

DNA Barcoding as a Steppingstone to Next Generation Sequencing

Sequence-a-genome

- 2021- Duckweed (Biofuels, Hudson Alpha)
- 2022- Jamaican Broom (Endemic/Endangered to PR, UPR)
- 2023- Assorted plant chloroplasts
- 2024- Whole bacterial genomes



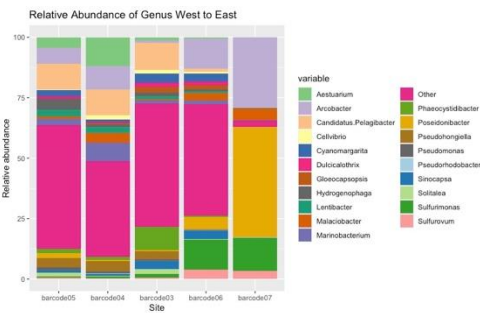
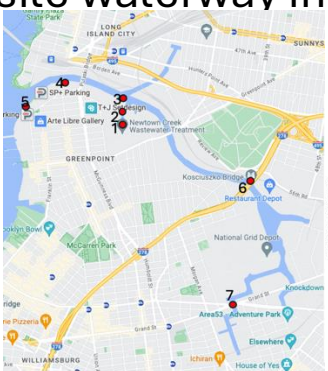
Wastewater Epidemiology

- 2024- Wastewater Bootcamp
- CC Transfer students sequenced phages from isolated from hospital wastewater.
- Phages relevant to phage therapy research



Exploring the Biodiversity of Newtown Creek

- 2023- CUNY City tech
- 16S Metabarcoding of eDNA from superfund site waterway in NYC



Using a high-throughput long read sequencing to sequence an amplicon ?

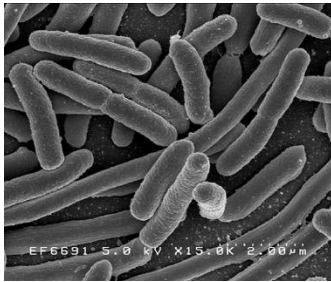
Using DNA Barcoding as an entry way to Nanopore Sequencing and next generation sequencing data

- Many faculty are trained in DNA Barcoding CURES
- The price is right (~\$3 a sample)

The Year of the Summer of Nanopore 2024

- Developing teacher training materials and schedule for three sequencing experiments in three days– Barcoding, Metabarcoding and Small Genome sequencing.

16S Metabarcoding



DNA extraction from Swabs

↓
16S PCR

↓
16S Barcoding Kit

DNA Barcoding

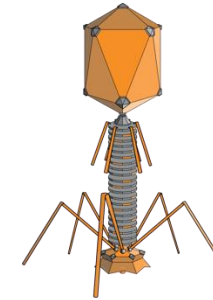


DNA Extraction from Plants

↓
RbCl PCR

↓
?

Whole Genome



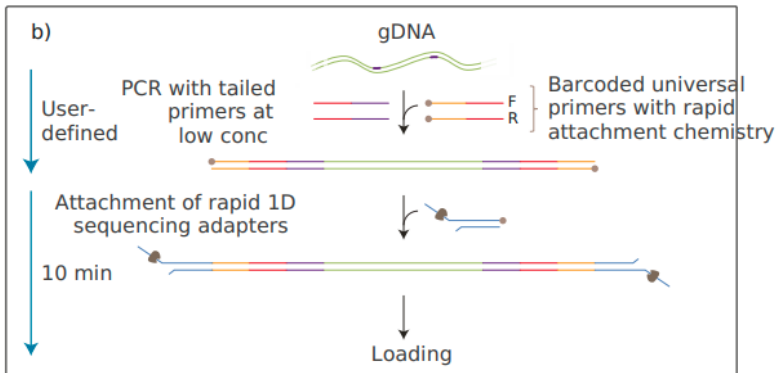
Phage Genome



Ligation Barcoding Kit

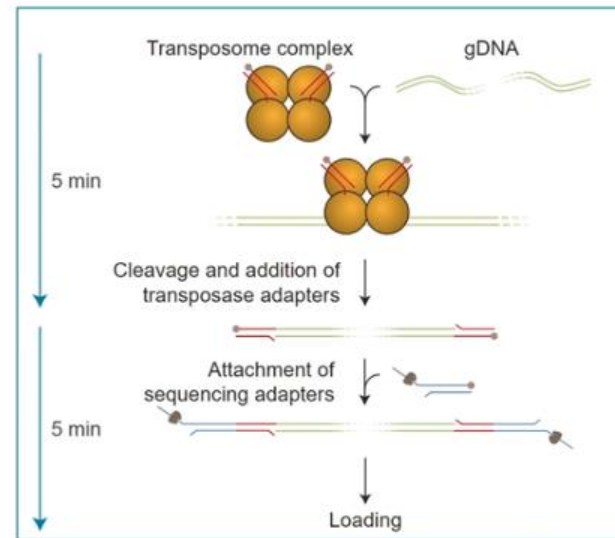
DNA Barcoding with Oxford Nanopore

Four Primer Protocol



- Barcoded amplicon libraries without fragmentation
- Can be used with metabarcoding and barcoding
- No ligation

Rapid Barcoding Kit



Flongle



- Rapid barcoding kit quick enough to do during class - add rapid barcode directly to PCR product
- Flongle - No need for very high throughput
- Even with fragmentation can get consensus of amplicons ~600bps
- Fragmentation - not for use with eDNA/metabarcoding

Summer of Nanopore 2024

May 15–17:

Spelman College, Atlanta, GA

May 29–31:

Arecibo C3 STEM Center, Arecibo, PR

June 10–12:

Skyline College, San Bruno, CA

June 17–19:

DNA Learning Center NYC at City Tech, Brooklyn, NY

June 18–20:

University of Hawaii at Manoa, Honolulu, HI

June 20–22:

Granite Technical Institute and Natural History Museum of Utah, Salt Lake City, UT

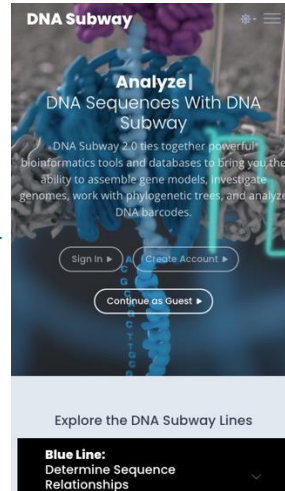
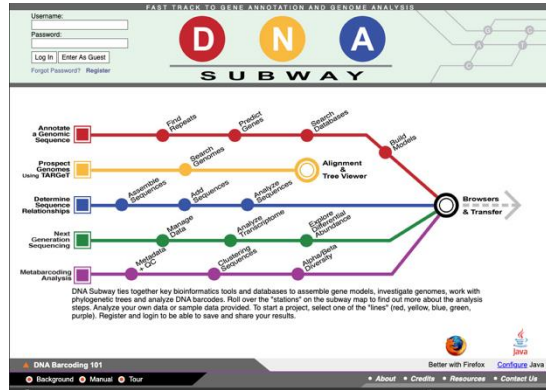
June 24–26:

James Madison University, Harrisonburg, VA

****133 faculty from over 100 institutions**

Design and Backend Considerations and Challenges

Mobile First



Scale/Real Estate

- Wrapping text, vertical scrolling
- ## Dexterity
- Relying on fingertips rather than a mouse/trackpad.

Interface

- Uploading from cloud rather than hard drive

Accessibility

- Meeting Web Content Accessibility Guidelines (WCAG)



Sue Lauter



Chun-hua Yang



Skyla Robinson



Daniel Jacobs



- Transition to no-cost NSF supported Jetstream2 infrastructure



- Containerization of workflows with Docker

Forensics Crime Lab 2024

- Forensic Entomology – using the identity of insects found on a cadaver to estimate the post-mortem interval (PMI)
- Specimens field collected from students of the Forensic Anthropology Center (FAC) at the University of TN, Knoxville.



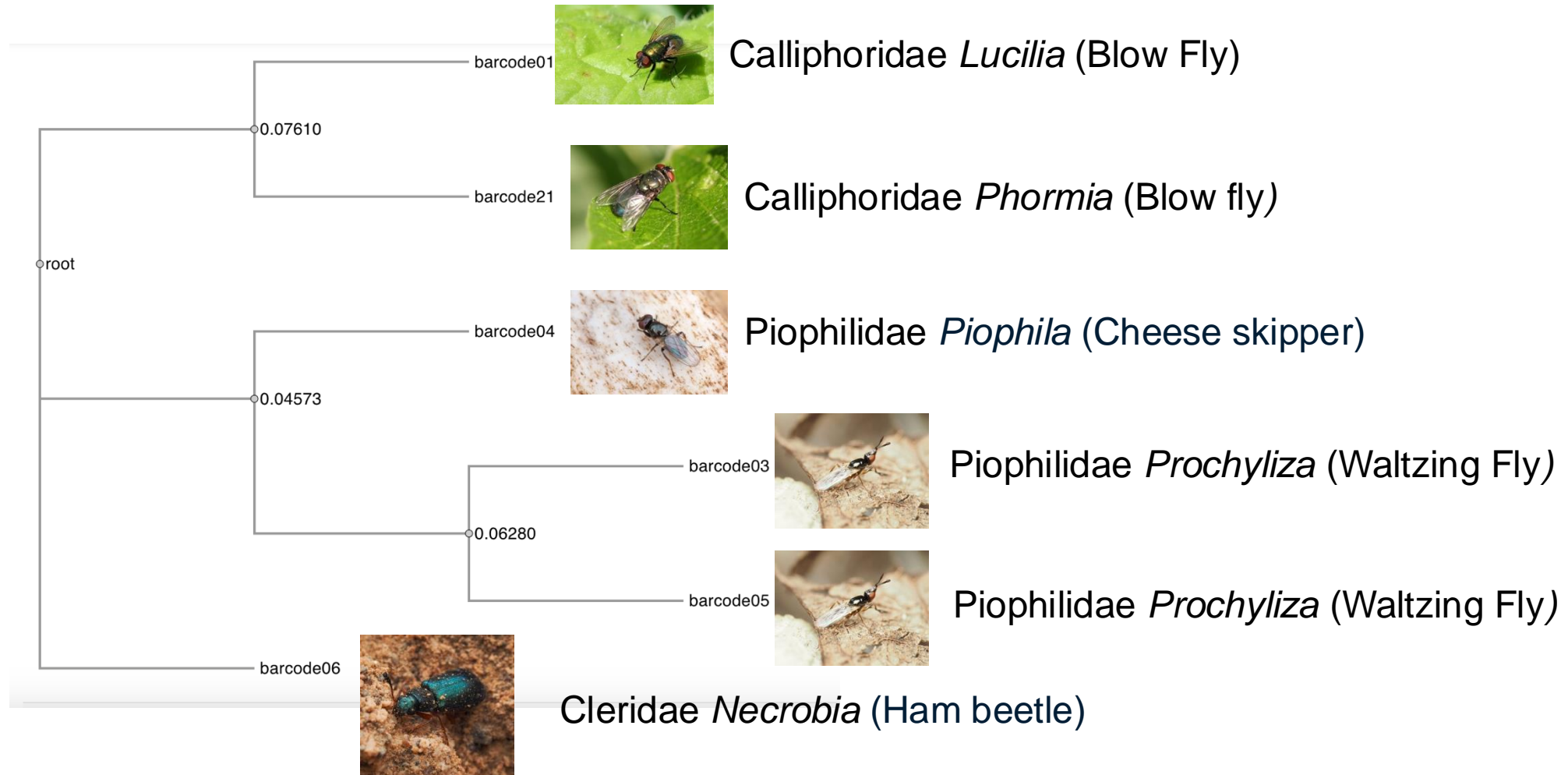
- Use DNA Barcoding to identify samples from the Forensics Anthropology Center to help determine post-mortem interval.

Sanger Sequences didn't come back in time!



Jeffrey Petracca

Forensics Crime Lab 2024



Next Steps...

Integrate capability to analyze ONT reads in DNA Subway lines



Features:

- GPU base calling
- Metabarcoding (**Purple line**)
- Small Genome assembly (**Orange line**)
 - Phage, chloroplasts, plasmids

Thanks!

- NSF DUE 2301207



- Oxford Nanopore Technologies



- Texas Advanced Computing Center

