



PROPOSAL NARRATIVE

(Maximum of 5 double-spaced pages, 1" margins, 12-point font)

I. Abstract

The goal of this project is to determine the source of fecal contamination in Pennington Creek. Dangerously high numbers of *E. coli* were recorded in the creek by the Morro Bay National Estuary Program (MBNEP) over the last 10 years. This is of particular concern because the creek runs directly through an outdoor school for K-12 education. One potential source of the contamination is the cattle in fields on either side of the creek for several miles, though they are supposedly kept out by fences. Another possibility is local wildlife—specifically wild turkeys, which roam around in flocks of over 100 and commonly roost directly over the creek.

The Cal Poly Pyroprinting Project, housed in the Chevron Center for Applications in Biotechnology, is building a comprehensive database of genetic fingerprints (pyroprints) of *E. coli* from local wildlife and domestic animals for use in bacterial source tracking. Strain-specific pyroprints of *E. coli* isolates from the water body of interest are matched against wildlife pyroprints in the database. The two are matched up in a way similar to the matching of fingerprints, hence the term “pyroprints”. We will collect *E. coli* from two locations along Pennington Creek (labeled CPN and PEN) over a 16-month period, with additional bimonthly monitoring of overall *E. coli* counts. The two sites, along with an extended sampling period, will provide a good idea of the spatiotemporal fluctuations in concentration and sources of *E. coli* in the creek. The MBNEP has offered to help with the project by providing half of the supplies and reagents. The results of this project will be ultimately used by Cal Poly and the MBNEP to guide management decisions regarding Pennington Creek.

II. Introduction

E. coli, bacteria common in the intestines of warm-blooded animals, are used as an indicator of fecal contamination that might include disease causing bacteria and viruses. Microbial source tracking (MST) is a relatively new field of study focused on the tracking of fecal contamination in foods and resource waters. Our research group, the Cal Poly Pyroprinting Project (CPPP), uses a database approach to MST. The idea is to collect strain-specific fingerprints (we call pyroprints) for large numbers of *E. coli* collected from a spectrum of fecal sources, then compare these pyroprints to those from *E. coli* isolated from specific water bodies, such as Pennington Creek. In the pyroprinting process we use PCR to amplify both rRNA Intergenic Transcribed Spacer (ITS) regions in the *E. coli* DNA. Pyrosequencing (a DNA sequencing method) these amplicons produces graphs, called pyrograms, which we convert to numerical sequences called pyroprints. We create two pyroprints per *E. coli*, one each from the 16-23 and 23-5 ITS regions. This maximizes the potential to discriminate between closely related *E. coli* strains, and was thus the chosen method for the CPPP.

An important aspect of the CPPP is its interdisciplinary approach. The biology and biochemistry students design projects and collect data (pyroprints), and the computer team develops programs and clustering algorithms to compare pyroprints and match them. Additionally, the computer group has created a website, the Cal Poly Library of Pyroprints (CPLOP), that allows internet users to access and compare any group of pyroprints that we have produced. The collaboration between the “pyrotechies” and “compooter” groups is what makes our project functional and unique.

Within the CPPP there are several student led research projects. The goal of this specific project, the Pennington Creek Pyroprinting Project, is to determine the source of fecal contamination in Pennington Creek. Four Cal Poly students Charles Moritz, Matthew Parry, Makenna Marty, and Dillon Shapiro will be investigating the source of contamination using the pyroprinting methods described above. We chose to give this site our attention because of both the high *E. coli* counts recorded by over the past decade, and use of the creek by the local outdoor nature school Rancho El Chorro. The creek meanders through the school’s campus and, as a valuable educational resource, is incorporated into the school’s curriculum. Children as young as six use this creek to learn about ecology and biology. Because

young children are more susceptible to water-borne pathogens and because these vulnerable students come in contact with the creek, safe water is of great concern.

Cal Poly is a major landowner along Pennington Creek, with active cattle operations in the watershed. Cattle are fenced out of the sensitive riparian corridor, and their access to the creek is limited to a few times per year when they are transferred between pastures. Despite these efforts, bacteria levels are often elevated on Cal Poly property, located upstream of the Outdoor School campus. Understanding bacterial sources is crucial to addressing the contamination issues on this creek. Another potential source is the abundant wildlife in the creek and surrounding riparian corridor. To best manage the land to minimize bacterial levels, understanding the impact from cattle versus wildlife is extremely valuable.

The Morro Bay National Estuary Program (MBNEP) has similar concerns about creek contamination, and are now an integral part of the project. MBNEP is a local non-profit dedicated to protecting and restoring Morro Bay and its watershed. They are providing over \$2,000 of supplies for the project, as well as training to the project members and expertise on the subject of water monitoring. They also created a fellowship for the primary investigator of the project, which includes a quarterly stipend and requires weekly, quarterly, and final reports from the project.

III. Objective(s)

- (1) Determine the source of fecal contamination in Pennington Creek.
- (2) Fortify the CPLOP with local wildlife that could be sources of fecal contamination.
- (3) Showcase the pyroprinting method for future applications in the county.

IV. Methodology

Every 2 weeks, 5 water samples will be taken from CPN (above the bridge) PEN (at the school) sites. After confirmation Tests (Macconkey isolations, Citrate Tests, Indole Test) two isolates will be selected from the 4 isolates of each original sample. These two isolates will be frozen down for storage, and used for PCR amplification of two specific sites the ITS 23-5, and 16-23 regions. A pyrosequencer is then used to create unique pyroprints from each of these amplicons, and ultimately 2 pyroprints will be

added to the database from each of the original CPN/PEN water samples. By the end of the project, a minimum of 200 pyroprints will be added to the database from each of the two sampling sites (CPN and PEN on the image attached).

At the same times of this sampling, IDEXX tests will be performed on water samples from APN, UPN, CPN, and PEN sites on Pennington creek. These results will allow us to track the severity and variability in E. coli concentrations in Pennington Creek over the span of the project.

The pyroprints are added to the Cal Poly Library of Pyroprints where they can be universally accessed. Clustering algorithms designed by the computer team are used to compare the environmental E. coli pyroprints to the reference database pyroprints. Analysis of matches should give an indication of the source of the E. coli in Pennington Creek.

V. Timeline

Jan 29th	Begin creek sampling and IDEXX testing
March - June 2014	Spring Sampling. Quarterly report due to MBNEP
July - Sep 2014	Summer Sampling + IDEXX testing. Quarterly report due to MBNEP.
Sep - Dec 2014	Fall Sampling + IDEXX testing. Quarterly report due to MBNEP.
Jan - March 2015	Winter Sampling + IDEXX testing. Final Report due to MBNEP.

VI. Final Products and Dissemination

The final product of the project will take several forms. It will serve as a senior project for several of the group members, and will hopefully be published. It will also be presented at any available poster sessions held by Cal Poly or other California biology departments. Finally, the fellowship agreement between MBNEP and whoever is taking the leading role in the project (Charles until at least winter 2015) requires both quarterly reports and a post-fellowship proposal, along with several presentations to the

MBNEP on the progress of the project. As mentioned earlier, the findings of our study may be used by MBNEP or Cal Poly to influence changes in the management of the Pennington Creek area in order to ensure the safety of students at Rancho El Chorro, and downstream in Morro Bay.

VII. Budget Justification

The only section we are applying for on the budget sheet is non-computer supplies and materials. We have requested \$3000 in funding for these materials in order to purchase all necessary reagents and supplies needed to complete the project (Excel sheet attached). At least 200 pyroprints are required from each sampling site in order to get conclusive results. It costs about \$10 to take a sample all the way to a pyroprint in the database, so our original estimate was around \$4000. Although we have received generous contributions from MBNEP, and the students working on the project have requested money from their departments through CBF funds, we are still well short of the money required to finish the proposed project in a thorough manner. The IDEXX testing and turkey sampling that must also be done is not taken into account with the "\$10" estimate. Certain Procedures, especially pyrosequencing, do not have a %100 success rate and may need to be done several times over to produce satisfactory results. Moreover, with extra funding we can extend and intensify the sampling of Pennington Creek. Increasing the sample size will drastically increase the quality and accuracy of our results. We are therefore requesting \$3000 from the Warren J. Baker Endowment in order to properly complete the project, and provide the much-needed results to those who can take measures to mitigate the serious contamination issues.

Qiagen Reagents (http://www.qiagen.com)	PN	Cost		
PyroMark Annealing Buffer (250 ml)	979009	\$49.90		
PyroMark Binding Buffer (200 ml)	979006	\$48.80		
PyroMark Gold Q24 Reagents (5 x 24)	970802	\$314.00		
PyroMark Q24 Cartridge (3) (good for 60-100 runs)	979202	\$420.00		
PyroMark Q24 Plate (100)	979201	\$204.00		
PyroMark Q24 Vacuum Prep Troughs (12) reuseable	979206	\$41.10		
PyroMark Vacuum Prep Filter Probe (100)	979010	\$290.00		
Wash Buffer 10X (200mL)	979008	\$48.80		
Denaturation Solution (500 mL)	979007	\$48.80		
GE HealthCare Reagent				
Streptavidin Sepharose 5 mL	17-5113-01	\$408.00		
Colony PCR				
NEB 2X Taq Master Mix	M0271L	\$224.00		
PCR tube (Phenix)	MPC-425Q	\$66.00		
Fisher Scientific				
Simmons Citrate Agar 500 grams	B11620	\$130.48		
LB agar 500grams	DF0445-17-4	\$80.10		
IDEXX reagents:				
Quanti tray (97 trays)	WQT-2K	\$135.00		
Colilert-18	WP2001-18	\$748.00		
THESE PRICES DO NOT INCLUDE TAX OR SHIPPING AND HANDLING				
Qiagen has high shipping costs				
NEB has very reasonable shipping cost, free if over \$300 otherwise \$19				
Fisher has free shipping on most items				



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Dear Dr. Christopher Dicus,

I'm writing to you to support Charles Moritz' proposal for this inaugural round of the Warren J. Baker Endowment: Excellence in Project-Based Learning. In my opinion Charles' proposal is precisely the kind of work Dr. Baker had in mind when he put this endowment together.

The project Charles is proposing evolved out of the larger *E. coli* Pyroprinting Program initiated by myself and three other faculty in 2010 with support from the W.M. Keck Foundation, CSUPERB and the National Science Foundation. Our initial work was focused on the development of a database of *E. coli* fingerprints (called pyroprints) to be used as a method for tracking sources of fecal contamination in the environment.

One of the long-term goals of the Pyroprinting Program is the use of this database to address local water quality issues and we've been in contact with the Morro Bay National Estuary Program (MBNEP) for some time, trying to get funding for work in the Morro Bay watershed. Charles agreed to start this work on a shoestring and his efforts have already garnered \$2000 of support from MBNEP along with matching time for training in the MBNEP volunteer monitoring effort. Consequently, this project is already in progress, has the backing (both in terms of equipment and space) of the Center for Applications in Biotechnology and will continue without further funding. However, support from the Warren J. Baker Endowment will give Charles the recognition he deserves and will also allow us to expand the project gathering more data for more solid results.

Charles is leading a group of 4 students in a study of the sources of fecal contaminants found in Pennington Creek. This creek is a tributary to Chorro Creek and the waters eventually exit into Morro Bay. In addition, Pennington creek borders on Cal Poly land which is used to house cattle and the study will help us understand if recent cattle exclusion fencing undertaken by Cal Poly has been effective in keeping cattle feces out of the creek.

As for Charles himself, I am really impressed. This is one of those independent, self-motivated and resourceful students that exemplify Cal Poly's style. Charles came to me over a year ago, interested in working on the Pyroprinting Project. We had a full plate at the time so I asked him if he'd be willing to do some independent work. He agreed to start up our "wild-life poop" program and coordinated with several animal rescue operations, collecting feces and isolating *E. coli*. Some time later he said he was enjoying the work enough to want to build a senior project out of it. I suggested Pennington Creek as a focus and he stepped right up to coordinate with Ann Kitajima at the MBNEP and immediately got a sampling plan and budget together we both could work with. In addition, without help, Charles collected a group of students to work with him on the project and he is now serving as the coordinator and leader of the project.

It's clear to me that Charles is going to go far, and we'll be glad we supported him. Although the funds he's requesting are small, the prestige of support from the Warren J. Baker Endowment will give this young go-getter a resume line he can stand on to reach even further.

Sincerely,



Chris Kitts