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Commentary

Brief Note on Human DNA Sequencing Technique

Melanie Nakatsu*

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710, USA

DESCRIPTION

Sequencing begins with biological material, almost always blood, in all human molecular techniques. The DNA from blood cells is extracted and purified through a number of stages that we don't need to worry about. After that, a very small sample of this DNA is used for testing.

The extraction and purification process physically breaks the DNA in a significant number of random places. As a result, restriction enzymes are frequently used to break the DNA into more manageable size fragments. The region to be sequenced is subsequently amplified using Polymerase Chain Reaction (PCR).

Sequencing begins with the same procedures as PCR does. The double-stranded helix of DNA gets broken apart into two single strands when it is heated (a process called denaturing). Then a primer is added, which is the same as the one used in the PCR. This primer is a short strand of single-stranded nucleotides that will bind to the complementary portion of DNA amplified in the PCR.

Starting with the primer, the rest of the double-stranded DNA must be synthesized. This is essentially the same as DNA replication; however it takes place in a test tube rather than a cell nucleus. The two most important components of replication are:

- A large quantity of free nucleotides.
- A polymerase enzyme capable of constructing the chain.

In the nucleotide "soup" of DNA, the majority of nucleotides in the soup are chemically identical to those found in ordinary DNA. The addition of a little amount of specifically engineered nucleotides with two critical properties is the brilliant trick in this step of the process. The first is that these nucleotides finish chains. That is, anytime one of these unique nucleotides is added to the chain, the process of constructing the double helix stops. The second key feature is that each unique nucleotide is "color-coded" through a chemical tag, allowing it to fluoresce into a certain color when exposed to the right light. Each of these particular nucleotides has its own color, such as green for adenine, yellow for thymine, and so on.

Following that, a huge number of polymerase molecules are introduced to the mix. The polymerase is a complex enzyme that helps the cell duplicate DNA once the hydrogen bonds are broken and the double helix is split into two single strands. To give polymerase some sentience, one could say it collects free nucleotides, inspects the next nucleotide in the single-stranded chain, and then glues the proper nucleotide partner into the other DNA strand. If the single strand's next nucleotide is A, for example, the polymerase will place a T on the developing strand.

The next step is to sit back and wait for nature to take its course. Many thousands of double stranded DNA molecules will be created since there are millions of single stranded DNA fragments with primers attached millions of polymerase molecules, and several billion nucleotides. Due to the possibility of including unique nucleotides, these complementary DNA strands will be of various lengths. When one of these unique nucleotides is added to the chain, further DNA strand synthesis is stopped. As a result, there are many millions of copies of double-stranded DNA, each of which is varied in length.

If the DNA mixture is heated, the double-stranded DNA breaks down into single-stranded DNA molecules of varied lengths. The single-stranded DNA is then loaded onto an electrophoretic gel. The new electrophoresis techniques are so sensitive that they can distinguish between two DNA strands that differ by only one nucleotide in length.

The bands will fluoresce when the gel is viewed under the right lighting. Because the special nucleotides are color-coded, the nucleotide sequence of the DNA can be deduced by reading the sequence of colors.

This type of sequencing method has become more automated in recent years. Specialized sequencing machines, which are effectively computerized robots, automate operations like as timing, heating and chilling, and pipetting mixes. The newest models additionally include a unique capillary tube that allows for automated electrophoresis. A computer, rather than a human observer, can read the colors thanks to laser lighting and optical scanning. Specialized software analyses the sequence, highlights areas of uncertainty, and stores the data.

Thousands of small sections of DNA have been sequenced at this point. The next step is to join these sections together to create the human genome sequence. The trick here is to return

Correspondence to: Melanie Nakatsu, Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710, USA, E-mail: melanie.nakatsu@duke.edu

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to the original breaking of the DNA into smaller pieces for sequencing. Consider cutting the DNA using a variety of restriction enzymes. The resulting sections will have some overlap because one kind will cut the DNA at one nucleotide sequence while the other will cut the DNA at a different nucleotide sequence. One can reassemble the entire sequence by determining whether sequences overlap with others.