

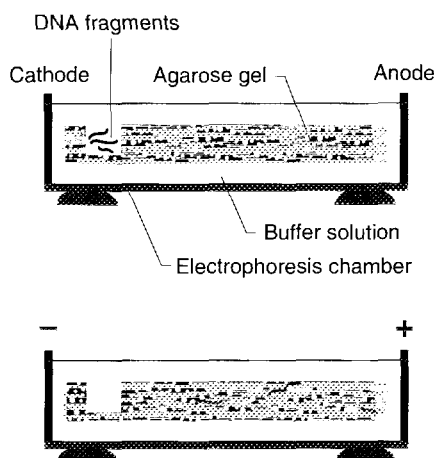
GEL ELECTROPHORESIS

Historically gel electrophoresis was first applied to separating proteins essentially according to mass, but the technique was adapted to separating fragments of DNA (or RNA) essentially according to fragment length. The technique works on DNA because the phosphate groups of a DNA fragment are negatively charged, and therefore, under the influence of an electric field, the fragment migrates through a gel (a porous, semisolid medium) in a direction opposite to that of the field. Furthermore, the rate at which the fragment migrates through the gel is approximately inversely proportional to the logarithm of its length.

Gel electrophoresis of DNA is carried out with two types of electric field. Conventional gel electrophoresis employs a field that is temporally constant in both direction and magnitude. In contrast, pulsed-field gel electrophoresis employs a field that is created by pulses of current and therefore varies periodically from zero to some set value. More important, the direction of the electric field also varies because different pulses flow through pairs of electrodes at different locations. (Note, however, that the time-averaged direction of the electric field is along the length of the gel.) The advantage of such a pulsed field is that it prevents long DNA fragments, fragments longer than about 50,000 base pairs, from jackknifing within the structural framework of the gel and thus allows the long fragments to migrate through the gel in a length-dependent manner, just as shorter fragments migrate in a constant electric field.

The gel employed is usually a solidified aqueous solution of agarose, a purified form of agar. By varying the concentration of agarose in the gel, conventional gel electrophoresis can be applied to samples containing DNA fragments with average lengths between a few hundred base pairs and tens of thousands of base pairs. (Another gel used for conventional electrophoresis is polyacrylamide, which is particularly suited

(a) Conventional Gel Electrophoresis



to separating fragments with lengths less than about a thousand base pairs and is therefore the gel of choice for sequencing.) Conventional gel electrophoresis in an agarose gel is illustrated in (a); details of the technique are as follows.

Agarose is dissolved in a hot buffer solution, and the gel solution is allowed to solidify into a thin slab in a casting tray in which the teeth of a comb-like device are suspended. After the gel has solidified, the comb is removed. The “wells” formed by the teeth of the comb are the receptacles into which the samples of DNA are loaded. The thickness of the gel is about 5 millimeters; its length and width are much greater and vary with the purpose of the electrophoresis. Before being loaded with the DNA sample(s), the gel is immersed in a conducting buffer solution in an electrophoresis chamber.

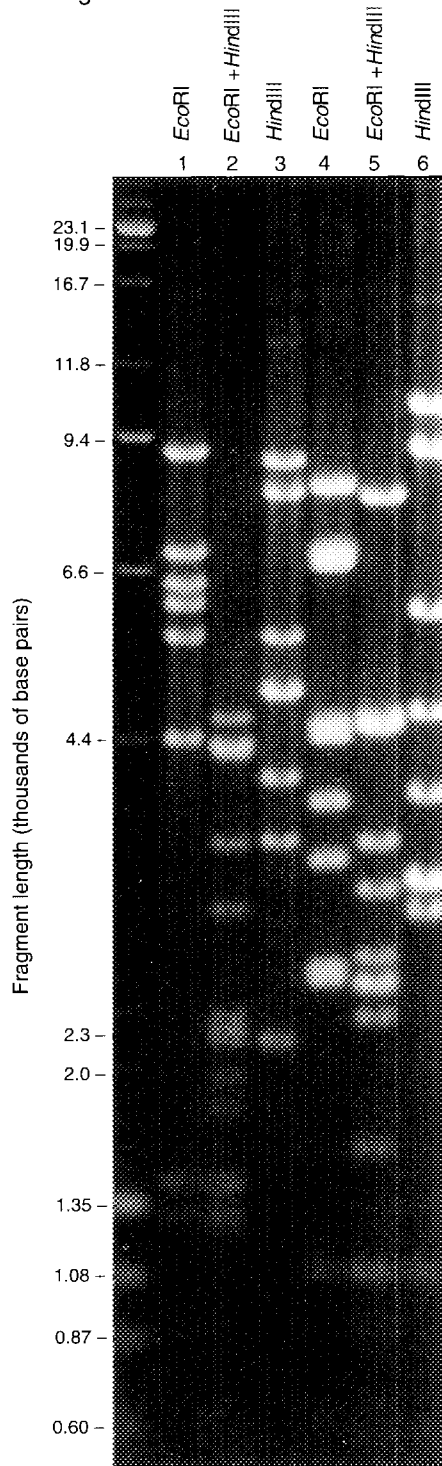
Before a DNA sample is loaded into a well, it is mixed with a dense solution of sucrose or glycerol to prevent the DNA from escaping into the buffer solution. Into one well is

loaded a gel-calibration sample, a sample containing fragments of known lengths. As shown in (a), the flow of electricity through the gel causes the fragments to migrate toward the positive electrode. The shorter fragments move more easily through the gel and therefore travel farther.

The positions of the fragments after electrophoresis can be detected by soaking the gel in a solution of ethidium bromide, which binds strongly to DNA and emits visible light when illuminated with ultraviolet light. In a photograph of the ultraviolet-illuminated gel, the fragments appear as light bands. The ethidium-bromide visualization technique makes the positions of all the fragments in the gel visible. An alternative visualization technique detects only certain fragments (see “Hybridization Techniques”).

The above description of gel electrophoresis might suggest that the sample of DNA contains but one copy of each fragment. In reality the sample must contain many copies of each fragment, and each band seen in the image of the length-separated fragments contains many fragments, all of which have the same length but not necessarily the same sequence.

(b) Conventional Gel Electrophoresis of Fragmented Human DNA Segments



Shown in (b) are the results of conventional gel electrophoresis of six different samples of human DNA. Samples 1, 2, and 3 consisted of the restriction fragments produced by cutting the same cloned segment of human DNA with *EcoRI* alone (a 6-base cutter), with both *EcoRI* and *HindIII* (another 6-base cutter), and with *HindIII* alone, respectively. Samples 4, 5, and 6 consisted of the restriction fragments produced by cutting a different cloned segment of human DNA again with *EcoRI* alone, with both *EcoRI* and *HindIII*, and with *HindIII* alone, respectively. The leftmost lane of the gel contains fragments of the lengths indicated. Note that all the restriction fragments are well resolved.

Shown in (c) are the results of pulsed-field gel electrophoresis of three identical samples, each containing all sixteen of the intact DNA molecules that compose the genome of the yeast *Saccharomyces cerevisiae*. The four longest chromosomal DNA molecules are not resolved; all four are located in the topmost band. The remaining twelve chromosomal DNA molecules, however, are well resolved. The indicated lengths of the resolved DNA molecules were determined from the positions, in the rightmost lane of the gel, of the fragments in a calibration sample. Even longer fragments, fragments with lengths up to about 5 million base pairs, can be separated by increasing the duration of the pulses.

(c) Pulsed-field Electrophoresis of Intact DNA Molecules of *Saccharomyces cerevisiae*

