Our Phage Phriends: Isolation & Characterization of Audrick & Porcelain
Samantha Donovan, Shivsai Gongalla, Sameer Akhtar, Noelle Dayal, Jacob Dietzel, Rita-Anne Falconio, Katlin Farver, Matthew Marshall, Alexis Schirling, Anifa Varkey, Madison Weston, Daniel Crosby, Trevor Cross, Dana Pape-Zambito, C. Nicole Sunnen
Department of Biological Sciences, University of the Sciences, Philadelphia, PA

Abstract
As part of HHMI's SEA-PHAGES (Science Education Alliance - Phage Hunters Advancing Genomics and Evolutionary Sciences) program, USciences students were able to isolate, characterize, and annotate two novel bacteriophages this year, Audrick and Porcelain. The phages both infected Mycobacterium smegmatis mc²155, but while Audrick was isolated using the enrichment protocol, Porcelain was isolated using a direct plating method. These phages were further characterized through the annotations of their genomes using DNAStar, Starterator, Phamerator, and HHPred. It was revealed that Audrick is a C1 cluster, lytic phage that is part of the Myoviridae family with a genome length of 150,205 bp, and that Porcelain is a J cluster, lysogenic phage that is part of the Siphoviridae family with a genome length of 109,575 bp. Further observations with electron microscopy, and additional experiments such as purification through ultracentrifugation using CsCl, gradient, lysogeny tests, and cloning of a putative repressor were conducted to obtain a greater understanding of these phages and the similarities and differences between them. For Audrick, an interesting phenomenon that occurred during ultracentrifugation was the presence of three Schlieren lines; we expected only one band of extremely pure phage. After analyzing the different bands with electron microscopy, we visualized empty heads in the top band, intact phage particles in the middle band, and a unidentified substance speculated to be ribosomes in the bottom band. Images from the top and middle bands also showed evidence of tail activation similar to that observed in phage T4.

Introduction
Porcelain and Audrick are currently 2 of 1513 novel Actinobacteriophages that have been annotated this year. Our Phage Phriends: Isolation & Characterization of Audrick & Porcelain

Materials and Methods
Purification of Audrick
- CsCl gradient of 2.4, 4, and 5.6 M
- Ultracentrifugation for 2.5 hours at 24,000 RPM
- Components of Audrick’s HTL were separated into Schlieren lines based upon density
Electron Microscopy
- Audrick’s isolated Schlieren lines were placed on separate EM grids
- Ultracentrifugation
- The Cloning of Porcelain’s Putative CI Repressor
- Bioinformatics to determine the repressor sequence and putative promoter regions
- Restriction digests to insert repressor sequence into vector & gel purification of product
- Ligation of plasmid with repressor sequence, and transformation of plasmid into E. coli MACH cells
Homology Tests
- Viruses were collected and plated
- Spot test of serially diluted Porcelain onto MACH lawn

Future Experiments
Audrick
- Ultracentrifugation of Audrick gives 3 Schlieren lines, but only the middle band contains intact, infectious phage.
- Based on the pictures obtained from EM, Audrick’s infection process is likely similar to phage T4’s infection process.

Porcelain
- Porcelain was successful in infecting a lysogen of the same cluster.
- Putative CI repressor gene was successfully transformed into E. coli MACH cells.

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Fig. 4: Ultracentrifugation Results from Audrick: a. Schlieren lines showing phage separation based on density. b. EM of top band at 75000x, revealing empty capsids and contracted tail sheaths. c. Spot test of top band showing no plaques at any dilution. Tiny plaques were only seen at full concentration. d. EM of middle band at 120000x, showing intact phage. e. Another EM of middle band at 120000x showing Audrick’s tail sheath contracted. f. Spot test of middle band reveals plaques at all dilutions. g. EM of bottom band at 75000x reveals a material that resembles ribosomes. h. Spot test of bottom band showing tiny plaques only at highest concentration.

Fig. 5: EM pictures representing Audrick’s infection process: a. Location of putative riveting mechanism where a rivet-like protein binds the head and tail b. Capsid with DNA, c. Empty capsid, after DNA injection d. Tail with base plate e. Core and contracted tail sheath ready for infection f. Cross section of a hollow sheath. g. J: 23, 1.5%

Fig. 6: Heteromorphinuty of MiaZeal and Porcelain: a. Porcelain successfully formed plaques on MiaZeal’s lysogen b. MiaZeal did not produce plaques on MiaZeal’s lysogen c. The complete clearing of MiaZeal’s lysogen plate upon heat shock at 42°C indicates the presence of lysogen.

Fig. 7: Porcelain’s gp93 shares homology with the lambda CI transcriptional repressor. Using HHPred with the plasmid database, the predicted structure of gp93 matched the crystal structure of the lambda CI repressor with a 99.4% probability. This was the third hit; the top two hits were also for HTN (heli-turn-helix) motifs present in lambda Cro/CI transcriptional regulators (99.6% and 99.5% probability).

Fig. 8: Plasmid map showing ligated putative CI repressor gene in red.

Fig. 9: Porcelain’s Positive Plasmid Transformation. a. Colonies resulting from plasmid transformation containing the putative repressor. b. Negative control plate containing colonies possibly from incomplete digestion and re-ligation.

Fig. 10: EM images of Porcelain showing putative CI repressor gene in red.