**PCR Lab Background Information**

**Objectives:** At the end of this lab, students should be able to:

* Explain how the PCR procedure works.
* Identify chemical components necessary to perform PCR and the role played by each.
* Prepare a PCR reaction to amplify a specific DNA segment.
* Use gel electrophoresis to separate PCR-prepared DNA fragments.
* Analyze an agarose gel, and use the reference standard curve to determine the size of DNA fragments.

**How Does PCR Work?**

**Polymerase Chain Reaction (PCR)** technology is now the standard way to prepare specific sequences of DNA. PCR procedures are accomplished with a piece of equipment called a **thermocycler (or “PCR machine”)**. This machine uses alternating temperature cycles to separate DNA strands and copy the target DNA segment. In essence, the machine automates the natural steps in DNA replication – separation of strands, priming and replication to produce complimentary DNA.

Specific DNA primers that bind selectively to the specified DNA sequence are designed and used along with a special heat-tolerant DNA polymerase enzyme called Taq polymerase. The PCR machine then goes through a series of heating/cooling cycles, and ONLY the targeted DNA is copied.

Millions of copies of the target DNA can be made relatively quickly in a thermocycler, so it is said that the DNA is **“amplified”** by PCR. The diagram below shows how the heat cycling separates DNA strands, anneals primers and then copies the primed DNA sequence.

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After about 30 cycles, you have over 1 billion copies of the target DNA. You also make a few copies of incorrect pieces, but these are small in number and don’t show up during analysis. The diagram above is by nature static, and you need to visualize the process in order to develop a good understanding of PCR. Watch this excellent animation!

Go to: <http://static.dna.gov/flash/pdi_s04_m01_01.swf>

In a PCR machine, the temperature of the reaction is cycled between different values to achieve different results as follows:

|  |  |  |
| --- | --- | --- |
| **Step** | **Temperature** | **What Happens?** |
| **denature** | high (94-95 oC) | DNA strands are separated by breaking the hydrogen bonds – two parent strands come apart. |
| **anneal** | low (50-70 oC) | Primers bind to their complementary bases on the template strand. |
| **extend/amplify** | medium (70-74 oC) | Taq polymerase adds dNTP nucleotides to the parent strands, making complementary DNA. |
| **hold** | Cold (4 oC) | Stops the reaction, preserves the amplified DNA. |

The first three steps make up one complete cycle of PCR, but it requires about 25-40 cycles to create enough copies of the target DNA to analyze.

The enzyme chemistry of DNA replication is modified to work inside a thermocycler. To set up a PCR reaction, you need to add the following chemical components to each reaction tube:

* Template DNA containing the segment of DNA you want to amplify.
* Primers, small pieces of DNA that bind to the DNA sequence. Two primers are required – one for each end of the target DNA sequence.
* Taq polymerase, a special heat-tolerant DNA polymerase obtained from Thermus aquaticus bacteria. Taq adds complimentary base pairs onto the 3’ end of the primed DNA sites.
* MgCl2 provides magnesium ions, which are an essential cofactor for DNA polymerases like Taq.
* dNTPs – deoxynucleoside triphosphate bases – these modified DNA bases are added as complimentary bases to the target DNA reaction. The dNTP mix contains adenine, thymine, cytosine and guanine dNTPs.
* Buffer maintains the pH of the reaction to ensure optimal Taq polymerase activity.

Our lab is a simulation of how PCR would be used to solve the food contamination question while avoiding any safety concerns about working with Listeria DNA. In the field, scientists would design primers that are specific to Listeria and then run PCR to amplify any Listeria DNA present in the food samples. We are simulating Listeria genes with safer bacterial plasmid DNA.

The **quality of your PCR outcome is highly technique-dependent.** Follow these technique tips to produce good PCR results:

* Add reagents to the bottom of the tube and mix by pipetting up and down.
* Keep all reagents on ice.
* Pipet carefully. Hold the reagents at eye-level, verify you have liquid in your pipette and it appears to be the correct amount, and watch the drop as it is added to the reaction tube.
* Change pipette tips between each delivery.