Department of Botany
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Aim: to study Restriction Digestion of plasmid DNA through photographs

Restriction Digestion is the process of cutting DNA molecules into smaller pieces with special enzymes called Restriction Endonucleases (sometimes just called Restriction Enzymes or RE's). The actual reaction conditions vary from one enzyme to the next, and include temperature, NaCl and/or MgCl2 concentration, pH, etc. All of these variables except temperature are optimized by mixing the enzyme and DNA with a buffer specific for the enzyme of choice. Once the Restriction Digest is completed, agarose gel electrophoresis is performed to separate the digest fragments by size and visualize the fragments and perhaps purify them for further experiments.

Agarose Gel Electrophoresis

Gel electrophoresis is a technique used to separate DNA fragments (or other macromolecules, such as RNA and proteins) based on their size and charge. Electrophoresis involves running a current through a gel containing the molecules of interest. Based on their size and charge, the molecules will travel through the gel in different directions or at different speeds, allowing them to be separated from one another. Using electrophoresis, we can see how many different DNA fragments are present in a sample and how large they are relative to one another. We can also determine the absolute size of a piece of DNA by examining it next to a standard "yardstick" made up of DNA fragments of known sizes. Mobility of a molecule under the influence of an electric field "is determined by its charge, its formula weight, the pore size of the matrix material and the strength of the electric field". The DNA molecules of various sizes (sometimes called DNA fragments) are separated by the molecular sieving action of the pores in the agarose gel. Agarose is used as the matrix in DNAelectrophoresis and The lower the percentage of agarose, the larger the pores.

Detection of Nucleic Acids in Gels:

The most popular method for the visualization of double stranded DNA (or RNA) in agarose gels is the fluorescent dye ethidium bromide . This dye is a planar molecule and intercalates between the stacked base pairs of DNA. The dye absorbs UV light at 300 to 360 nm and emits light at 590 nm in the red-orange region of the visible spectrum. Once the fragments have been separated, we can examine the gel and see what sizes of bands are found on it. When a gel is stained with a DNA-binding dye and placed under UV light, the DNA fragments will glow, allowing us to see the DNA present at different locations along the length of the gel. The number of base pairs in a DNA (or RNA) molecule is determined by comparison to the mobilities of standards. (Ladder/DNA marker).

Besides their size, the electrophoretic mobility of DNA molecules is also significantly affected by their shape. Plasmid DNA migrates differently depending on whether it is circular or linear.

Circular, Single Stranded Plasmid

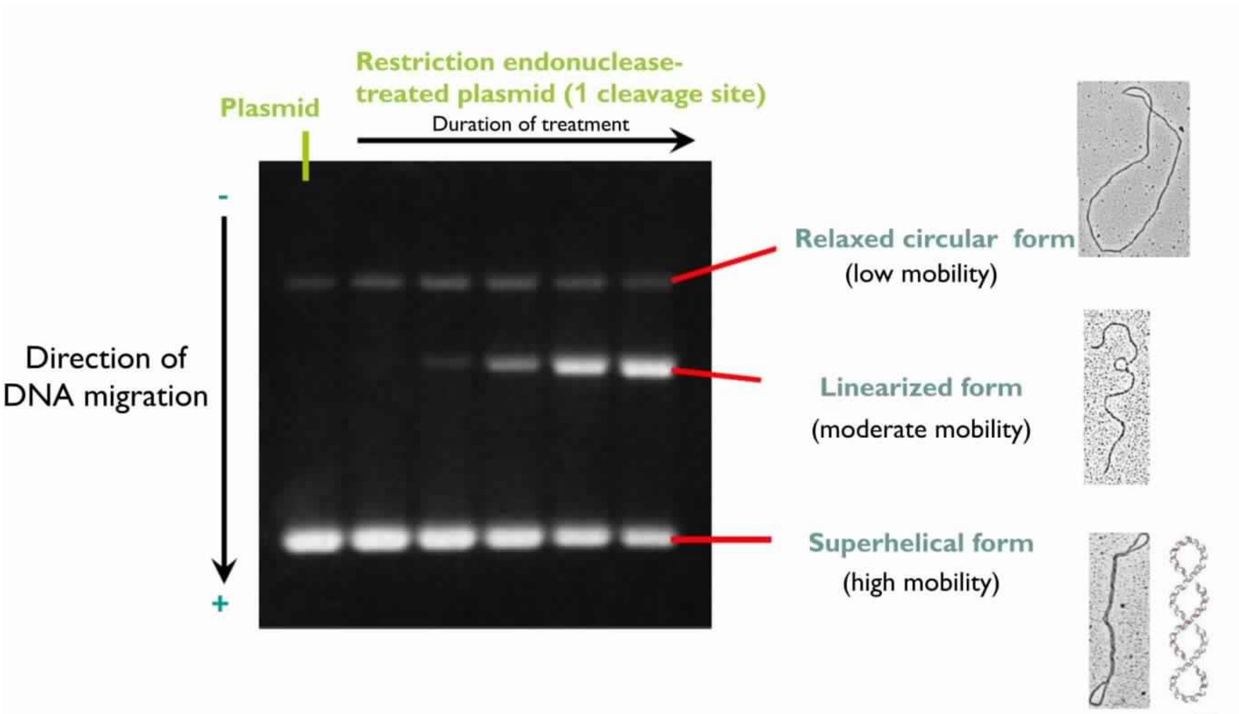
There are primarily two types of circular DNA, closed and nicked. Closed circular DNA has all of its nucleic acids linked with phosphodiester bonds and is supercoiled. Nicked circular DNA has at least one broken phosphodiester linkage. Nicked DNA is sometimes also called relaxed DNA because some of the tension present incovalently closed and superhelically twisted DNA has been released. When one of the strands of the superhelical plasmid DNA is cut, it will adopt a so-called relaxed circular form. The mobility of this form is slower than even that of linear DNA. This happens when the alkaline lysis step is overly harsh (e.g. it is incubated for too long)

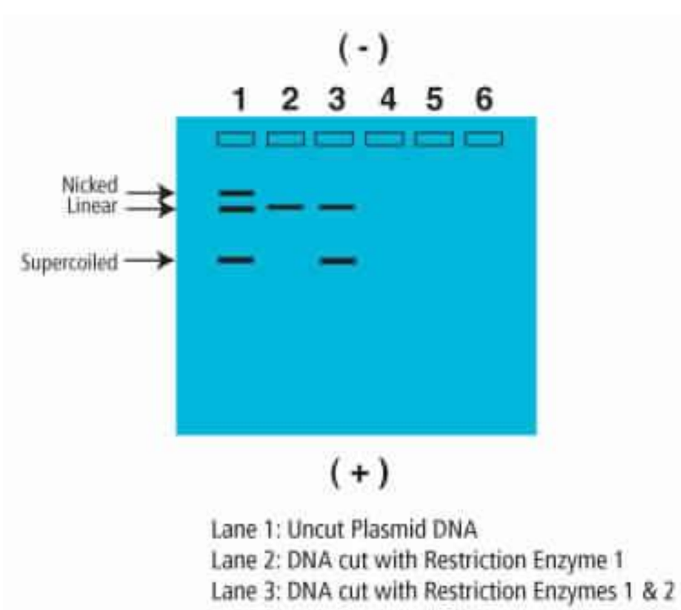
Linear form

Linearized DNA occurs when the DNA helix is cut in both strands at the same place. Linear DNA generally migrates between the nicked circle and the supercoiled forms. Linear DNA almost always migrates more slowly than circular supercoiled DNA. We can identify the linear DNA form on an agarose gel by comparing uncut plasmid DNA with a sample of the plasmid that has been linearized using a restriction enzyme. If you get linear DNA when you are hoping for supercoiled (e.g. after a plasmid prep) it is due to nuclease contamination or harsh treatment during purification.

Supercoiled Plasmid

Supercoiled DNA is the native confirmation found *in vivo* and occurs when extra twists are introduced into the double helix strand. Superhelically packed (Circular supercoiled DNA) is more compact and migrates through the pores more easily. Its hydrodynamic size is much smaller—and its electrophoretic mobility is therefore greater—than that of linear DNA molecules of the same size. . Supercoiled DNA migrates fastest than other forms in agarose gel due to its conformation. Supercoiled DNA is the desired species when isolating plasmid DNA.





Gel electrophoresis of plasmid DNA restricted with 1 restriction

Discussion of the gel picture:

Even though all of these DNA forms have the same number of nucleotides and the same formula weight, they migrate differently during gel electrophoresis.

From the intensity of the various bands appearing in the plasmid preparation run in the left lane, it can be inferred that the preparation largely contains the superhelical DNA form that is most abundant within the cells.

A smaller amount of the plasmid DNA is in the relaxed circular form.

In the other samples, treatment with a restriction endonuclease enzyme that cuts both DNA strands of the plasmid at a single recognition site results in the appearance of the linearised form of the plasmid, which has medium mobility. Some times improper handling or storage of the isolated DNA may degrade, which can be detected as a smear when run on the agarose gel.