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Biotechnology Kit©

PCR Activity Guide

Exploring PCR:

In this multi-part activity, students will explore the chemical mechanisms involved in PCR as well as how it can be used to identify diseases and other biotechnological markers.

Targeted Subject(s):

The following courses would benefit from this activity

- Introductory level biology - CP/Honors
- AP Biology
- Biotechnology

Intended Grade Level(s):

This activity has been written at the high school level. However, this activity can be tailored to your student population and differentiated as needed.

Prior Student Knowledge Required:

It is expected that students understand the following concepts prior to working through this model:

- Hydrogen and covalent bonding
- DNA structure
- Chromosomes, genes, and alleles
- Base pairing rules
- DNA replication
- Point mutations (eg. sickle cell disease)

Key Words:

This is the list of keywords that the students will need to be familiar with prior to the model:

- PCR - Polymerase Chain Reaction
- DNA
- Gene
- Nucleotides (dNTP)
- Base pairing
- Complementarity
- DNA polymerase
- Hydrogen bonds
- Covalent bonds
- Taq polymerase
- Buffer
- Thermal cycling
- Target DNA sequence / short product
- Primers
- Denaturation
- Annealing
- Extension
- Electrophoresis

For advanced classes, the following items vocabulary items can be explored through this model to differentiate and enhance understanding of the chemical process involved in PCR:

- $MgCl_2$ / $MgSO_4$
- oligonucleotides
- Buffers to increase specificity of the reaction
  - Ammonium Sulfate - (NH4)2SO4
  - Potassium Chloride - KCl

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Learning Objectives:

- **Identify** the three steps of a PCR protocol.
- **Model** the first three cycles of the PCR process, including the directionality (5' and 3' ends) of the primers and single-stranded PCR products.
- **Model** how each of the cycles are interconnected, using former cycles as template strands for subsequent PCR products.
- **Calculate** the number of desired-length PCR products will occur for each amplification cycle
- **Explain** why the reaction runs for 30 cycles.
- **Explain** the importance and function of enzymes.
- **Describe** the effect of temperature on enzymatic reactions.
- **Compare** the process of DNA Replication and PCR.
- **Describe** how the resulting strands will appear on an agarose gel.
- **Explore** how PCR can be utilized to identify a mutation within a gene segment.
- **Describe** how PCR is utilized in diagnosis and potential treatment of patients.

Materials Needed:

- 80 Red Adenosine Nucleotides
- 80 Yellow Thymine Nucleotides
- 80 Blue Cytosine Nucleotides
- 80 Green Guanine Nucleotides
- 7 Primer #1
- 7 Primer #2
- Bag of 16 Cycle Pins (2 extra)
- Primer Stickers

Instructions for the Activity:

Pre-Lesson Set Up

Prior to conducting the lesson, the teacher should set up the student workstations in the following manner.

- **Assemble sets of nucleotide bags.** These bags should have the four nucleotides in separate bags. This pre-assembly greatly aids in student completion of the task, as well as increases the processing time for students to create base pairs through the model process.
- **Assemble primers.** It is suggested that the teacher pre-assemble the primer sequences ahead of time and also attach the primer stickers. For re-use of the same primer sequences, you can place a strip of tape along the back of the nucleotide sequence.
    - Primer 1: **TGAC**
    - Primer 2: **TTCT**
- **Gather/Assemble Cycle Pins.** Dissecting pins are to be labeled with Cycle 1, Cycle 2, and Cycle 3 stickers. You will need two Cycle 1 pins, four Cycle 2 pins, and eight Cycle 3 pins.

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During Part 1: Exploring PCR

This first part of the exercise will introduce students to the methods of PCR and how this type of protocol will flow directly into DNA electrophoresis. The hyperlinked document is a pictorial guide for what your students will be modeling. It will be helpful to review this prior to the student exploration of the model.

Cycle 1

1. **Denature** the double stranded DNA (dsDNA). By separating the strands, you are representing the thermocycler heating up to **95°C** and breaking the hydrogen bonds that hold together the base pairs of the tarjget DNA.
2. **Anneal** the primers (Primer 1 and Primer 2) to the matching complementary strands of template DNA. These primers provide a 3' starting point so that the polymerase can elongate and add free nucleotides (dNTPs). The thermocycler will reduce the temperature to **55°C** for annealing.
3. **Extend** the sequence by adding free nucleotides (dNTPs) to the 3' end of Primer 1 and Primer 2. Add bases along the template DNA until you reach the end of the strand. The thermocycler increases the temperature during this phase to **72°C**.
4. Place a "Cycle 1" pin in each of the grey primers. This will help you keep order as the cycles progress.

Cycle 2

1. **Denature** both dsDNAs produced in Cycle 1 by pulling apart the foam strands.
2. **Anneal** primers. This time, pick up two grey Primer 1 pieces, and two grey Primer 2 pieces. Find a binding site for all four primers on the separated DNA strands. Place the "Cycle 2" pins in the four grey primers that you just annealed.
3. **Elongate** the DNA strands. Working one strand at a time, add dNTPs to the 3' end of the growing nucleotide chain.

Cycle 3

1. **Denature** all four dsDNAs produced in Cycle 2 by pulling apart the foam strands.
2. **Anneal** primers. This time, pick up four grey Primer 1 pieces, and four grey Primer 2 pieces. Find a binding site for all eight primers on the separated DNA strands. Place the "Cycle 3" pins in the eight grey primers that you just annealed.
3. **Elongate** the DNA strands. Working one strand at a time, add dNTPs to the 3' end of the growing nucleotide chain.

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During Part 2: PCR as a Medical Tool

This final component of the module will introduce the students how the method of PCR can be utilized for diagnostic purposes. This activity will look at the section of the β-globin gene where the sickle cell mutation is found.

Through this activity, a new way of using PCR is investigated. Students will explore ARMS-PCR (amplification refractory mutation system). This method of PCR employs two different primers to determine if an individual has a specific point-mutation. In the hyperlinked activity, several resources and links to papers that have utilized this protocol are listed.

Prior to running this extension activity, it is suggested that teachers complete the following pre-lesson steps:

- **Assemble both the wild-type (normal) and mutant β-globin template strands**. This will allow students to enter into the simulation without any delay. In addition, it will ensure that the template strand is copied exactly, as any alteration in the sequence will greatly impact the success of the simulation.
- **Assemble the primers**. It is highly suggested that the teacher pre-assemble the primers. For this extension, you will only be utilizing the grey nucleotides. For re-use of the same primer sequences, you can place a strip of tape along the back of the nucleotide sequence. It is highly suggested that teachers **do not** tell the students the identity of the primers as this will "give away" the answer at the onset of the activity and can potentially discourage the students from working through the modeling activity.
  - Wild-Type/Normal Primer 1: 5' CTGA 3'
  - Wild-Type/Normal Primer 2: 5' GCAG 3'
  - Mutant Primer 1: 5' ACTC 3'
  - Mutant Primer 2: 5' TCCA 3'
- **Gather/assemble identifying pins**. Cycle 1, 2, and 3 pins from Exploring PCR will be utilized in this module as well. In addition, it is suggested that the teacher create flags for the primers.

Running this component of the activity will look very similar to the "Exploring PCR" module. There are several options that the teacher can select, depending on the level of the students.

- **Provide an unknown patient DNA sequence**. Through this exploration, students identify which set of primers to utilize, model how PCR would run, and will then be able to diagnose their patients based upon the primers utilized by the size of the short PCR products that are produced.
- **Provide multiple DNA sequences**. With this, the teacher can provide students with two copies of unknown template, dsDNA. The students will then have to identify if their patient is homozygous (for either wt or mutant form of the sickle cell gene) or heterozygous.

Additionally, teachers can also opt to pair student groups together at the end to determine what the homologous form of the chromosome would exhibit (homozygous dominant/recessive, or heterozygous).

Both of these explorations allow for a continued discussion of the importance of gel electrophoresis and its paired usage with PCR based technology. In addition, students can examine their PCR products and model their predictions for electrophoresis banding patterns as a part of this activity.

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Teacher Tips or Notes for Successful Implementation:

- It is suggested that all pre-lesson set up protocols be followed. These steps will aid in student execution of the activity.
- Having a group of four allows for all members to participate by acting as Taq polymerase to complete the strand.
- It is suggested that students work on one strand at a time and refrain from simply matching their "assigned color" to its corresponding base pair. This will better illustrate the role of the polymerase and to help the students understand the enzymatic process happening.
- If your students are having trouble with time management, an online stopwatch may prove helpful in keeping the students on task, and working in an efficient manner. Again, be careful that the students do not resort to the faster/easier method of simply matching base pairs in a haphazard manner that would not reflect the method of Taq polymerase.
- The pins utilized in this activity are traditional dissecting pins. If you lose a cycle pin, they are easily replaced.
- Students sometimes have difficulty determining what the intended target sequence is. While it becomes apparent after cycle three with the short sequences, it may be advantageous to point this out to students as the cycles are progressing. Additionally, more pins can be added to clarify misconceptions for students. Some pins you might want to add:

  -Template DNA
  -Target Sequence

Resources:

- *Rahman, S.A., Rahaman, Md.S.E., Shipa, S.A., Rana, Md.M., Khan, R., Mahmud, M.G.R., Noor, J., Sultana, F. and Miah, Md.F. (2017) Detection of β-Hemoglobin Gene and Sickle Cell Disorder from Umbilical Cord Blood. Journal of Biosciences and Medicines, 5, 51-63. https://doi.org/10.4236/jbm.2017.510006*
- *F. Q. Alenzi and D. S. AlShaya, Biochemical and Molecular analysis of the beta-globin gene on Saudi sickle cell anemia, Saudi Journal of Biological Sciences, https://doi.org/10.1016/j.sjbs.2019.03.003*
- *Singh, P. J., Shrivastava, A. C., & Shrikhande, A. V. (2015). Prenatal diagnosis of sickle cell disease by the technique of PCR. Indian journal of hematology & blood transfusion : an official journal of Indian Society of Hematology and Blood Transfusion, 31(2), 233–241. doi:10.1007/s12288-014-0427-8*
- *"PCR Setup-Six Critical Components to Consider." Thermo Fisher Scientific - US. 21 Aug. 2019 <https://www.thermofisher.com/us/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/pcr-education/pcr-reagents-enzymes/pcr-component-considerations.html>.*

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