

# Bacterial genomics: Sequencing and Bioinformatics Training

14 – 18 July 2025



# Sequencing and Bioinformatics Training Course

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## Course Overview

This one-week intensive training course provides participants with a comprehensive introduction to sequencing workflows and bioinformatics analysis, with a focus on bacterial genomics. The course is designed for laboratory scientists, researchers, and students who wish to understand the end-of-the-end process of Bacterial DNA sequencing and Bioinformatics analysis. Participants will engage in both hands-on wet-lab activities and practical bioinformatics sessions.

## Course Contents

### Lectures

- DNA Extraction: Theory
- Bacterial Genomics and Applications

### Wet-Lab

- DNA Extraction: Laboratory Protocols
- DNA Quality Control and Assessment
- Library Preparation for Sequencing
- Illumina Next-Generation Sequencing

### Bioinformatics analysis

- Introduction to Unix Command Line and Conda Environments
- Sequence Data Retrieval and Preprocessing
- Genome Assembly and Quality Metrics
- Species Identification and Typing
- AMR and Virulence Gene Screening
- Phylogenetic Analysis and Interpretation
- Overview of Online GUI Bioinformatics tools

## Learning Outcomes

1. Understand the key steps in DNA extraction, library preparation, and sequencing.
2. Navigate and use Unix command line tools and manage software using Conda.
3. Retrieve, assess, and preprocess sequencing data for downstream analysis.
4. Assemble bacterial genomes and interpret assembly metrics.
5. Identify bacterial species and determine clonal complex, strain types, spa types, SCCmec types etc
6. Detect antimicrobial resistance and virulence genes
7. Construct and interpret phylogenetic trees to assess genomic relatedness.

# Training Timetable

Time	Monday	Tuesday	Wednesday	Thursday	Friday
8:00 - 9:00 AM	Welcome & Introduction DNA Extraction Lecture	Library Preparation		Lecture: Bacterial Genomics	AMR & Virulence Gene Screening
9:00 - 10:30 AM	DNA Extraction Lab	Library Preparation		Bacterial Genomics	AMR & Virulence Gene Screening
10:30 - 11:00 AM	Tea Break				
11:00 - 1:00 PM	DNA Extraction Lab	Library Preparation	Finish Library Prep & Start Sequencing	Dataset Retrieval, QC & Preprocessing	Phylogenetics
1:00 - 2:00 PM	Lunch				
2:00 - 3:30 PM	DNA Quality Control Check	Library Preparation	Intro to Unix & Conda	Genome Assembly, Species ID & Typing	Online Tools Overview
3:30 - 5:00 PM		Library Preparation	Intro to Unix & Conda	Genome Assembly, Species ID & Typing	Feedback & Closing

## DNA Extraction Using Zymo BashingBead™ Lysis Kit

### Materials Needed

- Fungal or bacterial cells (50–100 mg wet weight)
- Water or isotonic buffer (e.g., PBS)
- ZR BashingBead™ Lysis Tube (0.1 mm & 0.5 mm beads)
- BashingBead™ Buffer
- Bead beater (e.g., TerraLyzer™, FastPrep®-24, Disruptor Genie™, vortex)
- Microcentrifuge
- Zymo-Spin™ III-F Filter
- Zymo-Spin™ IICR Column
- Collection tubes
- Genomic Lysis Buffer
- DNA Pre-Wash Buffer
- g-DNA Wash Buffer
- DNA Elution Buffer
- 1.5 ml microcentrifuge tube

### Procedure

#### Sample Preparation

1. Add 50–100 mg of fungal or bacterial cells (wet weight) resuspended in up to 200 µl of water or isotonic buffer (e.g., PBS) into a ZR BashingBead™ Lysis Tube (0.1 mm & 0.5 mm).
2. Add 750 µl BashingBead™ Buffer to the tube.
3. Cap tightly to prevent leakage.

#### Cell Disruption

4. Secure the tube in a bead beater fitted with a 2 ml tube holder.
5. Process at maximum speed for ≥ 5 minutes.
6. Processing time varies by device:
  - High-speed disrupters: ~3 minutes (e.g., TerraLyzer™, FastPrep®-24)
  - Lower-speed devices: up to 20 minutes (e.g., Disruptor Genie™, vortex)

#### Centrifugation

7. Centrifuge the lysis tube at 10,000 × g for 1 minute.

#### Filtration

8. Transfer up to 400 µl of supernatant to a Zymo-Spin™ III-F Filter in a Collection Tube.
9. Centrifuge at 8,000 × g for 1 minute.

#### Lysis Buffer Addition

10. Add 1,200 µl Genomic Lysis Buffer to the filtrate in the Collection Tube.

#### DNA Binding

11. Transfer 800  $\mu$ l of the mixture to a Zymo-Spin™ IICR Column in a Collection Tube.
12. Centrifuge at 10,000  $\times$  g for 1 minute.
13. Repeat with the remaining mixture.
14. Note: Max column capacity is 800  $\mu$ l.

#### Pre-Wash

15. Discard the flow-through and place the column in a new Collection Tube.
16. Add 200  $\mu$ l DNA Pre-Wash Buffer, then centrifuge at 10,000  $\times$  g for 1 minute.

#### Wash

17. Add 500  $\mu$ l g-DNA Wash Buffer to the column.
18. Centrifuge at 10,000  $\times$  g for 1 minute.

#### DNA Elution

19. Transfer the column to a clean 1.5 ml microcentrifuge tube.
20. Add 100  $\mu$ l (minimum 35  $\mu$ l) of DNA Elution Buffer directly to the column matrix.
21. Centrifuge at 10,000  $\times$  g for 30 seconds to elute the DNA.

## DNA Extraction Worksheet

SNo.	Sample ID	A260/280	A260/230	Nanodrop Conc (ng/ul)	Qubit Conc (ng/ul)
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