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Perspective

Applications of Polymerase Chain Reaction in DNA Sequencing

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DESCRIPTION

The Deoxyribo Nucleic Acid (DNA) is found in all our cells serves as a template for building cells. The Human Genome Project, which reads the DNA code in cells completed in 2003, is undoubtedly one of the great achievements of modern life science. Our ability to accomplish this and to further understand and manipulate DNA is closely related to understanding of the bacterial and viral world. Outside of academia, the ability to understand and manipulate this code has great implications for society. Some of the fundamental techniques that allow us to read, copy, and manipulate DNA sequences, and briefly look at some of their implications for society. DNA is the fundamental store house of information in cells, and the ability to understand and manipulate its contents is a key way to understand our world. DNA itself is made up of nucleotides that can form hydrogen bonds with each other and form base pairs. Nucleotides are joined by a phosphate group that connects between carbons 5 and 3 of the sugar portion of the nucleotide. DNA is made up of two strands that run in opposite directions to each other.

DNA can be isolated from cells in a few simple steps. First, cells are disrupted with either detergents or forces (e.g. sound waves) to disrupt cell membranes. A protease enzyme is then used to degrade the protein from the cells so that it does not co-precipitate with the DNA in the next step. Cold alcohol is then added to turn the DNA into a solid precipitate. Then separate the fixed DNA by centrifugation and collect the clumped DNA at the bottom of the tube. One of the greatest achievements of our time is the Human Genome Project. This was determined using Sanger's DNA sequencing, a Polymerase Chain Reaction (PCR) based method. To perform Sanger sequencing, primers and some nucleotide triphosphates needed. PCR is performed on DNA extended by primers and DNA polymerase.

Since DNA is invisible to the naked eye, we need to visualize it. This can be done by using several methods. One method is to measure DNA by taking advantage of the fact that DNA absorbs UV light at 260 nm and measuring its absorbance. Dyes such as Ethidium Bromide, SYBR Green, and SYBR Safe can also be used. These dyes bind to DNA and the dye can be visualized to see the DNA. One method of using these is DNA electrophoresis. DNA is separated by size using an algae-derived agarose gel. Agarose is adjusted to form a porous mesh, and the size of the mesh depends on the amount of agarose used. 0.8%-2% agarose gels are commonly used. It also contains a staining agent as above to allow visualization. Place the DNA to be isolated in the wells of the gel and connect the tank to a power supply to apply a voltage to the gel. As mentioned above, DNA is negatively charged and migrates through the gel to the positive electrode. The mesh size of the gel, coupled with the length of the DNA, makes DNA migration more difficult. This is because larger DNA fragments move more slowly. This separates the DNA by size. The exact length can be determined by running DNA fragments of known size along with the sample. After the run the DNA is visualized using the correct staining light source and the DNA appears as bands on the gel.

In normal PCR, extend by adding nucleotide tri-phosphates, within the sequencing by adding so the reaction terminates. If those nucleotides are labeled, then the fragments produced can be separated by size using capillary electrophoresis (this works on the same principle as gel electrophoresis but on a smaller scale) and then this used to read the sequence. When the technique was first developed, the di-deoxynucleotides were labeled using radioactive phosphate, but more modern versions of the technique use different colored fluorescent dyes.

Regular PCR extends by adding nucleotide triphosphates, but if a sequencing reaction incorporates dideoxys, it cannot be further extended by PCR and the reaction terminates. Once these dideoxy nucleotides are labeled, the generated fragments are separated by size using capillary electrophoresis, which works on the same principle as gel electrophoresis, but on a smaller scale and sequenced used for reading, but more modern versions of the technique use fluorescent dyes of different colors. Gels are made by melting and solidifying agarose.

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