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Laboratory Exercise

The "Frankenplasmid" Lab*[S]

AN INVESTIGATIVE EXERCISE FOR TEACHING RECOMBINANT DNA METHODS

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We describe an investigative laboratory module designed to give college undergraduates strong practical and theoretical experience with recombinant DNA methods within 3 weeks. After deducing restriction enzyme maps for two different plasmids, students ligate the plasmids together in the same reaction, transform *E. coli* with this mixture of ligated DNA, and plate the cells on media that specifically select for hybrid plasmids. The main goal of the assignment is for students to deduce the gene map of one hybrid "Frankenplasmid" using the LacZ phenotype of its transformants, PCR, and restriction mapping. Our protocol results in a number of possible outcomes, meaning that students are mapping truly unknown plasmids. The open-ended nature of this assignment results in an effective module that teaches recombinant DNA procedures while engaging students with its investigative approach, increasing complexity, and puzzle-like quality. Moreover, the modular design of the activity allows it to be adapted to a more limited schedule, introductory courses, or more advanced courses.

Keywords: Recombinant DNA, restriction mapping, plasmids, PCR.

INTRODUCTION

The limited time frame of a standard college laboratory course presents a significant challenge to science instructors. A laboratory section may meet only once a week for several hours, and, in that window, the instructor must decide how to prioritize potentially competing objectives, such as reinforcing material covered in lecture, giving students experience with particular techniques and equipment, teaching investigative science, and challenging students—all while allowing them to succeed at a reasonable rate and enjoy the process.

It is particularly difficult to juggle these objectives when teaching the subject of molecular biology. Students typically hear and read of advanced molecular biology techniques early in their college careers, yet it takes considerable experience to master even the basic methods, such as micropipetting and sterile technique. The discord between what students are prepared to learn on a theoretical versus a practical level makes it difficult to reinforce lecture material in laboratory, let alone implement a multiweek, investigative project. In addition, many standard molecular techniques require long incubation and purification steps that can easily exceed an allotted time slot [1].

Other molecular biology instructors have addressed the challenges described above by designing exercises that repeat an experimental approach with increasing complexity over multiple class periods (*e.g.* [2, 3]). Here, we describe a 3-week laboratory activity that uses a similar strategy. Our goal was to design a coherent, investigative-style module that improves student skill and confidence in using molecular biology techniques and in visualizing what occurs on a molecular level when synthesizing and analyzing a recombinant plasmid. In this module, students first use restriction mapping to deduce the sequence of a given plasmid, and then ligate this molecule in the same reaction with a second plasmid to synthesize a novel and unknown recombinant plasmid (*i.e.* a "Frankenplasmid"). In later classes, students deduce their Frankenplasmid sequence with bacterial plating, another set of restriction digests, and PCR. Although our students had a high rate of success with this exercise, we implemented backup measures to ensure that all students were able to perform a complete analysis. Here, we provide the details necessary to implement this laboratory activity, assess the technical and educational effectiveness of the experiments, and discuss changes that could be made to suit the needs of other classes.

MATERIALS AND METHODS

The experiment described in this manuscript formed part of the curriculum for the Introductory Genetics course (BIOL 202) at Williams College. BIOL 202 has a prerequisite of two introductory biology courses with laboratory (one with a cellular and

[S] Additional Supporting Information may be found in the online version of this article.

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molecular focus, and one with an organismal focus), and it is a required component of our biology major. As such, we assume in this exercise that students are already familiar with the basic biochemistry of DNA (*i.e.* phosphodiester bonds, DNA bases, and hydrogen bonding between complementary base pairs). Although we believe the exercises described here could be adapted to an upper level, or even a first-year introductory course, initially, it would also be necessary to train students in the basic equipment and practices used for molecular cloning (*e.g.* micropipettors, microcentrifuges, sterile technique)—at Williams College, these techniques are introduced in the prerequisites to BIOL 202. Also note that a theoretical background in plasmids, restriction enzymes, DNA ligase, agarose gel electrophoresis, and PCR is assumed. In our class, all laboratory procedures were done with students working in pairs. However, working individually would be logistically feasible with a manageable class size.

We have divided the Materials and Methods into four sections. The first section provides a brief overview of the scope and content of the laboratory activity. The second contains information that is intended to assist the instructors and technical staff in preparing materials for class. To further assist implementing this module, a detailed checklist of supplies needed for the students during each week of the exercise is provided

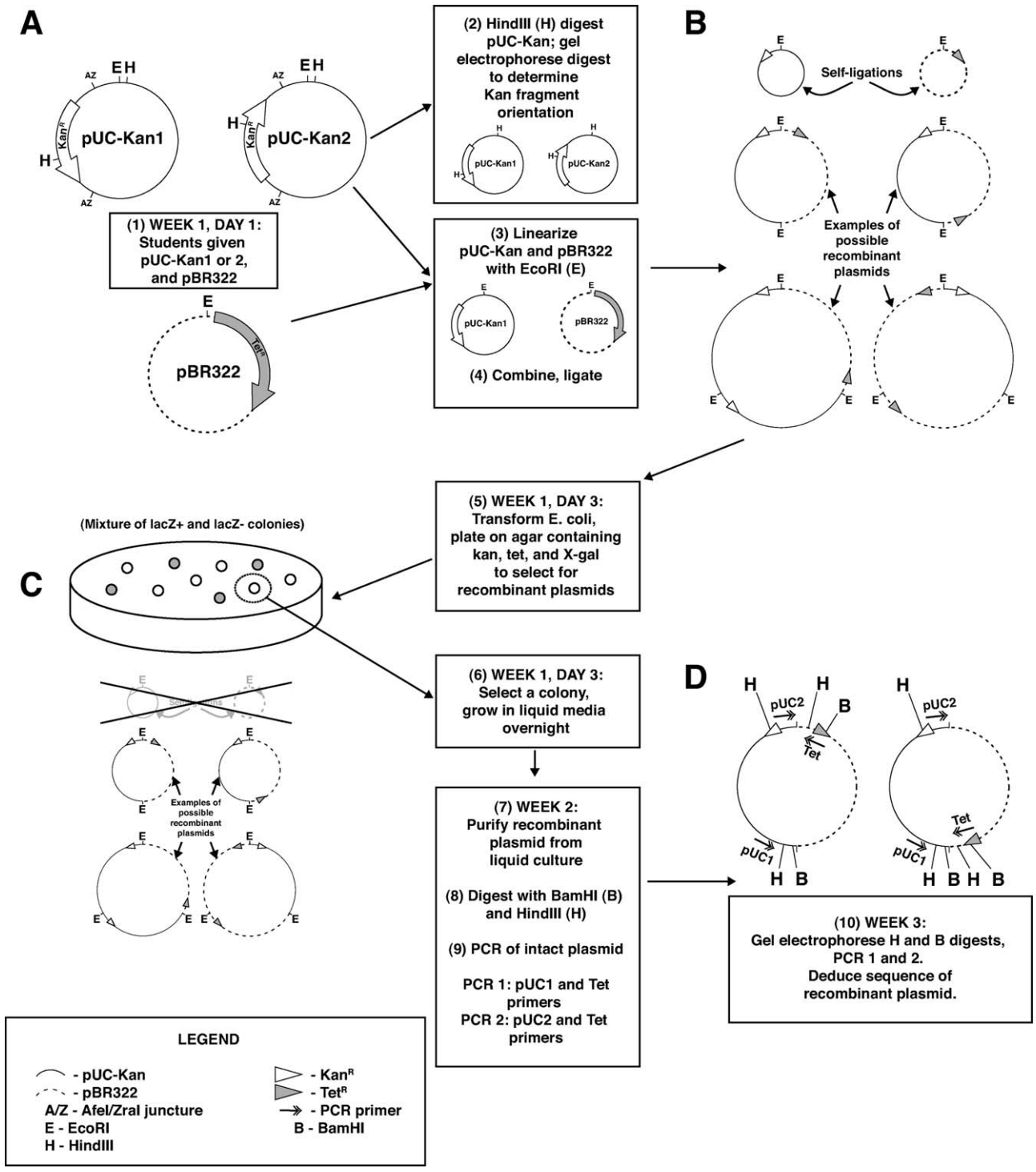

FIGURE 1.

online (http://biology.williams.edu/faculty-staff/ddean/). A copy of the experiment as written in the Williams College BIOL 202 student laboratory manual, complete with background information and further procedural details, may also be found at this link. The third section of this article details the protocols followed by the students and the scheduling of these exercises over 3 weeks, assuming a typical college laboratory course that meets one afternoon per week for ~3 h per session (see Results and Discussion section for alternative suggestions). Finally, we describe how we assessed the success of this activity in the fourth section.

Part 1. Experimental Overview

The overall goal of this laboratory is for students to investigate the structure of both known and unknown plasmids over the course of 3 weeks. Figure 1 provides an overview of this experiment, which we will also briefly discuss here.

Week 1—Students are initially provided with one of two premade plasmids (pUC-Kan1 or pUC-Kan2), both of which contain a kanamycin resistance gene (*Kan*^{R}) and a functional *LacZ* gene; the plasmids differ only in the orientation of *Kan*^{R} (Fig. 1*a*). The first task students undertake is to determine the orientation of *Kan*^{R} in their given plasmid relative to reference *Hin*dIII and *Eco*RI restriction cut sites (the locations of which have been determined by students in a prelaboratory exercise).

The second goal during Week 1 is for students to create a novel composite plasmid composed of pUC-Kan1 (or pUC-Kan2) and a second plasmid, pBR322. The latter vector contains a tetracycline resistance gene (*Tet*^{R}). To create the hybrid plasmid, both starting molecules are linearized with *Eco*RI, mixed, and then ligated together. This can result in self-ligations, as well as in several possible recombinant outcomes that are each composed of one or more pUC-Kan1 (or pUC-Kan2) molecules and one or more pBR322 molecules (Fig. 1*b*). All recombinant molecules will have functional *Tet*^{R} and *Kan*^{R} genes. However, these recombinant molecules may or may not show *LacZ* function, because the *Eco*RI site in pUC-Kan1 and pUC-Kan2 falls within the *LacZ* gene. (In order for *LacZ* function to be preserved, two pUC-Kan1 or pUC-Kan2 vectors would have to be conjoined in the same orientation.)

After the ligation step, students transform *E. coli* with their ligated DNA, and then plate the cells onto media containing kanamycin, tetracycline, and X-gal. Such media selects for DNA molecules that contain at least one copy of pUC-Kan1 or 2 and at least one copy of pBR322. Two days later, students select a single blue or white colony for further analysis (Fig. 1*c*). This colony is grown by laboratory staff in liquid culture in preparation for Week 2.

Week 2—During the second week, students map the structure of their novel composite plasmid using both PCR and restriction digests. These two different methods should provide complementary results that allow students to determine the composition of their plasmid, as well as the orientation of its composite parts (Fig. 1*d*).

Students begin Week 2 by purifying plasmid DNA from their liquid cultures. A subset of this DNA is digested with the restriction enzymes *Bam*HI and *Hin*dIII. The remaining intact DNA serves as the template for an amplification reaction using primers of known sequence. In a prelaboratory assignment, students will have deduced the location of these primers in the initial plasmids.

Week 3—On the final week of the exercise, students perform gel electrophoresis of the Week 2 PCR products and enzyme digests. Using these data, students are asked to propose a map of their unknown Frankenplasmid.

Part 2. Material Preparation Performed by Staff

Bacteria Culturing and Preparation of Competent Cells—*E. coli* of the DH5α strain was used in all preparations and experiments. Unless otherwise noted, cultures were grown in LB or on LB-agar at 37°C, and liquid cultures were shaken at 200 rpm during incubation. When selective agents were necessary, ampicillin (100 μg/mL), kanamycin (50 μg/mL), tetracycline (12.5 μg/mL), and/or X-gal (60 μg/mL) were added to the growth

FIG 1. **Flow chart depicting the key procedural steps in the laboratory exercise.** Steps (boxed text) are numbered sequentially in the order that they are performed by students. A figure legend (bottom left) identifies the symbols used. (*a*) On the first day of Week 1, each student pair is given aliquots of either the pUC-Kan1 or the pUC-Kan2 vector (solid circles), as well as of pBR322 (dotted circle). The two pUC-Kan vectors differ in the orientation of a pACYC177-derived *Afe*I fragment which contains the kanamycin resistance gene (*Kan*^{R}, white arrow). The junctures between this *Afe*I fragment and the *Zra*I site that it was ligated into are indicated (AZ). pUC-Kan plasmids also contain a *LacZ* gene (not shown), which spans the *Eco*RI site (E). pBR322 encodes tetracycline resistance (*Tet*^{R}, grey arrow). A *Hin*dIII (H) digest of the assigned pUC-Kan vector is run through a gel to determine the orientation of the Kan fragment. In separate reactions, both pUC-Kan and pBR322 are linearized with *Eco*RI (E), combined into the same tube, and ligated. (*b*) Examples of possible results from the ligation reaction. Self-ligated, individual plasmids are shown, as well as recombinant plasmids consisting of two or three individual vectors and at least one of each vector type. As before, pUC-Kan plasmids are depicted as solid lines and pBR322 plasmids are represented as dotted lines. Ligated sites are indicated (E), and for simplicity, the *Kan*^{R} genes (white triangles) and *Tet*^{R} genes (grey triangles) are reduced. (*c*) The ligation mix is transformed into *E. coli*, and cells are plated on agar containing kanamycin (kan), tetracycline (tet), and X-gal. Plating on this selective media selects against self-ligation events and DNA molecules other than those containing both pUC-Kan and pBR322. Colonies will contain a mix of blue (*LacZ*$^+$) and white (*LacZ*$^-$) colonies, depending on whether their recombinant plasmid has an intact *LacZ* gene. (Given that the *LacZ* gene was disrupted by the *Eco*RI digest of pUC-Kan, *LacZ* function can only be restored if the recombinant plasmid contains two pUC-Kan vectors that are conjoined in the same orientation.) Each student pair selects a single colony from their agar plate, inoculates a liquid culture containing kan and tet, and this culture is grown overnight to produce large quantities of a specific recombinant plasmid. On Week 2, this recombinant plasmid is isolated from the liquid culture. Aliquots of this plasmid are then digested with *Bam*HI (B) or *Hin*dIII (H). In a separate pair of tubes, intact recombinant plasmids are subjected to two separate polymerase chain reactions, one using the primers pUC1 and Tet (PCR 1) and one using the primers pUC2 and Tet (PCR 2). (*d*) On Week 3, the restriction digests and polymerase chain reactions are run through a gel to deduce the recombinant plasmid map. To illustrate the mapping process, detailed maps of two of the many possible recombinant plasmids are presented. Restriction sites are shown (B, H), along with the positions and directionalities of the PCR primer sequences (double arrows). Each plasmid yields a unique combination of colony color, *Bam*HI fragments, *Hin*dIII fragments, and PCR products: digests will yield fragments of different sizes in different plasmids, and only PCR primers in opposing orientations will yield a detectable product. With the information given in the laboratory manual, students can derive expected plasmid maps and match their observed results to one of these maps.

media. (See our online Supporting Information files for antibiotic stock formulations.)

To create competent cells, a single *E.coli* colony was added to 1 mL of LB and grown overnight. This culture (0.5 mL) was added to 100 mL of LB the next morning, and this secondary culture was grown until mid-log phase (OD_{600} of ~0.4–0.5 in a 1 cm cuvette), which typically took 2.5–3 h under our culturing conditions. The mid-log culture was chilled on ice for at least 15 min, then the cells were pelleted by centrifugation. Keeping the sample on ice as much as possible, the supernatant was removed, and the cells were gently resuspended in 100 mL of ice-cold, sterile 30 mM $CaCl_2$. After chilling the cells for an additional 15 min, the sample was recentrifuged, supernatant removed, and the cells were gently resuspended in 10 mL of ice-cold, sterile 85% 30 mM $CaCl_2$/15% glycerol. Aliquots (0.1 mL) were placed into chilled microfuge tubes and stored at −80°C until use. (Alternatively, these aliquots may be kept on ice and used immediately.) Aliquots were thawed on ice shortly before class.

Plasmids, Backup Measures, and Transformation Controls—To synthesize the pUC-Kan plasmids, a 1.43 kb *Afe*I fragment from pACYC177 containing the kanamycin resistance gene was ligated into the *Zra*I site of pUC19 using T4 DNA ligase. DH5α *E. coli* were transformed with the ligation mix, and two clones, "pUC-Kan1" and "pUC-Kan2," were isolated that exhibited opposite orientations of the pACYC177 *Afe*I fragment relative to pUC19. Both pUC-Kan1 and pUC-Kan2 retain the ampicillin resistance and LacZ coding functions of the original pUC19 plasmid. To prepare the pUC-Kan1 and 2 vectors for class, each clone was grown up in LB containing ampicillin and kanamycin. Plasmids were then isolated with the GenElute High Performance Maxiprep Kit (Sigma), and diluted to ~100 ng/μL in sterile ddH_2O. pACYC177, pUC19, enzymes, and protocols used for preparation of pUC-Kan1 and pUC-Kan2 were from New England Biolabs. pBR322 plasmid (1 μg/μL) was also purchased from New England Biolabs and diluted to 100 ng/μL by adding 9 volumes of sterile ddH_2O.

We took several steps to ensure that students had a recombinant plasmid to analyze at the conclusion of this module, even if errors were made as they performed the experiment. First, while testing the Week 1 procedure, the staff isolated recombinant (kanamycin- and tetracycline-resistant) bacterial colonies on LB-agar plates containing kanamycin and tetracycline, some of which were *lacZ*$^+$ and some *lacZ*$^-$. These plates were set out on Week 1, Day 3 for the students to use in case their ligation or transformation was not successful on Week 1. Second, between Weeks 1 and 2 of the module, some colonies from these plates were grown overnight in LB containing kanamycin and tetracycline, and a subset of the cultures was stored at 4°C in case students had not achieved growth of their selected colony in liquid culture. Finally, the remaining liquid cultures that the staff had set up were used to isolate recombinant plasmids as described in Week 2 of the student protocol. These plasmids were stored for the students to use as positive controls for transformation during Week 1 and as color references for LacZ enzyme function (Fig. 2*e*, *f*).

pUC-Kan1, pUC-Kan2, pBR322, and the positive control plasmids are available from the authors on request.

Part 3. Student Protocol

Week 1—Prelaboratory Exercise 1. Determining Restriction Site Locations in pUC-Kan and pBR322—This computer-based exercise familiarized students with some DNA sequence analysis tools available on the internet, allowing students to predict the possible restriction maps that they could observe in laboratory on Week 1. Before the first laboratory, students were given the Genbank accession numbers of the plasmids pUC19 (L09137), pACYC177 (X06402), and pBR322 (J01749) and directed to the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/). They used this database to locate the antibiotic resistance and *lacZ* genes in each vector. As shown in the laboratory manual (see online Supporting Information files), some assistance was given in cases where we deemed the sequence annotation to be confusing to students. For each vector, students were also asked to locate the restriction enzyme sites that are relevant to this experiment using the NEBCutter tool from New England Biolabs (www.neb.com; *Afe*I for pACYC177, *Zra*I for pUC19, and *Bam*HI, *Eco*RI, and *Hin*dIII for all three vectors). The prelaboratory exercise was turned in and corrected by teaching assistants at the start of the Week 2 class. This exercise provided a guide for students to predict the two possible *Hin*dIII restriction maps for their pUC-Kan clone.

Day 1. Restriction Mapping of pUC-Kan and Generation of a Recombinant Plasmid—Students were given 100 ng/μL aliquots of pBR322 and either pUC-Kan1 or pUC-Kan2. 1 μg (10 μL) of each plasmid was digested with FastDigest *Hin*dIII in one set of reactions and with FastDigest *Eco*RI in a second set, for a total of four reactions (Fermentas). The total reaction volume in each case was 40 μL. An additional 0.4 μg (4 μL) of each plasmid was added to buffer without enzyme to serve as a negative control for digestion (10 μL total volume). FastDigest Green buffer, which contains gel loading dye, was used for all of these samples, allowing them to be directly loaded into an agarose gel after digestion. The reactions were carried out at 37°C, and incubations were extended for 25 min to ensure complete digestion and to accommodate other procedures that the students were performing in parallel.

To estimate restriction fragment sizes, 10 μL of each restriction digest was run through a 0.8% agarose standard-sized minigel (~8 cm long) in TBE buffer at ~100V. Both the gel and TBE contained 0.5 μg/mL of ethidium bromide. We found that fragments were sufficiently separated when the lighter (yellow) dye had reached the far end of the gel. UV-illuminated photographs were taken of each gel and given to the students for analysis.

While gel electrophoresis proceeded, the remaining 32 μL of *Eco*RI-digested pBR322 and 32 μL of *Eco*RI-digested pUC-Kan were combined into one tube. This mix of DNA was purified using the DNA Clean & Concentrator Kit-5 (Zymo Research), then treated with 1-μL T4 DNA ligase (Fermentas) in a total reaction volume of 20 μL for at least 15 min at room temperature.

To transform cells with the ligated mixture of DNA, 10 μL of the ligation reaction was added to a 0.1 mL aliquot of competent cells that had been prepared as described above. The DNA and cells were incubated on ice for at least 15 min, then placed into a 37°C heating block for 3 min. Immediately after heat shock, 0.5 mL of LB was added, and the sample was incubated for an additional 30 min at 37°C on a heating block with no agitation. After this incubation, cells were pelleted by centrifugation, and 0.45 mL of the supernatant was removed and discarded. This reduced the overall volume without losing cells, allowing students to fit all of their transformed cells on one plate. The remaining culture (~160 μL) was resuspended and plated on LB-agar containing kanamycin, tetracycline, and X-gal. Plates were incubated for 2 days at 37°C to ensure the presence of large colonies.

Each pair of students was also assigned a transformation control to prepare in parallel. Approximately 20–50 ng of undigested pUC-Kan1 (Fig. 2*b*; Control C, c.f. laboratory manual), pUC-Kan2 (Fig. 2*c*; Control D), pBR322 (Fig. 2*d*; Control E), or

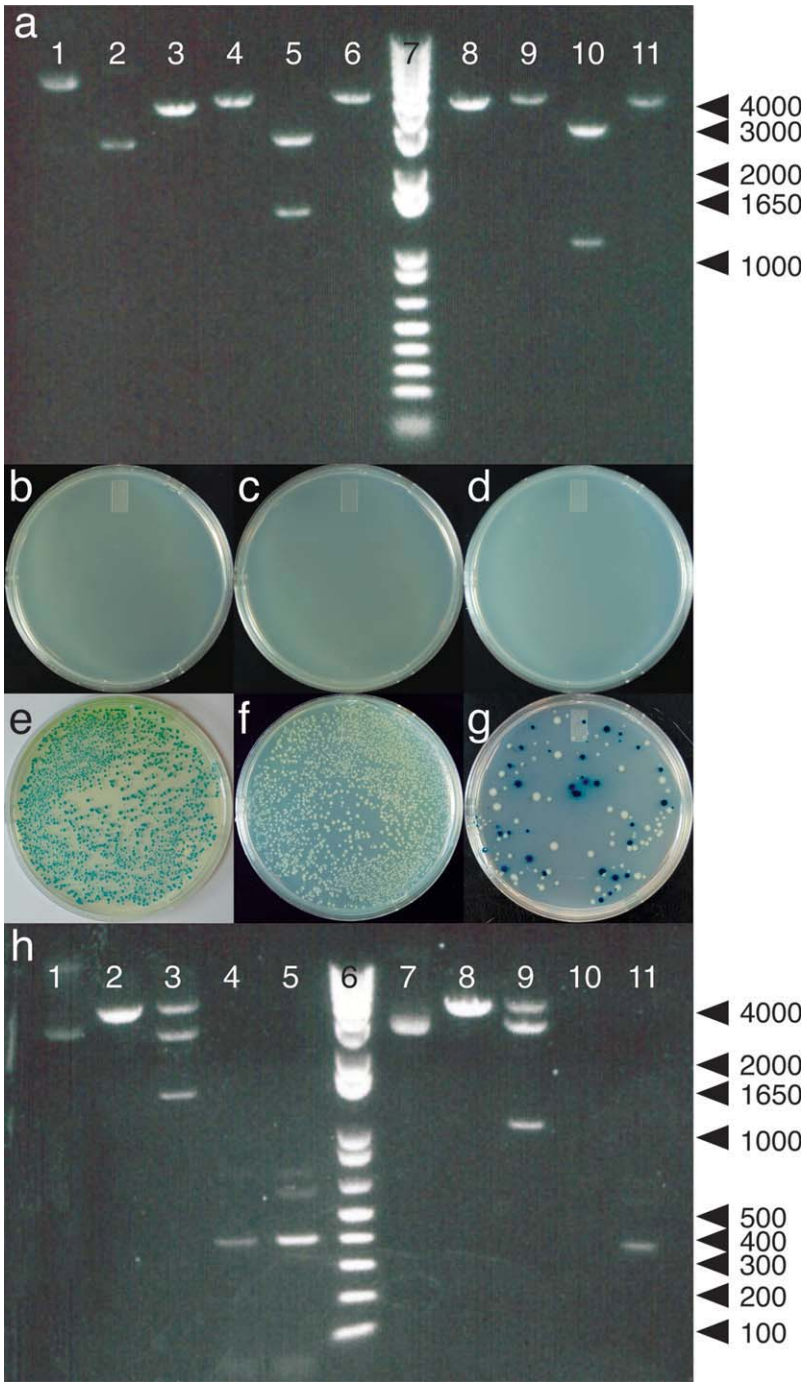

FIG. 2. **Examples of student data.** (*a*) Week 1 agarose gel, showing restriction analysis of the starting vectors. Two pairs of students shared each gel, with one pair loading their digests in Lanes 3–6 and the other pair using Lanes 8–11. Lane 1, undigested pUC-Kan1. Lane 2, undigested pUC-Kan2. Lane 3, *Eco*RI-digested pUC-Kan1. Lane 4, *Eco*RI-digested pBR322. Lane 5, *Hin*dIII-digested pUC-Kan1. Lane 6, *Hin*dIII-digested pBR322. Lane 7, molecular weight standard (MWS; standard sizes to the right of panel). Lane 8, *Eco*RI-digested pUC-Kan2. Lane 9, *Eco*RI-digested pBR322. Lane 10, *Hin*dIII-digested pUC-Kan2. Lane 11, *Hin*dIII-digested pBR322. The two pairs of students sharing a gel were given different pUC-Kan vectors because students had to match their observed results to one of two expected results, and seeing the differences between the *Hin*dIII digests of pUC-Kan1 and pUC-Kan2 (Lanes 5 and 10 respectively), facilitated this decision. (*b*-*g*) Week 1, Day 3 results. Transformed *E. coli* cells were plated on media containing kanamycin, tetracycline, and X-gal. (*b*) No growth by cells that were transformed with pUC-Kan1 due to the lack of tetracycline resistance. (*c*) No growth by cells that were transformed with pUC-Kan2, also due to the lack of tetracycline resistance. (*d*) No growth by cells that were transformed with pBR322 due to the lack of kanamycin resistance. (*e*) Growth of cells that were transformed with a $Kan^{R}Tet^{R}LacZ^{+}$ plasmid. The blue color demonstrates *lacZ* function. (*f*) Growth of cells that were transformed with a $Kan^{R}Tet^{R}LacZ^{-}$ plasmid. The white color shows a lack of *lacZ* function. The plasmids used in (*e*) and (*f*) were isolated by the staff before class using the Week 2 protocol (see Materials and Methods). (*g*) Cells transformed with a ligated mixture of pUC-Kan and pBR322. Cells are able to grow if they contain a plasmid that is a hybrid between the two starting vectors. Colonies may be blue or white depending on whether a *lacZ* gene is intact after ligation. (*h*) Week 3 agarose gel, showing restriction analysis and PCR of hybrid plasmids that were isolated from plates such as the one shown in (*g*). Similar to Week 1, two pairs of students shared a gel. Lanes 1–5 belong to one group, while Lanes 7–11 belong to the second group. As in (*a*), molecular weight standard sizes are shown to the right of the panel. Lanes 1/7, undigested recombinant plasmid. Lanes 2/8, *Bam*HI-digested recombinant plasmid. Lanes 3/9, *Hin*dIII-digested recombinant plasmid. Lanes 4/10, PCR1 of intact recombinant plasmid. Lanes 5/11, PCR2 of intact recombinant plasmid.

the positive control plasmids (Fig. 2*e*, *f*; Controls F and G) were transformed into competent cells and plated using the same procedure described above.

Day 3. Selection of a Recombinant Colony—On the morning of Day 3, our staff added kanamycin and tetracycline to LB, dispensed this solution into culture tubes in 5 mL aliquots, then stored these aliquots at 4°C for students to access. Students came into the laboratory later during Day 3, selected a single colony from their experimental plates, noted whether it was blue (*lacZ*$^+$) or white (*lacZ*$^-$), then used this colony to inoculate an aliquot of LB/kanamycin/tetracycline. If a student pair had not obtained transformants, they were instructed to select and grow a colony from a backup plate, which was generated by the same methods used by the students to generate recombinant colonies. Students then placed their liquid cultures, experimental plates, and control plates into a refrigerator. At the end of Day 3, liquid cultures were removed from the refrigerator, grown by our staff overnight under standard culturing conditions, and stored again at 4°C until the Week 2 laboratory period.

Week 2—Restriction Mapping and PCR of a Recombinant Plasmid—The results of all five sets of control plates were pooled and shared among the class. Students then isolated recombinant plasmid from their liquid cultures using the Qiaprep Spin Miniprep Kit (Qiagen). This isolated plasmid (10 μL), which contained between 300 and 600 ng in our hands, was digested with FastDigest *Bam*HI in one reaction and with FastDigest *Hin*dIII in a second reaction (Fermentas). An additional 10 μL of plasmid was added to buffer only to serve as a negative control for digestion. The total reaction volume in each case was 20 μL, and as in Week 1, reaction conditions were extended to 25 min and the FastDigest Green buffer was used. On completion of the digests, samples were heated to 70°C for 10 min to deactivate enzyme activity and then stored frozen until Week 3.

Recombinant plasmids were also analyzed by two polymerase chain reactions, each using a primer specific for pBR322 (Tet: 5′-CGCCATAGTGACTGGCGATGCTG-3′) and one of two primers specific for pUC19 and consequently, pUC-Kan (pUC1: 5′-TCACTCATTAGGCACCCCAGGC-3′ or pUC2: 5′-ATCAGGCGC CATTCGCCATTC-3′). Reactions were driven with GoTaq Flexi DNA polymerase (Promega). GoTaq Flexi Green buffer, which contains loading dyes of the same molecular weights as the FastDigest Green buffer, was used so that completed reactions could be loaded into the gel directly. Reaction conditions were 1× buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP (from the 10-mM PCR Nucleotide Mix, Promega), 0.05 U/μL DNA polymerase, 1 μM of each primer, and 1:20,000 template. PCR began with a hot start at 94°C for 3 min, followed by 35 cycles of (94°C for 30 s, 55°C for 30 s, 72°C for 60 s). A final extension phase of 72°C for 5 min was added at the end of these cycles, then samples were stored frozen until Week 3.

Week 3—Prelaboratory Exercise 2. Determining the Annealing Sites of the PCR Primers—Students were given the sequences for the three PCR primers used in this experiment and then directed to the BLAST website at the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih. gov/Blast.cgi). Using the bl2seq tool, each primer sequence was entered as a query and, depending on the specificity of the primer, the pUC19 or pBR322 sequence was entered as a subject. Students recorded the location and directionality of each primer sequence relative to its complementary vector. This exercise provided the means for students to predict the size of a PCR product if two primers were facing each other when annealed to the template.

Agarose Gel Electrophoresis of Week 2 Restriction Digests and PCR—Restriction digest and PCR samples from the Week 2 exercise were run through an agarose gel as described in the Week 1 procedure. UV-illuminated photographs were taken of each gel and given to the students for analysis. Expected results for possible plasmids will be provided to instructors on request.

Part 4. Assessment of Educational Value

In their final reports, students used their findings from Prelaboratory Exercise 1 to derive maps for pUC19, pACYC177, pBR322, and the two possible pUC-Kan plasmids. They then used their Week 1 *Hin*dIII digests to determine which pUC-Kan map best applied to their clone. Finally, they were asked to outline at least two possible recombinant plasmids, then to use their colony color, Week 3 *Bam*HI and *Hin*dIII digests, and PCR results to determine which map best fit their data.

To improve our instruction in the future, we determined the student experimental success rate at each of the major steps in the experiment. In addition, to gauge how students felt about the pedagogical value of this module, we administered a self-assessment survey after they had turned in their laboratory report, using a format and 5-point rating scale that has been reported previously (Table I; [4, 5]).

RESULTS AND DISCUSSION

Assessment of the Laboratory Procedures

Students were, on the whole, very successful with the laboratory work in this module. Figure 2 exhibits representative student data for the exercise. After Week 1, 97% of laboratory groups had obtained transformant colonies on their experimental plates (Fig. 2*g*; averages of 30 ± 15 SD *lacZ*$^-$ colonies and 22 ± 12 SD *lacZ*$^+$ colonies per plate, $n = 30$ plates). With an exception of one group that used the wrong incubator, all students were able to isolate sufficient amounts of recombinant plasmid from Week 2 liquid cultures to complete the experiment. After Week 3, 93% of laboratory groups had results from their colony color, restriction map, and PCR that, when taken together, were sufficient to suggest a single recombinant plasmid sequence (Fig. 2*h*; $n = 30$); the two exceptions were groups whose Week 3 *Bam*HI and *Hin*dIII restriction digests had both failed. Over the course of Weeks 1 and 3, 96.0% of restriction digests were sufficiently completed to be interpretable (Fig. 2*a*, *h*; $n = 175$).

As outlined in the Materials and Methods section, many measures were taken to provide backups for students. On Week 1, our staff provided extra plates of transformants in case students had not obtained colonies (however, there were sufficient numbers of colonies on the plates of most students for sharing to be possible, making this backup measure somewhat unnecessary). As a class, we set up multiple copies of each negative (Fig. 2*b–d*) and positive control plate (Fig. 2*e*, *f*) and pooled our results in case a mistake was made on a particular plate. Backup liquid cultures were made in case a transformant colony had not grown to saturation. Finally, if Week 1 or 3 gel results were uninterpretable, it was a simple task to give students a photo of a gel from another group that had started with their same pUC-Kan vector. In the end, all students had data that were sufficient to perform a complete analysis of a recombinant plasmid, even if an error had occurred as they performed the experiment themselves.

TABLE I
Self-assessment questions and responses

Statement	Strongly disagree (%)	Disagree (%)	Neutral (%)	Agree (%)	Strongly agree (%)	Mean score* ± SD ($n = 50$)
1. Please indicate your level of agreement or disagreement with each of the following statements:						
The "recombinant DNA analysis" laboratory enhanced my understanding of the polymerase chain reaction (PCR).	4	8	36	34	18	3.5 ± 1.0
The "recombinant DNA analysis" laboratory enhanced my understanding of restriction mapping.	0	2	6	42	50	4.4 ± 0.7
The "recombinant DNA analysis" laboratory enhanced my understanding of how to approach scientific questions experimentally.	0	4	14	58	24	4.0 ± 0.7
The "recombinant DNA analysis" laboratory enhanced my ability to integrate multiple lines of evidence to thoroughly answer a scientific question.	0	4	8	54	34	4.2 ± 0.7
The "recombinant DNA analysis" laboratory write-up improved my ability to present scientific hypothesis-testing in written form.	0	6	22	52	20	3.9 ± 0.8
The "recombinant DNA analysis" laboratory was a valuable educational experience.	0	2	6	50	42	4.3 ± 0.7

2. Compared to other course-related laboratory activities you have taken part in at Williams College (*i.e.* labs associated with BIO 101, BIO 102, or other courses) please rate the "RECOMBINANT DNA ANALYSIS" laboratory with respect to the following:

	Much worse than other labs (%)	Worse than other labs (%)	About the same as other labs (%)	Better than other labs (%)	Much better than other labs (%)	Mean score** ± SD ($n = 49$)
Teaching of useful laboratory skills	0	2	42.9	42.9	12.2	4.0 ± 0.7
Illustration of course-related content	0	0	16.3	55.1	28.6	3.9 ± 0.7
Illustration of the scientific process	0	0	36.7	40.8	22.4	3.9 ± 0.8
Overall educational value	0	2	18.4	53.1	26.5	4.0 ± 0.8

* 1 = Strongly disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, 5 = Strongly agree.
** 1 = Much worse than other labs, 2 = Worse than other labs, 3 = About the same as other labs, 4 = Better than other labs, 5 = Much better than other labs.

Empirically, it appeared that students were most challenged by having to pipet and mix small volumes of liquid effectively. We believe that these challenges account for the few restriction digests that failed and perhaps for the lack of transformants on one experimental plate. In consideration of this issue, students were frequently reminded of good pipetting technique and, quite simply, to "watch what they were doing", that is to visually confirm that a particular volume had been drawn out of the first tube, into the pipet tip, that this liquid had been completely dispensed into the bottom of the second tube, and that the components had been well mixed. This advice appeared to be helpful to them in many instances.

We also found it necessary to monitor students as they prepared and handled agarose gels. In a few cases, molten agarose leaked out of a poorly sealed gel mold, or gels were torn while being moved or loaded, causing bands to smear while migrating or reducing the holding capacity of a loading well. In anticipation of this, backup flasks of molten agarose were made available.

All student groups obtained at least one positive result from their two PCR tubes and, with two exceptions, the product sizes were consistent with their restriction map ($n = 60$). However, in cases where a restriction map for a recombinant plasmid predicted only one PCR product between the two primer sets, an unexpected product of the same size was frequently seen in the other PCR lane of the gel (*e.g.* Lane 4, Fig. 2*h*). Generally, this product was significantly lower in quantity, and, therefore, was attributed to cross contamination between the two PCR samples. Although this sort of error illustrates the sensitivity of PCR to a student without severely weakening their conclusions, in the future, we intend to purchase PCR barrier pipet tips for use in class and to encourage the students to pipet more carefully while making their PCR master mixes and samples.

Assessment of Pedagogical Value

Our assessment of student perception of this laboratory exercise was encouragingly positive. Table I shows the results of individual survey questions from 49 to 50 respondents. The table is broken into two sections: one section pertains to skills-related aspects of the laboratory activity and a stand-alone assessment of educational value, and the second section asks the students to compare the exercise to other comparable college-level activities. The majority of students agreed with the state-

ments that this activity enhanced their understanding of PCR and restriction mapping, although there appears to be a substantially higher level of comfort with the latter skill following this laboratory: 92% of respondents either agreed or strongly agreed that that the module enhanced their understanding of restriction mapping, as opposed to 52% who felt the same with regards to PCR ($n = 50$). While most students were able to interpret the results of their PCR data correctly (as judged by their laboratory write-ups), our assessment indicates that some students may have had difficulty understanding the basic mechanism of PCR. It may be particularly important that discussion of PCR in lecture is tightly coupled with its practice in the laboratory to ensure student success in this area. In the future, we also intend to give students more opportunity to visualize PCR by exposing them to more illustrations and online tutorials which depict the process. In terms of enhancing student understanding of the mechanics of carrying out scientific research (*i.e.* approaching questions experimentally, integrating multiple lines of evidence, and defending a scientific interpretation in writing), as well as the overall educational value of this activity, our assessment suggests that students found this laboratory to be a positive educational experience. Indeed, this activity compared extremely favorably to other college-level laboratories in which our students had previously participated. For instance, with respect to overall educational value 77.6% of respondents ($n = 49$) rated this activity as "better" or "much better" than other labs (*i.e.* scores of 4 or 5 in Section 2 of Table I); only a single student rated the laboratory as less valuable than other course-related laboratory activities.

CONCLUSIONS

In this report, we have described a new laboratory exercise that illustrates the principles and puzzle solving involved when implementing standard recombinant DNA techniques. While restriction enzymes and PCR are used cooperatively for DNA mapping in primary research (*e.g.* [6]), we have designed this module more as a teaching tool to help students visualize how DNA mapping works than to exhibit a research environment, cutting edge technology, or how cloning may be done under more controlled conditions. This in mind, the outcomes appear to be positive. First, this is an arguably challenging exercise for third semester biology students, yet students had a high rate of success in the laboratory on their own. Second, the few consistent sources of error illustrated the importance of good technique, yet with our suggested backup measures, every student was able to complete a full analysis within the limited time frame. Third, it was seen how multiple lines of evidence (*i.e.* plating, restriction mapping, and PCR) may be used collectively to answer the same question and strengthen a conclusion. Finally, according to our formal and informal assessments, students found the module to be an engaging and fulfilling assignment.

As alluded to in the previous paragraph, we excluded some steps from the student laboratory manual that other instructors may wish to add to the procedure, that is the steps one would take if they wished to custom design a specific plasmid in a more controlled fashion, much as one would do within a research setting. Specifically, we did not have the students determine DNA concentration (although our students had done this on multiple occasions in an introductory class), purify a restriction fragment from a gel, or treat one of the two fragments with calf intestinal alkaline phosphatase (CIAP). Including these measures would have been more representative of research-based techniques, but would require more time and funding, and would almost certainly reduce success. Instead, we chose to prioritize a high success rate, a high yield of recombinant plasmids, and for the students to have experience filtering through more than two possible recombinant plasmids within our available time frame so that they could firmly visualize what was occurring at the molecular level.

Finally, we feel that the cyclical design of this module is an effective and versatile educational tool. Students perform a restriction fragment analysis, then perform a second, more complex analysis at a later date, adding independent lines of evidence from their colony phenotypes and PCR. While we think that this repetition with increasing complexity is valuable, this exercise also has a modular capacity. For example, 1) if the instructor wishes to adapt the experiment for first-year biology students, or if 3 weeks are not available within a semester, Week 1 or Weeks 2 and 3 could be administered in isolation. 2) For more advanced classes, students could be made responsible for more steps in the exercise, such as making their own buffers, agarose gels, or competent cells, excising restriction fragments from a gel and purifying them, and even treating these fragments with CIAP to have more control over the possible ligation events. 3) If expenses or equipment availability are limited, PCR could be eliminated from the exercise without significantly weakening the conclusions made by most students.

We are happy to provide all plasmids, cells, maps, and expected outcomes to interested instructors on request.

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