

Mar 21, 2024

Version 3

GenomeTrakr WGS Protocol Collection and Workflow for MiSeq V.3

DOI

<https://dx.doi.org/10.17504/protocols.io.3byl4bwyjvo5/v3>



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Protocol Citation: Tina Lusk Pfefer, Julie Haendiges, Maria Balkey, Candace Bias, Ruth Timme 2024. GenomeTrakr WGS Protocol Collection and Workflow for MiSeq. protocols.io <https://dx.doi.org/10.17504/protocols.io.3byl4bwyjvo5/v3> Version created by [Tina Pfefer](#)



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Protocol status: Working

We use this protocol and it's working

Created: March 19, 2024

Last Modified: March 21, 2024

Protocol Integer ID: 96944

Keywords: GenomeTrakr, whole genome sequencing, enteric pathogens, surveillance, MiSeq, genometrakr wgs protocol collection, bacterial pathogen genomic data at ncbi, bacterial pathogen genomic data, dry lab workflow for sequence qc, lab workflow for illumina miseq section, genometrakr, maxwell rsc cultured cells dna kit, workflow for miseq, sample extraction to ncbi submission, dna extraction from inoculated broth, dna extraction, dry lab workflow, lab workflow, ncbi submission, new protocol, miseq, pulsenet lab, links to protocol, illumina miseq section, added new protocol, protocol, sequence qc

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.



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 Submission

- 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