Chapter 8

GENE SEQUENCING STRATEGIES

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◆ Introduction

Since the existence of mankind, he wondered about the events around him, asked questions and made new discoveries with the answers he found. Identification of nucleotide sequences within a gene and the findings obtained as a result of the analyzes have radically changed the development of biology. Supportive and complementary researches carried out by scientists from past to present form the basis of today's scientific technologies. In parallel with the development of recombinant DNA techniques that enable obtaining large amounts of pure DNA from an organism, DNA sequence analysis methods have also evolved.

Today two methods are used in DNA sequencing analysis. These two methods are different from each other: These two methods are;

1. Chemical sequencing method of Maxam and Gilbert [(1)].
2. Chain termination method of Sanger-Coulson [(2)].

◆ 1. Chemical sequencing method of Maxam and Gilbert

Maxam and Gilbert used hydrazine, dimethyl sulfate, or formic acid to determine the nucleotide sequencing of the DNA molecule to change the bases in the DNA. In this application, the nucleotides in the tubes change with the effect of piperidine, which is added to the tubes with DNA molecules in them. Thus the DNA chains are broken using chemicals [(3)]. The DNA whose nucleotide sequence will be detected is first marked with ^{32}P radioactive isotopes of phosphorus at the phosphate end (5') or with a fluorescent dye. Then DNA double helix are separated from each other or DNA is cut with a suitable restriction enzyme. Thus, only one end of the DNA is marked. In the second step, DNA molecules are allocated to four tubes and the certain reactions are carried out to replace and break the A, C, G or T nucleotides. By giving a limited time for the reaction, fragments of DNA from selected nucleotides at distinct points are obtained in each tube. As a result, a set of DNA fragments, all of which are marked from the 5'-positions, but different

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nucleotides in a single run, i.e. 3000 Gb, 48 human genomes can be easily read within 44 hours at the same time (33, 34).

The advantages of next generation sequencing technologies compared to existing ones are:

- Higher efficiency,
- Faster turnaround time; i.e. obtaining a high methylated genome sequence within minutes,
- Long reading rates and even shorter reading of the entire chromosome,
- To be able to reproduce the starting material correctly,
- Small amounts of starting material, i.e. a single molecule, is sufficient for sequencing,
- It is lower cost.

With the next generation sequencing approaches, the sequencing cost has decreased and access to the nucleotide data that only certain centers can produce and access has become easier.

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