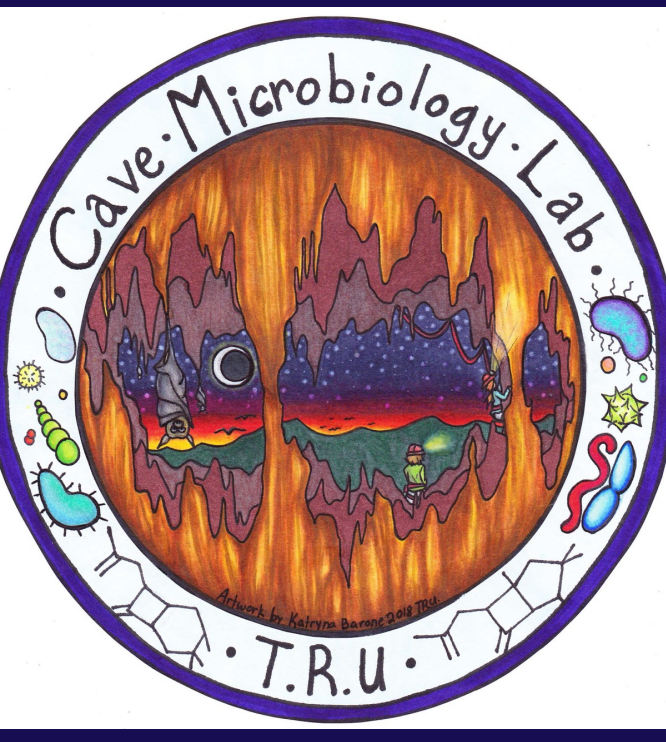


Isolation of Bacteriophages from Sewage Water Samples and their Effectiveness in Combating Multi-drug Resistant Bacteria



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Introduction

- In a preliminary study conducted in our lab isolated and purified three strains of bacteriophages that were successful in infecting two clinical strains of multi-drug resistant *Escherichia coli*.
- The bacteriophages were isolated from the municipal and hospital sewage of Kamloops and Kelowna in the Interior of British Columbia, Canada.

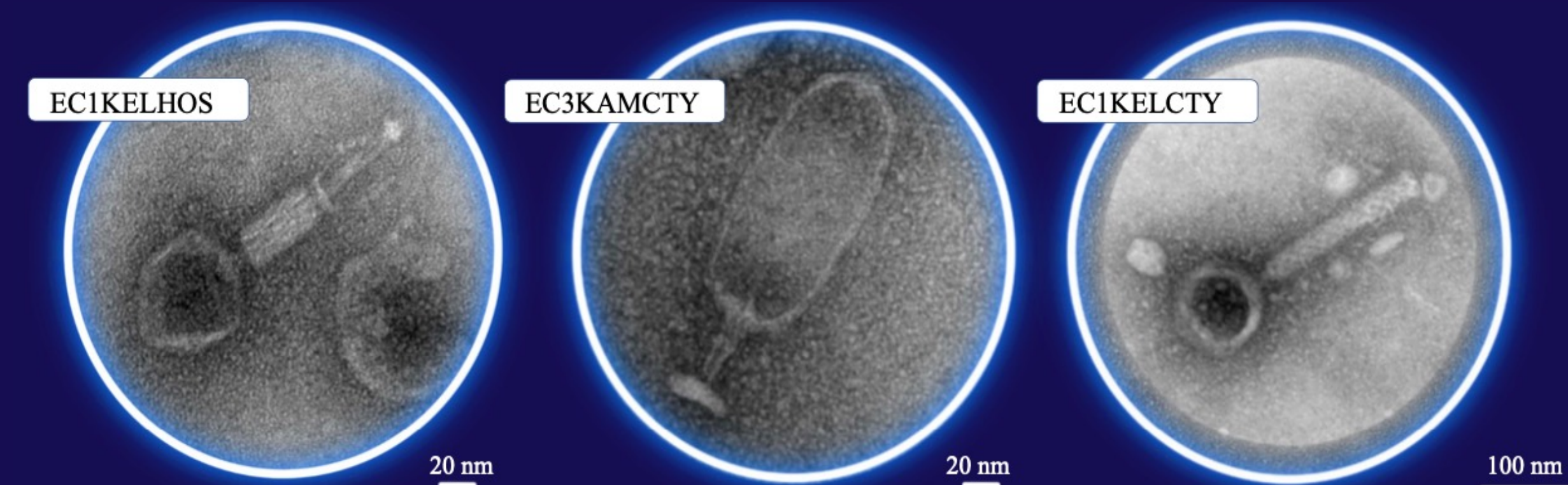


Figure 1. Transmission Electron Microscopy images of the three bacteriophages isolated in the preliminary study (UBC Bioimaging Facility RRID:SCR_021304).

Objectives

The objectives of this project were to

- Repeat the experiment to confirm the presence of the three strains of bacteriophages and their effectiveness on *Escherichia coli* (*E. coli*).
- Explore the impact of these three bacteriophages on other multi-drug resistant bacteria like *Pseudomonas aeruginosa* (PA) and Methicillin-resistant *Staphylococcus aureus* (MRSA). Additionally, seek out additional bacteriophages to provide further evidence supporting the development and efficacy of phage therapy in addressing antibiotic resistance.
- Investigate the temporal distribution pattern of bacteriophages in the interior of British Columbia and how their effectiveness in inhibiting multi-drug resistant bacteria in the lab setting differs or not.

Methods

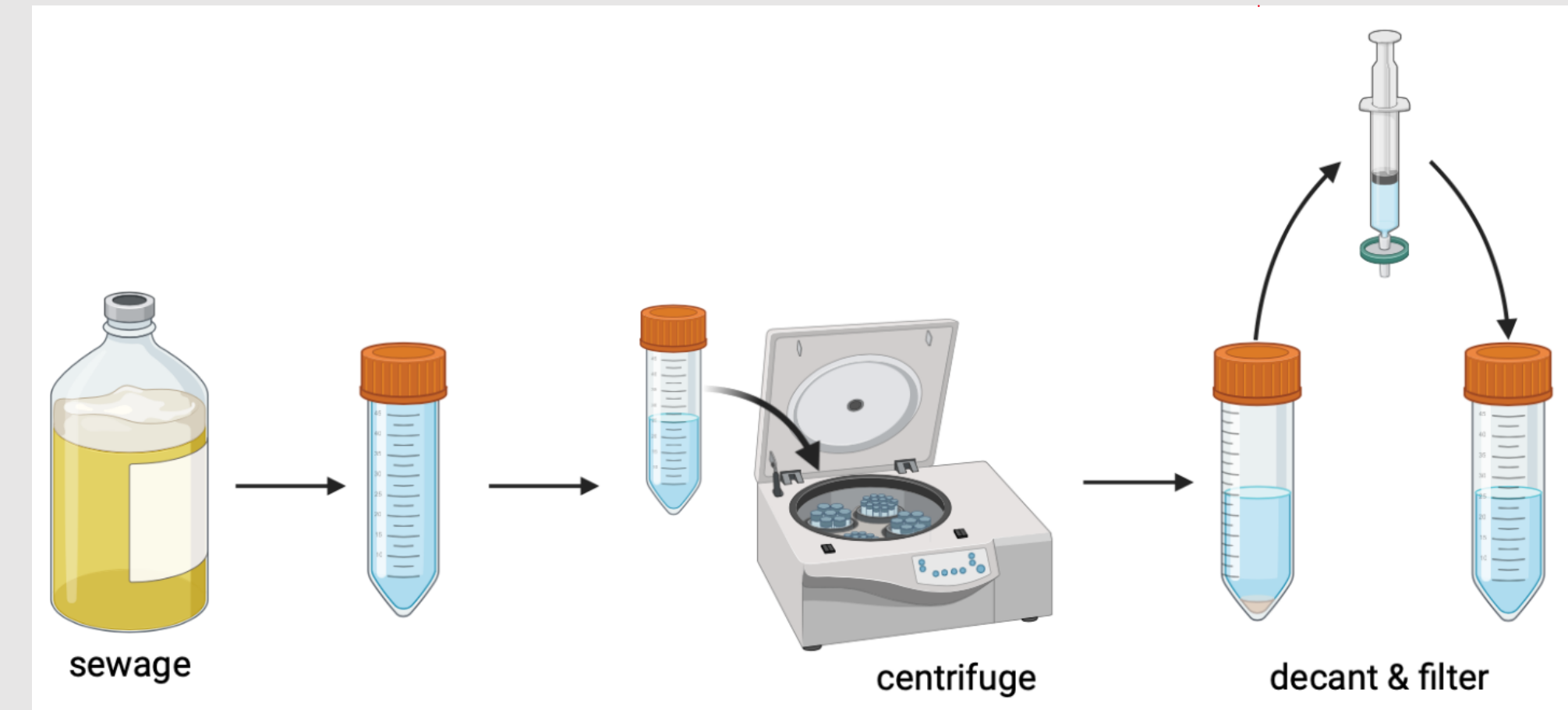


1. Sewage sample collection: Sewage samples were collected from the Kamloops Sewage Treatment Centre, Vernon Water Reclamation Center, and the Kelowna Wastewater Treatment Facility.

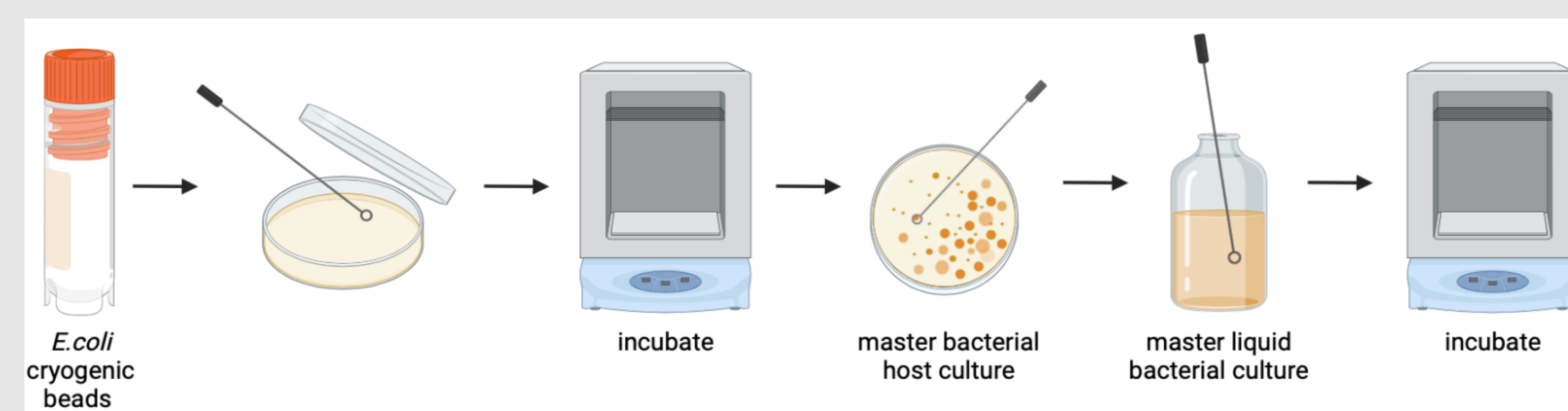
Figure 2. The areas from which the sewage samples were collected (Galymov *et al.*, 2022).

Methods

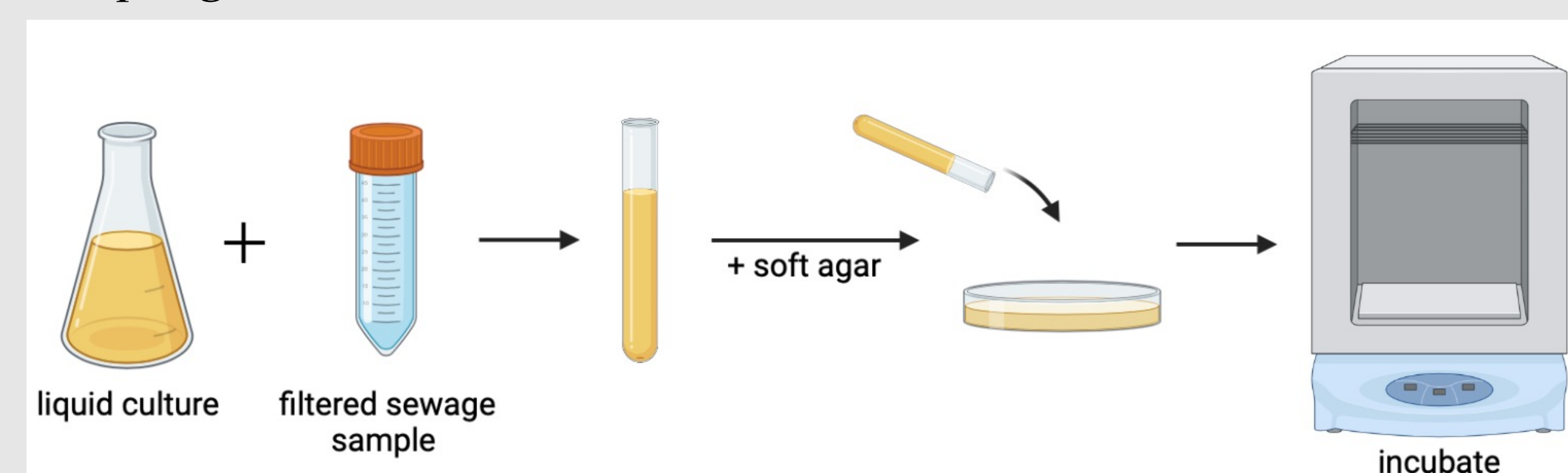
2. Sewage Sample Preparation:



3. Cultivating Bacterial Hosts and Liquid Bacterial Cultures:



4. Screening for Bacteriophages:



- We used the standardized methodology that was developed in our lab (Galymov *et al.*, 2022). A traditional double-layer agar method was used to screen, purify, and propagate bacteriophages from the local sewage samples.

Results

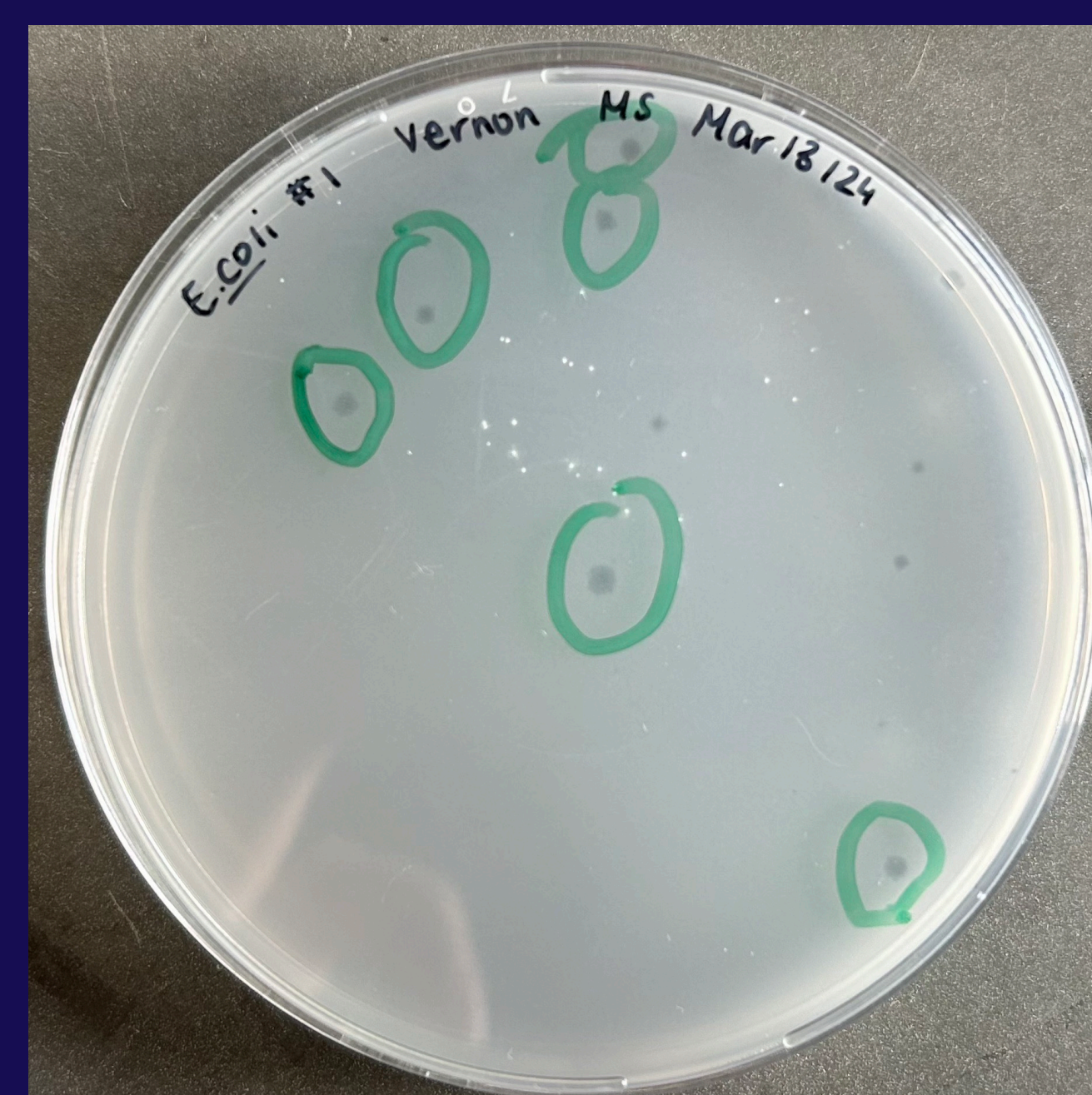


Figure 3. Plaques formed by bacteriophages from the Vernon sample in the double layer agar assay with MDR bacterial host *E. coli* #1.

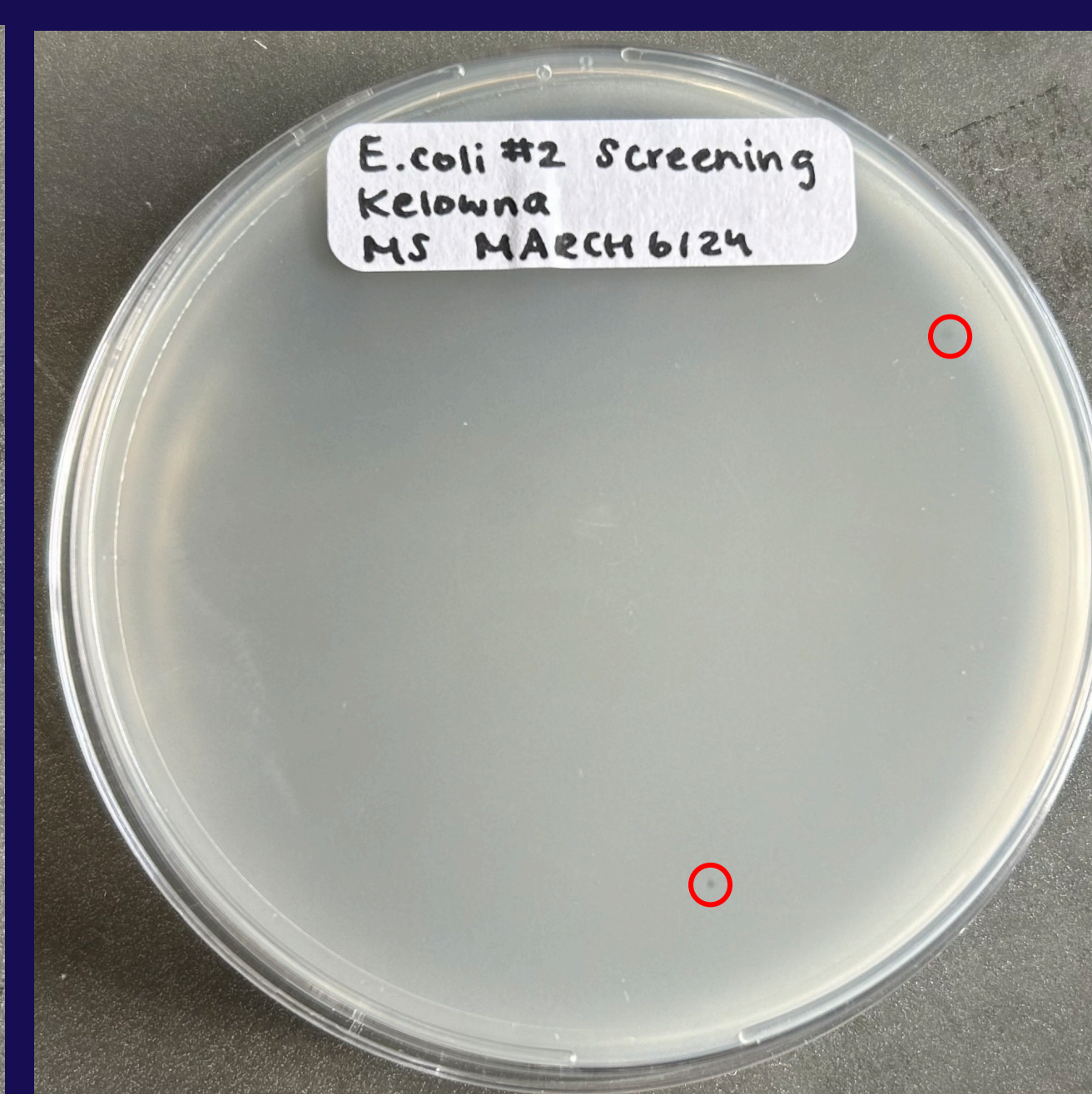


Figure 4. Plaques formed by bacteriophages from the Kelowna sample in the double layer agar assay with the MDR bacterial host *E. coli* #2.

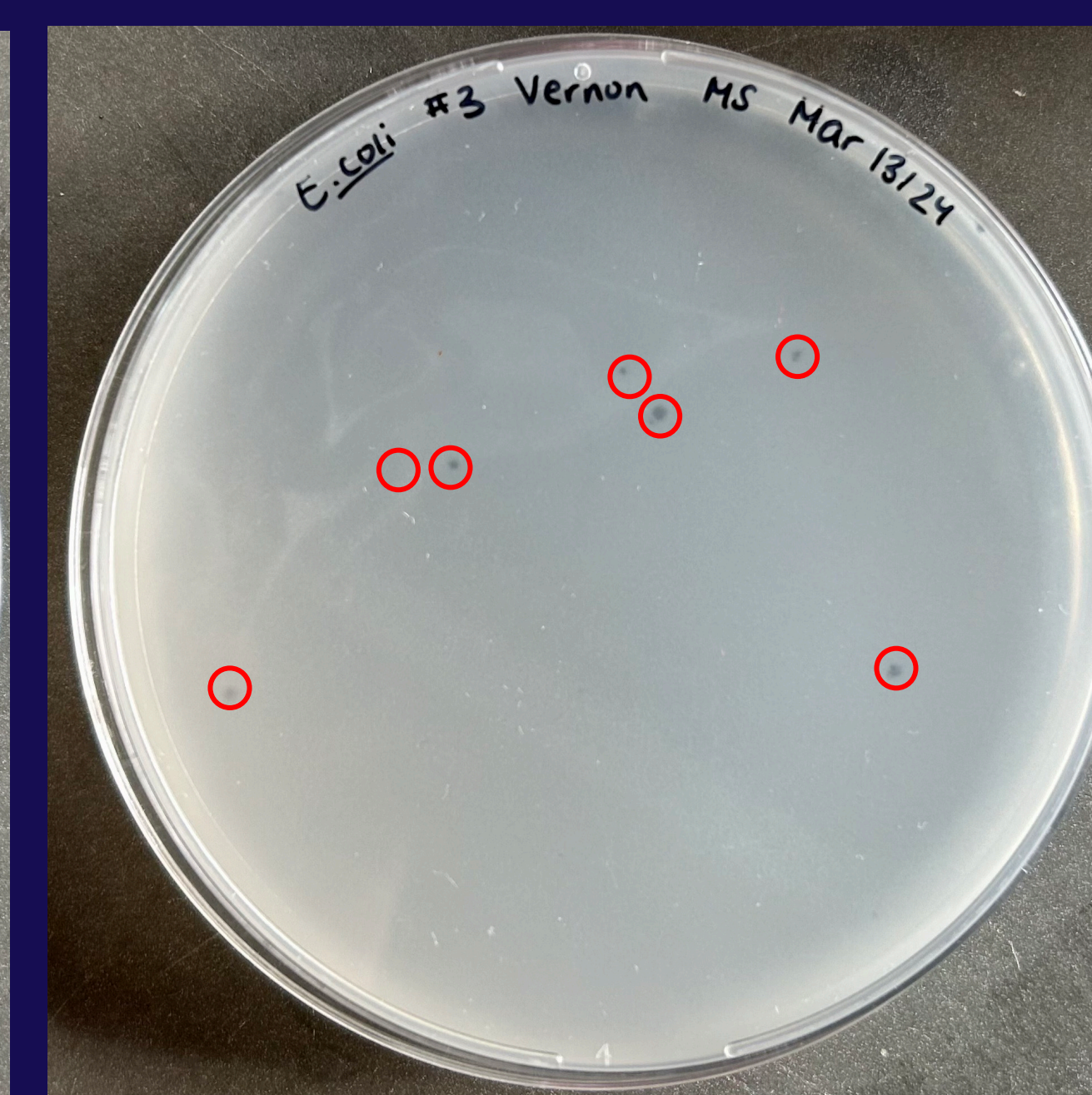


Figure 5. Plaques formed by bacteriophages from the Vernon sample in the double layer agar assay with the MDR bacterial host *E. coli* #3.

Discussion

- In comparison to the preliminary study, this study showed lysis from samples taken from the Vernon Water Reclamation Center. This could be due to the difference in the season in which both samples were obtained. The change in the population diversity of the bacteriophage due to the change in temporal pattern confirms that bacteriophages are season specific.
- Unlike the previous study, plaques in *E. coli* #2 were seen confirming that, during the winter, bacteriophages were found that were able to lyse *E. coli* #2 bacteria.
- Additionally, plaques were observed in all three stains of MDR bacteria tested, showcasing the diverse range of target bacteria that these bacteriophages strains can be used against.

Conclusions

- This study shows that bacteriophages could be potential candidates for the use in phage therapy. There is still an abundance of uncharacterized bacteriophages that could be used as an alternative and more targeted solution to battle MDR bacteria, given the specificity between the bacteriophage and its bacterial host.

Future Work

- Since this is an ongoing project, we plan to re-streak the isolated plaques for further confirmation of bacteriophage presence, and further isolate the bacteriophages, and specify the strains prevalent in each sewage sample.
- We would also like to see if these strains of bacteriophages have a similar effect on Gram-positive bacteria.

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References

- Galymov, A., Rehan, M., Urban, J., and Cheeptham, N. (2022). Identification and characterization of bacteriophages isolated from sewage water samples effective against two clinical strains of multi-drug-resistant *Escherichia coli*. Poster Presentation Abstract Submitted and accepted to the Canadian Association for Clinical Microbiology and Infectious Diseases (CACMID AMMI) Annual Conference. Sheraton Wall Hotel, Vancouver, April 5 to 8, 2022.
- Ghosh S, Persad E, Shue T-Y, Lam C, Islam A, Mascibroda LG, Sherman MB, Smith T, Cheeptham N. Explorative Study on Isolation and Characterization of a Microviridae G4 Bacteriophage, EMCL318, against Multi-Drug-resistant *Escherichia coli* 15-318. *Antibiotics*. 2018; 7(4):92. <https://doi.org/10.3390/antibiotics7040092>
- The methodology diagrams were created with the help of BioRender.com