BIO208 Genetics Lab

What is PCR and How Does It Work? (12 points)

EXPERIMENT OBJECTIVE: The objective of this experiment is for students to gain hands-on experience in the principles and practice of Polymerase Chain Reaction (PCR)**.**

BACKGROUND:

Polymerase Chain Reaction (PCR) has had an extraordinary impact on various aspects of biotechnology. PCR has revolutionized research and diagnostics-based molecular biology. PCR is a simple, accurate, and highly reproducible procedure. The technology introduced an important advantage to molecular biology. It provides the ability to start with a small amount of DNA and to be able to amplify it so that there will be a sufficient amount of DNA to perform experiments. It is analogous to a radio or stereo amplifier where radiowave signals which are normally not heard are amplified so we can hear music.

Since the first application of PCR to detect sickle cell anemia, a large number of diagnostic tests have been developed and are becoming routine tests. PCR is also used in genome projects for DNA mapping and sequencing and is being applied to forensics, paternity determinations, as well as the determination of evolutionary relationships. In all these cases the DNA samples that are extracted are limited and PCR amplifies segments of DNA that become the subject for further analysis and study.

In a PCR reaction, the first step is the preparation of the DNA sample that is extracted from tissues or various biological sources. In PCR experiments, the DNA or gene to be amplified is referred to as the target and the synthetic oligonucleotides used are referred to as primers. A set of two primers (a forward and reverse primer) usually ranging between 20 and 45 nucleotides are chemically synthesized to correspond to the two ends of the gene to be amplified. Each primer binds to one of the two DNA strands and is the initiation point of the amplification. The primer concentrations are always in excess of the target gene to make possible subsequent priming. The exact nucleotide primer sequences for a specific amplification reaction are determined to yield the best conditions (hybridization) for template-primer formation.

The specificity of DNA synthesis is dictated by the Watson - Crick base pairing rules and is directed by the template DNA. The strand being synthesized is complementary and antiparallel to the template DNA strand. *De novo* DNA synthesis catalyzed by DNA polymerase cannot occur without a primer having a free 3' terminal hydroxyl group, which is required for the addition of the next nucleotide. The primer is anti-parallel and is base paired to the template strand. An overview of the PCR reaction is shown on the next page.

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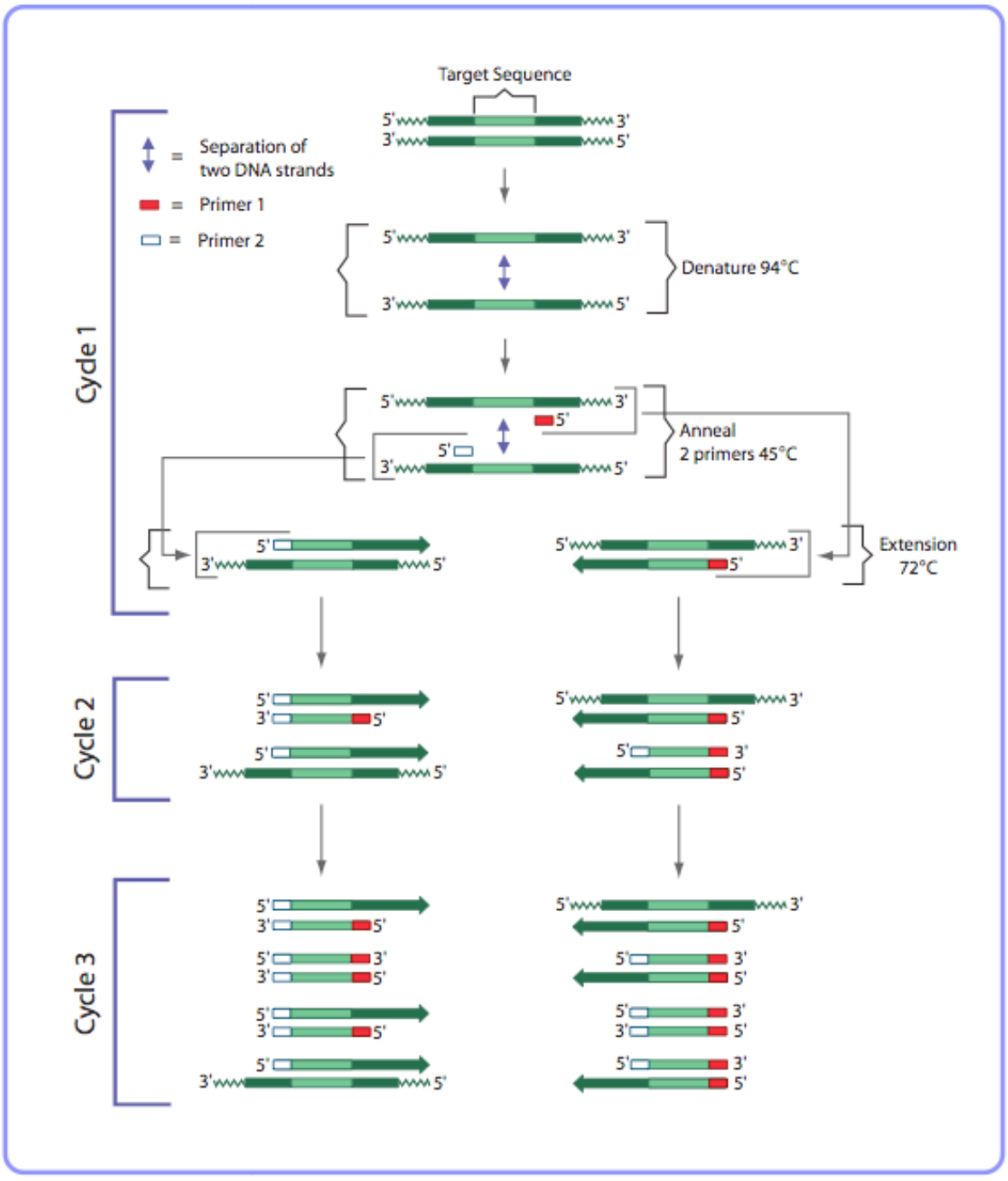
Polymerase Chain Reaction (PCR)



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A typical PCR reaction mixture contains template DNA, 2 primers (forward and reverse), *Taq* DNA polymerase, and the four deoxynucleoside triphosphates (dATP, dTTP, dCTP, dGTP) in the appropriate buffer. The incubation mixture is then exposed to a three step temperature cycle which is repeated. The first temperature is 94°C to melt the hydrogen bonds between the two strands of DNA. The temperature is then dropped to between 42° and 60°C to hybridize the two primers on the two DNA target strands. The temperature is then increased to 72°C, which is the optimum temperature for *Taq* DNA polymerase. At this temperature, the DNA polymerase synthesizes the opposite strand of DNA using the original strands as templates. These temperature cycles are repeated 20 to several hundred times. This process is made efficient by placing the reaction tubes in specifically designed thermal cyclers which are programmed to alternate temperatures rapidly and accurately. The amplified product is then detected by separating the reaction mixture by gel electrophoresis and analysis. *(Background adapted from Edvotek protocols)*

MATERIALS:

i) DNA template: (conc= 12.5ng/ul)
ii) Primer 1 (12.5 µM)
iii) Primer 2: (12.5 µM)
iv) Primer 3: (12.5 µM)
v) Water
vi) BIOMIX – contains Taq DNA polymerase, dNTPs, Mg^{2+}, and buffering salts

Note: **Primer pair 1,2 yields a product of ~ 550bp product while Primer pair 1,3 gives ~ 1025bp product.**

BIOMIX is a complete ready-to-use 2x reaction mix containing an ultrastable Taq DNA polymerase*. Developed to perform PCR assays of many common genomic and cDNA templates, the user has simply to add water, template and primers. BioMix dramatically reduces the time required to set up reactions, thereby minimizing the risk of contamination. Greater reproducibility is ensured, by reducing the number of pipetting steps that can lead to errors.*

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PROCEDURE:

DAY 1:

1) Label tubes 1-4. Make sure to use 0.2 ml thin walled PCR tubes for this step.

2) Add reagents as shown in the table below.

	TUBE 1	TUBE 2	TUBE 3	TUBE 4
DNA template	2 µl	2 µl	2 µl	2 µl
Primer 1	2 µl	2 µl	2 µl	2 µl
Primer 2	2 µl			2 µl
Primer 3		2 µl		2 µl
Water	6.5 µl	6.5 µl	8.5 µl	4.5µl
BIOMIX	12.5 µl	12.5 µl	12.5 µl	12.5 µl
Final volume	25 µl	25 µl	25 µl	25 µl

3) Add one drop of mineral oil to each tube to avoid sample from condensing on the top of the tube.

4) Add tubes to the preprogrammed PCR machine. Run the program BK 120.
Initial Denaturation: 94℃ for 5 min
25 cycles:
Denaturation: 94℃ / 30 sec; Annealing: 55℃ / 30 sec; Elongation: 72℃ / 90 sec
Final Elongation: 72 ℃ for 4 mins.
Maintain indefinitely at 4℃.

DAY 2:

1) Retrieve your tubes from the machine/refrigerator. Label 4 regular walled eppendorf tubes 1-4.

2) Using a micropipettor, carefully insert a tip to the bottom of the tube and withdraw 20 ul of sample. Be careful not to disturb the oil.

3) Label another 4 tubes (1-4). Add 10 ul of each sample to the corresponding tubes. Add 4 ul of loading dye to each sample. Heat your samples at 70℃ for 2-3 minutes and then load into a 1% agarose gel. The instructor will load a DNA marker alongside your samples to allow size determination of the final product.

4) Run gel at 120V for 45 minutes, stain with ethidium bromide, and visualize the bands using a UV box.

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WORK TO BE SUBMITTED: SHORT REPORT (12 points).
You are working independently on this project and therefore you are solely responsible for the quality of the work submitted. While not all students have had the opportunity to perform PCR before, remember that the success in this experiment will stem from your ability to understand the methodology, follow instructions, and pipet accurately. All students should have run a DNA agarose gel before. Please see the instructor if you have not done so. You will NOT be required to program the PCR machine.

Submit a SHORT lab report (1-2 pages). Points will be assigned for the accuracy of the data obtained. Use the information on the next page to help prepare the lab report.

LAB REPORT FORMAT (SHORT)

Cover page:
Title
Due Date
Name

Objective (3 point)

- State the reason for doing the experiment. Include the major objective(s) for this lab along with a summary of what you learned or concluded from your results. This should be in paragraph form.

Results (Actual data: 3 points, ***Make sure you show the instructor your results and get his/her initials)*** (Written results as outlined below: 3 points)

- Summarize all of your data in tables and/or figures. Make sure to assign a number, title and a label to the tables and/or figures.
- State all your observations. Refer to your Figures in your text as you would see in a scientific paper. (e.g. Data presented in Fig.1)
- List any unexpected results.

Discussion (3 points)

- State your conclusion. Make sure that your data supports your conclusion!
- List sources of errors in your experiment.
- Discuss any unexpected results.
- State any recommendations you may have for the future.
- If you know of any additional experiments that might clarify your results, mention them.

Extra credit: Describe an appropriate “negative” control for this experiment. Do not state a negative control that has already been provided in the text of this experiment. (1 point)