Bi1x – Spring 2010
Week 3 Prelab: Understanding PCR

One of the key steps in our experiment is amplifying the environmental genomic DNA that we have extracted from the Caltech ponds. We will achieve this amplification using the ubiquitous process of Polymerase Chain Reaction (PCR). As shown in Fig. 1 below, through a repetitive series of temperature cycles, DNA polymerase acts as a molecular Xerox machine copying molecules again and again. The goal of this Prelab is to get you acquainted with the concept of PCR.

Problem 1

A. Templates, amplicons and anchored products in a PCR

While it is true that the total number of molecules in a PCR reaction increases exponentially as a function of the cycle number, closer inspection reveals that this population is actually the sum of three distinct populations at any given moment in the PCR tube: there is the "original template", the "amplicons" and the "anchored products", all of which are targets for further amplification. The original template is simply the original molecule(s) from which we are trying to amplify a certain sequence. The amplicon is the double stranded DNA that stretches from the sequence of the forward primer to the sequence of the reverse primer; this is the desired end product. The anchored product is different from the amplicon in that only one side is bounded by the forward or reverse primers—there is no cue to tell the DNA polymerase when to stop replicating on the other side. Therefore, anchored products have an undetermined length that will be longer than the amplicon length. Note that anchored products are amplified off of the original template, whereas amplicons are actually formed from replication of either anchored products or other amplicons and not of the original template. Figure 1 below depicts the PCR amplification for the first three cycles and shows the original template, the anchored products and the amplicons.

i. **How many original templates, anchored products, amplicons and total molecules exist in the PCR tube at the start of the reaction and at the end of the first, second and third cycles (i.e. n=0,1,2 and 3) in Figure 1?** For example, for n=1 (at the end of the first cycle) there is one original (double stranded) template molecule, one anchored product (comprised of two single stranded DNAs) and zero amplicons, for a total of two molecules. You may assume that the amplification efficiency is 100%, i.e., at the end of every cycle the number of molecules exactly doubles (as depicted in Figure 1) and that we start out with one original template molecule and primers in excess.

ii. **How many molecules of the various classes exist at the end of the fourth (n=4) cycle? Can you write down a general formula for the number of various molecules (template, amplicon, anchored product and total number**

of molecules) as a function of the cycle number n? (You can make the same assumptions as in part i.).

iii. **What is the percent of anchored products out of the total number of molecules after n=35 cycles? Given your result, can anchored products be neglected after 35 cycles?**

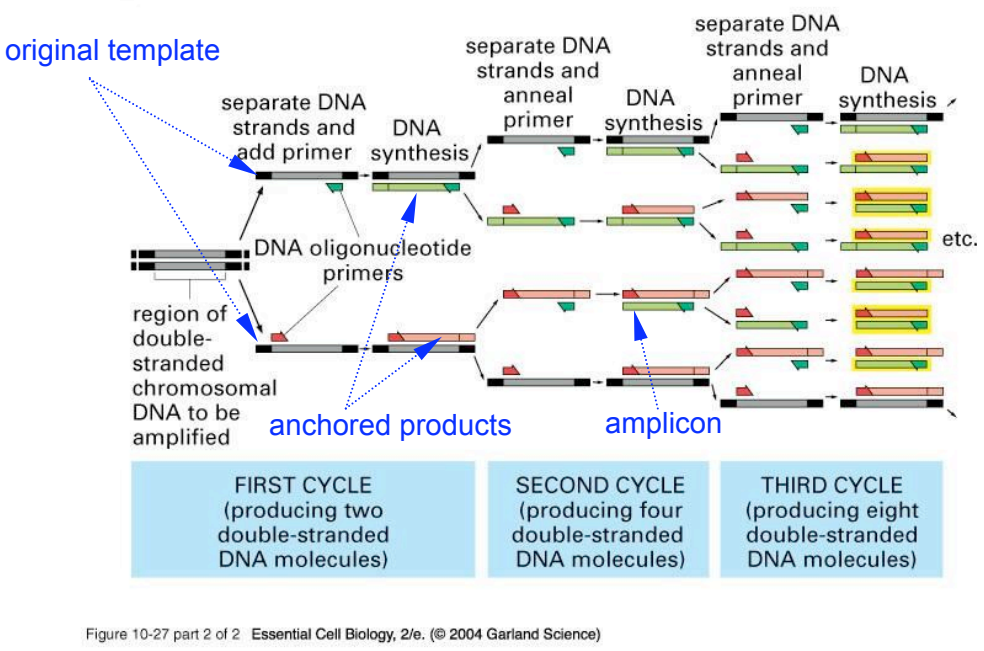

Figure 10-27 part 2 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Figure 1. The first three cycles in a PCR reaction.

B. The PCR program

Each cycle in the PCR consists of three precisely timed phases that are executed at certain carefully chosen temperatures. Figure 2 is a graph of the imposed reaction temperature as a function of time for any given cycle.

i. **Based on Figure 2, draw a schematic diagram of the temperature profile imposed on the PCR reaction as a function of time for the first two cycles.**

ii. **<u>Briefly</u> explain the purpose of each of the three phases and why the specific temperature was chosen for each phase.**

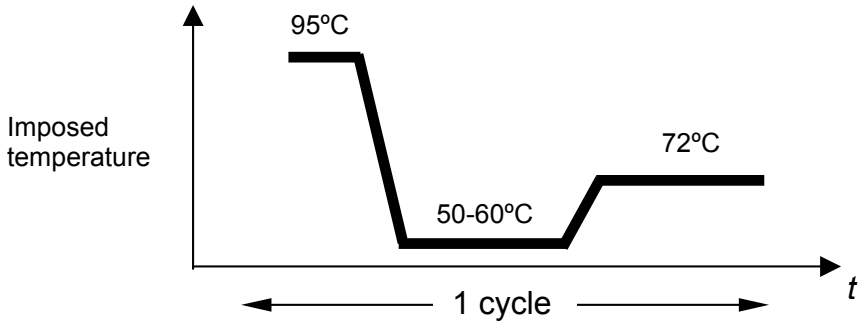

Figure 2. A typical imposed temperature profile during one cycle of a PCR reaction.

Problem 2: Exponential amplification

PCR is an exponential process: for an ideal reaction, the total number of molecules doubles every cycle. Exponential amplification adds up very quickly and allows us to amplify and detect a minute quantity of template, even a single molecule.

A. Assuming you start out with a single copy of the template, compute the minimum number of cycles required to generate at least 100 ng of amplicon DNA, which is the minimum amount of DNA needed in order to detect the product on an agarose gel. Assume that the template is 1000 base pairs long (1 base pair has a mass of 650 daltons; 1 dalton = $1.66 \cdot 10^{-24}$ g). You may also assume that the amplification is ideal, i.e., the number of molecules doubles every cycle.

B. Plot a graph of the total mass of amplified molecules (in units of ng) at the end of cycle 35 as a function of the number of initial template molecules for 1,2,3 … 10 initial template molecules. How many template molecules are required to pass the threshold for detection on an agarose gel?