



VERSION 3

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GenomeTrakr WGS Protocol Collection and Workflow for MiSeq V.3

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ABSTRACT

Here we have created a collection of all the protocols used for WGS using the MiSeq, in order, from sample extraction to NCBI submission.

This collection has three sections:

- **Section 1: WGS Wet lab workflow for Illumina MiSeq**
- **Section 2: Dry lab workflow for sequence QC and NCBI submission - Direct Submission**
- **Section 3: Dry lab workflow for sequence QC and NCBI submission - PulseNet labs**

Version history:

V3:

- Links to protocols updated with latest version,
- Added new protocol: DNA Extraction from Inoculated Broth for WGS using the Maxwell RSC Cultured Cells DNA Kit,
- Added new protocol: Querying for Bacterial Pathogen Genomic Data at NCBI.

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

Created: Mar 19, 2024

Last Modified: Mar 21, 2024

PROTOCOL integer ID: 96944

Keywords: GenomeTrakr, whole genome sequencing, enteric pathogens, surveillance, MiSeq

WGS Wet Lab Workflow for Illumina MiSeq

1 DNA Extraction - Manual

Protocol



NAME

Manual DNA Extraction using Qiagen DNeasy Blood and Tissue Kit

CREATED BY

Julie Haendiges

PREVIEW

2 DNA Extraction - Automated

Protocol



NAME

DNA Extraction from Inoculated Broth for WGS using the Maxwell RSC Cultured Cells DNA Kit

CREATED BY

Julie Haendiges

[PREVIEW](#)

3 DNA Quantification

Protocol



NAME

DNA Quantification using the Qubit Fluorometer

CREATED BY

Julie Haendiges

[PREVIEW](#)

4 Library Preparation

Protocol



NAME

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

CREATED BY

Julie Haendiges

[PREVIEW](#)

5 Sequencing

Protocol



NAME

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

CREATED BY

Julie Haendiges

PREVIEW

Dry Lab Workflow for Sequence QC and NCBI Submission - Direct Submis...

6 The following protocols are also included in a Springer Methods book chapter collection: [Utilizing the Public GenomeTrakr Database for Foodborne Pathogen Traceback \(protocols.io\)](#)

7 Check sequence quality:

Protocol



NAME

Quality control assessment for microbial genomes: GalaxyTrakr MicroRunQC workflow

CREATED BY

Ruth Timme

PREVIEW

8 Populate BioSample AND SRA metadata templates:

Protocol



NAME

Guidance for populating and validating GenomeTrakr metadata templates (BioSample and SRA)

CREATED BY

Ruth Timme

PREVIEW

9 Submit sequence and metadata to NCBI:

Protocol



NAME

NCBI submission protocol for microbial pathogen surveillance

CREATED BY

Ruth Timme

PREVIEW

10 Querying data at NCBI

Protocol



NAME

Querying for Bacterial Pathogen Genomic Data at NCBI

CREATED BY

Maria Balkey

PREVIEW

11 Update, retract, or replace your submissions at NCBI:

Protocol



NAME

NCBI Bacterial Pathogen Data Curation Protocol: SOP for Editing GenomeTrakr Submissions

CREATED BY

Ruth Timme

PREVIEW

Dry Lab Workflow for Sequence QC and NCBI Submission - PulseNet Labs

12 Check sequence quality:

Follow the CDC's guidance for assessing QC in BioNumerics

13 Populate NCBI template:

*****NOTE:** For NCBI submission for fields not included in the BioNumerics Template, please remember to include the name of the laboratory sequencing the isolates and the surveillance effort name in the **sequenced_by** and **project_name** fields as described in Step 5 of this protocol.

Protocol



NAME

Populating NCBI template for submissions using BioNumerics

CREATED BY

Maria Balkey

PREVIEW

14 Submit sequence and metadata to NCBI:

Follow the CDC's guidance for NCBI submissions through BioNumerics.

15 Query data at NCBI

⇒ [go to step #10](#) "Querying Data at NCBI"

16 Update, retract, or replace these records in NCBI databases, if necessary:

⇒ [go to step #11](#) Data curation protocol