Weeks 3 and 4 : DNA Science - Techniques

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1 Introduction

During these two weeks you will be working with DNA. Even though DNA is a very robust molecule, there are many techniques that have been developed which facilitate the process of working with DNA. These techniques are, in general, very popular in biochemistry and molecular biology. Below, we list these techniques and give a brief description for each. At the end of each section, we provide a source list, where more details can be found.

2 PCR - Polymerase Chain Reaction

PCR stands for Polymerase Chain Reaction. This is a technique used to amplify (i.e. make many copies of) specific regions of a DNA strand. The regions to be amplified are usually small, around $10kb$. In order for the DNA to be amplified, there are several components that go into the reaction:

- DNA template: This, of course, is the region of the DNA to be amplified
- The two primers: They determine the beginning and the end of the DNA fragment that will be amplified. Usually, they are between $18 - 25$ base pairs long. They are complementary to the beginning or end of the DNA fragment to be amplified. The primers is where the DNA polymerase will bind in order to start the synthesis of the new DNA strand.
- DNA polymerase: This does the actual copying of the region to be amplified
- dNTPs: They are the four nucleotides that the DNA consists of. In order to amplify the DNA fragment, we will need nucleotides that the DNA polymerase can use
- Buffer solution: This provides the right environment for the DNA polymerase to operate (contains ions, the right pH, etc.)

The PCR procedure is composed of several steps. We briefly describe each one of these steps below:

- Denaturing: In order for the DNA to be amplified, it needs to be broken apart into its two strands (denaturing). This happens when it is heated at a high temperature, 96^oC, which breaks apart the hydrogen bonds that connect the two DNA strands. Before the first cycle, we denature for an extended time period (5min), to ensure that both the template DNA and the primers are single stranded. During this step certain polumerases are also activated.

- Annealing: After the denaturing process, the temperature is lowered so that the primers can anneal (i.e. attach) to the single DNA strands. The temperature of this step is normally 5^oC lower than the melting temperature of the primers. Normally, this temperature is in the range $45 - 65^oC$. This temperature is important because if it too high, then the primers might not bind at all and if it is too low, they will bind randomly. The duration of this step is around 30sec.

- Elongation: Now that the primers are annealed, the DNA polymerase can attach to the primer and use the dNTPs in order to synthesize the new strands, as it moves along the DNA strand of the template. The temperature for this step depends on the DNA polymerase and the time depends both on the polymerase and on the length of the DNA to be amplified. The latter translates to about 1 minute per thousand base pairs. After the elongation is complete, the newly formed DNA strands can be used as a template for a second cycle of DNA amplification. Consequently, the denaturing, annealing and elongation steps can be repeated for a number of cycles (around 30 cycles) in order to obtain a large amount of DNA copies of the initial template. After the final cycle, a final elongation time of about 10 minutes is used in order to finish copying any remaining single stranded DNA.

References:

- Wikipedia: Polymerase chain reaction
- http://users.ugent.be/ avierstr/principles/pcr.html
- http://people.ku.edu/ jbrown/pcr.html
- http://www.dnalc.org/ddnalc/resources/pcr.html

3 Restriction and Ligation

Restriction refers to the process of using enzymes (called restriction enzymes) that can cut the DNA into fragments. Restriction enzymes are sequence specific: they recognize and bind only to specific DNA sequences and cut the sugar-phosphate backbones of the DNA strands. For example, EcoRI recognizes and binds only to the sequence GAATTC. A different enzyme, called DNA ligase, can attach or rejoin DNA fragments with complementary ends. It does that by catalyzing the chemical reaction that rejoins the DNA sugar-phosphate bonds. This process is called ligation.

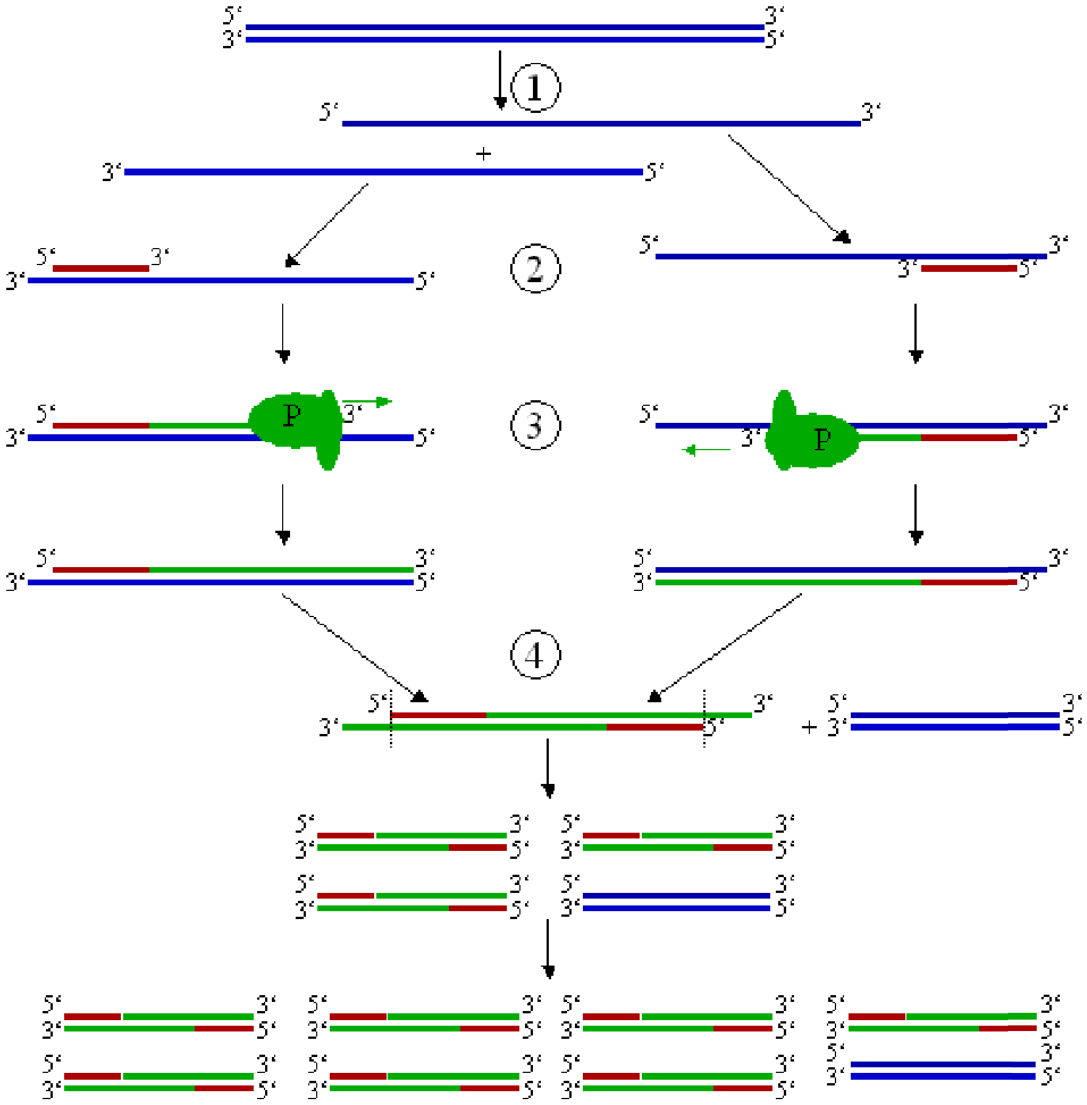

Figure 1: Schematic drawing of the PCR cycle. (1) Denaturing at 94-96C. (2) Annealing at (eg) 68C. (3) Elongation at 72C (P=Polymerase). (4) The first cycle is complete. The two resulting DNA strands make up the template DNA for the next cycle, thus doubling the amount of DNA duplicated for each new cycle (a total of three cycles is shown above). Image is taken from Wikipedia.

References:

- http://www.dnalc.org/ddnalc/resources/restriction.html

4 Electroporation and Transformation

DNA transformation is a naturally occurring event in which DNA can be transferred into bacteria. This event, however, is very rare and, for this reason, a method has been developed in order to make bacterial cells more "competent" for transforming foreign DNA.

The cell membranes of bacteria have hundreds of pores (i.e. adhesion zones) but the membranes themselves are made of lipid molecules that have negatively charged phosphates. So, even though the pores are large enough to admit small loops of DNA, the negatively charged DNA is repelled by the lipids of the cell membrane. In order to shield the negative charges, calcium is added (Ca^{2+}). Calcium interacts with the lipids' and the DNA's negative charge, creating a neutral situation. The lowering of the temperature congeals the membrane and makes it easier to shield the negative charges of the lipids. Then, a "heat shock", (i.e. a quick rise in temperature), creates a temperature imbalance on either side of the bacterial membrane and sets up a current. The DNA can then be swept through the adhesion zone, since there are no electrostatic interactions to prevent that any more. The transfer of the DNA into the bacterial cell has successfully taken place.

Electroporation is also used instead of a heat shock. When the voltage across the membrane exceeds its dielectric strength, then pores are formed. If the electric field and/or the duration of exposure to this field are really short, then the pores can reseal after a short period of time. However, this period of time is long enough for the DNA to enter into the cell and, therefore, for the transformation to take place!

References:

- http://en.wikipedia.org/wiki/Electroporation
- http://www.dnalc.org/ddnalc/resources/transformation2.html

5 Gel Electrophoresis

Gel electrophoresis is a method used to separate DNA fragments and other macromolecules based on their size, electric charge and other physical properties. The word electrophoresis comes from the Greek words "electro", which refers to the electric energy, and the word "phoros", which means to carry across. So, as the word denotes, this method uses an electric field in order to force the negatively charged DNA fragments to move across a gel. The smaller the DNA fragments, the further and faster they will move over a given period of time. The shape of the molecules also affects the distance they will travel as they move across the porous gel.

Agarose gels are made from agar (highly purified seaweed). The gel is constructed is such a way so that there are several wells on the top, where the DNA molecules are placed. The gel is submersed in a tank filled with salt solution, so as to conduct electricity. The DNA molecules are then loaded in the wells of the gel with some dye, that helps track their progress as they move across the gel.

After the gel has finished running (when the small fragments have almost reached the anode) the gel is stained in Ethidium bromide in order to make the fragments visible. The DNA

samples that were loaded in the wells are now seen in their respective lanes forming distinct bands. Each band in each lane corresponds to one size of DNA fragments. Bands at different lanes that end up at the same distance from the top (well) of the gel have molecules that have travelled with the same speed and, therefore, are approximately the same size and shape. In order to be able to quantify what this size is, one of the well is usually loaded with a ladder, a mixture of DNA fragments whose sizes are known.

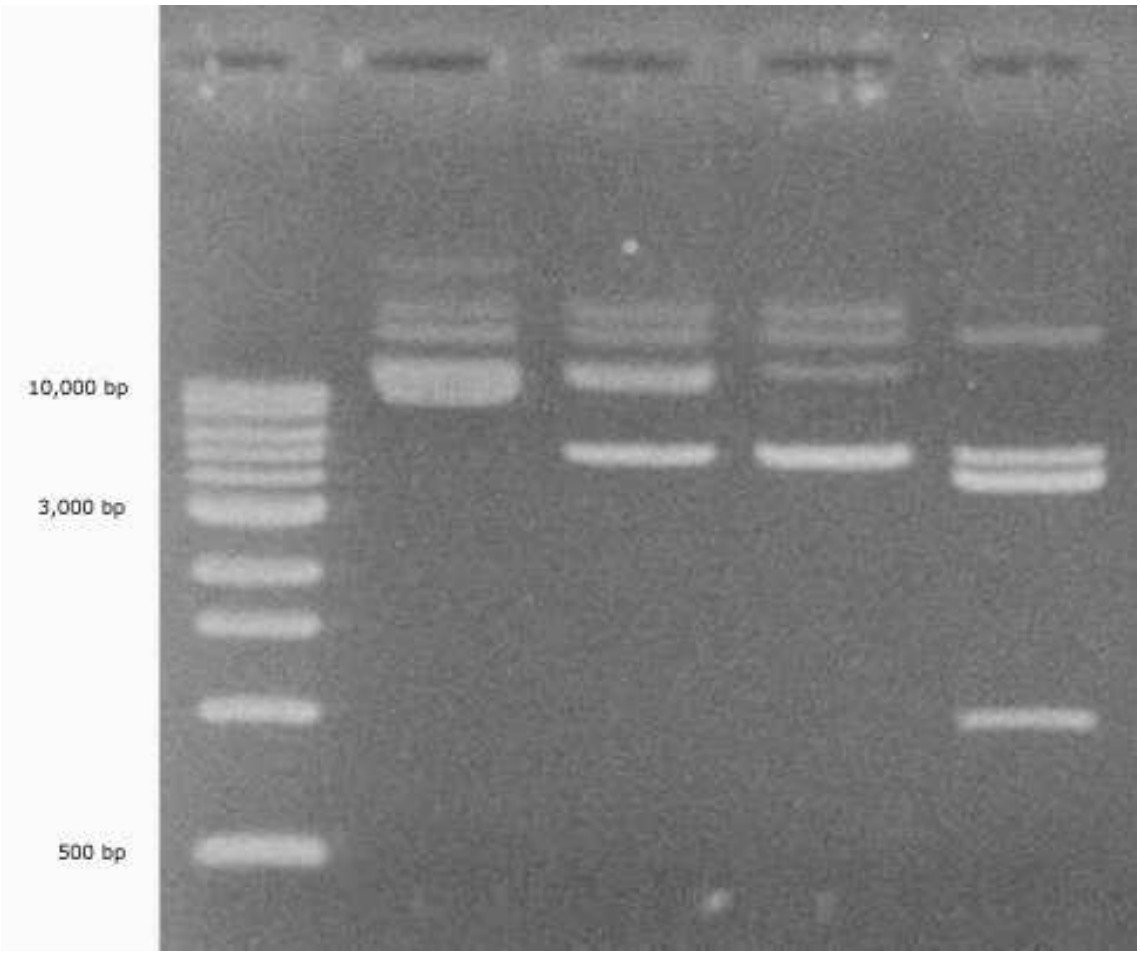

Figure 2: DNA agarose gel. The first lane contains a DNA ladder for sizing, and the other lanes show variously-sized DNA fragments that are present in some but not all the samples. Image taken from Wikipedia

References:

- http://www.dnalc.org/ddnalc/resources/electrophoresis.html
- http://www.bergen.org/AAST/projects/Gel/
- http://en.wikipedia.org/wiki/Gel electrophoresis