Journal of Industrial Microbiology & Biotechnology (2000) 24, 339–344

www.nature.com/jim

Integrated cell biology/biochemistry/molecular genetics laboratories: the cytoplasmic genome projects

SD Verhey[1,2]

[1]*Visiting Faculty (Biology), The Evergreen State College, Olympia, WA 98505, USA*

This paper describes an integrated laboratory project for intermediate to advanced undergraduate students. The project spans an entire academic quarter (10 weeks) and involves a series of operations that give students experience with fundamental techniques in cell biology, molecular biology, biochemistry, genomics, and bioinformatics. In the process, the student learning community is strengthened, students gain increasing confidence in their abilities in the laboratory, and data are collected toward the eventual sequencing of a cytoplasmic genome. The culmination of the project is the preparation by students of a paper written in the format of a particularly accessible online journal. *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 339–344.

Keywords: mitochondrial DNA; mitochondrial genome; plastid DNA; plastid genome; undergraduate education; genomics

Introduction

Students learn better in situations where their interactions with the material under study, and with one another, are collaborative, connected, active, and applied. Numerous strategies are in use to deliver such experiences to students, from group contracts and complex coordinated study programs to linked courses and learning communities [8,22]. Group contracts are full- or nearly full-time (10–16 quarter hours), formal learning contracts between 15–25 students and one instructor. At The Evergreen State College (TESC), coordinated study programs typically involve 3–4 faculty members from diverse fields who collaborate to integrate material from each discipline in a way that increases student awareness of the interconnectedness of the information under study [8]. Because group contracts and coordinated study programs are typically full-time, there are few problems with scheduling events such as occasional day-long laboratory sessions and field trips. Learning communities involve efforts to increase the students' interactions with one another, to encourage co-teaching/learning, and can be employed with many types of instruction. Linked courses are an approach to applying the coordinated study concept at schools that use more traditionally organized and scheduled courses [22].

Because of demands on the instructor's time and the inherent complexity of the setting, the laboratory portion of a science course can be the place where it is the most difficult to create an optimal learning experience for students. As a result, students may find themselves working in confusion on disembodied, cookbook exercises. Such exercises can be particularly frustrating if they are not connected to the material presented in the lecture portion of the course, and if the exercises themselves do not 'work'. This state of affairs, along with various initiatives aimed at improving the quality of science education, has led to the rise of inquiry-based courses and laboratories [5,9,20].

Through inquiry-based laboratories, students learn how science is actually done, in addition to learning techniques and concepts important to a particular field of study. The use of inquiry-based laboratories in biology has lagged behind the physical sciences for a variety of reasons, including student lack of confidence, organizational skills, and subject background at the freshman level [5]. The problem is compounded in the intermediate-level study of cell biology, biochemistry, and molecular biology, since the techniques for study in these areas can be complicated and expensive, in addition to being much less conceptually direct than those found in some other disciplines, such as classical physics. For more advanced students, confidence and organizational skills may be less of a problem.

Increasingly rapid advances in the biological sciences, coupled with increasing industrialization, bring their own challenges. The graduating student may reasonably be expected by prospective employers to have hands-on experience with cell biology, biochemistry, and molecular biology, as well as with genomics and other relatively new areas. Such experience is likely to be the most useful to students if it is presented in an integrated fashion, either in a series of linked courses or in a single course taken near the end of their college career [1,14].

With these issues in mind, a coordinated series of laboratories, spanning several disciplines, for junior/senior level biology students was developed. The laboratories described have been employed twice in the past 2 years, in the group contracts 'Structure of life' (SOL, Winter 1998) and 'Molecule to Corporation' (M2Co, Spring 1999). The lecture portion of both courses generally covered biochemistry, cell biology, molecular biology, and biotechnology. Rather than ask the students to participate in a full open-ended inquiry, an environment that was similar to a bounded-inquiry or a guided-inquiry situation was provided [5]. This gave the students enough direction early in the project to allow them to build confidence and gradually take responsibility for

[2]Correspondence to SD Verhey, Assistant Professor, at his present address: Department of Biological Sciences, Central Washington University, 400 E 8th Avenue, Ellensburg, WA 98926–7537, USA. E-mail: verheys@cwu.edu
Received 2 April 1999; accepted 22 November 1999

more control later in the project. The projects involved isolating mitochondria from carrots (*Daucus carota* L, in SOL), or chromoplasts from daffodil flowers (*Narcissus* sp, in M2Co), then cloning the organellar DNA into a plasmid vector to prepare a genomic library. Students sequenced random clones from the library and analyzed their sequences using various bioinformatics tools.

The final project was a written report of their findings in the style of the *Electronic Plant Gene Register* (*EPGR*), an online publication of the American Society of Plant Physiologists [20]. Undergraduates often find reading primary literature in a jargon-intensive field such as molecular genetics to be daunting [7]. The *EPGR* is a particularly accessible publication for undergraduates because the articles are all relatively short, and all have similar themes. At the completion of this project, the students had actually completed most or all of the procedures described in a typical *EPGR* article. This greatly enhanced their ability to read the literature, as well as their sense of a broader scientific community.

At the time of these projects, neither the carrot mitochondrial genome nor the daffodil plastid genomes had been sequenced. Working together toward the common goal of eventually sequencing a novel genome gave the students a sense of the amount and type of work involved in genome-scale sequencing. More important, it linked a series of unrelated techniques in a way that illustrated the usefulness of each individual approach and stimulated thinking about other ways to use different methodology in research. Finally, this approach provided numerous opportunities for cooperation and collaborative problem-solving and built considerable *esprit de corps*.

Materials and methods

Approximately twenty students were involved in each project. All students were in the junior- or senior-level. Most had been introduced to basic techniques in laboratory biology during earlier courses, but none had significant experience with the techniques encompassed by the project. For most meetings, the group was split into two sections to minimize equipment demands and maximize each student's involvement in the project. During most weeks, each lab group met once for 3 to 4 hours.

Laboratory instructions were made available to students via the Internet approximately 5 days before laboratory work was to be carried out, and students were expected to have printed and read the instructions before class. Table 1 lists URLs for all laboratory instructions. Early in the project, instructions were fairly detailed and supervision was relatively close; later in the project students were expected to find protocols and execute them with more autonomy. Generally, the instructions included a discussion of safety issues specific to the work being performed. Unless otherwise noted, students carried out all procedures working in pairs.

All molecular biological enzymes and supplies were purchased from or were the generous gift of Promega Corporation (Madison, WI, USA). Other chemicals were of the highest quality available.

Table 1 WWW links to instructions for laboratory work and other activities

Week	Activity	URL
1	Overview	http://www.cwu.edu/~verheys/cpgp/laboverview.html
2	Cell fractionation via sucrose density centrifugation	http://www.cwu.edu/~verheys/cpgp/lab1.html
3	DNA isolation	http://www.cwu.edu/~verheys/cpgp/lab2.html
	Restriction digestion and ligation	http://www.cwu.edu/~verheys/cpgp/lab3.html
4	Bacterial transformation	http://www.cwu.edu/~verheys/cpgp/lab5.html
5	Plasmid DNA isolation, restriction digestion, agarose gel electrophoresis	http://www.cwu.edu/~verheys/cpgp/lab6.html
6	DNA sequencing reactions	http://www.cwu.edu/~verheys/cpgp/lab7.html
9	DNA sequencing analysis	http://www.cwu.edu/~verheys/cpgp/seqanal.html
10	Lab report assignment	http://www.cwu.edu/~verheys/cpgp/labreport.html
	Field trip to UWGC	http://www.genome.washington.edu/UWGC

Week 1

In SOL, Week 1 was used to familiarize the students with some aspects of the biology of mitochondria during a two-session workshop. In the first session the instructor introduced the project and a list of questions about mitochondria was handed out; students were dispatched in small groups to seek answers to the questions in the library. In the second session, the questions were used to shape a Socratic dialogue and discussion of mitochondrial biology. In M2Co, which involved students who had previously spent time learning about mitochondrial biology, Week 1 was used to introduce and discuss the project ahead, and to orient the students to laboratory equipment and safety.

Week 2

Carrots or daffodils were obtained from a local grocery store. In a day-long laboratory meeting, the organelles were extracted as follows.

Mitochondria were extracted from carrots using a modi-

Table 2 mtDNA ORFs identified by BLAST searches

Sequence	Organism with highest DNA homology [references]
Chloroplast NADH-ubiquinone oxidoreductase	*Mutisia acuminata* [10,18]
Mitochondrial cytochrome oxidase subunit 1	*Solanum tuberosum* [12,17]
Chloroplast DNA-directed RNA polymerase, subunit beta	*Nicotiana tabacum* [3,15]

fication of procedures described by Cooper [2], Patel [13], Heidcamp [6], and Querol and Barrio [16]. Homogenization was carried out using a centrifugal type fruit/vegetable juicer (Singer Juice Extractor, Model 774). All juice was collected in a beaker packed in ice. Juice to be used for preparation of mitochondria was immediately diluted with 1 volume of grinding buffer (0.4 M sucrose, 0.165 M Tris-Cl pH 7.5, 10 mM KCl, 10 mM MgCl, 10 mM EDTA); and 10 mM 2-mercaptoethanol was prepared. The homogenate was distributed to prechilled Oak Ridge centrifuge tubes for low-speed centrifugation (Beckman F0605 rotor, $300 \times g$, 10 min, 4°C). The students were expected to calculate the amount of 2-mercaptoethanol to add, as well as the speed necessary for centrifugation at the required G-force.

The supernatants from the low speed centrifugation were decanted into fresh Oak Ridge tubes for high-speed centrifugation ($10\,800 \times g$, 30 min, 4°C). During the high-speed spin, sucrose step gradients were prepared using sucrose solutions of 33%, 44%, 50%, 57%, 60% (w/v, in 0.01 M EDTA, pH 7.5, see [21]). Each pellet from the high speed centrifugation was resuspended in 2 ml of grinding buffer and layered on top of a sucrose gradient. The gradients were centrifuged in a Beckman SW-27 rotor at $113\,000 \times g$ for 4 h at 4°C. After sucrose density gradient centrifugation, well-defined, tan-colored mitochondrial bands were collected from between the 33% and 44% sucrose steps using a syringe equipped with a wide-bore tip made from a capillary pipette and a short length of Tygon tubing. Mitochondria were pooled and stored undiluted at 4°C overnight.

In M2Co, daffodil chromoplasts and chromoplast DNA were extracted by a team of two students using methods researched by one of the students. Chromoplasts were extracted by the method of Liedvogel [11]. Briefly, 100 coronas were harvested from daffodils and homogenized in a blender with three volumes of 0.47 M sucrose, 5 mM $MgCl_2$, 0.2% polyvinylpyrrolidone, and 67 mM phosphate buffer pH 7.5. After a low-speed centrifugation as described above, chromoplasts were sedimented from the supernatant by centrifugation for 20 min at $16\,500 \times g$. The bright yellow pellet was resuspended in a solution containing 50% (w/v) sucrose, 5 mM $MgCl_2$, and 67 mM phosphate buffer pH 7.5. The resuspended organelles were pipetted into the bottom of empty centrifuge tubes (~8 ml $tube^{-1}$) in preparation for sucrose-density centrifugation. The chromoplast layer was overlaid with equal volumes of 40%, 30%, and 15% (w/v) sucrose in the same buffer. After centrifugation for 1 h at $50\,000 \times g$ the chromoplasts were collected as described above. DNA was extracted as described below for mitochondria.

Because of scheduling constraints and the need for a good yield, isolation of mitochondrial DNA (mtDNA) during SOL was completed by the instructor as follows. (During M2Co, students extracted the mtDNA using the method described below, but because each student used relatively little mitochondrial preparation as starting material, DNA yields were too low to be useful.) The mitochondrial preparation was diluted with TE buffer (10 mM Tris pH 7.5, 1 mM EDTA), and the mitochondria were aliquotted to Oak Ridge tubes and pelleted as described above. The supernatant was removed, each pellet was resuspended in approximately 1 ml of grinding buffer and the mitochondria were frozen to await DNA extraction. DNA was isolated as described by Querol and Barrio [16]. Briefly, a tube of mitochondria, representing about 150 g of carrot tissue, was thawed and the volume was adjusted to 2 ml by addition of 10 mM Tris pH 7.5, 1 mM EDTA, 50 mM NaCl. Mitochondria were disrupted by addition of 10% SDS to a final concentration of 0.5% (w/v) and by the addition of Proteinase K to a final concentration of 100 μg ml^{-1}. The mixture was incubated for approximately 3 h at 37°C. Then 0.25 ml of 5 M KOAc (5 M KOAc, pH adjusted to 7.5 with a few drops glacial acetic acid) was added, and the tube was stored at −20°C overnight. After thawing, centrifugation ($17\,000 \times g$, 10 min, 4°C) yielded a pale yellow supernatant. The supernatant was decanted to a clean tube, and 1 volume (2 ml) isopropanol (IPA) was added. After incubation for ~30 min at room temperature, the mixture was centrifuged ($17\,000 \times g$, 10 min, RT) to yield a clearly visible off-white pellet. After being washed with 70% ethanol and air-dried for 30 min, the pellet dissolved easily in 100 μl TE. Students were given the choice of two methods of quantitation: the plastic wrap/ethidium bromide method or the agarose plate/ethidium bromide method [19]. After quantitation, DNA was stored frozen to await library production.

In situations where scheduling a day-long lab on a weekday would be problematic due to conflicts with other classes, it should be possible to schedule a day-long Saturday lab in much the same way that field biology classes take field trips on weekends. In addition, use of the more rapid daffodil chromoplast preparation, which involves a 1-h sucrose-gradient centrifugation step as opposed to the 4-h centrifugation to prepare carrot mitochondria, should greatly reduce the time required for this step.

Week 3

In preparation for library production, students calculated the number of clones necessary to cover the genome in question, assuming a genome size of 200 kb, and carried out DNA concentration calculations to determine optimum conditions for ligation. Restriction enzymes *Bam*HI and *Hin*dIII were selected because they can be inactivated by heat. Standard protocols were used for all cloning work [4]. After a discussion of the relative merits of both approaches, the students chose whether to use one or two restriction enzymes to prepare the DNA for ligation into the cloning vector. Students set up the appropriate restriction digests, incubated the samples, and then heat-inactivated the restriction enzymes before setting up the appropriate ligation reactions. The vector plasmid was pGEM-3Z (Promega Corporation). The ligation reactions were incubated at 4°C overnight, then moved to −20°C to await transformation.

Week 4

Using aliquots from the ligation mixtures prepared in Week 3, students transformed *Escherichia coli* strain JM109, plated the bacteria on medium containing X-gal and IPTG, and screened the transformants for white (insert-containing) colonies. The students chose one or two colonies for growth in liquid media for use in plasmid DNA isolation.

Week 5

Plasmid DNA was isolated using Wizard Miniprep kits (Promega), following the manufacturer's instructions [4]. After quantitation of DNA concentration, restriction enzyme digests were prepared, and the digested DNA was run on agarose gels to identify inserts and insert sizes. Plasmid DNA was stored at −20°C to await sequencing. Because the project was viewed as a group effort, students obtained low DNA yields to obtain plasmid DNA from students who had done two preparations that worked well.

Week 6

Students set up sequencing reactions in a total volume of 20 μl, using 500 ng of plasmid DNA and 3.2 pmol of T7 primer added to a sequencing cocktail from PE Biosystems, Foster City, CA, USA (dye terminator cycle sequencing Ready Reaction Kit using Amplitaq DNA polymerase FS). The sequencing cocktail was provided by the Washington State University Laboratory for Biotechnology and Bioanalysis in Pullman, WA. Each student carried out sequencing reactions on one or two clones.

Following cycle sequencing as directed by PE Biosystems, DNA fragments were precipitated by the addition of 2 μl of 3 M sodium acetate and 50 μl of 95% ethanol. After incubation on ice for at least 10 min, the fragments were pelleted by centrifugation in a microfuge for 20 min. Pellets were washed with 70% ethanol and air-dried overnight in the dark. Samples were shipped to the Laboratory for Biotechnology and Bioanalysis at Washington State University to be read using a PE Biosysems 373 automated DNA sequencer.

Weeks 7–8

While waiting for sequencing results, students learned to operate the Amray Model 1810 scanning electron microscope, and participated in a demonstration of sample preparation, including critical point drying and tungsten coating of carrot tissue collected and fixed during Week 2. In the laboratory, students prepared and ran standard SDS-PAGE gels to examine the proteins present in mitochondria. In preparation for a field trip, students read and discussed a paper describing a strategy for large-scale DNA sequencing [23].

Once the students received their sequence data, they analyzed the results using various web-based bioinformatics tools, including GenBank, SwissProt, and the Brookhaven Protein Data Bank. They performed searches for nucleic acid homology, carried out computer-based conceptual translations, and searched for amino-acid homologies. They attempted to model protein products using Swiss-Model. Students whose sequencing results did not include recognizable open reading frames (ORFs) used ORFs from data generated by other members of the group.

Week 9

The class went on a field trip to the University of Washington's Genome Center to observe genome-scale sequencing, listened to a presentation on the Center's sequencing projects, and participated in a question-and-answer session with Center personnel.

Week 10

Students turned in their written reports and presented oral reports of their results in a symposium-style meeting. Since the assignment was to discuss an ORF, and not all students' sequences originally contained ORFs, students shared sequencing results when necessary. Students were responsible as individuals for writing their reports, but most reports contained the contributions of more than one student. This was reflected in the authorship of the reports: the individual who wrote the report was first author, and students who made other contributions (such as supplying clones for sequencing, or sequence results, for example) were additional authors. One paper had only a single author: the student had successfully completed each step of the process, and his sequence results contained an ORF [12].

Students were evaluated based on their participation in the laboratory work, their laboratory notebooks, their mock *EPGR* article, their presentations, and their cooperative work in groups. Missed lab sessions were generally not a problem, but when this occurred there was no provision for make-up of the session.

Results

Although the students had been prepared for the possibility that it would be necessary to repeat certain procedures if any step in the process was unsuccessful, all procedures worked adequately the first time except for the isolation of mtDNA during M2Co. When done in a single batch, processing of mitochondria for DNA yielded approximately 15 μg DNA per 150 g carrots, but dividing the mitochondria among the students greatly reduced the efficiency of DNA recovery. For this reason, in M2Co the decision was made to proceed with plastid DNA, which was obtained (when processed in a single batch) with a yield of 30 μg kg^{-1}. Restriction of organellar DNA and ligation with pGEM-3Z produced ligation products that transformed *E. coli* JM109 sufficiently to yield approximately 100 insert-containing colonies. Although transformation with 'homemade' competent cells was relatively inefficient, enough ligation mixture was left over to produce a complete library in highly competent cells. Most of the colonies tested were found to contain inserts ranging in size from 500–2000 bp and 75% of the mtDNA sequencing reactions yielded sequence data, for a total of approximately 7 kb of mtDNA sequence. As shown in Table 2, sequences from three mitochondrial clones were found to encode recognizable ORFs. Two of these contained greatest homology to chloroplast genes, suggesting either that the mtDNA was contaminated with plastid DNA (the most likely possibility), or that the ORFs identified by the students are the first of their kind to be isolated from mitochondria.

Most students and student groups experienced failure with one or more of the steps. However, since the entire class worked together, it was a simple matter to distribute DNA, clones, or sequences so that everyone could complete the final project. For unknown reasons most chromoplast sequencing reactions did not provide data; in this case students shared the chromoplast sequences that were obtained,

and in addition used the mitochondrial sequences obtained by the earlier class.

Discussion

From a technical standpoint, each of these projects was successful: mtDNA and plastid DNA libraries were prepared and used as a source of clones for sequencing; and the sequencing produced data on several ORFs. None of the carrot ORFs had been previously described in carrot. But what did the students learn?

In the course of carrying out the laboratory work, students learned numerous cell and molecular biology techniques in a coordinated sequence during which each step relied upon the success of the previous steps. They used mathematics to calculate concentrations, dilutions and transformation efficiencies in a real-world setting. They experienced first-hand that ambitious projects can be broken down into a series of smaller, more accessible steps, and that teamwork makes the steps even more accessible. They found that the instructions issued by instructors are generally adapted from the primary scientific literature, and that they themselves could find relevant information in the literature and adapt it to their needs. The best example of students taking the initiative to derive methods from the literature is the student-originated isolation of daffodil chromoplasts and plastid DNA.

Analyzing their data, students identified numerous homologous sequences from a variety of organisms, animals as well as plants. They came to recognize, in a way that made sense to them, the unity and diversity of genes and proteins. They began to think of computers as actual, useful tools, and acquired computer skills that few of them imagined existed before they participated in this exercise. On the field trip to the genomics laboratory, they were able to ask intelligent, even pointed, questions.

While working on their reports, they were empowered to think that they might actually be able to publish in the primary scientific literature (indeed, one group attempted to clone and sequence the other half of the cox1 ORF during a later independent research project). They were introduced to mores governing how authorship is distributed on scientific papers, and they discovered that luck can be as important as skill, but that, as has been famously stated, 'chance favors the prepared mind'. They participated in the production of new data and saw how scientific information is disseminated.

These projects generated considerable enthusiasm among the students, and propelled most graduating students into post-college jobs in academic laboratories. In fact, every student who sought such a job has now found one, and several reported that the person responsible for hiring them had expressed interest in the project.

The data generated during the completion of these projects cover only a fraction of either genome, but data obtained over a series of projects, or by projects at a number of institutions, would add up quickly. A multi-institution effort to pool and assemble sequence data is possible. Requests for information are welcome.

Acknowledgements

The assistance of the Promega Corporation, Betty Kutter and Barbara Anderson of the T4 Laboratory at The Evergreen State College, Derek Pouchnik of The Laboratory for Biotechnology and Bioanalysis at Washington State University, Lowell Brady, and members of the Group Contracts 'Structure of Life' and 'Molecule to Corporation' is gratefully acknowledged. Ben Harrison originated and carried out the initiative to extract DNA from daffodil plastids, and was assisted by Toby Schroeder.

References

1 Campbell M. 1999. One enzyme fits all. In: HMS Beagle: The BioMedNet Magazine, Issue 47 (Feb 5). Available: http://www.biomednet.com/hmsbeagle/47/labres/adapt.htm
2 Cooper TL. 1977. The Tools of Biochemistry. Wiley & Sons, New York, NY.
3 Dark A and D Miller. 1998. DNA sequence highly homologous to tobacco DNA directed RNA polymerase beta-subunit, isolated from *Daucus carota*. Final project prepared for 'Structure of Life' at The Evergreen State College, presented 10 March 1998.
4 Doyle K. 1996. Protocol and Applications Guide, 3rd edition. Promega Corporation, Madison, WI.
5 Grant BW and I Vatnick. 1998. A multi-week inquiry for an undergraduate introductory biology laboratory. J Coll Sci Teach 28: 109–112.
6 Heidcamp WH. 1996. Cell fractionation. In: Cell Biology Laboratory Manual [Online]. Available: http://www.gac.edu/cgi-bin/user/-cellab/phpl?indexl.html
7 Herman C. 1999. Reading the literature in the jargon-intensive field of molecular genetics. J Coll Sci Teach 28: 252–253.
8 Jones RM. 1981. Experiment at Evergreen. Schenkman Publishing, Cambridge, MA.
9 Keefer R. 1999. Criteria for designing inquiry activities that are effective for teaching and learning science concepts. J Coll Sci Teach 28: 159–165.
10 Kim K-J and RK Jansen. 1995. NdhF sequence evolution and the major clades in the sunflower family. Proc Natl Acad Sci USA 92: 10279–10383.
11 Liedvogel B. 1987. Isolation of membraneous chromoplasts from daffodil flowers. Meth Enzymol 148: 241–246.
12 Lin A. 1998. A gene, found in *Daucus carota*, with highly conserved homology to coxI. Final project prepared for 'Structure of Life' at The Evergreen State College, presented 10 March 1998.
13 Patel AS. Undated. Isolation of mitochondria from *Saccharomyces cerevisiae* using the Avanti J high performance centrifuge. Beckman Instruments Application Information, Part Number A-1803A.
14 Obringer JW and JS Kent. 1998. The senior biology seminar—a capstone course. J Coll Sci Teach 28: 263–266.
15 Ohme M, M Tanaka, J Chunwongse, K Shinozake and M Sugiura. 1986. A tobacco chloroplast DNA sequence possibly coding for a polypeptide similar to *E. coli* RNA polymerase beta-subunit. FEBS Lett 200: 87–90.
16 Querol A and E Barrio. 1990. A rapid and simple method for the preparation of yeast mitochondrial DNA. Nucleic Acids Res 18: 1657.
17 Quinones V, S Zanlungo, L Holuigue, S Litvak and X Jordana. 1995. The coxl initiation codon is created by RNA editing in potato mitochondria. Plant Physiol 108: 1327–1328.
18 Rickert E, L Brady and K Budinich. 1998. Isolation of a mitochondrial sequence from *Daucus carota* with homology to chloroplast NADH dehydrogenases. Final project prepared for 'Structure of Life' at The Evergreen State College, presented 10 March 1998.
19 Sambrook J, EF Fritsch and T Maniatis. 1989. Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
20 Staswick P. 1999. The Electronic Plant Gene Register [Online]. The American Society of Plant Physiologists. Available: http://www.tarweed.com
21 Stukus P and JE Lennox. 1995. Use of an investigative semester-length laboratory project in an introductory microbiology course. J Coll Sci Teach 25: 135–139.

22 Tichenor LL and JP Kakareka. 1995. An interdisciplinary teaching approach integrating cell biology and biochemistry: crossing disciplinary boundaries in an upper-level scientific learning community. J Coll Sci Teach 25: 144–149.

23 Wong GK-S, J Yu, EC Thayer and MV Olson. 1997. Multiple-complete-digest restriction fragment mapping: generating sequence-ready maps for large-scale DNA sequencing. Proc Natl Acad Sci USA 94: 5225–5230.