MORRO BAY
NATIONAL ESTUARY PROGRAM

Morro Bay National Estuary Program
Community Project Application Cover Sheet

Project Title: Role of Labyrinthula spp. in the development of eelgrass wasting dise

Applicant: Silvio Favoreto and Laurie McConnico

Address: Cuesta College HW1, San Luis Obispo, CA 93403-8106

Contact Person(s): Silvio Favoreto & Laurie McConnico

Phone: (805) 546-3100 Ext 2718 **Fax:** (805) 546-3232

Email: silvio_favoreto@cuesta.edu; laurie_mcconnico@cuesta.edu

Amount Requested (cannot exceed $5,000**):** $4998.03

Total Estimated Project Cost: $25,000

Amount & Source of Other Funding (if relevant):

Cuesta College Foundation and Biology Division: $20,000.00

Project Description Summary (fill in here or attach additional document; word limit 300**):**

Biology Faculty, Drs. Favoreto and McConnico will work with students at Cuesta College to assess the presence, distribution and abundance of the eelgrass pathogen Labyrinthula throughout Morro Bay Estuary. This pathogenic slime mold is suspected to be present in Morro Bay, based on visual evidence of tissue necrosis, however, no data exist to confirm its presence or understand its potential impact on the declining eelgrass population in the Estuary.

Using samples collected during a June 2018 summer course, students enrolled as Research Assistants at Cuesta College will work throughout late fall 2018 and early spring 2019 to extract DNA, begin qPCR techniques to quantify Labyrinthula and finish visually quantifying the extent of necrotic tissue on eelgrass blades photographed during June 2018. These student researchers will present preliminary results at a conference in spring 2019 and help with public outreach via MBNEP Blog postings. During summer 2019, twenty students enrolled in Applied Environmental and Microbiology (210M) will collect eelgrass from throughout the Estuary to culture and quantify Labyrinthula using standard microbiological and genetic techniques. Students will also help to determine the entire genome of Labyrinthula and assess its genetic variability within Morro

Check off the Comprehensive Conservation and Management Plan Priority Issue(s) addressed by the proposed project:

- [x] Sedimentation.
- [ ] Bacterial contamination.
- [x] Elevated nutrient levels.
- [x] Toxic pollutants.
- [x] Scarce freshwater resources.
- [x] Preserving biodiversity.
- [ ] Environmentally balanced uses.

Briefly describe how your project addresses the priority issue(s) you checked above.

Eelgrass habitats provide food, protection, and serve as nursery grounds for a m

The proposed project must work to implement at least one of the Action Plans that address the Priority Issues listed above. The Action Plans are detailed beginning on page 23 of the Management Plan, which can be found at MBNEP.org.

In the space below, please list the relevant Action Plan(s) and briefly describe how they will be addressed by this project.

The proposed project is in direct alignment with ECR-7, ECR-8, EO-1 and E0-4. E

Title: Role of *Labyrinthula* spp. in the development of eelgrass wasting disease in the Morro Bay Estuary.

Applicants: Silvio Favoreto and Laurie McConnico

Summary:

Biology Faculty, Drs. Favoreto and McConnico will work with students at Cuesta College to assess the presence, distribution and abundance of the eelgrass pathogen *Labyrinthula* throughout Morro Bay Estuary. This pathogenic slime mold is suspected to be present in Morro Bay based on visual evidence of tissue necrosis, however, no data exist to confirm its presence or understand its potential impact on the declining eelgrass population in the Estuary.

Using samples collected during a June 2018 summer course, students enrolled as Research Assistants at Cuesta College will work throughout late fall 2018 and early spring 2019 to extract DNA, begin qPCR techniques to quantify *Labyrinthula* and finish visually quantifying the extent of necrotic tissue on eelgrass blades photographed during June 2018. These student researchers will present preliminary results at a conference in spring 2019 and help with public outreach via MBNEP Blog postings. During summer 2019, twenty students enrolled in Applied Environmental and Microbiology (210M) will collect eelgrass from throughout the Estuary to culture and quantify *Labyrinthula* using standard microbiological and genetic techniques. Students will also help to determine the entire genome of *Labyrinthula* and assess its genetic variability within Morro Bay. Such data would be key in evaluating if more than one strain of *Labyrinthula* was present and perhaps could account for some of the variation in perceived infection within Morro Bay.

Given the extreme decline in Morro Bay's eelgrass population, this work is timely and key to evaluating best practices in the conservation of the limited and important natural resource. The unique approach of engaging with local college students to tackle understanding of this environmental problem and communicating research results to the public via the MBNEP Blog and scientific meetings/publications will ensure a wide range of public and scientific literacy surrounding the eelgrass decline.

Priority Issues:

Eelgrass habitats provide food, protection, and serve as nursery grounds for a myriad of species. The high biodiversity associated with eelgrass beds is compromised by sedimentation, excess nutrients and toxins within the Morro Bay Estuary. Each of these factors may influence the virulence of *Labyrinthula* and impact eelgrass health/persistence. This study will shed light on the extent to which eelgrass beds are impacted by the *Labyrinthula* pathogen and help evaluate how to best protect eelgrass and its associated biodiversity.

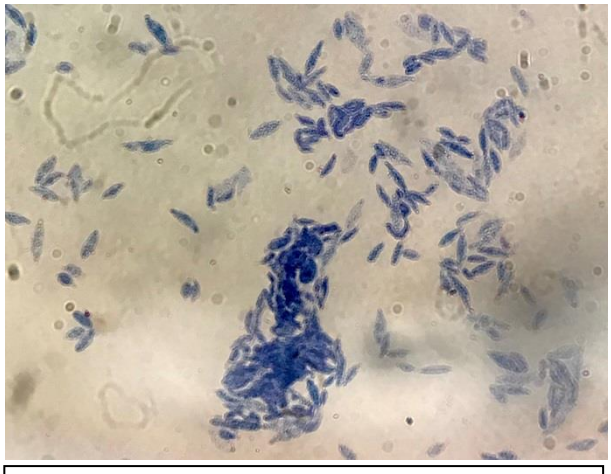
Action Plans:

The proposed project is in direct alignment with ECR-7, ECR-8, EO-1 and E0-4. ECR-7 covers eelgrass data and research. This is the premise of our entire proposal. ECR-8 is focused on Eelgrass restoration. Without a clear understanding of why eelgrass is on the decline in Morro Bay, there is no way to effectively restore the plant community. Our project results will shed light on whether the *Labyrinthula* pathogen is present in the estuary and begin to assess what role it has played in observed eelgrass declines. This knowledge will be very helpful in future restoration efforts. E0-1 is focused on public outreach and education. Since we will be working with local community college students to gather and disseminate research results in classes at Cuesta College, at local conferences and via the MBNEP Blog we will be able to engage with the public in a variety of formal and informal settings. This is key to making sure the public is aware of the eelgrass decline and the variety of ways the MBNEP has reached out to community partners to understand its decline and attempt restoration. Finally, E0-4, which aims to offer formal education programs is an integral part of the proposed community project, as students enrolled in Applied and Environmental Microbiology and Research Assistant courses at Cuesta College will help to collect and analyze data, contributing to the understanding of *Labyrinthula* induced wasting syndrome in Morro Bay Estuary.

Project Title: Role of *Labyrinthula spp.* in the development of eelgrass wasting disease in the Morro Bay Estuary.

Full Project Description:

Labyrinthula spp. is considered an important pathogen of Eelgrass, the overgrowth of this slime mold in association with plant tissues causes wasting disease (Short et al.1987). *Labyrinthula* caused a pandemic decline in eelgrass in Europe and North America in the 1930's and 1980's, leading to massive population loss in a short period (Bergmann et al. 2011). Now, necrotic leaf tissue indicative of wasting disease is present throughout Morro Bay. It appears most prevalent in the back bay (south of the Marina) where eelgrass is now extremely sparse. *Labyrinthula* is often present in estuarine environments, but physical factors may also influence its virulence. These findings are important in Morro Bay because recent studies have shown that the back bay exhibits higher salinity, lower light levels, increased dry season temperatures and reduced eelgrass density than the forebay. Thus, it is critical to determine if the pathogen *Labyrinthula* is present in Morro Bay, whether the observed necrotic tissue is associated with the presence of *Labyrinthula*, and whether there is a higher prevalence of the pathogen in regions of the estuary with unfavorable physical conditions (like the back bay).



Giemsa stain of *Labyrinthula zosterae* in culture. Note typical spindle shaped cells.

With Morro Bay National Estuary Program (MBNEP) support, Drs. Favoreto and McConnico of Cuesta College will address these principle questions and others while determining the role *Labrinthula* may play in local eelgrass declines. They will continue work initiated in the summer course Applied and Environmental Microbiology (Bio 210M) offered at Cuesta College (June 2018). During this course, students collected and preserved eelgrass blades from throughout Morro Bay and began culture work to determine the extent of *Labyrinthula*. This labor-intensive effort was the first step to understanding the distribution and potential impact of *Labyrinthula* in the Bay.

Specifically, funds from MBNEP will be used to purchase reagents (via Fisher Scientific and ChaiBio) and provide tuition assistance for Research Assistants at Cuesta College. This tuition assistance is essential to attract and retain highly motivated students and ease their financial burden while volunteering their time in the unpaid internship. These Research Assistants will work during Fall 2018 and Spring 2019 to extract DNA from previously collected eelgrass samples (June 2018) and initiate quantitative polymerase chain reaction (qPCR) to determine the number of *Labyrinthula* cells per gram of eelgrass. During May and June 2019 additional MBNEP funds will be used to support a second sample collection of eelgrass throughout the Bay. These samples will be processed identically to those collected in June 2018 and will allow for determination of temporal patterns in *Labyrinthula* distribution and abundance. Repetition of the collection and sampling protocol will allow for a better understanding of the interannual dynamics of *Labyrinthula* and its potential impacts on eelgrass. The funding from MBNEP will also support resources that will allow for the determination of the entire *Labyrinthula* genome within Morro Bay eelgrass (this disposable DNA sequencer will be purchased from Nanopore). Such an effort to assess the spatial variation in metagenomics of *Labyrinthula* would be the first work of its kind and play a key role in determining the interconnectedness of *Labyrinthula* populations throughout the Bay and how such genetic variation might play a role in plant infection rates and processes.

As in summer 2018, students will collect 20 eelgrass blades from each of four sites in Morro Bay (located in the forebay near the channel mouth, two mid-bay locations near the Marina and channels, and back bay). Students will evaluate ten healthy (purely green) and ten necrotic blades using an established, visual wasting index (Burdick et al. 1993) at permanent plots monitored by Cal Poly and the MBNEP. Leaves will be sectioned longitudinally from tip to base. Half of the longitudinal section will be preserved for genetic analysis and the other half cut and processed for culture. While cultured samples will allow for a rapid (<1 week of growth required) qualitative assessment of *Labyrinthula* presence and infection characteristics, qPCR of preserved leaf material will quantitatively assess total microbial load on green and necrotic tissue that is not easily determined via culture techniques. Ultimately, cultured strains of *Labyrinthula* from throughout the Bay will be cryopreserved and a MiniIon sequencer will be used to determine differences in *Labyrinthula* genomes at the front and back of the bay.

Using the approaches described above, this project will address five critical questions:

1) Is the *Labyrinthula* pathogen present in Morro Bay?
2) Are areas of necrotic tissue found on eelgrass leaf blades in Morro Bay associated with a higher prevalence of *Labyrinthula* pathogen than found on healthy blades? If yes, this would indicate that *Labyrinthula* is a likely cause of necrotic tissue and blade loss in the bay. If not, *Labyrinthula* may be present in the bay, but not at virulent levels.
3) How well does the visual wasting index predict presence and abundance of *Labyrinthula* on eelgrass leaf blades?
4) Is *Labyrinthula* prevalence different in different parts of the bay that are subject to dramatically different physical water conditions, as determined by Dr. Walter's research?
5) How diverse is the population of *Labyrinthula* in the Morro Bay Estuary and are there any patterns between *Labyrinthula* species present and prevalence of necrotic tissue in eelgrass blades?

The proposed investigation is a pioneering effort in understating the impact of *Labyrinthula* on eelgrass health in the Morro Bay Estuary. The research will not only provide a wealth of novel information about local microbial biodiversity and virulence; it will serve to educate a new generation of young scientists using cutting-edge field and laboratory techniques. The opportunity for students to present their research findings at a technical conference will enhance their exposure to scientific communication beyond the classroom, while working to share project results via the NEP Blog will bridge the final gap between science and the public.

Community Engagement:

Community engagement will involve two components: 1. student-centered research and 2. public outreach in the weekly MBNEP Blog

1. This research will involve community engagement with students at Cuesta College. Students, including lifelong learners, transfer students, summer students from Cal Poly, High School Enrichment Students and those looking to retool for the workplace, will participate by enrolling in Research Assistant courses (Bio 241 and 242) or Applied and Environmental Microbiology (Bio 210M). Twenty to twenty-five students will ultimately work to help collect samples, extract DNA, quantify and analyze DNA using qPCR and metagenomic techniques. Students will also help to prepare data for a scientific meeting and publication.
2. Along with Drs. McConnico and Favoreto, students enrolled in Bio 241/242, and Bio 210M will prepare a series of 2-3 blogs focused on the research they are conducting. These blog posts will highlight the research techniques necessary to understand *Labyrinthula* infection within Morro Bay. The end goal of the blog entries will be to educate the public about eelgrass declines and the need to evaluate the role *Labyrinthula* may play in the loss of this valuable local resource. A special effort will be made to coordinate with MBNEP to ensure that the content is at an appropriate level for the targeted audience.

Audience:

Target audience of the project: Cuesta College Students, General Public, and the Scientific Community.

Mode of Contact: Classes, Scientific and local Meetings, and MBNEP Blog entries.

Number of people reached: Taking into account the Students (Biology, Microbiology, Environmental and Applied Microbiology, Marine science and High School Enrichment Students), Research Assistants, Members of the Central Coast Biology Society, Cal Poly eelgrass research group and MBNEP Blog Readers, the project will impact approximately 1000 individuals/ year.

Benefits:

There have been no evaluations of the incidence of *Labyrinthula* in Morro Bay. **This proposal involves a systematic assessment of the prevalence of eelgrass wasting disease in Morro Bay. The project will elucidate *Labyrinthula*'s possible role in the recent drastic eelgrass decline and in preventing recovery. Additionally, by evaluating the genetic variability of *Labyrinthula* found in Morro Bay, it will be possible to determine if pathogens collected from the forebay are genetically distinct from the back bay. This analysis will address the hypothesis that genetic variation in the pathogen is responsible for the increased incidence of necrosis.**

This project requires extensive knowledge in field ecology and microbiology, as well as cell culture and genomics, the expertise of Drs. Favoreto and McConnico. Also, to make this work relevant to the local scientific and general community, work done by Cuesta students and faculty will be in close collaboration with the MBNEP, Cal Poly faculty (Dr. Ryan Walter - Physics), The Nature Conservancy (Dr. Jennifer O'Leary) and regular contact with the faculty of the Biological Sciences Division at Cuesta College and members of the Central Coast Biological Society. Such extensive collaborations will ultimately benefit the community as information will be exchanged among many local partners with a stakehold in healthy eelgrass ecosystems. Having students lead the sampling and analytical processes to address these complex issues provide real-world skills for both their education and future careers.

Project Budget:

Item and vendor	Projected expenditure date	Value
Permits		
Collection Permit for eelgrass required for subsequent field sampling (Application fee and permit fee) – California Department of Fish and Wildlife	Ideal deadline for application September 30th, 2018	$433.67
Supplies		
Reagents – ChaiBio See the list on attached appendix	Ideal before December 31st, 2018	$1,757.00
Qiagen DNA Isolation Kits – Qiagen. See the description on attached appendix	January 2019	$495.00
Culture media supplies – Fisher Scientific	December 2018	$278.36
MinIon DNA Sequencer –Oxford Nanopore Technologies. Single use instrument. See the description on attached appendix	Ideally, January-February 2019	$1,050.00
Conference Fees		
Student Conference Registration –Fee for one student. Community College Undergraduate Research Initiative.	April – July 2019	$450.00

Printing Fee		
Poster Printing Fee – Kinko's	April – July 2019	$150.00
Classe Fee		
Student Course Registration Fee for four students – Cuesta College	January and May 2019	$384.00
	Total Funds Requested	**$4,998.03**

Other funding sources:

- Cuesta College Foundation agreed to fund the purchase of a qPCR machine ($5,000.00) in addition to $2,000.00 in supplies to support coursework during summer 2018 and 2019.
- The Biological Sciences Division agreed to fund the purchase of a regular thermocycler (PCR) ($3,000.00).
- Drs. McConnico and Favoreto volunteer their expertise in the execution of this proposal and to mentor students enrolled as Research Assistant's dedicated to this project (~500hours/semester)
- Cuesta College supports Drs. McConnico and Favoreto during Summer at an hourly rate of $80.00 for 1 week of field and lab work.

Evaluation: Success of this project will be dependent on a few key factors:

1. The number of students completing Applied and Environmental Microbiology (Bio 210M) and Research Assistant courses (Bio 241/242) at Cuesta College: ~20 students will participate and benefit from this applied research project given enrollment history and course caps.
2. Following the project timeline: Research Assistant students will extract DNA from previously collected samples throughout the fall and spring. Drs. Favoreto and McConnico will then work with 210M students during May/June 2019 to collect additional samples and repeat the experimental protocol.
3. Ability to address the key questions posed in the proposal (see Project Description pages 1-2): Ultimately, addressing fundamental questions about the biology and infection potential of *Labyrinthula* will help understand the potential to mitigate eelgrass loss in Morro Bay. Given the aesthetic, environmental and economic value of the eelgrass resource in Morro Bay, this is one of the most important metrics in evaluating project success.
4. Presentation: Students will present the research results at a scientific meeting (2019/2020) to raise awareness about the importance of healthy eelgrass beds, their local and global population decline, and the role genetic tools can play in evaluating environmental problems. Disseminating research results to the public is key to project success.
5. Publication: Results will be submitted to a peer-reviewed journal during 2019/2020 and publication is anticipated by 2020. Publication of the project results in an international journal will highlight the local impacts of *Labyrinthula* and facilitate a global conversation about pathogenic impacts to a vital marine resource.

Schedule:

	2018	**2019**											
Task/ Activities	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
Visual Scoring of eelgrass wasting	X	X	X					X	X	X			
Blade Collection	X					X	X						
Culture & DNA isolation	X	X				X	X	X	X	X			
Genomic DNA analysis			X	X	X			X	X				

	2018	2019											
Task/ Activities	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
Quantitative PCR		X	X	X	X		X	X	X	X			
Report results to NEP and public (Blog news)		X					X					X	
Student Conference					X								
Final NEP Report											X		
Prepare publication											X	X	X

Qualifications:

Drs. Silvio Favoreto and Laurie McConnico will serve as co-PIs on this grant. They have co-taught the course in Applied and Environmental Microbiology (Bio 210M) at Cuesta College 6 times and combined have 20+ years of teaching experience at the undergraduate level. In particular, they will have access to classrooms and laboratory supplies afforded by Cuesta College and the Biological Sciences Department. These supplies combined with student interest in learning practical field and laboratory techniques and the skill sets of the co-investigators and associates at Cal Poly will guarantee project success. Please, see a summary of respective faculty expertise below.

Silvio Favoreto has a Ph.D. in Microbiology and 12 years of experience in practical applications of this field while employed as a Researcher/Faculty at the University of California and Northwestern University. Current teaching responsibilities and prior research experience qualify Silvio in the areas of host-microbe interaction and pathogen detection. All of these lines of inquiry require expertise in DNA sequencing and analysis, microbial culture techniques, microbial identification, quantitative PCR, metagenomics and cell biology. During his career as a researcher and faculty member, Silvio has led five fully funded independent research projects, mentored 25 students, and published 46 peer-reviewed scientific articles.

Laurie McConnico has a Ph.D. in Marine Sciences and 18 years experience working as a marine ecologist along the east and west coasts of North America. She has extensive experience working in algal and plant dominated ecosystems and was mentored by Drs. Isabella Abbott and Michael Foster. Her research requires expertise in the identification and quantification of marine macrophytes and associated fauna, as well as experimental design and statistical analyses (uni and multivariate). She is also experienced in the collection, preservation and chemical analysis of seawater samples. She has served as Principal Investigator in five research projects, including those in the Northwestern Hawaiian Islands and Mexico. Laurie mentored 17 graduate and undergraduate students, and published eight peer-reviewed articles.

Researchers Drs. Ryan Walter and Jennifer O'Leary, while not PI's on this grant, have spearheaded work on the oceanographic conditions in Morro Bay Estuary and/or helped document eelgrass population dynamics alongside the MBNEP. Professional collaborations with these individuals and MBNEP Biologists, along with thoughtful data exchange will enhance project success.

References:

Bergmann N, Fricke B, Schmidt MC, Tams V, Beining K, Schwitte H, Boettcher AA, Martin DL, Bockelmann AC, Reusch TB, Rauch G (2011) A quantitative real-time polymerase chain reaction assay for the seagrass pathogen *Labyrinthula zosterae*. Mol Ecol Resour 11:1076–1081

Short FT, Muehlstein L, Porter D (1987) Eelgrass wasting disease: cause and recurrence of a marine epidemic. Biol Bull:557–562

Silvio Favoreto and Laurie McConnico's Application, #72-18RF-C's Missing data

Project Title:

Role of Labyrinthula spp. in the development of eelgrass wasting disease in the Morro Bay Estuary.

Project Description Summary

Biology Faculty, Drs. Favoreto and McConnico will work with students at Cuesta College to assess the presence, distribution and abundance of the eelgrass pathogen Labyrinthula throughout Morro Bay Estuary. This pathogenic slime mold is suspected to be present in Morro Bay, based on visual evidence of tissue necrosis, however, no data exist to confirm its presence or understand its potential impact on the declining eelgrass population in the Estuary.

Using samples collected during a June 2018 summer course, students enrolled as Research Assistants at Cuesta College will work throughout late fall 2018 and early spring 2019 to extract DNA, begin qPCR techniques to quantify Labyrinthula and finish visually quantifying the extent of necrotic tissue on eelgrass blades photographed during June 2018. These student researchers will present preliminary results at a conference in spring 2019 and help with public outreach via MBNEP Blog postings. During summer 2019, twenty students enrolled in Applied Environmental and Microbiology (210M) will collect eelgrass from throughout the Estuary to culture and quantify Labyrinthula using standard microbiological and genetic techniques. Students will also help to determine the entire genome of Labyrinthula and assess its genetic variability within Morro Bay. Such data would be key in evaluating if more than one strain of Labyrinthula was present and perhaps could account for some of the variation in perceived infection within Morro Bay.

Given the extreme decline in Morro Bay's eelgrass population, this work is timely and key to evaluating best practices in the conservation of the limited and important natural resource. The unique approach of engaging with local college students to tackle understanding of this environmental problem and communicating research results to the public via the MBNEP Blog and scientific meetings/publications will ensure a wide range of public and scientific literacy surrounding the eelgrass decline.

meetings/publications will ensure a wide range of public and scientific literacy surrounding the eelgrass decline.

Briefly describe how your project addresses the priority issue(s) you checked above.

Eelgrass habitats provide food, protection, and serve as nursery grounds for a myriad of species. The high biodiversity associated with eelgrass beds is compromised by sedimentation, excess nutrients and toxins within the Morro Bay Estuary. Each of these factors may influence the virulence of Labyrinthula and impact eelgrass health/persistence. This study will shed light on the extent to which eelgrass beds are impacted by the Labyrinthula pathogen and help evaluate how to best protect eelgrass and its associated biodiversity.

In the space below, please list the relevant Action Plan(s) and briefly describe how they will be addressed by this project.

The proposed project is in direct alignment with ECR-7, ECR-8, EO-1 and E0-4. ECR-7 covers eelgrass data and research. This is the premise of our entire proposal. ECR-8 is focused on Eelgrass restoration. Without a clear understanding of why eelgrass is on the decline in Morro Bay, there is no way to effectively restore the plant community. Our project results will shed light on whether the Labyrinthula pathogen is present in the estuary and

begin to assess what role it has played in observed eelgrass declines. This knowledge will be very helpful in future restoration efforts. E0-1 is focused on public outreach and education. Since we will be working with local community college students to gather and disseminate research results in classes at Cuesta College, at local conferences and via the MBNEP Blog we will be able to engage with the public in a variety of formal and informal settings. This is key to making sure the public is aware of the eelgrass decline and the variety of ways the MBNEP has reached out to community partners to understand its decline and attempt restoration. Finally, E0-4, which aims to offer formal education programs is an integral part of the proposed community project, as students enrolled in Applied and Environmental Microbiology and Research Assistant courses at Cuesta College will help to collect and analyze data, contributing to the understanding of Labyrinthula induced wasting syndrome in Morro Bay Estuary.

fisher scientific
part of Thermo Fisher Scientific

Item	Price	Qty.	Availability	Item Subtotal
50-413-117 HORSE SERUM FILTERED 100ML by LAMPIRE Biological Laboratories SDP 7333400100ML	$73.25 / Each	2	Ships from manufacturer - Usually Ships in 2 Business Days	$146.50
10-378-016 Penicillin-Streptomycin-Glutamine (100X) PEN STREP GLUTAMINE 100X by Gibco™ 10378016	$30.12 / Each	2	Ships from manufacturer - Usually Ships in 2 Business Days	$60.24

Subtotal	$206.74
Estimated Shipping *	$51.61
Container ice charge	$33.00
Shipping charge	$18.61
Estimated Tax	$20.01
Sales tax	$20.01
Cart Total	$278.36

*Price and availability are subject to change.

Silvio Favoreto

From: Oxford Nanopore <noreply@nanoporetech.com>
Sent: Monday, September 03, 2018 10:58 AM
To: Silvio Favoreto
Subject: Nanoporetech Quotation

Oxford NANOPORE Technologies	**Quotation** Date: 03 Sep 2018
Billing Address Silvio Favoreto Cuesta College HWY1 Building 2300, Room 2307 HWY1 Building 2300, Room 2307 San Luis Obispo, 93421 United States	**Shipping Address** Silvio Favoreto Cuesta College HWY1 Building 2300, Room 2307 HWY1 Building 2300, Room 2307 San Luis Obispo, 93421 United States

Account No.		Quotation No.	ONT-98173
VAT No.		Valid Until	03 Oct 2018

Item No	Description	Qty	Unit price	Net price
StarterPack	Basic Flow Cell Wash Kit x1 SpotON Flow Cell (R9.4) x2 MinION Sequencing Device x1 Ligation Sequencing Kit x1	1	$1,000.00	$1,000.00

Subtotal	$1,000.00
Shipping (DAP) - based on 1 shipment(s) to United States	$50.00
Tax @ 0%	$0.00
Total	$1,050.00

Please click here to order the items on this quote

Tax/VAT Rates UK Customers will be charged UK standard rate VAT @ 20% where applicable	VAT Registration Details UK shipment: UK VAT No GB 874642494 EU Shipment: Dutch VAT No NL823865599B01 For an intra-EU supply this is subject to the VAT in the country of acquisition
Exchange Conversion Currently all prices are quoted in USD.	Bank Details USD Payments ONLY - Barclays Bank, Sort Code: 20-65-26, Account No. 78228533 IBAN: GB35 BARC 2065 2678 2285 33, SWIFT/BIC: BARCGB22 GBP Payments ONLY - Barclays Bank, Sort Code: 20-65-26, Account No: 60104302 IBAN: GB39 BARC 2065 2660 1043 02, SWIFT/BIC: BARCGB22 Cheques payable to Oxford Nanopore Technologies Ltd.

Information about this quotation

This quotation is provided in conjunction with the following conditions:

- Minimum order quantities apply, as described in the conditions of sale
- Quotations provided are valid until the date specified
- All orders resulting from this quotation received by Oxford Nanopore should be placed in USD, unless otherwise stated and agreed
- Customers shall remain responsible for all shipping and freight charges incurred by shipment. Any charges included within the quotation of for illustration purposes only and are subject to change
- In the event of any questions related to this quotation, please email us at support@nanoporetech.com
- Pricing for consumables applies only to the products specified herein
- The tax and shipping figures shown are based on the information you provided and may be subject to change
- All orders placed are subject to the Oxford Nanopore's condition of sale and associated terms found here

Delivery information and shipping

Whilst shipping information varies dependent on your location, there are some uniform rules that can be applied to all deliveries

- All orders placed must have a defined ship schedule, for at least the first 90 days of the period of the contract
- Shipments associated with bulk orders should be split over the period of the contract and should be complete before expiry
- Any changes to shipping schedules must be communicated to Oxford Nanopore with a minimum notice period of 28 days. ONT accepts no responsibility for any losses caused by such changes inside of this period
- Orders received without ship schedules will have a shipping schedule defined for them by Oxford Nanopore, for which the above terms will apply to
- Our standard Inco Terms are DAP (Delivered At Place), although this can differ depending on destination country. Please contact us should you have any questions related to this
- We have four distribution hubs located in the UK, US, and Netherlands, from which your order will be fulfilled

Oxford Nanopore Technologies Ltd. Gosling Building Edmund Halley Road Oxford Science Park Oxford OX4 4DQ UK
Financial enquiries email: accounts.receivable@nanoporetech.com
Order enquiries email: support@nanoporetech.com
Company Registration No. 05386273

Reagents & consumables ship free on orders above $300

Your order qualifies for free consumable shipping!

Product		Quantity	Subtotal
PCR Master Mix 2X (/pcr-master-mix) 25 µL reactions (volume): 1,000 reactions (10 x 1.25 mL) Catalog #: R02100L	$120.00	1 ✕	$120.00
PCR Master Mix with Hot Start 2X (/pcr-master-mix-hot-start) 25 µL reactions (volume): 1,000 reactions (10 x 1.25 mL) Catalog #: R02150L	$205.00	3 ✕	$615.00
PCR Tube & Cap Strips, 8-Well Strips, 100 µL, Optically Clear (/pcr-tube-cap-strips-8-well-100-ul-optically-clear) Box: 1 Box Catalog #: S02132B	$114.00	3 ✕	$342.00
PCR Master Mix + Hot Start DRY (/pcr-master-mix-hot-start-dry) Reactions: 200 reactions (4 x 50) Catalog #: R02160S	$170.00	4 ✕	$680.00

Order Total: $1,757.00 [before shipping]

Begin Checkout

Shipping Estimator

93403

PCR Master Mix 2X (1,000 reactions (10 x 1.25 mL))
PCR Master Mix with Hot Start 2X (1,000 reactions (10 x 1.25 mL))

Shipping Method	Price
Standard Overnight	~~$30.00~~ FREE
Priority Overnight	$50.00

PCR Tube & Cap Strips, 8-Well Strips, 100 µL, Optically Clear (1 Box)
PCR Master Mix + Hot Start DRY (200 reactions (4 x 50))

Shipping Method	Price
Ground	~~$23.76~~ FREE
Two-Day	$27.67
One-Day	$38.93

Estimate

QIAGEN

Your Shopping Cart - QUOTE

Product	Quantity	Your Price	Subtotal
 QIAamp DNA Mini Kit (50) Cat No./ID: 51304 show details	3	$165.00	**$495.00**

Total Price $495.00

Does not include applicable taxes, shipping and handling, or other charges.

Add Products to Your Cart

Enter items 1 at a time via **Manual Entry** or upload multiple items via **Mass Upload**

© QIAGEN 2013–2018. All rights reserved.

Sample to Insight

Location: United States Language: English