HyperLadder™ | Nucleic Acid Electrophoresis

Nucleic acid electrophoresis is a widely performed molecular biology technique and is used to separate, identify and purify nucleic acids based on the principle of charge migration. Nucleic acid molecules are separated by applying an electric field to migrate the negatively charged molecules to positive electrodes through a matrix.

Migration is determined by both size and conformation, allowing nucleic fragments of different sizes to be separated. However, the relationship between fragment size and migration rate is non-linear, since larger fragments have greater frictional drag and are less efficient at migrating through the polymer.

Applications of nucleic acid electrophoresis include analytical techniques such as restriction enzyme mapping, sequence analysis, confirmation of plasmid construction and PCR products, detection of DNA polymorphisms, Northern and Southern blotting, separation of fragments for recovery and cloning as well as other downstream techniques.

This application note provides useful hints for effective gel analysis of genomic DNA.

AGAROSE GELS

Agarose is a natural polysaccharide purified from seaweed that forms flexible gels of sufficient mechanical strength at percentages as low as 0.5%. Agarose gel electrophoresis is the most commonly used method for separating DNA fragments between 0.1 and 25 kb.

- Standard high melting point agarose is used in routine DNA electrophoresis for high clarity separation of a wide range of DNA fragments (Agarose, Molecular Grade, BIO-41025).
- Separated DNA fragments can be isolated and purified from TAE and TBE agarose gels using a silica-membrane based DNA purification kit (ISOLATE II Gel and PCR Kit, BIO-52059).

DNA LOADING DYES/BUFFERS

DNA loading dyes are used to prepare samples for loading on agarose or polyacrylamide gels. Components include:

- Glycerol, which increases sample density relative to the surrounding buffer to facilitate easy loading.
- EDTA, which binds divalent metal ions that may interfere with electrophoresis. By complexing metal ions, EDTA also inhibits metal-dependent enzymatic reactions e.g. DNA degradation by nucleases.
- Tracking dyes to monitor the progress of electrophoresis by the migration of the dyes. Loading dyes are supplied with each DNA ladder/marker and are also available separately.

The Meridian DNA Loading Buffer Blue (BIO-37045) is a ready-to-use solution premixed with bromophenol blue. Table 1 describes the speed of migration of this dye at various gel concentrations.

Table 1. Migration of bromophenol blue at various agarose gel concentrations.

AGAROSE GEL CONC.	BROMOPHENOL BLUE
0.70%	600 bp
1.00%	400 bp
1.50%	250 bp
2.00%	120 bp
3.00%	50 bp

DNA MARKERS AND LADDERS

Meridian offers a broad selection of convenient ready-to-use DNA ladders/markers ranging from 25 bp to 10 kb for accurate analysis of linear double-stranded DNA in agarose or polyacrylamide gels (Table 2).

Table 2. DNA ladders/markers

Ladder	Cat. No.	Separation Range	Loading Dye Color
HyperLadder 1kb	BIO-33025 (200 lanes) BIO-33026 (500 lanes)	200bp-10,000bp	Blue
HyperLadder 50bp	BIO-33039 (200 lanes) BIO-33040 (500 lanes)	50bp-2000bp	Blue
HyperLadder 100bp	BIO-33029 (200 lanes) BIO-33030 (500 lanes)	100bp-1000bp	Blue
HyperLadder 25bp	BIO-33031 (200 lanes) BIO-33032 (500 lanes)	25bp-500bp	Blue

Features:

- High intensity bands for easy identification
- Ready-to-use DNA ladders/markers are ideal for accurate sizing
- Optional mass determination
- Stable at room temperature for at least 6 months
- Supplied with additional 5x sample loading buffer

To measure the size of a linear DNA fragment and to troubleshoot any electrophoresis issues, a DNA marker should be run alongside your experimental sample (e.g. HyperLadder 1kb). Table 3 highlights the recommended agarose percentages for separating different fragment sizes of DNA.

RECOMMENDATIONS FOR DNA ELECTROPHORESIS

Table 3 DNA electrophoresis. Recommended gel concentrations for Different DNA fragment sizes.

Effective DNA Separation Range - Agarose Gels	
Recommended % Agarose (w/v)	Effective Separation Range of Linear DNA* (bp)
0.3	5,000-60,000
0.5	1,000-20,000
0.7	800-12,000
1.0	500-10,000
1.2	400-7,000
1.5	200-3,000
2.0	100-2,000
2.5	50 -100
3.0	10-75

ELECTROPHORESIS CONDITIONS

The electrophoretic mobility of DNA molecules depends on the voltage and the composition of the electrophoresis buffer, as well as gel concentration.

Voltage

The applied voltage depends upon the purpose of gel electrophoresis. For Southern hybridization the applied voltage should be adjusted to 1-3 V/cm. This results in the slower migration of fragments in the gel and hence better resolution. For routine analysis of DNA fragments, an applied voltage of 5-8 V/cm is recommended. Moreover, if electrophoresis is done for the purification of the fragments from the gel, then the applied voltage is adjusted to 3-5 V/cm.

Buffers

For electrophoresis of nucleic acids, Tris-Acetate-EDTA (TAE) and Tris-Borate-EDTA (TBE) buffers are commonly used. TAE has the lowest buffering capacity and is more quickly exhausted during extended runs, but provides the best resolution for larger DNA fragments. TBE Buffer should be used for pulse-field gel electrophoresis to ensure adequate buffering power due to the high voltages used in this procedure.

Gel concentration

Agarose gels can be used to separate and visualize different sizes of linear DNA. The higher the percentage of agarose, the smaller the linear DNA fragment that can be resolved. The sugar polymers that constitute the agarose gel matrix act like a sieve. The greater the agarose concentration, the smaller the pores created in the gel matrix, and the more difficult it is for large linear DNA molecules to move through the matrix. Changing the agarose concentration changes the size of the sieve matrix of the gel. However, there is an upper and lower limit to accurate separation of DNA molecules using agarose gel electrophoresis (Table 3).

It should be noted that secondary and tertiary DNA structures present in nicked, supercoiled and dimeric molecules will always display different mobilities on a gel compared to linear DNA standards of the same size.

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