

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

FINAL REPORT

I. Project Title

Application of Pyroprinting for Source Tracking of *E. coli* in Pennington Creek

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

Award Recipient: Charles Moritz (Biological Sciences & Biochemistry)

Dillon Shapiro (Biological Sciences)

Christopher Pann (Biological Sciences)

III. Faculty Advisor and Department

Dr. Chris Kitts, Chair of the Biological Sciences Department

IV. Cooperating Industry, Agency, Non-Profit, or University Organization(s)

Cal Poly Biological Sciences Department

Morro Bay National Estuary Program

Chevron Center for Applications in Biotechnology

V. Executive Summary

This project's goal was identifying sources of fecal contamination in Pennington Creek. The creek is part of the Morro Bay watershed and runs through an outdoor educational facility, making the unsafe levels of *E. coli* reported a concern. This study was partly funded the Morro Bay National Estuary Program with the purpose of informing management strategies for the creek. Bi-monthly water samples were collected at three locations over a 14-month period from January 2014 to March 2015, from which *E. coli* were isolated and strain-typed using a novel DNA fingerprinting method called pyroprinting. Pyroprints from the creek *E. coli* were matched to a database of >7000 pyroprints from *E. coli* collected from various hosts including local wildlife, humans, and domesticated animals. Out of 398 *E. coli* isolated from Pennington Creek, 87 (22%) were successfully assigned to unique host species. Overall, the dominant source of *E. coli* was cows (44%), followed by turkeys (15%), humans (11%), and a variety of lesser contributors. Spatial and temporal analysis indicated that cows were contributing year round and at all three sites, and turkeys were contributing mainly at the site farthest upstream. It was not surprising that cows were the largest contributor considering the creek borders Cal Poly land which is used to house cattle. Turkeys were also expected to be a major source of contamination as they are often seen in large pods in the area. The human influence remains a mystery; however it may be a reflection of proportion of the *E. coli* reference database that human isolates account for (35%). Overall, the findings of this project suggest that the best targets for managing the fecal contamination in Pennington Creek are cows and turkeys. Pertaining specifically to the cows, the results indicate that the recent exclusion efforts are insufficient.

VI. Major Accomplishments

- (1) Production of 398 Pyroprints from Pennington Creek, which were successfully used to identify major sources of fecal contamination.
- (2) Results presented at the American Society of Microbiology General Assembly
- (3) Results to be presented at the CSU Council on Ocean Affairs, Science and Technology (COAST) and Water Resources and Policy Initiatives (WRPI) annual Student Research Poster Reception

VII. Expenditure of Funds

The entire award was used to buy reagents for growing and finger printing *E. coli*. A summary of costs is provided below. Charles Moritz received a stipend from the Morro Bay Estuary Program, the other students were not funded.

Description	Cost
PyroMark Sequencing Reagents (fingerprinting)	\$1,900
Colony PCR reagents	\$600
<i>E. coli</i> sampling, growth, and confirmation	\$500

VIII. Impacts to Student's Learning

This project was applied in nature, serving as a means of identifying the source of a worrisome contamination. Additionally, the project was important for the development of a novel strain typing method—one that has a lot of potential in the field of Microbial Source Tracking. However, beyond these practical objectives, this project was an invaluable learning experience for myself and the other members of the team. In the midst of rigorous academic coursework, and while working other jobs, executing this project pushed our limits. It was not without challenges. These included one of the sample sites running dry during the drought, necessitating the addition of another upstream, and the discovery that a majority of the samples had to be re-done because of an issue with the water after the lab relocation. Personally, this was my first time coordinating a project of this scale, and so it took a while to organize the students and responsibilities involved. We eventually developed a successful system to ensure that all work was being completed and recorded, and we learned a lot through the process.

This project has had a deep effect on my career path. I currently work as a research assistant in a medical microbiology laboratory in UC Berkeley's School of Public Health, where I use many of the same techniques that I learned as a part of this project. My current position also draws from non-technical skills I developed through this project—how to design, adapt, and complete a large-scale project. This aspect was actually far more important for both my success in my current role, and for what I plan to do in the future. The knowledge that a student can take something from an idea, stumbled upon in casual conversation, and bring it to fruition is empowering. In the future I aspire to be a part of many more such experiences, either in academia or in industry.

A great debt of gratitude is owed to those that made this project a possibility. Without the Warren J. Baker Endowment this project would not have been the success that it was. The extra funding allowed the project to extend an extra 6 months; without which the number of samples needed to draw conclusions would not have been reached. Many other groups and individuals also made major contributions to the project. Specifically, I would like to thank Dr. Chris Kitts and Jennifer VanderKelen. Jennifer trained the entire team in the necessary lab skills, and she tirelessly, often repetitively, answered our questions. Chris reached far beyond his role as the “big picture” overseer to provide extensive managerial advice on all aspects of the project. There was never a question or request of him that was not responded to in a timely manner, despite his commitment to running the department. He also took time out to help with producing the final poster, and to accompany me to New Orleans where it was presented to the American Society of Microbiology. And finally, much credit is deserved by all of the students on the team and in the lab, whose dedication and grit pushed the project through to the finish.