SEA-Phages Lab: Discovery of FunIvenBio101A

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Abstract
We began this lab on May 16 and concluded it on June 8, 2022. Phage was present and we found a siphoviridae that was about 220 nm in total. Its head was approximately 25 nm, and its tail was approximately 195 nm. While it was not perfectly pure, we isolated its DNA at a relatively consistent concentration.

Introduction
The SEA-PHAGES (Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science) Project is sponsored by the Howard Hughes Medical Institute. The goal of our lab is to isolate, purify, and amplify new phages from environmental samples that we collected. This is to be done by using a specific bacterial host, initially Mycobacterium Smegmatis and then Arthrobacter Sp, coupled with electron microscopy and DNA isolation and analysis. This experiment has already been conducted many times by other students, and the past research is all publicly available at https://phagesdb.org/ (Links to an external site.).
The program aims to increase interest in biology through immediate immersion in authentic, valuable, yet accessible research. To find and name our own bacteriophages. The SEA-PHAGES program has also produced useful research that has assisted doctors and medical research.
This lab was all about searching for bacterial phage, also called phage or bacterial virus. Frederick W Twort discovered bacterial phage in 1915 and around the same time was also independently discovered by Felix D'Herelle. It was named bacterial phage after the Greek word for bacteria either. This describes how the phage, a virus, attacks and infects the bacteria. The infection may or may not lead to the imminent death of the bacteria, depending on the conditions. Well many phages has been discovered. It's estimated that there are as many phages as there are people and therefore, there are many many more yet to be discovered. The goal of this lab is to discover bacterial phage in the soil sample collected.

Methods and Materials Used
- Cleaning Bench
    - Goal
        - Create zone of sterility
    - Materials Used
        - Plastic gloves
        - Antibacterial wipes
        - Alcohol-based solution
        - Bunsen burner
    - Methods
        - Place plastic gloves on hands
        - Wipe down bench with antibacterial wipes

        - Spray bench with alcohol-based solution
        - Light Bunsen burner
- 5.1 Collecting Environmental Samples
    - Material Used
        - Plastic sandwich bags for collecting soil samples
        - Shovel
        - Clean plastic bottle for liquid samples
        - Labeling pen
        - Smart phone or tablet with GPS capabilities or computer
    - Method
        - Grab a handful of soil, keeping the plastic bag between your hand and the sample
        - Remove your hand, inverting the bag with the soil to the inside, and seal the bag
        - Label the sample bag
        - Record the GPS coordinates of your sample collection site
        - Record the date, time, and weather of when sample was collected
- 5.2 Direct Isolation
    - Goal
        - To extract phages from an environmental sample
    - Materials Used
        - Environmental sample
        - Phage Buffer (5 ml/sample)
        - Sterile 3 ml or 5 ml syringe
        - 0.22 μm syringe filter
        - 5 ml serological pipettes
        - Microcentrifuge tubes
        - 15 ml conical tube
    - Method
        - Fill 15 ml conical tube one-third to one-half way full with soil
        - Add PB until the sample is submerged beneath 2–3 ml of liquid
        - Incubate the tube while shaking vigorously in a shaking
- 5.3 Plaque Assay
    - Goal
        - Detecting the presence of phages on bacterial lawns
    - Materials Used
        - Phage samples for isolation, purification, or titering
        - Host bacteria (Initially M. Smeg then Arthrobacter Sp. (250 μl/plate)

        - Agar plates
        - Phage buffer
        - Top agar, molten (between 55 - 60 ˚C)
        - Microcentrifuge tubes
        - 5 ml serological pipettes
    - Method
        - Combine 250 µl of Arthrobacter Sp. with 10 µl of each dilution using micropitetter
        - Wait 10 minutes
        - Plae sample with TA
            - Using 5 ml pipette combine 3ml of TA with bacteria+dilution
            - Suck up to mix
            - Plated with PYCaTA
        - Wait 10 mintues
        - Invert plate
        - Repeat process for all plates
- 5.4 Picking a Plaque
    - Goal
        - To retrieve phage particles from a plaque and create a liquid sample
    - Materials Used
        - Agar plates with plaques of interest
        - Phage buffer
        - Microcentrifuge tubes
    - Method
        - Using a labeling pen, mark the plaques you intend to pick by drawing a small circle around the plaque on the *bottom* of the plate
        - Label each tube according to the identifier you used for each plaque.
        - Using aseptic technique, place 100 µl of phage buffer into each microcentrifuge tube
        - Identify a well isolated plaque
        - Using P200 place tip in center of plaque and place tip into PB
        - Vortex (label as new 0 [10^0])
- 5.5 Soil Enrichment

- Goal
  - To amplify phages present in your environmental samples
- Materials Used
  - Solid environmental sample
  - 0.22 μm Corning ® Tube-Top Vacuum Filter Systems or syringe filters
  - Liquid media
  - Conical tube
  - Microcentrifuge tubes
- Method
  - Fill 50 ml conical tube with your sample to the 15 ml mark
  - Add liquid media to 35 ml mark
  - Vortex
  - Filter Solution using pipette transfer Athrobacter Sp (.5 μm) in filtrate
  - Vortex; incubate with shaking for 2-5 days
  - Get 2 microcentrifuge tubes
  - Label them team 4 #1 and #2
  - Transfer 1.4 ml of culture into each microcentrifuge tube
  - Centrifuge for 60 seconds to pellet bacteria
  - Transfer supernatant into new microcentrifuge tube (being careful not to pick up pellet)
    - (Make sure to keep tubes)
  - Spot 10 μm
  - Conduct Spot Test 5.6

- 5.6 Spot Test
  - Goal
    - Test a sample for the presence of phage
  - Materials Used
    - Liquid Phage sample
    - Ager Plate
    - Host Bacteria ((Initially M. Smeg then Arthrobacter Sp.)
    - Top Agar
    - Phage Buffer
    - 5 ml pipette
  - Method

        - Prepare bacterial lawn Label bottom of the plate Team 4 then Date and Spot Test
        - Combine 250 µl M. Smeg with 3 ml of TA
            - (Later Different Bacteria was used)
        - Using sterile 5ml pipette transfer 3ml of molten TA to a culture tube containing host bacteria then draw back up solution to mix
        - Plate
        - Allow to solidify
            - Sit for 10 min
        - Spot 10 µl of filtrate onto plate
        - Do not invert when incubating
- 6.1 Plaque Assay for Purification
    - Goal
        - To generate well-isolated plaques
    - Materials Used
        - Phage samples for isolation, purification, or titering
        - Host bacteria (Initially M. Smeg then Arthrobacter Sp. (250 µl/plate)
        - Agar plates
        - Phage buffer
        - Top agar, molten (between 55 - 60 ˚C)
        - Microcentrifuge tubes
        - 5 ml serological pipettes
    - Method
        - Follow Protocol 5.4 - Picking a Plaque
        - Follow Protocol 6.2 - Serial Dillution
        - Plaque Assay and Plate each dilution
        - Follow Protocol 5.3 - Plaque Assay
- 6.2 Serial Dilution
    - Goal
        - To prepare liquid phage samples of decreasing concentrations
    - Materials Used
        - Phage samples
        - Phage buffer
        - Microcentrifuge tubes
        - Pipettes

    - Method
        - Arrange and label tubes 10^0, 10^-1, 10^-2 ...
        - Add 90 µl of PB in each tube
            - Appendix 1
        - Add 10 µl of sample and vortex
        - Repeat
- 6.3 Collecting Plate Lysates
    - Goal
        - To generate a highly concentrated liquid phage sample
    - Materials Used
        - Webbed plate(s) with clonal phage population
        - Phage buffer (8 ml/plate)
        - 0.22 µm filter
        - 5 ml syringe
        - 15 ml sterile conical tube for lysate storage
    - Method
        - Plate Sample with TA using 5 ml pipette
        - Combine 3ml of TA with bacteria solution
        - Wait 10 minutes
        - Invert plate
        - Repeat steps for all plates
        - Incubate at 27 degrees C
- 6.4 Spot Titer
    - Goal
        - To determine the concentration of phage particles in a lysate using a spot test
    - Materials Used
        - Lysate for titering
        - Host bacteria (Initially M. Smeg then Arthrobacter Sp. (250 µl/plate)
        - Agar plates
        - Phage buffer
        - Top agar, molten (between 55 - 60 ˚C)
        - Microcentrifuge tubes
        - 5 ml serological pipettes

    - Method
        - This is done to calculate the titer of our sample
        - Label plate
            - Appendix 2
        - Prepare a bacterial lawn using an aseptic technique using a 5 ml pipette transfer 3 ml of molten ager
        - Conduct Protocol 6.2 - Serial Dilution
        - Spot the dilution and controls on the prepared bacterial lawn
        - Transfer 3 µl of all samples onto the bacterial lawn
        - Allow to solidify
            - 10 min
        - Incubate plate at 27 degrees C
        - Don't invert plate
- 9.1 Phage DNA Extraction
    - Goal
        - To isolate genomic DNA from phage
    - Materials Used
        - Phage lysate
        - Microcentrifuge tubes
        - 5 ml serological pipettes
        - Nuclease mix
        - 12 ml 80 % isopropanol, freshly prepared
        - 2 ml DNA clean-up resin (Promega Wizard DNA Clean-Up Kit)
        - 2 DNA clean-up columns (Promega Wizard DNA Clean-Up Kit)
        - ddH2O pre-warmed (95 °C)
    - Method
        - Degrade bacterial DNA/RNA in high titer lysate
            - Aseptically transfer 1 µl of phage Lysate into tube
            - Add 5 nµ nuclease mix to the lysate
            - Mix gently thru inversion - don't vortex
            - Begin incubation at 37 degrees C for 10 min
        - Denature the protein capsid to release phage DNA
            - Add 2 ml of DNA Cleanup resin
            - Transfer your nuclease treated phage lysate from the microcentrifuge tube to 10 ml conical tube
            - Mix gently by repeatedly inverting for 2 mins
        - Isolate the phage genomic DNA

- Label to wizard kit columns
- Remove plungers for the 2 and 3 ml syringe
    - Set column and syringe barrel on a new microcentrifuge tube
    - Transfer 1.5 ml of phage DNA resin solution using pipette
    - Do not discard empty 15 ml conical tube
    - Insert plunger into syringe and push liquid thru
    - Unscrew column and release plunger
    - Reattach syringe to colum
- Wash Salt from DNA
    - Add 2 ml 80% isopropanol to each syringe barrel
    - Repeat 2 more times
- Remove additional isopropanol
    - Spin for 5 mins
    - Transfer to new microcentrifuge tube
    - Spin for 1 min
    - Transfer to new tube
    - Use P200 to transfer 50 µl of DIY water
        - Let sit for 1 minute
    - Vortex for 1 min
- Determine concentration of phage using DNA Spectrometer
- Store at 4 degrees C for short term
    - -20 degrees C for long term

Results

The dirt which we initially collected and used was from David's backyard in Roseland, New Jersey, at @40.8080,-74.2852.

Our class initially attempted this trial with M Smagnatis; however, we experienced poor bacterial growth, and after one last spot test, we switched bacteria. The initial bacteria m Smeg is an acid-fast bacterial species that is known for its relatively high growth rate. (American Society for Micro Biology) That being said, due to poor growth, we switch to Arthrobacter sp. Arthrobacter Sp. is one of over 70 species that make up the genus Arthrobacter classified within the phylum actinobacteria. Arthrobacter will grow and

divide on the nutrient-rich media and is tan to yellow in color. (Seaphages) This is merely a revision, and the goals, procedures, and materials have not changed. Changing the bacteria only changed one input, at which point we commenced redoing all previous steps done.

We then conducted a spot test which revealed no phage in our soil. We waited one more day, but the results were still negative. Although we did not have any phage present, we did experience unexpected bacterial growth, as seen in appendix 3. we believe the reason for this unexpected bacterial growth was due to leaving our zone is sterility.

Due to this lack of phage, we revised our experiment yet again. Switch to using group twos dirt then had already tested positive for phage. Was group 2's, which was Collected on: 5/16/22

The coordinates are: @ 40.977297, -74.1540369

The temp was: 70-75 Fahrenheit

The location: Kaya's garden

We proceeded with an attempt to purify our new soil. We experienced bacterial contamination on all plates but our 10^0, and due to this, no phage was visible. This was due to our Top Ager being contaminated. Our 10 to the 0, there was some phage present, but it was mostly concentrated on one side. The plaques were all about the same size, and they were very tightly gathered. We attempted to pick one of these bacteriophages and purify it further. This resulted in improvement. Our 10^0 showed improvement as the phage was more plentiful and spread out. However, by comparison, our 10^0 still was very clustered and had phages of varying sizes. Our 10^-1 show phages of similar size, but they were still close together. This would become our new 10^0. We again continue to dilute and purify our sample. We can experience bacterial

contamination on 5/31/22 and required the professor's help in attempting you select a phage and salvage our plate. However, we could not pull any phage due to bacterial contamination spreading, and we can no longer proceed with our current sample.

Due to the aforementioned extensive bacterial contamination, we were no longer able to continue working with our sample. To get phages, we will be picking a sample from team 3s 10^-3 plate.

Team threes dirt was also from team 2, i.e., Kaya's Garden. In order to take a different plaque than team 3, we chose the opposite of what they chose. Team 3 chose the smaller plaque, while team 4 chose the larger ones.

We proceeded to generate any concentrated liquid page sample and ended up having our plate flushed with 5 ml of phage buffer. We attempted to begin a spot titer test; however, due to further bacterial contamination, we were not able to complete this. At this point, we would learn that our phage buffer caused our continued bacterial contamination.

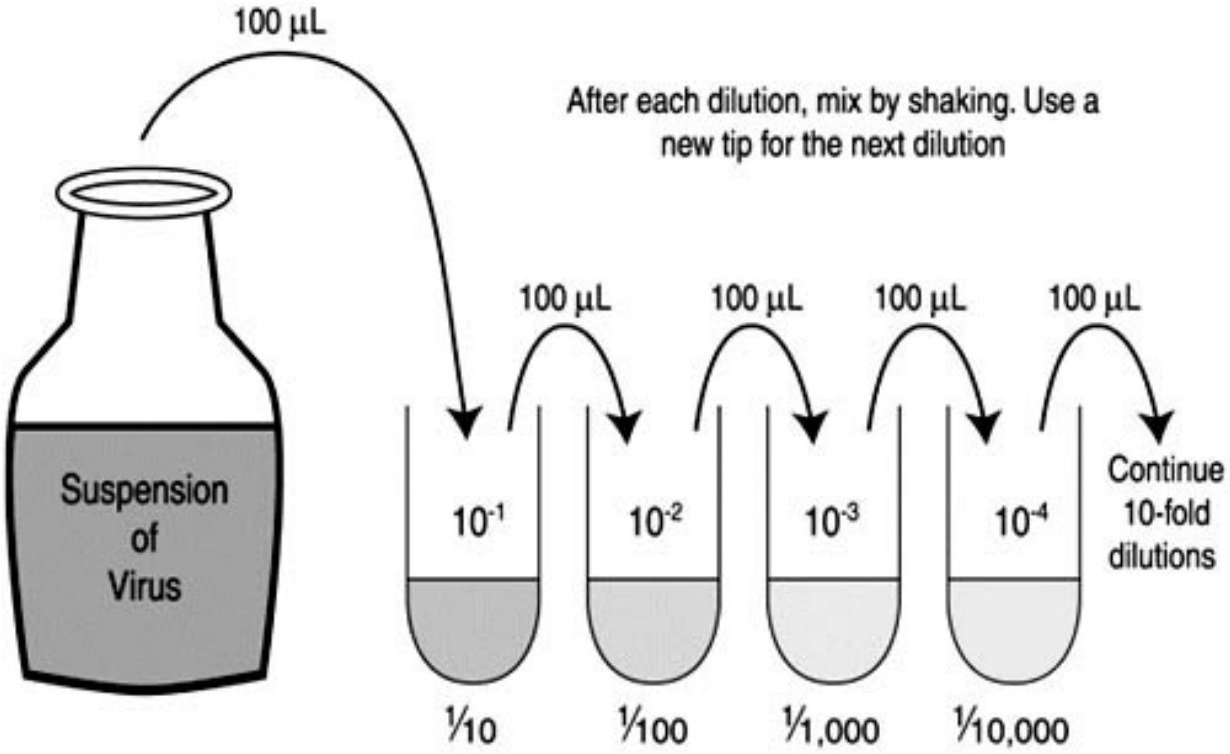
We proceeded to extract the DNA of our Phage, which we were successful in. We named our phage using a random name generator and the word bio, which resulted in: FunIvenBio101A. This type of phage is a Siphoviridae. It is about 220 nm, with its head being approximately 25 nm and its tail being approximately 195 nm.

Images of our phage are below (Appendix 4,5,6).

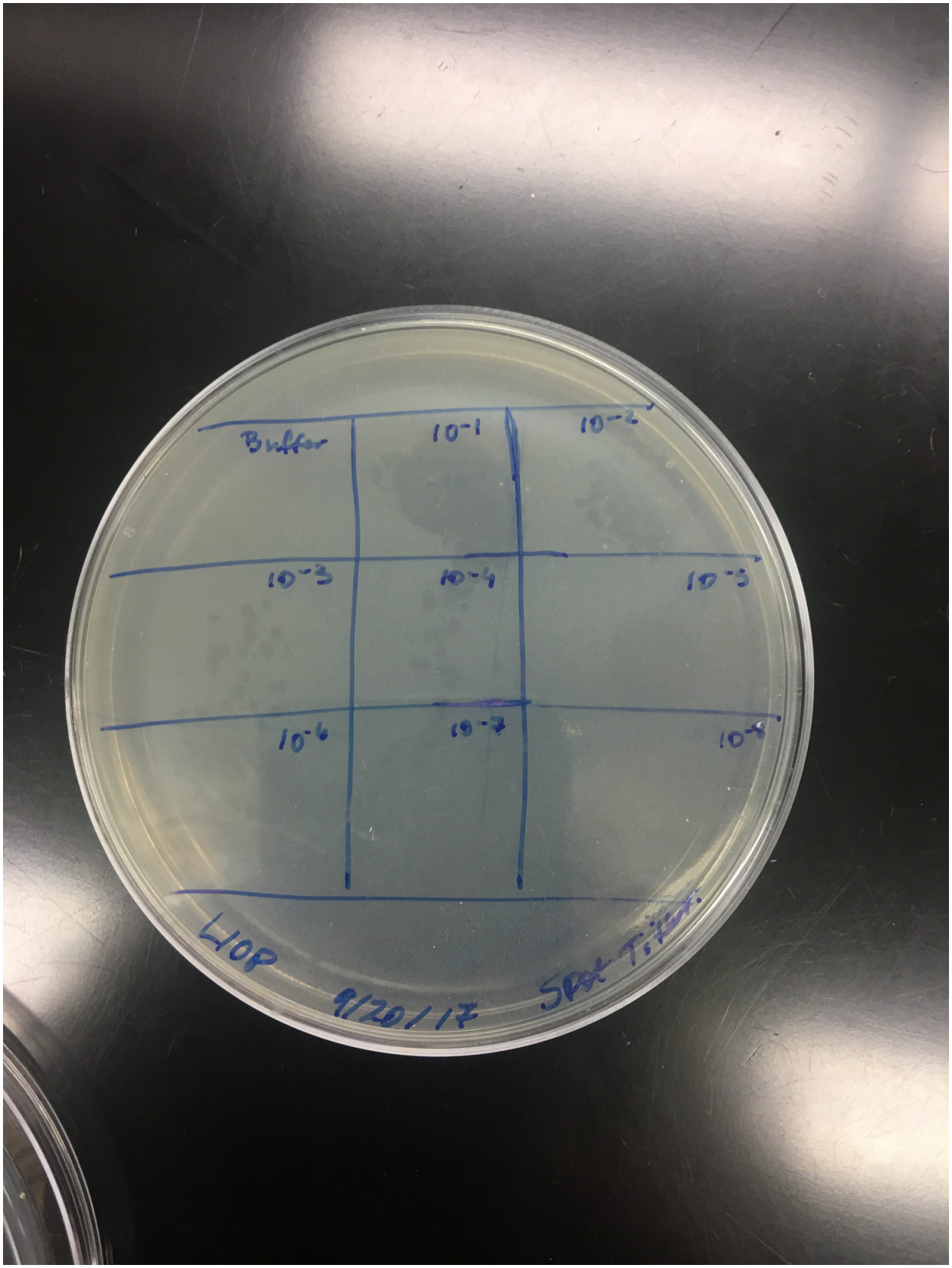
We obtained the DNA information from our spectrophotometer results (Appendix 7, 8, 9). while our phage purification was not perfect as there is a goal of 1.8, we had results of 1.9 and 1.92, which are still very good. Further, our concentration was also very good at 72.4 and 77.3. We then registered or phage on the website mentioned above.

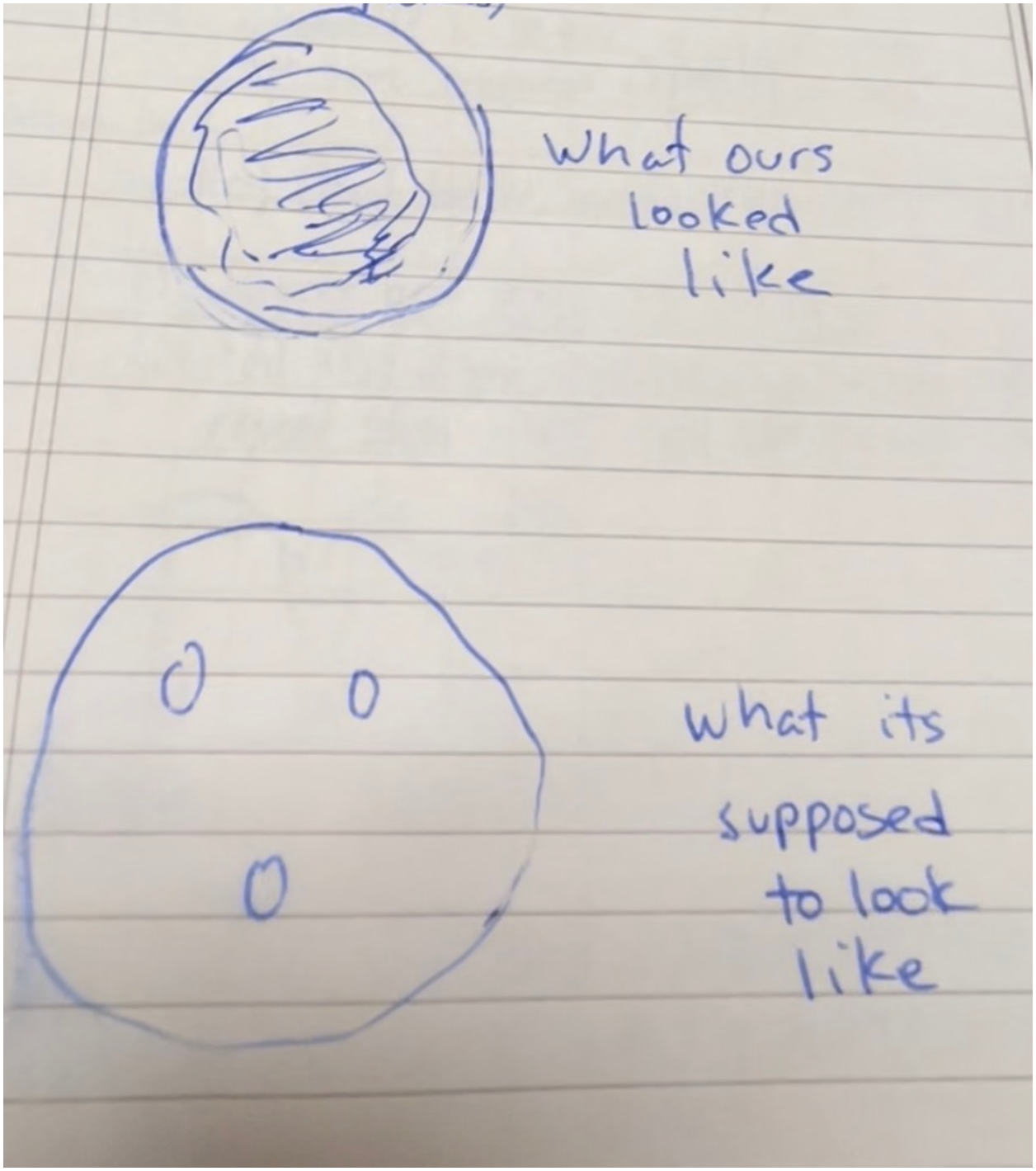
Apendix

1

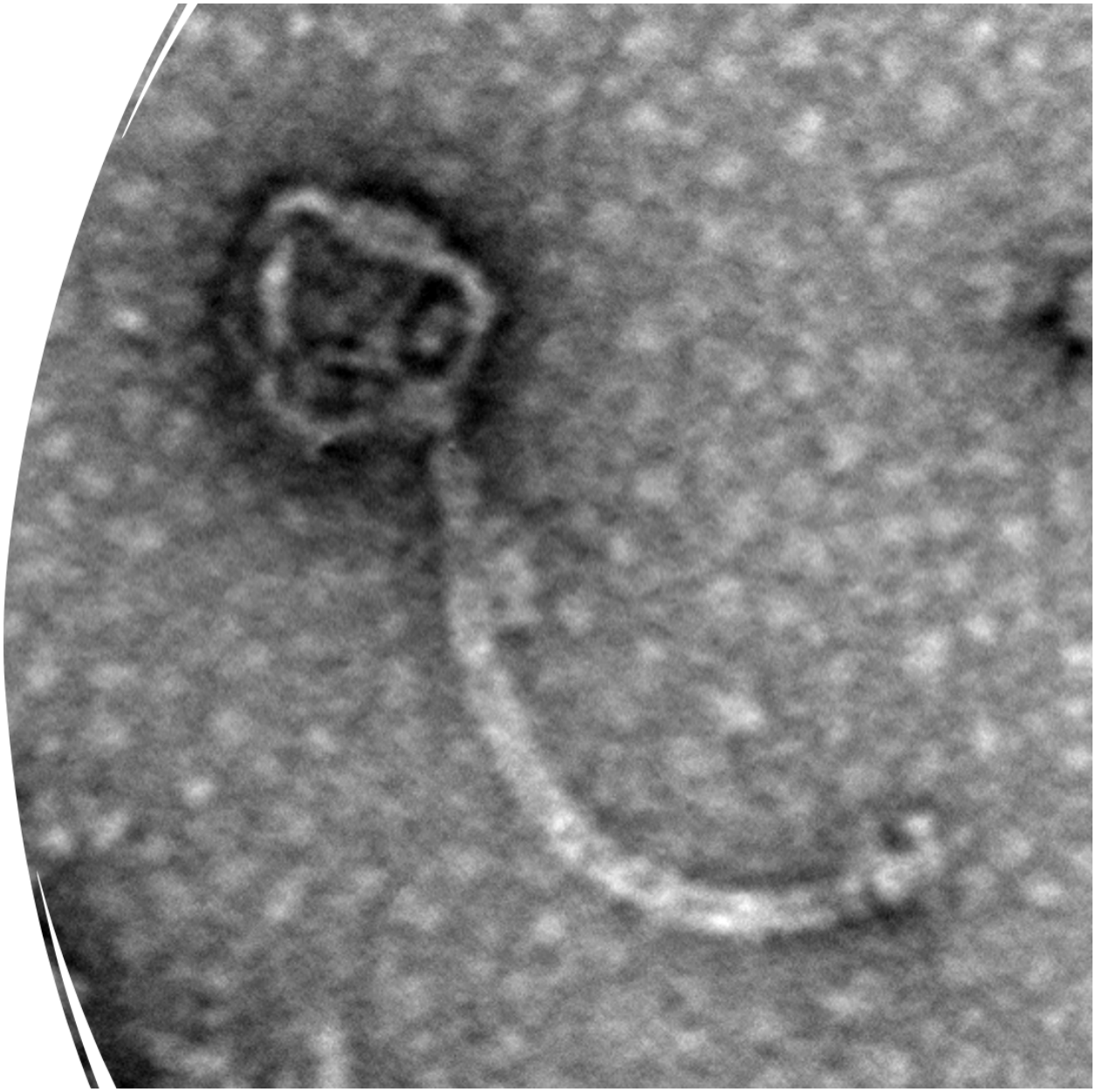

100 µL
After each dilution, mix by shaking. Use a new tip for the next dilution
Suspension of Virus
100 µL
100 µL
100 µL
100 µL
10^{-1}
10^{-2}
10^{-3}
10^{-4}
Continue 10-fold dilutions
1/10
1/100
1/1,000
1/10,000

2

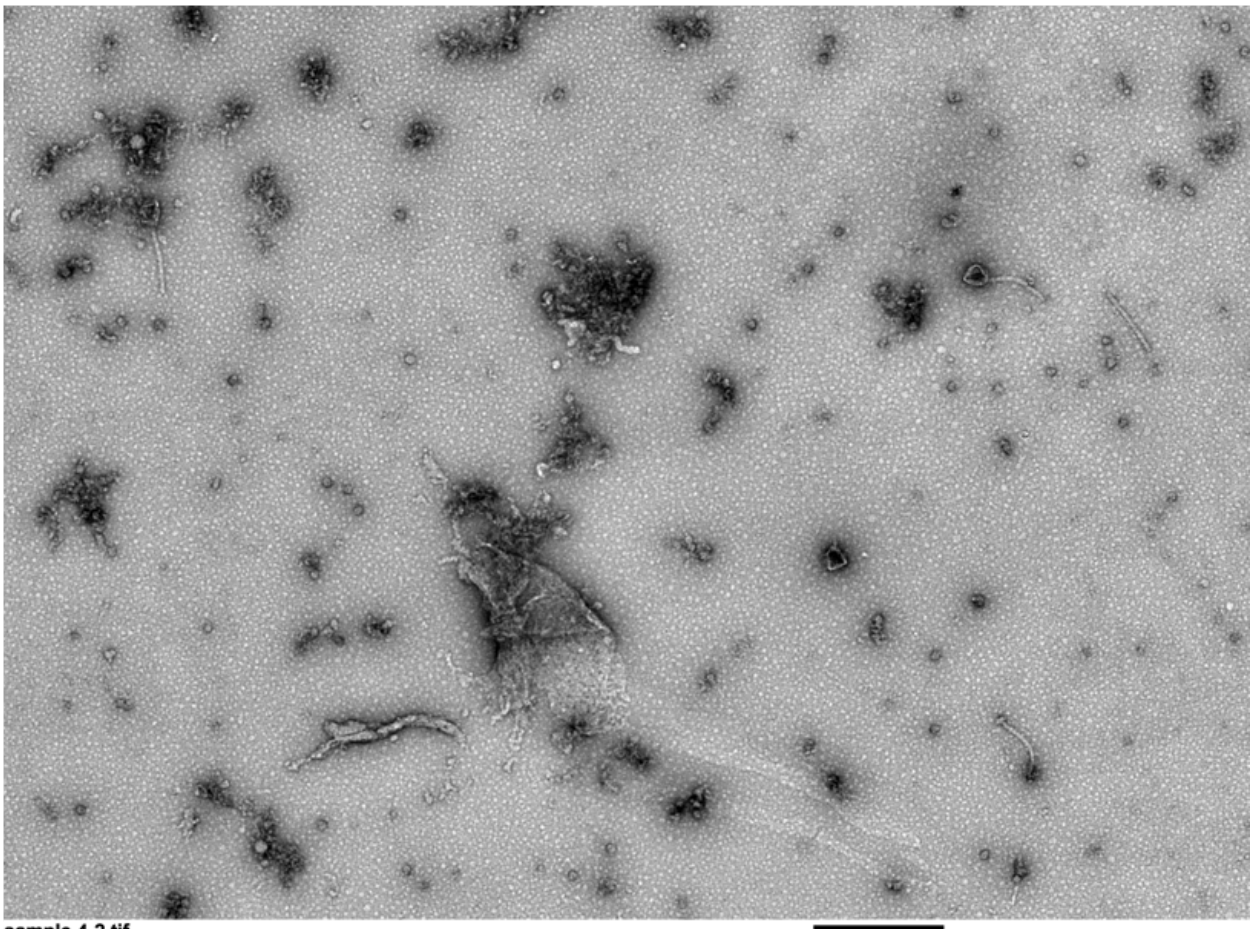



What ours looked like
what its supposed to look like

4



5

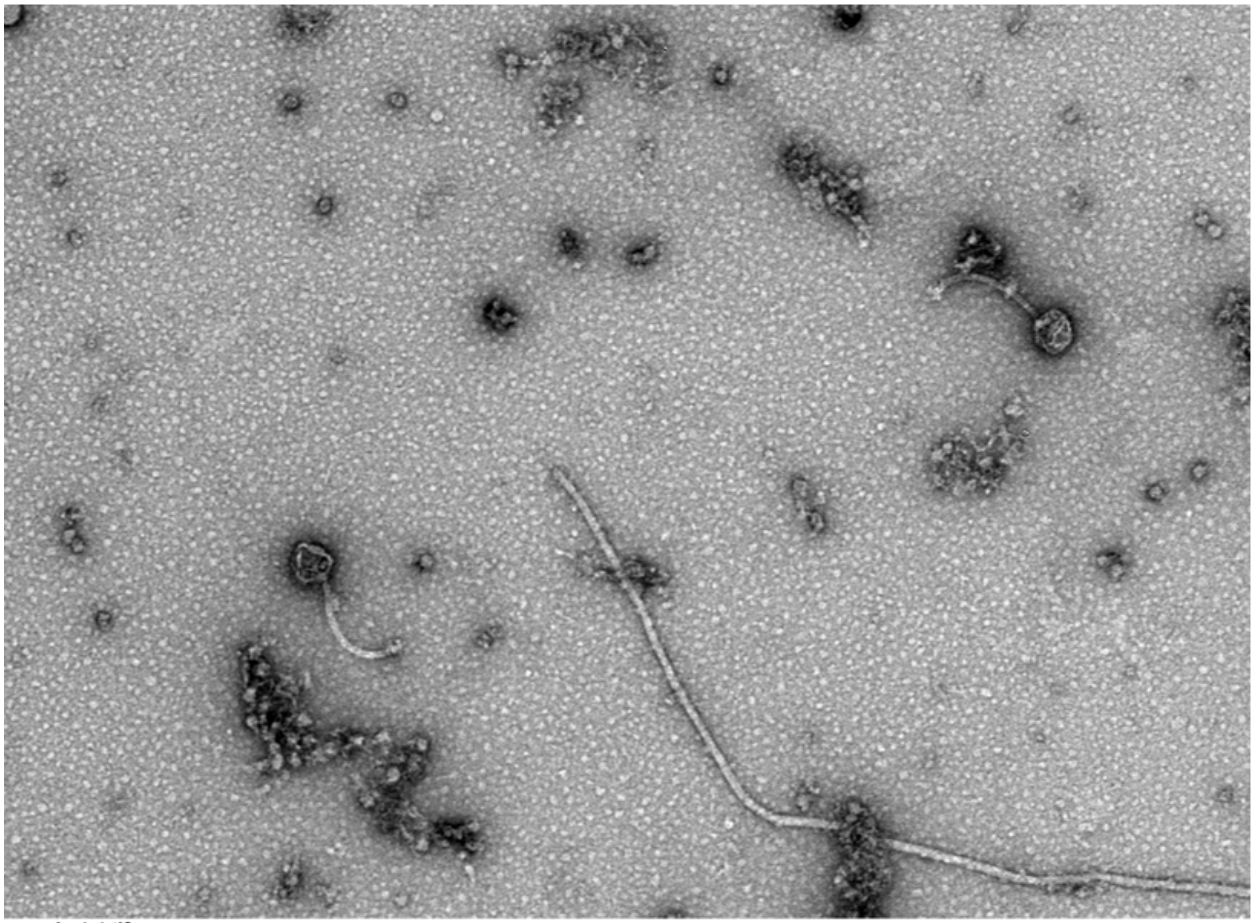


sample 4-2.tif
Cal: 0.931590 nm/pix
12:22 6/3/2022

400 nm
HV=100kV
Direct Mag: 7000 x

Camera: NANOSPRT12, Exposure: 1600 (ms) x 4 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

6

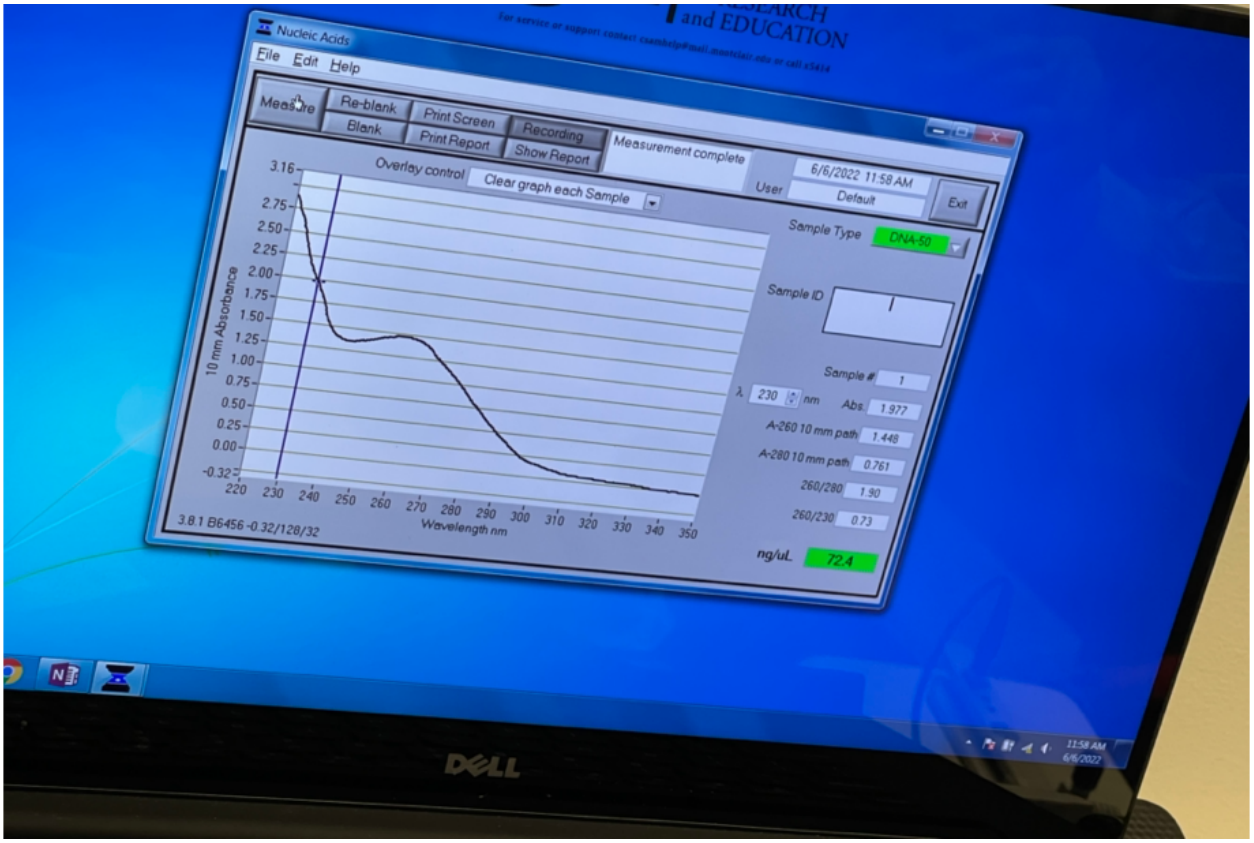


sample 4-4.tif
Cal: 0.543427 nm/pix
12:25 6/3/2022

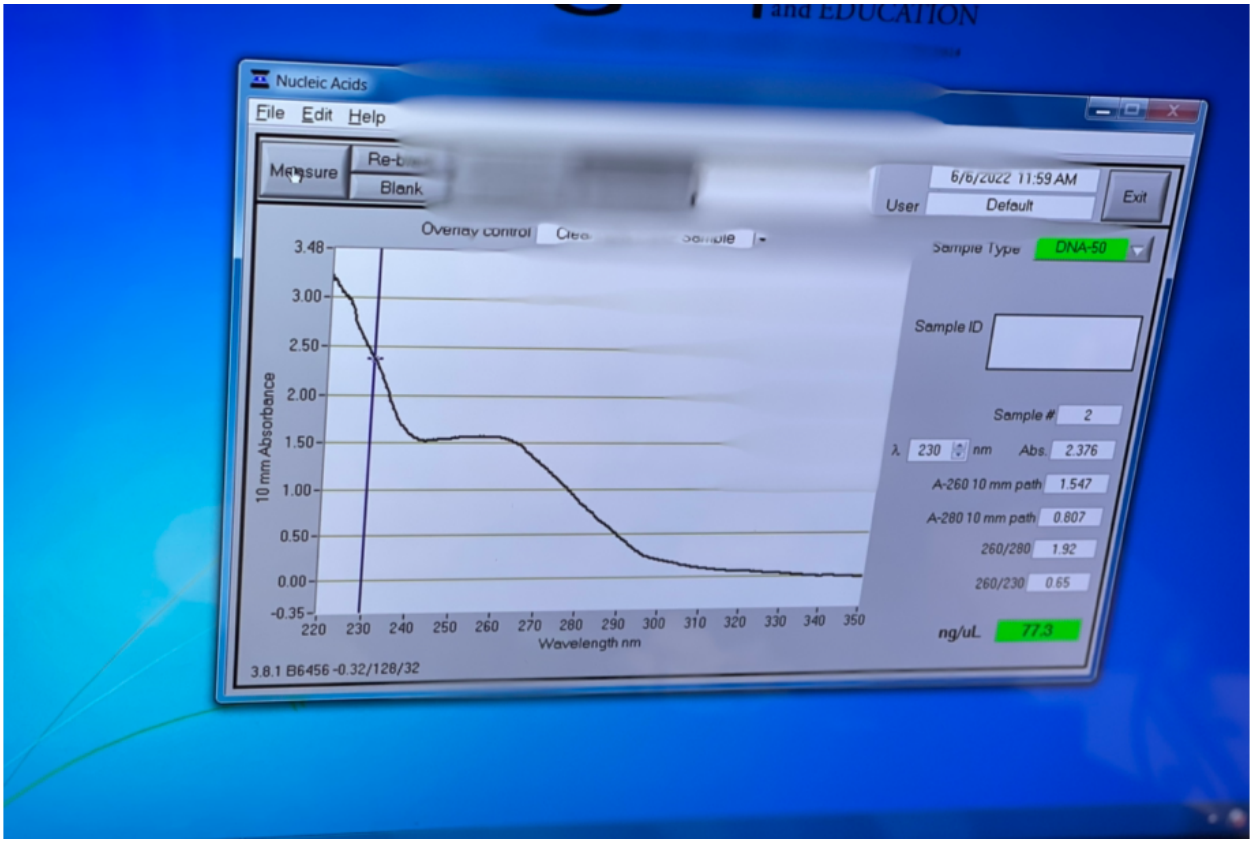
200 nm
HV=100kV
Direct Mag: 12000 x

Camera: NANOSPRT12, Exposure: 1600 (ms) x 4 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

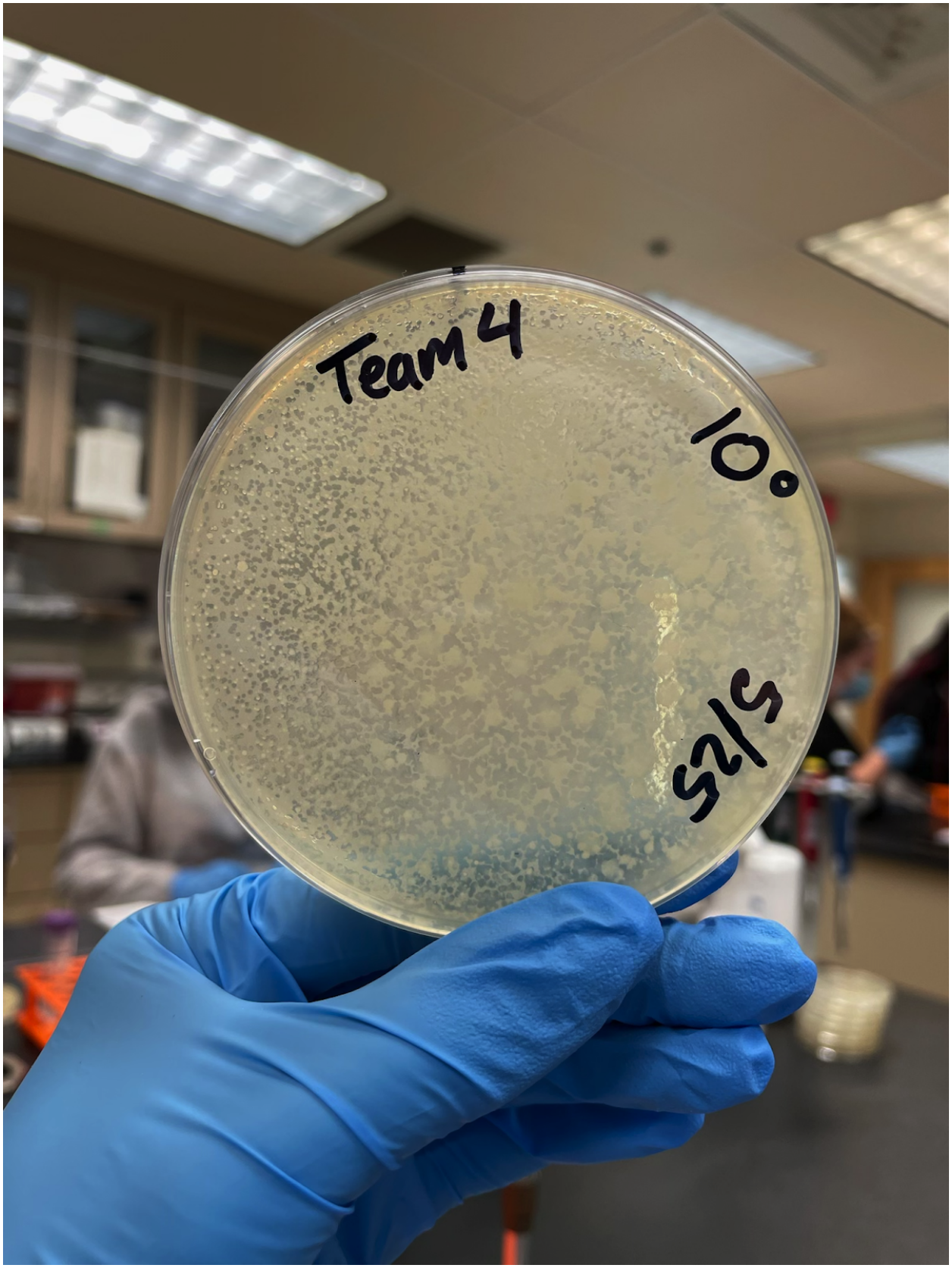
7

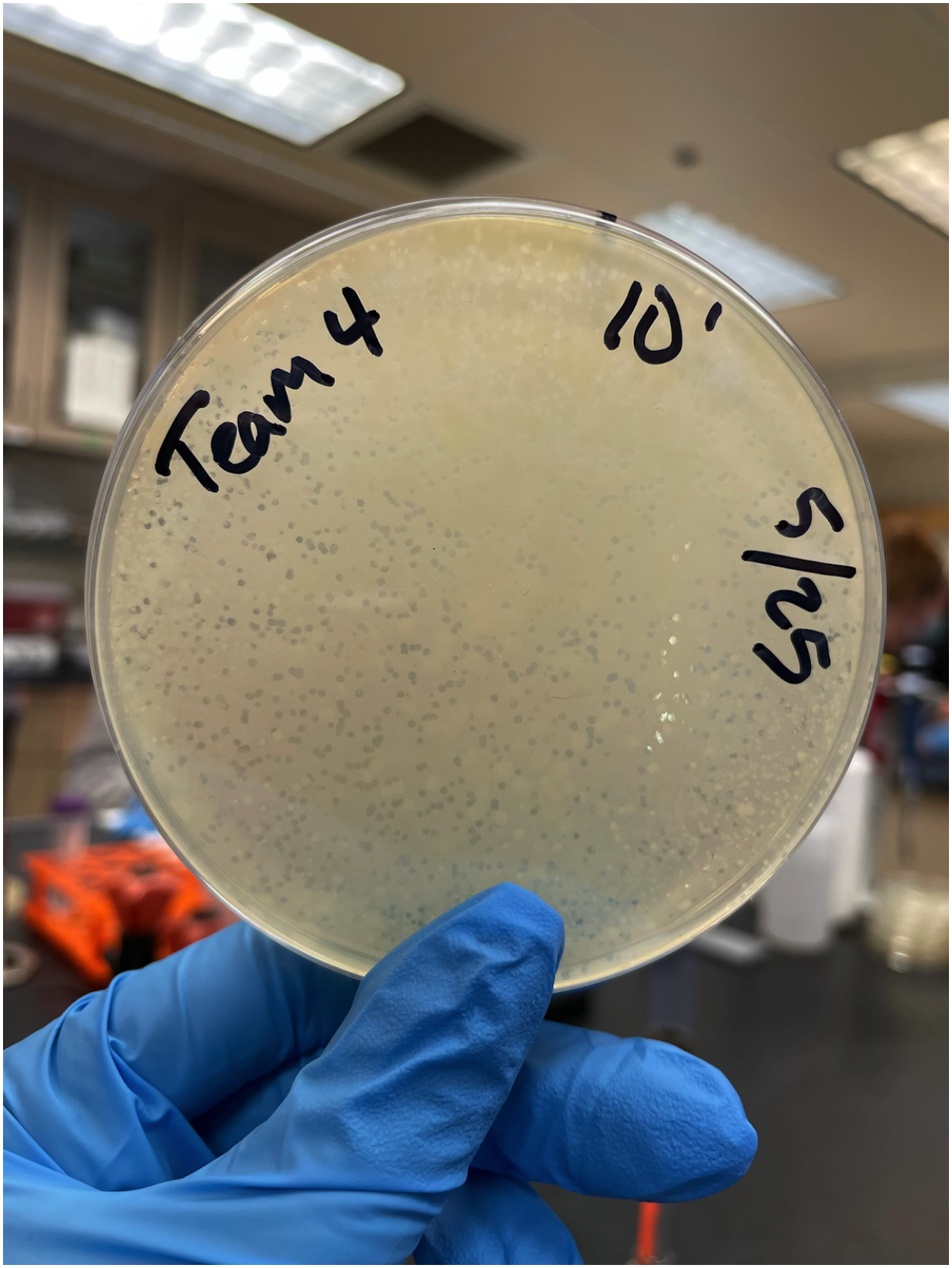

Nucleic Acids
File Edit Help
Measure
Re-blank
Blank
Print Screen
Print Report
Recording
Show Report
Measurement complete
6/6/2022 11:58 AM
User
Default
Exit
Overlay control
Clear graph each Sample
Sample Type
DNA-50
Sample ID
Sample #
1
λ 230 nm
Abs 1.977
A-260 10 mm path 1.448
A-280 10 mm path 0.761
260/280 1.90
260/230 0.73
ng/uL 72.4
10 mm Absorbance
3.16
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.32
220 230 240 250 260 270 280 290 300 310 320 330 340 350
Wavelength nm
3.8.1 B6456 -0.32/128/32
DELL

8



9 Inclass breakdown of results
IMG_4524.mov


Team 4
10°
S2/S


Team 4
10⁻
5/5/25


Team 4
5/25
10^{-2}
Team 4
10^{-2}
5/25

Citations

https://phagesdb.org/

https://seaphagesphagediscoveryguide.helpdocsonline.com/

Mastering Bio (the text book)

Dr. Adams

https://journals.asm.org/doi/10.1128/AEM.01318-12

https://seaphages.org/