Horizontal Gel Electrophoresis

Gel concentration

The range of fragment sizes to be separated will determine the choice of agarose concentration for a gel. Typical agarose concentration is 0.5% to 3.0%. For large DNA fragments low-percentage gels are required, while for small DNA fragments, high-percentage gels are recommended. Weak gels (0.5% agarose) should be electrophoresed at low temperatures (e.g. -4°C).

Agarose gels of 0.75% to 1.0%, for routine electrophoresis, are recommended for a wide range of separations (0.15 to15 kb). 2…4% agarose gels are usually selected for PCR fragment resolution. If the gel has to be subsequently photographed, thin gels (2 to 3 mm) with low-percentage agarose are better than thick or high-percentage gels. The latter produce increased opaqueness and autofluorescence.

Electrophoresis buffer

TAE buffer provides optimal resolution of fragments >4 kb in length, while for 0.1 to 3 kb fragments, TBE buffer should be selected. TBE has both a higher buffering capacity and lower conductivity than TAE and therefore should be used for high-voltage electrophoresis. Additionally, TBE buffer generates less heat than TAE at an equivalent voltage and does not allow a significant pH drift. Note: because of its lower buffering capacity, TAE should be circulated or mixed from time to time for full-length electrophoresis, especially at higher voltages.

Temperature influence

Electrophoresis at high voltages produces heat. Additionally, high-conductivity buffers such as TAE generate more heat than low-conductivity buffers. Care should be taken in agarose gel electrophoresis with voltages greater than 175 V, as heat build up can generate gel artifacts such as S-shaped migration fronts, and in extended electrophoresis runs, can even melt the agarose gel. With high voltage electrophoresis, the use of low-melting-point agarose gels should be avoided.

RNA mobility

Either before or during electrophoresis, RNA should be denatured. For example, RNA fragments which have denatured with glyoxal and dimethyl sulphoxide can be separated on neutral agarose gels, or RNA can be fractionated on agarose gels containing methylmercuric hydroxide or formaldehyde. RNA samples usually require longer runs or buffers that are easily depleted, so it is necessary to circulate the buffer. Northern analyses should not normally be run on a mini gel tank.

Separation performance

Gel concentration, running buffer, voltage, temperature, conformation, and the presence of ethidium bromide all affect separation results. To establish progress of double-stranded DNA, ethidium bromide (0.5 µg/ml) is often added to running buffer.

The dye's fluorescence properties allows the band to be visualised under a UV lamp. However, ethidium bromide may slow the DNA migration rate by approx. 15%. As an alternative, after electrophoresis, the gel may be stained in an ethidium bromide solution (0.5 µg/ml H20) for 15 to 60 minutes and then viewed or photographed on a UV trans-illuminator.

Note: staining time should be minimised to prevent small nucleic acid fragments from diffusing out of the gel. Background fluorescence of unbound ethidium bromide can be minimised through destaining by soaking the gel for 5 minutes in 0.01 M MgCl2, or for 30 minutes in de-ionised water. Caution! Ethidium bromide is a known mutagen. Always wear gloves when handling. Wear UV safety goggles and protect skin when using any UV light source.

Enhancing resolution

2 x TAE buffer can be used in units with low buffer volume to enhance resolution during extended runs.