Biochemical Techniques in DNA Computing

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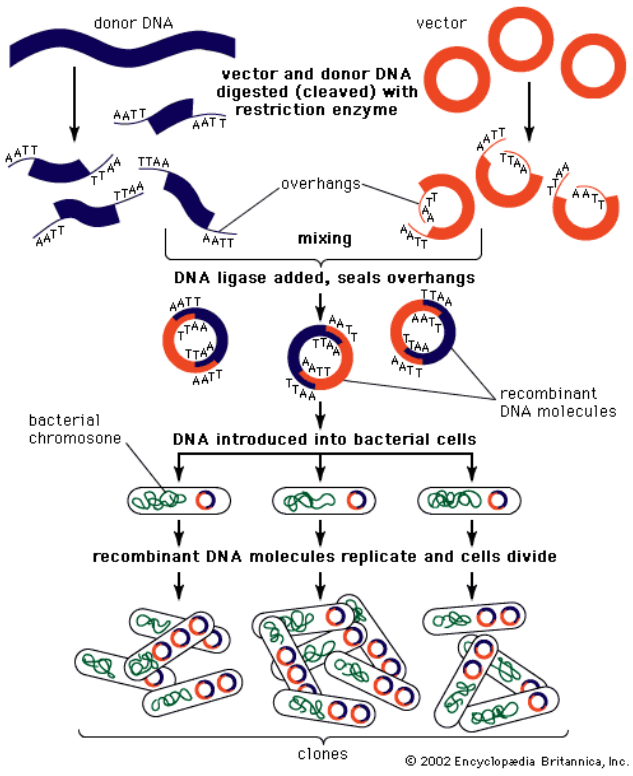
Introduction

In our molecular algorithms course we focus on the various algorithms and computations to which DNA computing can be applied. In order to better understand the research papers in the area of DNA computing; here I outline some of the important commonly used biochemical techniques and what they can accomplish.

Recombinant DNA

Often written rDNA, recombinant DNA is a mix of two molecules of DNA. Each molecule of DNA is comprised of two strands with complementary bases attached to each other, and so two types of breaks can form, single strand breaks, and also double strand breaks. Single strand breaks can be repaired by DNA polymerase using the complementary strand, whereas, to fix the double strand breaks, DNA ligase is required. DNA ligase is still required in the case of single strand break to completely fix it for the last phosphodiester bond.

What are 5' and 3'? Pronounced 'five-prime' and 'three-prime' they are the ends of the nucleotide sequence in a sugar ring molecule where more molecules can bind with the help of phosphodiester bonds.



So, to make an rDNA we first need to break two molecules with the same endonuclease, so that they have compatible ends, and then simply seal them using DNA ligase. This is known as a ligation reaction. I have used this procedure in the past to add DNA to E. Coli bacteria so they began to produce lactic acid.

Polymerase Chain Reaction (PCR)

This is a crucial technique that allows us to easily and quickly replicate (often referred to as amplifying) a DNA template, a given strand of DNA. Often, we use an oligonucleotide as a template because they can be used in many techniques. "Oligonucleotides are short sequences of nucleotides (RNA or DNA), typically with twenty or fewer bases. Automated synthesizers allow the synthesis of oligonucleotides up to 160 to 200 bases. The length of a synthesized base is usually denoted by 'mer' (from 'Greek' meros "part"). For example, a fragment of 25 bases would be called a 25-mer. Oligonucleotides are often used as probes for detecting complementary DNA or RNA because they bind readily to their complements." [8].

The PCR procedure consists of cycles of temperature changes that perform deanaturation, annealing, and elongation. It melts the DNA template and primers by disrupting the hydrogen bonds between complementary bases of the DNA strands, yielding single strands of DNA. As the name implies, the annealing step allows the DNA template and primers to anneal. Only closely matching stable bonds are allowed to anneal by controlling the temperature and the timing. This step lets the DNA polymerase synthesize the complementary DNA. Together, sequences of these steps allow the PCR procedure to output the replicated DNA strands.

Gel Electrophoresis

In this technique we take a DNA sample and cleave it into smaller segments using restriction enzymes. The small DNA segments are put into wells in a porous Agarose gel floating in a salty buffer solution contained in a chamber with electrodes on the ends. The electric current causes the DNA segments to move toward the cathode, and of course, the smaller fragments move faster and get farther than the larger segments, thereby getting sorted in the chamber. This is a standard technique which I have also had a chance to perform in a biochemistry lab in Worcester before. Figure 1 shows a schematic diagram of the Gel and DNA, while Figure 2 is an actual photograph of the DNA illuminated in the gel under ultraviolet light.

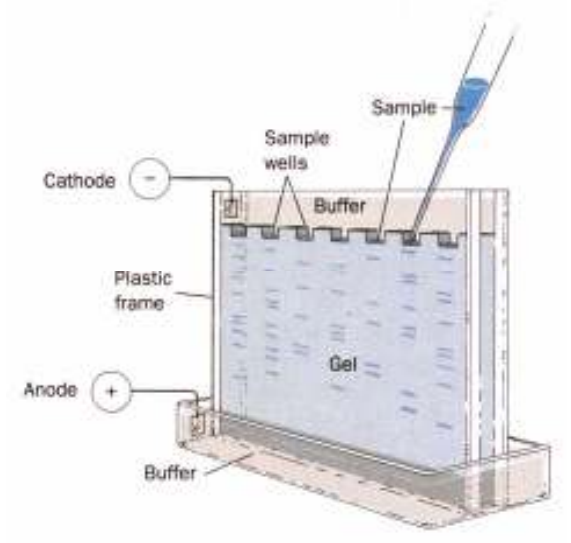


Figure 1

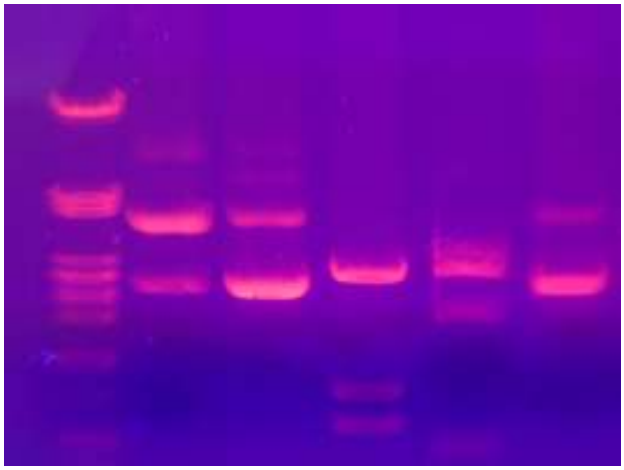


Figure 2

Blotting

Southern Blotting

This is a procedure used to check if a certain sequence exists in a DNA sample. It begins by using Gel Electrophoresis described above to first separate the DNA by size. Then it uses a radioactively marked hybridization probe (a variable length DNA fragment denatured to a single strand so it can bind to target DNA) for the actual sequencing. The radioactivity used for tagging helps detect the DNA segment being sequenced. I have skipped the details for this procedure involving the movement of the DNA from the Gel to a nylon membrane using blotting, since they are standard lab procedures unnecessary for our purpose.

Northern Blotting

This is a procedure similar to the Southern Blotting procedure for RNA sequencing.

Western Blotting

This is a procedure similar to the above and also starts with Gel Electrophoresis but is used to detect the presence of specific proteins in a tissue. This method was invented by George Stark here at Stanford.

dsDNA refers to Double Stranded DNA
ssDNA refers to Single Stranded DNA
ddH_2O – double distilled H_2O

Summary

Overall, I have discussed techniques for cutting, copying, identifying and pasting DNA strands together. These are really the standard lab techniques forming the basis for our computational ability with the

DNA strands and molecules. Cleaving used restriction enzymes, copying was done via PCR, identifying by gel electrophoresis and blotting and pasting or mixing by DNA ligase to form rDNA.

Some Very Basic Terms

- Amino acids are the building blocks of proteins. There are 20 natural amino acids.
- Antigen is a substance that, after take-up by an organism, elicits an immune response.
- Antibody is a protein produced by the immune system in order to protect the body against a foreign substance (antigen).
- Aptamer Oligonucleotides with important biological applications
- Base Pair - two nucleotides on opposite complementary DNA or RNA strands that are connected via hydrogen bonds
- Chromosome Components in a cell that contain genetic information. Each chromosome contains numerous genes. Chromosomes occur in pairs: one obtained from the mother; the other from the father. Chromosomes of different pairs are often visibly different from each other (see also DNA).
- DNA The material inside the nucleus of cells that carries genetic information. The scientific name for DNA is deoxyribonucleic acid.
- Epitope is the smallest part of an antigen that can be recognized by an antibody.
- Gene The fundamental physical and functional unit of hereditary.
- Morpholino oligos have non-natural backbones, which do not activate RNase-H but can knockdown gene expression or modify splicing.
- Polymorphism The appearance in a population of the same gene in multiple forms because of mutations.
- Polynucleotide
- Recombinant DNA is DNA formed by the artificial combination of several existing DNA strands.
- Watson and Crick Complementary – A complementary sequence such that in DNA, Adenine (A) is complementary to Thymine (T), and Cytosine (C) to Guanine (G). In RNA, the nucleotide Thymine is replaced by nucleotide Uracil (U).

Image Resources

Figure 1: http://www.science.fau.edu/chemistry/Mari/biochemlab/05_023.jpg

Figure 2:
http://upload.wikimedia.org/wikipedia/commons/thumb/6/60/Gel_electrophoresis_2.jpg/800px-Gel_electrophoresis_2.jpg

Works Cited

[1] http://www.dartmouth.edu/~cbbc/courses/bio4/bio4-1996/RecombinantDNA1.html

[2] http://www.howstuffworks.com/dna-computer.htm

[3] http://www.med.unc.edu/wrkunits/3ctrpgm/pmbb/mbt/GLOS.htm - Glossary of selected molecular biology terms

[4] http://www.stanford.edu/group/hopes/diagnsis/gentest/s7.html

[5] http://crypto.stanford.edu/~dabo/biocomp.html - Papers on DNA computing from Princeton

[6] http://en.wikipedia.org/wiki/3%27_end – Directionality of a molecule

[7] http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/R/RecombinantDNA.html - Details of DNA recombining and Gene cloning

[8] http://en.wikipedia.org/wiki/Oligonucleotide - Oligonucleotides and source for basic terms section

[9] http://en.wikipedia.org/wiki/Polymerase_chain_reaction