

This article describes the development of a curriculum in a hands-on biomanufacturing laboratory environment. Data are presented on the production, purification, and analysis of a recombinant protein performed by undergraduate students at James Madison University.

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Development of Biotechnology Curriculum for the Biomanufacturing Industry

by Robert L. McKown, PhD and George L. Coffman, PhD

Introduction

The biotechnology industry has come of age and is delivering on its promise to produce new drugs for the treatment of human disease. The biopharmaceutical product development pipeline is full, and the demand for new manufacturing capacity is expected to triple in the next five years. As the industry gears up for increased production, the demand for a skilled workforce to manufacture these products also will increase. The unique technological skills required for biomanufacturing are not readily found in traditional academic programs.

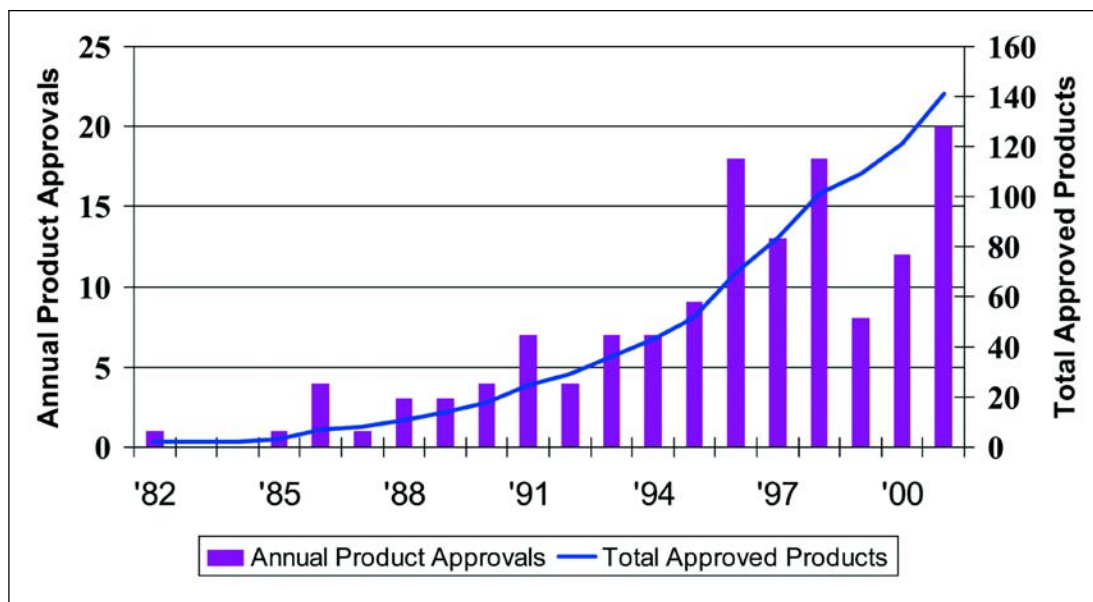
Historical Perspective

The term “biotechnology” was first introduced in 1917 by Karl Ereky and defined as “All lines of work by which products are produced from raw materials with the aid of living things.”¹ Although the use of living systems to make a product has a long and established history, the modern definition of biotechnology is usually associated with genetic engineering and recombinant DNA technology. G. Steven Burrill, CEO, Burrill & Company (San Francisco, CA), de-

fining biotechnology as “Recombinant genetic engineering...using biological processes to develop products,” thereby preserving the original notion that biotechnology uses living systems to make products. Stanley Cohen and Herbert Boyer published the founding principles of recombinant DNA technology in 1973,² and in 1980, a US patent was issued describing a “Process for Producing Biologically Functional Molecular Chimeras.” On April 7, 1976, the first independent biotechnology company, Genentech, Inc. (South San Francisco, CA), was founded to commercialize the newly discovered technology and the adventure began.³

Although many applications of recombinant DNA technology were considered, the first biotech product created with this technology was human insulin (Humulin) produced in the bacteria *Escherichia coli* to treat diabetes. Lacking manufacturing capabilities, Genentech licensed recombinant insulin to Eli Lilly and Company (Indianapolis) for production and marketing. Humulin was a major success (worldwide sales in 1999 reached \$880 million) and set a paradigm that is still the driving force of the

Figure 1. Annual biopharmaceutical product approvals from 1982-2001.



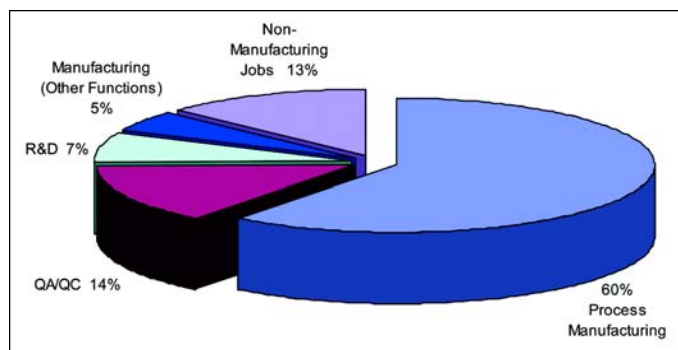


Figure 2. Distribution of the scientific and technical workforce in a typical biomanufacturing facility.

biotech industry today. Brandon Price, President, Goodwin Biotechnology, Inc. (Plantation, FL) captured the focus of the industry in the statement “The promise of biotechnology is the bioproduction of drugs so complex they can only be made in living systems.”

As the first human therapeutic products generated through recombinant DNA technology began to emerge from research laboratories, it became apparent that a manufacturing infrastructure was needed to produce material for clinical trials and the commercial market. Unlike traditional pharmaceutical drugs that use chemical synthesis to make product, most biopharmaceutical drugs require viable biological host cells for the production of recombinant proteins. Using living systems as vehicles for manufacturing human therapeutics introduced issues of reproducibility, product identity, product purity, product potency, and possible contamination with human pathogens. These issues also resulted in new federal regulations governing the manufacture of biopharmaceuticals (biologics) that are still evolving. The first biopharmaceutical products to enter clinical trials utilized the established manufacturing technology of microbial fermentation; however, it soon was realized that certain products require mammalian cells grown in culture to produce a biologically active molecule. Although mammalian cell culture technology has an established history in laboratory research, it had never been scaled up to manufacture the quantities of product needed for commercial use. In addition, new purification technologies were needed to isolate a protein of interest from the complex mixture of molecules found within the cell. The unique requirements for production of pharmaceuticals in living systems presented a challenge to invent new manufacturing technologies. The early pioneers of the biotechnology industry not only discovered and cloned new genes; they also invented the technology needed to bring these products to market and in doing so provided a foundation for the biomanufacturing industry.

In the last five years, the biotech industry has come of age and is delivering on its promise to produce new drugs for the treatment of human disease. Since the approval of recombinant insulin in 1982, more than 100 new biopharmaceutical products have been approved for the market (Figure 1) and the pipeline is full. Approximately 400 new drugs requiring biological systems for production are in clinical development and the recent sequencing of the human genome combined with high throughput screening technologies will fuel the pipeline well into the future. As more new drugs enter the product development pipeline, additional biomanufacturing capacity will be needed to bring these products to market. Recent analysis suggests that demand for manufacturing capacity for protein-based drugs will triple in the next five years.⁴ Cer-

tainly, the industry will respond and build the infrastructure needed to meet market demand, but bricks, mortar, and stainless steel tanks are only half the equation. A trained and technologically skilled workforce will be needed to execute the complex process of manufacturing a biopharmaceutical product.

Biotechnology Education

Biomanufacturing is a labor-intensive endeavor requiring unique skills that are not readily found in traditional academic programs. A common error made by academics, especially in the biological sciences, is that biotechnology means just molecular and cell biology.⁵ While molecular and cell biology may provide a solid foundation for the research side of the biotech industry, additional highly technical skill sets are needed for the manufacturing side of the industry. Figure 2 shows the distribution of the scientific and technical workforce in a typical large biomanufacturing facility.⁶ More than 90% of the jobs are in production-related areas while only 7% are in research and development. The biotech industry was founded on technology derived from basic academic research; however, it is the application of this technology that fuels the industry. The challenge in developing new curriculum in biotechnology is to find the correct balance between basic science education and the application of this knowledge to develop a market product.

Development of biotechnology curriculum is problematic in that it is an applied science rather than a basic science. Although techniques founded in biotechnology permeate research protocols in the life sciences, very few academic programs are devoted to biotechnology. In a recent survey of college programs, only 15 Bachelor of Science degree programs in biotechnology could be identified in the United States.⁷ Although a few highly specialized training programs in bioprocessing have been established, biomanufacturing programs in the traditional academic sense are virtually nonexistent. This fact does not bode well for the biotech industry at a time when record numbers of new product candidates are entering the pipeline and manufacturing capacity is expanding.

A New Approach to Higher Education

In 1993, an innovative approach to higher education was launched with the enrollment of the first freshman class in the Integrated Science and Technology Program (ISAT) at James Madison University.⁸ In response to a national call for fundamental change in science education, the ISAT Bachelor of Science degree program was created. A new curriculum was designed to provide students with a breadth of knowledge and skills across a variety of scientific and technological disciplines. Formal training in collaborative and leadership methods, problem-solving techniques from many disciplines, and use of the computer as a problem-solving tool were integrated into the curriculum. Strategic sectors were identified that reflect national critical technologies and include Biotechnology, Energy, Engineering and Manufacturing, Environment, Information and Knowledge Management, Health Systems, and Telecommunications. Highly qualified faculty members with industry experience in each of these strategic sectors were recruited to develop and teach this novel curriculum.

In May of 2001, the fifth ISAT class graduated and the program currently enrolls more than 800 students. By every index, the ISAT program has been a success. ISAT graduates are recruited for positions that are often filled by graduates of



Figure 3. JMU students working on a production campaign in the biomanufacturing laboratory. Clockwise from top right; Dr. George Coffman and Curtis Jones, Maria Scherer (front) and Jo Maillet, Melissa Orr, Carl Randecker and Laura Pillor (front), and Megan Barber and Kevin Carlton (rear).

the traditional sciences, engineering, and business programs. Employers have been particularly impressed with the interdisciplinary skills and the project-orientated team approach to problem solving of ISAT graduates. It is in this environment of applied interdisciplinary education that the notion of creating a new curriculum in biomanufacturing has evolved.

The Biomanufacturing Laboratory at JMU

In considering the development of a new curriculum in biomanufacturing, it became apparent that experiential laboratory work would be critical for effective education, and the idea of a functional facility for the production and purification of recombinant proteins emerged. Planning for a Biomanufacturing Laboratory at JMU began in 1997 as an integral component of a proposed Center of Manufacturing Innovation to be located on the JMU campus. In 1998, Virginia's Manufacturing Innovation Center⁹ was founded and awarded a Center Grant from Virginia's Center for Innovative Technology.¹⁰ In 1999, the College of Integrated Science and Technology (CISAT) at JMU dedicated 2,300 square feet of wet laboratory space in three adjoining rooms for the creation of a functional biomanufacturing facility. CRB Consulting Engineers (Cary, NC) became a partner in the project and provided both design plans for the laboratory layout and a detailed list of equipment needs. JMU students were engaged to participate

in the project and played an active role in the design and set-up of the laboratory. An ISPE JMU Student Chapter was founded and students attended training workshops and participated in international meetings.

In the fall of 2001, the Biomanufacturing Laboratory became operational. The laboratory has established capabilities for cloning and expressing genes in bacterial systems, pilot-scale fermentation, purification of recombinant proteins, and analytical testing - *Figure 3*. In addition, the laboratory supports basic and applied research in the areas of molecular biology, genetics, and biochemistry. Besides education and workforce development, the Biomanufacturing Laboratory offers pilot-scale production of recombinant proteins for research and product development on a contractual basis. Four ISAT faculty members currently use the facility for basic and applied research in the areas of biomanufacturing process and product development.

Curriculum Development: The Production Campaign

The production "campaign" is the manufacturing strategy of choice for most biopharmaceuticals. With this strategy, a production run is carefully planned from beginning to end. Genetically engineered cells are retrieved from a master cell bank, grown to produce a working cell bank, and tested for production

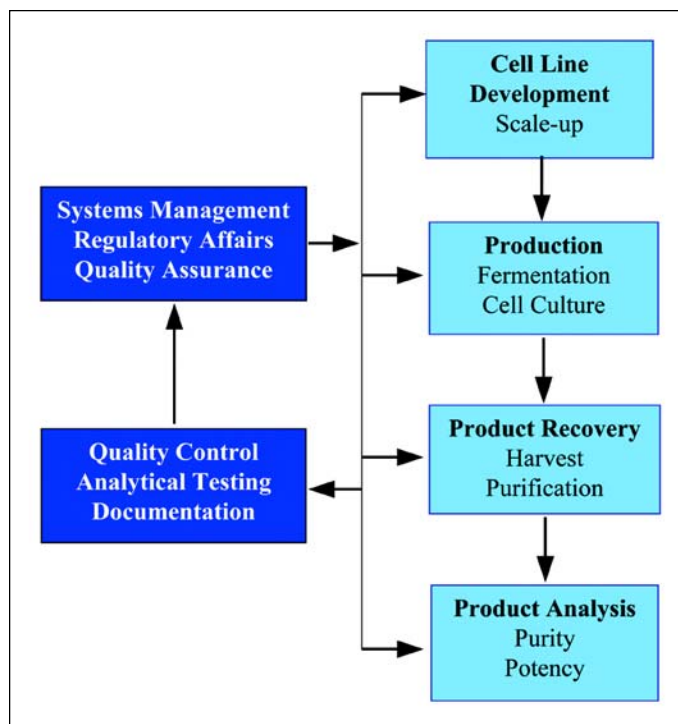


Figure 4. Components and organizational structure of the production platform and management systems in the biomanufacturing laboratory.

capabilities. The facilities and equipment are sanitized and validated. Media are prepared, the production platform is set up, and the entire process is tested and validated. The cell line is scaled up in increasing increments to inoculate the bioreactor. Cell growth within the bioreactor is carefully monitored, and at a precise moment, the cells are induced to express the protein product. The product is then harvested and isolated through multiple separation steps to achieve a high degree of purity. The production campaign itself is a highly orchestrated event with numerous checkpoints for quality control and quality assurance. The final product is subjected to an array of analytical tests and quarantined for safe storage. The entire process is carefully documented to assure quality control and regulatory compliance with current Good Manufacturing Practices (cGMP). Experiencing a production campaign is critical in understanding the purpose and function of each step involved and was a focal point in the development of a new curriculum.

For the purpose of curriculum development, the production campaign can be divided into a number of distinct interrelated components that are shown in Figure 4. The production train is a sequential series of steps that begin with the cell line, move through production, product recovery, and end with a purified product. Each of these steps is monitored for compliance with Standard Operating Procedures (SOPs), and product development is recorded with analytical testing for quality control. Detailed documentation is collected for regulatory compliance and standards are set for quality assurance. A systems management structure provides oversight for the entire manufacturing process.

A primary goal for any new curriculum development is to place abstract concepts in the context of practical applications. The biomanufacturing laboratory is the classroom, and the students are active players in the manufacturing process. The pedagogical approach involves the formation of teams to plan and execute specific aspects of the manufacturing process. Five

teams with two to four members each are organized into the task related categories of cell line development, production, product recovery, product analysis/quality control, and systems management/regulatory affairs. Each team develops a detailed plan of action for the execution of their specific task and then the teams coordinate their plans to develop a campaign protocol. In the process of developing this protocol, teams learn how to create and follow SOPs, how to operate and monitor sophisticated equipment, and how to document the process for cGMP compliance. Troubleshooting and problem solving become an integral part of the learning experience. The production campaign is the high point of the learning experience and is executed according to the protocol. Following the production campaign, the student teams prepare written and oral reports for the class to review and analyze. In this manner, the students gain a global perspective of the manufacturing process and understand the role and importance of the various components.

Proof of Concept: The Green Fluorescence Protein (GFP)

A challenge in developing any biotechnology laboratory curriculum has been visualization of the molecular processes involved. It usually requires a leap of faith to believe that DNA has been cloned, genes have been expressed, and proteins have been purified when the evidence is bands on a gel or numbers on a graph. In considering a gene to clone, express, and purify for an educational experience in biomanufacturing, the Green Fluorescence Protein (GFP) from the jellyfish *Aequorea victoria* became an ideal candidate. The bright green fluorescence of the jellyfish is a distinctive phenotypic characteristic. Cloning and expression of a single gene transfers this phenotype to bacteria and the protein can be visualized throughout the purification process - Figure 5.

E. coli strain HB101 was transformed with pGLO, an ampicillin-resistance conferring plasmid in which the gene for GFP is under the control of the arabinose operon. Ampicillin resistance, restriction mapping, and visualization of bacterial colonies under long-wave ultraviolet light established proof of successful transformation. A master cell bank was created and stored for future use. A working cell bank was developed and scaled up for a production campaign in a 10-liter fermentation bioreactor. From the time of inoculation, the cells were induced to produce GFP by the addition of L-arabinose. The culture was grown to late log phase, and the cells were harvested by centrifugation and lysed by sonication. After the removal of cell debris by high-speed centrifugation, solid ammonium sulfate was added to the cleared lysate to 45% salt saturation. Precipitated proteins were removed by centrifugation, leaving a supernatant which glowed bright green under long-wave UV. This supernatant was loaded onto a hydrophobic interaction chromatography column, the column was washed with 1.5 M ammonium sulfate solution to remove the non-binding protein fraction, and the GFP was eluted with a linear salt gradient from 1.5 M ammonium sulfate to 10 mM Tris-HCl, 10 mM EDTA, pH 8. Fractions containing GFP were pooled and loaded onto a chromatography column, and proteins were eluted with a solution of 10 mM Tris-HCl, 10 mM EDTA (pH 8.0) - Figure 6. The crude cell lysate and the two chromatography purification steps were analyzed by SDS polyacrylamide gel electrophoresis - Figure 7. The entire production process was documented and reviewed by the campaign teams.

In recent work, the gene encoding GFP was cloned into an

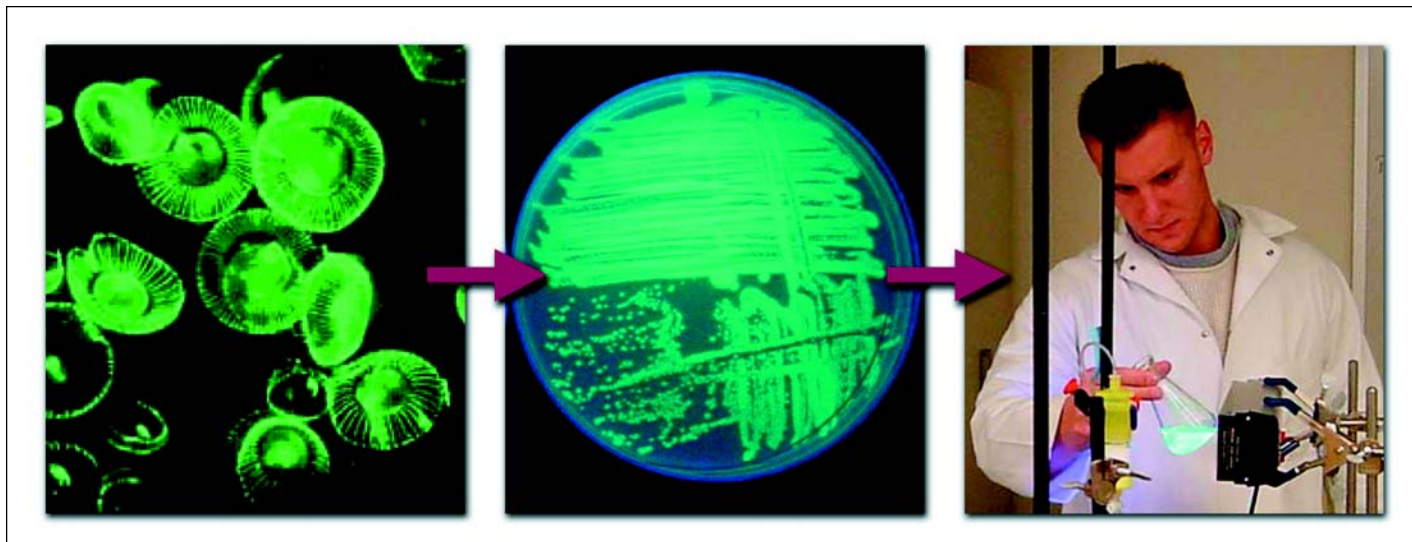


Figure 5. Cloning, expression, and purification of the Green Fluorescence Protein from the jellyfish *Aequorea victoria*.

inducible expression vector and was transformed into *E. coli*. Small-scale cultures were grown, and after cell lysis by sonication, crude (cleared) lysate was loaded onto a small column of chitin beads. After column washing, the chitin-bound intein-GFP hybrid protein was treated with a reducing agent and allowed to self-cleave. Finally, GFP was eluted from the column, and its purification was demonstrated by SDS-PAGE. A scaled up production and purification of GFP campaign is planned to allow further student experience in the art of protein production and downstream processing.

Over the past year, eight ISAT students have been working in three separate teams to develop and execute the GFP production protocol as part of their senior project requirement for graduation. The hydrophobic interaction chromatography step of the GFP purification protocol has been integrated into an ISAT laboratory course (ISAT 305) and performed by 114 ISAT students during the fall, 2001 semester. The entire campaign production curriculum will be introduced as a laboratory component to an existing ISAT lecture course (ISAT 451, Biotechnology in Industry and Agriculture) in the fall, 2002 semester that services approximately 48 students annually. A new ISAT course in biomanufacturing that includes cGMP regulatory issues will be introduced in the spring, 2003 semester. Biomanufacturing education has become an important component of the biotechnology concentration within the ISAT program. The Bachelor of Science ISAT degree program currently enrolls more than 800 students and approximately 170 students will be awarded a degree this year.

Summary

The promise of biotechnology to discover and produce a new generation of drugs to treat human disease is now a reality. The first 100 biopharmaceutical products to reach the market have demonstrated that the notion of manufacturing biological molecules in living systems to produce human therapeutic agents is a viable alternative to chemical synthesis.

The product development pipeline is full and the biotech industry is expanding manufacturing capacity to meet production demands. The rapidly evolving technology of biomanufacturing and the increasing demands for capacity present a challenge and an opportunity for the development of new curricula. The Integrated Science and Technology Program at James Madison University has taken an experiential

approach to biomanufacturing education with the development of a functional bioprocessing facility. The biomanufacturing laboratory is the classroom and the students have taken an active role in the design process, set up, and development of a new curriculum.

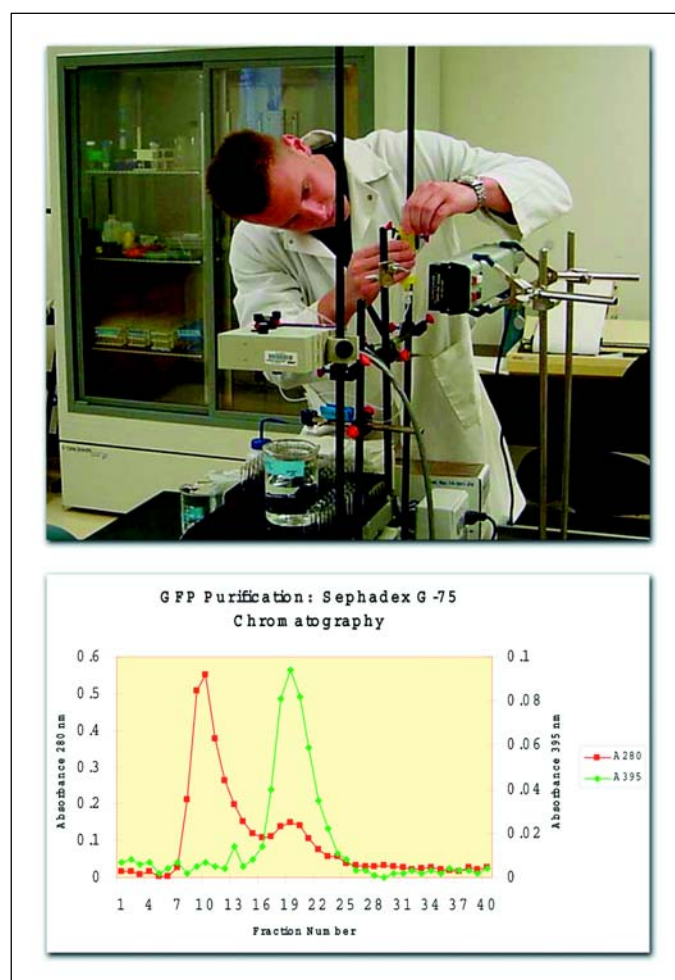


Figure 6. Purification of Green Fluorescence Protein with a chromatography column. JMU student Luke McGinty.

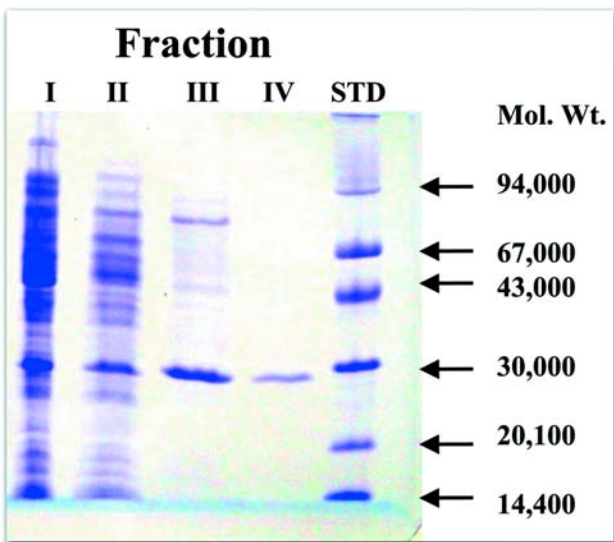


Figure 7. SDS Polyacrylamide Gel Electrophoresis of the purification fractions of GFP. JMU student Megan Barber.

References

1. Glick, B. R. and Pasternak, J. J. 1998. *Molecular Biotechnology; Principles and Applications of Recombinant DNA*, 2nd Ed., American Society for Microbiology, publishers, Washington, DC, 5-6.
2. Cohen, S., A. C. Y. Chang, H. W. Boyer, and R. B. Helling, 1973. Construction of Biologically Functional Plasmids in Vitro. *Proc. Natl. Acad. Sci USA* 70: 3240-3244.
3. <http://www.genentech.com>.
4. Odum, J. N. 2001. Biotech Manufacturing: Is the Crisis Real? *Pharmaceutical Engineering*, 21: 22-33.
5. Dahms, A. S. 2001. The US Biotechnology Industry: The Importance of Workforce Quality in the Maintenance of Corporate Competitive Advantage. *Biochemistry and Molecular Biology Education*, 29 206-208.
6. Windows on the Workplace. 1997. A report published by North Carolina Biotechnology Center.

7. The College Board College Handbook 2001, 38th Edition. College Board Publications, New York, NY.
8. <http://www.isat.jmu.edu>
9. <http://www.vmic.org>
10. <http://www.cit.org>

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About the Authors



Robert L. McKown, PhD is a Professor of Biotechnology in the Department of Integrated Science and Technology at James Madison University. He received his PhD in molecular biology and biochemistry from the University of California at Irvine and completed a Postdoctoral Fellowship in molecular genetics at the University of California, San Francisco.

As a Senior Research Scientist for DNA Plant Technology in Oakland, California, he worked on the development of genetically engineered bacteria, yeast, and plants expressing foreign genes. In 1994, he took a faculty position in the Department of Biological Sciences at California State University, Hayward and became a consultant to the biotechnology industry. In 1996, he accepted a faculty position at James Madison University to develop a biotechnology program for the new College of Integrated Science and Technology. He is currently a member of the Board of Directors for the Virginia Biotechnology Association and faculty advisor to the JMU Student Chapter of ISPE. He can be contacted at: 1-540/568-2776, mckownrl@jmu.edu



George L. Coffman, PhD is an Assistant Professor in the Department of Integrated Science and Technology at James Madison University. In addition to being Laboratory Coordinator for the department, he is involved with method development and student training in the “downstream processing” aspects of the Biomanufacturing Laboratory. Prior to coming to James Madison, he was president and analytical chemist for an environmental testing laboratory. He holds an MS from Clemson University and a PhD from the University of Alabama in Birmingham, both in microbiology. He can be contacted at: 1-540/568-2767, coffmagl@jmu.edu.

James Madison University, CISAT MSC 4102, Harrisonburg, VA 22807. 