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A Publicly Available PCR Methods Laboratory Manual and Supporting Material †

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INTRODUCTION

A laboratory manual and supporting material have been developed that use 24 different laboratory problems to illustrate basic polymerase chain reaction (PCR) concepts, to promote independent problem-solving skills, and to reinforce the scientific method. This material is appropriate for those interested in science in general or PCR in particular; i.e., a high school student wanting to learn basic research or a post-doc unfamiliar with PCR would both find portions of this material useful. In order to maximize reliability, most labs use Lambda (λ) bacteriophage DNA and a master mix with loading dye (Promega #D1501 and #M7122).

There are currently several textbooks and technical manuals that detail basic (and advanced) PCR techniques (1–3). Although these are excellent resources, they do not provide the hands-on troubleshooting experience needed to learn the technique. The materials described herein force each student to design his or her own experiment and to troubleshoot it if it does not work.

The student edition of this manual is 117 pages long and includes 24 labs. Appendices in the manual allow reproduction of procedures by providing primer sequences and catalog numbers (typically VWR) for all supplies. The student version is freely available as a supplemental file (Appendix I) or at http://ans.latech.edu/BasicPCRMethods.html.

The instructor's lab book includes all of the student lab book material, with additional appendices containing suggested lab setup diagrams, answers to end-of-lab questions and pictures showing suggested tray setup and picture distribution. A total of 51 PowerPoint slides include 22 lecture-format and 29 for specific labs. A test bank including 41 multiple choice, 20 true/false, and 21 short answer/fill in the blank questions is provided. A lab practical includes safety protocols, PCR programming, agarose gel loading, pipettor accuracy testing, and pipettor settings knowledge. In addition, six different final lab practical exercises are included. Instructor resources are available from the corresponding author at no charge.

PROCEDURE

An 11-question safety checklist is provided in the manual. Safety concerns include ethidium bromide exposure, electrical shock from gel rigs, or burns from gel preparation. To reduce the danger from ethidium bromide, only the instructor handles un-diluted stock, and students are required to wear gloves when working with prepared gels. The hazard of electrocution is reduced by utilizing modern, electronic power supplies and shielded-wire gel rigs. We provide safety glasses to each student, which, along with closed-toed shoes, are required during gel preparation.

The first four labs introduce the student to pipetting and PCR, agarose gel setup, primer design and rehydration. Labs 5 to 17 each answer a specific question about PCR or the detection of products. Lab 18 increases reaction complexity, while lab 19 requires the preparation of additional samples to act as unique subject DNA. Labs 20 to 24 require additional components (20, 24), extended thermal cycler programming (21, 23), or the possession of a gradient thermal cycler (22). A comprehensive, lab-by-lab listing of material is provided in Appendix E, which includes required quantities for all labs. If implementing all of the labs, a class of 12 to 24 students will require 1 to 2 days of setup, including all tube labeling and distribution of primers, DNA, and reagents into trays for each student (Appendix L). Once prepared, the materials are stored in a freezer. After competency in core techniques and safety have been assured, each student is provided with the material required for the whole course and allowed to proceed at his or her own pace. A lab fee of $45 is collected from each student that easily covers the cost of a 24-student lab (assuming adequate hardware is already in place).

Lab operation involves the preparation of four 20- × 25-cm 1.5% agarose gels with 72 to 96 wells per gel and thawing of student material trays one hour prior to class. Class starts with loading the agarose gels, followed by lecture/discussion (typically no more than 10 to 20 minutes per day). Students are then free to set up labs, perform documentation of previous labs, etc. Toward the end of the class, each gel row is documented using a UVP Gel

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†Supplemental materials available at http://jmbe.asm.org

DOC system with a thermal printer, creating a copy for each student who loaded on that row. We use a large glass cabinet with tape to distribute these pictures (Appendix L). By maintaining a strict lab time of 2.5 hours, the common practice of "throwing time at a problem" is reduced.

Completed student reactions are placed in 96 well trays with a small run slip (Appendix F). This slip tells the instructor which program and machine to run the samples in. Most labs use primers designed for Lambda DNA that function at the annealing temperature of 60° C, which allows most samples to be run on the same machine (Appendix L).

Each student determines the order to perform experiments and plans each lab (Fig. 1). If the experiment fails, the student performs it again until it is successful. Because of the "attempt until success" nature of the course, an excess supply of reagents is always given (Appendix E), and a "Master" tube of reagent is used to increase each student's supply as needed (Appendix L).

Having 20 to 24 students performing a wide array of protocols under a time deadline is like herding cats. There are two basic rules that are followed. First, a student may not ask the instructor for assistance until he or she has attempted to solve it with at least two other students. Second, once guidance is given, all similar questions are referred to the student(s) who received guidance on how to solve the problem. An exception is provided when the question concerns safety.

Although this manual has been tested over several years by upperclassmen and graduate students and taught by multiple instructors, it will contain errors. Suggestions for a new or improved exercise are always welcome. In addition, if you have created "how-to-program" instructions for a particular model of thermal-cycler, please do email the information to the corresponding author so that it can be included in the next revision. "On-the-spot" revisions are possible using a DOCX version of the manual available to instructors.

CONCLUSION

We have designed PCR learning materials based on the use of Lambda (λ) and self-extracted human DNA. By using this DNA and minimal equipment, we have created a simple, student-driven research experience. All of the course materials (except instructor resources, available from the author) are online and freely available.

SUPPLEMENTAL MATERIALS

Appendix 1: PCR Methods Lab Manual (Student version)

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The authors declare that there are no conflicts of interest.

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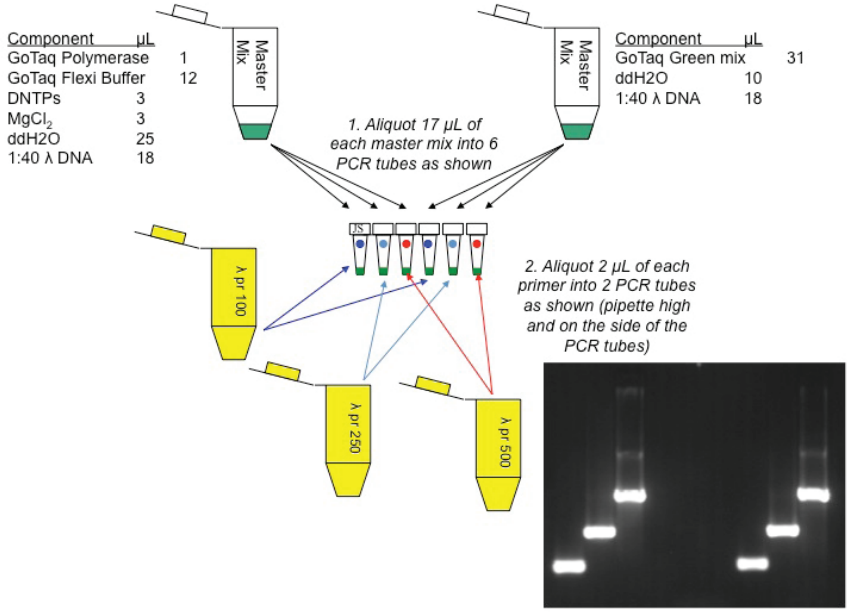

FIGURE 1. Example of master mix setup diagram. Note that this is provided to students as an example—subsequent master mix diagrams are only provided in the instructor materials. Inset shows student generated gel picture of lab.