(2)

Performance Report

Title: Molecular Probes in Marine Ecology: Concepts, Techniques and Applications

Office of Naval Research
Program: Advanced Training in Molecular Marine Biology

Grant Number N00014-89-J-1933

Scientific Officer: Randall S. Alberte

Principal Investigator and Course Director: J. Woodland Hastings
Professor of Biology, Harvard University

Institution: Marine Biological Laboratory

Contact: Leslie D. Garrick, Office of Sponsored Programs
(508) 548-3705, ext. 218

Date: March 12, 1990

Marine Ecology Course
Report, Summer, 1989

I. OBJECTIVES OF THE COURSE

A. Philosophy: Ecology interfaces with and depends upon many scientific disciplines, ranging from geophysics and atmospheric chemistry to population genetics and behavioral biology, to name just a few. Likewise, ecological issues touch all of humanity, with concerns ranging from sewage disposal and recycling to the ozone hole and the greenhouse effect. Again, the actual list is a much longer one, and the concerns are compelling.

How can scientists address and provide guidance in the solution of such problems? There is we think, no single or simple approach. Many significant steps have already been taken, involving many scientists and many initiatives. Among these, the investigation and understanding of marine ecosystems can be viewed as one of the most important. By virtue of its buffering capacity for many physical and chemical variables, and because of its biological productivity, the ocean is central to both the realities of the issues and the understanding of the problems. Ecology, in whatever disguise, embraces the many questions and seeks workable solutions. The 1989 MBL marine ecology course, with its program in molecular marine biology, provided training in the use of molecular probes towards the solution of these problems. At the same time, it attempted to formulate a conceptual framework as to how these techniques may be applied to help resolve of the more global ecological issues.

B. History and Tradition: The Marine Ecology course at MBL was introduced in 1956 and has been offered each year since that time. In more recent years its interaction and cooperation with the MBL Marine Ecosystems program has been of continuing value.

C. Training Goals: During the past decade, developments in the area of molecular biology, biotechnology, immunology and microsensing techniques have revolutionized many areas of biological sciences. We designed this course for graduate students, postdoctoral fellows and established investigators who wish to learn these several techniques and their applications to physiological and ecological investigations.

II. COURSE ORGANIZATION

A. Structure of the Course: The nine week course was organized as three modules of three weeks each. The first was centered around techniques concerned with the construction and use of DNA probes, under the direction of Dr. Dennis Powers, with Drs. T.T. Chen, Rob Rowan, J.W. Hastings and Head Course Assistant Laura Brezinsky. The second section was directed by Dr. Hans Paerl, with Drs. Bess Ward and Steve Giovononi, along with assistants Julie Kirshstein and Carolyn Currin. Students employed immunological techniques for the detection of proteins and cell types, as well as microsensing techniques for measuring pH and oxygen. The third module involved individual research projects based on individual interests; seven students participated in this.

B. Students: Twenty-four students (see Appendix) were accepted in the course, the number being based on laboratory layout and facilities. This is certainly a maximum number for the type of instruction given, since much of the space is occupied by sea water tables that have little use.

In selecting students we attempted to achieve diversity in as many ways as possible, ranging from specific interests and educational background to career stage and geographical location. The small numbers involved make it difficult to assess minority participation; every attempt was made to solicit applications from such individuals.

Because of the advanced nature of the course, and its specific applicability to individual problems in Ecology, no undergraduates were accepted in the course. The participants included 15 graduate students, 7 at the postdoctoral level and two faculty members. One of the postdoctoral students was a member of the science writing program.

C. Lectures and Seminars:

(1) Daily: Lectures by staff and special visitors were given 6 days per week, usually at 0930, with some additional lectures on certain days. The speakers and titles are given in the appendix. Outside speakers are also listed separately in the appendix.

(2) Student Seminars: A special and very successful feature of this course was the student seminar program. Organized and led by student Gisele Muller-Parker, eleven of the students presented research seminars to the group at 8PM on four evenings per

week during the second three week period. This allowed students to discuss the applicability of techniques learned in the course to their individual research projects. Titles and speakers are giving in the appendix.

III. DESCRIPTION OF THE COURSE

A. Overview: The lectures and laboratories introduced participants to the theory and practice of several key tools of molecular biology, and their application to the study of important ecological problems.

B. Laboratory: In the first module of the laboratory, work was centered around two undertakings both utilizing material from fish: the preparation of cDNA libraries and isolation of individual cDNAs, and the isolation of genomic DNA and restriction mapping. In the course of these exercises, a number of other procedures were utilized, including dot blots, electrophoresis, and Northern and Southern blotting. DNA sequencing was also carried out by most students; oligonucleotide synthesis was used to create synthetic probes, and the polymerase chain reaction (PCR) technique was used to amplify specific DNAs.

In the second module, experimental studies were focused on the use of immunological techniques for the detection of specific proteins and cells. Immunoassays for nitrogenase were used, and immunofluorescence illustrated the power and versatility of the technique. With cell and tissue extracts the quantitation of specific proteins by Western blotting was possible, and affinity purification of antibody was illustrated. An exercise with the cell sorter illustrated the identification and isolation of specific cell types or species by immunofluorescence. Species identification by *in situ* hybridization with fluorescent-labeled DNA probes were also covered. Finally, students carried out measurements of O_2 and pH with high spatial resolution using micoelectrodes.

In the third module, students carried out individual projects. Students participating in this module, are designated by asterisks in the student list. Their projects related to their individual research interests.

Acknowledgments: MBL personnel in many different departments were cooperative and helpful. We wish to acknowledge in particular the help of Les Garrick and Florence Dwane in organizing the course and registering students, and Linda Huffer in obtaining equipment on loan.

Marine Ecology - Summary of Student Research

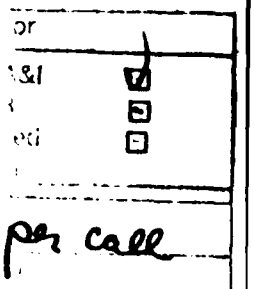
Mary Alice Coffroth, SUNY Buffalo.

I am interested in the population structure and population dynamics of benthic marine invertebrates, focusing specifically on scleractinian corals and gorgonian soft corals. I have initiated a study of genetic diversity and population structure of a clonal gorgonian, Plexaura sp. I am proposing to use DNA fingerprinting to resolve the clonal structure of populations of Plexaura sp. in the San Blas Islands, Panama. During the course I used techniques introduced in the course to refine my protocols for extraction of genomic DNA for Plexaura sp. and have obtained high molecular weight DNA. I have digested this DNA with a number of enzymes (EcoR I, BamH I, Sau3A, Hae III) which will be appropriate for cloning the DNA into lambda vectors. In the postcourse I plan to continue this work by generating a partial genomic library for Plexaura sp. DNA. I hope to screen clones for probes that detect repetitive DNA sequences that can be used in the DNA fingerprinting. I also plan to synthesize an oligomer of the 15bp repeat in wild-type M13 bacteriophage that has been used in fingerprinting studies. I would like to test the ability of this oligomer to detect repetitive DNA in Plexaura sp. by probing several Southern blots of gorgonian DNA that I have brought with me to the course. Finally, during the course I have amplified 18s rDNA from Plexaura sp. using primers provided by the instructors. I am now cloning the amplified DNA and will sequence this segment of DNA.

Lynne Gilson, Harvard University

I plan to study cellular protein export. The dogma for protein transport involves an N-terminal 'signal - peptide', which is recognized by the cellular machinery. Recently, four instances of transport of molecules across membranes (two proteins in E. coli, polysaccharide secretion in rhizobial, nodule - forming bacteria and drug efflux in mammalian tumor cells which leads to multi-drug resistance) without 'signal - peptide' sequences have been found. One of the proteins necessary in each of these transport systems shows homology. This protein is highly conserved in a C-terminal domain. Preliminary sequence analysis indicates the recent evolution of this protein group.

I am interested in how extensive this family of protein is, both within a given cell and across diverse organisms. I plan to use synthetic oligonucleotides designed to hybridize with the conserved domain to do a northern blot of tautog (black fish) mRNA to see how many genes may have this sequence. I could also screen a cDNA Library (from tautog liver) or use PCR to amplify and clone the DNA of interest. Restriction analysis with already determined enzymes will give a



preliminary sense of relatedness between analogous genes which can be extended with sequencing.

Peter L. Starkweather, University of Nevada, Las Vegas

My project is a pilot study of zooplankton population structure using ribosomal genes which will have applications to marine systems. The original biogeographic questions demand characterization of a nested series of population units, from individual clones of parthenogenetic species within a single lake, through several isolated lake clusters, to a comparison of zooplankton genetics and life histories throughout an extensive geographic province. The hypothetical structure of the work proposes that planktonic populations, both sexual and asexual, will show increasing genetic distance from one another across geographic space and that the genetic representation of isolated groups will show associations with both Rocky Mountain and Sierra Nevadan conspecifics. This problem will remain an important research focus of my laboratory, but I plan to expand this work to marine systems.

I have already used the Polymerase Chain Reaction (PCR) and subsequent restriction enzyme fragment analysis of the amplified 18s ribosomal RNAs to differentiate among 6 crustacean taxa representing 4 taxonomic orders. These include the clear separation of 2 widely distributed copepod from each other and from more distantly related marine or euryhaline species. Even with this fairly low resolution procedure, it appears that we may develop very sensitive (possibly species specific) genetic markers with some ease. With subsequent sequencing of the variable domains of the 18s RNAs (or, even more likely, the 28s ribosomal genes) of various taxa, it should be possible to construct individual species probes for both holo-and meroplanktonic species.

In specific terms, further laboratory development could result in:
1. Development of a series of isotopic or fluorescent probes to distinguish early life stages of numerous planktonic taxa, perhaps to the species level. This technique should allow routine and simple quantitation of such stages, providing better estimates of population size, larval recruitment and species distribution. This technique would also be applicable for recognition of very early life cycle stages of settling organisms, including those characterized as "fouling".
2. Development of genetic markers for water masses which contain distinctive zooplankton populations.

John F. Stolz, University of Massachusetts

My plan is to construct a genomic library from Chlorobium sp. (packaged into lambda) and to synthesize an oligonucleotide probe for the gene encoding the bacteriochlorophyll *c* binding protein for

Chlorobium sp. based on existing protein structure. The reason this work is important is that green phototrophic bacteria are important constituents of microbial communities found both in intertidal marshes (microbial mats) and stratified coastal waters (bacterial plates). Although they are known to be significant primary producers and nitrogen fixers in anaerobic communities, their role in the establishment and maintenance of anoxia is poorly understood.

IV. Administrative Functions of the Course

Advertising:
The Marine Ecology course is advertised widely. From a mailing list made up of the society members of ASLO and ESA, U.S. Marine Stations, the National Marine Educators Association, 11,000 course announcements were distributed. A copy of the announcement for 1990 is appended. In addition, paid advertisements were taken in Science, Nature, and the AWIS Bulletin.

Other Support for the Marine Ecology Course:
Private funds totalling $50,000 were obtained by the MBL for the course in 1989. The loan of high quality research equipment is critical to the success of MBL courses. In 1989, 20 companies loaned equipment to the Marine Ecology course. A list of that equipment is appended.

Under-represented Minorities in Science:
The MBL is committed to the increased participation of under-represented minority students in our educational programs. Each course director is aware of this commitment and the course admissions committees have made diversity of students an important criterion when the classes are chosen.

Vertebrate Animals:
Use of vertebrate animals by the Marine Ecology course will be approved the MBL Institutional Animal Care and Use Committee.

V. Plans for 1990

The Marine Ecology course will be offered from June 17 to July 28, 1990 for a class of 24 graduate students, postdoctoral fellows and established investigators who wish to learn the techniques of molecular biology and their application to physiological and ecological investigations. Lectures and discussions will accompany the laboratories and will focus on the technologies being learned and applications to specific problems in marine ecology. In addition, a mini-symposium on global ecological issues is planned in conjunction with this course.

The laboratory portion of the course will be divided into three repeating modules running concurrently. Students will take two of the three laboratory sections, to be offered concurrently in two cycles during the 6 weeks. These modules are:
1. Cloning, manipulation and analysis of nucleic acid probes.
2. Analysis of populations and species with nucleic acid probes.
3. Symbioses: identification of organisms and functional interrelationships

Module 1 - Cloning, manipulation and analysis of nucleic acid probes
Staff: T. Chen, C.M. Lin and C. Cheng

Using fish and other marine organisms as experimental material, nucleic acids will be isolated, characterized and used in the construction of specific probes. Students will utilize a variety of procedures and techniques, including isolation, purification and quantitation of RNA and DNA; dot blots, electrophoresis, Northern and Southern blotting and restriction mapping; construction of cDNA libraries; oligonucleotide synthesis and the creation of synthetic probes; screening cDNA libraries and DNA sequencing.

Module 2 - Analysis of population and species with nucleic acid probes
Staff: D. Powers and others to be appointed

This section will capitalize on the use of mitochondrial DNA, chloroplast DNA and DNA fingerprinting, along with the amplification of 16S-RNA and other specific genes, to address questions of genetic variability within and between marine species and populations. The laboratory techniques will include isolation, purification, and restriction analysis of DNA; oligonucleotide synthesis, Southern blotting and *in vitro* gene amplification by the polymerase chain reaction; DNA sequencing.

Module 3 - Symbioses: identification of organisms and functional interrelationships
Staff: *K.H. Nealson, J.W. Hastings , C. Wimpee and B.Wimpee*

Functional associations involving specific biochemical capabilities contributed by one organism that benefit a second organism will be analyzed experimentally. The projects will be designed to identify both the organisms (which often involve non-culturable symbionts) and their genetic potentials with regard to specific biochemical activities. Biochemical techniques, including enzyme assays, will be complemented with immunochemical analyses, including Western Blots. The use of specific nucleic acid probes will involve Southern and Northern blotting, as well as PCR amplification. The symbioses to be examined will include those that contribute energy from photosynthesis and sulfur oxidation, carbon fixation, nitrogen fixation, and bioluminescence.

Student Seminars:
Students will have the opportunity to give presentations to the class of their own research projects underway at home institutions. Attention will be given as to how the techniques employed in the course may be applicable, but also to the fundamental scientific issues being addressed.

Director: J.W. Hastings, Harvard University

Faculty: Dennis A. Powers, Stanford University; Thomas T. Chen, University of Maryland; Kenneth H. Nealson, University of Wisconsin; Charles Wimpee, University of Wisconsin; C.M. Lin, University of Maryland; John Hobbie, Ecosystems Center,MBL.

Mini-Symposium: "Sources and Biotic Controls of Trace Gases"

This theme was chosen because molecular probes are an important tool for studying the sources and controls of the trace gases entering the Earth's atmosphere. For example, methane is found in the sea at saturated at saturated concentrations. However, it is unclear whether the organisms producing the methane are located inside paticles of organic matter or inside zooplankton guts. The location problem must be solved before controls and feedbacks may be studied. And it is obvious that molecular probes are useful tools to study this problem.

In the absence of many examples of the use of these probes, we will instead set the ecological/biogeochemical stages and discuss the origin, rates of production, and controls of various trace gases(carbon dioxide, methane, sulfur gases, nitrogen gases) in the ocean and terrestial ecosystems.

Participants in this symposium include:

Mary Scranton (SUNY Stony Brook) - Methane in the Sea
John Dacey (WHOI) - DMS in the Sea
Ellen Druffle/Peter Brewer/David Glover/Katherine Goyet (WHOI *Carbon Dioxide Study Group*)
Patrick Crill (University of New Hampshire) - Methane from Freshwater Wetlands
Paul Steudler (MBL) - Sulfur gases from Forest Ecosystems

Potential Participants, who have not yet accepted the invitation:

Mary Firestone (University of California, Berkeley) - N_2O Molecular Probes
Steve Wofsy (Harvard University) - Dynamic Diffusion Methods of Analysis

Appendices

A. Staff of 1989 Marine Ecology Course

B. Students enrolled in the 1989 Marine Ecology Course

C. Lecture schedules, 1989 Marine Ecology Course

D. List of student seminars, 1989 Marine Ecology Course

E. Laboratory Exercises 1989

F. 1989 and 1990 Course Announcements

G. List of loaned equipment, 1989

(A)

MARINE BIOLOGICAL LABORATORY

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MARINE ECOLOGY

Molecular Probes: Concepts,Techniques and Applications

Staff

June 18 - July 29, 1989

J. Woodland Hastings, Instructor and Director, Harvard University, Cambridge, MA Marine dinoflagellates: molecular biology of circadian control and ecology of bioluminescence in the marine environment.

Dennis Powers, Instructor, Stanford University, Hopkins Marine Station, Pacific Grove, CA Molecular probes in the study of environmental adaptation.

Thomas Chen, Instructor, University of Maryland and Center of Marine Biotechnology, Baltimore, MD. Developmental genetics: molecular cloning & sequencing of cDNAs from marine eukaryotes.

Rob Rowan, Instructor and Course Coordinator, Stanford University, Hopkins Marine Station, Pacific Grove, CA. Molecule genetics; ecology and molecular basis of symbiosis.

Hans W. Paerl, Instructor, University of North Carolina at Chapel Hill, NC. Aquatic nitrogen cycling, eutrophication and regulation of microbial growth and production in aquatic habitats.

Bess B. Ward, Instructor, University of California at Santa Cruz, Santa Cruz, CA. Marine nitrogen cycling, immunological and molecular methods in ecological studies.

Steve Giovannonni, Instructor, Oregon State University, Corvallis, OR. Phylogenetic and ecological relationships; applications of 16S ribosomal RNA problems to studies of symbiosis and phylogeny.

Laura Brezinsky, Head Course Assistant, Module, University of Hawaii.

Julie Kirshtein, Course Assistant, Module 2, University of North Carolina at Chapel Hill, NC.

Carolyn Currin, Course Assistant, Module 2, University of North Carolina at Chapel Hill, NC.

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MARINE BIOLOGICAL LABORATORY
1989 Accepted Students

Marine Ecology

Dror Angel, Graduate Student
CUNY

Barbara Best, Post-Doctoral
Columbia University

Daniel Brazeau, Graduate Student
SUNY at Buffalo

Robert Browne, Faculty
Wake Forest University

Mary-Alice Coffroth, Post-Doctoral
SUNY at Buffalo

Hudson DeYoe, Graduate Student
Bowling Green State University

Lynne Gilson, Graduate Student
harvard University

Alan Groeger, Post-Doctoral
Murray State University

Matthew Hoch, Graduate Student
University of Delaware

Jen-jen Lin, Graduate
University of California at San Diego

Michael Montgomery, Graduate Student
University of Colorado

Gisele Muller-Parker, Post-Doctoral
University of Maryland

David Penny, Graduate Student
University of Southern California

Carol Reeb, Graduate Student
University of Georgia

Christopher Scholin, Graduate Student
Woods Hole Oceanographic Institution

Steven Sczekan, Post-Doctoral
North Carolina State University

MARINE BIOLOGICAL LABORATORY
1989 Accepted Students

Marine Ecology

Jeffrey Silberman, Graduate Student
University of Miami

David Smith, Graduate Student
University of California, San Diego

Peter Starkweather, Faculty
University of Nevada

Tracy Stevens, Graduate Student
Portland State University

John Stolz, Graduate Student
University of Massachusetts

Tzung-horng Yang, Graduate Student
University of California, San Diego

Science Writing Fellowship Program:

Susan Okie, M.D.
Washington Post Science & Medical Reporter

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1989 LECTURE SCHEDULE: MARINE ECOLOGY COURSE: PROBES IN MARINE ECOLOGY

Homestead First Floor Lecture Hall

WEEK 1

June 19	9:00 Extraction of Nucleic Acids Robert Rowan, Hopkins Marine Station, Stanford University, Pacific Grove, CA
June 20	9:00 Gel Electrophoresis and Enzymes for Recombinant DNA Techniques Robert Rowan, Hopkins Marine Station, Stanford University, Pacific Grove, CA
June 21	9:00 Construction of DNA Libraries Genomic Libraries: Robert Rowan, Hopkins Marine Station, Stanford University, Pacific Grove, CA cDNA Libraries: Tom Chen, Center of Marine Biotechnology, University of Maryland, Baltimore, MD
June 22	9:00 Nucleic Acid Hybridization and Screening of DNA Libraries: I Tom Chen, Center of Marine Biotechnology, University of Maryland, Baltimore, MD
June 23	9:00 Nucleic Acid Hybridization and Screening of DNA Libraries: II Tom Chen, Center of Marine Biotechnology, University of Maryland, Baltimore, MD
June 24	TO BE ANNOUNCED
June 25	NO LECTURE

C-2

1989 LECTURE SCHEDULE: MARINE ECOLOGY COURSE: PROBES IN MARINE ECOLOGY

Homestead First Floor Lecture Hall

WEEK 2

June 26 — 10:30 Analysis of Chemically induced Biological Change in the Marine Environment

John Stegeman, WHOI, Woods Hole, Mass.

June 27 — 10:30 Molecular Probes for Aquatic Toxicants

Tom Chen, Center of Marine Biotechnology University of Maryland, Baltimore, MD

June 28 — 10:30 The Genetic Architecture of a Model Marine Organism: Biogeography of isozymes, allozymes and mtDNA

Dennis A. Powers, Hopkins Marine Station, Stanford University, Pacific Grove, CA

June 29 — 10:30 Evolutionary and Physiological Strategies for Adapting to Changes in Environmental Temperature and Oxygen

Dennis A. Powers, Hopkins Marine Station, Stanford University, Pacific Grove, CA

June 30 — 10:30 Fish Growth Hormone Genes and their Evolutionary and Ecological Aspects

Tom Chen, Center of Marine Biotechnology, University of Maryland, Baltimore, MD

July 1 — 10:30 A Translational Regulatory Mechanism for Circadian Rhythms in a Marine Dinoflagellate

WoodY Hastings, Harvard University, Cambridge, Mass

11:15 A Circadian Rhythm in a Procaryote

Beatrice Sweeney, University of California at Santa Barbara

July 2 — NO LECTURE

C-3

1989 LECTURE SCHEDULE: MARINE ECOLOGY COURSE: PROBES IN MARINE ECOLOGY

Homestead First Floor Lecture Hall

WEEK 3

July 3 — 10:30 The Polymerase Chain Reaction and its Application to the Study of Symbiotic Dinoflagellates

Robert Rowan, Hopkins Marine Station, Stanford University, Pacific Grove, CA

July 4 — NO LECTURE

July 5 — 10:30 Analysis of Growth and Differentiation of Marine Phytoplankton and Bacteria

Penny Chisholm, MIT, Cambridge, Mass

July 6 — 10:30 Molecular Probes for Studying Symbiotic Invertebrates

Colleen Cavanaugh, Harvard University, Cambridge, Mass

NOTE CHANGE OF TIME FROM 10:30 TO 9:30:

July 7 — 9:30 Analyzing Microbial Diversity with Ribosomal RNA Probes

Stephen Giovannoni, Oregon State University, Corvalis, OR

July 8 — 9:30 Phylogenetic Analyses of Marine Picoplankton by Ribosomal RNA Gene Cloning and Sequencing

Stephen Giovannoni, Oregon State University, Corvalis, OR

July 9 — NO LECTURE

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1989 LECTURE SCHEDULE: MARINE ECOLOGY COURSE: PROBES IN MARINE ECOLOGY

Homestead First Floor Lecture Hall

WEEK 4

July 10	90:30 The Inference of Phylogenetic Trees from Molecular Data Stephen Giovannoni, Oregon State University, Corvalis, OR
July 11	9:30 The Phylogeny of Oxygenic Phototrophs: Cyanobacteria, Prochlorophytes and Chloroplasts Stephen Giovannoni, Oregon State University, Corvalis, OR
July 12	9:30 Analyzing Microbial Diversity with Ribosomal RNA Probes Stephen Giovannoni, Oregon State University, Corvalis, OR
July 13	9:30 A Phylogeny of Metazoa form 18S rRNA Seqeunces Katherine G. Field, Oregon State University, Corvalis, OR
July 14	9:30 Transposable Elements in Bacteria Bess Ward, University of California, Santa Cruz, CA
July 15	9:30 Transposable Elements in Eukaryotes Bess Ward, University of California, Santa Cruz, CA
July 16	NO LECTURE

C-5

1989 LECTURE SCHEDULE: MARINE ECOLOGY COURSE: PROBES IN MARINE ECOLOGY

Homestead First Floor Lecture Hall

WEEK 5

July 17 — 9:30 *Transposon Mutagenesis*

Bess Ward, University of California, Santa Cruz, CA

July 18 — 9:30 Non-radioactive Methods for Nucleic Acid Probes

L. Kerkhoff, Scripps Institution of Oceanography, La Jolla, CA

13:00 DNA Hybridizations using Homologous and Heterologous nif gene probes

J. Kirshtein, University of North Carolina, Chapel Hill, NC

July 19 — 9:30 Immunological Methods in Marine Microbiology

Bess Ward, University of California, Santa Cruz, CA

July 20 — 9:30 Needs and Desired Applications ofImmunology from the Ecologists Perspective

Hans Paerl, University of North Carolina, Chapel Hill, NC

July 21 — 9:30 Immunoassays: Applications, Pros and Cons

Carolyn Currin, University of North Carolina, Chapel Hill, NC

July 22 — 9:30 Flow Cytometry: Its Applications to Marine Ecology

R. Olson, WHOI. Woods Hole, Mass

July 23 — COURSE PICNIC (No Lecture)

C-6

1989 LECTURE SCHEDULE: MARINE ECOLOGY COURSE: PROBES IN MARINE ECOLOGY

Homestead First Floor Lecture Hall

WEEK 6

July 24

9:30 Strategies and methodologies for use of synthetic DNA in molecular cloning
Richard Ogden, Agouron Institute, La Jolla,CA

July 25

90:30 Design, Construction and Use of Microelectrodes for measurement of ions and Dissolved Gasses in Micro-environments

R. Carlton, Michigan State University

13:00 Application of O_2 Microelectrodes in Diverse Marine Environments

B. Bebout, University of North Caroline, Chapel Hill, NC

July 26

9:30 Investigation of Micro-environments: Practical Considerations in Experimental Design and Data Interpretations

R. Carlton

July 27

9:30 Micro-physiological Probes: Progress and Application in the Past Decade

Hans Paerl, University of North Caroline, Chapel Hill, NC

July 28

9:30 Integrating Microchemical, Microautoradiographic and Immunochemical Analyses of Community Productivity/Metabolism

Hans Paerl, University of North Caroline, Chapel Hill, NC

July 29

9:30 The use of molecular probes for the identificatic of luminous species in the Black Sea
Ken Nealson, Great Lakes Research Institute University of Wisconsin, Milwaukee

MARINE BIOLOGICAL LABORATORY

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MARINE ECOLOGY STUDENT SEMINARS
Summer 1989
8 pm Conference Room, Loeb 301A

7/10	Carol Reeb	Discontinuous distribution of American oyster mtDNA genotypes.
7/11	Gisele Muller-Parker	Symbioses between cnidarians and zooxanthellae.
7/12	Dror Angel	The ecology of colonial radiolarians.
7/13	Dan Brazeau	Sex ratio evolution in clonal organisms.
7/17	John Stoltz	Stratified microbial ecosystems.
7/18	Tracy Stevens	Genetic variation in killer whales using mtDNA.
7/19	Steve Sczekan	Molecular controls of gene expression at transcriptional and translational levels: Applications to circadian control.
7/20	Robert Browne	Molecular approaches for investigating sexual and asexual brine shrimp.
7/24	Barbara Best	To sink and swim: Potential use of molecular probes to larval dispersal studies.
7/25	Mary-Alice Coffroth	Population dynamics of a clonal gorgonian.
7/26	Peter Stark-weather	Zooplankton communities: Prospects for molecular probes in lake and ocean.

E-1

6

MARINE BIOLOGICAL LABORAT ORY
MARINE ECOLOGY LABORATORY - 1989

FIRST MODULE LABORATORY

The class was broken into two groups of 12 students. Within each group, students worked in pairs on either:

Group 1. Genomic Library (Rob Rowan and Laura Brezinsky).

Extract and purify nuclear genomic DNA from a local fish -- the Tautog. Learn about agarose gel electrophoresis.

Partially digest nuclear DNA with a restriction enzyme, and size the DNA on sucrose gradients.

Ligate sized fish DNA to bacteriophage lambda vector DNA, package same, and plate on E. coli to obtain genomic libraries.

As a demonstration, mitochondrial DNA was prepared from Tautog ovaries, by conventional methods (isolate and lyse mitochondria, purify mitochondrial DNA by CsCl density gradient centrifugation).

Randomly picked nuclear genomic clones, and purified mitochondrial DNA, were used as hybridization probes to examine genetic variability among 20 individual Tautogs (Southern Blots).

Group 2. cDNA Library (Tom Chen and Dennis Powers)

RNA was isolated from Tautog liver, purified, and fractionated by oligo-dT cellulose chromatography.

cDNA was prepared from the poly(A)+ RNA, and ligated to a bacteriophage lambda vector. This DNA was packaged to produce cDNA libraries.

cDNA libraries were screened using an LDH (Lactate Dehydrogenase) cDNA clone from another fish -- Fundulus. Tautog LDH cDNA clones were isolated by repeated screening.

Other bacteriophage lambda clones (provided by Tom Chen) were grown, and their DNA was isolated and cut with restriction enzymes. This excercise demonstrated how to characterize a cloned DNA.

E-2

POLYMERASE CHAIN REACTION LABORATORY

Students amplified small-subunit ribosomal RNA genes from either mixed bacterial populations (Steve Giovannoni) or from symbiotic dinoflagellates or their favorite small marine organism (e.g., algae, coral, copepods, microflagellates, dinoflagellates) (Rob Rowan).

Isolate organisms and prepare DNA.

Amplify rDNA using universal (or zooxanthella-specific) primers by PCR.

Analyze amplified DNA with restriction enzymes. Specific organisms are identified by their digest pattern.

E-3

E. Laboratory

SECOND MODULE LABORATORY

July 6	Nucleic Acid Fractionation Using Quiagen Ion Exchange Columns (SG)
July 7	Labeling DNA Probes Using Polynucleotide Kinase (SG)
July 8	Probing Ribosomal RNAs from Natural Microbial Populations (SG)
July 10,11	The Selective Amplification of 16S and 18S rNAs with Phylogeneitc Group-Specific Primers (SG)
July 12,13	RNA and DNA Sequencing (KGF)
July 14,15	Transposon Mutagensis: Conjugation and Extraction of Genomic DNA and Plasmids from Bacteria; agarose gel electrophoresis of extracted/ restriction digested DNA; Southern Blotting (BBN)
July 17	Hybridization with biotinylated probes; mutant screening (BBW)
July 18	Signal Development for Biotinylated Probes (LK) and Applications of Non-Radioactive Nucleic Acid Probe Labeling Techniques (JK)
July 19	Rocket Immunoelectrophoresis (BBW)
July 20	Immunofluorescence Assays for External/Internal Antigens (CC & BBW)
July 21	Immunoelectrophoresis/Blotting (Western Blotting Techniques)
July 22	Assessment of Importance of PicoPlankton by Flow Cytometry and Analysis of Bacteria by Immunofluorescence/Flow Cytometry (RO)
July 24	Applications Immunology in Ecology: Immunoblotting, Immunocytochemistry, Microautoradiography (HWP and CC)
July 25	Microelectrodes Applications (RC and BB)
July 26	Microelectrode Applications for Marine Macrophytes, Symbioses (RC); Combining Immunological, Microautoradiographic and Microchemical Techniques to Characterize Marine Microhabitats (HWP)

Molecular Probes in Marine Ecology: Concepts, Techniques and Applications

Marine Biological Laboratory
Woods Hole, Massachusetts

Modules 1 and 2: **June 18 - July 27, 1989**
Optional Module 3: **July 31 - August 19, 1989**

Limited to 24 students.
Director: J. W. Hastings, Harvard University
Faculty: Dennis A. Powers, Stanford University; Hans W. Paerl, University of North Carolina; Thomas T. Chen, University of Maryland; Stephen Giavannoni, Oregon State University; Bess Ward, University of California, Santa Cruz; Robert G. Rowan, Center for Marine Biotechnology (University of Maryland and Johns Hopkins University).

Financial Aid: Most students who request financial aid receive awards approximately equal to the cost of tuition. Students who are non-U.S. residents generally receive a travel supplement.

For further information and application forms, contact:

Admissions Coordinator
Office of Sponsored Programs
Marine Biological Laboratory
Woods Hole, MA 02543

Application Deadline:
March 1, 1989

The MBL is an EEO Affirmative Action Institution.

This is an intensive course for graduate students, postdoctoral fellows and established investigators who wish to learn the techniques of molecular biology and their application to physiological and ecological investigations. Lectures and discussions will accompany the laboratories and will focus on the technologies being learned and applications to specific problems in marine ecology. In addition, a MINI SYMPOSIUM ON GLOBAL ECOLOGICAL ISSUES is planned in conjunction with this course.

There will be two technique modules of 3 weeks each:

Module 1 - Nucleic Acid Probes

Staff: D. Powers, T. Chen, R. Rowan, J.W. Hastings

Objective: To gain theoretical practical experienceand competence in a variety of techniques using fish and other marine organisms as experimental material. Students will learn the following techniques: Isolation, purification, and quantitation of RNA and DNA; dot blots, electrophoresis, Northern and Southern blotting, mtDNA isolation and restriction mapping; constructing cDNA libraries; oligonucleotide synthesis and the creation of synthetic probes; screening cDNA libraries; DNA sequencing.

Module 2 - Immunochemical Probes, Molecular Biology and Microphysiological Techniques

Staff: H. Paerl, B. Ward, S. Giavannoni

Objective: Building on the techniques in Module 1 students will learn the procedures and limitations of immunological methods for the detection of specific proteins (enzymes) and also for detection of specific cells and cell types as well as newer microtechniques for measurement of pH and O_2.

Students will learn the following techniques: transposon mutagenesis; botin labelled DNA probes, a sensitive and safe alternative to ^{32}P labelling of DNA probes; group-specific DNA probes for organism identification; species identification by *in situ* hybridization with fluorescent-labelled DNA probes; amplification of 16S-RNA genes using the polymerase chain reaction; measurements of N^2 fixation; immunoassays for nitrogenase; Western blots, affinity purification of antibody; cell sorting by immunofluorescence.

Module 3 - Independent Projects in Molecular Marine Ecology

An **optional** third three-week module will be comprised of independent research-oriented projects utilizing the techniques learned in modules 1 and 2. Possible topics include: recruitment, environmental adaptation and physiological ecology, and food chain studies.

Marine Ecology: Concepts, Techniques and Applications of Molecular Probes

June 17-July 28, 1990

This is an intensive course, intended primarily for graduate students, postdoctoral fellows and established investigators who wish to learn the techniques of molecular biology and their application to physiological and ecological investigations. Lectures and discussions will accompany the laboratories and will focus on the technologies being learned and applications to specific problems in marine ecology. In addition, a MINI SYMPOSIUM ON GLOBAL ECOLOGICAL ISSUES is planned in conjunction with this course.

Students will rotate through two of the three following laboratory sections, to be offered concurrently in two cycles.

Module 1 - Cloning, manipulation and analysis of nucleic acid probes

Using fish and other marine organisms as experimental material, nucleic acids will be isolated, characterized and used in the construction of specific probes. Students will utilize a variety of procedures and techniques, including isolation, purification and quantitation of RNA and DNA; dot blots, electrophoresis, Northern and Southern blotting and restriction mapping; construction of cDNA libraries; oligonucleotide synthesis and the creation of synthetic probes; screening cDNA libraries and DNA sequencing.

Staff: T. Chen, C.M. Lin and C. Cheng

Module 2 - Analysis of population and species with nucleic acid probes

This section will capitalize on the use of mitochondrial DNA, chloroplast DNA and DNA fingerprinting, along with the amplification of 16S-RNA and other specific genes, to address questions of genetic variability within and between marine species and populations. The laboratory techniques will include isolation, purification, and restriction analysis of DNA; oligonucleotide synthesis, Southern blotting and in vitro gene amplification by the polymerase chain reaction; DNA sequencing.

Staff: D. Powers and others to be appointed

Module 3 - Symbioses: identification of organisms and functional interrelationships

Functional associations involving specific biochemical capabilities contributed by one organism that benefit a second organism will be analyzed experimentally. The projects will be designed to identify both the organisms (which often involve non-culturable symbionts) and their genetic potentials with regard to specific biochemical activities. Biochemical techniques, including enzyme assays, will be complemented with immunochemical analyses, including Western Blots. The use of specific nucleic acid probes will involve Southern and Northern blotting, as well as PCR amplification. The symbioses to be examined will include those that contribute energy from photosynthesis and sulfur oxidation, carbon fixation, nitrogen fixation, and bioluminescence.

Staff: K.H. Nealson, J.W. Hastings, C. Wimpee and B. Wimpee

Student Seminars:

Students will have the opportunity to give presentations to the class of their own research projects underway at home institutions. Attention will be given as to how the techniques employed in the course may be applicable, but also to the fundamental scientific issues being addressed.

Limited to 24 students.

Director:
J. Woodland Hastings, Harvard University

Faculty:
Dennis A. Powers, Stanford University; Thomas T. Chen, C.M. Lin, University of Maryland; Kenneth H. Nealson, Charles Wimpee, University of Wisconsin; John Hobbie, Ecosystems Center, MBL.

Financial Aid:
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Office of Sponsored Programs
Marine Biological Laboratory
Woods Hole, MA 02543
(508) 548-3705, EXT. 216

Application Deadline:
March 1, 1990

PLEASE POST

1989 LOANER EQUIPMENT - MARINE ECOLOGY

AMERICAN BIONETICS

1 Slot blot system

APPLIED BIOSYSTEMS

1 one column DNA Synthesizer

BECKMAN

1 LS 3801 Scintillation Counter
2 Microfuges w/ standard and horizontal rotors
1 GPR Centrifuge w/buckets & assortment of adapters for
10ml and 50ml tubes

(SHARED W/EMBRYOLOGY)
1 L8 M80 Ultracentrifuge w/Ti 70.1, SW28, SW28.1 buckets, SW60Ti,
Vti80, 80Ti, Vti65, SW40, SW55 rotors
1 J2-21 Refrigerated Centrifuge w/JA-14, JA-20, JS-13 rotors

BRINKMANN

1 Model 5415 Eppendorf Microfuge

BETHESDA RESEARCH LABS

6 Model H5 Electrophoresis Units, Cat. No. 1087EJ

COY LABORATORY PRODUCTS

2 Thermal Cyclers

DIAMOND GENERAL

1 Microsensor (Model to be confirmed by Hans Paerl)

DUPONT

1 Technospin R refrigerated Centrifuge w/H-5094 rotor, H5091
Buckets, w/variety of adapters

EG & G

1 DNA Synthesizer System

FOTODYNE

1 UV Transilluminator(Fotodyne UV 300)
1 Transilluminator Polaroid Camera(Fotodyne FCR-10) w/5-5343
Camera Hood

INTERNATIONAL BIOTECHNOLOGIES, INC.

4 MPH Electrophoresis Units(Cat. No. 5200) w/ standard combs for loading DNA
4 CPS 500 Power Supplies
1 Microfuge w/rotor
2 MBP 3000 Power Supplies
2 STS Sequencing Apparatuses w/shark tooth combs & Spacer sets for 0.4mm gels(80310, 80444) & extra xet of glass plates
5 Horizontal mini gel boxes

ISCO

4 Model 452 Power Supplies

LAB LINE

1 Model 3540 Shaker Water Baths w/gable cover & platform for 125ml, 250ml flasks
1 Model 3528-5CC/LB Environmental Shaker w/cooling coil and illuminators
1 Model 3524-5 Platform for 40 250ml flasks
1 Model 3524-7 Platform for 24 500ml flasks

NESLAB

1 Model RTE 220 refrigerated circulator

NEW BRUNSWICK

1 R76 Water Bath Shaker

OLYMPUS

3 SZ-III Zoom Stereo Microscopes

SAVANT

2 Model HSC10K High Speed Tabletop Centrifuge w/HSR10-1795 & HSR24 rotors
2 Gel Dryers
(SHARED WITH PHYSIOLOGY OR EMBRYOLOGY)

1 Speed Vac System

SEQUOIA TURNER

1 Model 450 Fluorometer(for chlorophil analysis)

STRATEGENE

1 Stratalinker 1800

ZEISS

2 Zoom Stereo Microscopes w/video
1 compound equipped for epifluorescence
1 compound for DIC