Experimental Section

Recombinant DNA Techniques — A Laboratory Course

H STEWART HENDRICKSON* and JOHN GIANNINI†

** Department of Chemistry*
and
† Department of Biology
St Olaf College
Northfield, MN 55057, USA

Introduction

The academic calendar at St Olaf College includes a 4-week interim in January when students take just one course. This term is ideally suited for an intensive laboratory course since students can spend successive full days in the lab. They are able to do experiments which are not feasible during the regular term when the lab may meet only once or twice a week for three- to four-hour periods. This is certainly true for recombinant DNA techniques where cells have to be grown up one day and then harvested and the DNA isolated the next. Many techniques require large blocks of time and have to be done on successive days. In designing a course on recombinant DNA techniques, we made use of this unique term to allow students to experience an intensive research-like laboratory project. The first course was taught during January, 1992, and was repeated in 1993 with minor revisions. We designed the course so that students would work on an integrated project to answer a specific question (is a particular gene present in different bacteria?). Students were involved in all aspects of the laboratory, from making up all the solutions to setting up all the experiments. We wanted the students to learn some basic techniques in the context of a research experience.

One of us (HSH) uses the expression plasmid, pIC,[1] in his research to obtain large amounts of recombinant phosphatidylinositol-specific phospholipase C (PI–PLC) from *Bacillus cereus* in an *E coli* host. The objective of this laboratory course was to transform this plasmid into *E coli*, isolate large amounts of plasmid, cut out the PI–PLC gene with restriction endonucleases, purify it by gel electrophoresis, and make a biotinylated probe. This probe would then be used in a Southern blot and hybridization to determine the presence of a similar gene in the genomic DNA of other bacteria.

Educational Objectives

Our main goal for this course was to give students a realistic idea of what it is like to work in a molecular biology laboratory. Semester-long laboratory courses usually meet only once or twice a week for only four hours a day. This greatly limits the type of experiments students can do and students lack a sense of continuity in what they are doing. Efficient use of laboratory time necessitates making up of reagents and setting up experiments for the students. We wanted our students to be involved in all aspects of their laboratory work and to achieve a sense of self-sufficiency. By meeting every day for 6–8 hours the students were exposed to a realistic laboratory situation and were able to focus completely on what they were doing. In addition, we wanted to expose our students to as many current molecular biology techniques as possible. This was facilitated by our morning lectures and discussions.

Organization of the course

The class met for 1.5 h each morning for lecture and discussion of protocols, and the remainder of the day was spent in the laboratory. *Basic Methods in Molecular Biology* by Davis *et al*[2] was used as the laboratory text and *Recombinant DNA* by Watson *et al*[3] was used as the lecture/discussion text. The lecture/discussion topics are listed in Table 1. We began with a discussion of DNA structure and later covered various aspects of molecular genetics and genetic engineering. During the last half hour we often discussed the experiment we would do in lab that day. The laboratory schedule is given in Table 2. The first day was devoted to organization, cleaning and sterilizing glassware. The next day we did an exercise on the use of pipetting devices and the spectrophotometric determination of DNA. On the third day the students made up most of the solutions and then began the project. Eight students were enrolled in the course and they worked in pairs on the project.

Table 1 Lecture/discussion topics

Day	*Topic*
1, 2	DNA structure and replication
3, 4	The genetic code, transcription and translation
5	Enzyme tools for genetic engineering
6	*E coli* cloning vectors
7, 8	Gene cloning and construction of pIC plasmid
9	The polymerase chain reaction
10	Site-directed mutagenesis
11	DNA diagnostics
12	DNA profiling and forensics
13	Mapping and cloning human disease genes
14	The human genome project

Table 2 Laboratory Schedule

Day	*Experiment*
1	Organize lab, clean and sterilize glassware
2	Measurement exercise (pipetting and spectrophotometry)
3	Prepare stock solutions, start overnight *E coli* culture
4	Prepare competent cells, transformation
5	Plasmid mini-prep, reculture transformed cells, glycerol stocks
6	Restriction mapping, agarose gel electrophoresis
7–9	Large scale alkaline lysis plasmid prep
10	Restriction cutting and gel electrophoresis of PI–PLC gene
11	Isolation of gene from gel (GeneClean)
11	Nick translation — prepare biotinylated probe
12	Prepare genomic DNA from bacterial cells
13–15	Southern blot and hybridization
16–18	Prepare posters

Equipment

Most of the equipment needed for this course is common to many biochemistry or cell biology laboratories. We had an incubator with an orbital shaker for growing cells. Five-ml cell cultures were routinely grown in 18 mm × 150 mm test tubes set at an angle of 20° on the orbital shaker for proper aeration. A hood was used as an area for sterile transfers and an autoclave was available for sterilizing solutions and glassware. A variety of water baths, with and without shakers, were also available. Several mini- and two medium-sized horizontal gel electrophoresis systems with power supplies were available along with a UV-transilluminator and Polaroid camera system for photographing gels. Two high-speed microcentrifuges (one located in the cold room), several clinical benchtop centrifuges, and a large refrigerated centrifuge (20 000 rpm) were used. Students used a variety of Rainin and Eppendorf automatic pipettors. A common refrigerator/freezer and a −70°C freezer were available

in addition to dry ice and crushed ice. Most of the equipment was available in a single laboratory where the students worked.

Experiments

Competent cells of *E coli* MM294 were prepared according to the procedure in Davis *et al*[2] (section 8–1) and transformed with the pIC plasmid. Cells were plated on LB/amp agar. Cells were recultured to mid log phase and glycerol stocks were made. A plasmid mini-prep was done according to the method of Grimberg *et al*[4] and the plasmids were characterized by restriction mapping (*Nhe*I, *BamH*I, and *EcoR*I) and agarose gel electrophoresis (Davis *et al*,[2] sections 5–4 and 5–5).

A large scale alkaline lysis plasmid preparation was done according to Davis *et al*[2] (section 8–3) using Sepharose chromatography to separate plasmid from small ribonucleotides. The plasmids were again characterized by restriction cutting and gel electrophoresis, and the DNA concentration was determined by UV spectroscopy. The PI–PLC gene fragment was cut from the plasmid using *BamH*I, and purified by agarose gel electrophoresis. The gene fragment was recovered from the gel using the Geneclean II kit.[5]

A biotinylated probe was prepared from the PI–PLC gene fragment using the BioNick labeling kit from GIBCO BRL.[6] Genomic DNA was isolated from *B thuringiensis* and *B subtilis* according to Unit 2.4 in *Short Protocols in Molecular Biology*.[7] The genomic DNA was digested with *BamH*I and electrophoresed on an agarose gel. The DNA was transferred to a nylon membrane by the Southern blot technique (Davis *et al*,[2] section 5–6) using 0.4 M NaOH as the transfer solution. Hybridization with the biotinylated probe and detection by a dye-coupled alkaline phosphatase reaction was carried out according to the protocol provided with the GIBCO BRL BluGENE Detection System.[8] A control was included with the intact pIC plasmid. Blue bands indicating hybridization were observed with the control and both *Bacillus* species.

Writing and Posters

We required students to keep a detailed lab notebook of all the procedures performed. We checked the notebooks weekly and made constructive comments. The students were responsible for turning in a weekly progress report which included a complete data set for each experiment and a summation of the results. A final full-length paper was required as a summation of the entire project. During the final week of class, we instructed students in the preparation of scientific posters. They then constructed posters and presented their projects at a poster session on the last day of class; other faculty and students of the Biology and Chemistry Departments were invited to view the posters.

Grading and Evaluation

We evaluated students on all aspects of the project. Lab notebooks, progress reports, final papers and posters were all graded. A final oral exam was required in which students were tested on their comprehension of the project they had just completed. We asked them to describe what they did in lab, why they did it, and the theory behind it. By far the most important aspect of the evaluation was their lab work; their enthusiasm and hard work in the lab were clearly the major criteria for grading.

Course Appraisal

This course involved very intensive interactions between the students and instructors. A ratio of eight students to two professors was not unreasonable since we were both quite busy every day. This course could also be run by one instructor. In their course evaluations the students spoke favorably of the small student/teacher ratio and individual attention. They learned a great deal in lab by being involved in all aspects of the work including making up solutions and setting up experiments; they had not had this experience in any other lab course outside of independent research. They particularly enjoyed preparing posters at the end of the term; they felt a great deal of satisfaction in putting their results together and having a tangible product of their work to display. After the poster session the posters were displayed for several weeks in the hallways of the Chemistry Department. Students enjoyed the relaxed atmosphere of the course. There were often periods of waiting during experiments when we would gather for informal discussions of diverse topics such as the ethics of genetic research; this was only possible under the unique interim schedule. Several students later obtained jobs directly because of the experience they had in this course.

Conclusions

The success of this course was due in large part to the interim schedule and the opportunity of the students to spend successive full days in the lab. We designed the course so that the students were involved in all aspects of their work. We set very modest goals and designed a project with a specific question to answer. The amount of work required fit nicely into the available time. We chose to focus on a specific plasmid, pIC, because one of us had experience with it in his research and had first-hand knowledge of its development at the University of Oregon. We could then give the students a personal history of this plasmid. A similar course could be set up with another plasmid, depending on the experience of the instructor. We carefully went through all the experiments ourselves before the course. We thus avoided any major problems; only minor problems were encountered due to student error. The authors will be happy to provide a copy of their laboratory manual containing all protocols and references on a floppy disk (PC/Word Perfect) to anyone interested. In the future we plan to develop another laboratory course using this same model on protein science.

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References

[1] Koke, J A, Yang, M, Henner, D J, Wolwerk, J J and Griffith, O H (1991) 'High-level expression in *Escherichia coli* and rapid purification of phosphatidylinositol-specific phospholipase C from *Bacillus cereus* and *Bacillus thuringiensis*', *Protein Expression and Purification* **2**, 51–58

[2] Davis, L G, Digner, M D and Battey, F F (1986) *Basic Methods in Molecular Biology*, Elsevier, New York

[3] Watson, J D, Witkowski, J, Gilman, M and Zoller, M (1992) *Recombinant DNA*, second edition, W H Freeman, San Francisco

[4] Grimberg, J, Maguire, S and Belluscio, L (1989) 'A simple method for the preparation of plasmid and chromosomal *Escherichia coli* DNA', *Nucleic Acids Research* **17**, 8893

[5] BIO 101, Inc, PO Box 2284, La Jolla, CA 92038–2284

[6] BioNick Protocol Labeling System no 8247SA, GIBCO BRL, PO Box 68, Grand Island, NY 14072–0068

[7] Ausubel, F M, Brent, R, Kingston, R E, Moore, D D Seidman, J G, Smith, J A and Struhl, K (1992) *Short Protocols in Molecular Biology*, second edition, Wiley, New York

[8] BluGENE Detection Systen no 8279SA, GIBCO BRL, PO Box 68, Grand Island, NY 14072–0068