Two Laboratory Activities Using Conventional or Real-Time PCR to Simulate Pathogenic E. coli Detection †

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INTRODUCTION

This lab activity is designed as an extension to a case study activity in which students design a PCR-based diagnostic test for a pathogenic strain of E. coli (3). In the case study, students use bioinformatics to discover that the Shiga toxin genes (stx1 and stx2) are unique to the Enterohemorrhagic E. coli (EHEC) strain O157:H7 and come to the conclusion that doing PCR with primers designed for Shiga toxin will differentiate O157:H7 from other strains of E. coli. In this activity, students actually perform the PCR assay. Two lab activities are provided, one using conventional PCR and gel electrophoresis and the other using real-time PCR. The activities are designed to complement one another, but either could be used alone. Each activity takes two lab or class periods, although there is enough overlap that when both are done the time needed can be as little as two lab periods plus one lecture (see Appendix 5). The intended audience is Microbiology and Biology majors taking an introductory level course in general microbiology or biology, as well as upper level students.

While conventional PCR and gel electrophoresis are easily within the scope of many teaching laboratories, real-time PCR is used less frequently. However, real-time PCR has widespread applications in disease diagnosis and management, gene expression studies, food testing, and forensics (1, 4) and students are likely to encounter it in the workplace or in subsequent schooling. Therefore, it is a valuable addition to any curriculum.

The real-time PCR lab uses SYBR green to detect the amount of PCR product produced. SYBR green is a dye that fluoresces when bound to double-stranded DNA. Fluorescence is detected by a camera attached to a thermocycler after each round of amplification, providing a measure of the relative number of amplicons. While real-time PCR using SYBR green lacks the sensitivity of other detection methods, such as TAQMAN probes, its straightforward methodology makes for a good introduction to the technique, especially for undergraduates.

This lab continues a case study scenario in which students are asked to determine if any E. coli isolates from a local beach are the strain O157:H7. Students perform colony PCR on various strains of E. coli using primers specific to the stx1 gene. Students can test isolates they obtain from the environment, or the E. coli strains can be provided for them, depending on the situation (see Appendix 5).

PROCEDURE

In this lab, colony-PCR using non-pathogenic strains of E. coli is employed. E. coli O157:H7 has two Shiga toxin genes, stx1 and stx2 (5); testing could be done for either one. Testing for stx1 is described here, since a nonpathogenic strain of E. coli with stx1 cloned on a plasmid, TOP10 pSTX1 (2), is available. It was used as a positive control strain and as a mock positive unknown, eliminating the risk associated with using O157:H7. E. coli TOP10 was used as the negative control. Detailed instructions for PCR, gel electrophoresis, and real-time PCR are provided on the student and instructor handouts (Appendices 1 and 5). Typical student results for conventional and real-time PCR are provided in the instructor handout (Appendix 5). Safety issues involve proper handling of microorganisms and general precautions surrounding gel electrophoresis and are discussed in the instructor handout (Appendix 5).

CONCLUSION

The conventional PCR and gel electrophoresis lab has been used twice in an undergraduate microbiology course for biology majors. When used in conjunction with the virtual PCR case study (3), students found this lab reinforced the concepts of PCR and gel electrophoresis. Comments included “This step solidified what we had been learning in the previous parts. Virtually doing PCR is good, but the hands-on experience is even better. The more times I do PCR, the better I understand the process,” and “I enjoyed this very much, it was interesting and exciting.”

When compared with students who had only done the virtual PCR, students who did the PCR laboratory...
performed better on assessment questions testing their understanding of PCR and gel electrophoresis. The average class score on a question asking what happens at 95°C during PCR was 75% for students who had only done the virtual PCR, while it was 95% for students who had done both the virtual and hands-on lab. Similarly, when asked to identify the correct size of a band compared to a molecular mass ruler run on an agarose gel, the average scores were 75% and 100%, respectively.

On one occasion, students performed the real-time PCR lab following the conventional PCR lab. Between the two labs, instruction was provided on the theory of real-time PCR. These students, half of whom were freshmen, found real-time PCR to be challenging to understand, but comparing the real-time PCR results with the gel electrophoresis results increased comprehension. Therefore, if time allows, it is recommended that students perform both lab activities and that sufficient in-class time be allowed for explanation and interpretation of the results.

The lab handouts have several questions pertaining to PCR and gel electrophoresis that can be used to assess student learning. Keys are provided in Appendices 2 and 4. Possible modifications to this lab could include having students design primers to amplify the Shiga toxin gene rather than providing pre-designed primers for the students. In addition, the lab could easily be modified by the instructor to incorporate alternate scenarios, such as testing patient specimens or food products. Students could also find, read, and summarize primary literature in which real-time PCR is utilized.

SUPPLEMENTAL MATERIALS

Appendix 1: Student handout – Conventional PCR and gel electrophoresis
Appendix 2: Student handout – Conventional PCR and gel electrophoresis – answer key
Appendix 3: Student handout – Real-time PCR
Appendix 4: Student handout – Real-time PCR – answer key
Appendix 5: Instructor handout

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REFERENCES