Pyrosequencing

Principle

Methodology

Application

Advantage

Limitations

Continue from Page 18

Pyrosequencing / Pyrosequencing

Date 7/8/21
Page

Introduction

In Pyrosequencing, a Sequencing Primer is hybridized to a single-stranded template and sequencing is based on detection of the pyrophosphate (ppi) released during DNA synthesis.
The Pyrosequencing is simple and robust; it contains (in addition to the standardized ~~Seq~~ Reagent mixture) the DNA template Sequencing primer, APS (adenosine-5'-phosphosulphate), luciferin, and the enzymes DNA Polymerase (Klenow fragments of E. coli DNA polymerase I), ATP ~~sulf~~ sulfurylase, Luciferase and apyrase. To this reaction mixture, the four dNTPs are added sequentially one after the other e.g. – in the sequence G, C, T, A,

Pyrosequencing Reaction

Pyrosequencing technology is a recently established non electrophoretic, Sequencing-by-synthesis technique which uses an enzymatic system based on Luciferase to monitor DNA production.

Principle

$$(DNA)_n + dXTP \xrightarrow{DNA\ Polymerase} (DNA)_{n+1} + PPi$$

$$PPi + APS \xrightarrow{ATP\ sulfurylase} ATP + SO_4^{2-}$$

Date 7/8/21
Page

$ATP + Luciferin + O_2 \xrightarrow{Luciferase} AMP + PPi +$ Oxyluciferin + CO_2 + Light

$ATP + dXTP \xrightarrow{APyrase} ADP + dXDP + 2Pi$

$ADP + dXDP \xrightarrow{APyrase} AMP + dXMP + 2Pi$

Methodology

1. DNA fragmentation
2. Adapter binding to ends of DNA fragments.
3. Denaturation of fragments
4. fix to a solid surface, sepharose beads of streptavidin - coated magnetic beads (Beads which are surrounded by single stranded sequence).
5. Beads has sequences complementary to adapters sequences.
6. Loads the beads into sequencing wells (small DNA grooves).
7. Chemical process.

Date 7/8/21
Page

I. Add DNA polymerase and single dNTP (polymerization).

II. Add sulfurylase and APS (ATP synthesis).

III. Add Luciferin and Luciferase (Light production)

IV. Detection (Light sensor).

V. Washing or add enzyme apyrase enzyme.

Application of Pyrosequencing.

(1) Both for confirmatory sequencing of, say, disease genes or even entire genomes, and also for de novo sequencing.

(2) Genotyping of individuals, including detection of SNPs (single Nucleotide polymorphisms).

(3) Determination of sequences of difficult secondary structures (e.g. - hairpins) DNA segments that are not readily sequenced by other sequencing techniques.

(4) Identification of Bacterial species and strains by analysing between 20 and 100 nucleotides of 16S rRNA gene.

Date 7/8/21
Page

(5) Identification of genes represented in cDNA library.

(6) Comparative sequencing to identify variations across those genes that have been sequenced. pyrosequencing shows excellent accuracy for analysis of polymorphic DNA fragments.

Advantages

(1) Very small Quantities of DNA are required; even one pmol (picmole) of DNA generates enough light to be detected by a, say, charge-couple device (CCD) camera.

(2) It is very rapid; the overall reaction from polymerization to light detection takes only 3-4 sec at room temp.

(3) It has over 99% accuracy in SNP genotyping with automated systems.

(4) The entire process; including loading of pyrograms, has been automated.

(5) It is highly cost competitive; the cost of sequencing one sample is arounds 70 cent using standard pyrosequencing techniques.

Limitations:-

- It can be used for sequencing relatively short segments of DNA, but now up to 400 nucleotides can be sequenced.

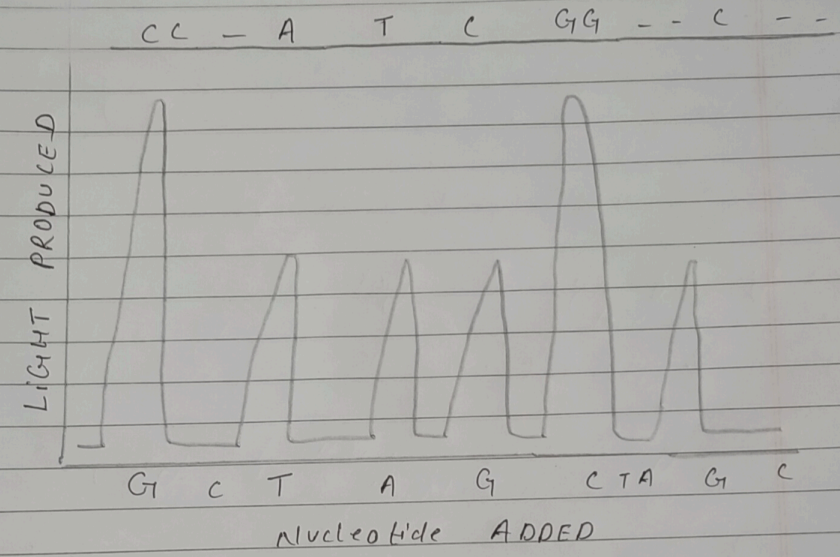

fig - A Pyrogram pattern and the nucleotide sequence derived from it. production of light indicates nucleotide incorporation. A stronger light signal e.g - in response to addition of G and C reveals incorporation at two consequtive sites while lack of signal, e.g- for C and T shows lack of incorporation.

The END