Buffers and Reagents for Electrophoresis

Electrophoresis is defined as the movement of ions and charged macromolecules through the medium when an electric current is applied. Agarose and polyacrylamide are the primary stabilizing media used in the electrophoresis of macromolecules. Agarose is mainly used for the nucleic acid electrophoresis; polyacrylamide is used for the protein electrophoresis.

Nucleic Acid Gel Electrophoresis

Agarose Gel : Agarose gel is a macroporous matrix which allows rapid diffusion of high molecular weight (1000kDa range) macromolecules without significant resistance. It is a non-toxic polysaccharide with high gel strength which allows the use of concentration 1% or less. Agarose gel are thermoreversible it's low-gelling-temperature and low-melting temperature permits easy recovery of samples even one which are heat labile like DNA/RNA.

For the DNA electrophoresis 0.5-1% gel of low EEO agarose **(MB002)** is used in submerged horizontal electrophoresis and the buffer system, usually used is TAE **(ML016, ML010)** or TBE. **(ML017, ML022).** With the change in concentration of gel and the type of buffer DNA upto 20-50000bp can be separated. For larger bp DNA, TAE buffer system is the best choice whereas TBE is preferred for the small DNA molecules (<1kb).

There is change in the buffer system used for electrophoresing RNA due to its molecular composition and the size of it being smaller than DNA. RNA is likely to form secondary structure which makes it difficult to migrate through the agarose gel matrix therefore a denaturing system is most frequently used which contains Formaldehyde, MOPS and Formamide. The MOPS buffer system **(ML050)** in combination with Formaldehyde creates and a denaturing environment avoiding secondary structures of the sample loaded and its easy separation. For all the RNA works it is recommended to use DEPC treated water **(ML024)** for preparing buffers to inhibit the RNase action as DEPC inactivates the RNase completely.

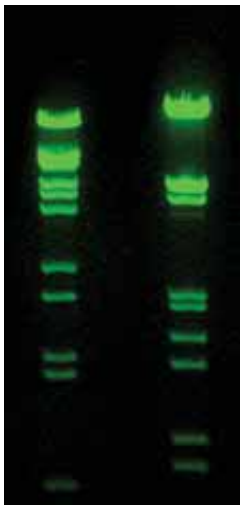

Fig. 37 a : Gel stained using Hi-SYBr Safe Gel Stain ML053

For loading of the DNA/RNA on the gel and tracking the run, it is important to avoid the loss of the loaded sample and its separation; various DNA & RNA loading dyes serve the purpose. Loading buffers increase the density of the sample ensuring that DNA/RNA settles evenly into the well, add colour to the sample which visually simplifies loading, they contain dye like Xylene cyanol, Bromophenol blue **(ML015)**, Orange G **(ML092)** which under the electric field migrate towards anode, enabling electrophoretic processes to be monitored.

eg. Glycerol based dye, sucrose based dye, alkaline buffered dye.

At the end of the electrophoretic run there has to be visualization of the DNA/RNA sample separated in the processes; but for which our normal vision is not sufficient. The DNA bands are observed in UV light for which the band has to fluoresce, there are special fluroscent dye which associate themselves to the molecule of DNA reversibly. Very common of all is Ethidium bromide (MB074) , which detects both single and double strand DNA and RNA. Various other method of DNA staining are available like Silver stain **(ML123)**, Propidium Iodide **(ML067),** non hazardous SYBr Green staining **(ML053)** etc.

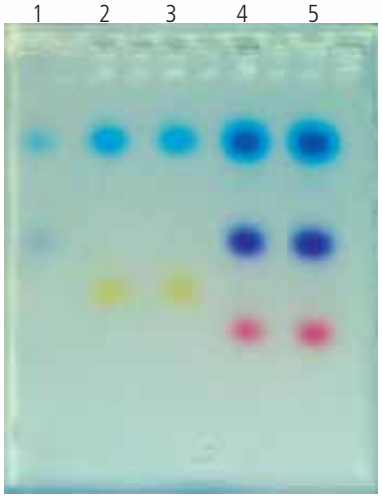

Fig. 37 b : DNA Gel electrophoresis using diffrent loading dyes
Lane 1 : ML015
Lane 2 & 3 : ML092
Lane 4 & 5 : ML086