Warren J. Baker Endowment
for Excellence in Project-Based Learning
Robert D. Koob Endowment *for Student Success*

FINAL REPORT

Final reports will be published on the Cal Poly Digital Commons website (http://digitalcommons.calpoly.edu).

I. Project Title
Marine Virus Isolation for Use in Phage Templating of Nanoparticles

II. Project Completion Date
June 2017

III. Student(s), Department(s), and Major(s)

(1) Pranav Santan, Biological Sciences, Microbiology

(2) Morgan Smith-Boeck, Biological Sciences, Microbiology

(3) Siddharth Prabhu, Biological Sciences, Microbiology

IV. Faculty Advisor and Department

Dr. Nathaniel Martinez (Biological Sciences)

V. Cooperating Industry, Agency, Non-Profit, or University Organization(s)
Not Applicable

VI. Executive Summary

Over the course of the past year, we experimented with bacteriophage (phage) isolation from both fresh and marine water. We also tested two different chemical flocculation techniques, polyethylene glycol (PEG) and iron chloride. Furthermore, we tested different vesicles in which chemical flocculation occurs, such as separation funnels, centrifuge tubes, and Erlenmeyer flasks. We analyzed the effectiveness of flocculation via florescent microscopy. Each flocculated sample was visually compared to the original unconcentrated sample by staining the DNA with SYBR-Green. A successful concentrated viral sample yielded a >10 fold increase in overall fluorescence.

We found that both iron chloride and PEG were equally as effective in precipitating phage from water. Unfortunately, while the original protocols we read were designed for marine water, we had little success in precipitating phage from marine water. We tested samples of marine water from various locations, and used both methods of chemical flocculation. Despite this experimentation,

we did not succeed in precipitating phage from sea water. Fresh water appeared to have a large viral load, as florescence microscopy showed large amounts of virus, even on the unconcentrated sample. Most experiments were done with fresh water since the viral load is high.

We are now in the process of utilizing our library of phage pull-downs to isolate, classify and categorize phage by sequence analysis. In addition, we are exploring ways to expand our phage libraries through the isolation of host bacterial cells. Finally, we are developing strategies for screening a variety of panels of nanoparticles to determine phage-particle interaction strengths.

VII. Major Accomplishments

(1) We developed protocols for bacteriophage isolation that would be effective given the resources available. Our protocols were derived from protocols given in published research papers.

(2) We used florescent microscopy to analyze the effectiveness of our bacteriophage isolation procedures.

(3) We designed biopanning assays to bind bacteriophage to carbon nanotubes.

VIII. Expenditure of Funds

Provided by Ellen Calcagno (Biological Sciences)

IX. Impact on Student Learning

Undergraduate students played all the significant roles in the design and application of this project. Students learned many microbiology and molecular biology techniques, including bacteriophage and bacterial inoculation, incubation, isolation and identification, Biopanning techniques and overall good laboratory practices. Students maintained laboratory notebooks and presented their findings at the 2017 CalPoly COSAM Student Research Findings.