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The Amplification and Characterization of Mycobacteriophages MyPhage and SolarFlare

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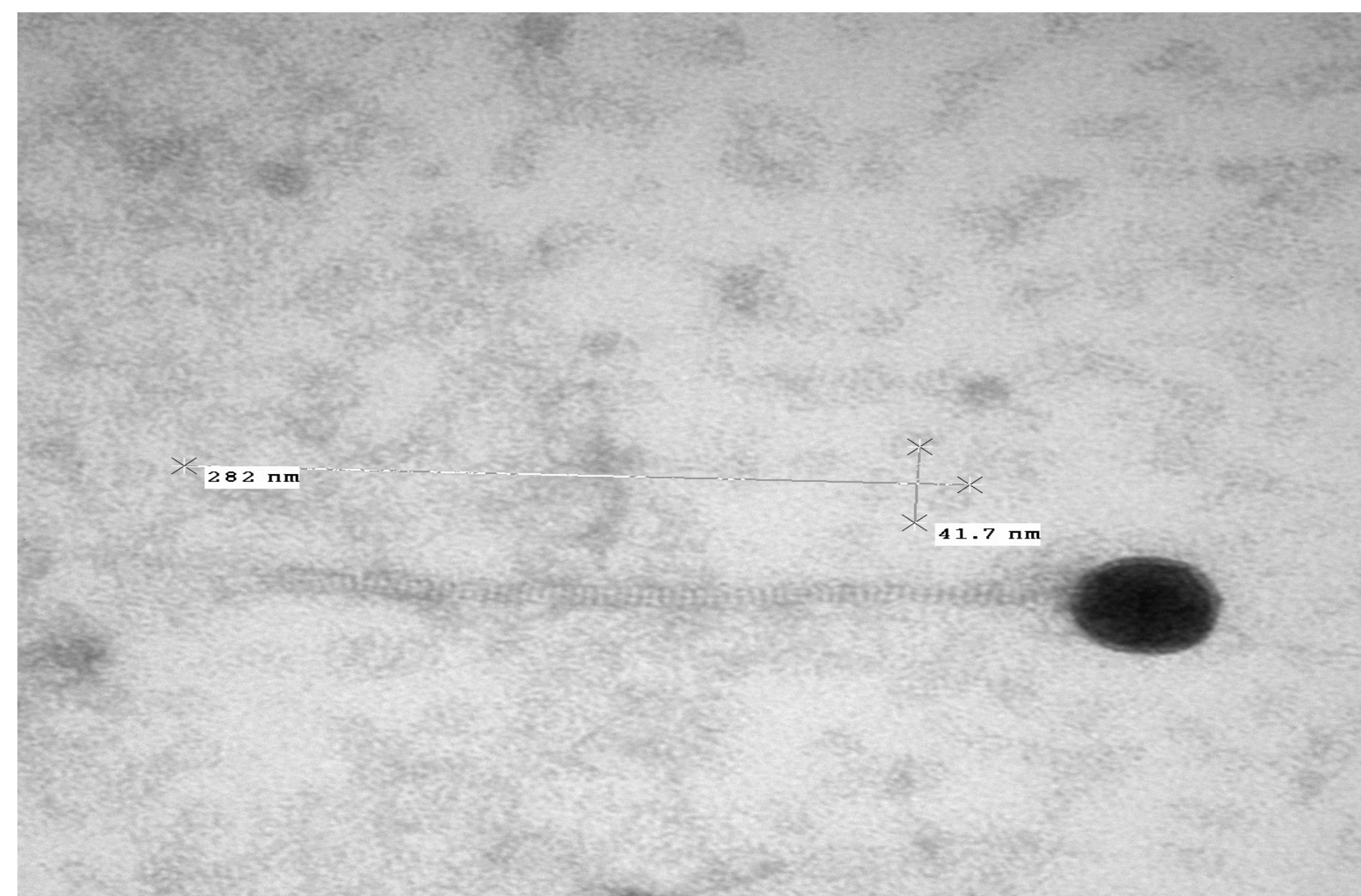
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The Amplification and Characterization of Mycobacteriophages MyPhage and SolarFlare

Erin S. Fogarty, Hannah E. Sparks, Tavi R. Wise, Dr. Marisa L. Pedulla



Solar flare 3.111
Solar flare phage 7-16-18
Print Mag: 105000x @ 51 nm
11:17:07/16/18
TEM Mode: Imaging
Microscopist: J Driver

100 nm
HV: 75kV
Direct Mag: 200000x
University of Montana

Figure 1. TEM Image of SolarFlare, taken at the University of Montana.

Background

Bacteriophages are viruses that infect and replicate in bacteria and are being researched for medical applications. MyPhage is a Mycobacteriophage discovered in Anaconda, Montana in May of 2009. The student found the phage in soil from a potted plant. The background of SolarFlare is unknown.

Methods

- Spot Titer
- Plaque Assay
- Streaking Plates
- Harvest Plates
- Creating a new lysate
- DNA Extraction
- Restriction Enzyme Digest
- Gel Electrophoresis of Restriction Enzyme Digest
- Polymerase Chain Reaction (PCR)



Figure 2. SolarFlare Plaque Assay

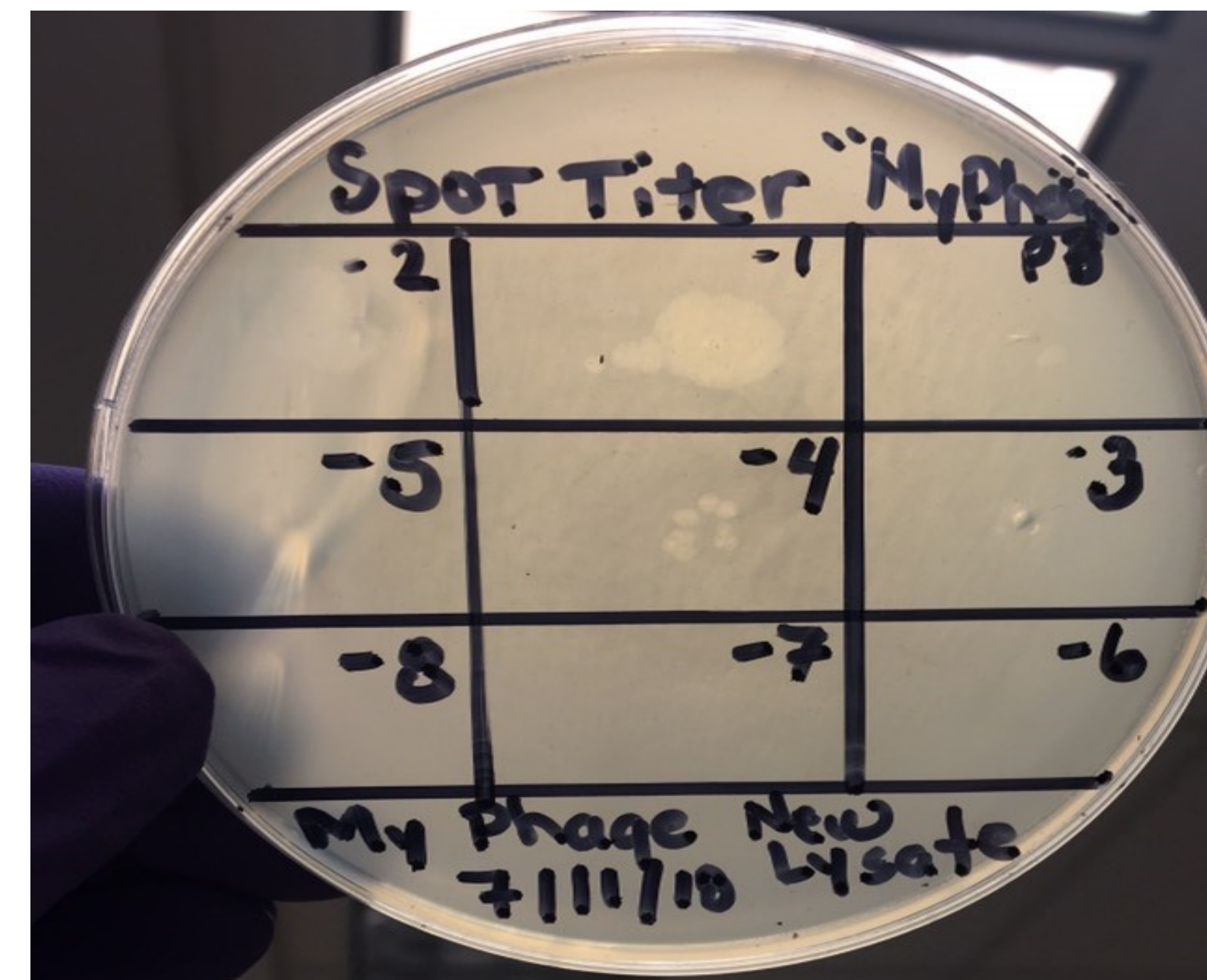


Figure 3. Spot Titer of MyPhage to calculate the amount of plaque forming units per microliter

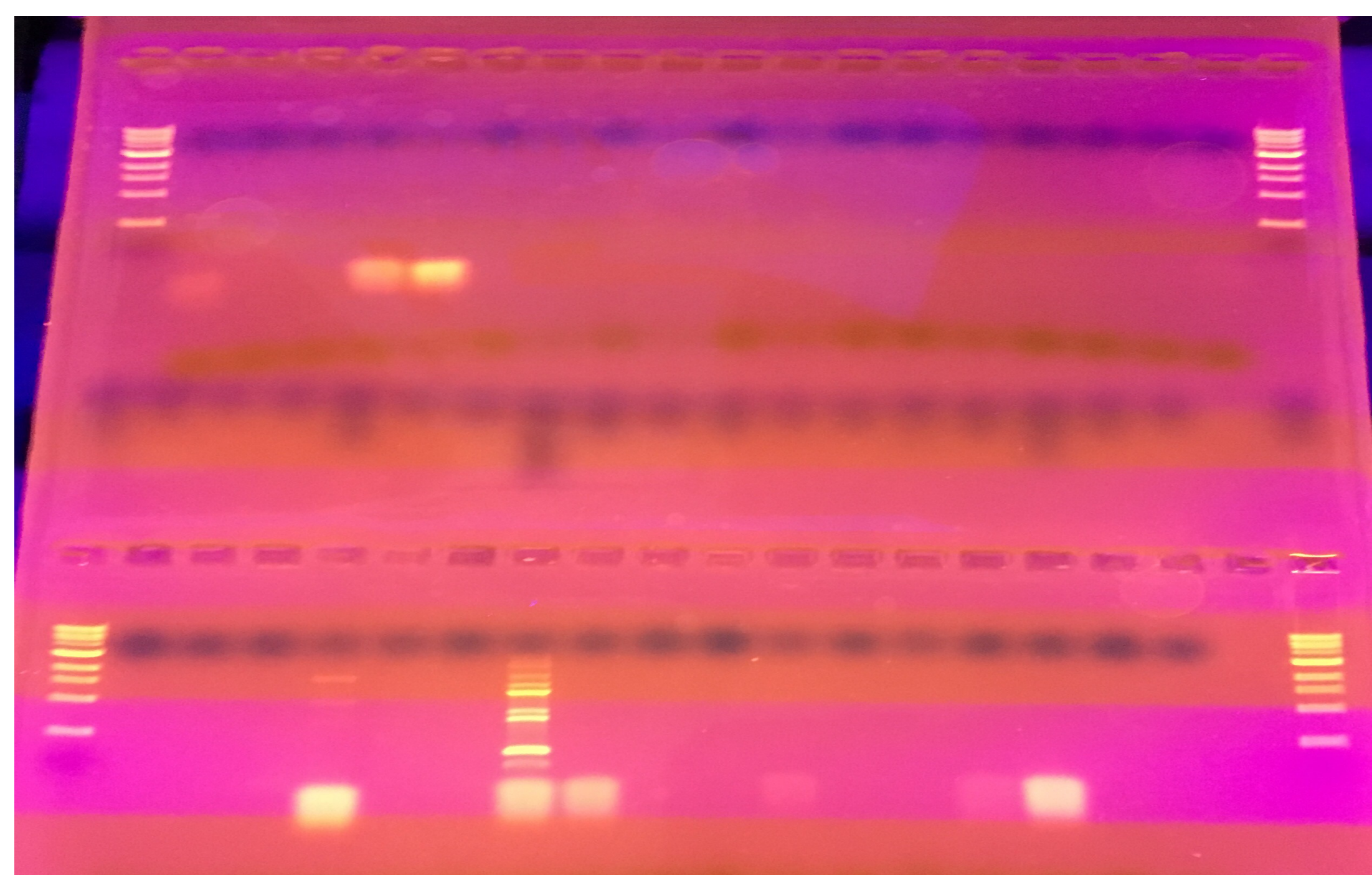


Figure 5. Polymerase Chain Reaction (PCR) of MyPhage

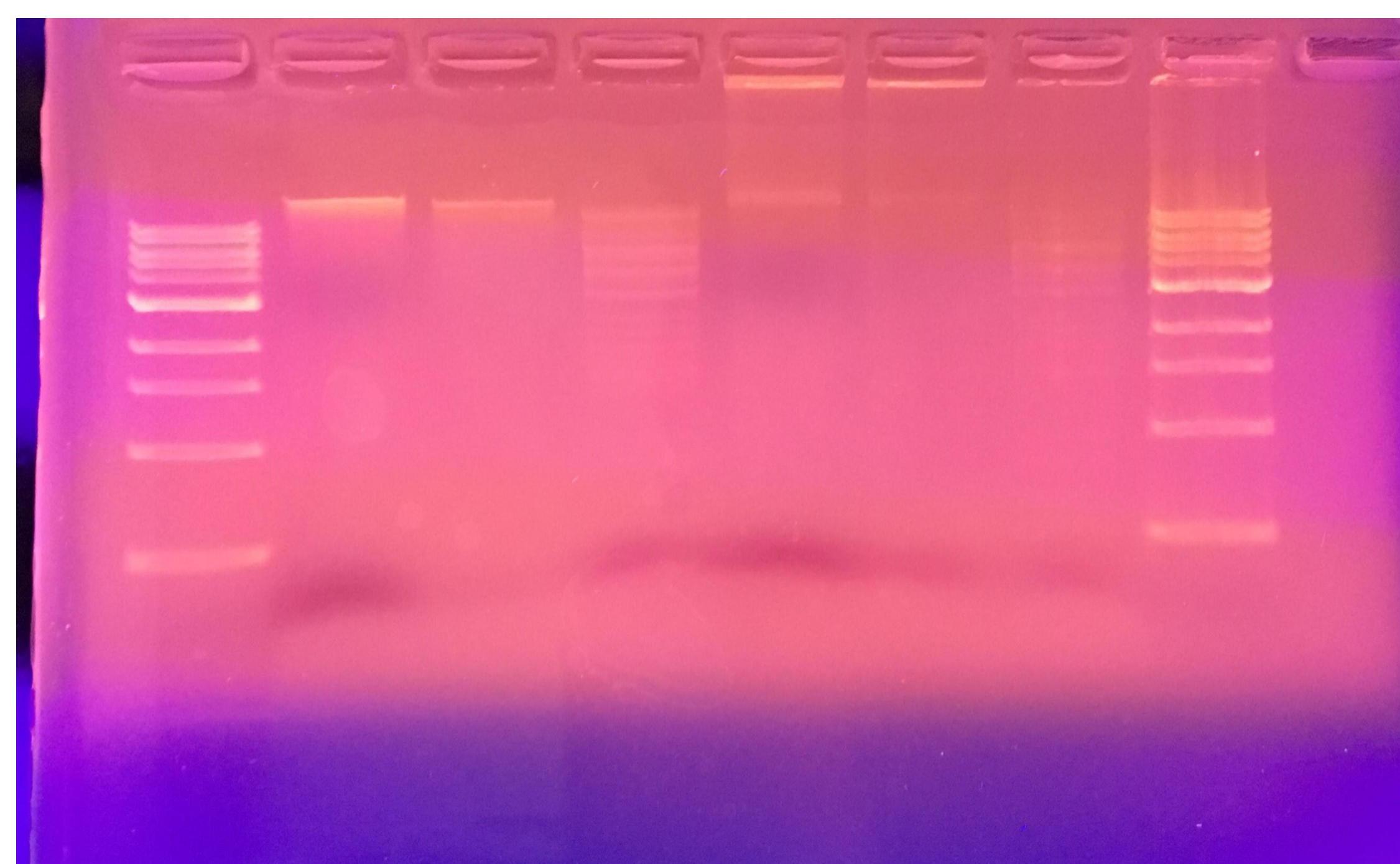


Figure 6. Gel Electrophoresis of Restriction Enzyme Digest of MyPhage.

Results

- MyPhage Titer: $(10\text{pfu}/3\text{uL}) \times (1000\text{uL}/1\text{mL}) \times 10^4 = 3.33 \times 10^7$ pfu/mL
- SolarFlare Titer: $(4\text{pfu}/3\text{uL}) \times (1000\text{uL}/1\text{mL}) \times 10^7 = 1.3 \times 10^{10}$ pfu/mL
- The PCR of MyPhage and SolarFlare is a unique fingerprint in which some enzymes digest DNA and others don't
- The Gel Electrophoresis of MyPhage shows a possibility of it belonging to cluster B4 based on comparisons of other phages' DNA.
- The Gel Electrophoresis of SolarFlare indicated it could belong to clusters A1, A2, B1, B3, D, E, F1, F2, G, I1/I2.

Conclusion

- Transmission electron microscopy indicated siphoviridae morphology of both phages.
- Restriction Enzyme Digests gives each phage characterization and a "fingerprint" to help identify it.

Future Work

- Adopting and Purifying other phages from Montana Tech.
- Research in the Montana State University Labs

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