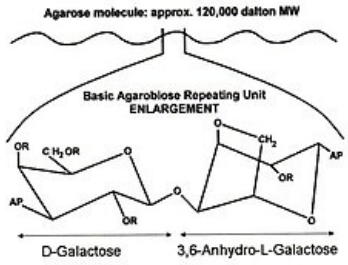
Electrophoresis of nucleic acids

Sections of DNA , PCR reaction products or DNA grafts can basically be separated by chromatography or electrophoresis. Electrophoretic separation is much more widely used. It is usually performed in a gel, either agarose or polyacrylamide. In both cases, molecules that carry a negative charge in an alkaline environment move in an electric field from the cathode to the anode. Gels form a relatively dense network, which larger molecules pass through more slowly than smaller molecules - this is why we speak of the **molecular sieve** technique .

Agarose gel electrophoresis

Agarose is a polysaccharide consisting of D-galactose and anhydro-L-galactose, which is produced by some seaweeds and under the name agar is used to produce gels in the food industry, microbiology, immunology or biochemistry. Gels containing 0.5 to 4% agarose are used for nucleic acid electrophoresis. The higher the polysaccharide content, the better the distinguishing ability of the gel, but this also means that the electrophoresis process is slower and the preparation of the gel is more technically demanding.



Agarose

Selection of agarose gel concentration for DNA electrophoresis

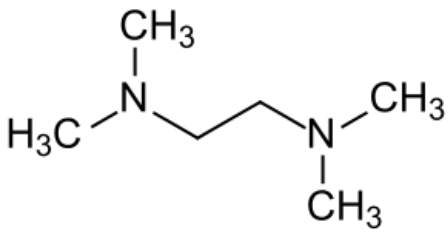
Agarose content in the gel	DNA length
0,5 %	1–30 kbp
0,7 %	0,8–12 kbp
1,0 %	0,5–10 kbp
1,2 %	0,4–7 kbp
1,5 %	0,2–3 kbp
2,0 %	50 bp–2 kbp
3–4 %	10 bp–1 kbp

Ethidium bromide is usually added directly to the agarose gel , so that after the electrophoresis the individual fractions can be visualized by UV radiation . Another option for detection is blotting on a membrane and subsequent staining of nucleic acids or hybridization with labeled probes.

Polyacrylamide gel electrophoresis

Another support used in nucleic acid electrophoresis is **polyacrylamide gel** . Polymerization of acrylamide produces linear polyacrylamide molecules . These are connected by cross bridges, which are formed by copolymerization with N,N'-methylenebisacrylamide . Both acrylamide and methylenebisacrylamide are relatively stable substances; polymerization takes place in the absence of atmospheric oxygen (removed by deaeration using a vacuum) and begins by mixing the catalysts of [[ammonium peroxydisulfate **(known under the older name ammonium persulfate, APS for short) and N,N,N',N'-tetramethylethylenediamine (TEMED).**

APS in aqueous solution with TEMED releases free oxygen radicals , which attack acrylamide and bisacrylamide molecules and thus trigger their polymerization.



TEMED

The molecular sieve of the polyacrylamide gel is quite dense, so it is suitable for the separation of shorter fragments.

Choice of polyacrylamide gel concentration for DNA separation

Polyacrylamide	DNA length
3,5 %	1–2 kbp
5 %	75–500 bp
8 %	50–400 bp
12 %	35–250 bp
15 %	20–150 bp
20 %	5–100 bp

Because polyacrylamide is less reactive than agarose, DNA fragments can be stained by other techniques in addition to the methods used in the agarose gel. Among the classical methods, silvering is among the most sensitive, with which the amount of DNA can be detected even several orders of magnitude lower than with ethidium bromide.

Links

Related articles

- Restriction fragment length polymorphism
- Conformation polymorphism of single chains
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