Dna sequencing methods: a history

DNA sequencing is the process of reading the correct order of base pairs, which builds up a DNA. It is a fundamental requirement for modern gene manipulation technique in the molecular field. Evaluation of 3 billion base pairs of 24 different chromosomes was a great success in the Human Genome Project. It has helped to reveal 20, 000 to 25, 000 human genes and also about the controlling regions (Human Genome project Information, 2008).

The sequencing of the human genome is a milestone in the scientific research, which came easier by evaluating related organisms with human genome. In 1990, the Human Genome Project has been achievable because of modern computer facilities. Perhaps, the comparison and characterization of genome between organisms, which contains millions of nucleotides has become reality only with the modern advancement of microchips and processors. Bioinformatics is the only stepping stones for each and every progress of DNA sequencing projects over past two decades (Edmund Pillsbury, n. a.). This overview discusses about the growth in DNA sequencing project with new technologies at different periods and how it has reduced the cost of sequencing project.

With an improvement Sanger used plus and minus method to sequence 5386 bp phage Đ¤X 174 genome (Primrose et al., 2001), but due to the large amount of sample requirement, time and labour intensive nature, genome sequencing research has limited the attempts of sequencing project. Later, Sanger has invented a new method of “ shotgun” sequencing, in which random fragments of DNA from the host genome was isolated and it was used as primers for the PCR amplification of the whole genome. Then the

contiguous transcripts are formed by assembling of amplified parts of DNA to the overlapping regions and then the convention primers are exposed at the gaps between the contigs by giving complete sequenced genome. Five years later, Sanger used “ shotgun” method to sequence 48, 502 bp, which helped sequencing project to grow at a faster rate. This rooted a strong foundation to sequence few viral and organellar genomes like 229kb of CMV, 192 kb of vaccinia, 187 kb mitochondrial and 121kb chloroplast genome of Marchantia polymorpha, and the 186 kb genome of smallpox (Edmund Pillsbury, n. a.).

Maxam and Gilbert method

In 1977, Maxam and Gilbert method was widely used in the sequencing project, which involves the basic-specific cleavage of DNA by the usage of chemical reagents. With this Maxam and Gilbert method, most large sequences can be sequenced except bacteriophage T7.

Chain terminator or dideoxy method

The chain terminator or dideoxy method depends on the two properties of DNA polymerases such as their ability to synthesize a single stranded complementary copy of DNA template and also depends upon their ability to use 2′, 3′-dideoxynulceotides as substrates. After the incorporation of analogue at the growing point of the DNA chain but the 3′ end lacks an OH group and making the substrate unfit for the chain elongation. As a result, the growing DNA chain is terminated. In this process, dideoxy-nucleotides act as chain terminators (Primrose et al., 2001).

Edward David Hyman method

In 1988, Edward David Hyman has introduced new method for the DNA sequencing, which is apart from the polyacrylamide gel electrophoresis of single stranded DNA fragments that was generated by any one of the following method such as, chain terminator or dideoxy method, selective enzymatic fragmentation of RNA or selective chemical degradation of DNA and also it does not involves any of the radioactivity or fluorescence method. The Edward David Hyman method has introduced luciferase-Sepharose column for the first time to sequence DNA. This method involves measuring of pyrophosphate generated by DNA polymerization reaction. The solutions containing dNTPs are pumped into a DEAE-Sepharose column, in which DNA and DNA polymerase are held together. As a result pyrophosphate is generated, and it is measured continuously by a device containing series of columns that are packed with enzymes covalently linked to Sepharose (Eward David Hyman, 1988). This method is fully depends on manual operations and calculations and also requires large amount of chemicals.

TIGR assembler method

The Institute of Genomic Research (TIGR) has introduced TIGR assembler software for DNA sequencing in 1990. In this method, genome is fragmented into smaller segments, each of up to 40 kb of DNA. Then these fragments are further broken down to smaller pieces with the help of shotgun method and then they are sequenced to reconstruct the genome. Finally, the research group has met their success by sequencing 1. 8 Mb of H. Influenzae genome. In this project, the task of TIGR assembler software was to reassemble approximately 24, 000 DNA fragments into the whole genome and this entire

process took 30 hours for the computer with 512 Mb RAM (Edmund Pillsbury, n. a.).

Automated DNA sequencing method

The large polymerase gene of Sendai virus was used for this method. This method involves primer with fluorescent dye-labelled dideoxynucleotide terminators was run on an automated ABI DNA sequencer. The final data of L gene of Sendai virus has revealed that it contains exactly 6800 nucleotides and the amino acid sequence of a protein was found to be 252. 876 kDa. As the automated sequencing method was so fast in the identification of complete sequence, this technique is less economic and time conserving at those periods (Gieseckea et al., 1992).

Pseudo-coulometric loading in capillary electrophoresis DNA sequencing

Generally in capillary electrophoresis, the sample volume for the injection should be in the range of nano litre, which is so difficult in loading the sample in such a small volume and also it is hard to measure the samples so lesser than micro litre. Due to this problem we cannot load the entire sample for the analysis, only fraction of the sample can be added to the capillary tube and moreover, this will be a great problem in the DNA sequencing projects because the preparation of sequencing sample is so expensive to stack on-column. This method engages the usage of very low ionic strength formamide to re dangle the DNA after the precipitation of ethanol because the formamide increases the voltage drop and decreases the electric field in the capillary tube and thus preventing the migration of sample DNA, which helps in long loading periods of the sample without band broadening.

Perhaps, the formamide increases the movement of large number of DNA and as the loading becomes a coulometric process, the whole process has reduced the cost of the whole project. (Daniel et al., 1996)

Replaceable polymers in DNA sequencing by capillary electrophoresis

Few years before the maintenance of high-performance DNA sequencing by capillary electrophoresis is highly economic because the capillary tubes are so sensitive to the impurities and more costly to replace it. Due to the advancement in recent technologies, this problem has been solved by the development of replaceable sieving polymers and capillary coatings for the electrophoresis. Moreover, the development of electro-osmosis-inhibiting matrix polymers has made the capillary coating process quite easier and also facilitated the automation of high-throughput parallel systems for sequencing DNA in large-scale (Mark A Quesada, 1997).

Microfluidic devices for DNA sequencing

When the human genome project has become reality, all researchers have turned their attention towards reducing the cost and time of the genome project. Few latest technologies like microfabricated DNA sequencing, sample processing and analysis devices have reached their success in reducing the time and cost. The integrated microfluidic processing has really reduced the costs, reagents, time and the usage of robotics or any other apparatus. The microfabricated device involves clone isolation, template amplification, purification and electrophoretic analysis in its own fabricated circuit itself and thus reducing the cost and time to the maximum extent (Brian et al., 2003).

Multiplexed DNA sequencing-by-synthesis

In the multiplexed DNAsequencing-by-synthesis method, the sequences of DNA templates are hybridized to a surface of an immobilized array of DNA primers. They are determined by detecting the number of nucleotides. As each array is encoded with a Xuorescein-labelled deoxyribonucleoside triphosphate (dNTP) species, the DNA sequencing can be determined by detecting the number of nucleotides by which primers extend in sequential DNA polymerase-catalyzed nucleotide reaction. The labelling on dNTP will be destroyed after each screening with photostimulated reaction by dipheyliodonium chloride. The incorrect binding effects were found to be small in this experiment and also the low level contamination of dNTPs with other nucleotides will produce negative signals. The impurities are cleared by polymerase-catalyzed integration into complementary cleaning duplexes. With this method we can readout 6000 bases per minute (Sergei et al., 2006)

Conclusion

The Human Genome Project is an impossible one before three decades but it become real soon with the automated sequencing systems and bioinformatics. Moreover, the cost, labours, chemicals, time and apparatus has been drastically reduced to its maximum extent with the help of computers. This overview has clearly signifies that the DNA sequencing method is growing each and every year. Many scientists across the world are proving with their researches, that DNA sequence project is no more expensive at present and it will be cheaper, reduce time in future.