Lecture 30: DNA sequence analysis methods-II

Fluorescence DNA Sequencing:

Fluorescence DNA sequencing or automated DNA sequencing implies the same procedure as in Sanger's method but with one major difference. In Sanger's method, detection is done by developing autoradiograph with radiolabelled primer which makes it lengthy and time consuming and it can be used as an ideal throughput procedure due to its health hazardousness. To get rid of these problems, in automated DNA sequencing, fluorescent molecules are being used instead of radiolabelled. Fluorescent dye labels are incorporated into DNA extension products using 5' labelled primers (dye primers) or 3' labelled dideoxynucleotide triphosphates i.e. Dye terminators. Usually the fluorescent labelling is done at 3' end of dideoxyNTP so different type of fluorochromes can be used for each ddNTP and whole reaction can be carried out in a single tube. Each dye may have a different emission wavelength when excited with an argon ion laser. Thus all four bases can be distinguished by the emission of four different colors, in a single gel lane (Fig. 5A) or by capillary electrophoresis (Fig. 5 B,C,D). Capillaries are small, a 50 µm inner diameter, and they dissipate heat very efficiently due to their high surface area to volume ratios. Thus a capillary based system can be run with much higher voltages and it dramatically lowering the run time. Most importantly, capillary systems can be automated which is a major limitation in gel based systems.

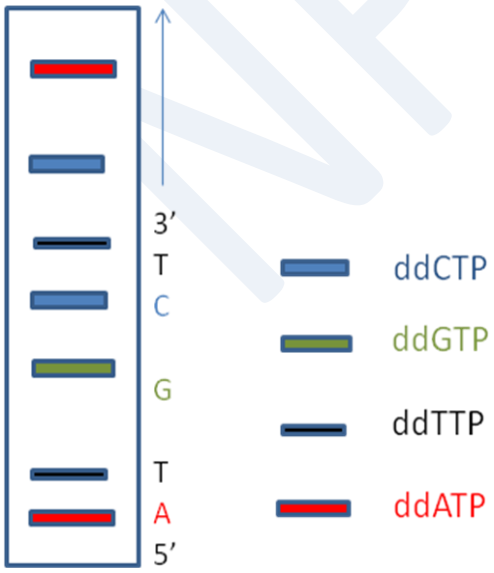

Figure 5A: A Sequencing SDS PAGE gel with 4 different fluorochrome tagged ddNTP products run in a single lane.

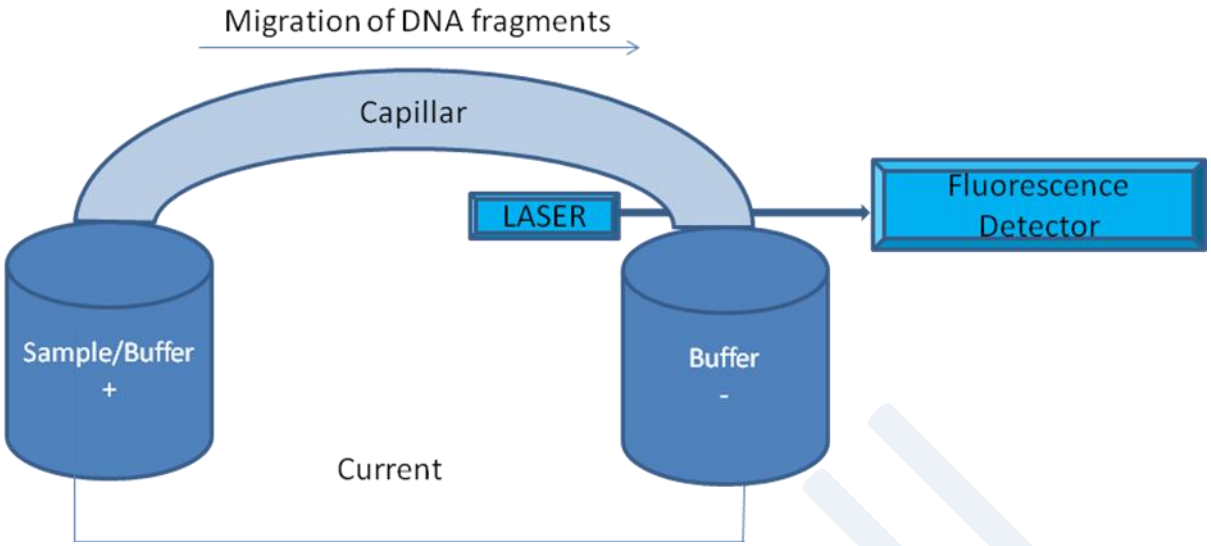

Figure 5B: A schematic representation of basic set up of capillary based DNA sequencing System.

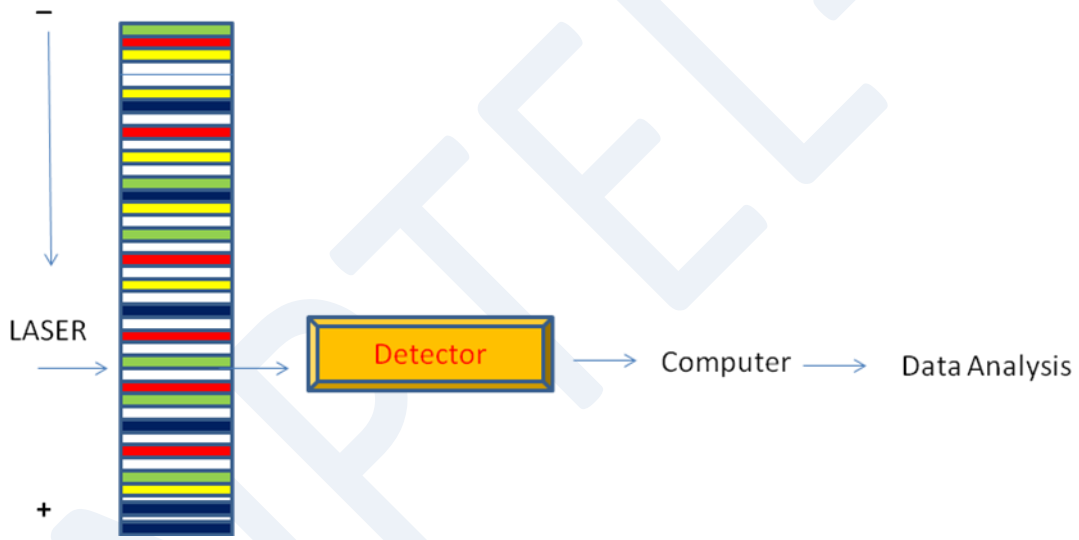

Figure 5C: False color representation of resolved DNA fragments, being excited by LASER.

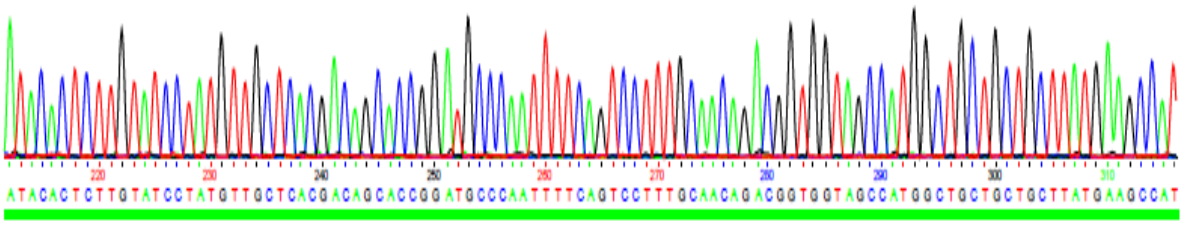

Figure 5D: The Final electrogram Output of DNA.

Benefits of Fluorescence DNA sequencing:

Fluorescent DNA sequencing eliminates the use of radioactive isotopes for DNA labelling and thus reduces the amount of radioactive waste.

Due to its automation, it is also time saving procedure because here we don't need to generate autoradiograph or any associated tasks which are required to work with radioactive material as radiation survey, inventory or regarding its disposal documentation work.

And also it provides more reliable results than Sanger manual DNA sequencing.

Automated DNA Sequencers which works on Fluorescent labelling basis are more efficient in working as they can sequence more than 350 samples in a single run and 24 runs in a day.

Interesting article to read

http://www.nytimes.com/2011/12/01/business/dna-sequencing-caught-in-deluge-of-data.html