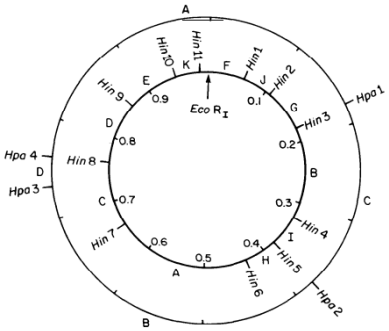
Restriction Enzyme Digest & Gel Electrophoresis

Field Trip Background HS

Background Information

Restriction enzymes are proteins that cut double-stranded DNA at specific recognition sites. They are utilized by bacteria as protection against the invasion of foreign DNA from viruses. In 1968, W. Arber and S. Linn, and then M. Meselson and R. Yuan purified similar enzymes that were able to cut DNA, but these early enzymes cleaved the DNA at random positions. In 1970, H.O. Smith, K. W. Wilcox and T. J. Kelly purified and characterized the recognition and cleavage site of a more useful enzyme, *Hind II*, an enzyme that cuts at a specific recognition site every time. This enzyme was used by Daniel Nathans to cut the circular genome of Simian Virus 40 (SV40) to generate the first **restriction map**. Arber, Smith, and Nathans were awarded a Nobel Prize in physiology or medicine in 1978. The ability to cut DNA at specific sequences became the first step toward molecular cloning.

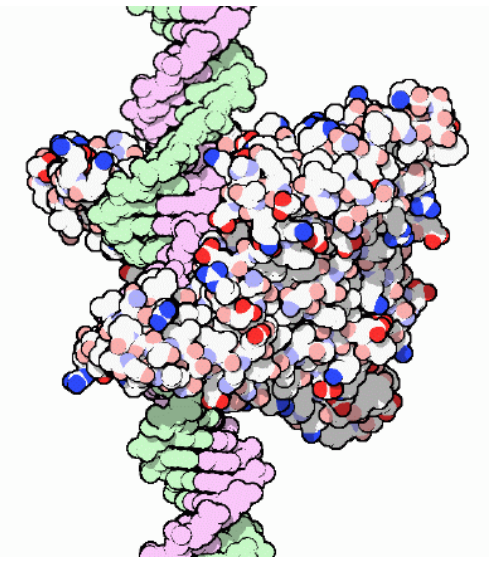


https://www.nobelprize.org/uploads/2018/06/nathans-lecture.pdf

Think of a restriction enzyme cutting DNA like a pair of scissors

Restriction of DNA Using Restriction Enzymes

In the **Restriction Enzyme Digest & Gel Electrophoresis** field trip, students cut plasmid DNA with 4 different restriction enzymes and use gel electrophoresis to visualize the DNA fragments. Molecular biology laboratory skills and equipment, as well as laboratory safety, will be discussed and used in this lab.



PDB Molecule of the Month
https://pdb101.rcsb.org/motm/8

Plasmids are small circular pieces of DNA naturally found in bacteria and some other microscopic organisms. They are separate from the bacteria's larger chromosome. They are relatively small: usually 1000-6000bp and typically have a small number of genes, frequently one associated with antibiotic resistance. Scientists use restriction enzymes to help create plasmids containing a particular gene of interest.

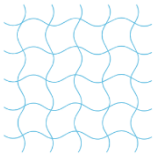
In this lab, students cut a particular plasmid with four different restriction enzymes in the appropriate buffers. This process of enzymatically cutting DNA is known as **restriction digest**. Restriction enzymes act like scissors to cut DNA into pieces. Different restriction

enzymes (and there are hundreds) recognize and cut different DNA sequences. When DNA is cut in a restriction digest, the sizes of the resultant DNA fragments correspond to the distances (in base pairs) between restriction sites. The reaction is incubated at the enzyme's optimum temperature to digest the DNA. This takes at least 30 minutes. During this time students will learn about **restriction enzymes**, **restriction digest** and **gel electrophoresis** in a laboratory which incorporates kinesthetic learning.

Agarose Gel Electrophoresis of DNA

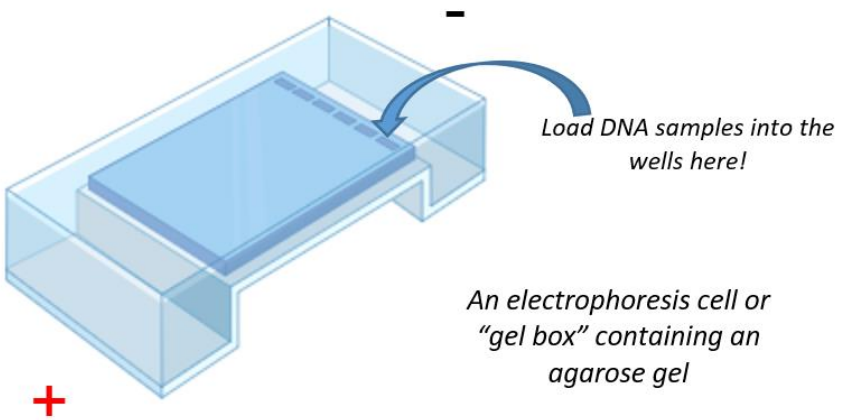
Electrophoresis is a laboratory technique that is used to separate charged molecules from one another based on size and charge. DNA is negatively charged and will move under the force of an electric current through a gel matrix made from the sugar, **agarose**. DNA molecules separate by size when electrophoresed through an agarose gel, with the smaller ones moving more rapidly through the gel than the large ones.

Purified **agarose** is a powder that is insoluble in water (or buffer) at room temperature but melts in boiling water (or buffer). After it's heated, agarose undergoes **polymerization** as it cools, where the liquid agarose sugar molecules solidify into a gel (matrix of agarose). 1% agarose gels in a sodium borate running buffer are used for this field trip, which creates a matrix with pores of appropriate size to separate the DNA fragments produced by the restriction digest of an approximately 5kb plasmid. Molten agarose is cooled to 55°C prior to pouring into casting trays around a six-well comb, and allowing it to solidify (instructors prepare the agarose gel). The comb is removed from the solidified gel to form small pockets called wells.



A matrix of agarose

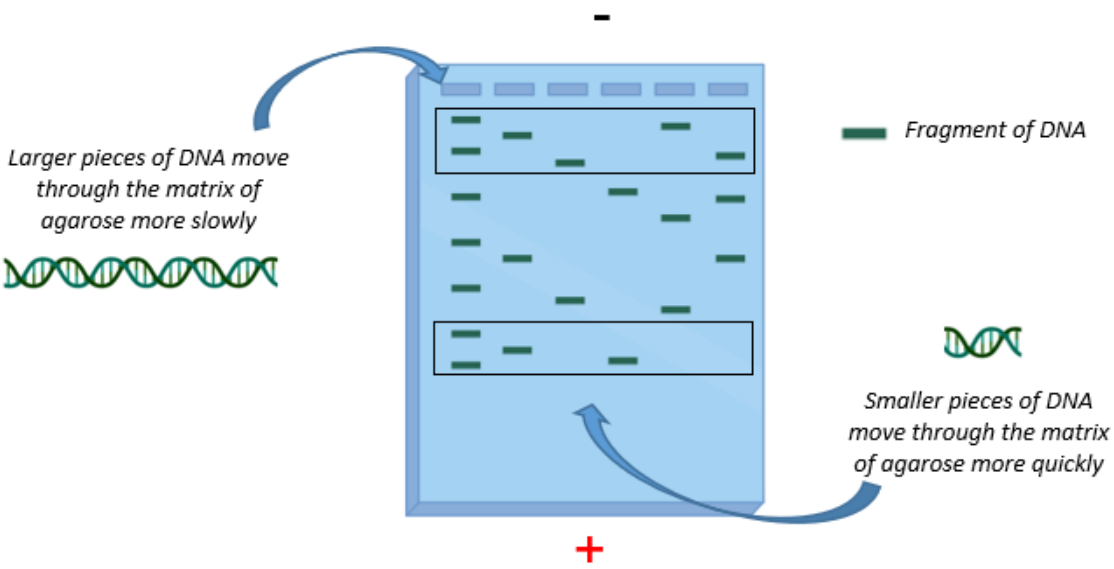
To perform **gel electrophoresis**, an agarose gel is placed into an electrophoresis cell that contains both positive and negative electrodes and a buffered solution (sodium borate) to act as an electrically-conductive medium. Restriction enzyme-digested DNA samples are mixed with a loading dye containing tracking dyes, a thickening agent and a chemical that serves as a DNA stain. The prepared samples are then loaded into the wells of the agarose gel. When the electrophoresis cell is connected to a power supply to establish an electric current, DNA fragments migrate through the agarose gel.



An electrophoresis cell or "gel box" containing an agarose gel

Visualization of DNA in a Gel

The electrophoresis chamber used also contains a built-in short wave blue light. A dye added to the agarose gel binds to double-stranded DNA and fluoresces under this blue light. Students can track the migration of their DNA bands over the course of the lab. A photograph of the gel is taken so that it is easier to study the banding pattern.



If you have any questions or would like more information before you bring your students to the BTCI for this field trip, please contact us. Alternatively, bring your questions along and we can discuss them during the lab. We look forward to seeing you and your group on your scheduled field trip day. Thank you for your interest in the BTC Institute's Biotechnology Field Trips program!

Images for this background obtained from Biorender.com

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