Metagenomics Pre-Meeting Course:
Using Metagenomics to Survey Vector-Based Pathogens
(Collaborative effort by ACAV, ACGH, ACMCIP and ACME)
October 30, 2022; 8:30 a.m. – 5 p.m. PT
Washington State Convention Center, Seattle, Washington, United States
This course will be held in-person only.

Metagenomic next-generation sequencing (mNGS) is a powerful tool that enables sequencing all the genetic material in a sample. Sequencing all the microorganisms in a sample enables researchers to identify pathogens and novel taxa without *a priori* knowledge. This course will cover the basics of mNGS, from experimental design to data analysis, and its importance in global health. The data analysis portion of the course will be hands-on, focused on analyzing an mNGS dataset from mosquitoes collected from California using the tool Chan Zuckerberg ID (CZ ID). Chan Zuckerberg ID (CZ ID) is a free, open-source tool that enables researchers to analyze metagenomic datasets from their laptops without the need for computational skills.

Although this course focuses on mNGS data acquired from mosquitoes, it should be noted that mNGS can be used to characterize the microbes in any sample type, so the tools learned in this course can be translated to other sample types of interests. Case studies of how mNGS has contributed to global health policies will also be covered.

Who is this course for?

This in-person course is for those new to mNGS and who would like to use Illumina sequencing technologies. Those who would like to learn about using Chan Zuckerberg ID (CZ ID) for their analysis will benefit from this course. Laptops are required to attend this course. Attendees are responsible for bringing their own laptops.

About Chan Zuckerberg ID (CZ ID) (formerly IDseq)

Chan Zuckerberg ID (CZ ID) is a free, cloud-based pipeline and analysis software that accepts raw mNGS sequencing data for fast, push-button processing to provide actionable information on the state of microbes in a given set of samples. This allows scientists to make data-driven decisions about when to deploy antibiotics, where to prioritize immunization campaigns, how to shape vector-borne disease surveillance and control efforts, and more. Our publication can be found here.

What you will learn

- What is mNGS, and what can you do with it?
- Proper experimental design to get the most out of your data
- How to prep samples in the wet lab for Illumina sequencing
- Bioinformatic basics - fastq files, pipeline overview, QC
- Analyzing mNGS data using Chan Zuckerberg ID (CZ ID)

Course Organizer:
Amy Krystosik, PhD, MPH, Data Scientist, Chan Zuckerberg Initiative, Redwood City, California, United States

Instructors:
Vida Ahyong, PhD, Scientist, Chan Zuckerberg Biohub, San Francisco, California, United States

Liz Fahsbender, PhD, Product Application Scientist, Chan Zuckerberg Initiative, Redwood City, California, United States

Juliana Gil, PhD, Scientist, Chan Zuckerberg Biohub, San Francisco, California, United States

Katrina Kalantar, PhD, Computational Biologist, Chan Zuckerberg Initiative, Redwood City, California, United States

Jessica Manning, MD, MSc, Laboratory of Malaria and Vector Research, NIAID, NIH, Rockville, Maryland, United States & International Center of Excellence in Research, NIAID, NIH, Phnom Penh, Cambodia

AGENDA

8 a.m. Light Continental Breakfast

8:30 a.m. **Case Study: Real-World Application of mNGS**
Jessica Manning, MD, MSc

9 a.m. **Wet Lab Methods**
Vida Ahyong, PhD and Juliana Gil, PhD
Introduction to mNGS

- Why is it useful?
- How does it differ from other methods such as 16s rRNA sequencing or whole-genome sequencing?
- Detection of viruses!

Sequencing technologies/wet lab methods

- Proper lab setup
- Sample collection and preparation
- Loading the sequencer

11 a.m. **Bioinformatics Intro**
Liz Fahsbender, PhD and Katrina Kalantar, PhD
Bioinformatics basics

- FASTQ files
- Assemblies
- Public databases
- BLAST

mNGS pipeline

- Available tools
- What is needed to run them

Noon Lunch

1 p.m. **Chan Zuckerberg ID (CZ ID) Introduction and Hands-On Activity (Coffee break included)**
Liz Fahsbender, PhD and Katrina Kalantar, PhD
Quality control

- How to analyze your dataset
- mNGS pipeline

Analyze dataset

- Create and apply a background model
- Identify pathogens of interest
- Identify contaminants
- Identify false positives
- Build consensus genome/pipeline overview

Heatmap - how to identify pathogens of interest, contamination, patterns
Creating and reading a phylogenetic tree

5 p.m. Course Adjourns

Faculty Bio’s

Vida Ahyong, PhD, is a Scientist on the Rapid Response Team at the Chan Zuckerberg Biohub with a background in malaria genomics, host-pathogen interactions, bacterial metabolism, and bioinformatics. Vida is training scientists worldwide to use new tools and technologies to identify and detect pathogens through metagenomic sequencing.

Liz Fahsbender, PhD, is a Product Application Scientist on the Chan Zuckerberg ID (CZ ID) team at the Chan Zuckerberg Initiative, where she trains scientists on how to use Chan Zuckerberg ID (CZ ID) and relays their needs back to the team. She has a background in viral metagenomics and pathogen identification, with experience identifying novel viruses.

Juliana Gil, PhD, is a scientist on the Rapid Response Team at the Chan Zuckerberg Biohub. Her background is in molecular host-microbe interactions in plant systems. Her research has focused on functional genomics of agricultural relevant diseases and ecological genomics in food models. She is now applying her experience in detecting human infectious diseases and training teams to apply metagenomic sequencing to investigate the etiology of diseases.

Katrina Kalantar, PhD, is a Computational Biologist on the Chan Zuckerberg ID (CZ ID) Team at the Chan Zuckerberg Initiative, where she works on the bioinformatics pipeline. She has a background in metagenomics and host transcriptomics, with specific research experience in using these tools to understand infectious diseases.

Jessica Manning, MD, MSc, is an infectious diseases physician-scientist based in Cambodia, where she leads NIAID's International Center of Excellence in Research as part of its Laboratory of Malaria and Vector Research. The center is a collaborative team with the Cambodian Ministry of Health's National Center for Entomology, Parasitology and Malaria Control and is primarily focused on translational and clinical research surrounding vector-borne diseases such as dengue and multi-drug resistant malaria in Southeast Asia.

Teaching Assistant Bio’s

Sophana Chea, MSc, is the Laboratory Lead at the NIAID International Center of Excellence in Research in Cambodia. He joined the Manning lab in 2019, where his roles include coordinating sample collection, processing and sequencing, and overseeing sequencing operations.

Sreyngim Lay, BSc, is the Sequencing Team Leader for the NIAID International Center of Excellence in Research in Cambodia. She has led sequencing efforts in the Manning lab since 2019 and has an intricate knowledge of Illumina sequencing platforms and protocols.

Christina Yek, MD, is a critical care medicine and infectious disease fellow at the National Institutes of Health Clinical Center in Bethesda, MD. She joined the Manning lab in 2021 and has been involved in project planning and bioinformatic analysis of sequence data.