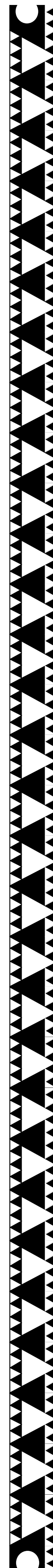

AGAROSE GEL ELECTROPHORESIS OF DNA

Why would anyone want to study DNA? Scientists have learned that the incredible amount of information stored in DNA can answer many questions and solve problems, which affect people daily. For example, the forensic analysis of DNA can help convict (or free) suspected criminals, solve cases of poaching of endangered species, and determine which species of salmon is migrating up the Columbia River. The ability to identify specific bacterial strains using a DNA profile allows meat to be tested for the presence of harmful strains of E. coli, preventing fatal food poisoning. People can be tested for the presence of harmful genes, and someday those genes will be repaired through gene therapy. Biotechnology uses genes to make products humans need for fighting disease. These are all cases in which the analysis of DNA is required.

You will be working on the problem of conserving a species with the help of biotechnology and the analysis of genomic DNA. The species of organism involved is *Loxodonta africana* or the African elephant. Because it is difficult to obtain this elephant DNA for classroom use, you will be using the DNA of a virus named lambda. Since all DNA is composed of the same four nucleotides and lambda DNA is a well-known and readily accessible form of DNA, it provides an excellent model for you to use in this lab. You will be working with a biotechnology technique called gel electrophoresis to analyze your genomic DNA.

Pieces of genomic DNA are too big to analyze on the kind of agarose gel that we (and many other research scientists) are using. Proteins called restriction enzymes are used to cut DNA at specific DNA sequences creating smaller fragments that can be visualized on an agarose gel. Each restriction enzyme cuts DNA at a particular nucleotide sequence acting like molecular scissors. Keep in mind that DNA from any organism can be studied using these same concepts and technologies. The analysis of DNA by gel electrophoresis is a necessary step in many experiments using DNA.

In your lab you will find that some samples (those from known locations) have already been cut by a restriction enzyme and are ready for analysis by electrophoresis. One sample, the model ivory sample, you will have to cut yourself. This sample of genominc DNA will be cut by the restriction enzyme BamHI. BamHI, cuts DNA at the sequence

5′…G\GAT C C…3′
3′…C CT AG\G…5′

This sequence of DNA is called the **recognition site** for BamHI

Whenever BamHI encounters this sequence in a strand of DNA, it cuts, breaking the sugar-phosphate backbone between the two Gs on both strands of the DNA. Each enzyme cuts DNA in a predictable and reproducible manner. Since the early I970s, hundreds of restriction enzymes have been discovered and catalogued according to their recognition sites. Thus, it is possible to choose from a library of these enzymes to cut DNA at chosen sequences.

Restriction enzymes are produced by, and derived from, various bacteria. Their funny names come about from the following rules:

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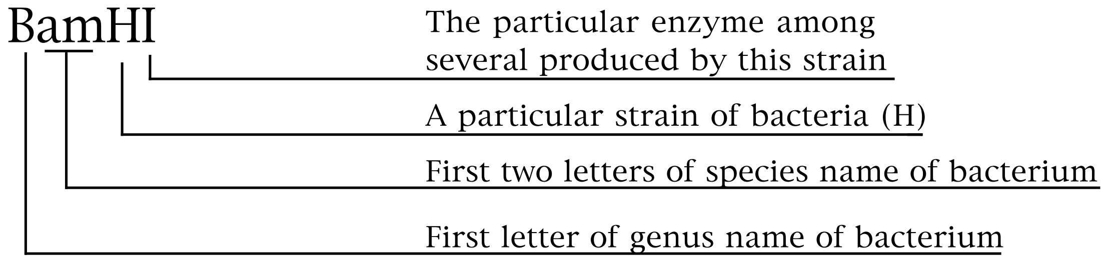
SEP Server:Users:group:*SEP Files:*Uber SEP = Index:Kits:*Kits:Elephant Trunk:Elephant Project V3.0:*EP word docs:ElephantprojectV3.0.doc 1/12/2010 9:18 AM

An enzyme derived from *Bacillus amyloliquefaciens*

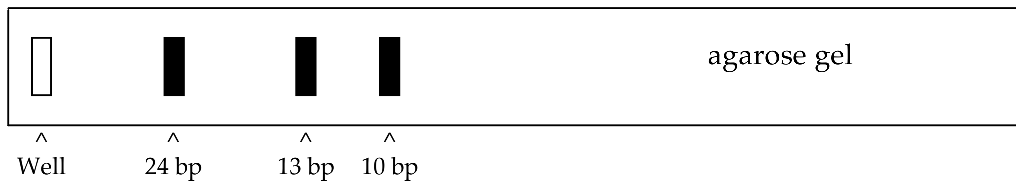


Why do bacteria have restriction enzymes? They use them to disable "alien" DNA of bacteriophages (viruses which infect bacteria). The bacteria give their own DNA a cloak of protection by modifying the recognition sequence DNA in their own genome. Then the restriction enzyme in the cell cuts the unprotected DNA of the invader.

After the DNA is cut by restriction enzymes the pieces are sorted out by size using agarose gel electrophoresis. Gel electrophoresis is an example of a tool that uses the properties of molecules (DNA in this case) to isolate and study them. In this lab, you will see how this process works using genomic DNA samples.

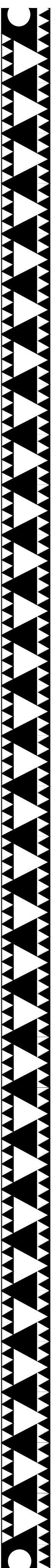
The following diagram is an example of the restriction digest followed by electrophoresis:

ELEPHANT GROUP A

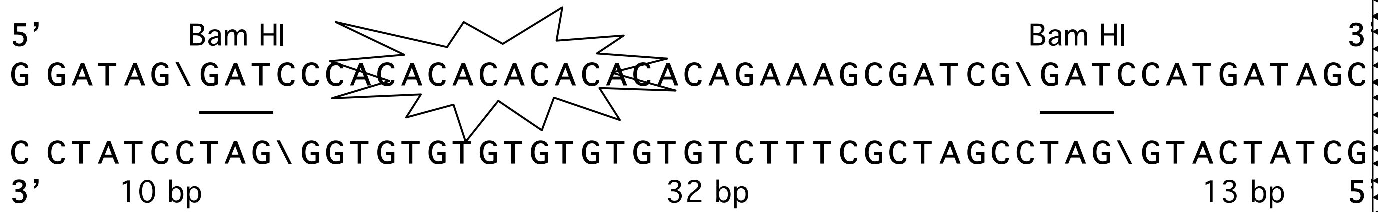


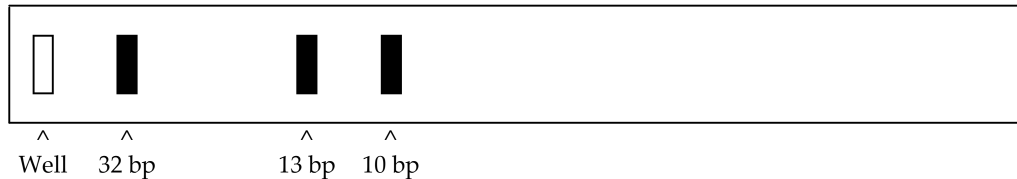
When DNA is cut by restriction enzymes we see a distinct banding pattern on the electrophoresis gel. This pattern represents the different size fragments which have resulted from the digestion process and the different number or recognition sites in the DNA Since most organisms of a particular species have nearly identical genomic DNA, how can you use this technique to identify a specific source of DNA?

One method deals with finding sites that are polymorphic or variable in the organism you are studying. These polymorphic areas usually contain either insertions, deletions or substitutions of nucleotides (A, C, G or T). They can also demonstrate a tandem repeat (AGAGAGAGAG, etc.) These polymorphisms are often called mutations and usually occur in an area of the DNA that does not code for a necessary function of the organism but is passed down to the offspring. See diagram below:



ELEPHANT GROUP B





Thus, different mutations can cause a difference in the fragment lengths of the DNA and this difference is detectable on the gel electrophoresis-banding pattern. Since it is inheritable, offspring can be traced by finding the patterns in family lines.

What do you think would happen to the length of DNA fragments and/or the banding pattern if a mutation occurred at the recognition site of a restriction enzyme? Use the above diagram to explain.