INFORMATION ABOUT PRINCIPAL INVESTIGATORS/PROJECT DIRECTORS(PI/PD) and co-PRINCIPAL INVESTIGATORS/co-PROJECT DIRECTORS

Submit only ONE copy of this form **for each PI/PD and co-PI/PD** identified on the proposal. The form(s) should be attached to the original proposal as specified in GPG Section II.B. Submission of this information is voluntary and is not a precondition of award. This information will not be disclosed to external peer reviewers. ***DO NOT INCLUDE THIS FORM WITH ANY OF THE OTHER COPIES OF YOUR PROPOSAL AS THIS MAY COMPROMISE THE CONFIDENTIALITY OF THE INFORMATION.***

PI/PD Name: Jay D Keasling

Gender: ☒ Male ☐ Female

Ethnicity: (Choose one response) ☐ Hispanic or Latino ☒ Not Hispanic or Latino

Race:
(Select one or more)

- ☐ American Indian or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Native Hawaiian or Other Pacific Islander
- ☒ White

Disability Status:
(Select one or more)

- ☐ Hearing Impairment
- ☐ Visual Impairment
- ☐ Mobility/Orthopedic Impairment
- ☐ Other
- ☒ None

Citizenship: (Choose one) ☒ U.S. Citizen ☐ Permanent Resident ☐ Other non-U.S. Citizen

Check here if you do not wish to provide any or all of the above information (excluding PI/PD name): ☐

REQUIRED: Check here if you are currently serving (or have previously served) as a PI, co-PI or PD on any federally funded project ☒

Ethnicity Definition:
Hispanic or Latino. A person of Mexican, Puerto Rican, Cuban, South or Central American, or other Spanish culture or origin, regardless of race.
Race Definitions:
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Black or African American. A person having origins in any of the black racial groups of Africa.
Native Hawaiian or Other Pacific Islander. A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.
White. A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

WHY THIS INFORMATION IS BEING REQUESTED:

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PI/PD Name: Adam Arkin

Gender: ☒ Male ☐ Female

Ethnicity: (Choose one response) ☐ Hispanic or Latino ☒ Not Hispanic or Latino

Race:
(Select one or more)
- ☐ American Indian or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Native Hawaiian or Other Pacific Islander
- ☒ White

Disability Status:
(Select one or more)
- ☐ Hearing Impairment
- ☐ Visual Impairment
- ☐ Mobility/Orthopedic Impairment
- ☐ Other
- ☒ None

Citizenship: (Choose one) ☒ U.S. Citizen ☐ Permanent Resident ☐ Other non-U.S. Citizen

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PI/PD Name: David Jenkins

Gender: ☒ Male ☐ Female

Ethnicity: (Choose one response) ☐ Hispanic or Latino ☒ Not Hispanic or Latino

Race:
(Select one or more)

- ☐ American Indian or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Native Hawaiian or Other Pacific Islander
- ☒ White

Disability Status:
(Select one or more)

- ☐ Hearing Impairment
- ☐ Visual Impairment
- ☐ Mobility/Orthopedic Impairment
- ☐ Other
- ☒ None

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COVER SHEET FOR PROPOSAL TO THE NATIONAL SCIENCE FOUNDATION

PROGRAM ANNOUNCEMENT/SOLICITATION NO./CLOSING DATE/if not in response to a program announcement/solicitation enter NSF 00-2

NSF 00-49 05/15/00

FOR NSF USE ONLY

NSF PROPOSAL NUMBER

FOR CONSIDERATION BY NSF ORGANIZATION UNIT(S) (Indicate the most specific unit known, i.e. program, division, etc.)

ENG - Directorate for Engineering

DATE RECEIVED	NUMBER OF COPIES	DIVISION ASSIGNED	FUND CODE	DUNS# (Data Universal Numbering System)	FILE LOCATION
				153881537	

EMPLOYER IDENTIFICATION NUMBER (EIN) OR TAXPAYER IDENTIFICATION NUMBER (TIN): 946002123

SHOW PREVIOUS AWARD NO. IF THIS IS
☐ A RENEWAL
☐ AN ACCOMPLISHMENT-BASED RENEWAL

IS THIS PROPOSAL BEING SUBMITTED TO ANOTHER FEDERAL AGENCY? YES ☐ NO ☒ IF YES, LIST ACRONYMS(S)

NAME OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE: University of California-Berkeley

ADDRESS OF AWARDEE ORGANIZATION, INCLUDING 9 DIGIT ZIP CODE:
University of California-Berkeley
336 Sproul Hall
Berkeley, CA. 947205940

AWARDEE ORGANIZATION CODE (IF KNOWN): 0013128000

NAME OF PERFORMING ORGANIZATION, IF DIFFERENT FROM ABOVE

ADDRESS OF PERFORMING ORGANIZATION, IF DIFFERENT, INCLUDING 9 DIGIT ZIP CODE

PERFORMING ORGANIZATION CODE (IF KNOWN)

IS AWARDEE ORGANIZATION (Check All That Apply) (See GPG II.D.1 For Definitions) ☐ FOR-PROFIT ORGANIZATION ☐ SMALL BUSINESS ☐ MINORITY BUSINESS ☐ WOMAN-OWNED BUSINESS

TITLE OF PROPOSED PROJECT: DNA arrays for assessing pollutant removing potential of environmental systems

REQUESTED AMOUNT	PROPOSED DURATION (1-60 MONTHS)	REQUESTED STARTING DATE	SHOW RELATED PREPROPOSAL NO., IF APPLICABLE
$ 198,356	24 months	09/01/00	

CHECK APPROPRIATE BOX(ES) IF THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW

☐ BEGINNING INVESTIGATOR (GPG 1.A.3)
☐ DISCLOSURE OF LOBBYING ACTIVITIES (GPG II.D.1)
☐ PROPRIETARY & PRIVILEGED INFORMATION (GPG II.D.10)
☐ NATIONAL ENVIRONMENTAL POLICY ACT (GPG II.D.10)
☐ HISTORIC PLACES (GPG II.D.10)
☐ SMALL GRANT FOR EXPLOR. RESEARCH (SGER) (GPG II.D.12)
☐ VERTEBRATE ANIMALS (GPG II.D.12) IACUC App. Date ____
☐ HUMAN SUBJECTS (GPG II.D.12)
Exemption Subsection ____ or IRB App. Date ____
☐ INTERNATIONAL COOPERATIVE ACTIVITIES: COUNTRY/COUNTRIES ____
☐ FACILITATION FOR SCIENTISTS/ENGINEERS WITH DISABILITIES (GPG V.G.)
☐ RESEARCH OPPORTUNITY AWARD (GPG V.H)

PI/PD DEPARTMENT: Department of Chemical Engineering

PI/PD POSTAL ADDRESS:
116 Gilman Hall
MC 1462
Berkeley, CA 947201462
United States

PI/PD FAX NUMBER: 510-643-1228

NAMES (TYPED)	High Degree	Yr of Degree	Telephone Number	Electronic Mail Address
PI/PD NAME: Jay D Keasling	Ph.D.	1991	510-642-4862	keasling@socrates.berkeley.edu
CO-PI/PD: Adam Arkin	Ph.D	1992	510-495-2366	aparkin@uclink4.berkeley.edu
CO-PI/PD: David Jenkins		1960		jenkins@ce.berkeley.edu
CO-PI/PD				
CO-PI/PD				

CERTIFICATION PAGE

Certification for Principal Investigators and Co-Principal Investigators:

I certify to the best of my knowledge that:

(1) the statements herein (excluding scientific hypotheses and scientific opinions) are true and complete, and
(2) the text and graphics herein as well as any accompanying publications or other documents, unless otherwise indicated, are the original work of the signatories or individuals working under their supervision. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if an award is made as a result of this proposal.

I understand that the willful provision of false information or concealing a material fact in this proposal or any other communication submitted to NSF is a criminal offense (U.S.Code, Title 18, Section 1001).

Name (Typed)	Signature	Social Security No.*	Date
PI/PD Jay D Keasling			
Co-PI/PD Adam Arkin			
Co-PI/PD David Jenkins			
Co-PI/PD			
Co-PI/PD			

SSNs are confidential and are not displayed
ON FASTLANE SUBMISSIONS

Certification for Authorized Organizational Representative or Individual Applicant:

By signing and submitting this proposal, the individual applicant or the authorized official of the applicant institution is: (1) certifying that statements made herein are true and complete to the best of his/her knowledge; and (2) agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this application. Further, the applicant is hereby providing certifications regarding Federal debt status, debarment and suspension, drug-free workplace, and lobbying activities (see below), as set forth in Grant Proposal Guide (GPG), NSF 00-2. Willful provision of false information in this application and its supporting documents or in reports required under an ensuring award is a criminal offense (U. S. Code, Title 18, Section 1001).

In addition, if the applicant institution employs more than fifty persons, the authorized official of the applicant institution is certifying that the institution has implemented a written and enforced conflict of interest policy that is consistent with the provisions of Grant Policy Manual Section 510; that to the best of his/her knowledge, all financial disclosures required by that conflict of interest policy have been made; and that all identified conflicts of interest will have been satisfactorily managed, reduced or eliminated prior to the institution's expenditure of any funds under the award, in accordance with the institution's conflict of interest policy. Conflict which cannot be satisfactorily managed, reduced or eliminated must be disclosed to NSF.

Debt and Debarment Certifications

(If answer "yes" to either, please provide explanation.)

Is the organization delinquent on any Federal debt? Yes ☐ No ☒

Is the organization or its principals presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency? Yes ☐ No ☒

Certification Regarding Lobbying

This certification is required for an award of a Federal contract, grant, or cooperative agreement exceeding $100,000 and for an award of a Federal loan or a commitment providing for the United States to insure or guarantee a loan exceeding $150,000.

Certification for Contracts, Grants, Loans and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, ''Disclosure Form to Report Lobbying,'' in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

AUTHORIZED ORGANIZATIONAL REPRESENTATIVE	SIGNATURE	DATE
NAME/TITLE (TYPED) Patricia Gates		

TELEPHONE NUMBER	ELECTRONIC MAIL ADDRESS	FAX NUMBER
510-642-8109	pgates@uclink.berkeley.edu	510-642-8236

*SUBMISSION OF SOCIAL SECURITY NUMBERS IS VOLUNTARY AND WILL NOT AFFECT THE ORGANIZATION'S ELIGIBILITY FOR AN AWARD. HOWEVER, THEY ARE AN INTEGRAL PART OF THE INFORMATION SYSTEM AND ASSIST IN PROCESSING THE PROPOSAL. SSN SOLICITED UNDER NSF ACT OF 1950, AS AMENDED.

PROJECT SUMMARY

The treatment of liquid and solid wastes of domestic and industrial origin and the clean up of polluted ground and water bodies relies heavily on the activities of microorganisms. Many of the metabolically important microorganisms (or consortia) responsible for mediating pollutant transformations are currently unidentified, in large measure, because of system complexity and for the reason of their inability to grow outside of their natural habitats in laboratory cultures. It is important to determine the identity and population density of microorganisms significant to pollutant transformation reactions to allow the reliable design of treatment processes and an accurate assessment of their transformation rates, and of factors that affect these.

In the past, researchers have relied on indirect microbiological techniques such as pure culture isolation and most probable number estimates to detect and enumerate microorganisms in environmental cultures. These culture-based methods often provide an inaccurate description of community composition because they rely on indirect measurement of often-variable physiological properties. Newly developed molecular techniques based on comparative sequence analysis of DNA and RNA hold the promise of providing tools to develop direct methods for environmental community characterization. Several recent studies have employed ribosomal RNA (rRNA)-based methods to analyze activated sludge communities. However, the techniques employed in these studies are time consuming and expensive. The high-density DNA arrays recently developed for high-throughput measurement of gene expression may allow rapid assessment of microbial populations in various environments. The application of DNA array technology to activated sludge and contaminated environments will be a major advance towards the development of marketable kits for diagnostic analyses of pollutant transforming communities.

We propose to lay the groundwork for the development of DNA array technology for the rapid detection of microorganisms in pollutant transforming cultures and the quantitative measurement of their activities. Specifically, we will (1) develop targets for DNA oligonucleotide probes using small and large subunit rDNA genes and transcribed spacer regions between those genes; (2) fabricate macroarrays of oligonucleotide probes for microorganisms present in a laboratory-scale wastewater treatment reactor performing enhanced biological phosphorus removal and in a constructed co-culture that degrades *p*-cresol and 2-chloroethanol; (3) calibrate the DNA array by performing quality control tests: optimizing target preparation and hybridization conditions, calibrating signals, and optimizing data analysis; and (4) use the DNA array to assess changes in community structure and metabolic activity as a function of changes in environmental conditions. Given the short time scale and the high-risk nature of the proposed work, we have chosen to test and apply the array technology to two systems with which we have significant experience. The first system is a mixed culture enriched from a wastewater treatment plant that performs enhanced biological phosphorus removal. The second system is a defined dual-species culture that degrades *p*-cresol and 2-chloroethanol.

Many areas of microbial ecology and environmental assessment and treatment would benefit greatly from well-developed and standardized array methods. Besides their use in community structure profiling, array technology could be used to assess functional activity in various populations of microbes and provide significantly more information about the capacity of a given community to effectively process pollutants than available by current methods. For example, "activity arrays" designed to detect important metabolic genes would be extremely useful as diagnostic tools and for industrial wastewater "treatability" assessment. In addition, these arrays could be used to track the spread of antibiotic resistance genes (or of any recombinant gene) in natural and engineered systems. Finally, our understanding of the microbial world is embarrassingly undeveloped, largely because adequate tools to study micro-scale ecosystems have not been available until very recently. The array technologies proposed here will eventually lead to high-density arrays for assessing extant microbial diversity.

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For font size and page formatting specifications, see GPG section II.C.

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B	Table of Contents (NSF Form 1359)	1	
C	Project Description (plus Results from Prior NSF Support) (not to exceed 15 pages) **(Exceed only if allowed by a specific program announcement/solicitation or if approved in advance by the appropriate NSF Assistant Director or designee)**	15	
D	References Cited	5	
E	Biographical Sketches (Not to exceed 2 pages each)	6	
F	Budget (NSF Form 1030, plus up to 3 pages of budget justification)	4	
G	Current and Pending Support (NSF Form 1239)	6	
H	Facilities, Equipment and Other Resources (NSF Form 1363)	1	
I	Special Information/Supplementary Documentation	0	
J	Appendix (List below.) **(Include only if allowed by a specific program announcement/ solicitation or if approved in advance by the appropriate NSF Assistant Director or designee)**		

Appendix Items:

*Proposers may select any numbering mechanism for the proposal. The entire proposal however, must be paginated. Complete both columns only if the proposal is numbered consecutively.

PROJECT DESCRIPTION

INTRODUCTION

The treatment of liquid and solid wastes of domestic and industrial origin and the clean up of polluted ground and water bodies relies heavily on the activities of microorganisms. Many of the metabolically important microorganisms (or consortia) responsible for mediating pollutant transformations are currently unidentified, in large measure, because of system complexity and for the reason of their inability to grow outside of their natural habitats in laboratory cultures. It is important to determine the identity and population density of microorganisms significant to pollutant transformation reactions to allow the reliable design of treatment processes and an accurate assessment of their transformation rates, and of factors that affect these.

In the past, researchers have relied on indirect microbiological techniques such as pure culture isolation and most probable number estimates to detect and enumerate microorganisms in environmental cultures. These culture-based methods often provide an inaccurate description of community composition because they rely on indirect measurement of often-variable physiological properties. Newly developed molecular techniques based on comparative sequence analysis of DNA and RNA hold the promise of providing tools to develop direct methods for environmental community characterization. Several recent studies have employed ribosomal RNA (rRNA)-based methods to analyze activated sludge communities (6, 14, 32, 35, 38, 57). However, the techniques employed in these studies are time consuming and expensive. The high-density DNA arrays recently developed for high-throughput measurement of gene expression may allow rapid assessment of microbial populations in various environments. The application of DNA array technology to activated sludge and contaminated environments will be a major advance towards the development of marketable kits for diagnostic analyses of pollutant transforming communities.

GOALS

We propose to lay the groundwork for the development of DNA array technology for the rapid detection of microorganisms in pollutant transforming cultures and the quantitative measurement of their activities. Specifically, we will:

1) develop targets for DNA oligonucleotide probes using small and large subunit rDNA genes and transcribed spacer regions between those genes;
2) fabricate macroarrays of oligonucleotide probes for microorganisms present in a laboratory-scale wastewater treatment reactor performing enhanced biological phosphorus removal and in a constructed co-culture that degrades *p*-cresol and 2-chloroethanol;
3) calibrate the DNA array by performing quality control tests: optimizing target preparation and hybridization conditions, calibrating signals, and optimizing data analysis;
4) use the DNA array to assess changes in community structure and metabolic activity as a function of changes in environmental conditions.

Given the short time scale and the high-risk nature of the proposed work, we have chosen to test and apply the array technology to two systems with which we have significant experience. The first system is a mixed culture enriched from a wastewater treatment plant that performs enhanced biological phosphorus removal. The second system is a defined dual-species culture that degrades *p*-cresol and 2-chloroethanol.

SIGNIFICANCE

Many areas of microbial ecology and environmental assessment and treatment would benefit greatly from well-developed and standardized array methods. Besides their use in community structure profiling, array technology could be used to assess functional activity in various populations of microbes and provide significantly more information about the capacity of a given community to effectively process pollutants than available by current methods. For example, "activity arrays" designed to detect important metabolic genes would be extremely useful as diagnostic tools and for industrial wastewater "treatability" assessment. In addition, these arrays could be used to track the spread of antibiotic resistance genes (or of any recombinant gene) in natural and engineered systems. Finally, our understanding of the microbial world is embarrassingly undeveloped, largely because adequate tools to study micro-scale ecosystems have not been available until very recently (42). The array technologies proposed here will eventually lead to high-density arrays for assessing extant microbial diversity.

BACKGROUND

MICROBIAL COMMUNITIES IN POLLUTANT TRANSFORMATION

The treatment of liquid and solid wastes of domestic and industrial origin and the clean up of polluted ground and water bodies relies heavily on the activities of microorganisms. The diversity of activities is staggering – from the suicide metabolism of methanotrophs oxidizing chlorinated hydrocarbons during bioremediation, to the chemolithotrophic oxidation of ammonia to nitrate by nitrifying bacterial consortia in activated sludge, to the strict syntrophy of interspecies hydrogen transfer in anaerobic food webs. Many of the metabolically important microorganisms (or consortia) responsible for mediating pollutant transformations are currently un-identified, in large measure because of system complexity, and for the reason of their inability to grow outside of their natural habitats in laboratory cultures. It is important to determine the identity and population density of microorganisms important in pollutant transformation reactions. This type of information will allow the reliable design of treatment processes and an accurate assessment of their transformation rates, and of factors that affect these.

Two areas where understanding the numbers and types of organisms present could significantly impact a process are biological nutrient removal and bioremediation of toxic pollutants. Both of these remediation processes have significant economic impacts on society and would greatly benefit from a tool to measure the types and number of organisms present.

1) **Biological nutrient removal.** In wastewater treatment parlance, biological nutrient generally refers to the removal of the various forms of nitrogen (N) and phosphorus (P) utilizing the relatively uncontrolled microbial cultures that develop in secondary treatment processes (e.g., activated sludge). Nutrient removal is required for a variety of reasons such as the eutrophication of inland and marine waters, the toxicity of ammonia to many receiving water biota, and the oxygen demand placed on receiving waters by ammonia oxidation and algal respiration requirements. The latter two can lead to anoxia with its accompanying elimination of aerobic biota, production of odorous anoxic metabolites, and release of sediment bound metals.

   A sustainable P removal treatment method is the so-called enhanced biological phosphorus removal (EBPR) process, in which anaerobic/aerobic cycling of the biomass in an activated sludge plant produces microorganisms that can store inorganic polyphosphate. Because this elevates biomass P content, it results in greater than normal P removal per unit amount of biomass formed. Microorganisms that perform this metabolism have never been isolated and were only recently characterized using cultivation independent molecular phylogenetic techniques. A knowledge of the organisms' intrinsic activity will be useful in determining the reasons for the frequently

observed and unexplained upsets/failures of EBPR in prototype plants. It will also be useful as an early warning detection method for potential process upset conditions.

Biological nitrogen removal consists of ammonification of organic nitrogen, then nitrification of the ammonia not used for microbial growth, by a two-step oxidation, first to nitrite and then to nitrate using molecular oxygen as the electron acceptor. The culture is recycled through anoxic conditions where the nitrate is removed by reducing it to the sparingly soluble N_2 gas using the biodegradable carbon in the wastewater as the reductant. The limiting step of the sequence is nitrification, and wastewater treatment plants that must nitrify are usually overdesigned considerably because of the uncertainty in predicting nitrification rates in wastewater. Indeed, Parker has estimated that some $2 billion of extra capacity is designed into existing activated sludge plants in the USA for this reason (43).

2) **Bioremediation of toxic pollutants.** Most biodegradative transformations result from the concerted effort of multispecies microbial consortia (5, 56) acting on single pollutants and mixed wastes in both manmade and natural systems. The species within such consortia can interact in a variety of ways, including competing for common substrates, forming syntrophic chains where different species are responsible for carrying out sections of a long degradative pathway, and mutualistic interactions where one species provides growth factors or removes inhibitory substances to benefit another species. For example, reductive dehalogenation of a variety of chlorinated pollutants (PCBs, HCB, PCE, PCP, etc.) can often only be performed by mutualistic anaerobic consortia: a fermentative organism provides the reducing equivalents and the degrading organism synthesizes a necessary cofactor for survival of the fermentative organism (41). Most environments that are contaminated have not just one, but several, different pollutant present. Unfortunately, one contaminant may be a carbon/energy source for a particular organism but very toxic to another organism that is responsible for degradation of a third contaminant. To better design remediation strategies, it is important to understand how individual contaminant(s) affect the growth of various organisms in the environment and whether specific degradative microorganisms are present in a contaminated site.

We have chosen one example from each of these areas as test cases for the DNA array.

MOLECULAR MICROBIAL ECOLOGY

The advent of molecular tools has revolutionized the fields of microbial ecology and applied microbiology. These tools have several advantages over traditional culture based techniques (e.g., pure culture isolation, selective enrichments, and most-probable-number estimates), which have classically been used to characterize microbial community structure and are known provide a biased measure of microbial diversity (27). Methods based on nucleic acid (both DNA and RNA) sequence comparison allow for the direct identification and enumeration of microbes in complex environments and therefore avoid distortions created by the selective forces of the culture-based methods. Specifically, comparative sequence analysis of the small subunit ribosomal RNA (SSU rRNA) gene has been used extensively for the characterization of microbial diversity in natural and engineered systems, including activated sludge (2).

With the development of the polymerase chain reaction (PCR) and automated sequencing, SSU rDNAs have been retrieved, sequenced, and cataloged en masse in recent years. However, PCR based methods are not quantitative because of factors such as amplification bias, cloning and sequencing artifacts, and gene copy number bias (25, 44, 70). Hence, oligonucleotide probes targeting the SSU rRNA have been used mostly for quantitative studies of population abundance and community structure (47, 59, 60). Probes can be used to detect either rRNA in whole cells using fluorescent *in situ* hybridization (FISH) and epifluorescent microscopy (3) or community rRNA extracted from a biomass sample and immobilized on a solid support using quantitative

"dot" or "slot blot" hybridization (47). Both applications have been used to quantitatively characterize microbial communities in biological waste treatment systems and link structure with performance (2, 13, 14, 21)

ARRAY TECHNOLOGY

Nucleic acid blotting techniques have been used in molecular biology for decades. Indeed, as described above, dot blot hybridization with rRNA-targeted probes is a popular method for quantitative microbial ecology. However, blotting methods are notoriously time-consuming and not very amenable to wide application in the waste treatment industry. Array technology holds the promise of combining the power of molecular probes with streamlined, standardized and packagable protocols. The recent explosion of interest by molecular biologists in array technologies is linked to two important advances (33). First, the use of non-porous supports, such as glass, has allowed miniaturization and the use of fluorescent markers, allowing rapid analysis of small quantities of sample without the need for radioactive labels. Second, the development of methods for the *in situ* synthesis of high-density oligonucleotides has promoted the use of combinatorial and massively parallel approaches to sequence analysis. Simply stated, it is now possible in a matter of hours to measure the presence or absence of hundreds to thousands of genes (or gene products) in a biological sample.

Currently, DNA arrays, also known as "DNA microchips", are primarily used by biologists studying expression profiling of organisms at the genome scale, by geneticists searching for signatures ("polymorphisms") in gene families correlating with phenotypic anomalies, and by pharmaceutical companies investigating drug development. DNA macroarrays (low density arrays of DNA oligonucleotides attached to membranes) have also been used to analyze changes in gene expression caused by changes in culture conditions (62). Thus, the extension of these techniques to quantitative and determinative microbial ecology is a natural one.

Array fabrication. The first step in array fabrication is to select the "probes" that will be robotically printed on the array (17). In general, one of two formats is chosen: long probes or short probes. Current technology requires that long probes be pre-synthesized and spotted by either using pins that rely on contact with the support, or non-contact piezoelectric devices. "Traditional" cDNA array technology developed by Pat Brown's laboratory at Stanford University uses quill-like pins and a robot that can be assembled with off-the-shelf parts. Short probes may be synthesized *in situ* using photolithography or ink-jet delivery of nucleotide precursors to the surface (58); they can also be pre-synthesized and spotted using traditional methods.

The selection of appropriate probes for detection and quantification of microbial populations will be critical to the success of the proposed application. Only one previous report on the use of arrays for microbial community characterization could be found in the literature (23). Guschin and co-workers attempted to use oligonucleotide probes previously designed for FISH to rRNA, but identified several problems that need to be addressed before this technique can be applied quantitatively. These problems stem from the fact that two different oligo sequences will bind target nucleic acid quite differently, resulting in widely different signals from what should be equal quantities of two targets. Similar observations have been made using orthogonal sets of oligos targeting the same gene. This is a challenge that will have to be overcome before the proposed technology can be successfully developed.

Target preparation and labeling. The preparation of target nucleic acids appears to be one of the largest obstacles to the application of microchip technology to quantitative microbial ecology. Labeling must be efficient and reproducible, and it must not interfere with quantification by producing uncontrolled bias. In addition, the purity of the nucleic acid target is critical to hybridization performance, since cellular lipids, proteins, and carbohydrates can cause significant non-specific binding to slide surfaces (17).

A potential limitation of the quantitative application of microarray technology is the lack of a reliable method for labeling target nucleic acids. In current protocols used in biological

laboratories studying gene expression, mRNAs are extracted, reverse transcribed to make cDNA, and the cDNA is enzymatically labeled with a fluorescent molecule ("fluor") (17). Sometimes mRNA is chemically biotinylated directly (15). When genomic DNA from eukaryotes is used for sequence polymorphism detection, an amplification step is often required (33), possibly making quantification impossible. Efforts have been made to improve direct chemical labeling techniques utilizing fluors with attached hydrazine groups that can couple to aldehyde groups on partially depurinated DNA or oxidized 3'-terminal ribonucleosides on RNA (46). These methods can be optimized to produce the maximum yield of labeled product (around ~60%).

New chemically based labeling techniques have been developed for DNA, and will soon be available for RNA. These methods are based on platinum chemistry developed by Kreatech Diagnostics and marketed by Molecular Probes in kit form. The fluorophore reagent in the kits reacts with the N_7 of guanine residues to form a stable coordination complex and the labeled probe can then be purified with a simple spin column. This labeling technique is faster and simpler than the hydrazine/aldehyde chemistry and could potentially be optimized for labeling of nucleic acids extracted from pollutant-degrading cultures.

Hybridization. Hybridization conditions are critical for quantitative assessment of the sample of interest. For DNA microarrays, hybridization is carried out in small chambers that hold a microscope slide. Because the chamber is small, less sample is needed than for DNA macroarrays and the sample may be concentrated more. For DNA macroarrays on membranes, hybridization is carried out in conventional systems for used for Northern, Southern, and dot blots, i.e., roller bottles. Determining the hybridization condition that achieves the most uniform hybridization of all probes to their respective targets will be an iterative process of design as described below.

Detection. Detection is the next step in the array technology. Several companies offer high-quality fluorescence detection instruments for imaging microarrays. Most of the systems use one or more lasers and filter sets to provide detection of a wide range of fluors. Spatial resolution is one of the most important parameters in microarray detection (48). Because of the high-density of oligonucleotides in microarrays, the spatial resolution of the detector must be very small.

Because macroarrays have significantly lower density oligonucleotide packing, the detector need not have the resolution required for microarrays. In this case, phosphoimagers and fluorimagers developed for Northern, Southern, and dot blots typically have resolution great enough to image fairly large macroarrays. The latest imagers are equipped with multiple lasers and filter sets to allow simultaneous imaging of multiple fluors.

Data analysis. Data analysis is the last step in the life cycle of an array. Micro- and macroarrays generate enormous amounts of data. Fortunately, many detector manufactures supply software for analyzing the data from high-density microarrays. The software must have the capability to average over the area of the spot containing the oligonucleotide probe and to compare the average intensities in each of the spots. Additionally, the software must be able to cluster those spots (corresponding to genes, or in our case, organisms) that respond similarly to a particular environmental change.

RESULTS FROM PRIOR NSF SUPPORT

The PIs are involved in several NSF grants. The work performed under one of these grants (BES-9612840) relates directly to the work proposed herein, so the results of this work are presented below. The research funded under this grant investigated the following three major areas related to EBPR: characterization of the nature and metabolic activity in activated sludge microorganisms that carry out EBPR; characterization of the enzymes and genetic systems associated with polyphosphate and polyhydroxyalkanoate accumulation in model organisms; and establishment of process-related stoichiometry and kinetics. The details follow.

TITLE	Mechanism of Enhanced Biological Phosphorus Removal
AWARD NUMBER	BES-9612840
P.I.	David Jenkins & Jay D. Keasling
AMOUNT	$413,077
PERIOD	04/15/97 - 04/14/00

HUMAN RESOURCES

Ilana Aldor is a fourth-year PhD student in the Department of Chemical Engineering. Ilana is currently reconstituting the PHB degradation pathways.

Douglas Bolesch was a PhD student in the Department of Chemical Engineering. Doug received his PhD in 1997 and is currently working for Chiron. Doug studied the enzymes involved in PP metabolism.

Willie Harper is a third-year graduate student in the Department of Civil and Environmental Engineering. Willie is currently working on the use of EBPR to treat nutrient-deficient wastewater.

Katherine McMahon is a second-year PhD student in the Department of Civil and Environmental Engineering. Katherine is currently studying microbial community structure of EBPR.

Jaya Pramanik was a PhD student in the Department of Chemical Engineering. Jaya received her PhD in August 1997 and is currently working for IBM. Jaya developed the flux-based model of EBPR metabolism.

Andrew Schuler was a graduate student in the Department of Civil and Environmental Engineering. He received the CH_2M-Hill/AEESP Dissertation Award for this PhD thesis. Following a one-year post-doctoral fellowship in Prof. T. Mino's lab at the University of Toyko, Andy was appointed Assistant Professor of Civil and Environmental Engineering at Duke University. He studied the effect of influent P:COD ratios on EBPR.

Piper Trelstad is a fifth-year PhD student in the Department of Chemical Engineering and will graduate in August 2000. Piper is currently determining enzyme activity and control of gene expression in *Acinetobacter*. When she completes her Ph.D., she will work at Merck.

Steve Van Dien was a graduate student in the Department of Chemical Engineering. Steve received his PhD in May 1998 and is currently a post-doctoral fellow with Professor Mary Lidstrom at the University of Washington. Steve studied PP metabolism in genetically-engineered *Escherichia coli*.

NEW COURSES DEVELOPED

David Jenkins and Jay Keasling developed a new course on Bioflocculation and Biofilms for graduate students. The class met once per week to discuss recent literature and to hear from guest lecturers. The following lectures were given: (1) introduction to bioflocculation in wastewater treatment plants; (2) troubleshooting activated sludge bioflocculation performance; (3) visualizing biofilms colonization; (4) genetics and metabolism of bacterial alginates; (5) importance of divalent metal cations to bioflocculation; and (6) bioflocculation processes in biofilms.

PUBLICATIONS RESULTING FROM THIS AWARD

1) **Keasling, J. D.** 1997. Regulation of intracellular toxic metals and other cations by hydrolysis of polyphosphate. Ann. N. Y. Acad. Sci. **829:**242-249.
2) **Keasling, J. D., S. J. Van Dien, and J. Pramanik.** 1997. Engineering polyphosphate metabolism in *Escherichia coli:* implications for bioremediation of inorganic contaminants. Biotechnol. Bioeng. **58:**231-239.
3) **Schuler, A. J., and D. Jenkins.** 1997. The effect of varying activated sludge phosphate content on the enhanced biological phosphorus removal metabolism. Presented at the Water Environment Federation 70th Annual Conference and Exposition, Chicago, IL. pp.

4) **Van Dien, S. J., S. Keyhani, C. Yang, and J. D. Keasling.** 1997. Manipulation of independent synthesis and degradation of polyphosphate in *Escherichia coli* for investigation of phosphate secretion from the cell. Appl. Environ. Microbiol. **63**(5)**:**1689-1695.
5) **Pramanik, J., P. L. Trelstad, and J. D. Keasling.** 1999. A flux-based stoichiometric model of enhanced biological phosphorus removal metabolism. Wat. Sci. Tech. **37**(4-5)**:**609-613.
6) **Pramanik, J., P. L. Trelstad, A. J. Schuler, D. Jenkins, and J. D. Keasling.** 1998. Development and validation of a flux-based stoichiometric model for enhanced biological phosphorus removal metabolism. Water Research. **33**(2)**:**462-476.
7) **Van Dien, S. J., and J. D. Keasling.** 1998. Control of polyphosphate metabolism in genetically engineered *Escherichia coli*. Enz. Microbiol. Technol. **24**:21-25.
8) **Van Dien, S. J., and J. D. Keasling.** 1998. Optimization of polyphosphate degradation and phosphate secretion using hybrid metabolic pathways and engineered host strains. Biotechnol. Bioeng. **59**(6)**:**754-761.
9) **Van Dien, S. J., and J. D. Keasling.** 1998. A dynamic model of the *Escherichia coli* phosphate-starvation response. J. Theor. Biol. **190:**37-49.
10) **Trelstad, P. L., P. Purdhani, W. Geissdorfer, W. Hillen, and J. D. Keasling.** 1999. Polyphosphate kinase *of Acinetobac*ter sp. Strain ADP1: purification and characterization of the enzyme and its role during changes in extracellular phosphate. Appl. Environ. Microbiol. **65**(9):3780-3786.
11) **Van Dien, S. J., and J. D. Keasling.** 1999. Effect of polyphosphate metabolism on the *Escherichia coli* phosphate-starvation response. Biotech. Prog. **15**(4)**:**587-593.
12) **Keasling, J. D.** 1999. Tools for metabolic engineering of bacteria. Trends in Biotechnology **17**:452-460.
13) **Bolesch, D. G., and J. D. Keasling.** 1999. The effect of anions and cations on equilibrium binding of polyphosphate by *Escherichia coli* exopolyphosphatase. J. Biol. Chem. Submitted.
14) **Bolesch, D. G., and J. D. Keasling.** 1999. Polyphosphate binding of *Escherichia coli* exopolyphosphatase. Biochemistry. Submitted.
15) **Crocetti, G. R., P. Hugenholtz, P. L. Bond, A. Schuler, J. Keller, D. Jenkins, and L. L. Blackall.** 2000. Identification of polyphosphate accumulating organisms and the design of 16S rRNA-directed probes for their detection and quantitation. Applied and Environmental Microbiology **66**(3)**:**1175-1182.
16) **Schuler, A. J., D. Jenkins, and P. Ronen.** 2000. Microbial storage products, biomass density, and settling properties of enhanced biological phosphorus removal activated sludge. Presented at the 1st World Congress of the Intl. Water Assn., Paris, France.
17) **Schuler, A. J. and D. Jenkins.** 2000. The effect of transient upsets on enhanced biological phosphorus removal in sequencing batch reactors. Presented at the Intl. Water Assn. Conf. on SBRs, Narbonne, France.
18) **Schuler, A. J. and D. Jenkins.** 2000. Enhanced biological phosphorus removal from wastewater by biomass with different phosphorus contents, Part I: experimental methods and results. Submitted to Water Environ. Res.
19) **Schuler, A. J. and D. Jenkins.** 2000. Enhanced biological phosphorus removal from wastewater by biomass with different phosphorus contents, Part II: anaerobic ATP utilization and acetate uptake rates. Submitted to Water Environ. Res.
20) **Schuler, A. J. and D. Jenkins.** 2000. Enhanced biological phosphorus removal from wastewater by biomass with different phosphorus contents, Part III: anaerobic sources of reducing equivalents. Submitted to Water Environ. Res.
21) **McMahon, K. D., N. R. Pace, D. Jenkins, and J. D. Keasling.** 1999. Microbial community structure in laboratory scale enhanced biological phosphorus removal systems. In preparation.

AWARD FOR RESEARCH SUPPORTED BY NSF

In October 1999, Andrew J. Schuler and David Jenkins received the Association of Environmental Engineering and Science Professors Annual Award for the best doctoral

dissertation in wastewater treatment research for the 1998-1999 academic year for the Ph.D. dissertation of Andrew J. Schuler.

RESULTS

See section in *Preliminary Work* devoted to EBPR.

PRELIMINARY WORK

As mentioned in the introduction, we will develop DNA macroarrays to assess microbial communities involved in pollutant degradation/removal. We will focus our development work on two microbial communities with which we have significant experience: the first community is a mixed culture enriched from a wastewater treatment plant that performs enhanced biological phosphorus removal and the second is a defined dual-species culture that degrades *p*-cresol and 2-chloroethanol.

ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL

EBPR metabolism in laboratory scale SBRs. Lab-scale sequencing batch reactor (SBR) experiments using an acetate-, Casamino acids-, mineral salts-feed were conducted at a wide range of influent P:COD ratios on a culture derived from City of San Francisco, CA activated sludge. These cultures are described in more detail in Schuler et al (50-53). This enrichment culture, which we continue to maintain in our laboratory for ongoing EBPR research, will serve as the primary test of the DNA arrays proposed herein.

Attempts to obtain a pure culture. Initially, we attempted to isolate polyphosphate-accumulating microorganisms (PAMs) in pure culture. Biomass from a SBR carrying out EBPR was homogenized, serially diluted, and spread-plated onto solid medium made from autoclaved SBR effluent and 2% agar. Plates were incubated at room temperature for 5 – 10 days until colonies appeared. Single colonies were further purified on King's B and/or TSA medium (31) for typing with the BIOLOG system. The most commonly retrieved isolates were identified as members of the γ subclass of the Proteobacteria. Close relatives of the recently identified *Rhodocyclus*-like PAM (i.e., members of the β subclass of the Proteobacteria) were not obtained. These results are consistent with previous studies that document the bias of traditional culturing techniques (29, 54, 65, 66). After several attempts we abandoned these techniques in favor of the SSU rDNA clone library technique described below.

Characterization of microbial communities in laboratory-scale SBRs. We have conducted preliminary work to characterize the microbial community structure in both PAM- and glycogen accumulating microorganism (GAM)-dominated lab-scale SBR cultures. Through collaborations with Norman Pace at UC Berkeley and Linda Blackall at the University of Queensland, Australia, we constructed SSU rDNA clone libraries using Bacteria-specific PCR primers and 3 biomass samples corresponding to low P_x (LP), high P_x (HP), and very high P_x (VHP) (**Table 1**). Two additional libraries were constructed using LP and HP biomass, and "universal" primers to help eliminate bias of PCR primer specificity (70). Approximately 100 clones from each library were screened by restriction fragment length polymorphisms (RFLP) and representatives of each RFLP type were sequenced. Based on comparison to public databases using BLAST Version 2.0 (1) and the ARB software package (61), sequence types were assigned to commonly recognized phyla. The distribution of assigned sequence types is presented in Table 1 for libraries generated with Bacteria-specific primers. Libraries constructed with "universal" primers did not differ significantly. Sequence types corresponding to organisms closely related to *Rhodocyclus* spp. were correlated with high P_x and the characteristics of a PAM-dominated sludge. Sequences associated with the Actinobacteria (high GC Gram-positive (HGCGP) bacteria) were not observed in appreciable numbers in any of the libraries. These results contradict those of previous studies which suggested that HGCGP bacteria may play major roles in EBPR (11, 29, 30, 66) but they are consistent with those reported by researchers operating similar lab-scale SBRs (6, 26).

Table 1. Distribution of sequence types in clone libraries constructed with Bacteria-specific PCR primers and their phylogenetic associations.

Phylogenetic group[a]	% in library[b]		
	1 % P_x LP	16% P_x HP	25% P_x VHP
α Proteobacteria (subclass) [c]	**18**	**47**	**6**
Rhodobacter group	4	22	5
Rhizobiaceae group	4	15	0
Other α Proteobacteria	9	10	1
β Proteobacteria (subclass)	**63**	**30**	**58**
Rhodocyclus spp.	0	22	47
Zoogloea spp.[d]	52	0	9
Acidovorax spp.	12	9	0
γ Proteobacteria (subclass)	**5**	**4**	**4**
Cytophagales (Division)	**7**	**16**	**29**
Actinobacteria (Division)	**5**	**1**	**1**
Others and those unaffiliated with currently defined Divisions	**1**	**1**	**2**

[a] Sequences less than 96% identical to a cultivated genus were assigned to a phylogenetic group as defined by the NCBI Taxonomy database , and those less than 93% identical to a cultivated organism were assigned only to a putative Bacterial Division (27) or subclass.
[b] Values do not add up to 100% because of rounding.
[c] Totals for the subclasses of the Proteobacteria and Divisions are shown in bold.
[d] *Zoogloea* is not a phylogenetically coherent genus. Our clone sequences were most closely related to *Zoogloea ramigera* strain ATCC 19324, which is associated with the Rhodocyclus group in the β Proteobacteria (55)

We used fluorescent *in situ* hybridization (FISH) to verify the community structure inferred from sequence types present in the SSU rDNA clone libraries and to quantify key populations carrying out EBPR transformations. We used probes developed by Hesselman and coworkers (26) to detect the *Rhodocyclus*-like organism, including a very specific probe (RHX991) targeting only the uncultivated sequence type and a more general probe (RHC439) for *Rhodocyclus* spp. and close relatives. We found that these organisms dominated the system at 16% P_x, comprising 70% of DAPI-stained cells. They were virtually absent at 1% P_x. Through our collaboration with Linda Blackall we documented a linear correlation between the number of *Rhodocyclus*-like organisms and P_x in activated sludge from similar lab-scale SBRs (13). These organisms may be the dominant PAMs in full-scale systems since preliminary experiments have detected significant levels of organisms binding probes RHC439 and RHX991 in full-scale EBPR activated sludge. This work is described in McMahon et al (39) and Crocetti et al (13).

DUAL-SPECIES CULTURE FOR MIXED ORGANICS DEGRADATION

We have developed and studied a dual-species culture of *Pseudomonas putida* DMP1, a *p*-cresol degrader, and *Pseudomonas* sp. GJ1, a 2-chloroethanol (2CE) degrader, as a model system for biodegradation of mixed organics (12). *P.* sp GJ1 will not mineralize *p*-cresol, and its growth is severely inhibited by the presence of *p*-cresol (Figure 1). *P. putida* DMP1 will not mineralize 2CE, but its growth is only slightly inhibited by the presence of 2-CE. The best growth of the two organisms and biodegradation of the two organics is achieved when the two organisms are co-cultured either in biofilms or in planktonic cultures. In addition, GJ1 was engineered with the gene for the green fluorescent protein to distinguish it from DMP1 in biofilms. By adding the fluorescent dye SYTO 59, one can visualize both organisms using fluorescence microscopy (or cell sorting) and yet distinguish them.

PROPOSED WORK

We propose to lay the groundwork for the development of DNA array technology for the rapid detection of microorganisms in pollutant transforming cultures and the quantitative measurement of their activities. Specifically, we will:

1) develop targets for DNA oligonucleotide probes using small and large subunit rDNA genes and transcribed spacer regions between those genes;

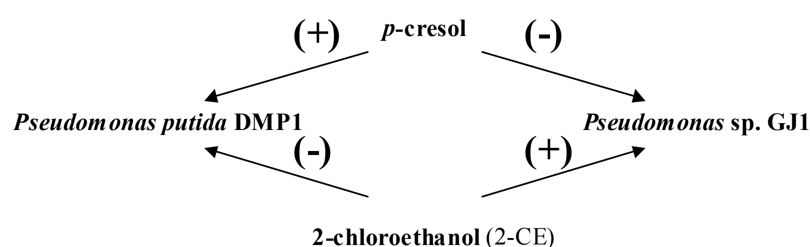

Figure 1. Relationship between strains DMP1 and GJ1 and their respective carbon sources.

2) fabricate macroarrays of oligonucleotide probes for microorganisms present in a laboratory-scale wastewater treatment reactor performing enhanced biological phosphorus removal and in a constructed co-culture that degrades *p*-cresol and 2-chloroethanol;
3) calibrate the DNA array by performing quality control tests: optimizing target preparation and hybridization conditions, calibrating signal, and optimizing data analysis;
4) use the DNA array to assess changes in community structure and metabolic activity as a function of changes in environmental conditions.

As described above, we have chosen two model systems: a defined co-culture degrading a mixed organic waste and an enrichment culture containing a relatively diverse group of microorganisms performing EBPR. The constructed co-culture will be used to calibrate the DNA array, whereas the enrichment culture presents a diverse system to test the flexibility and resolution of the array.

ARRAY DESIGN AND FABRICATION

System design

Most studies on microbial diversity and community structure have focused on rRNA molecules and their genes, particularly the small subunit (SSU), since this molecule was identified as a suitable chronometer for organism evolution by Woese (71). Although the methods exploiting SSU rRNA comparative sequence analysis have been extremely useful for such studies by virtue of its short length and high degree of conservation, these features limit its application to array technology because more sequence space is required for the design of suitable oligonucleotide probes. Recently, microbial ecologists have begun to catalog sequences of other regions in *rrn* operons, including an intergenic spacer region (ISR) which often codes for one or more tRNAs and will be defined here as the sequence separating the SSU and LSU rDNAs on the genome (Figure 2). The ISR, also known as "internal transcribed spacer" or ITS, is more variable than SSU or LSU rDNA and these variations can be intercistronic as well as interstrain (20). Hence, the ISR has been used primarily for more fine-scale differentiation between closely related groups of microorganisms, mainly in the clinical setting (for Bacteria) and in population genetics studies (for Eucarya). A handful of microbial diversity surveys have used ISRs to characterize mixed communities (7, 19, 20).

A recent survey of sequence databases indicated that the ISR varies in length from 143 to 1,529 bp within the dataset available in 1999 (19). This dataset does not represent an even sampling of microbial diversity, since clinically significant organisms are disproportionately represented (80% of the 307 ISRs surveyed were from Gram-positive phyla or the γ-Proteobacteria), but in the interest sampling the largest number of sequences, all available ISRs from Archaea and Bacteria were included. With Gram-positives excluded, the mean ISR size was 533 ± 233 bp. Many Gram positives do not have tRNA included in the ISR (22), thus the mean length was 327 ± 111 bp for the 191 sequences in the dataset (19). Since SSU and LSU rDNA are approximately 1,540 bp and 2,900 bp in length, respectively, the total length of *rrn* operons varies

from about 4.58 Kbp to 5.97 Kbp. This sequence space should be long enough and divergent enough to design appropriate oligonucleotide probes for detection of activated sludge bacteria.

Acquisition and set-up of arrayer and scanner. Due to the limited time and resources available in this program, we propose the development of macroarrays to prove the concept of large, very dense DNA microarrays to probe community structure. By developing DNA macroarrays, we will be able to make use of a new Amersham Typhoon imaging system that can detect radionuclides (^{32}P, ^{33}P, ^{35}S, ^{14}C) as well as fluorescence. This imaging system is already available in the Keasling laboratory. Thus, we propose the purchase of a BioRobotics μTAS spotter (or similar spotter from another manufacturer). This system can be used to construct high-density microarrays on glass slides or low-density macroarrays on membranes. Hence, it will be flexible enough for the eventual development of microarrays if the proof-of-concept experiments proposed here are successful.

Scanning and detection. One level of the protocol development and data analysis will be to determine the limits and response curves of the scanner. This entails determining when the detectors saturate, and the shape of standards curves. Standards curves will be a fundamental aspect of data analysis (see below). The most important curves for this section will be taken with a positive control probe/target pair. A set of experiments that hybridize increasing amounts of target to a probe spot will given us some idea of the hybridization/detection response and saturation curves.

Probe development

Database mining and generation of libraries. Probe libraries will be generated using PCR amplification of target genes. Well-established protocols are routinely used to harvest SSU rDNA sequences from mixed microbial communities using PCR (6, 16, 18, 26, 57) and these are available in our laboratory (39). Initially, primer pairs will be designed to amplify a portion of the 3' end of the SSU gene, the intergenic spacer region, any tRNAs located in the operon, and the 5' end of the LSU gene. "Universal" SSU-targeted primers are available (16, 34) and LSU-targeted primers will be designed based on multiple alignments of all available LSU sequences (approximately 400). Several combinations of primers will be tested for their ability to amplify potentially large (> 3 Kb) fragments of the *rrn* operon from a selection of representative bacteria available in pure culture. Suitable sets of primers will then be applied to biomass from laboratory scale SBRs performing EBPR. Libraries from this culture will be used to construct an EBPR array.

If the methods described above provide promising preliminary data and if we have sufficient time, we will make clone libraries for activated sludges from one or more prototype wastewater treatment plants, including plants that incorporate EBPR (e.g., San Jose, CA) and those that do not (e.g., East Bay Municipal Utility District, Oakland, CA). Since activated sludge communities are known to be extremely diverse (57), libraries will be pre-screened using restriction fragment length polymorphism (RFLP) analysis. Representative clones from each RFLP type will be sequenced partially using SSU-specific primers to provide a phylogenetic typing of the clone and the results will be used to choose a subset of sequence types for inclusion on the array. This subset will contain enough sequences to adequately represent the diversity of types found in the libraries, with an emphasis on phylogenetic groups associated with activated sludge in previous studies (6, 38, 57, 64, 65, 67), including β-Proteobacteria, Actinobacteria (high GC Gram positives), and Cytophagales.

Selection and preparation of probes. We will perform a "top-to-bottom" approach (4) directly on the arrays. Previously designed SSU and LSU rRNA FISH probes (for a good summary see Snaidr et al. 1997) will be incorporated, possibly with some modifications in order to standardize hybridization conditions across the array. These will serve as a rough estimate of division- or phylum-level community composition. More specific probes will be designed to detect the sequences acquired by clone library generation. With this format, we will be able to determine how well the specific probes capture the diversity within the more widely defined groups, allowing for another measure of quality control. For example, discrepancies between the

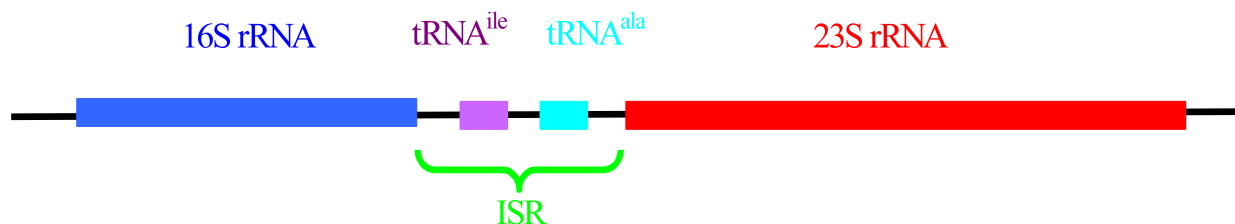

Figure 2. Schematic of the rRNA operon showing the relative locations of genes coding for 16S rRNA, 23S rRNA, and in this case, two tRNAs. The "intergenic spacer region" is marked in green. The number and kind of tRNAs located in the ISR varies among organisms and among operons in the same organism.

signal from a probe targeting "all" of the β-Proteobacteria and the sum of the signals from all probes targeting organisms within the β-Proteobacteria can be noted and addressed.

Probe selection can be a complex iterative process. For large sequences, there is little design that can be done and specificity is obtained mainly because hybridization occurs over a very long and specific DNA sequence and the concentrations of probe and target are set to minimize non-specific binding. For short probes more rational approaches are available. Ideally, the probe set is selected so that each probe is specific for its target and orthogonal (non-homologous) to any region of any other target. There should be high confidence that each probe sequence is sufficiently rare that there is no other DNA in the target organisms that may hybridize to it. There should be little or no secondary structure in each probe if possible in order to maximize rapid hybridization dynamics. Finally, the high the melting temperature of the probe/target duplex and the more uniform the melting temperature among all the probe/target pairs, the better the reproducibility and quantification of the hybridization. Each of these requirements may be optimized through the use of the computer-based predictions. However, it is usually impossible to meet all these constraints closely enough to be sure that there will not be significant variation in probe efficiency. Further, the computer programs that calculate each of these constraints are imperfect at best. Thus, experimental measurement and array redesign will be necessary. This means that we must develop individual estimates of how well a particular probe/target pair can be used to estimate population levels (see below). Detection of "dud" probes will force redesign of that member of the array.

ARRAY CALIBRATION

Source of calibration target

Cloned library DNA. Libraries constructed from various sludges and used to generate probe sequences will serve as target during array calibration. Several different mixtures of known quantities of cloned partial *rrn* operons will be hybridized to fabricated arrays and the resulting signal intensities will be used to optimize hybridization and wash conditions. The arrayed probe specificity will be evaluated using a matrix of mixtures, each containing different subsets of all possible targets. If certain probe sequences do not give satisfactory signal or confer adequate specificity, new probes will be designed. We anticipate that several iterations will be required to hone in on an optimal array design. The final array design will be used to generate a database correlating the concentration of target nucleic acid with a signal intensity for each spot in the array. Work with the Davis laboratory with Affymetrix-based DNA chips for monitoring the population of different deletion strains of yeast, indicate that reproducibility of population estimates can be very high if proper quality control procedures are followed (even achieving chip-to-chip intensity correlation coefficients of over 0.99 (Adam Arkin, Guri Giaever, Ron Davis,

unpublished results)). The database will serve as a reference standard for each array experiment conducted with native nucleic acids extracted from defined and natural communities.

Constructed co-cultures. As mentioned in the *Preliminary Work* section, we have constructed a co-culture of *Pseudomonas putida* DMP1 and *Pseudomonas* sp. GJ1 that degrades *p*-cresol and 2-chloroethanol. In addition, there is a significant amount of cross-inhibition in this system to generate very interesting culture dynamics. Once we have constructed an array for this co-culture, we will perturb the culture by adding various amounts of the two organics or by initiating the culture with different amounts of the two organisms. Possible experiments include:

- Develop co-cultures by mixing equal amounts of DMP1 and GJ1, but add different amounts of 2CE and *p*-cresol. Then, examine the effect of the difference concentrations of organics on cell numbers. At the same time that a sample is taken to analyze using the array, one could plate cells on different media or do flow cytometry to count the numbers of GJ1 and DMP1.
- Develop co-cultures by mixing different amounts of DMP1 and GJ1, but keep the amounts of the two organics added to each culture the same. Examine the effect of initial population on the final population.

We will use the array to quantify changes in the population in response to changing environmental condition. The results from the array will be checked by plating the co-cultures on solid medium selective for each microorganism and/or by counting GFP-producing and non-producing organisms on non-selective solid medium or by flow cytometry.

Target preparation and labeling

When analyzing co-cultures and activated sludge, we will optimize nucleic acid extraction procedures for hybridization to arrays. Several published extraction protocols (6, 8, 28, 35, 69) will be evaluated and, if necessary, modified. We will investigate the use of commercially available nucleic acid extraction kits whenever possible, keeping in mind that array technology may one day be developed in kit format.

Newly developed nucleic acid labeling kits based on platinum chemistry (Molecular Dynamics, Sunnyvale, CA) will be used to label target DNA and RNA. Some modifications may need to be made to the kit protocols in order to achieve reproducible and predictable labeling efficiency. In the interest of quality control, nucleic acids produced in vitro will be included in each labeling reaction to evaluate the efficiency of labeling, the integrity of labeled products, and to serve as an internal standard during hybridization. A parallel labeling reaction will be carried out using a calibrated mixture of target DNA and a different fluor, as described below.

Initially, we will only test hybridization of DNA to the arrays. The presence of a particular DNA sequence in a sample will correspond to the presence of the organism in the community, giving us "presence/absence" information. If DNA can be successfully hybridized to give quantitative results (as determined during quality control tests), we will then attempt to detect rRNA extracted from the same samples. The quantification of rRNA abundance would be extremely powerful, since this would provide a measure of the metabolic activity of the target organisms (45). As described previously, measurement of both the RNA and DNA components in a sample (potentially in the same experiment using two different fluorescent tags), would give an even more defined measure of relative physiological status (24). The data analysis and interpretation required to determine how hybridization signal is related to relative activity will be extremely challenging, but should not be impossible.

Calibration of signal

Methods exploiting the specificity of oligonucleotide probes usually require the optimization of hybridization conditions for each probe (4, 47). Although the massively parallel format of arrays is part of what makes the technology so attractive, it also creates difficulties in the

calibration of hybridization signal. The only published study on the application of array technology to the analysis of microbial communities emphasized the need to normalize hybridization signals to achieve true quantitative measures of sequence abundance (24).

The goal of calibration is to relate the measured intensity of fluorescence from a given spot to the size of the original bacterial subpopulation in the reactor. However, between the sampling from the reactor and the final scanner reading of intensity there are a number of systematic and random transformations of the original signal. There is an initial sampling error in the extraction of bacteria from the reactor. Depending on the volume extracted and the number density of each cell type, the actually density of cells extracted will deviate more or less with the density in the reactor. The amount of RNA expressed, on average, by each bacterial subtype might also be different. The next error introduced is in the isolation of RNA from the mixed bacterial sample. This leads to random loss of signal from each of the different RNA populations. Differential amplification and amplification error introduces still more variance in the different RNA population. Then differences in probe/target hybridization efficiency (thermodynamics and rates) leads to still more variant readouts of RNA population levels. The scanning and image analysis stages introduce still more generally nonlinear transformation and error in the signal. Finally, inhomogeneities in the array structure introduced during fabrication and washing may lead to systematic and random errors in hybridization.

To approach a robust estimate of original population densities we can design simultaneous experimental controls and statistical analyses that address each of these issues. As an example, duplicate probes can be uniformly distributed around the array to estimate hybridization variability and array inhomogeneities. Doping in a known amount of a control bacterium at the sampling stage of the process can lead to estimates in experiment-to-experiment variability in the overall process. Synthetic control probes introduced at the amplification and hybridization stages can separate errors introduced during these steps. Different control probes, each at a difference concentration, can be used to probe the dynamic range of each chip reading separately. Pilot experiments in which RNA from increasing sample sizes from the reactor are hybridized to arrays leads to standard curves for each organism that may be used as a reference to which other experimental measurements can be compared. Finally, duplicate experiments and cross-analysis of chips under many conditions can be used to develop statistical models for each tag that allow classification of a particular intensity reading into a number of "effects" classes such as "high", "low", and "unchanged" with confidence levels assigned. We have had encouraging results in the yeast haploinsufficiency trials we have been analyzing for the Davis laboratory (unpublished).

Data analysis

Once chips have been calibrated, that is, once the transformation from population density to intensity for each tag has a statistical model based on the controls and standard experiments above, then it becomes possible to ask how each of the bacteria in a microbial population is responding to different culture conditions. There are a number of interrelated types of experiments to be analyzed. The first is steady-state experiments wherein the bacteria in the reactor are allowed evolve to final unchanging (or in rare cases, oscillating) population densities under particular culture conditions. For each steady-state the population density average for each strain is identified and databased. These experiments may be extended by measuring the temporal response (growth/death curves) for each strains during a condition shift. By associating these dynamics with process variables such as amount of phosphate accumulated, pH, and various organic products, one can identify with each steady-state experiment a fitness for long term process control. Associations with temporal response data may indicate which strains are responding to changes in which changing process variables. In the end, each population/process variable measurement forms a profile that may be compared across conditions. By applying standard multivariate analysis techniques such as principle component or factor analysis it will be possible to identify which culture conditions, or population composition provides the most temporally stable and overall robust process control.

APPLICATION TO MICROBIAL CONSORTIA

Population dynamics in laboratory-scale EBPR systems

Successful EBPR operation requires fully developed, robust populations of organisms that can carry out the metabolic transformations associated with polyphosphate accumulation. Hence, many studies have attempted to identify the types of organisms present in systems performing well and in those exhibiting poor performance. Polyphosphate accumulating microorganisms (PAMs) were recently identified using rRNA-based techniques and EBPR cultures maintained in laboratory-scale SBRs (6, 13, 26). Now that PAMs have been conclusively identified, the effects of operating parameters on PAM populations can be directly assessed. In addition, these and other studies suggest that a number of other types of bacteria are active in EBPR systems, possibly contributing to the process in as yet unidentified ways (40). Thus, it is desirable to have the capacity to track several kinds of organisms simultaneously. We will use the EBPR array to simultaneously monitor all unique microbial populations present in lab scale SBRs highly enriched for EBPR communities.

Initially, we will characterize the steady-state microbial community structure at various influent P:COD loadings and sludge ages. These two parameters are known to affect the sludge P content and, in turn, the bulk EBPR activity (36, 37, 49, 68). Then, we will investigate the relationships between other operating parameters, microbial population dynamics, and process performance. Previous studies have examined the effects of anaerobic phase pH, anaerobic retention time, aerobic retention time, and feed composition on EBPR with limited attention given to effects on community structure. For example, an excessively long aerobic phase can cause poor performance (9, 10, 63); EBPR activity can be "washed out" from activated sludge at low sludge ages, and the washout sludge age is temperature dependent (37). Since these researchers did not perform quantitative microbial ecology studies, it is unclear whether these and other effects on performance are due to shifts in community structure or to changes in metabolism in otherwise stable populations. While monitoring key kinds of organisms as well as physical and chemical parameters, we plan to, in various experiments, (i) gradually lengthen the aerobic phase in the SBRs; (ii) gradually increase/decrease the sludge age; (iii) introduce O_2 and/or nitrate into the anaerobic zone; (iv) gradually change the composition of the feed to include other C sources. The data we obtain from these experiments will indicate which operating parameters are critical to the maintenance of enriched and robust PAM populations. Other essential/detrimental kinds of non-PAMs will be identified by correlating their absence/presence with poor performance.

These experiments will be carried out in parallel with other molecular techniques already employed in our laboratory, including rRNA-based FISH and dot blot hybridization to extracted DNA and RNA using labeled oligonucleotide probes. The combined results from these analyses and data generated using the EBPR array will be integrated to provide a more complete understanding of the EBPR process.

Survey of full-scale wastewater treatment plants

Although it is unlikely that there will be time available in this phase of the investigation, we eventually plan to use the developed arrays as diagnostic tools in prototype wastewater treatment plants. At first we would select plants with different P removal capabilities, including EBPR plants that function well and those that have a history of erratic performance. Using our arrays we will determine the ranges of populations that exist in these plants under good and poor EBPR performance. By examining the full range of process modifications (there are over a dozen) that are used to achieve EBPR, we will be able to identify those process features that are associated with the development of high and stable polyphosphate accumulating microorganism populations.

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1. S. J. Van Dien, S. Keyhani, C. Yang, and J. D. Keasling. 1997. "Manipulation of independent synthesis and degradation of polyphosphate in *Escherichia coli* for investigation of phosphate secretion from the cell." *Appl. Environ. Microbiol.* **63**:1689-1695.
2. J. Pramanik, P. L. Trelstad, A. J. Schuler, D. Jenkins, and J. D. Keasling. 1998. "Development and validation of a flux-based stoichiometric model for enhanced biological phosphorus removal metabolism." *Water Research* **33**:462-476.
3. R. Brent Nielsen and J. D. Keasling. 1999. "Reductive dechlorination of chlorinated ethene DNAPLs by a culture enriched from contaminated groundwater." *Biotechnol. Bioeng.* **62**:160-165.
4. P. L. Trelstad, P. Purdhani, W. Geibdorfer, W. Hillen, and J. D. Keasling. 1999. "Polyphosphate kinase of *Acinetobacter* sp. Strain ADP1: purification and characterization of the enzyme and its role during changes in extracellular phosphate." *Appl. Environ. Microbiol.* **65**(9):3780-3786.
5. S. E. Cowan, E. Gilbert, A. Khlebnikov, and J. D. Keasling. 2000. "Dual labeling with green fluorescent proteins for electron microscopy." *Appl. Environ. Microbiol.* **66**:413-418.

Other publications:

1. C. L. Wang, P. C. Michels, S. Dawson, S. Kitisakkul, J. A. Baross, J. D. Keasling, and D. S. Clark. 1997. "Cadmium removal by a new strain of *Pseudomonas aeruginosa* in aerobic culture." *Appl. Environ. Microbiol.* **63**:4075-4078.
2. T. A. Carrier, K. L. Jones, and J. D. Keasling. 1998. "mRNA stability and plasmid copy number effects on gene expression from an inducible promoter system." *Biotechnol. Bioeng.* **59**:666-672.
3. S. J. Van Dien and J. D. Keasling. 1998. "Optimization of polyphosphate degradation and phosphate secretion using hybrid metabolic pathways and engineered host strains." *Biotechnol. Bioeng.* **59**:754-761.
4. T. A. Carrier and J. D. Keasling. 1999. "Library of synthetic 5' secondary structures to manipulate mRNA stability in *Escherichia coli*." *Biotechnol. Prog.* **15**:58-64.
5. J. D. Keasling. 1999. "Gene-expression tools for the metabolic engineering of bacteria." *Trends in Biotechnology* **17**:452-460.

List of collaborators:

Former collaborators on research projects include: LeRoy Bertsch, Ted Hupp, Jon Kaguni, Ken Raymond

Current collaborators on research projects include: Douglas Clark, David Jenkins, Harvey Blanch

Current and former students:

Jaya Pramanik	Doug Bolesch	Robert Pape	Natalya Eliashberg
Stephen Van Dien	Salomeh Keyhani	Trent A. Carrier	Kristala Jones
R. Brent Nielsen	Piper Trelstad	Clifford Wang	Ilana Aldor
David Reichmuth	Jessica Hittle	Andrew Walker	Nichole Goeden
Neil Renninger	Christina Smolke	Sundiep Tehara	Stacie Cowan
Katherine McMahon	David Lubertozzi	Cynthia Gong	Michel Maharbiz

Total number = 24

Current and former post-doctoral scholars:

Susan T. Sharfstein	Wubin Pan	Doug Bolesch	Sang-Weon Bang
Eric Gilbert	Artem Khlebnikov	Yet-Pole I	Seon-Won Kim
Vincent Martin			

Total number = 9

Thesis advisors: Bernhard Palsson & Stephen Cooper
Post-doctoral advisor: Arthur Kornberg

Synergistic activities

1. Developed a metabolic engineering toolkit for bacteria. All tools in the kit are available to the public at no charge.
2. Developed a relatively complete metabolic model of *E. coli* that also serves as a metabolic database.
3. Developed a web-based course for biochemical engineering.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Adam P. Arkin, Ph.D.	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Carleton College, MN	B.A.	1988	Chemistry
Massachusetts Institute of Technology, MA	Ph.D.	1992	Physical Chemistry
Stanford University (Chemistry), CA	Postdoc	1992-95	Nonlinear Chem. Systems
Stanford University (Developmental Biology), CA	Postdoc	1995-1997	Modeling Development

Professional Experience

July 1999- Present Assistant Professor, Departments of Bioengineering and Chemistry, University of California, Berkeley

Faculty Scientist, Computational and Theoretical Biology Department, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA

January 1998-July 1999 Staff Scientist, Computational and Theoretical Biology Department, Physical Biosciences Division, E.O. Lawrence Berkeley National Laboratory, University of California, Berkeley, CA

Address

1 Cyclotron Road, MS 3-144, Berkeley, California, 94720

Honors

M.I.T. Technology Review Top 100 Young Innovator Award Recipient.

RESEARCH INTERESTS

Adam Arkin's research interests include: 1) physical chemical analysis of elementary biochemical processes, 2) genetic and biochemical network deduction, analysis and simulation, 3) development of advanced tools for bioinformatics, statistical data analysis and pathway analysis, 4) experimental implementation of novel gene expression control systems in microbes, and 5) instrumentation for biochemical measurements

Teaching

Chemistry 130A: Biophysical Chemistry

Selected Publications

1. **Arkin, A.P.**, Youvan D.C. (1992) An Algorithm for Protein Engineering: Simulations of Recursive Ensemble Mutagenesis. *Proc. Natl. Acad. Sci. USA* **89**(16):7811-7815.
2. **Arkin, A.P.**, Youvan, D.C. (1992) Digital Imaging Spectroscopy. In: The Photosynthetic Reaction Center J. Deisenhofer & J.R. Norris eds. 133-154.
3. **Arkin, A.P** ,.Shen, P.-D., Ross, J. (1997) A Test Case of Correlation Metric Construction of a Reaction Pathways from Measurements. *Science*. **277**(5330): 1275.

4. McAdams, H.H., **Arkin, A.P** (1999) Genetic Regulation at the Nanomolar Scale: It's a Noisy Business! *TIGS.* **15**(2): 65-69.

Publications Related to Proposal

1. McAdams, H., **Arkin. A.P.** (1997) Stochastic Mechanisms in Gene Expression. *Proc. Natl. Acad. Sci., USA* . **94**(3):814.
2. Swanson, C., **Arkin, A.P.**, Ross, J. (1997) An Endogenous Calcium Oscillator May Control Early Embryonic Division. *Proc. Natl. Acad. Sci., USA* . **94**(4):1194.
3. McAdams, H. H., **Arkin, A.P.** (1998) Simulation of Prokaryotic Genetic Networks. *Annu. Rev. Biophys. Biomol. Struct.* **27**: 199-244
4. **Arkin, A.P**., Ross, J., McAdams, H.H. (1998) Stochastic Kinetic Analysis of a Developmental Pathway Bifurcation in Phage-λ *Escherichia coli*. *Genetics.* **149**(4):1633-1648.
5. **Arkin, A.P.** (1999) Signal Processing by Biochemical Reaction Networks. In: Biodynamics. J. Walleczek, ed. Cambridge University Press, Cambridge.In Press.

LIST OF GRADUATE ADVISOR, GRADUATE AND POST GRADUATE ADVISEES

Doctoral Advisors: Dr. Doug Youvan, President/CEO or Kairos, Inc (was assistant professor M.I.T.,main dissertation advisor); Profs. Keith Nelson, Department of Chemistry, M.I.T. Post-doctoral Advisors: Prof. John Ross, Department of Chemistry, Stanford University, Prof. Lucy Shapiro & Dr. Harley McAdams, Developmental Biology, School of Medicine, Stanford University. *Post-Doctoral Advisees:* Dr. Alex Gilman (chemistry) '98/-, Dr. Denise Wolf (engineering) '98-, Dr. Chris Rao (chemical engineering) 00

LIST OF COLLABORATORS IN THE PAST 48 MONTHS OTHER THAN THOSE CITED ABOVE

McAdams, H. Stanford University, Doyle, J. *California Institute of Technology,* Antje Hofmeister, *University of California, Berkeley,* Guri Giaever, Ron Davis, *Stanford University,* Dan Rokhsar, *University of California, Berkeley and LBNL*, Stephen Holbrook, *LBNL,* Roger Brent, *Molecular Sciences Institute.*

SYNERGISTIC ACTIVITIES

(1) Developed software suite for simulation of genetic networks that has been made available on the web and donated to a number of off-site groups for further development. Also forms the core of the Bio/Spice biological simulation and analysis tool.

(2) Helped develop and write a proposal for a multisite Alliance for Cellular Signaling out of University of Texas, Southwestern Medical Center dedicated to forming a collaboration among more than forty experimentalist, computer scientists and computational biologists to understand G-protein signal transduction in B-Cells and cardiomyocytes.

(3) Coordinating development, with Peter Karp (Stanford Research Institute), Milton Saier (UCSD), Fernando Valle (Genecor, Intl.), and Tyrrell Conway (U. Oaklahoma) of EcoReg and database of primary and secondary data on the kinetics, regulation, and expression in *E. coli* to create a synergy with EcoCyc, a knowledge base of *E. coli* pathways.

David Jenkins

Professor in the Graduate School
Department of Civil and Environmental Engineering
University of California
Berkeley, CA 94720-1710
(510) 642-5337
jenkins@ce.berkeley.edu

Education
Ph.D. Public Health (Sanitary) Engineering, 1960, Kings College, University of Durham, England
B.Sc. Applied Biochemistry,1957, Birmingham University, England

Honors and Awards
Foyle Prize, Birmingham University, 1957
Post Doctoral Research Fellow, Harvard University, 1969-70
Harrison Prescott Eddy Medal, Water Environment Federation (WEF), 1974, 1985 and 1988
Engineering-Science Award, Association of Environmental Engineering and Science Professors (AEESP), 1978, 1982
Distinguished Service Award, WEF, 1981
Japan Society for the Promotion of Science Fellowship, 1982
Thomas Camp Medal, WEF, 1988
Honorary Life Member, WEF, 1988
Simon Freeze Award and Lectureship, American Society of Civil Engineers, 1988
Distinguished Lecturer in Environmental Engineering, AEESP, 1988
George Bradley Gasgoine Medal, WEF, 1989
Republic of China, National Research Council Fellow, March 1992
Samuel H. Jenkins Medal, the International Water Association (IWA) 1992
Honorary Life Member, IWA, 1994
Gordon Maskew Fair Medal, WEF, 1995
Outstanding Publication Award, AEESP, 1995
CH2M-Hill/AEESP Doctoral Thesis Award (Andy Schuler), 1999
Berkeley Citation, 1999

Professional Affiliations
Fellow, Chartered Institution of Water Engineering and Management, England
Member, California Water Environment Association
Member, AEESP

Professional Service

Member, Joint Editorial Board, 15th and 16th editions of Standard Methods for the Examination of Water and Wastewater, 1974-1981
Member, State of California, Water Resources Control Board, Operator Certification and Advisory Board, 1980-present
Director, AEESP, 1972-1975 and 1983-1986
Member, Governing Board, IWA, 1990-1992

Member, Programme Committee, IWA, 1987-1992
Chair, Specialist Group on Nutrient Removal, IWA, 1980-1990
Member, Research Council, WEF Research Foundation, 1989-1991
Director, Board of Control, WEF, 1989-1992
Chair, USA National Committee to IWA, 1990-1992
Member, Editorial Board, Water Environment Research, 1992-1997
Member, International Science Advisory Committee, Technion University, Israel, 1995-present
Member, Editorial Board, Water 21, 1996-present

Research and Professional Activity in the Fields of:
Wastewater and Sludge Treatment Processes
Mechanism and Control of Activated Sludge Bulking and Foaming
Chemical and Biological Removal of Nitrogen and Phosphorus from Wastewaters
Operation and Management of Wastewater Treatment Plants
Water and Wastewater Chemistry

5 Relevant Publications

1. **Schuler, A. J., and D. Jenkins.** 1997. The effect of varying activated sludge phosphate content on the enhanced biological phosphorus removal metabolism. Presented at the Water Environment Federation 70th Annual Conference and Exposition, Chicago, IL. pp.
2. **Pramanik, J., P. L. Trelstad, A. J. Schuler, D. Jenkins, and J. D. Keasling.** 1998. Development and validation of a flux-based stoichiometric model for enhanced biological phosphorus removal metabolism. Water Research. **33**(2)**:**462-476.
3. **Schuler, A. J., and D. Jenkins.** 1999. Anaerobic ATP utilization and acetate uptake rates in enhanced biological phosphorus removal from wastewater. Water Environment Research. in press.
4. **Crocetti, G. R., P. Hugenholtz, P. L. Bond, A. Schuler, J. Keller, D. Jenkins, and L. L. Blackall.** 2000. Identification of polyphosphate accumulating organisms and the design of 16S rRNA-directed probes for their detection and quantitation. Applied and Environmental Microbiology **66**(3)**:**1175-1182.
5. **D. Jenkins and V. Tandoi.** 1991. The Applied Microbiology of Enhanced Biological Phosphate Removal - Accomplishments and Needs. *Water Res*. **25**:53-56.

List of Collaborators

Former collaborators on research projects include:	R. E. Selleck, B. Beaman
Current collaborators on research projects include:	J. D. Keasling, D. Sedlak, A. Ekster, S. ghosh, R. R. Trussell
Former Postdoctoral associates:	none
Current and Former Graduate Students: (Going back 5 years)	K. R. Pagilla A. Schuler P. Daniel A. Fainsod (M. Eng.) L. Jinkins (M. Eng.) W. F. Harper B. Narayanan K. D. McMahon C. A. DeLeon

SUMMARY PROPOSAL BUDGET — YEAR 1

ORGANIZATION	FOR NSF USE ONLY		
University of California-Berkeley	PROPOSAL NO.	DURATION (months)	
		Proposed	Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR	AWARD NO.		
Jay D Keasling			

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-mos. CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Jay D Keasling - Associate Professor	0.00	0.00	0.00	$ 0	$
2. Adam Arkin - Assistant Professor	0.00	0.00	0.00	0	
3. David Jenkins - Professor	0.00	0.00	0.00	0	
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (3) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	0.00	0	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (1) GRADUATE STUDENTS				20,000	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				20,000	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				4,614	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				24,614	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.) Arrayer $ 72,000					
TOTAL EQUIPMENT				72,000	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				0	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				18,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				18,000	
H. TOTAL DIRECT COSTS (A THROUGH G)				114,614	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) % of MTDC (Rate: 50.4000, Base: 38345)					
TOTAL INDIRECT COSTS (F&A)				19,325	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				133,939	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.D.7.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 133,939	$

M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $		
PI / PD TYPED NAME & SIGNATURE*	DATE	FOR NSF USE ONLY	
Jay D Keasling		INDIRECT COST RATE VERIFICATION	
ORG. REP. TYPED NAME & SIGNATURE*	DATE	Date Checked	Date Of Rate Sheet / Initials - ORG

SUMMARY PROPOSAL BUDGET

YEAR 2

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months)	
	Proposed	Granted
AWARD NO.		

ORGANIZATION
University of California-Berkeley

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR
Jay D Keasling

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-mos. CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Jay D Keasling - Associate Professor	0.00	0.00	0.00	$ 0	$
2. Adam Arkin - Assistant Professor	0.00	0.00	0.00	0	
3. David Jenkins - Professor	0.00	0.00	0.00	0	
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (3) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	0.00	0	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (1) GRADUATE STUDENTS				20,800	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				20,800	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				4,798	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				25,598	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				0	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				18,720	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				18,720	
H. TOTAL DIRECT COSTS (A THROUGH G)				44,318	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) % of MTDC (Rate: 50.4000, Base: 39879)					
TOTAL INDIRECT COSTS (F&A)				20,099	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				64,417	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.D.7.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 64,417	$

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI / PD TYPED NAME & SIGNATURE*	DATE	FOR NSF USE ONLY		
Jay D Keasling		INDIRECT COST RATE VERIFICATION		
ORG. REP. TYPED NAME & SIGNATURE*	DATE	Date Checked	Date Of Rate Sheet	Initials - ORG

SUMMARY PROPOSAL BUDGET — Cumulative

ORGANIZATION	FOR NSF USE ONLY		
University of California-Berkeley	PROPOSAL NO.	DURATION (months)	
		Proposed	Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: Jay D Keasling	AWARD NO.		

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-mos. CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Jay D Keasling - Associate Professor	0.00	0.00	0.00	$ 0	$
2. Adam Arkin - Assistant Professor	0.00	0.00	0.00	0	
3. David Jenkins - Professor	0.00	0.00	0.00	0	
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (3) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	0.00	0	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (2) GRADUATE STUDENTS				40,800	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				40,800	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				9,412	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				50,212	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.) $ 72,000					
TOTAL EQUIPMENT				72,000	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				0	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				36,720	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				36,720	
H. TOTAL DIRECT COSTS (A THROUGH G)				158,932	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
TOTAL INDIRECT COSTS (F&A)				39,424	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				198,356	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.D.7.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 198,356	$

M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $		
PI / PD TYPED NAME & SIGNATURE* Jay D Keasling	DATE	FOR NSF USE ONLY — INDIRECT COST RATE VERIFICATION	
ORG. REP. TYPED NAME & SIGNATURE*	DATE	Date Checked / Date Of Rate Sheet	Initials - ORG

NSF Form 1030 (10/99) Supersedes all previous editions C*SIGNATURES REQUIRED ONLY FOR REVISED BUDGET (GPG III.B)

BUDGET JUSTIFICATION

3 Principal Investigators: The 9-month salaries for the PI's are paid by the University of California. No additional salary is requested.

1 Graduate Student: Salary for one graduate student is proposed. Fringe benefits are calculated at 1.3% for the academic months and 3.0% for the summer months (escalated at 4.0% per year). Student Health Insurance is $226/semester (escalated at 7.5% per year) and full fee remission is $1,978.25/semster (escalated at 10% per year).

Overhead is not charged on student health insurance or fee remission.

Equipment: Funds are requested for a DNA arrayer. The BioRobotics µTAS arrayer will allow macro- and microarray construction. The total cost of this equipment is $72,000.

Supplies: Major supplies for these studies include molecular biological reagents, general chemicals, growth media, etc. Supplies are escalated at 4% per year to adjust for inflation.

Escalation: Graduate student salaries have been escalated at 4.0%. Graduate Student Health has been escalated at 7.5% and Fee remission has been escalated at 10% also as mandated by the University of California. Supplies and have been escalated at 4.0% per year. Other escalation rates are noted above.

Overhead: Overhead is calculated at 50.4% as mandated by The University of California. Graduate student health, fee remission, and equipment are not subject to overhead.

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal

Investigator: **Jay D. Keasling**	Other agencies (including NSF) to which this proposal has been/will be submitted. None

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Applied Biology and Bioprocess Engineering Research Training Grant (Harvey W. Blanch, P.I.)**

Source of Support: **National Institutes of Health**

Total Award Amount: **$1,028,174** Total Award Period Covered: **07/01/94 - 06/30/99 (no students supported)**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. **0** Cal: Acad: Sumr:

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Mechanism of Enhanced Biological Phosphorus Removal (David Jenkins, P.I.)**

Source of Support: **National Science Foundation**

Total Award Amount: **$413,077** Total Award Period Covered: **04/15/97 - 04/14/00**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Metabolic engineering of marine microorganisms for heavy metal removal (Douglas S. Clark, P.I.)**

Source of Support: **Department of Energy**

Total Award Amount: **$587,157** Total Award Period Covered: **10/01/97 - 09/30/00**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Marine Bioproducts Engineering Center (Univ. of Hawaii and UC Berkeley) (with Harvey W. Blanch, Douglas S. Clark, Clayton Radke, and Tasios Melis at UCB)**

Source of Support: **NSF**

Total Award Amount: **$3,305,000** Total Award Period Covered: **03/01/98-02/28/03**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Metabolic engineering of bacteria for degradation of organophosphate contaminants**

Source of Support: **NSF & ONR**

Total Award Amount: **$419,870** Total Award Period Covered: **11/01/98-10/31/01**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: **Jay D. Keasling**	Other agencies (including NSF) to which this proposal has been/will be submitted. None

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: **Engineering mRNA stability for coordinated expression of multiple genes in new operons**
Source of Support: **NSF**
Total Award Amount: **$374,804** Total Award Period Covered: **04/01/00-03/31/03**
Location of Project: **University of California at Berkeley**
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: **Metabolic engineering of Terpenoid Biosynthesis**
Source of Support: **NSF**
Total Award Amount: **$374,765** Total Award Period Covered: **04/01/00-03/31/03**
Location of Project: **University of California at Berkeley**
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: **Metabolic engineering of filamentous fungi**
Source of Support: **Merck**
Total Award Amount: **$500,276** Total Award Period Covered: **10/20/99-10/19/03**
Location of Project: **University of California at Berkeley**
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: **Intelligent Bioassay Arrays (with R. Howe and S. Smith)**
Source of Support: **DARPA**
Total Award Amount: **$2,069,913** Total Award Period Covered: **0401/2000 – 0331/2003**
Location of Project: **University of California at Berkeley**
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: **Jay D. Keasling**	Other agencies (including NSF) to which this proposal has been/will be submitted. None

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Development of novel organisms for heavy metal and actinide removal**

Source of Support: **Department of Energy**

Total Award Amount: **$785,689** Total Award Period Covered: **10/01/2000 – 09/30/2003**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Biocomplexity: Analysis, Rational Design, and Random Evolution of Complex Gene Circuits**

Source of Support: **National Science Foundation**

Total Award Amount: **$1,127,909** Total Award Period Covered: **9/01/2000 – 08/31/2003**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Mechanism of Enhanced Biological Phosphorus Removal (David Jenkins, P.I.)**

Source of Support: **National Science Foundation**

Total Award Amount: **$558,706** Total Award Period Covered: **04/15/00 - 04/14/03**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **DNA arrays for assessing pollutant removing potential of environmental systems (with Adam Arkin and David Jenkins)**

Source of Support: **National Science Foundation**

Total Award Amount: **$198,356** Total Award Period Covered: **9/01/2000 - 8/31/2002**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: Arkin, Adam

Other agencies (including NSF) to which this proposal has been/will be submit- None

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: Biocomplexity: Analysis, Rational Design, and Random Evolution of Complex Gene Circuits (this proposal)

Source of Support: NSF

Total Award Amount: 1,127,909 Total Award Period Covered: 9/1/00-8/31/03

Location of Project: Berkeley

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr: 1.0

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: Alliance for Cellular Signalling

Source of Support: ONR

Total Award Amount: $ 140,327 Total Award Period Covered: 9/1/00-8/31/05

Location of Project: Berkeley

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr: 0.20

Support: Sumr:

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: Molecular Design Institute II: Construction of Genetic Circuitry in Saccharomyces Cerivisiae

Source of Support: ONR

Total Award Amount: $ 100,000 Total Award Period Covered: 10/1/00-9/30/00

Location of Project: Lawrence Berkeley National Laboratory

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr: 0.20

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: Instant Cell Analysis, BioSpice, Cellular Device and Exquisite Detection: Towards an Interactive Biology

Source of Support: ONR/DARPA

Total Award Amount: $251,069 Total Award Period Covered: 9/1/99-12/31/01

Location of Project: Lawrence Berkeley National Laboratory

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr: 0.25

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

NSF Form 1239 (10/99) USE ADDITIONAL SHEETS AS NECESSARY

Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: Arkin, Adam

Other agencies (including NSF) to which this proposal has been/will be submit-
None

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: Bio/Spice: A Tool for Integrated Cellular Bioinformatics and Dynamical Genomics
Computational Tool for Simulating Cellular Development and Genetic Pathways

Source of Support: DOE
Sumr: 0.25

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: DNA arrays for assessing pollutant removing potential of environmental systems

Source of Support: National Science Foundation
Total Award Amount: $198,356 Total Award Period Covered: 9/01/2000 - 8/31/2002
Location of Project: University of California at Berkeley
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

NSF Form 1239 (10/99) USE ADDITIONAL SHEETS AS NECESSARY

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: **David Jenkins**

Other agencies (including NSF) to which this proposal has been/will be submitted.

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Mechanism of Enhanced Biological Phosphorus Removal (with Jay D. Keasling)**

Source of Support: **National Science Foundation**

Total Award Amount: **$413,077** Total Award Period Covered: **04/15/97 - 04/14/00**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Mechanism of Enhanced Biological Phosphorus Removal (with Jay D. Keasling)**

Source of Support: **National Science Foundation**

Total Award Amount: **$558,705** Total Award Period Covered: **03/01/00 – 02/28/03**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **The use of EBPR to treat P-deficient wastewaters**

Source of Support: **Water Environment Research Foundation**

Total Award Amount: **$100,000** Total Award Period Covered: **07/01/00 – 06/30/01**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: **1** Acad: Sumr:

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **DNA arrays for assessing pollutant removing potential of environmental systems (with Adam Arkin and Jay Keasling)**

Source of Support: **National Science Foundation**

Total Award Amount: **$198,356** Total Award Period Covered: **9/01/2000 - 8/31/2002**

Location of Project: **University of California at Berkeley**

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

FACILITIES, EQUIPMENT & OTHER RESOURCES

FACILITIES: Identify the facilities to be used at each performance site listed and, as appropriate, indicate their capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Use "Other" to describe the facilities at any other performance sites listed and at sites for field studies. USE additional pages as necessary.

Laboratory: **The Keasling group has a laboratory of approximately 2000 square feet supplied with modern equipment required for current biotechnology: ultracentrifuge, high-speed centrifuge, UV/Vis spectrophotometer, balances, pH meters, -80 C freezer, -20 C freezer, chromatography refrigerators, thermocyclers, Hewlett-Packard and Varian gas**

Clinical:

Animal:

Computer: **Keasling's office is equipped with a Dell Pentium Pro computer and a Hewlett-Packard LaserJet. The laboratory is equipped with PC-compatible and Macintosh computers and printers. The College of Chemistry Graphics Facility contains Silicon Graphics, DEC Alpha, IBM RS6000, Intel, Macintosh, Ardent, and Vax computers, Macintosh Laserwriters,**

Office: **Keasling has an office in Latimer Hall, Arkin in the Melvin Calvin Laboratory, and Jenkins in Davis Hall.**

Other: ____________________

MAJOR EQUIPMENT: List the most important items available for this project and, as appropriate identifying the location and pertinent capabilities of each.

The Keasling laboratory has a new Molecular Dynamics Typhoon imaging systems that will be used extensively for this project.

High-end computational and databasing facilities dedicated to biocomputation are available at the National Energy Super Computer Center at LBNL. In addition, we have limited access to microarray and microscopy facilities.

OTHER RESOURCES: Provide any information describing the other resources available for the project. Identify support services such as consultant, secretarial, machine shop, and electronics shop, and the extent to which they will be available for the project. Include an explanation of any consortium/contractual arrangements with other organizations.

Continuation Page:

LABORATORY FACILITIES (continued):

chromatographs, a Hewlett-Packard HPLC with mass spectrometry detector, peristaltic pumps, fractions collectors, columns, flo-thru UV monitor, incubators, waterbaths, water purification equipment, electrophoresis equipment, scintillation counter, computers and laserwriters, microcentrifuges, pipetmen, and other small equipment. Other equipment available in adjacent laboratories includes autoclaves, constant temperature rooms, HPLC, fermenters, a Coulter counter, microscopes, large-scale chromatography equipment, NMR, EPR, cell culture laboratories, glove box and other equipment for anaerobic cell culture, and media preparation facilities.

The Arkin research group has a laboratory of approximately 1300 square feet supplied with modern equipment required for current molecular biology: ultracentrifuge, high-speed centrifuge, UV/Vis spectrophotometer, balances, pH meters, -80 C freezer, -20 C freezer, chromatography refrigerators, thermocyclers, incubators, waterbaths, water purification equipment, electrophoresis equipment, Macintosh computers and laserwriters, microcentrifuges, pipetmen, and other small equipment. Other equipment available in adjacent laboratories includes autoclaves, constant temperature rooms, HPLC, shakers, radiation handling facilities, phosphorimagers, etc.

COMPUTER FACILITIES (continued):

and Color Printers. DNA sequence analysis software is available.

The Arkin laboratory is equipped with two silicon graphics 2 process R10000 octane computers, seven Pentium-based NT machines and a four node, 8 processor IBM SP2. In addition, there are access to three laserwriters and a Tektronix Phase 360 printer. The NT workstations are fully loaded with composition, drawing, and statistical data analysis and development software. The SGI computers are set up with MYSQL databases and APACHE web-servers and a complete development environment.