Understanding Primers and Polymerase Chain Reaction by Using a Kinesthetic Paper Model

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INTRODUCTION

This kinesthetic PCR lab is appropriate for an advanced high school or any undergraduate lab in which students have learned the structure of DNA. It requires students to manually arrange a paper DNA template and primers as would be found in various steps of PCR and then play the role of DNA polymerase (DNAP) in synthesizing new DNA. They write out two cycles, facing and overcoming misconceptions, then explore how to design primers. As they do this, they reinforce the importance of three rules of DNAP: template requirement, 3’-hydroxyl requirement, and 5’→3’ directionality.

PCR is a core molecular biology technique with applications across many research questions and fields of study. Given the proper components and a thermocycler, PCR is a simple procedure, albeit one that requires accurate micropipetting. As such, instructors might teach students PCR in many courses, sometimes without much previous coverage of molecular biology. Many PCR labs involve students adding a few labeled liquids to a tube, perhaps with a student-isolated DNA template, and then the instructor starting a thermocycler. Some inquiry-based labs might allow students to design and troubleshoot their own primers, but this is time-consuming and expensive.

Students have misconceptions about PCR that are not addressed by such a wet lab. For example, they may believe that primers cut DNA, that both primers bind to the same strand of DNA, or that all DNA is amplified during PCR (1). In general, they may have difficulty determining how primers relate to the sequence to be amplified and the purpose of each temperature in a cycle.

A physical model can help students learn (2, 3). Physical models have been explored previously for PCR by using yarn (4) and foam-printed nucleotides (5); these approaches were found to be effective. Here, I present a simplified and inexpensive paper model in a system with relevance to health and an emphasis on DNAP function.

PROCEDURE

Preparation

Students should be made familiar with the structure of DNA (including complementary bases, “base pairs” as a unit, and antiparallel strands) and heterozygosity. The assignment assumes basic familiarity with Huntington’s disease, which was chosen due to its health impact and because it can be identified using PCR with a procedure similar to that simulated by the students (6).

Preparation involves color printing and cutting double-stranded DNA template and primers (see Appendix S1 in the supplemental material). The lab requires chalk and large flat surfaces that can be written on with chalk (e.g., lab benches). Papers are not consumed and can be reused. Each group needs access to a camera, which can typically be a cell phone but might be provided by the instructor. Each group will also need a red pen and a pencil.

Activity

The exercise (see Appendix S2) takes approximately 1.5 h. It might be taught during a lab on PCR or during the next lab section, for example, as a gel is running, to help the students interpret their results. The lab can be easily made up outside of class time.

Each group receives two complementary template strands and three of each primer. Students reproduce two cycles of PCR, with denaturation, annealing, and extension, taking a picture of each step (Fig. 1) and answering questions. Finally, they describe why other primer orientations would not function correctly and design some primers of their own for a sample sequence (Appendix S3).

In detail, the first step is to line up the template strands to make double-stranded DNA. This may be difficult for students until they consider the antiparallel nature of DNA. They separate these strands, then bind one primer to each at its complementary
sequence. Next, they use chalk to write new bases extending from the 3’ ends of each of the primers complementary to the template. A common error is to fill in bases on the 5’ end of the primer, which indicates a misconception about DNA polymerase activity (a misconception that would prevent students from understanding the actual outcomes of PCR). Students may realize this on their own or may require prompting. They separate the strands again and bind new primers. They then use chalk to extend the DNA from newly synthesized strands of DNA.

The previous steps illustrate how PCR works, but students likely will still need help designing their own primers. Students complete three figures by drawing lines for new DNA synthesis through two cycles. The first figure is a correct orientation of primers and results in successful PCR. The other figures illustrate orientations of primers that result in failed PCR (two primers with 3’ ends pointing outwards, or two primers bound to the same template strand), because the newly synthesized DNA will not bind to any primers. Students may need help understanding why these do not work. Finally, students apply this information to design a pair of working primers when presented with only one strand of DNA sequence.

**Safety issues**

There are no safety issues in this lab, as the only materials are paper and chalk, both of which are nontoxic.

**CONCLUSION**

Doing PCR can be an ineffective method of fully teaching students how PCR works. This exercise greatly improved my students’ abilities to identify the steps of PCR and their purpose. It also helped them understand the rules of DNA polymerase that cause PCR to work as it does (and which help explain other diverse biological concepts, from telomeres to Okazaki fragments). Students typically enjoy the “arts and crafts” feel of the lab, and some have expressed that it helped them visualize the process of PCR.

The lab could easily be modified to deal with another gene of interest rather than Huntingtin, though the amplified region needs to be short for the lab to be practical. A few questions deal with variable-length alleles, and these might need to be reconsidered for another gene. Any new primers and template sequences made should be in a monospaced font such as Courier New and with font sizes between 44 and 72. It would be possible to extend this lab by having students simulate, with chalk, DNA replication from incorrect primer orientations, rather than drawing lines on figures. This would likely improve learning but would take substantially more time. A complete third cycle could also be added, though this presents some logistical difficulties, as by the end of cycle 2 some double-stranded DNA molecules have two chalk portions that are difficult to separate.

One might use this lab to guide students toward designing primers to use for an experiment, with the caveat that it does not emphasize primer optimization. After this exercise, students would be prepared to use and understand the results of a program for automated primer design (such as [https://www.ncbi.nlm.nih.gov/tools/primer-blast/](https://www.ncbi.nlm.nih.gov/tools/primer-blast/)).

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, PDF file, 0.8 MB.

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