



**Using Cell-free Systems to Synthesize and Express Bacteriophage Genomes:
Practical Biomanufacturing for Bioscience Educators**

DNA Learning Center NYC at City Tech, Brooklyn, NY
June 3-6, 2024

Monday, June 3

- 8:30 am Workshop Objectives, Concepts, and Workflows: Red, Green, Blue, Purple
- 9:00 am Phage T7 Genome Assembly and Tail Fiber Mutagenesis (Part 1)
- Set up Long PCR Reactions (~1 hour)
- 11:00 am TXTL of Reporter Genes (Part 1)
- Set up Reactions of Linear and Plasmid DNA
- TXTL Production of Phage T7 (Part 1)
- Set up Reactions at Different Concentrations
- 12:30 pm LUNCH, Vincent Noireaux
- 1:30 pm Phage T7 Genome Assembly and Tail Fiber Mutagenesis (Part 2)
- PCR Clean-up, DNA quantitation, and Gel Electrophoresis
 - PCR Mutagenesis (~2 hours)
- 3:00 pm TXTL Production of Phage T7 (Part 2)
- Set up Spotting Assay and Overnight Incubation
 - Set up Plate Reader
- 4:30 pm Phage T7 Genome Assembly and Tail Fiber Mutagenesis (Part 3)
- PCR mutagenesis: Clean-up, DNA quantitation, and Gel Electrophoresis

Tuesday, June 4

- 8:30 am Phage Genome Assembly and Tail Fiber Mutagenesis (Part 4)
- DNA Assembly Reactions and TXTL Reactions of T7 Wild Type and T7 Mutants
- 10:30 am TXTL of Reporter Genes (Part 2)
- View Results Under UV Light
- TXTL Production of Phage T7 (Part 3)
- Results, Calculate Plaque Forming Units (PFUs)
 - Results from Plate Reader
- 12:00 pm LUNCH
- 1:00 pm Seminar: Phage Biology, Steve Bowden
- 2:00 pm Phage Genome Assembly and Tail Fiber Mutagenesis (Part 5)
- Electrophoresis of T7 Genome Assemblies (optional)
 - Set up Spotting Assay and Overnight Incubation
- 4:00 pm Questions and Answers



Wednesday, June 5

- 9:00 am **Phage Genome Assembly and Tail Fiber Mutagenesis (Part 6)**
• Pick and PCR of Amplified Tail Fiber Mutants
- 10:00 am Seminar: Nanopore DNA Sequencing, Anna Feitzinger
- 11:00 am **Phage Genome Assembly and Tail Fiber Mutagenesis (Part 7)**
• Gel Electrophoresis of Amplified Tail Fiber Mutants
• Spotting Mutant and Wild-type Plaques
- 12:30 pm LUNCH
- 1:00 pm **Nanopore DNA Sequencing of Tail Fiber Mutants (Part 1)**
• Rapid Adaptor Library Preparation
• Load Nanopore Flow Cell and Begin Sequencing
• Review Early Results

Thursday, June 6

- 9:00 am **Phage Genome Assembly and Tail Fiber Mutagenesis (Part 8)**
• Calculate Efficiency of Plating by Spotting Mutant and Wild-type Plaques
- 9:30 am **Nanopore DNA Sequencing of Tail Fiber Mutants (Part 2)**
• Create Consensus, Align Sequences, and Analyze Mutations in Tail Fiber Sequence
- 11:30 am Classroom Implementation: Settings, Challenges, Bottlenecks, Support
Post-Workshop Survey
- 12:30 pm Graduation
- 1:00 pm LUNCH (optional)