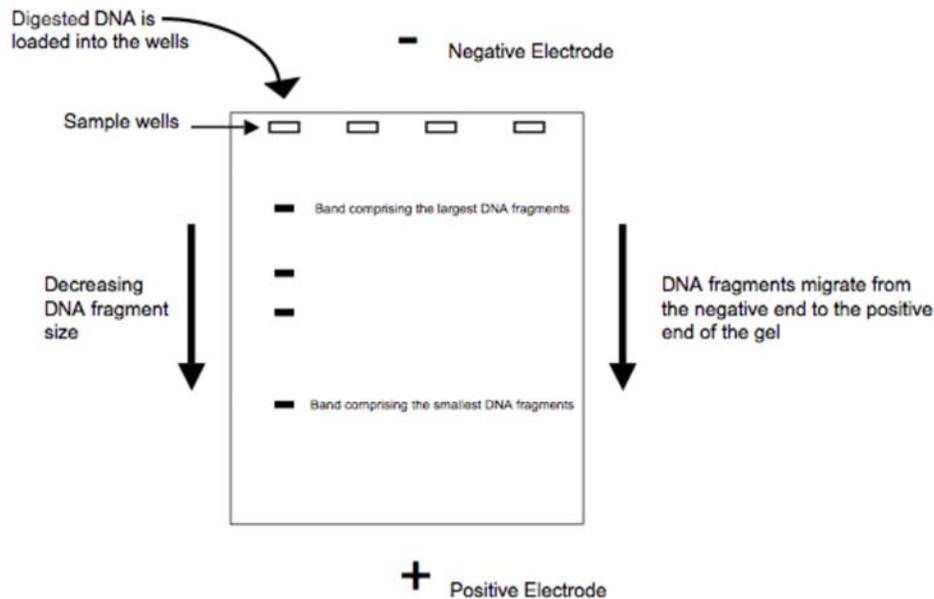


Restoring the American Chestnut

Comparing DNA:

DNA samples can be compared by a method called gel electrophoresis. Gel electrophoresis is a technique used to separate DNA fragments based on size which produces a “fingerprint.” DNA, which has been cut with restriction enzymes, is placed in a gel (looks and feels like clear Jello). The gel is sometimes called a matrix since it contains small holes for the DNA to travel through. If the DNA is too large, then it will not be able to fit through the holes. A restriction enzyme recognizes a particular sequence of bases on the DNA helix and cuts the DNA at that point. The DNA pieces, or fragments, are different sizes and will travel through the gel at different speeds. The smaller the fragment, the faster it can travel and vice versa. (It’s like having a 180 lb quarterback versus a 300 lb quarterback on your football team.) In order to make the DNA run through the gel, electricity is supplied. Gel electrophoresis uses positive and negative charges to separate charged particles. Electricity travels through a buffer solution in the electrophoresis chamber. The buffer conducts electricity allowing the electric current to flow from the cathode (negative electrode) to the anode (positive electrode). (Remember that buffers also maintain the pH of the solution.) DNA is negatively charged; therefore it travels toward the positive electrode. The result will be a pattern of “bands” of DNA that can be compared to each other and a control. Bands in different lanes that end up at the same distance from the top contain molecules that passed through the gel with the same speed, which usually means they are approximately the same size. There are molecular weight size markers that contain a mixture of molecules of known sizes, these serve as the control. If the control is run on one lane in the gel parallel to the unknown samples, the bands observed can be compared to those of the unknown in order to determine their size. DNA is often stained because, by itself, it is colorless and the bands would not be visible.



In this simulation you will examine evidence to determine if a gene is present that may possibly be able to supply resistance to the Chestnut blight (*Cryphonectria parasitica*). You will model the process of electrophoresis and DNA fingerprinting. The control in this case is a segment of DNA from a Chinese Chestnut tree that is known to contain a gene for resistance. Tree 1 is a 7/8 American: Chinese cross, Tree 2 is a 15/16 American: Chinese cross, Tree 3 is a 100% American chestnut. By comparing the DNA fingerprints, we should determine which tree’s DNA segment is most likely to contain a gene for resistance- the tree with the most DNA fragments in common with the control.

Restoring the American Chestnut

Procedure:

1. The first restriction enzyme cuts at every point it finds G A A T C , always cutting between the G and the G. (GA/ATTC) Draw lines on your DNA Sample sheet labeled EcoRI to separate the DNA through both the 5' and 3' strand for the control, tree 1, tree 2 and tree 3. An example is located at the bottom of the page.
2. Count the number of base pairs (bp) in each piece of DNA that you created for each DNA sample. Record the base pair number above each DNA fragment.
3. Place the fragments on the simulated gel by making a dark rectangular mark beside the appropriate bp number in the correct lane for each sample.
4. Compare the chestnut DNA to the control and indicate on your chart, which sample most likely contains the resistance gene.
5. The second restriction enzyme cuts where it finds G G A T T C, always cutting through the G and the A (G / GATTC). Repeat steps 1-4.
6. Compare the two gels to locate the sample that contains the resistance gene to be used for the creation of a transgenic American Chestnut tree.

Analysis:

1. On your chart, label the positive (+) and the negative (-) ends. Circle the tree's DNA that matches the DNA of the Chinese, or resistant, tree.
2. For each of the following tasks performed in the activity, describe what they are actually simulating.

Drawing lines between the DNA base pairs: _____

Making marks on the "gel": _____

3. For each word below, describe how it relates to DNA fingerprinting:

Polymerase Chain Reaction

Gel Electrophoresis

Restoring the American Chestnut

Restriction Enzyme

4. Which tree was most like the control? How do you know and why do you believe this is true:

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Gel for comparison:

Type of restriction enzyme used: EcoRI

Restriction enzyme recognition site sequence:



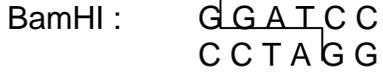
| | Control <input type="text"/> | Tree 1 <input type="text"/> | Tree 2 <input type="text"/> | Tree 3 <input type="text"/> |
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Restoring the American Chestnut

Gel for Comparison:

Type of restriction enzyme used: BamHI

Restriction enzyme recognition site sequence:



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