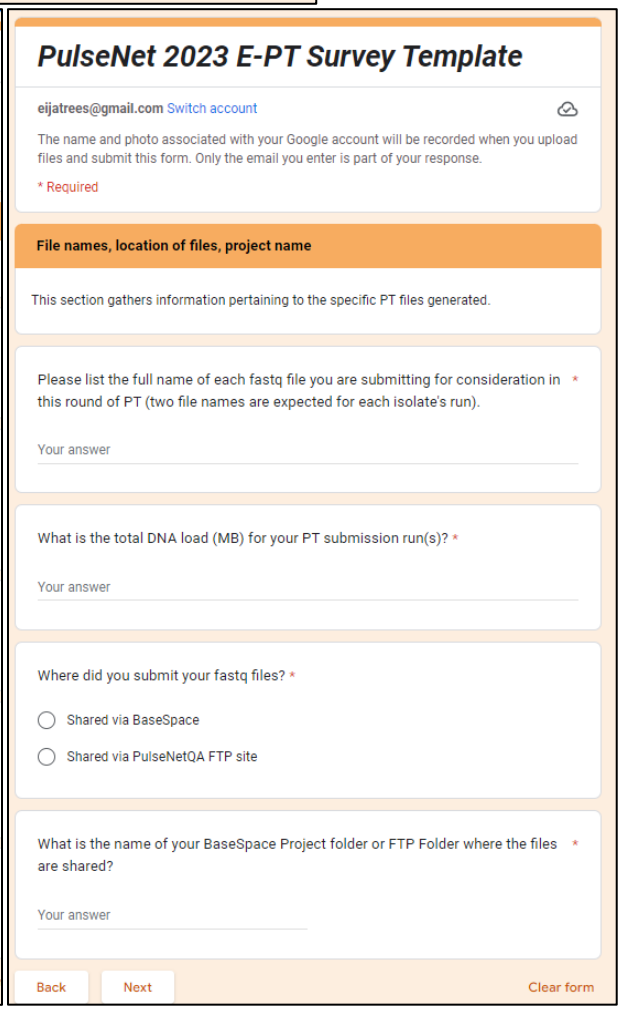
Yes. I will also share my results with FDA (or GT).

No

Please provide name of PT submission submitter(s) for this round. *

Your answer

Back Next Clear form



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File names, location of files, project name

This section gathers information pertaining to the specific PT files generated.

Please list the full name of each fastq file you are submitting for consideration in this round of PT (two file names are expected for each isolate's run). *

Your answer

What is the total DNA load (MB) for your PT submission run(s)? *

Your answer

Where did you submit your fastq files? *

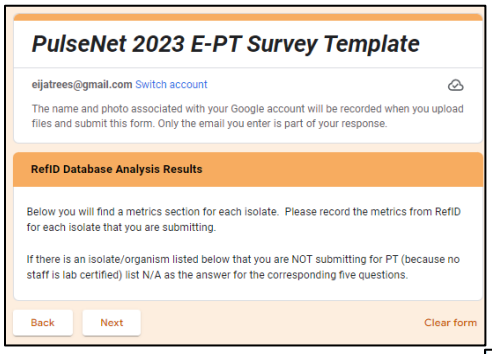
Shared via BaseSpace

Shared via PulseNetQA FTP site

What is the name of your BaseSpace Project folder or FTP Folder where the files are shared? *

Your answer

Back Next Clear form



PulseNet 2023 E-PT Survey Template

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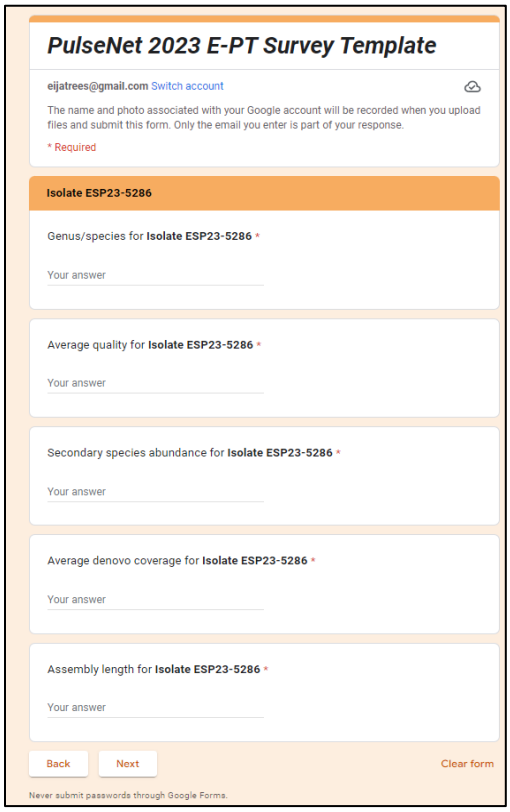
RefID Database Analysis Results

Below you will find a metrics section for each isolate. Please record the metrics from RefID for each isolate that you are submitting.

If there is an isolate/organism listed below that you are NOT submitting for PT (because no staff is lab certified) list N/A as the answer for the corresponding five questions.

Back Next Clear form



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* Required

Isolate ESP23-5286

Genus/species for **Isolate ESP23-5286** *

Your answer

Average quality for **Isolate ESP23-5286** *

Your answer

Secondary species abundance for **Isolate ESP23-5286** *

Your answer

Average denovo coverage for **Isolate ESP23-5286** *

Your answer

Assembly length for **Isolate ESP23-5286** *

Your answer

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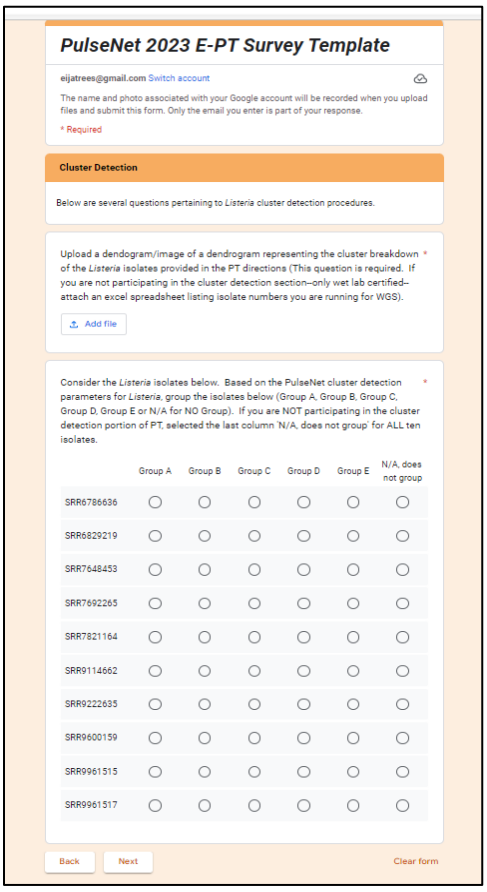

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* Required

Cluster Detection

Below are several questions pertaining to *Listeria* cluster detection procedures.

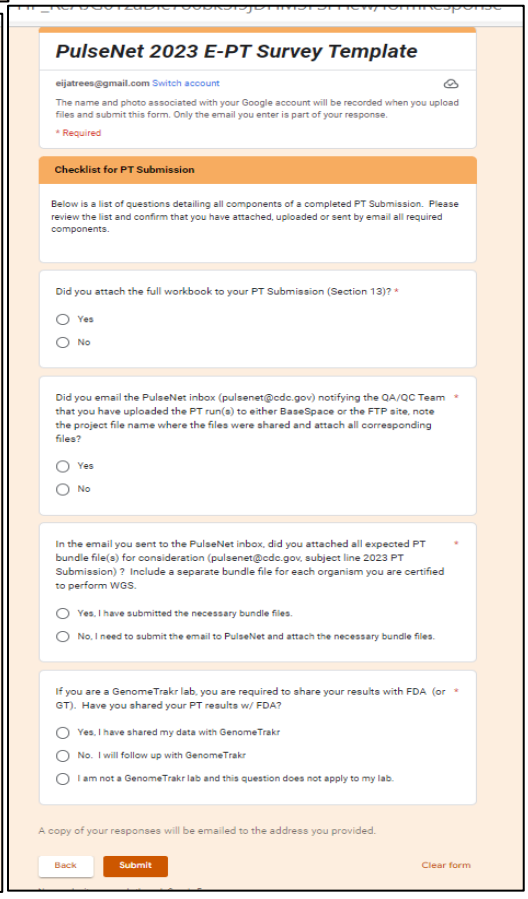
Upload a dendogram/image of a dendrogram representing the cluster breakdown * of the *Listeria* isolates provided in the PT directions (This question is required. If you are not participating in the cluster detection section--only wet lab certified--attach an excel spreadsheet listing isolate numbers you are running for WGS).

Add file

Consider the *Listeria* isolates below. Based on the PulseNet cluster detection * parameters for *Listeria*, group the isolates below (Group A, Group B, Group C, Group D, Group E or N/A for NO Group). If you are NOT participating in the cluster detection portion of PT, selected the last column 'N/A, does not group' for ALL ten isolates.

	Group A	Group B	Group C	Group D	Group E	N/A, does not group
SRR6786636	○	○	○	○	○	○
SRR6829219	○	○	○	○	○	○
SRR7648453	○	○	○	○	○	○
SRR7692265	○	○	○	○	○	○
SRR7821164	○	○	○	○	○	○
SRR9114662	○	○	○	○	○	○
SRR9222635	○	○	○	○	○	○
SRR9600159	○	○	○	○	○	○
SRR9961515	○	○	○	○	○	○
SRR9961517	○	○	○	○	○	○

Back Next Clear form



PulseNet 2023 E-PT Survey Template

eijatrees@gmail.com Switch account

The name and photo associated with your Google account will be recorded when you upload files and submit this form. Only the email you enter is part of your response.

* Required

Checklist for PT Submission

Below is a list of questions detailing all components of a completed PT Submission. Please review the list and confirm that you have attached, uploaded or sent by email all required components.

Did you attach the full workbook to your PT Submission (Section 13)? *

○ Yes
○ No

Did you email the PulseNet inbox (pulsenet@cdc.gov) notifying the QA/QC Team * that you have uploaded the PT run(s) to either BaseSpace or the FTP site, note the project file name where the files were shared and attach all corresponding files?

○ Yes
○ No

In the email you sent to the PulseNet inbox, did you attached all expected PT * bundle file(s) for consideration (pulsenet@cdc.gov, subject line 2023 PT Submission) ? Include a separate bundle file for each organism you are certified to perform WGS.

○ Yes, I have submitted the necessary bundle files.
○ No, I need to submit the email to PulseNet and attach the necessary bundle files.

If you are a GenomeTrakr lab, you are required to share your results with FDA (or * GT). Have you shared your PT results w/ FDA?

○ Yes, I have shared my data with GenomeTrakr
○ No. I will follow up with GenomeTrakr
○ I am not a GenomeTrakr lab and this question does not apply to my lab.

A copy of your responses will be emailed to the address you provided.

Back Submit Clear form

Appendix PNQ09-3. Acceptable Library Preparation Kits, Sequencing Platforms, Sequencing Chemistries and Validated DNA Loading Capacities

Nextera XT[1], Illumina (PNL34)

Illumina Sequencing Kit	DNA Load (Mb)
MiSeq v2, 300	60
MiSeq v2, 500	80
MiSeq v3, 600[2] no *E. coli/Shigella/Vibrio*	200
MiSeq v3, 600[2] *E. coli/Shigella/Vibrio*	175
MiSeq Micro, 300	35
MiSeq Nano, 500	13
MiniSeq Mid Output	60
MiniSeq High Output	100

[1]300 cycle chemistry not acceptable for *Escherichia, Shigella* or *Vibrio*
[2]DNA loads for v3 600 cycle cartridges are based on sequencing runs of 500 cycles using this chemistry

Illumina DNA Prep (DNA Flex) (PNL35)

Illumina Sequencing Kit	DNA Load (Mb)
MiSeq v2 300	90
MiSeq v2 500	100
MiSeq v3 600[1] no *E. coli/Shigella/Vibrio*	200
MiSeq v3 600[1] *E. coli/Shigella/Vibrio*	175
Micro 300 no *E. coli/Shigella/Vibrio*	35
Micro 300 *E. coli/Shigella/Vibrio*	30
Nano 500	13
Nano 300 no *E. coli/Shigella/Vibrio*	13
Nano 300 *E. coli/Shigella/Vibrio*	10
MiniSeq Mid Output	60
MiniSeq High Output	100
iSeq *E. coli/Shigella/Vibrio*	25
iSeq no *E. coli/Shigella/Vibrio*	35
NextSeq	400

[1]DNA loads for v3 600 cycle cartridges are based on sequencing runs of 500 cycles using this chemistry

QIAseq FX, Qiagen (PNL36)

Illumina Sequencing Kit	DNA Load (Mb)
MiSeq v2 300	80
MiSeq v2 500	100

KAPA HyperPlus, Roche (PNL37)

Illumina Sequencing Kit	DNA Load (Mb)
MiSeq v2 300, no *E. coli/Shigella/Vibrio*	80
MiSeq v2 300, *E. coli/Shigella/Vibrio*	75
MiSeq v2 500	100

NEBNext Ultra II FS (PNL42)

Illumina Sequencing Kit	DNA Load (Mb)
MiSeq v2 300, *no E. coli/Shigella/Vibrio*	90
MiSeq v2 300, *E. coli/Shigella/Vibrio*	75
MiSeq v2 500	100

Illumina DNA PCR-Free (PNL43)

Illumina Sequencing Kit	DNA Load (Mb)
MiSeq v2 300	90
MiSeq v2 500	100

Appendix PNQ09-4. Critical Quality Metric Thresholds/Acceptable Ranges for PulseNet Proficiency Testing

Organism	Average denovo coverage	Average quality (Q score)	Assembly length (MB)[1]	Secondary species abundance	% core present[2]	Allele difference compared to reference	Average read length (bp, 300c/500c)[5]
Listeria monocytogenes	≥ 20x	≥ 30	2.8-3.2	≤ 1.0	≥ 95	≤3	135/225
***E. coli* (most serotypes)**	≥ 40x	≥ 30	4.9-6.0	≤ 1.0	≥ 85	≤3	135/225
Shigella* spp./Rare *E. coli	≥ 40x	≥ 30	4.2-4.9	≤ 1.0	≥ 85	≤3	135/225
***Salmonella* spp.**	≥ 30x	≥ 30	4.4-5.7	≤ 1.0	≥ 85	≤3	135/225
***Campylobacter* spp.**	≥ 20x	≥ 30	1.4-2.2	≤ 1.0	≥ 85[3]	≤3	135/225
Vibrio cholerae	≥ 40x	≥ 30	3.8-4.3	≤ 1.0	NA[4]	NA[4]	135/225
Vibrio parahaemolyticus	≥ 40x	≥ 30	4.9-5.5	≤ 1.0	NA[4]	NA[4]	135/225
Vibrio vulnificus	≥ 40x	≥ 30	4.7-5.3	≤ 1.0	NA[4]	NA[4]	135/225

- All critical quality metrics, except % core present can be found in the RefID database (SOP PND20). % core present is determined in the organism-specific databases (SOP PND05).
- Rows shaded with the same color represent organisms transferred to the same organism-specific database.
- [1]The sequence assembly lengths for each species listed in the table apply for routine work. For proficiency testing, the assembly lengths are strain-specific based on the reference sequence generated by CDC with ± 5% margin of error.
- [2]The goal for percent core present is 95% or above. For proficiency testing, a percent core of 85% or above will pass for all other species ***except Listeria*** if all other required quality thresholds are met.
- [3]85% threshold applies to *C. jejuni* only. Other *Campylobacter* species may still pass quality with % core present <85%; please contact PulseNet@cdc.gov before resequencing
- [4]A core scheme for *Vibrio* is still under development
- [5]Average read length is not a critical quality metric, i.e., failing to meet the threshold results in points deduction, not an automatic failure of proficiency test submission.

Appendix PNQ09-5. Proficiency Testing Cover Letter Template

Month yyyy

Dear *Participant(s), Primary PulseNet Contact,* and *Lab Director*,

The *yyyy* round of WGS PulseNet Proficiency Testing for *Organism(s)* has been completed. The results for your laboratory are enclosed.

Below is the breakdown of the number of laboratories who have passed this round:

19/21 laboratories passed 2 are currently pending*

*Laboratories that did not pass were notified in *month* along with a request for resubmission.

A minimum 85% of all points available was required to pass this proficiency testing round. A thorough description of the scoring system is included in each report, as well as specific comments and suggestions for your laboratory, when applicable. Detailed summary reports are posted in PulseNet Announcements on SharePoint. PulseNet laboratory staff should have access to this forum. If your laboratory also participates in GenomeTrakr, your report has been shared with the GenomeTrakr PT contact(s).

Successful completion of the Proficiency Testing for the specified organisms maintains your standing as a certified PulseNet laboratory and satisfies the annual PT requirements set forth by GenomeTrakr. If you have any questions, please send an email to PulseNet@cdc.gov. We appreciate your participation and continued support of PulseNet.

Sincerely,

name
contact info

Cc: PulseNet Team, Centers for Disease Control and Prevention
Food Safety Team, Association of Public Health Laboratories

Appendix PNQ09-6. Proficiency Testing Notification of Repeated Failure and Loss of Certification Template

[Save on CDC Letterhead]

[Date]

[Name and info of lab director],
The [*laboratory*] PulseNet laboratory failed the [*yyyy*] round of the PulseNet WGS Proficiency Testing for [*organism]* three times. [*Insert reason for fail*]. The failure to pass Proficiency Test despite three attempts will result in the loss of the certification for [*organism*]. The *[laboratory*] laboratory must resubmit the certification for [*organism*] in order to reinstate their certification status and the privilege to submit data to the national [*organism*] database.

[*Person*] was notified on [*date*] and a certification resubmission was requested. Comments and suggestions for improvement were provided. PulseNet participants are always reminded to contact their designated PulseNet Area Laboratory or CDC with any questions or for troubleshooting assistance.

Please let us know if you have any questions or concerns.

Thank you,

Name
Contact info