REPORT DOCUMENTATION PAGE

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Service, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188) Washington, DC 20503.

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) 2. REPORT TYPE 3. DATES COVERED (From - To)

4. TITLE AND SUBTITLE
Syngenomics Applied to the Tryptophan Biosynthetic Pathway

5a. CONTRACT NUMBER
N00014-01-1-0148

5b. GRANT NUMBER
N00014-01-1-0148

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)
Miller, Jeffrey H.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California, Los Angeles
Office of Contract and Grant Administration
10920 Wilshire Blvd., Suite 1200
Los Angeles, CA 90024-1406

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

10. SPONSOR/MONITOR'S ACRONYM(S)
ONR

11. SPONSORING/MONITORING AGENCY REPORT NUMBER

12. DISTRIBUTION AVAILABILITY STATEMENT
Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
We have initiated a long range project aimed at engineering microorganisms with multiple new capacities, using *Escherichia coli* as the prototype organism. Our approach is to determine which organisms can serve as direct donors of genetic material, in that their genes are expressed by the *E. coli* cell, and which require additional engineering. We are developing methods for incorporating up to 300 kb fragments into the *E. coli* chromosome to allow whole pathways to be encoded by foreign DNA. Also, we are looking for dramatic phenotypic differences in *E. coli* generated by cloned fragments from foreign organisms, using the mutator phenotype as an example. We have identified genes from *Lactococcus lactis* and *Pseudomonas aeruginosa* that cause mutator phenotypes when overexpressed in *E. coli* and interestingly, one of these encodes a regulator for multiple drug resistance.

15. SUBJECT TERMS
New capacities, Foreign DNA, Mutator phenotypes, Gene expression

16. SECURITY CLASSIFICATION OF:
a. REPORT Unclass.
b. ABSTRACT Unclass.
c. THIS PAGE Unclass.

17. LIMITATION OF ABSTRACT
UL

18. NUMBER OF PAGES
4

19a. NAME OF RESPONSIBLE PERSON
Jeffrey H. Miller

19b. TELEPHONE NUMBER (Include area code)
(310) 825-8450

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI-Std Z39-18
INSTRUCTIONS FOR COMPLETING SF 298

1. REPORT DATE. Full publication date, including day, month, if available. Must cite at least the year and be Year 2000 compliant, e.g., 30-06-1998; xx-08-1998; xx-xx-1998.

2. REPORT TYPE. State the type of report, such as final, technical, interim, memorandum, master's thesis, progress, quarterly, research, special, group study, etc.

3. DATES COVERED. Indicate the time during which the work was performed and the report was written, e.g., Jun 1997 - Jun 1998; 1-10 Jun 1996; May - Nov 1998; Nov 1998.

4. TITLE. Enter title and subtitle with volume number and part number, if applicable. On classified documents, enter the title classification in parentheses.

5a. CONTRACT NUMBER. Enter all contract numbers as they appear in the report, e.g. F33615-86-C-5169.

5b. GRANT NUMBER. Enter all grant numbers as they appear in the report, e.g. 1F665702D1257.

5c. PROGRAM ELEMENT NUMBER. Enter all program element numbers as they appear in the report, e.g. AFOSR-82-1234.

5d. PROJECT NUMBER. Enter all project numbers as they appear in the report, e.g. 1F665702D1257; ILIR.

5e. TASK NUMBER. Enter all task numbers as they appear in the report, e.g. 05; RF0330201; T4112.

5f. WORK UNIT NUMBER. Enter all work unit numbers as they appear in the report, e.g. 001; AFAPL30480105.

6. AUTHOR(S). Enter name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. The form of entry is the last name, first name, middle initial, and additional qualifiers separated by commas, e.g. Smith, Richard, Jr.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES). Self-explanatory.

8. PERFORMING ORGANIZATION REPORT NUMBER. Enter all unique alphanumeric report numbers assigned by the performing organization, e.g. BRL-1234; AFWL-TR-85-4017-Vol-21-PT-2.

9. SPONSORING/MONITORS AGENCY NAME(S) AND ADDRESS(ES). Enter the name and address of the organization(s) financially responsible for and monitoring the work.

10. SPONSOR/MONITOR'S ACRONYM(S). Enter, if available, e.g. BRL, ARDEC, NADC.

11. SPONSOR/MONITOR'S REPORT NUMBER(S). Enter report number as assigned by the sponsoring/monitoring agency, if available, e.g. BRL-TR-829; -215.

12. DISTRIBUTION/AVAILABILITY STATEMENT. Use agency-mandated availability statements to indicate the public availability or distribution limitations of the report. If additional limitations/restrictions or special markings are indicated, follow agency authorization procedures, e.g. RD/FRD, PROPIN, ITAR, etc. Include copyright information.

13. SUPPLEMENTARY NOTES. Enter information not included elsewhere such as: prepared in cooperation with; translation of; report supersedes; old edition number, etc.

14. ABSTRACT. A brief (approximately 200 words) factual summary of the most significant information.

15. SUBJECT TERMS. Key words or phrases identifying major concepts in the report.

16. SECURITY CLASSIFICATION. Enter security classification in accordance with security classification regulations, e.g. U, C, S, etc. If this form contains classified information, stamp classification level on the top and bottom of this page.

17. LIMITATION OF ABSTRACT. This block must be completed to assign a distribution limitation to the abstract. Enter UU (Unclassified Unlimited) or SAR (Same as Report). An entry in this block is necessary if the abstract is to be limited.
FINAL REPORT

Grant #: N00014-01-1-0148

PRINCIPAL INVESTIGATOR: Dr. Jeffrey H. Miller

INSTITUTION: University of California, Los Angeles

GRANT TITLE: Syngenomics Applied to the Tryptophan Biosynthetic Pathway

AWARD PERIOD: 13 November 2000 - 30 November 2002

OBJECTIVE: To add new capacities to a standard microorganism, *Escherichia coli* by incorporating genes from different sources.

APPROACH: We have several approaches to reach the first milestone in a long ranging project. The first involves finding out which foreign organisms can express their DNA in *E. coli* without additional genetic engineering. In order to achieve this we decided to use the tryptophan synthetase A gene as a model gene to express and monitor. The second approach is to develop methods to incorporate large segments of foreign DNA into *E. coli*, initially via plasmids and subsequently as part of the chromosome. A third approach is to screen foreign genomes for DNA segments that when expressed in *E. coli* show dramatic effects.

ACCOMPLISHMENTS: First, we tested the expression of tryptophan synthetase A in *E. coli* from different genomic sources by examining cloned DNA fragments. We monitored the ability of each cloned gene to complement a tryptophan synthetase A deficient strain of *E. coli*. The microorganisms that by this and other criteria were able to express their genes with their own promoter operating in *E. coli* were: *Campylobacter jejuni*, *Lactococcus lactis*, *Helicobacter pylori*, and *Pseudomonas aeruginosa*. Those failing to express in *E. coli* under their own promoter include: *Caulobacter acetobutylicum*, *Aquifex aeolicus*, *Bacillus subtilis*, and *Haemophilus influenzae*.

We have completed a bioinformatics study of the existing sequenced microbial genomes, currently 86, including one sequenced in our laboratory. We researched the biosynthetic pathways to generate a database of the potential metabolic engineering pathways compatible with *E. coli*. From this we determined that ultimately, finding ways of incorporating large DNA fragments into *E. coli* is the best way to engineer strains with new multiple capacities. We are therefore
developing methods to generate *E. coli* strains with large inserts of foreign DNA. In addition to chromosome integration systems acquired from other investigators, we have also developed our own plasmid, pHybrid, that is a modified bacterial artificial chromosome vector. This would allow us to incorporate 100-300kb segments of foreign DNA into the chromosome via gene replacement of the 100 genes covered by a viable gpt-lac deletion. Initially, we have generated BAC clones from *Lactococcus lactis* genomic DNA (the entire sequence of *L. lactis* is known) as a proof of principle, with segments of up to 50 kb, sequenced the ends, and are now putting these into the *E. coli* chromosome.

As an additional approach, we screened Sau3A1 partial digest libraries, 3-5 kb in length, cloned into a multicopy vector, for expression in *E. coli* that resulted in dramatic phenotypic differences. We focused initially on those that created mutator effects by using an indicator strain that generates blue papillae in response to frameshift mutators. We examined large numbers of clones generated from the genomic digests of both *Lactococcus lactis* and *Pseudomonas aeruginosa* and found several clones from each organism that produced mutator effects. Sequencing these clones revealed the identity of the genes that increased the *E. coli* mutation rate when overexpressed in *E. coli*. The genes from *Lactococcus lactis* that showed these effects were the uvrA gene, rnhA, an unassigned open reading frame, and a truncated dnaA gene. The clones from *Pseudomonas aeruginosa* that created the mutator phenotype all expressed the nfxB gene, a regulator of a multi-drug resistance pathway. This latter result has some provocative implications.

**CONCLUSIONS:** DNA from a variety of organisms can be expressed in *Escherichia coli*, providing a treasury of genes from which to use to build new capacities for a multi-potent microorganism. Although additional engineering can allow the use of genes and sets of genes from an even wider array of microorganisms, this additional step or steps is not required at the present time. Analysis of pathways from many different sequenced genomes indicates that bringing large fragments of genomes (50-300 kb) into an organism such as *E. coli* is required to provide whole pathways for metabolic engineering. Genes from other organisms can generate interesting phenotypes in *E. coli* that can be used in screening and selection, as was done for mutators arising from inserting *Pseudomonas aeruginosa* and *Lactococcus lactis* DNA. This can be used as a shortcut for finding important genes.

**SIGNIFICANCE:** Our studies have laid the groundwork for building microorganisms with increased capacities, using *E. coli* as the sample microorganism. Genes from other
microorganisms can express important phenotypes, and methods for incorporating very large fragments can outline a plan for introducing multiple capacities into a single organism. The finding that expressing a regulator of multidrug resistance from another organism, in this case the nfxB gene from 
Pseudomonas aeruginosa, creates a mutator phenotype in 
Escherichia coli is tantalizing, since it suggests a relationship between acquisition of drug resistance and the induction of high mutation rates that may have profound implications for antibiotic use and disease control.

PATENT INFORMATION: None

AWARD INFORMATION: None

PUBLICATIONS: None