Topic: Polymerase Chain Reaction (PCR)

Learning Outcomes:

Upon completion of this lab, students will be able to:

- Explain the role of each key ingredient of PCR.
- Model the steps of PCR and describe the processes taking place at each step (denaturation, annealing, and extension).
- Draw and explain the first three rounds of PCR (template, primers, direction of amplification, and products).
- Propose an application of PCR in their field or personal life.

Introduction:

In this lab, students will learn the fundamental principles of PCR, how to perform PCR, what components are required and why they are necessary, how to depict the steps of PCR, and how PCR can be applied to biological research and real-world problems in their personal lives. This lab is meant to be the start of a series of DNA-based labs and could neatly lead into either restriction digests, sequencing, cloning, or other labs based on the PCR product. It would be ideal in a student-led research project series aiming to transform bacteria or identify an unknown organism.

Most approaches to teaching PCR, even recent active learning modules, begin with PCR directly through watching videos and simulations. However, this still lacks a source of personal motivation and context for students to connect PCR to their own experience. This lab takes a unique approach by starting the conversation with Sanger sequencing, since it is much easier for students to relate to applications of genetic sequencing. Prior to class, students will watch a video about Sanger Sequencing (Thermo Fisher) and complete a companion worksheet to help them identify the key concepts, rather than focusing on particular details. The worksheet will conclude with two discussion questions with which to start the lab: 1) how do we produce a strong enough light flash to be detectable, and 2) how could genetic sequencing be applied to their lives and/or careers. The worksheet will ensure that everyone watches the video and has a common experience from which to build.

The lab will begin with a discussion of their proposed applications to ensure their engagement with the material. This will be followed by discussion of ways to amplify the signal, leading students to the idea that we need a way to start sequencing with many copies of the DNA to be sequenced. The fundamentals of PCR are derived directly from DNA replication, thermodynamics, enzyme kinetics/biochemistry, and ecology. Students will be led through a discussion of DNA replication and how we could manipulate it to design PCR (denaturation with heat, heat tolerant polymerase, & Okazaki fragments/primers), along with the different required components (Magnesium, buffer, & nucleotides). Students will essentially reinvent PCR and build the “recipe” they will use. Students will be shown how to document PCR in their lab notebook and given the reagents discussed to set up their own PCR reactions using whatever template DNA is applicable to the lab series in which this lab is used. The reactions can be run in a thermal cycler while the remainder of the lab takes place.

Many students can describe the components of PCR, but fail to understand the details of the PCR cycles going on in the thermal cycler. To address this component as well, students will then be introduced to the concept of cycling and the “Chain Reaction” of PCR, to help them visualize what is happening inside their tubes in the thermal cycler. The instructor will lead students through a detailed discussion of the first three PCR cycles with drawings, verbal descriptions, and a physical representation using yarn (adapted from

Haydel *et al.*, 2015). These three different representations should help students of all learning modalities synthesize and absorb the material and give them the practice they will need to succeed in subsequent assessments and courses. As a summative exercise, students will be given a worksheet to complete using a virtual PCR tool (Cold Spring Harbor, or University of Utah). If possible, students could also be instructed to download a free trial of SnapGene and given a template sequence and primers with which to run a virtual PCR and other follow-up simulations of experiments, such as cloning or restriction digestion.

Lab Activities:

Before class – Engage (~15 min)

Students watch the video (https://www.youtube.com/watch?v=e2G5zx-OJIw) on Sanger sequencing and fill out a guided worksheet. They will turn this in as their pre-lab. It should be worth a few points to motivate completion, but not enough to over-penalize them for wrong answers (though that should be fairly difficult to accomplish).

In Class – Explore (15 min)

Class will start with a discussion of student's proposed applications for genetic sequencing. This will lead to a review of the video of Sanger sequencing and the problem of how to amplify the signal. Student ideas will be discussed and the students will be led to conclude that we need many copies of the DNA to be sequenced.

In Class – Explain (1 hour)

Students will be led through a discussion of DNA replication and how we could manipulate it to design PCR (denaturation with heat, heat tolerant polymerase, & Okazaki fragments/primers), along with the different required components (Magnesium, buffer, & nucleotides). Students will document the role of each component in their lab notebooks. Students will be shown how to record a PCR reaction in their lab notebook and given the reagents discussed to set up their own PCR reactions using whatever template DNA is applicable to the lab series in which this lab is used. The reactions can be run in a thermalcycler while the remainder of the lab takes place.

In Class – Elaborate (40 min)

Although the components of PCR have been described, the means of producing many copies have not been explained. To help them visualize what is happening inside their tubes in the thermal cycler, students will then be introduced to the concept of cycling and the "Chain Reaction" of PCR. The instructor will lead students through a detailed discussion of the first three PCR cycles with drawings, verbal descriptions, and a physical representation using yarn (adapted from Haydel *et al.*, 2015). They will draw the first three steps of PCR in their lab notebooks, including the template, primers, direction of amplification, and products.

In Class – Evaluate (5 min)

The instructor will lead student in discussion of the similarities and differences between DNA replication, PCR, and Sanger sequencing and they will collaboratively complete a Defining Features Matrix. This will help prevent them from confusing the three as the same processes.

After Class – Evaluate (~30 min)

As a post-class exercise, students will be given a worksheet to complete using a virtual PCR tool (Cold Spring Harbor, or University of Utah). This, in addition to their lab notebook records will compose their lab report for this class.

Assessments:

Formative assessments in this lab include:

- The pre-class worksheet
- The documentation of the PCR components and their roles.
- Their lab notebook models of the first three PCR cycles.
- The worksheet for their lab report.

Exam questions:

- Explain the role of each key ingredient of PCR.
- Draw the 3 steps of the PCR reaction and describe the processes taking place at each step.

References

Haydel, S.E. and Stout, V. 2015. A Kinesthetic Modeling Activity to Teach PCR Fundamentals. *CourseSource*. https://doi.org/10.24918/cs.2015.8

Thermo Fisher Scientific. 2015. How does Sanger Sequencing Work? – Seq It Out #1. https://www.youtube.com/watch?v=e2G5zx-OJIw

Cold Spring Harbor, Biology Animation Library. Polymerase Chain Reaction. https://www.dnalc.org/resources/animations/pcr.html

University of Utah, Genetic Science Learning Center. 2008. Virtual Labs, PCR. http://learn.genetics.utah.edu/content/labs/pcr/