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2024 GenomeTrakr Proficiency Testing exercise (PulseNet Harmonized) V.8

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Protocol status: Working
We use this protocol and it's working

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Abstract

Version Updates

v5: Added links to GalaxyTrakr_PT_exercise_report_2022.xlsx
v6: added 2023 PT strains; edited Steps 1, 2, 4, and 5 to include references to growth of Listeria on BHIA plates; Updated Tables in Steps 7.1 and 8.1 with sample information for 2023 PT strains.
v7: Section 9 and Section 11: deleted file GalaxyTrakr_PT_exercise_report_2023.xlsx; uploaded new file version GalaxyTrakr_PT_exercise_report_2023-v2.xlsx
v8: Added new guidelines for the 2024 PT exercise including strain identifiers for the 2024 isolates, steps for processing Camyplobacter isolates, links for sample processing and data quality assessment protocols. Removed requirements to submit spreadsheet containing output from GalaxyTrakr

This SOP outlines guidelines on how to process the isolates for the 2024 GenomeTrakr (GT) Proficiency Testing exercise.

This SOP is applicable to all GenomeTrakr labs participating in the 2024 GenomeTrakr Proficiency Testing exercise (PulseNet Harmonized).

The FDA GenomeTrakr program will ship the following proficiency testing isolates in April 2024.

ESP24-4709: Escherichia coli
SAP24-0369: Salmonella enterica
SAP24-0005: Salmonella enterica
CAP24-7320: Campylobacter jejuni

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 2024GT 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 2024 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 2024 GT PT exercise.

Materials

Materials Needed

Sterile sturdy forceps

- 1 ml pipetman
- 1 ml sterile pipet tips
- 1 µl and/or 10 µl sterile inoculating loop
- Gas Generator for cultivation of microaerophilic microorganisms
- Gas pack jar

Reagents Needed

- Trypticase Soy + 5% Sheep Blood Agar plates (BAP) or equivalent media
- Brain Heart Infusion agar + 5% Rabbit Blood (BHIRB) or equivalent for *Campylobacter*
- Sterile reagent grade water or Phosphate Buffered Saline (0.01M PBS; pH 7.4)
- Sterile grade reagent water
- 70% isopropyl alcohol

Safety warnings

Biological Safety Warning: *Escherichia/Shigella*, *Salmonella, Listeria* and *Campylobacter* 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.

Before start

There are four sections in this protocol:

Section 1: Culture preparation of lyophilized isolates.
Section 2: DNA Extraction
Section 3: Library Preparation and Sequencing
Section 4: Quality of Sequencing Data
Section 5: Data Transfer

Culture Preparation

1 ***Salmonella and Escherichia*** **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 plate; 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 plates don'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 plate as appropriate and incubate at 37°C overnight in aerobic conditions. On the next day check BAP plate and proceed as step 4.

6 ***Campylobacter*** **Lyophilized cultures:**

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.

7 Re-suspend the lyophilized cells with 1ml of sterile **phosphate-buffered saline**. Mix gently to produce a uniform suspension. Plate 100 µl of this suspension onto a non-selective BHIRB plate

plate and incubate microaerobically at 42°C for 48 hr. It is recommended to plate in duplicate to ensure adequate growth.

8 Check the BHIRB plate for purity and pick an isolated colony and streak it onto a fresh BHIRB plate.

9 Incubate microaerobically at 42°C overnight. Use the growth from this plate to make DNA extracts of the PT strains. Transfer culture to fresh medium and incubate at 42°C overnight; this will ensure that the same culture can be retested, if necessary.

DNA Extraction

10 ***- Each isolate for the proficiency testing exercise shall be processed as any routine isolate according to laboratory guidelines.*
** PulseNet Labs will process these isolates according to PulseNet guidelines.*

11 Perform DNA extraction according to lab's normal workflow described on GenomeTrakr and/or PulseNet SOPs.

Protocol

NAME

Manual DNA Extraction using Qiagen DNeasy Blood and Tissue Kit

CREATED BY
Julie Haendiges

PREVIEW

Library Preparation and Sequencing

12 *** The sequencing run for the PT exercise shall be loaded as any routine run following established loading requirements.*

*** Control reference strains shall be included in the run when there is not enough isolates to fill a sequencing run.*

13 Perform library preparation according to lab's normal workflow described on GenomeTrakr and/or PulseNet SOPs. Isolates must be processed along with routine isolates to meet the **genome loading requirements** for the sequencing cartridge described in protocol Illumina DNA Prep (M) Tagmentation Library Preparation for use on an Illumina MiSeq Sequencer V.3.

Protocol

NAME

Illumina DNA Prep (M) Tagmentation Library Preparation for use on an Illumina MiSeq Sequencer

CREATED BY

Julie Haendiges

PREVIEW

If you don't have enough isolates for your sequencing run, please run **reference control isolates** in the sequencing run.

14 Quantify DNA according to lab's normal workflow described on GenomeTrakr and/or PulseNet SOPs.

Protocol

NAME

DNA Quantification using the Qubit Fluorometer

CREATED BY

Julie Haendiges

PREVIEW

15 Sequence DNA libraries according to lab's normal workflow described on GenomeTrakr and/or PulseNet SOPs.

Protocol

NAME

Procedure for Operation and Maintenance of the Illumina MiSeq for Whole Genome Sequencing

CREATED BY

Julie Haendiges

PREVIEW

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

16.1 **Sample_ID or Sample_Name:** Include in these fields the strain identifiers as it is indicated in 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
		ESP24-4709	PR0506_2024_Proficiency_Testing_Exercise	Escherichia coli
	SAP24-0369	SAP24-0369	PR0506_2024_Proficiency_Testing_Exercise	Salmonella enterica
	SAP24-0005	SAP24-0005	PR0506_2024_Proficiency_Testing_Exercise	Salmonella enterica
	CAP24-7320	CAP24-7320	PR0506_2024_Proficiency_Testing_Exercise	Campylobacter jejuni

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

NextSeq 1000/2000 and MiSeq systems running Windows 10 (MCS v4 and up) have only Sample_ID available in the sequencing sample sheet, make sure to include the strain identifier as indicated in Table 1, columns Sample_ID and Sample_Name.

16.2 **Project:** Please fill out the project field with the project identifier PR0506_2024_Proficiency_Testing_Exercise.

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

16.4 PulseNet labs will fill out the sequencing sample sheet according to PulseNet guidelines.

Quality of Sequencing Data

17 Perform quality control of sequencing data according to lab's normal workflow described on GenomeTrakr and/or PulseNet SOPs.

Protocol

NAME

Quality control assessment for microbial genomes: GalaxyTrakr MicroRunQC workflow

CREATED BY

Maria Balkey

PREVIEW

Data Transfer

18 **Data transfer**

After checking the quality of your records, transfer the data with **acceptable quality** according to GenomeTrakr guidelines via BaseSpace or share drive.

18.1 **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, click share button, then select send invitation.
- Enter the email address for the FDA team (**gnometrakr@fda.hhs.gov**) under -invite a collaborator- option, and then click Add Collaborator.
- Click Share the associated Project as well.
- Click Save Settings. Your run will be automatically shared with the GenomeTrakr team.

18.2 **Sharing a project in BaseSpace without sharing a run**

- Click the Projects tab in the Illumina BaseSpace website .
- Select the project (**PR0506_2024_Proficiency_Testing_Exercise)** that you would like to share with the GenomeTrakr team.
- Click the share project button, then select send invitation.
- 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.

18.3

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

19 **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 “2024 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