Objectives: A) To familiarize the student with the polymerase chain reaction and B) to use this technique to estimate the size of the insert cloned into a vector.

Introduction: The polymerase chain reaction (PCR) is a powerful method by which one can amplify (increase) the quantity of a specific DNA from virtually any source without having to clone it from a segment of the surrounding DNA. This technique depends on the ability of DNA polymerases, isolated from organisms that normally live in conditions of high heat, to faithfully copy (replicate) the DNA under extreme conditions.

DNA replication is normally an in vivo process. The DNA to be copied is called the template. Before the template can be replicated, the normally double-stranded DNA must dissociate into the two single strands. In vivo, this step requires the action of several enzymes. Next, a short segment of complementary sequence (the primer) must pair with (anneal to) it. Under the appropriate cellular conditions, the DNA polymerase “reads” the template sequence beginning at the end of the annealed primer and copies it using the appropriate deoxyribonucleotide triphosphates [dNTPs] (extension).

PCR is an automated technique. It allows one to perform the replication process in vitro. The experimenter places a reaction tube containing the template, DNA primers, dNTPs, enzyme, buffer, and cofactor in a reaction tube. The reaction tube is then placed in a thermocycler. A thermocycler is a machine that controls the time and temperature of the amplification reactions. The amplification reactions consist of several rounds of the “denature, anneal, extend” reaction sequence. In PCR, the separation of the double strands (denaturation) is accomplished by heating the template almost to boiling. The mixture is then cooled slightly to allow annealing of the primer, and then the temperature is increased to that which allows the polymerase to efficiently copy the template. The amplification reactions are repeated until there are sufficient copies of the template for subsequent manipulations/analyses. Once the amplification reactions are completed, the sample is cooled, and the products electrophoretically analyzed.
Each PCR cycle consists of three steps: a denaturation step (melting) 95°C, an annealing step with variable temperatures typically around 50°C, and finally an extension step 72°C. The two parameters that are usually altered are the annealing temperature and the extension time. If the annealing temperature is too high the primers will not bind and no product is formed. If the annealing temperature is too low the specificity of binding is compromised (the primers will bind in multiple places and many bands will be seen on the gel). The extension time is also important. For each product to become template for the next round it must copy the DNA long enough to incorporate the binding site for the other primer. The general rule is 1 minute per every 1000 base pairs in the product. If you want a product of 3.5 kB, the extension time should be at least 3.5 minutes.

We will not be doing an actual experiment today. Your instructor will lecture and maybe have an in-class exercise for you to do.
Polymerase Chain Reaction STUDY GUIDE

1. What is the polymerase chain reaction?
2. Describe what is meant by “amplification sequence”.
3. Why is it not possible to have automated PCR without a heat-stable DNA polymerase?
4. What is the function of each component in the amplification reaction tube?
5. On the average, 1 bp = 635Da. Use this information to calculate the mass (in µg) of both pBluescript KS+ and Primer 1 (23 bases a.k.a. a 23-mer) that was used in the PCR reactions?
6. In both this and the previous experiment, an agarose gel was used to analyze the DNA. However, in this experiment the agarose concentration was more than twice that used previously (1.5% versus 0.7%). Why?

This is the End of the Experiments. Good job!

*Does this look like Booker T. Washington?*