

Laboratory Exercises

Using Restriction Mapping to Teach Basic Skills in the Molecular Biology Lab

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Digestion of DNA with restriction enzymes, calculation of volumes and concentrations of reagents for reactions, and the separation of DNA fragments by agarose gel electrophoresis are common molecular biology techniques that are best taught through repetition. The following open-ended, investigative laboratory exercise in plasmid restriction mapping allows students to gain technical expertise while simultaneously exploring the utility of gel electrophoresis and restriction mapping. Because of its interpretive nature, this project also provides data suitable for a written report, and can thus be used to reinforce lessons on figure presentation and science writing skills.

Keywords: Restriction mapping, gel electrophoresis.

Restriction digests of DNA and agarose gel electrophoresis are standard molecular biology techniques used for molecular cloning and DNA diagnostics; these frequently used techniques should be mastered by all students taking a course in molecular biology or genetics. Familiarity with these basic techniques can be taught in an open-ended, project-based curriculum, the approach endorsed in recent reviews of biology curricula such as “BIO2010” [1]. A project-based laboratory allows students to take ownership of their work, leaves room for innovation and creativity, and deepens the learning experience for individual students [1]. It also provides opportunities for student-led discussion of the application of these techniques in the rapidly advancing world of science.

The following lab project has been used as the first laboratory exercise in a Molecular Biology course at Lawrence University, a liberal arts college, since its introduction 12 years ago. The course serves sophomore through senior students and has both lecture and laboratory/discussion components. Lab sessions are three hours in length, once per week, but students are granted unlimited access to the lab and are expected to spend extra time on this project. Although prerequisites for the class ensure previous laboratory experience, students frequently enter the laboratory unsure of their skills. Despite initial hesitance in the laboratory, this exercise has received positive critique by the majority of those who have completed the course. Because it is repetitive in nature, this project allows students sufficient time and opportunity to become comfortable with each procedure.

Under the expectation that students become increasingly independent of written instructions, the following basic procedures are learned as a result of this lab exercise: calculation of buffer and reagent dilutions and reactant volumes, use of micropipetters, preparation and use of agarose gels to separate DNA fragments, production and use of standard curves to calculate DNA fragment sizes, the importance of controls when analyzing data from gel electrophoresis, interpretation and trouble shooting of agarose gel electrophoresis products, and problem-solving in the production of the restriction map. This project also lends itself very well to dissemination as a written laboratory report, giving students experience in the preparation of tables and figures as well as producing concise written prose.

This laboratory exercise has as its explicit goal the production of a restriction map of an “unknown” plasmid using four different restriction enzymes, though goals for student learning are much more encompassing. Restriction mapping is still used to compare clones [2], follow traits within and between species for evolutionary [3] and agricultural applications [4], and as the basis of RFLP and SNP genetic mapping [5]. Restriction mapping and RFLP analyses are also used in humans for prenatal diagnosis of disease and carrier analysis [6]. Students find their understanding of these more abstract applications is deepened by experience with the production of their own restriction maps.

A few others have published on the use of restriction mapping as a learning tool. Szebereny (2002) provides a paper and pencil problem-based approach that teaches the concepts of mapping without the laboratory experience [7]. Nielsen and Echols [8] include restriction mapping of chloroplast rDNA as one week of a 10–12 week sequence of laboratory exercises while Higgins *et al.* [9] provide instruction for a more instructor-directed lesson

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in restriction mapping. More recently, Wilterding and Luckie have used what they term an investigative DNA “stream” that includes a plasmid miniprep, and DNA fingerprinting via restriction digests and gel electrophoresis [10]. Only some of these exercises are active and open-ended, none of them require the students to determine the exact experimental design, and none build skills through repetition.

EXPERIMENTAL PROCEDURES

Chemicals, Enzymes, and other Reagents

Standard laboratory chemicals were obtained from Fisher Scientific. Plasmid DNA (pBR322) and restriction enzymes with BSA and 10× buffers were purchased from New England Biolabs (Beverly, MA); agarose was purchased from Seakem LE, and DNA size marker (HI-LO) from Minnesota Molecular. The loading dye recipe is 0.25 g bromophenol blue, 0.25 g xylene cyanol, 40 g sucrose brought to 100 mL with distilled water. Ethidium bromide stain was 5 µg/mL. Gel running buffer was 1× TBE¹ [11]. Plasmid DNA preparation by students could precede this lab exercise if time permits, but some preparations will undoubtedly be too contaminated to give clean restriction digest results.

All reagents are provided to students as one set per freezer box (Table I). Restriction enzymes can be diluted to the indicated concentrations in the manufacturer’s recommended buffers to reduce costs. Students are instructed to be judicious in their use of reagents, but additional reagents are provided as needed. Providing students with smaller volumes at the start minimizes the inevitable spilling or cross contamination of expensive reagents.

Hardware and Safety

Horizontal gel electrophoresis chambers, UV transilluminator, and photodocumentation system were from Fotodyne. Power supplies (EC105) were from EC Apparatus Corporation.

The primary safety issue in this exercise is the use of ethidium bromide (EtBr), a mutagen that must be handled while wearing gloves. Materials contaminated with EtBr, e.g. gloves, gels, pipette tips, are disposed of as hazardous waste. To minimize exposure to EtBr, only the dilute staining solution is available to students, a special area of the lab is given to gel staining, and students are instructed to leave all EtBr-contaminated materials in this area only. Latex and non-latex gloves of all sizes are provided. EtBr is not added directly to agarose gels during their preparation, thus eliminating contamination of the microwave, gel boxes, and running buffer.

Scheduling

It is important to note that this exercise is best assigned at the very beginning of the course. Pedagogically, the skills built here are basic and later labs can build on students’ abilities to set up reactions properly and to efficiently run agarose gels. In addition, students gain some good “lab sense” by working independently right away. A second, more practical, reason is that students will have more time to attend to the project before mid-terms and papers are assigned in other classes. Request of the written lab report in the third week of the course nicely distributes the course assignments. The suggested timeline below is based on lab sizes of 12–16 students. Times needed for the entire lab section to complete a step are given. Some students will complete tasks sooner.

TABLE I
Reagents provided per student group

Reagent	Concentration	Volume initially provided
Plasmid	300 µg/mL	30 µL, most need 60 µL by end
Restriction Enzyme	10× (as provided by N.E.B.)	50 µL
Buffers	10×	20 µL
BSA	10×	20 µL
Marker, HiLo	117 µg/ml	170 µL
Enzymes (as a set of 4)		
EcoRI	13,333 units/mL	20 µL
Rsa I, Hinc II	6,666 units/mL	20 µL
Ava I, Bam HI		20 µL
Sph I, BsrB I	10,000 units/mL	20 µL
NaCl	1 M	1 mL
Tris-HCl	1 M	1 mL

First Lab (3 h total)

45 min: Introductory remarks on plasmids, restriction enzymes, reaction conditions, use of micropipetters, and the importance of tube labeling.

5 min: Practice with micropipetters using colored water or loading dye.

30 min: Students prepare and incubate (1 h) 4 single restriction digests.

30 min, while digests incubate: Remarks on use of agarose gel electrophoresis, demonstration using sieves, and instructions on gel preparation and loading.

20 min: Students prepare a 1.0% agarose gel and load samples on the gel.

20 min, while gels are running: Remarks on how to use molecular size standards to construct a standard curve; instructions on staining and photographing gels.

20 min: Staining and photography of gels. Analysis to see that all reactions were successful. Students store their samples at –20°C for later use.

Second Lab (3 h)

30 min: Introductory remarks on the concept of restriction mapping and its applications (RFLPs). Instructions on setting up double restriction digests, suggestions on preparing larger volumes of restriction digests that use low salt buffers and suggestions for using last week’s results as the basis for the first round of double digests.

30–45 min: Students plan their approach, set up restriction digests, and pour a gel.

45 min: Student-led discussion of a related piece of primary literature.

45–60 min: Students load, run, and stain an agarose gel.

Independent Work (1 additional week on student’s own time – 3–10 h)

There is a range in the amount of time students spend finishing this project, depending on how well they think ahead, how well they plan their gels (did they remember to have single digests on the same gel as double digests for direct comparison?), and how well they label tubes for later use. The instructor will need to meet with some students to help them understand how to change the buffer conditions for double digests and later to help some students interpret gels. This is all time well-spent.

Week 3

The instructor likely will need 10 min during a lecture a few days before the written report is due to review how to use the

¹The abbreviations used are: TBE, Tris-Borate-EDTA (121.1 g Tris, 51.35 g boric acid, 3.72 g EDTA in 1 litre); EtBr, ethidium bromide.

data on DNA fragment sizes to construct a restriction map. Though this was covered in the pre-lab lecture in week 2, students need to hear it again now that they have data—the explanation will have been too abstract for many students the first time around.

Written Instructions to Students—Restriction Mapping of Plasmid DNA – You have been given 10 µg of plasmid DNA. Using the information provided below, map the restriction sites of four different enzymes: W, X, Y, and Z. The plasmid DNA is circular and contains approximately 4,350 base pairs. The single EcoRI/Bam HI site can be placed at base pair zero; all other restriction sites should be mapped relative to the EcoRI/Bam HI site.

Mapping will be accomplished by performing single and double restriction digests of the plasmid followed by separation of the DNA fragments using agarose gel electrophoresis. Be sure to run appropriate controls on each gel, including single digests to compare with double digests or doubles to be compared with each other. The more digests you have on each gel, the more information you will obtain by comparing fragment sizes directly. A standard curve of fragment sizes will be obtained by running marker DNA on each gel. Each gel will generate a standard curve for use with that gel only. Very small fragments may not be visible and their presence might need to be inferred.

General Protocol for Restriction Digests (recipe can be modified as needed, but buffer must always be 1/10th the total volume and total enzymes added must not exceed 1/10th total volume to sufficiently dilute the “anti-freeze”).

3 µL (0.9 µg) DNA
14 µL sterile water
2 µL 10× restriction buffer
1 µL restriction enzyme

Some enzymes require BSA for proper function. You have a 10× stock solution of BSA; when adding BSA, reduce the volume of water added accordingly. Be sure to add the restriction buffer that is appropriate for the enzyme being used. An attached page details the buffer conditions required of each enzyme. Stock solutions of 1 M NaCl and 1 M Tris HCl are provided—dilute them to the appropriate final concentration in your double digests when you need to change buffer conditions. For example, after a 1-h incubation of a 20 µL Apa L1 digest, remove 4 µL to run on the gel. Divide the rest into two tubes. In one tube, add 1 M NaCl to bring the NaCl concentration to 100 mM then add 1 µL of Bgl I. Bring NaCl concentration of the other tube to 50 mM NaCl and add Eco RI. Incubate both for another hour. An easy way to figure out the volume of stock solution needed (e.g. 1 M NaCl, in this case) is:

Final Volume desired × Final Concentration desired =
Stock Concentration × Stock volume to use

Solve this equation for whichever variable you don't know, in this case, volume of stock to use. Remember that NaCl is already in some of the buffers to begin with—so in going from Eco RI to Bgl I, for example, you only need to add to a final concentration of 50 mM, since you start at 50 mM and end at 100 mM. Please ask for help if you need it!

Restriction digests should be done in 0.5 mL eppendorf tubes. Add the enzyme last—*keeping the tube of enzyme out of the freezer for as brief a time as possible*. Digests are done at 37C for 1 hour. Finished reactions may be put into the freezer. Label the tubes—you can re-run digests on later gels thus saving your time and reagents. *Return enzymes to the freezer immediately*.

Following the 1-h incubation, remove 4 µL to a fresh tube and add 1 µL of loading dye. This mixture can be loaded onto a gel. Alternatively (to save tubes) you can put the dye and digest together on a piece of parafilm if you are ready to load the gel immediately. By NOT adding dye to your reaction tube, you can later add a second or third enzyme to the reaction rather than having to re-do the reaction.

NOTES

1. Always wear gloves when handling DNA and enzymes. Turn them inside out to dry out to reuse. Discard holey gloves in EtBr waste.
2. *Always* use a clean sterile pipette tip to pipette DNA, enzymes, and buffers.
3. Keep enzymes in the freezer or for minimal time on ice. Keep DNA on ice or at 4C (refrigerator); buffers should be at –20°C (freezer) when not in use.
4. You are responsible for keeping the lab clean. All gel boxes *must* be emptied and rinsed after each use. Turn them upside down on towels to dry. If left with buffer in them, the water will evaporate leaving salts encrusted on the electrodes.
5. *Allow agarose to cool* a bit before pouring the gel. Very hot agarose will create stress fractures in the gel beds.

Agarose Gel Electrophoresis

Prepare an agarose gel in 40 mL of 1× TBE (dilute the 10× TBE appropriately). You should microwave the agarose/buffer mixture until you no longer see bits of agarose floating in the mixture. Use medium power and do not allow the agarose to boil over. *Allow agarose to cool* so you can easily touch the flask. Gels should be allowed to set for about 15 min before use.

Use 1× TBE as running buffer just covering the top of the gel. Load the gel through the buffer. Connect the leads, recalling that DNA is negatively charged and will migrate to the positive pole. Set the power supply to 100 mamps constant current for 1 h. Stain the gel in a large weigh boat with 1 µg/mL ethidium bromide *Wear Gloves—Carcinogen* for 10 minutes. View gel on the UV box—take a picture *if* it is a useful gel.

Sample gel:

marker uncut Bam HI Bam/Hinc II Hinc II Hinc II/Bsr Bsr BI

Hints: You do not need to do all possible combinations of digests. You can save digests to re-run on later gels for division into two additional digests. Keep them in your freezer box and mark things well.

TABLE II
Restriction endonuclease sets

Set 1	Set 2	Set 3	Set 4	Set 5
Bam HI (1)	Eco RI (1)	Eco RI (1)	Eco RI (1)	Bam HI (1)
Ava I (1)	Ava I (1)	Hinc II (2)	Ava I (1)	Ava I (1)
Hinc II (2)	Hinc II (2)	Rsa I (3)	Bsr BI (2)	Bsr BI (2)
Rsa I (3)	Rsa I (3)	Sph I (1)	Rsa I (3)	Hinc II (2)

Each set contains either Bam HI or Eco RI as the reference site given at base pair zero of the plasmid. The number given in parenthesis indicates the numbers of endonuclease sites in pBR322.

Additional Materials

In addition to the earlier-mentioned laboratory instructions, students are given the manufacturer's instructions for each restriction enzyme and the overview on restriction endonucleases and their reactions from their catalog [NEB]. They are also given instructions on completing the required written report.

RESULTS

Upon completion of this lab project, students produce a report in manuscript form that summarizes their data. They must include gels that contain double digests, one standard curve, a table of all DNA fragment sizes seen and inferred, and a circular plasmid restriction map. Because the restriction map of pBR322 is known, students are not given the identity of the plasmid. Since this course is taught every year, plasmid identity is never divulged and the set of restriction enzymes used is changed each year; however, since students must produce representative gels and be able to explain the logic used to construct the map, knowing the identity of the plasmid would be of little use.

Each set of four restriction enzymes used in any one year contains two enzymes that cut the plasmid once and two that cut either two or three times. One of the single cutting enzymes is arbitrarily placed at base pair zero of the plasmid, and students are told to map other restriction sites relative to that enzyme site. Five different sets of enzymes used to cut pBR322 are given below (see Table II), these sets work well and have always given interpretable results.

TABLE III
Survey Questions

- Q3: Understanding of proper use of micropipettors
 Q4: Preparation of an agarose gel
 Q5: Use of an agarose gel to separate DNA fragments (loading, running, staining the gel)
 Q6: Calculating and making dilutions of stock solutions (as for double digests)
 Q7: Interpreting data from an agarose gel (including determining DNA fragment sizes)
 Q8: Understanding how restriction digests work
 Q9: Understanding the principles of gel electrophoresis
 Q10: Understanding restriction mapping

Respondents were asked to rank their skills (Q3-7) or comprehension (Q8-10) both before and after taking Molecular Biology on a scale from 0-6 in which 0 = no ability or no understanding and 6 = fully capable of the skill or complete understanding of the concept.

As is evident from representative gels, several bits of data routinely present challenges for the students, providing excellent examples of the ambiguity present in any type of data analysis, as well as the need for careful observation and prediction of expected results. In set 2, Hinc/Rsa double digests produce a 60 base pair fragment that is almost never visible, in addition this digest produces two DNA fragments of similar size (1,629 and 1,565), so students must notice the heavy EtBr staining or be able to resolve the two fragments. The same is true with the 931 and 879 base pair fragments produced by a BglI/Eco RI double digest. In this case, the doublet is more obvious because it stains brighter than the larger sized DNA fragment above it on the gel. Several sets of enzymes produce fragments of 124–160 base pairs, but their presence is usually easier to infer. The figure legends denote these and other challenges in the data.

While any endonuclease that cuts the plasmid only once may be used as "base pair zero," one must choose an enzyme that cuts very reliably. Both Eco RI and Bam HI serve this purpose well.

Assessment of Student Learning

Student learning was assessed by instructor observation of student laboratory work and written reports, by

FIG. 1. (A) Agarose gel electrophoresis of plasmid DNA digested with enzyme set 1. Plasmid pBR322 (2 µg) was digested with one restriction enzyme in the buffer provided by the manufacturer. Enzymes added after adjusting buffer conditions of initial reactions are indicated after the slash mark. One tenth of the total reaction was separated in a 1.0% agarose gel for 1 h at 40 mA. The Rsa/Bam HI double digest contains a 211 bp fragment whose presence can be inferred by comparing the size of the largest fragment (1905 bp) to that from an Rsa single digest (2116 bp). Also, the Rsa I/Hinc II double digest should display five separate pieces. Only three of these fragments are visible because the smallest (60 bp) is too small to be seen on the digest, and the two largest (1629 bp and 1565 bp) are too similar in size to be distinguished from one another. (B) Agarose gel electrophoresis of plasmid DNA digested with enzyme set 2. See Fig. 1 for methods. Rsa I/Hinc II double digest contains an unresolved doublet of 129 and 1565 bps; Rsa I/Eco RI double digest contains a 164 bp fragment that can be inferred by comparing the 515 bp fragment to the 680 bp fragment seen in the Rsa I single digest (Figure 3). (C) Agarose gel electrophoresis of plasmid DNA digested with enzyme set 3. See Fig. 1 for methods. Note that the Hinc II digest contains a partially digested fully linear fragment of 4361 bp. Two double digests, Rsa I/Eco RI and Sph I/Hinc II produce fragments that are often too small to see (164 and 89 bps, respectively), but their presence is easily inferred. Students will need help to realize that the Rsa I/Hinc II and the Rsa I/Sph I double digests each produce two fragments of similar size that are difficult to resolve (1629 and 1565 bps, and 1718 and 1565 bps, respectively). (D) Agarose gel electrophoresis of plasmid DNA digested with enzyme set 4. See Fig. 1 for methods. Students may need assistance to determine that the Rsa I/BsrBI digest creates two fragments (360 & 319 bps) that are difficult to resolve. Again, the smallest fragments of the Rsa I/Eco RI, Rsa I/BsrBI, and EcoRI/BsrBI digests are often not visible but are easily inferred. (E) Agarose gel electrophoresis of plasmid DNA digested with enzyme set 5. See Fig. 1 for methods. The Bam HI/Hinc II double digest produces a 276 bp fragment that is often difficult to see, but its presence is easily inferred by comparing the double digest to the Hinc II single digest.

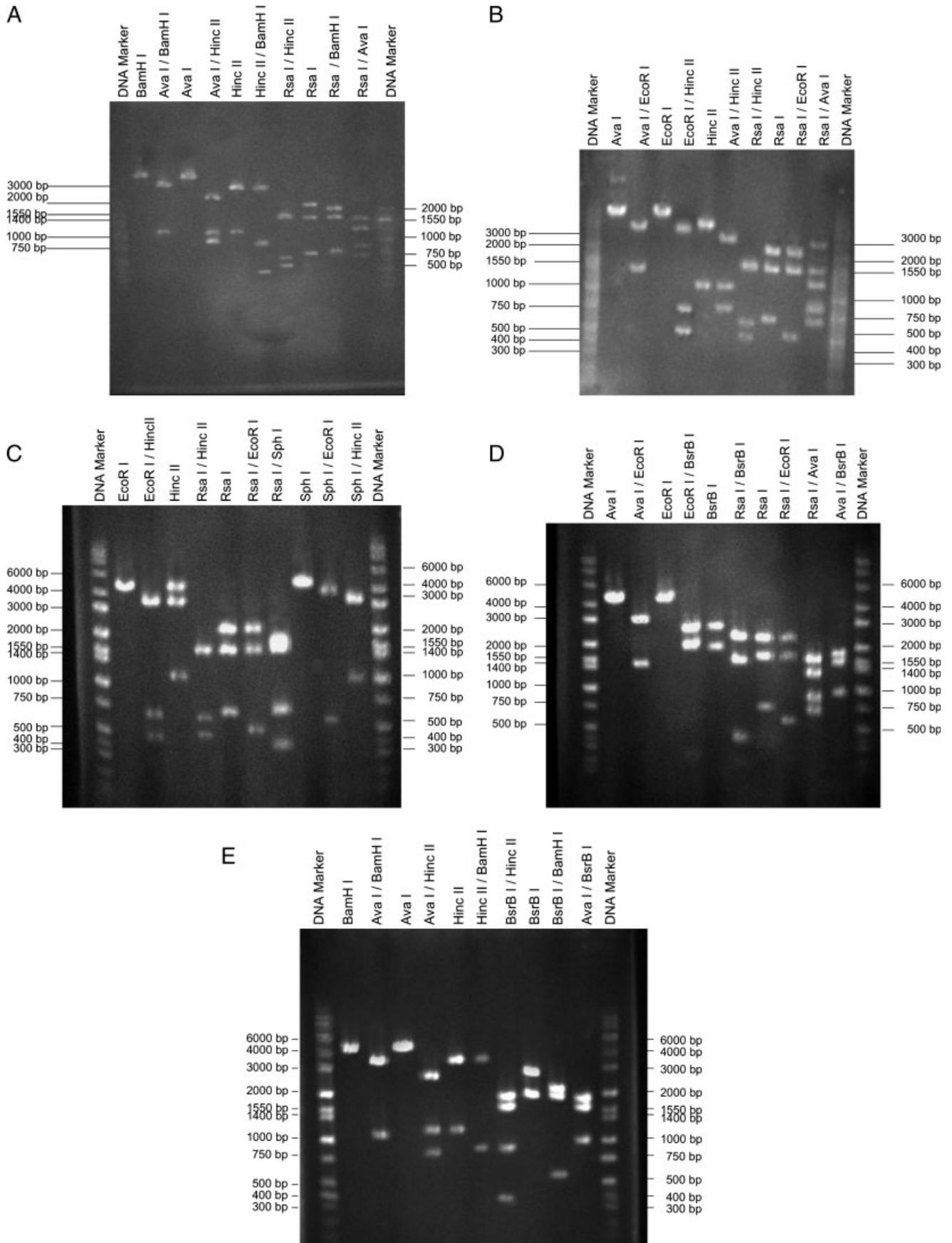


FIG. 1.

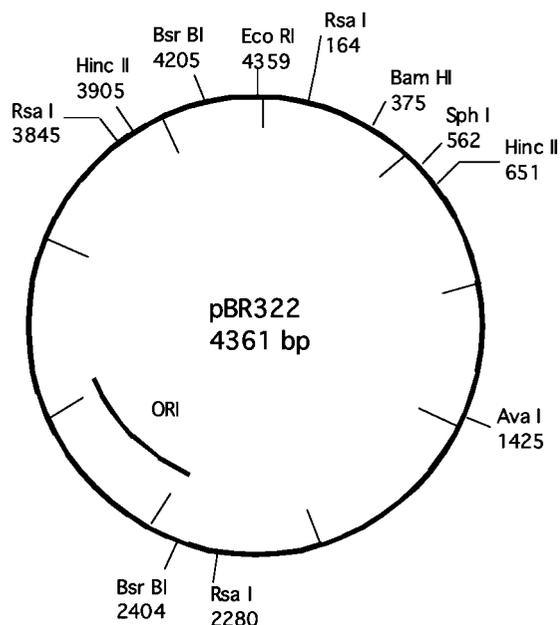


FIG. 2. **Restriction Map of pBR322.** Locations of restriction sites for each enzyme used in these experiments are shown, with the base pair location indicated. Total plasmid size is 4361 base pairs.

annual course evaluations, and by a web-based survey of students who took Molecular Biology at Lawrence University in the last three years.

Increases in student perception of their skills and understanding of the laboratory techniques and concepts presented by this laboratory project were assessed directly through a web-based survey of 33 recent students of the Molecular Biology course at Lawrence University. All students who had taken Molecular Biology in the past two years and who were still on-campus (21 students) and 12 recent graduates for whom email addresses were available were surveyed. The total response rate was 69%. Respondents were asked to rate their ability and confidence in undertaking particular laboratory skills as well as their conceptual understanding of the techniques on a scale of 0–6, retrospectively, prior to taking, and after having taken Molecular Biology (Questions are found in Table III). Students were also asked to provide written comments on positive and negative aspects of the laboratory experience. Sixteen of the 22 respondents chose to write lengthy responses, all of which were very positive.

Overall, student self-assessed understanding of common molecular biology laboratory techniques was greatly enhanced after completion of the restriction mapping project. This is made clear by the comparison of student rankings of each technique for before course enrollment and after project completion. Increase in confidence level ranged from 2.31 to 3.48 points, corresponding to a 39–58% increase in comprehension. On average, students ranked their understanding of each technique after completion of the lab exercise as 2.9 points higher than the ranking of the technique prior to enrollment in the course.

Separate assessment of graduate and undergraduate confidence level rankings mirrored the overall response

Student Self-Assessment of Learning

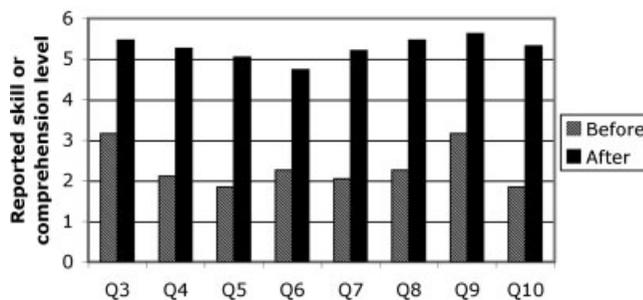


FIG. 3. **Self-assessment of student learning.** Thirty-three students who had undertaken this laboratory exercise as part of a molecular biology course in the previous two years completed a retrospective web-based survey assessing their skill and understanding of the techniques and concepts of the exercise before and after completing the course. Total response rate was 69%.

pattern (data not shown). Students currently enrolled at Lawrence University and students who have since completed their undergraduate requirements each reported an overall increase in their confidence level in every technique. Undergraduate responses show an increase in confidence level ranging from 2.64–4.09 points, or 44–68% increase in comprehension. Ratings by recent graduates display a more moderate increase of 23–44%, primarily because these students ranked their ability and comprehension prior to taking Molecular Biology as higher than did current undergraduates.

When asked for comments about the project, respondents provided extremely positive replies. Ten of the 16 voluntary responses came from students still attending the university, while the remaining six were those of recent graduates. On the whole, responses did not markedly differ between the two groups, though for the most part the undergraduates could not apply the skills that they had learned to other studies or aspects of their career. Because of this, the undergraduates continually commented on the usefulness of repetition of techniques and the importance of writing a coherent lab report. One student generously said “if there is one class that made me feel a competent biologist . . . it was Molecular, due largely to the hands-on experience required from performing the restriction lab” while another stated that “the repetition was highly valuable . . . and I have found that the techniques and concepts have stayed with me to a greater extent than I have experienced with other classes.” Graduates, too, appreciated the repetition of laboratory techniques and the exposure to scientific writing, but most also commented on the value of independent lab work as preparation for becoming a scientist. One respondent enthusiastically commented that “the skills I learned in molecular, particularly those from the restriction mapping lab, have made me very successful in my current position. In fact, the depth of understanding I gained through the repetition of the lab has allowed me to take over the job of teaching new graduate students those very same skills . . .” Another appreciatively stated “I am now a graduate student using molecular techniques on a daily basis. I have found that I was

MUCH more skilled than my counterparts from other universities. Other students had done similar techniques but didn't really understand them and were certainly not ready to work independently in a lab—I was." These typical comments underscore the importance of repetition to hands-on learning and to the development of problem-solving skills.

Written comments using a standard university course evaluation form were solicited each year. While no single question was designed to assess the effect of this one lab, several questions invited comments on laboratory work. Responses from 45 evaluations from 2001–2004 are summarized below. In response to the question "Did you experience in the laboratory advance your understanding of the principles and methods in the subject matter?" Most students simply answered "yes," but the following responses are typical of those who wrote additional comments:

"The restriction enzyme mapping lab was really neat and helpful in learning how to run gels and read them."

"I was very excited to do many of the protocols that I had only read about previously."

"Hands-on experience of major techniques made you feel empowered in some strange way."

In response to the question "What assignment(s) did you think most contributed to meeting the objectives of the course, and why?" the following responses were typical:

"The first lab report [restriction mapping] was tough. I probably was not prepared, but I think that is a result of my inexperience rather than the course. The lab reports did help us analyze what we had learned in lab."

"Our mapping lab was very helpful in my understanding of that subject."

"The structure of the lab reports forced us to think like a molecular biologist."

Instructor observations confirm student perceptions of increased skills and comprehension. Virtually all students are confused initially when confronted with their first electrophoresis results, and all students have questions about how to modify buffer conditions when adding a second restriction enzyme to a reaction. That students master these skills is evident, as all students are eventually able to produce and interpret complex restriction fragment patterns in agarose gels for their written reports.

CONCLUSION

As is evident from student responses, this investigative laboratory provides an excellent opportunity for students to become well acquainted with several techniques of molecular biology. It has been a successful and effective laboratory project because it not only prompts the individual to accurately execute each procedure in a timely manner using limited resources, but also provides ample data for analysis to allow students to build an understanding of the broader applications of each technique. Because a portion of the lab time is unsupervised, stu-

dents must troubleshoot on their own and take the time to think about their mistakes and the best ways to correct them. They also learn the importance of controls and the need to make direct comparisons between gels, and, in so doing, begin to understand how to obtain more data using fewer materials.

We have found the repetitive nature of this laboratory to be very effective in the instruction of lab technique. Students enter the course with little experience in molecular biology techniques. They exit the course with a concrete understanding of the broad concepts of gel electrophoresis, restriction mapping, digestion of DNA using restriction endonucleases, and reaction conditions. Based on over a decade of experience using this lab exercise, we are confident in saying that all students improve their skills and, in this last iteration of the course, 60% of the lab pairs produced gels of near-publishable quality. Through subsequent analysis of the data, students are also much more confident in their ability to interpret standard curves and ambiguity in gel data, to determine the most efficient course of action for a given laboratory protocol, and to put together a concise, well-written lab report without step-by-step guidance. The overall impact on student learning has been extremely positive.

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REFERENCES

- [1] National Academies of Science (2003) BIO2010: Transforming undergraduate education for future research biologists, National Academies Press, Washington, DC. Available at www.nap.edu/books/0309085357/html/.
- [2] M. Yamasaki, Y. Ikuto, A. Ohira, K. Chater, H. Kinashi (2003) Limited regions of homology between linear and circular plasmids encoding methylenomycin biosynthesis in two independently isolated streptomycetes, *Microbiology* **149**, 1351–1356.
- [3] A. Westerbergh, J. Doebley (2002) Morphological traits defining species differences in wild relatives of maize are controlled by multiple quantitative trait loci, *Evolution* **56**, 273–283.
- [4] J. V. Magalhaes, D. F. Garvin, Y. Wang, M. E. Sorrells, P. E. Klein, R. E. Schaffert, L. Li, L. V. Kochian (2004) Comparative mapping of a major aluminum tolerance gene in sorghum and other species in the Poaceae, *Genetics* **167**, 1905–1914.
- [5] M. Inoue, Z. Gao, M. Hirata, M. Fujimori, H. Cai (2004) Construction of a high-density linkage map of Italian ryegrass (*Lolium multiflorum* Lam.) using restriction fragment length polymorphism, amplified fragment length polymorphism, and telomeric repeat associated sequence markers, *Genome* **1**, 57–65.
- [6] G. S. Pandey, S. R. Phadke, B. Mittal (2002) Carrier analysis and prenatal diagnosis of haemophilia A in North India, *Int. J. Molec. Med.* **10**, 661–664.
- [7] J. Szeberenyi (2002) Restriction Mapping *Biochem. Mol. Biol. Educ.* **30**, 258–259.
- [8] B. L. Nielsen, S. D. Echols (2002) Use of a chloroplast rRNA gene to introduce basic molecular biology techniques, *Biochem. Mol. Biol. Educ.* **30**, 408–413.
- [9] S. J. Higgins, B. D. Hames, E. McIntosh, A. Colman (1990) Restriction enzyme mapping: A simple student practical, *Biochem. Educ.* **18**, 144–146.
- [10] J. H. Wilterding, D. B. Luckie (2002) "Stream"-lined education: Increasing student-initiated learning with an investigative DNA "stream," *J. Coll. Sci. Teaching* **31**, 303–307.
- [11] J. Sambrook, S. W. Russell (2001) *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.