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Review Article

A short review on DNA sequencing techniques

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ABSTRACT

A DNA sequence is a scientific instrument used to automate the DNA sequencing process. Given a sample of DNA, a DNA sequence is used to determine the order of the four bases: G (guanine), C (cytosine), A (adenine) and T (thymine). DNA can be sequenced by a chemical procedure that breaks a terminally labelled DNA molecule partially at each repetition of a base. DNA sequencing is very significant in research and forensic science. The main objective of DNA sequence generation method is to evaluate the sequencing with very high accuracy and reliability. DNA sequencing can solve a lot of problems and perform a lot of work for human wellfare A sequencing can be done by different methods. Automated DNA sequencing is based on usage of flouroscent label and takes less time. Ion torrent semiconductor sequencing is based on the release of hydrogen ions and the pH of the solution lowers. Shot gun sequencing is used for larger fragments of DNA and it is widely used during human genome project. This article provides a comprehensive overview on DNA sequencing techniques in bioanalysis.

Keywords: DNA sequencing, radioactive, human genome

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INTRODUCTION

The term DNA sequencing refers to methods for determining the order of the nucleotides bases adenine, guanine, cytosine and thymine in a molecule of DNA. The first DNA sequence were obtained by academic researchers, using laboratories methods based on 2- dimensional chromatography in the early 1970s.[1] By the development of dye based sequencing method with automated analysis, DNA sequencing has become easier and faster. The knowledge of DNA sequences of genes and other parts of the genome of organisms has become indispensable for basic research studying biological processes, as well as in applied fields such as diagnostic or forensic research. DNA can be sequenced by a chemical procedure that breaks a terminally labelled DNA molecule partially at each repetition of a base. The length of the labelled fragments then identify the position of that base.[2] We describe reactions that cleave DNA preferentially at guanines,at adenines,at cytosine and thymines equally, and at cytosine alone. When the product of these four reactions are resolved by size,by electrophoresis on a polyacrylamide gel, the DNA sequences can be read from the pattern of radioactive bands. The technique will permit sequencing of atleast 100 bases from the point of labelling. The purine specific reagent is dimethyl sulphate; and the pyrimidine specific reagent is hydrazine.[1,3]

DNA sequencing is very significant in research and forensic science. The main objective of DNA sequence generation method is to evaluate the sequencing with very high accuracy and reliability.[3]

There are some common automated DNA sequencing problems:[3,5]

1. Failure of the DNA sequence reaction.
2. Mixed signal in the trace (multiple peaks).
3. Short read lengths and poor quality data.
4. Excessive free dye peaks " dye blobs" in the trace.
5. Primer dimer formation in sequence reaction
6. DNA polymerase slippage on the template mononucleotide regions.

So, we should have to do the sequencing in such a manner to avoid or minimize these problems. DNA sequencing can solve a lot of problems and perform a lot of work for human wellfare A sequencing can be done by different methods:

Techniques of DNA Sequencing:[4-10]

- Sanger method
- Maxam and Gilbert sequencing
- Automated sequencing method
- Next generation sequencing
- 454 pyro sequencing
- Ion semiconductor torrent sequencing
- Shotgun sequencing

SANGERS SEQUENCING

The first DNA sequencing method devised by Sanger and Coulson in 1975. Regions of DNA up to 900 base pairs in length are routinely sequenced using this method.

Ingredients for Sanger sequencing: A DNA polymerase enzyme. A primer, which is a short piece of ssDNA that binds to the template DNA and acts as starter for the polymerase. The four DNA nucleotides (dATP, dTTP, dCTP, dGTP). The template DNA to be sequenced. Dideoxy ,or chain terminating versions of all four nucleotides (ddATP, ddTTP, ddCTP, ddGTP).

Procedure: The DNA sample to be sequenced is combined in a tube with primer, DNA polymerase, DNA nucleotides, Dideoxy nucleotides are also added, but in much lesser amount than ordinary nucleotides. The mixture is first heated to denature the template DNA , then cooled so that the primer can bind to single stranded template. Once the primer is bound, the temperature is raised again, allowing DNA polymerase to synthesize new DNA starting from the primer. DNA polymerase will continue adding nucleotides to a chain until it happens to add a dideoxy nucleotide instead of a normal one, at that time the strand will end with dideoxy nucleotide and chain termination takes place. By the time the process is completed, the dideoxy nucleotide will have been incorporated at every single position of target DNA in atleast one reaction. The tube will contain fragments of different lengths, ending at each of the nucleotide positions in the orginal DNA. After the reaction is done, the fragments are run through the long thin tube containing a gel matrix in a process called capillary gel electrophoresis.

MAXAM-GILBERT SEQUENCING METHOD

It is the method of sequencing developed by Allan Maxam and Walter Gilbert in 1976-1977. It is based on chemical modification of DNA and subsequent cleavage at specific nitrogenous bases.

Principle: Purification of DNA fragments that to be sequenced and labelled with radioactive material. Chemical treatment generates breaks at specific nitrogenous bases and thus a series of labelled fragments are generated. The fragments in four reactions are arranged side by side in gel electrophoresis for size separation. The fragments visualize in X-ray for autoradiography. To visualise fragments, the gel is exposed to X-ray film for autoradiography, yielding a series of dark bands each corresponding to a radiolabelled DNA

fragment, from which the sequence may be inferred.

Procedure: Maxam-Gilbert sequencing requires radioactive labelling at one 5' end of the DNA fragment to be sequenced (gamma-32P). Chemical treatment generates breaks at a small proportion of one or two of the four nucleotide bases in each of four reactions (G, A+G,C, C+T). For example, The purines (A+G) by using formic acid, guanines by dimethyl sulfate.

Advantages

- No premature termination due to DNA sequencing.
- Purified DNA can be read directly
- Can be used to analyze DNA protein interactions
- Can be used to analyze nucleic acid structure

Disadvantages

- Not widely used
- Use of radioactivity and toxic chemicals
- It requires extensive use of hazaardous chemicals

Automated DNA Sequencing

It is based on Sanger-Coulson method, with two notable differences from the standard procedure.

The first difference concerns the labelling of products of Polymerase chain reaction:

- It uses the fluorescent labels in place of radioactive labelling
- The florescent labels are usually attached to the four dideoxy nucleotides used for chain termination.
- In the four track system of automated DNA sequencing, each of the four dideoxy nucleotides used in the separate reaction and the products are run in 4 adjacent lanes of gel.
- If a different fluorochrome is attached to each of the 4 dideoxy nucleotides, all of them could be used in the same reaction in place of preparing a separate reaction for each dideoxy nucleotide. This is called single track system as reaction products aare run in a single gel lane.

The reaction products are subjected to polyacrylamide gel electrophoresis under denaturing conditions or loaded into a capillary filled with a sequencing gel.

- The bands produced in a polyacrylamide gel are identified with the help of fluorescence detector, which identifies the fluoroscent signal emitted by each band.
- The fluorochromes are excited by laser beam and the resulting fluoroscence signal is sensed by photovoltaic cell.
- The resulting data are fed into a computer, which in turn, converts these signals into the base sequence of DNA molecules.
- Automated DNA sequences can read up to 96DNA sequences in 2hrs period.

Advantages over manual DNA sequencing:

- Radioactivity is not used.
- Gel processing after electrophoresis and autoradiography are not needed.
- The tedious manual reading is not needed as data is processed in computer.
- The sequence data is directly fed into and stored in a computer.
- It is extremely fast
- The seperation of same reaction products can be repeated to recheck the result in case of doubt as they can be stored for long period of time.

Next Generation Sequencing

The principle behind next generation sequence (NGS) is similar to Sanger sequencing, which relies on capillary electrophoresis. The genomic strand is fragmented, and the bases in each fragment are identified by emitted signals when the fragments are ligated against a template strand. The NGS method uses array-based sequencing which combines the techniques developed in Sanger sequencing to process millions of reactions in parallel, resulting in very high speed and throughput at a reduced cost.

454 Pyrosequencing:

It is based on the ' sequencing by synthesis' principle, where a complementary strand is synthesized in presence of polymerase enzyme. In contrast to using dideoxy nucleotides to terminate chain termination, pyrosequencing detects the release of pyrophosphate when nucleotides are added to DNA chain. It intially uses the emulsion PCR technique to construct the colonies required for sequencing and removes the complementary strand. Next, a ssDNA sequencing primer hybridizes to the end of the strand, then the 4 different dNTP are then subsequently made to flow in and out of the wells over colonies. When the correct dNTP is enzymatically incorporated into the strand, it causes release of pyrophosphate. In presence of ATP sulfurylase and adenosine, the pyrophosphate is converted into ATP. The ATP molecule is used for luciferase-catalysed conversion of luciferin to oxyluciferin, which produces light that can be detected with camera. The relative intensity of light is proportional to the amount of base added.

Ion Torrent Semiconductor Sequencing

It is an approach in which, a new DNA strand, complementary to the target strand, is synthesized

one base at a time. A semiconductor chip detects the hydrogen ions produced during DNA polymerization. Following colony formation using emulsion PCR, the DNA library fragment is flooded sequentially, with each nucleoside triphosphate (dNTP), as in pyrosequencing. The Dntp is then incorporated into the new strand if complementary to the nucleotide on the target strand. Each time a nucleotide is successfully added, a hydrogen ion is released, and it is detected by the sequence' s pH meter.

Shotgun Sequencing: Shotgun sequencing, also known as shotgun cloning is a method used for sequencing long DNA strands or the whole genome. In this method, DNA is broken up randomly into numerous small segments and overlapping regions are identified between all the individual sequences are generated. Multiple overlapping reads for the target DNA are obtained by performing several rounds of this fragmentation and sequencing. Computer programs then use the overlapping ends of different reads to assemble them into a continuous sequence. The Shotgun approach was first used successfully with the bacterium Haemophilus influenzae. Craig venter used this method to map human genome project in 2001.

CONCLUSION

Sanger sequencing is based on the principle of chain termination and uses a radioactive labelled primer. Maxam - Gilbert sequencing is based on the principle of chemical cleavage and uses the chemicals and radioactive material. Automated DNA sequencing is based on usage of flouroscent label and takes less time. Pyrosequencing is based on the release of pyrophosphate and emission of light. Ion torrent semiconductor sequencing is based on the release of hydrogen ions and the pH of the solution lowers. Shot gun sequencing is used for larger fragments of DNA and it is widely used during human genome project.

REFERENCES

1. Sanger F; Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J. Mol. Biol. 94 (3): 441– 8.
2. Maxam AM, Gilbert W. A New Method for Sequencing DNA. Proc. Natl. Acad. Sci. U.S.A. 74 (2): 560– 4.
3. Nyrenet.al., Solid Phase DNA Minisequencing by an Enzymatic Luminometric Inorganic Pyrophosphate Detection Assay. Analytical Biochemistry 208 (1).
4. Kasper TJ et.al., Separation and detection of DNA by capillary electrophoresis. J Chromatogr A 458:303– 312.
5. Sanger Fet.al., DNA sequencing with chain-terminating inhibitors". Proc. Natl. Acad. Sci. U.S.A. 74 (12): 5463– 7. Bibcode1977PNAS...74.5463S. doi:10.1073/pnas.74.12.5463. PMC 431765.PMID 271968.
6. Gilbert, W. DNA sequencing and gene structure. Nobel lecture, 8 December 1980.
7. Maxam AM, Gilbert W. A new method for sequencing DNA. Proc. Natl. Acad. Sci. U.S.A. 74 (2): 560– 4. Bibcode 1977PNAS...74..560M.doi:10.1073/ pnas.74.2.560. PMC 392330. PMID 265521.
8. Pettersson E et.al., Generations of sequencing technologies. Genomics 93 (2): 105– 11.
9. Olsvik O et.al., Use of automated sequencing of polymerase chain reaction-generated amplicons to identify three types of cholera toxin subunit B in Vibrio cholerae O1 strains. J. Clin. Microbiol. 31 (1): 22– 5. PMC 262614.PMID 7678018.
10. Schuster, Stephan C. Next-generation sequencing transforms today's biology. Nature methods (Nature Methods) 5 (1): 16– 18.