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DNA Decoding

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Abstract

DNA decoding means determination of the order of arrangement of nucleotides in the DNA. The four nucleotides that makes up DNA are: Cytosine, Adenine, Guanine and Thymine. The different generations of DNA sequencing are: first generation that consists of Sanger sequencing method and Maxam-Gilbert sequencing method; second generation consisting Roche 454, Illumina Solexa and ABI-SOLiD techniques; third generation consists of Helicos, PacBio and Ion Torrent and fourth generation consists of Nanopore sequencers. The first-generation sequencing methods have limitation that it could sequence a small number of DNA sequence in one go and that the cost per base is very high. Therefore, development of the high throughput new generations of sequencing was required to sequence the genome of individuals or organisms for diagnosis and treatment in short period of time with low cost.

Introduction

Sequence of DNA can be read by different methods. Reading DNA sequence means determination of the order of arrangement of nucleotides in the DNA. The four nucleotides that makes up DNA are: Cytosine Adenine, Guanine and Thymine. The first or early DNA sequencing method includes Sanger Sequencing method and Maxam-Gilbert Sequencing method. DNA sequencing can help in determination of the sequence of individual genes, clusters of genes, full chromosomes or entire genomes of an organism. This can be applied in study of genomes and the proteins they encode to identify changes in genes, its associations with diseases, phenotypes and to identify potential drug targets; genetic testing to determine if an individual has any risk of genetic diseases and in identification of an organism from any source and in forensic study.

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Generation of Sequence

DNA sequencing can be done by using different generations. These different generations are: first generation that consists of Sanger sequencing method and Maxam-Gilbert sequencing method; second generation consisting Roche 454, Illumina Solexa and ABI-SOLiD techniques; third generation consists of Helicos, PacBio and Ion Torrent and fourth generation consists of Nanopore sequencers. The first-generation methods are the mostly used sequencing methods but have limitations. These limitations are that it could sequence a smaller number of DNA sequences in one go and that the cost per base is very high. Therefore, development of the high throughput new generations of sequencing are required to sequence the genome of huge number of individuals or organisms for the diagnosis and treatment in short period of time with low cost. While the advantages of the new generations include sequencing of larger number of sequences instantly with lower cost per base and provides high throughput data within lesser time and more accuracy.

First generation sequencing

Fredrick Sanger gave the first method for DNA sequencing called Sanger sequencing or chain termination method back in 1977. Allan Maxam and Walter Gilbert developed the chemical method of DNA sequencing the same year. Sanger sequencing depends on incorporation of specific chain-terminating dideoxynucleotides by DNA polymerase. In Sanger sequencing method, DNA primer is used for the DNA synthesis, this primer is complementary to the template DNA (that is the DNA to be sequenced) and the four deoxynucleotide triphosphates (dATP, dGTP, dCTP and dTTP) are used to extend the primer by adding the complementary dNTP to the template DNA strand using polymerase, then, the four terminating nucleotides – dideoxyucleotide triphosphates (ddNTPs: ddATP, ddGTP, ddCTP and ddTTP) labelled with a distinct fluorescent dye are used to terminate the DNA synthesis thereby sequencing the DNA. Whereas Maxam-Gilbert is based on chemical modification of DNA and subsequent cleavage at specific bases. Maxam-Gilbert sequencing method requires radioactive labelling at one end of the DNA to be sequenced, chemical treatment with specific chemicals for specific nucleotides break the DNA to produce small proportion of one or two of the four nucleotide bases in each of four reactions (G, A+G, C, C+T); different chemicals may be Dimethyl sulfate for Adenine and Guanine, Piperidine for Guanine, Hydrazine NaCl for Cytosine and Hydrazine for Cytosine and Thymine, therefore, a series of labelled fragments is generated from the radiolabelled end to the first cleaved site in each molecule. The sequencing is then done by gel electrophoresis.

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New Generation Sequencing Methods

Development of advanced sequencing is important with the increasing population and the increase in different disease conditions both in human and animals. The new generation methods are the advanced methods of DNA sequencing which run faster and more accurate with high throughput data.

Second-generation sequencing technology

They are known as next generation sequencing technology and includes Roche 454, Illumina Solexa and ABI-SOLiD. Roche 454 depends on the principle of pyrosequencing, Illumina Solexa depends sequencing by synthesis on and ABI-SOLiD depends on sequencing by ligation. They all follow four general steps for sequencing – Library preparation, cluster generation, DNA sequencing and Data analysis. They permit running of millions of sequencing reactions in parallel on the same solid surface which may be beads or glass slide and do not require the physical separation of reaction in different well or tube but spatially separated. Hence, thousands of different reactions can occur simultaneously. So, they are able to produce enormous amount of data at very economic cost and expenditure. Moreover, these technologies are more rapid than traditional method that can sequence whole genome of small organisms in a single day.

Third-generation sequencing

They are also known as next next-generation sequencing or Long Read sequencing and includes Helicos, PacBio and Ion Torrent. This generation refers to those technologies which do not require amplification step and are capable of sequencing single DNA molecule in real time. These platforms have the capability to provide single run at very low cost as well as made the preparation of sample easier. Additionally, third-generation systems often yield larger reads of around a kilobase length, which solves the issue of assembling the reads.

Fourth-generation sequencing

This generation includes Nanopore sequencers offered by Oxford Nanopore Technologies (ONT), namely GridION and MinION. They are based on principle of ligation chemistry. These methods have the ability to in situ (perform sequencing directly in the cell) sequence the fixed tissue cells and are able to offer simultaneous visualization and quantitative analysis of the transcriptome in the fixed tissues.

Conclusion

DNA sequencing can give the whole genetic information of the organism. This has a large range of application in human, animal and plant biotechnology – in studying the evolutionary biology

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to understand the mechanism of adaptation to the changing of the environments; in studying the genetic diseases of the different organisms; in genetic testing of the organism whether it has the genes for a disease or not and in development of personalized medicine as every individual has different genetic make-up, so each will have different treatment and management of the condition. Sequencing of the genes has a few challenges. The lack of computing skill, requirement of different hardwares and expensive equipment and reagents are the main challenges of the present sequencing methods. DNA sequencing is one of the foremost technologies required in the development of human, animal and plant biotechnology.

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