Berkeley Engineers and Mentors

Lesson Plan: Biotechnology (High School Part II)
Lesson Type:
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Project-based- 2 weeks
This is a two week lesson plan for the high school section. Week 1 introduces the concept of DNA, DNA extraction, and the transformation of DNA into bacteria. Week 2 introduces the concepts of PCR (Polymerase Chain Reaction) and DNA gel electrophoresis in the context of DNA finger printing/DNA forensics.

Materials
Gel Electrophoresis (split class into two groups) for one group
- 1 Rectangular soap box
- 1 piece of Stainless steel wire, gauge between 18 and 24
- 1 Wire cutter
- 5 9V batteries
- 2 alligator clips
- 1 flat piece of Styrofoam (thin)
- 1 pair of scissors

Setting the agarose gel:
- 1 spoon
- 1 measuring cup (in milliters)
- 1 bowl for mixing (microwave safe)
- ½ teaspoon baking soda
- 1 bottled water
- ¼ teaspoon agarose
- 1 microwave
- 1 knife
- 1 medicine dropper
- 2 bottles of food dye (enough for whole class)

Polymerase Chain Reaction/Primer Interactions (done in groups of 3-4)
12 small flat magnets
1 roll of clear tape (2 inches wide)

Agenda
The goal of the lesson plan is to familiarize students with the concept of gel electrophoresis and the polymerase chain reaction. Students will have a chance to build their own "gel box" and then to run an agarose gel. Students will also explore concepts of molecular primer design necessary for PCR. Students will examine the agar plates with GFP from last week.

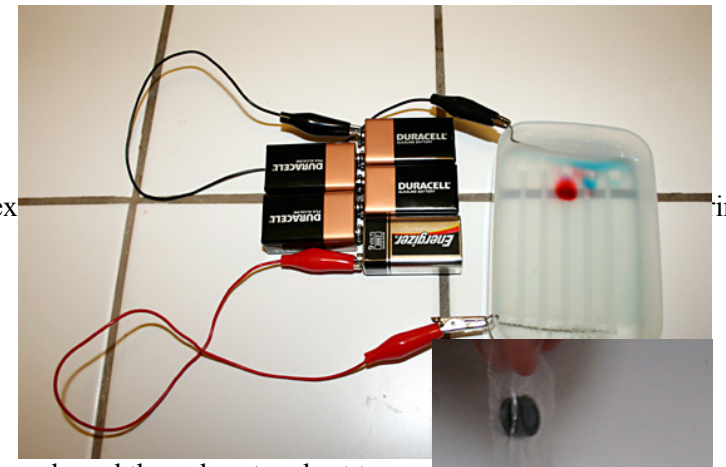
Agenda (1.5hours):
Introduction to Gel Electrophoresis – 15min
Gel Box Construction and Run Agarose Gel – 20min

Introduction to PCR – 10min
Magnet Primer Design activity – 20min
Observe the Results of the Electrophoresis – 5min
Examine the GFP plates from last week – 10min
Concluding Discussion/DNA forensics – 10min

Introduction

Gel Electrophoresis

Introduce the subject by asking if students had watched any kind of crime/detective tv shows such as CSI or Castle. Then explain the concept of DNA fingerprinting and how it is used in identifying suspects. Start a discussion of gel electrophoresis and how it is used in DNA fingerprinting. Explain the principles of gel electrophoresis, but first drawing a schematic of the gel electrophoresis set-up. The basic components of the gel box consist of a circuit, agarose, and DNA. The gel box apparatus is depicted in the photo. Explain that the students themselves will do some detective work by constructing their own gel box and exploring the way that electrophoresis works using food dye (instead of actual DNA).



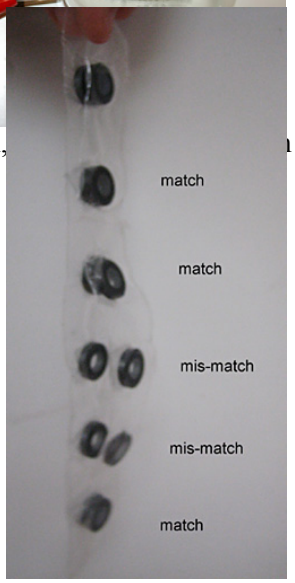
Polymerase Chain Reaction/Primer Interactions

Introduce PCR by talking about its relevance in DNA forensics. Another process is used in DNA forensics is PCR. PCR is used to amplify the amount of biological samples. Then ex imers used t

Activities

Gel Electrophoresis

In this activity, students will build their own gel box and then run a gel of food dye. It is important to start this activity right away since construction will take awhile. Once the gel box is made and the gel cast and set to run, the PCR/pri



Procedures:

1. Cut two pieces of stainless steel wire that is slightly longer than the width of the plastic box.
2. Bend the wires so that they hook over the sides of the plastic box and run the width of the box. Place the two wires at opposite ends of the lengths of the box
3. Connect the 5 9V batteries until only one positive and one negative terminal are exposed
4. Clip the alligator clips onto the positive and negative ends of the batteries and then each alligator clip will clip onto the respective wires. Do not complete the circuit until the gel is ready for running. Also, note which way is the positive and negative electrode, since you want the gel to run from the negative to the positive electrode.
5. Cut a comb out of the Styrofoam. This will be used to create the wells in the agarose gel.
6. Make the buffer solution that soaks the gel: combine ½ teaspoon of baking soda with 200ml of bottled water and mix well in a bowl
7. Make the agarose gel by combining ¼ teaspoon of agarose and 100ml of the buffer solution that was just made
8. Microwave the agarose solution until the powder has all dissolved and the solution is bubbling.
9. Remove the metal electrodes from the plastic box, and pour the gel into the box. Add the

Styrofoam comb to one end of the solution to create the wells. Do not pour the gel too thick or else it would be difficult to see the food dye separation.

10. Wait for 10minutes until the gel solidifies (during this time, it would be wise to start the discussion of the PCR reaction)
11. Pour the rest of the buffer over the solidified gel
12. Pull the comb out of the gel
13. Cut a thin slice at the top and bottom of the gel to make room for the metal electrodes, and reattach them to either ends of the box
14. Use the medicine dropper to put in 1 or 2 drops of food dye of different colors into each of the wells
15. Complete the circuits and wait about 15minutes before checking the gel (go on to do the PCR activity)
16. Record observations on worksheet

Polymerase Chain Reaction/Primer Interactions

In this short demo activity, a string of magnets will simulate the base pair interactions between the template DNA strand and the primer that anneals to it during a PCR reaction. The strength of the interaction depends on the number of "matches" between the "base pairs". This activity nicely explains why a certain number of base pairs in the primer is required to match the sequence on the template DNA.

Procedure:

1. Arrange 6 magnets all in one orientation (all N or all S). place them on a piece of tape with the sticky side up.
2. Place another piece of tape on top of the 6 magnets. This makes a strip of magnets that represents the template strand
3. Take 3 magnets and make the same magnet strip (to make the short primer), except this time, vary the N and S orientations on the magnets such that there would be a mismatch or two when the "template strand" and the "primer" stuck together
4. Take the remaining 3 magnets and make a variation from the previous step
5. Compare the strength with which the "primer" sticks to the "template strand"

Closing Discussion

For the electrophoresis activity ask questions such as: what is gel electrophoresis? What are the components of the gel box? What do you use electrophoresis for? For the PCR/primer activity, ask questions such as: What is PCR used for? What is the purpose of PCR? What are the components of a PCR reaction? Also ask students to discuss how DNA fingerprinting works. During the closing discussion, also allow students to ask questions about the topic.

Worksheet

See below

Index card take home question

What are some of the applications of PCR and gel electrophoresis? Have you encountered any of these applications and are they relevant to your life?

Background

Gel Electrophoresis

Gel electrophoresis is a process to separate pieces of DNA, RNA, and proteins by their sizes. This technique is very useful in molecular biology labs for verification of DNA sequences for cloning and DNA isolation purposes. Gel electrophoresis is also useful in forensics as a visualization tool to see the different pieces of DNA that would identify a suspect. The basic principle of electrophoresis is the

application of current that causes DNA or proteins to run in one direction. DNA is negatively charged because of its phosphate backbone. When placed in an electric field, the DNA will migrate from the negative to the positive end. The DNA is usually loaded into a gel medium. This is done both to hold the DNA in a solid medium (since DNA is in solution otherwise) and to allow the DNA to run in a manner that allows for separation based on size. The agarose gel used has many microscopic holes that allow DNA to run through. Larger pieces of DNA will go through the holes much slower than a smaller piece, and smaller pieces of DNA travel further than the larger piece relative to the start point at the negative electrode.

Mathematically, the mobility of the DNA through the agarose gel can be calculated using the following concepts. The effective force that charged ions (in this case, the DNA) feels in an electric field is

$F_{ef} = q*E$ where F_{ef} = effective force, q = charge of ion, and E = magnitude of electric field

The movement of ions is opposed by frictional forces

$F_{fr} = 6*\pi*r*\eta*V_{ep}$ where F_{fr} = frictional forces, r = radius of molecule, η = viscosity of the agarose, V_{ep} = migration velocity

In a constant electric field, equilibrium is reach, resulting in the effective force equaling the frictional force

$F_{ef} = F_{fr}$

$q*E = 6*\pi*r*\eta*V_{ep}$

solving for V_{ep},

$V_{ep} = (q*E)/(6*\pi*r*\eta)$

From this equation, it can be seen that the speed at which the DNA travels through the agarose is a function of the charge, q, and size, r, of the molecule, since E and η are constants.

Polymerase Chain Reaction/Primer Interactions

Polymerase chain reaction (PCR) is a method to amplify DNA usually for further analysis. PCR is very effective because it can increase the amount of DNA by millions of folds. The key component of PCR is a thermophilic DNA polymerase, an enzyme that adds bases to elongate a DNA strand, that can operate at greater than 70C. Other necessary components of PCR include the template DNA, two short DNA primers that flank the DNA region of interest, magnesium buffer, dNTPs and a thermocycler. The template DNA provides the original copies of DNA that the primers can attach to for amplification. There are two primers, the forward and reverse. Both primers anneal to the template DNA, however, they are positioned in the opposite directions due to the antiparallel nature of DNA. The magnesium buffer is used to stabilize the DNA polymerase and to keep it on the DNA strand. The dNTPs are the building blocks that the polymerase adds to the growing DNA strand. The thermocycler heats and cools the DNA in a fashion that allows the double stranded DNA to separate and re-anneal. The schematic shows one cycle of the repetitive PCR process, which include melting of the double strands at 95C, annealing of primers at 55-60C, extension of the DNA strands at 72C, and repeating the process. To calculate the amount of DNA made after N cycles of PCR, use the simple equation

Molecules of DNA after N cycles = $M*2^{(N+1)}$, where M = number of molecules in the original sample, N = number of cycles

From this equation, a standard 25 cycles of PCR can yield greater than $3X10^7$ molecules! The invention of PCR was so ground breaking that its inventor Kary Mullis won a Nobel Prize in Chemistry for it.

PCR is used in many medical applications such as detecting infectious agents (HIV, hepatitis, malaria, anthrax), cancer diagnostics, and paternity testing.

One of the advantages of PCR is that it can amplify any segment of DNA as long as it is present in the template DNA. The two flanking primers are designed so that there is a 20base pair overlap with

the template. The intermolecular interactions between template and primer are attributed to the specificity of base pairing: A-T and C-G. Only when a certain number of "matches" have been reaches can the primer securely anneal onto the template and amplification process begin.

DNA fingerprinting/Restriction Fragment Analysis

DNA fingerprinting actually does not require a fingerprint. All that is required is a sample of genetic material since every cell contains the same genetic materials. A DNA molecule consists of bases in certain order that constitutes the unique sequence for each person. A simple check swab will gather enough DNA for the fingerprinting. Restriction enzymes are proteins that specialize in cutting DNA by recognizing specific sequences of DNA. Since each individual has a unique sequence of DNA, these restriction enzymes will cut at different places for the DNAs of different individuals. The resulting fragments are called restriction fragment length polymorphisms (RFLPs). In order to visualize these RFLPs, gel electrophoresis is used. The sample is run through a gel and the gel patterns of two different individual will be different.

References

Gel Electrophoresis
http://www.sciencebuddies.org/science-fair-projects/project_ideas/BioChem_p028.shtml?fave=no&isb=cmlkOjg1NjI5NTYsc2lkOjAscDoxLGlhOkJpb0NoZW0&from=TSW
http://www.virtualmedicalcentre.com/healthinvestigations.asp?sid=60
Lectures from BioE111 by Professor S.W. Lee

PCR/Primers
http://www.sciencebuddies.org/science-fair-projects/project_ideas/BioChem_p017.shtml?fave=no&isb=cmlkOjg1NjI5NTYsc2lkOjAscDoxLGlhOkJpb0NoZW0&from=TSW

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Gel Electrophoresis and DNA Primer Lesson Worksheet

1. What is the purpose of PCR? What are some applications of PCR?

2. What is the purpose of gel electrophoresis? What are the components of the gel box? Draw a picture of the gel box set-up.

3. In DNA fingerprinting, _____ are used to make specific cuts in DNA and results in RFLPs that are able to identify the differences between individuals.

4. For the gel electrophoresis activity, record the observations from the results of the gel.

food dye color	number of bands	migration distance of the bands

5. What are the specific base pairings that allow DNA to zip into a double strand?

6. All individuals with different genetic material will show different DNA band patterns under DNA fingerprinting. However, there is one exception to the rule. What two individuals will have the same DNA fingerprinting band patterns?