BioPharmaceutical Technology Center
I N S T I T U T E

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 were first proposed in the early 1960's by W. Arber and D. Dussoix to explain the way bacteriophages (viruses that infect bacteria) could infect some strains of bacteria but not others. Restriction enzymes are isolated from bacteria. The bacteria use them as protection against the invasion of foreign DNA. 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, *HindII*, 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**. The ability to cut DNA at specific sequences became the first step toward molecular cloning.

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 lambda bacteriophage DNA with 3 different restriction enzymes and use gel electrophoresis to visualize the DNA. Molecular biology laboratory skills and equipment, as well as laboratory safety, will be discussed and used in this lab.

Lambda bacteriophage DNA is used in this experiment. The isolated DNA is linear, 48,502 base pairs long, and has recognition sites for many different restriction enzymes. It is commonly used for molecular weight size markers in gel analysis of DNA, as well a substrate in restriction enzyme activity assays.

In this lab, students cut lambda DNA with three 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 usually takes about 45 minutes. During this time students will learn about **REs**, **restriction digest** and **gel electrophoresis** in a laboratory which incorporates kinesthetic learning.

Revised December 2019

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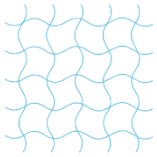
Agarose Gel Electrophoresis of DNA

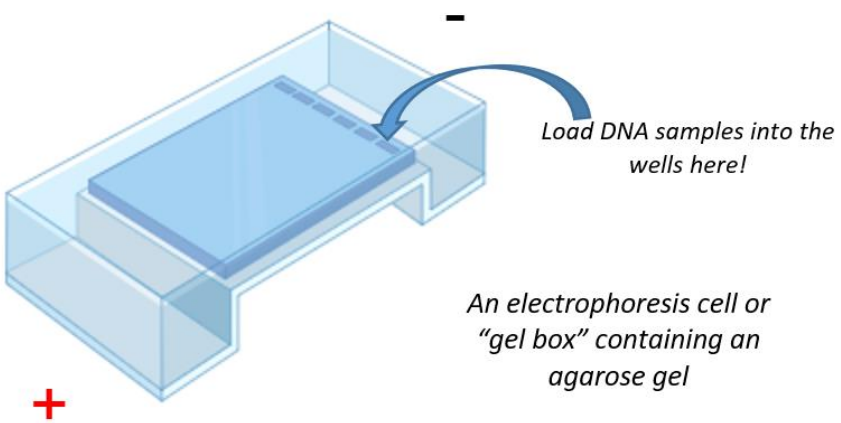
Electrophoresis is a laboratory technique that is used to separate charged molecules from one another based on size and charge. DNA is negatively charged and will move under the force of an electric current through a gel matrix made from the sugar, **agarose**. DNA molecules separate by size when electrophoresed 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). Higher concentrations of agarose produce firmer gels. 0.8% agarose gels in a sodium borate running buffer are used for this field trip. 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.

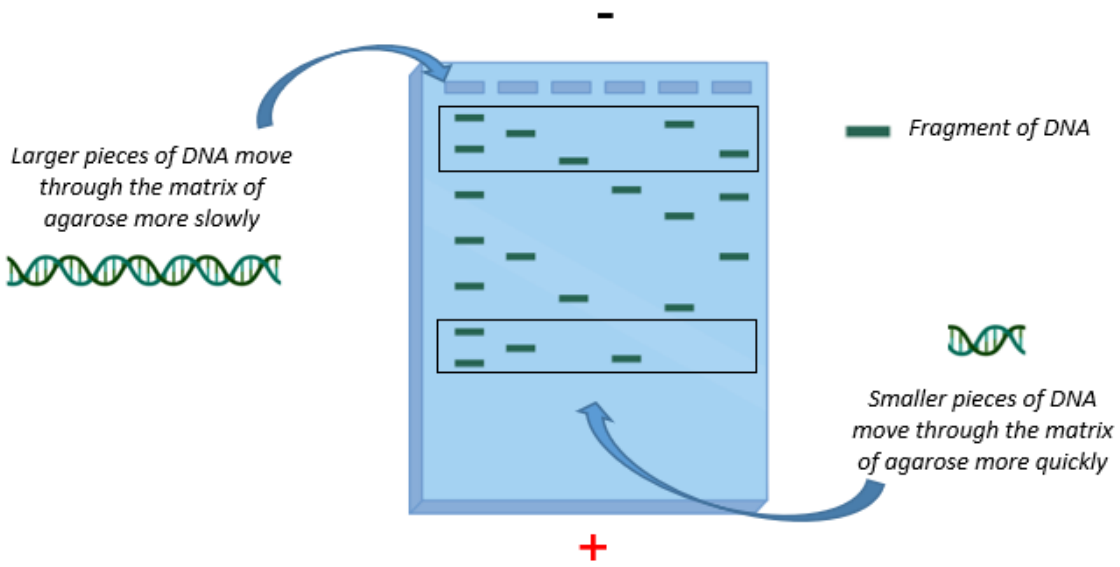


An electrophoresis cell or "gel box" containing an agarose gel

Visualization of DNA in a Gel

After running the gel, DNA is visualized by exposing the gel to a medium-wavelength ultraviolet (UV) light source*. The DNA stain component of the loading dye binds itself to the DNA molecule. When the gel is exposed to UV light, the DNA bands fluoresce in a distinctive pattern of DNA fragments, with one band for each different size fragment. A photograph of the gel is taken so that it is easier to study the banding pattern.

**Students should ALWAYS wear safety glasses while in the lab; ESPECIALLY when using a UV light source.*



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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Revised December 2019