2022 GenomeTrakr Proficiency Testing exercise (PulseNet Harmonized) V.1

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This SOP outlines guidelines on how to process the isolates for the 2022 GenomeTrakr (GT) Proficiency Testing exercise.

This SOP is applicable to all GenomeTrakr labs participating in the 2022 GenomeTrakr Proficiency Testing exercise (PulseNet Harmonized).

The FDA GenomeTrakr program will ship the following proficiency testing isolates on April, 2022.

ESP22-5191	*Shigella*
SAP22-0236	*Salmonella*
SAP22-0692	*Salmonella*
SAP22-1300	*Salmonella*

Completion of the entire proficiency test entails the following:

- GT participating laboratory generates sequencing data (fastq files) using the PT strains provided by CDC through FDA-CFSAN-WGS Program-GT in accordance to GT and/or PulseNet SOPs.

- Populate sample sheet according to 2022 GT Proficiency Testing exercise (PulseNet Harmonized) SOP

- Submission of sequencing records to the appropriate project on BaseSpace or Isilon according to GT SOPs

- By participating in the 2022 GT Proficiency Testing exercise (PulseNet Harmonized), GT labs provide consent to use the PT exercise data in subsequent analysis and manuscript publications. Participants will be acknowledged for their contribution on any publication that might require processing data from the 2022 GT PT exercise.

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Materials Needed
Sterile sturdy forceps
- 1 ml pipetman
- 1 ml sterile pipet tips
- 1 µl and/or 10 µl sterile inoculating loop

Reagents Needed
- Trypticase Soy + 5% Sheep Blood Agar plates (BAP) or equivalent media
- Sterile reagent grade water or Phosphate Buffered Saline (0.01M PBS; pH 7.4)
- BHI broth
- Sterile grade reagent water
- 70% isopropyl alcohol

Biological Safety Warning: *Shigella* and *Salmonella* strains are considered Level 2 biological agents by the U.S. Department of Health and Human Services. Use appropriate precautions when handling the vial or culture. Carry out laboratory work in a biological safety cabinet when applicable to ensure aseptic conditions and personal safety.

There are four sections in this protocol:

Section 1: Culture preparation of lyophilized isolates.
Section 2: Sequencing
Section 3: Data Transfer

Culture Preparation

1 *Salmonella* and *Escherichia/Shigella* Lyophilized cultures:

Day 1

Document the isolate number(s) and the lyophilized date(s) for your records. Wipe the aluminum cover and outside of the vial with isopropyl alcohol. Using sturdy forceps, aseptically remove the aluminum cover and rubber stopper from the vial containing the lyophilized culture. Wipe the outside of the rubber stopper and neck of the vial with isopropyl alcohol before removing the stopper.

2 Re-suspend the lyophilized cells with 1 ml of **sterile reagent grade water.** Allow to stand for a few minutes and/or mix gently to produce a uniform suspension. Plate **10 µl** of this suspension onto a blood agar plate (BAP) and incubate at 37°C overnight in aerobic conditions. It is recommended to plate in duplicate to ensure adequate growth.

3 Add the rest of the suspension to 5 mL of BHI broth and incubate the culture overnight at 37°C in aerobic conditions.

4 **Day 2 and 3**

Check the BAPs; if the culture appears pure, pick an isolated colony, and streak it on a fresh BAP; incubate at 37°C overnight in aerobic conditions. Use the growth from this plate to make DNA templates of the PT strains. Transfer culture to fresh medium and incubate at 37°C overnight; this will ensure that the same culture can be retested, if necessary.

5 **Optional**

If a BAP doesn't show bacterial growth, prepare a new plate by taking a loop from the BHI overnight culture (prepared at step 3), streak it on a BAP and incubate at 37°C overnight in aerobic conditions. On the next day check BAP and proceed as step 4.

Sequencing

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Perform DNA extraction, library preparation and sequencing according to lab's normal workflow described on SOPs posted at GenomeTrakr protocols.io.

https://www.protocols.io/view/manual-dna-extraction-using-qiagen-dneasy-blood-an-81wgbp31qvpk/v1
https://www.protocols.io/view/procedure-for-operation-and-maintenance-of-the-ill-rm7vz8z52vx1/v1
https://www.protocols.io/view/illumina-dna-prep-m-tagmentation-library-preparati-x54v9m7ezg3e/v1
https://www.protocols.io/view/dna-quantification-using-the-qubit-fluorometer-81wgbp3x3vpk/v1

PulseNet Labs will process isolates according to PulseNet Guidelines.

Isolates must be processed exactly as any routine isolate would be processed in the laboratory.

7   Sequencing sample sheets must be filled out according to Table 1.

7.1   **Sample_ID:** Include in this field the values from the **Sample_ID** column of Table 1, *do not modify these IDs*. You will also find this identifier in the vial of the lyophilized culture.

A	B	C	D
Sample_ID	Sample_Name	Project	Description
ESP22-5191	ESP22-5191-M0XXXX-20220324	PR0393_2022_Proficiency_Testing_Exercise	*Shigella*
SAP22-0236	SAP22-0236-M0XXXX-20220324	PR0393_2022_Proficiency_Testing_Exercise	*Salmonella*
SAP22-0692	SAP22-0692-M0XXXX-20220324	PR0393_2022_Proficiency_Testing_Exercise	*Salmonella*
SAP22-1300	SAP22-1300-M0XXXX-20220324	PR0393_2022_Proficiency_Testing_Exercise	*Salmonella*

Table 1: PT strain identifiers for Sequencing Sample Sheet (PT isolates processed on a sequencing run with historical and/or routine isolates)

Non PulseNet Labs: NextSeq 1000/2000 and MiSeq systems running Windows 10 (MCS v4 and up) have only Sample_ID available in the sequencing sample sheet, populate the Sample_ID column with the values in the **Sample_ID** column of Table 1.

PulseNet Labs: NextSeq 1000/2000 and MiSeq systems running Windows 10 (MCS v4 and up) have only Sample_ID available in the sequencing sample sheet, follow PulseNet guidelines to populate Sample_ID. Include the isolate identifier, lab ID, Instrument ID and run start date. (e.g. CJP19-D445-**GA**-M01001-20220324).

7.2   **Sample_name:**

Non PulseNet Labs: Fill out this field according to example provided in column Sample_Name of table 1. Include the isolate identifier, instrument ID (M0XXXX where "XXXX" corresponds to the instrument identifier) and run start date. (e.g. CJP19-D445_M01001_20220324).

PulseNet Labs: Include the isolate identifier, lab ID, Instrument ID (M0XXXX where "XXXX" corresponds to the instrument identifier) and run start date. (e.g. CJP19-D445-GA-M01001-20220324).

7.3 **Project:** Please fill out the project field with the project identifier PR0393_2022_Proficiency_Testing_Exercise.

7.4 **Description:** For your use only, we do not track this field. Organism names might be included in this field.

8   Replicates

Are you running more than one set of PT isolates in a run?

YES: proceed to Step 8.1
NO: proceed to Step 9.

If you don't have enough isolates for your sequencing run, you could run PT isolates multiple times, because MiSeq does not allow duplicate sample_IDs, please fill out the sequencing sample sheet using the instructions included in **Step 8.1**. See Table 2 (Non-PulseNet) and 3 (PulseNet) for examples. **Only one set of files shall be shared with GenomeTrakr.**

8.1

Modify Isolate identifiers (IDs at vial of lyophilized culture) to create unique identifiers for PT replicated isolates by adding suffixes such as: "_2" or "_3" to isolate identifiers.

Non-PulseNet Laboratories: If you choose to run replicates, the sample sheet must contain unaltered isolate identifiers for each PT strain in the **Sample_name** field. Identifiers in the sample_ID field must include the isolate identifier, replicate, instrument Id and start run date.

A	B	C	D
Sample_ID	Sample_Name	Project	Description
ESP22-5191-1-M0XXXX-20220324	ESP22-5191	PR0393_2022_Proficiency_Testing_Exercise	Shigella
SAP22-0236-1-M0XXXX-20220324	SAP22-0236	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
SAP22-0692-1-M0XXXX-20220324	SAP22-0692	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
SAP22-1300-1-M0XXXX-20220324	SAP22-1300	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
ESP22-5191-2-M0XXXX-20220324	ESP22-5191	PR0393_2022_Proficiency_Testing_Exercise	Shigella
SAP22-0236-2-M0XXXX-20220324	SAP22-0236	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
SAP22-0692-2-M0XXXX-20220324	SAP22-0692	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
SAP22-1300-2-M0XXXX-20220324	SAP22-1300	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
ESP22-5191-3-M0XXXX-20220324	ESP22-5191	PR0393_2022_Proficiency_Testing_Exercise	Shigella
SAP22-0236-3-M0XXXX-20220324	SAP22-0236	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
SAP22-0692-3-M0XXXX-20220324	SAP22-0692	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
SAP22-1300-3-M0XXXX-20220324	SAP22-1300	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
ESP22-5191-4-M0XXXX-20220324	ESP22-5191	PR0393_2022_Proficiency_Testing_Exercise	Shigella
SAP22-0236-4-M0XXXX-20220324	SAP22-0236	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
SAP22-0692-4-M0XXXX-20220324	SAP22-0692	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
SAP22-1300-4-M0XXXX-20220324	SAP22-1300	PR0393_2022_Proficiency_Testing_Exercise	Salmonella

Table 2: Strain identifiers for sequencing sample sheet (Case for runs that include replicates) (Non-PulseNet)

PulseNet Laboratories: Identifiers in the sample_ID and sample_Name fields must include the isolate identifier, replicate, lab ID, instrument Id and start run date.

	A	B	C	D
	Sample_ID	Sample_Name	Project	Description
	ESP22-5191_1_GA_M0XXXX_20220324	ESP22-5191_1_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Shigella
	SAP22-0236_1_GA_M0XXXX_20220324	SAP22-0236_1_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
	SAP22-0692_1_GA_M0XXXX_20220324	SAP22-0692_1_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
	SAP22-1300_1_GA_M0XXXX_20220324	SAP22-1300_1_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
	ESP22-5191_2_GA_M0XXXX_20220324	ESP22-5191_2_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Shigella
	SAP22-0236_2_GA_M0XXXX_20220324	SAP22-0236_2_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
	SAP22-0692_2_GA_M0XXXX_20220324	SAP22-0692_2_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
	SAP22-1300_2_GA_M0XXXX_20220324	SAP22-1300_2_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
	ESP22-5191_3_GA_M0XXXX_20220324	ESP22-5191_3_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Shigella
	SAP22-0236_3_GA_M0XXXX_20220324	SAP22-0236_3_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
	SAP22-0692_3_GA_M0XXXX_20220324	SAP22-0692_3_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
	SAP22-1300_3_GA_M0XXXX_20220324	SAP22-1300_3_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
	ESP22-5191_4_GA_M0XXXX_20220324	ESP22-5191_4_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Shigella
	SAP22-0236_4_GA_M0XXXX_20220324	SAP22-0236_4_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
	SAP22-0692_4_GA_M0XXXX_20220324	SAP22-0692_4_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella
	SAP22-1300_4_GA_M0XXXX_20220324	SAP22-1300_4_GA_M0XXXX_20220324	PR0393_2022_Proficiency_Testing_Exercise	Salmonella

Table 3: Strain identifiers for sequencing sample sheet (Case for runs that include replicates) (PulseNet)

8.2 **Project:** Please fill out the project field with the project identifier PR0393_2022_Proficiency_Testing_Exercise

8.3 **Description:** For your use only, we do not track this field.

9 **Quality of sequencing run**

-Data analysis results must be reported to GenomeTrakr in the spreadsheet included in the attachment. 📎 **GalaxyTrakr_PT_exercise_report_2022.xlsx**

- Check the quality of your sequencing records by following the SOP **Assessing sequence quality in GalaxyTrakr**. Note that Shigella isolates will be predicted as *Escherichia coli* with PubMLST scanning of contigs.

The result is acceptable. Report your findings in the spreadsheet GalaxyTrakr_PT_exercise_report_2022, sheet MicroRunQC.

- Run the tool **ShigaTyper** Shigella genoserotyping. Report your findings in the spreadsheet GalaxyTrakr_PT_exercise_report_2022, sheet ShigaTyper.

- Run the tool **SeqSero2** for *Salmonella* serotying**.** Report your findings in the spreadsheet GalaxyTrakr_PT_exercise_report_2022, sheet SeqSero.

Data Transfer

10   **Data transfer**

After checking the quality of your records, transfer the data and associated QC results to GenomeTrakr.

10.1   **Sharing a run in BaseSapce**

- Click the Runs tab in the Illumina BaseSpace website .
- Select the run that you would like to share with the GenomeTrakr team.
- Go to the summary tab and click at the share button.
- Enter the email address for the FDA team (**gnometrakr@fda.hhs.gov**), and then click Add Collaborator.
- Click Save Settings. Your run will be automatically shared with the GenomeTrakr team.

10.2   **Sharing a project in BaseSapce**

- Click the Projects tab in the Illumina BaseSpace website .
- Select the project (**PR0393_2022_Proficiency_Testing_Exercise)** that you would like to share with the GenomeTrakr team.
- Click the share project button.
- Enter the email address for the FDA team (**gnometrakr@fda.hhs.gov**), and then click Add Collaborator.
- Click Save Settings. Your project will be automatically shared with the GenomeTrakr team.

10.3
Labs inside the FDA network must share the the sequencing files by transferring the sequencing run folder to the isilon storage drive.

11   **PT exercise completion notification**

Notify GenomeTrakr of your completion of the PT exercise by sending an email to: **genometrakr@fda.hhs.gov**. The subject line should include "2022 WGS Proficiency Testing_YourLabName", attach sequencing sample sheet and include the following information in the body of the email:

a. Run name:
b. Sequenced by:
c. Results submitted by:
d. MiSeq ID:
e. Flow cell ID:

Select one of the options

f. SOP Protocol:
   - PulseNet SOP
   - GenomeTrakr SOP

Non-PulseNet Laboratories, please include 📊 **GalaxyTrakr_PT_exercise_report_2022.xlsx** in the attachments of the notification email.

PulseNet Laboratories must send the 2022 Proficiency Testing exercise data and results to PulseNet.