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19. ABSTRACT (Continue on reverse if necessary and identify by block number) The goal of this project was to make state of the art molecular biology instrumentation available to marine biologists. A DNA sequencer and an oligonucleotide synthesizer were purchased. The synthesizer is in full operation. It is heavily used and has proved to be quite cost-effective. The sequencer is in operation, but is still in the shakedown phase. We anticipate that it will be cost competitive with manual sequencing and will also be heavily used. Projects supported include marine symbiosis (Haygood, Felbeck), cell biology (Vacquier), pressure adaptation in marine bacteria (Bartlett), population genetics and evolution of marine organisms (Perrin, Newman).					
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FINAL REPORT
ONR GRANT N00014-89-J-1284

Principal Investigator: Margo G. Haygood

Grantee: University of California, San Diego
Scripps Institution of Oceanography

Grant Title: Central Equipment Facility for Molecular Marine Biology

Start Date: 1 December 1988

Project Objective:

The objective of this effort is to provide marine biologists with access to a state of the art molecular biology facility, and consequently to facilitate the integration of molecular biology into marine biology.

Accomplishments:

We combined the ONR DURIP award with SIO matching funds to purchase both an Applied Biosystems Model 370A DNA sequencer, and an Applied Biosystems Model 391 DNA synthesizer.

Synthesizer:

This instrument is located in a common area on the third floor of Hubbs Hall. Mr. Gary Moy, technician for Dr. Victor Vacquier, is in charge of the machine, with my technician, Ms. Deeanne Edwards as his deputy. The instrument has worked flawlessly and is heavily used. We began operation in September of 1989. We have had seven users (four major users) and 77 oligonucleotides have been synthesized to date. The oligonucleotides have been used for sequencing, as hybridization probes and as PCR primers. We have also used the machine to make biotinylated oligonucleotides as probes and for solid phase sequencing of PCR products.

Since we are a small, close-knit group, we do not use a formal recharge system. We charge users \$12 start-up and \$3 per base (with an additional \$8 for an OPC cartridge if purification is desired). This represents a 40% savings over the cost of synthesis at the UCSD Center for Molecular Genetics (CMG) oligonucleotide synthesis facility. When we need more reagents for the machine, users purchase reagents in proportion to the debt they have accumulated. The level of use has been high enough that we have not had problems with wasted chemicals or delays. Due to ever increasing UCSD discounts on reagents from Applied Biosystems, our actual costs are a little less than what we are charging, so we are comfortably in the black and have a little flexibility for charitable purposes (e.g. unfunded graduate students). I estimate we have saved about \$3000-\$4000 in oligonucleotide synthesis costs in the space of six months, which is about 20-30% of the list price of the instrument at the time we bought it. Thus this instrument appears to be quite cost effective even for a relatively small group such as ourselves.

Sequencer:

We decided to locate the sequencer at the Center for Molecular Genetics as a jointly managed SIO/CMG DNA sequencing facility. This was done for several reasons. First, the capacity of the machine is much greater than can be occupied by Marine Biology users alone, and the more heavily the machine is used, the more economically it can operate. Second, the machine requires a dedicated technician, and we did not have the resources to provide this, nor the experience in managing a big Core Facility. CMG has operated its oligonucleotide synthesis core facility for several years. Finally, we saw it as an opportunity to strengthen ties between Marine Biology and CMG.

The instrument arrived in August 1989, and a technician, Ms. Patty Tooker, was hired to run it. She has completed training and the machine is in a shakedown phase of operation. Approximately 50 test templates of various types have been sequenced. The results are comparable to manual sequencing, i.e. 350 -500 bases per run. Both single and double stranded templates work well. Ms. Tooker gave a presentation on the sequencer to Marine Biology members March 2, 1990, and several have given her templates to test. We are in the process of testing custom primers as well. When testing is completed, we will set price policies and open our doors to the public. Our preliminary experience is that the sequencer will be competitive in cost with manual sequencing.

Future Plans:

We plan to begin to provide sequence for paying customers within the next two months. Applied Biosystems has developed an upgrade to the sequencer for about \$20,000. We are seeking funds to purchase the upgrade, initially from within the university

For the synthesizer, we are investigating the possibility of adding an HPLC for oligonucleotide purification to our facility. HPLC is not necessary for ordinary unmodified oligonucleotides, but would be quite useful for separating biotinylated and fluorescently tagged oligonucleotides.

Projects supported:

Marine symbiosis:

Flashlight fish/luminous bacteria- M.G. Haygood

Chemolithotrophic symbionts of hydrothermal vent animals - H. Felbeck

Pressure

Adaptation of marine bacteria to hydrostatic pressure - D. Bartlett

Cell biology:

Calmodulin dependent adenylate cyclase in sea urchin sperm - V.D. Vacquier

Population genetics and evolution in marine animals

Cetaceans - P. Rosel (W. Perrin)

Barnacles - R. Van Syoc (W. Newman)

Publications:

Haygood, M.G. Relationship of the luminous bacterial symbiont of the Caribbean flashlight fish, *Kryptophanaron alfredi* (family Anomalopidae) to other luminous bacteria based on bacterial luciferase (*lux*) genes. submitted to Arch. Microbiol.

Bookbinder, L.H., G.W. Moy and V.D. Vacquier. Primary structure of the calmodulin binding adenylate cyclase from sea urchin sperm. in preparation.

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