Genetic Engineering

Among the most important techniques of modern biotechnology are those used to move individual genes from one organism to another. These techniques can move a specific gene among dissimilar organisms that cannot interbreed. For example, genes for cold tolerance can be moved from a fish to a plant. Collectively, the techniques for manipulating and moving genetic material are known as genetic engineering. In agriculture, genetic engineering is often applied as part of a traditional breeding program.

Genetic engineering begins with a gene and a host plant or animal. The end product is a new plant or animal with a gene from another organism, known as a transgenic organism. Eight major steps are required.

1. A source of genes which confer the desired trait is found (usually an organism).
2. DNA is removed from a donor organism cells and cut into fragments.
3. DNA fragments are grouped and sorted by size using electrophoresis, and a fragment containing the desired gene is isolated.
4. The fragment containing the gene is joined to other DNA that makes it possible to move the gene into a new organism.
5. The altered DNA is placed into cells.
6. The transformed cells are developed into a useful transgenic organism.
7. The transgenic organism is grown and tested.
8. The gene is transferred to the progeny.

Many techniques are used to accomplish these steps, including genemapping, DNA sequencing, genetic engineering, immunoassay, tissue culture, field test design, and many others.

The process of finding and selecting genes is critical and difficult. A fragment that contains a gene is minute, and part of a very long DNA molecule. Matching a known trait with a specific sequence of DNA is about like finding a particular straw in a mountain of hay. Nevertheless, a DNA sequence and a trait (or a protein responsible for a trait) can be matched, and the DNA that contains a gene can be isolated. Techniques for accomplishing this are discussed in Lesson 4, Selecting Genes.

A recombinant DNA overview

To be inserted into a host cell, an isolated gene is usually combined with additional DNA from another organism. The combination allows the foreign gene to function, replicate, and be inheritable. Often, the additional DNA is a self-replicating closed loop, called a plasmid, that comes from a bacterium. DNA that is combined from several different organisms is called recombinant DNA.

The process of recombining DNA begins when millions of copies of the original source DNA are
The Genetic Engineering Process

1. Find donor organism and identify genes

2. Remove DNA from donor and cut into fragments

3. Sort fragments by size and locate desired genes

4. Create recombinant DNA

5. Transfer recombinant DNA into cells

6. Grow transformed cells

7. Grow transgenic organism
cut into fragments. The fragments are created using enzymes, called **restriction enzymes**, which cleave the DNA double strand. Restriction enzymes leave special ends on the fragments they produce. The ends, called **sticky ends**, can be spliced to other DNA. The source DNA is cut in many locations, and the resulting fragments are sorted and grouped by size using a process called **gel electrophoresis**. The identity of the gene-containing fragments has been worked out during the process of selecting a gene. Once the fragments are grouped and sorted, the fragments containing the gene can be found and isolated.

The sticky ends of the fragments containing the gene are then spliced to other DNA, often a plasmid that has been cut to produce matching sticky ends. Another enzyme, called a **ligase**, brings the correct sticky ends together and fuses them. The result is recombinant DNA composed of DNA from sources in several different organisms.

**Restriction enzymes**
Restriction enzymes are specialized proteins found in bacteria, which use them as a defense against viruses. Each restriction enzyme recognizes a specific code sequence on DNA double strands, such as the CGAT/GCTA sequence in the example. At the **recognition sequence**, the enzymes break both strands’ phosphodiester bonds.
The hydrogen bonds release, and a fragment is generated. Many restriction enzymes are known which cut at different recognition sequences, and two different enzymes may be used to cut on both sides of a gene. Restriction enzymes offer two advantages in cutting DNA. First, the cut ends are usually “sticky,” so that they can often be rejoined to complementary ends of other DNA fragments that were cut with the same restriction enzyme. Second, the enzymes usually cut at specific, known base sequences on the DNA strand.

**Electrophoresis**
A restriction enzyme cuts the DNA strand at recognition sequences for that enzyme. Many recognition sequences may occur along the strand, so many fragments of different lengths may be generated. The desired gene occurs on only one of these fragments. To obtain fragments that contain the desired gene, the DNA must be grouped and sorted according to size. **Electrophoresis** is the process used to group and sort DNA fragments.

The DNA which has been cut into fragments with a restriction enzyme is called a **restriction digest**. The digest is made with millions of copies of DNA extracted from cells of the same organism, from a culture of bacteria, or from a strain of viruses. After the DNA is digested by the enzyme, the solution contains millions of copies of each size of fragment.

To group and sort these fragments, samples of the restriction digest are placed in wells at one end of an electrically charged gel. The wells are at the negatively charged end. The negative charge repels negatively charged DNA, and the positive charge at the far end attracts it. The charge moves the DNA fragments through the gel.

The gel resists the flow of strands through it, but it does not resist the movement of all strands equally. The larger the strand, the greater the resistance, and the more slowly it travels. The smaller the strand, the farther it travels in a given amount of time. After a time, the groups of short strands migrate to near the far end of the gel, and the groups of long strands remain nearer the wells. Strands of the same size move at the same rate, and so stay together. The end result is an array of fragments that are sorted according to size. This array provides important information about
the DNA that was cut, and grouping the fragments makes it possible to obtain many copies of the desired DNA.

Ligases
Once the gene is cut out, it must be combined with other DNA which will make it viable in the cell. The tool that allows DNA fragments to be recombined is another set of enzymes called ligases. Ligases are enzymes which repair DNA in the cell. Ligases establish a phosphodiester bond between 5' and 3' ends of complementary sequences, such as the CGAT and GCTA sequences in the example below. Fragment ends which were cut by the same restriction enzyme have such complementary sequences.

When fragments with complementary ends are placed in a solution containing a ligase, recombination occurs at random. Many recombinations are possible, and a fraction of these contain the desired recombinant DNA. For example, if one of the fragments with complementary ends was a loop-shaped plasmid which restriction enzymes have opened into a linear piece, ligases can join a new gene to both ends and remake a loop with the new gene in it.

Recombinant plasmids
DNA is often cut and spliced into special DNA molecules that can replicate and function inside the cells. The most commonly used special DNA molecule is a loop called a plasmid.

Plasmids are found naturally in bacteria and some yeasts. They contain a critical base sequence, called an origin of replication, where DNA replication can begin. The origin of replication and several genes allow plasmids to copy themselves inside the cell. When they copy themselves, they also copy genes that have been spliced into them.

Vectors
Recombinant plasmids can ferry a desirable gene into certain kinds of cells under special circumstances. For example, certain bacterial cells can accept and retain plasmids directly through the cell wall. Many kinds of cells, however, cannot accept foreign DNA directly, so other methods of moving DNA into cells are necessary. One method involves using organisms that inject DNA into cells. A biological system for carrying functional DNA into a cell is called a vector. Vectors can be plasmids alone, but they can also be viruses or
certain bacteria. Lesson 4, Gene Transfer, discusses vectors in greater detail.

** Reporter genes

Before scientists try to grow a transgenic plant or animal from transformed cells, they need to be sure the cells do incorporate the new DNA. Unfortunately, transformation is rarely obvious in cells. For example, a fungal resistance trait is not apparent until a plant is propagated and successfully resists fungal attack. Scientists require early evidence at the cellular level to assure that the cells are transformed successfully.

To identify transformed cells, a **reporter gene** may be added to a recombinant plasmid before it is inserted into the cell. When the plasmid replicates inside the cell and the reporter gene begins to function, the cell produces proteins encoded by the reporter gene. Reporter genes code for expression of a protein that produces an observable trait at an early stage, such as the ability to grow in the presence of an antibiotic or herbicide. A reporter gene may also encode a detectable protein the plant does not normally make. The reporter and desired genes are linked, so cells carrying the reporter gene probably also carry the desired gene.

** Reporter Gene**

- Gene which encodes herbicide resistance is isolated
- Resistance gene is inserted into plasmid, along with desired gene
- Plasmid replicates inside bacterial vector
- Bacterium injects herbicide resistance gene into plant cells, along with desired gene
- Only resistant plantlets grow in medium with herbicide—these plants also contain desired gene

**Terms**

- **gel electrophoresis** – an electrical process used to group and sort DNA fragments according to their size. The process uses a low voltage direct current to cause all DNA fragments of the same length to migrate at the same rate. Shorter fragments migrate through the gel more rapidly, and longer fragments migrate more slowly.

- **ligase** – an enzyme that can fuse complementary ends of DNA to form a recombinant molecule.

- **origin of replication** – a specialized base sequence on the DNA strand which enables DNA replication to begin.

- **plasmid** – a loop-shaped DNA molecule, separate from the chromosome, that can replicate inside a cell.
recombinant DNA – DNA formed by combining DNA from several sources.

reporter gene – a gene added to recombinant DNA which gives clear and early indication that an organism has been genetically transformed. Reporter genes commonly code for expression of a protein that produces an easily observable result.

recognition sequence – the DNA code sequence which is recognized and cut by a restriction enzyme.

restriction digest – DNA which has been cut into fragments with restriction enzymes.

restriction enzyme – an enzyme which cleaves DNA at a specific code sequence.

sticky ends – ends of DNA that have been cut with restriction enzymes. Sticky ends will stick to another end with a complementary base sequence, such as an end cut with the same restriction enzyme.

transgenic – having a gene from another non-interbreeding organism.

vector – living microorganisms or specialized pieces of DNA which carry foreign DNA into a cell and allow it to be integrated as a functional unit into a cell.

**Sources and Resources**

Videotapes on Recombinant DNA


**The Web of Life, 1995.** The Smithsonian Institution and WETA-TV. 50 minutes.

**References Works on Recombinant DNA**


**Recombinant DNA Information on the Internet**


The Iowa Biotech Educator – a quarterly newsletter for educators incorporating biotechnology into their curricula, [www.biotech.iastate.edu/ia_biotech_educator](http://www.biotech.iastate.edu/ia_biotech_educator).

National Centre for Biotechnology Education (NCBE) – University of Reading, United Kingdom.[www.ncbe.reading.ac.uk](http://www.ncbe.reading.ac.uk). Information and activities for teachers.

Extracting DNA from Wheat Germ

About this Activity
This demonstration allows students to see DNA and think about some of its properties. The procedure does not relate specifically to recombinant DNA, except that in most cases DNA manipulation requires extracting it from cells. To extract DNA for the purpose of making recombinant DNA, researchers use principles demonstrated in this activity. This procedure extracts enough DNA for students to wrap around a glass rod, to see, and to weigh on an analytical balance.

Objectives
- explore the structure of DNA and its relationship to the cell
- extract DNA from cells.

Relevant Skills Standards
- isolate nucleic acids
- follow protocol
- comprehend a technical vocabulary
- monitor physical properties of a solution
- use basic weighing and measuring techniques
- perform basic separation techniques.

Background Reading
Do this after the class has read Lesson 2, Cells and DNA, and Lesson 3, Recombinant DNA.

Materials/Equipment
Most classes will probably share water bath and balances so the class as a whole will need at least one each of:
- balances accurate to 0.1 g. Results of weighing DNA would be better with a balance accurate to 0.0001 g., so borrow an analytical balance if you can.
- a 55½ C. (131½ F.) water bath or access to water heated to 55½ C. (131½ F.) and maintained at that temperature ±2½ C. (5½ F.) for 10 minutes. A candy thermometer or other 140½ F (60½ C) thermometer can be used to monitor the temperature.
- 50g or more raw (untoasted) wheat germ
- tap water
- a box of baking soda
- 300 ml of 95% ethanol
- a bottle of Palmolive liquid detergent
- a bottle of Adolph's 100% Natural Tenderizer (unseasoned and with papain).
- one 500 ml beaker and one 50 ml graduated cylinder per lab station
- a cooler full of ice
- a box of small plastic spoons
- one glass stirring rod per lab station
- a dozen sheets of filter paper
Suppliers
Your local grocery store (for untoasted wheat germ and Palmolive liquid)
Carolina Biological Supply – 1-800-334-5551, www.carolina.com
Ward’s Biology – 1-800-962-2660, www.wardsci.com
Sigma – 1-800-336-9719, www.sigma.sial.com

Teacher Preparation
The materials and equipment below are for each lab station. Students can help to prepare the lab stations and walk through the activity on the day before the lab.

1. Set up a 55-60° C (131½-140½ F.) water bath. If you do not have a water bath, an alternative is to add hot water to a plastic dishpan of several gallons capacity, maintain several inches of depth, and assign students to carefully monitor and maintain a temperature of 55-60° C (131½-140½ F.) with a thermometer.

2. Just before class, set up ice baths. Separate Styrofoam coolers at each work station will work best, but some teachers use one large central ice bath. Keep the ethanol in the ice bath. It’s important that it be cold.

3. At each lab station, place:
   • at least 3 g raw wheat germ
   • at least 200 ml tap water
   • at least 16.8 g baking soda
   • at least 20 ml 95% ethanol
   • at least 10 ml Palmolive liquid detergent
   • at least 6g Adolph’s 100% Natural Tenderizer (unseasoned with papain).
   • 500 ml beaker
   • 50 ml graduated cylinder
   • ice in a cooler or a large mixing bowl
   • a small plastic spoon
   • a glass stirring rod
   • a sheet of filter paper.

Procedure
Divide the class into teams of about 3-4 students. If your class is small, students can work in pairs. Walk students through the steps before they begin.

Answer Key for Student Questions
1. What percent of the wheat germ weight was the DNA you extracted?
   **Answer:** Amounts vary from 1 - 5%.

2. Draw a picture of a wheat cell (a generic plant cell), labeling all cell parts. Next, using your picture, make a flow chart diagram of the process of extracting the DNA. Include the addition of the detergent, the meat tenderizer, the baking soda, and the ethanol.
   **Answer:** Charts should show the dissolution of the cell membrane with the detergent, the formation of complexes and precipitation of solids with the addition of the meat tenderizer, the shielding of the negative ends of DNA with the sodium ions, and the precipitation of DNA in the alcohol in which it is insoluble.

3. Why did we layer the ethanol on the top, and not mix it into the wheat germ solution?
   **Answer:** DNA is insoluble in concentrated
cold alcohol. Mixing the alcohol into the solution would have diluted the alcohol to the extent that DNA would have become soluble in it, and would not have precipitated.

Related Activities
The Paper Plasmid activity in Recombinant DNA and Biotechnology is the simplest way to understand the basic mechanics of recombinant DNA in a plasmid/bacterium model without conducting a wet lab. This activity is highly recommended.

In addition, students could repeat the activity, but bringing the temperature in steps 4-7 to near or above 60½, and measure the difference in weight of extracted DNA. The higher temperature will denature some or all of the DNA, causing less to be spooled.
About this Activity
To work with DNA, it must first be extracted from a cell and purified. The purpose of this activity is to extract DNA, observe it, and determine what mass of DNA has been extracted.

You will add Palmolive liquid to dissolve the wheat germ’s cell membranes (made of fats). When the membranes dissolve, DNA and other cell contents go into the solution.

A 10-minute heat treatment will form complexes with the detergent, fats, and proteins, causing them to settle out while the DNA stays in solution. Time is of the essence with this step. After 10 minutes at high temperature, too much DNA breaks down, making it more difficult to spool. Watch the time carefully, and never let water temperature reach 60½ C (140½ F). At this temperature, DNA breaks down rapidly. A quick cool-down is also important to save DNA, so swirl the beaker in the ice during cool-down.

The baking soda is added because its positive sodium ions shield the negative phosphate ends of the DNA strands, allowing them to move together and come out of solution.

Cold alcohol is poured over the solution because DNA is not soluble in it. When you float the cold alcohol on top of the wheat germ/detergent solution, an interface or boundary layer forms between the alcohol and the solution. Along that boundary layer, the DNA will come out of solution, or precipitate.

Materials
- at least 3 g UNTOASTED (Raw) wheat germ. Toasted wheat germ will NOT work.
- about 250 ml tap water
- at least 2.1 g baking soda
- ice in a cooler or a large mixing bowl
- 20 ml 95% ethanol (keep on ice)
- at least 10 ml liquid dish detergent
- at least 6 g meat tenderizer (unseasoned with papain).
- one 50 ml and one 500 ml beaker
- 50 ml graduated cylinder
- a plastic spoon
- glass rod
- a sheet of filter paper

Directions
1. On the lab balance, weigh out separately exactly 3.0 g of untoasted wheat germ, 6.0 g of the meat tenderizer, and 2.1 g of baking soda. Keep these weighed amounts in separate weigh boats or on separate pieces of clean paper.

2. Use the graduated cylinder to measure out 25 ml of water, pouring it into the 50 ml beaker. Add the 2.1 g of baking soda, and stir until dissolved.

3. Into the 500 ml beaker, pour 200 ml of hot tap water (but cooler than 55½ C or 131½ F) and place the beaker in the water bath. Be sure the water bath is at 55½ C (131½ F). With the bea-
ker in the water bath, stir in the 3g of wheat germ with the plastic spoon. Make the mixture as smooth as possible.

4. Measure out 10 ml of liquid dish detergent in the graduated cylinder. Check the time and record it, then pour the 10 ml of detergent into the beaker with the wheat germ. Maintain temperature at 55½ C and stir occasionally. Keep the temperature below 60½ C. Higher temperatures denature the DNA.

5. Immediately add the 6 g of Adolph’s meat tenderizer to the wheat germ/detergent solution and add the 20 ml of baking soda solution you made in step 2.

6. Maintain the temperature at 55½ C until exactly 10 minutes from the time the detergent was added (step 4).

7. At exactly 10 minutes from the time you added the detergent, put the beaker in ice and gently swirl to cool down rapidly. Record the time, and leave on ice for exactly 15 minutes. Place the graduated cylinder in the ice to cool it for the next step.

8. Toward the end of your 15 minute wait, measure out 20 ml of cold 95% ethanol in the cold graduated cylinder. Keep in the ice bath until ready. During your wait, carefully weigh and record the exact weight of a single sheet of filter paper (to the limit of accuracy of your balance).

9. After 15 minutes, slowly pour a layer of cold ethanol down the inside edge of the beaker, forming a top layer over the solution. Do not mix the alcohol and solution.

10. Gently push the glass rod through the boundary layer, but don't disturb the wheat germ slurry in the solution below. Slowly turn the rod, moving it in and out of the interface. A white substance will appear and stick to the rod. This is the DNA.

11. Place the weighed filter paper on a clean piece of paper, and as you pull DNA out of the solution, wipe it onto the filter paper. Continue doing so until no more DNA appears at the boundary layer. Allow the filter paper to dry in a clean place where it won't be disturbed. Record your observations of the DNA in your lab notebook.

12. When the filter paper is dry, weigh it with DNA, and subtract the weight of the paper. The remainder is the weight of DNA extracted from 3.0 g of wheat germ. Record the weight in your lab notebook.

Thought/Reflection Questions to Answer in the Lab Notebook

1. What percent of the wheat germ weight was the DNA you extracted?

2. Draw a picture of a wheat germ cell, labeling all cell parts. Next, using your picture, make a flow chart diagram of the process of extracting the DNA. Include the addition of the detergent, the meat tenderizer, the baking soda, and the ethanol.

3. Why did we layer the ethanol on the top, and not mix it into the wheat germ solution?
Ideas for Experiments and Further Research

If you decide to conduct an experiment, follow the guidelines in Appendices C, D, and E: The Scientific Method and Experimental Design, Lab Notebook Contents, and Writing a Research Report at the end of this manual. If you work in a small team of 3–4, record team members’ responsibilities. Presenting the results to the class is an important part of the experiment.

Wheat germ cells were used in this activity, but DNA may be extracted from other kinds of cells. Other protocols may be found in kits from educational suppliers, or at web sites such as Genentech’s Access Excellence, or the University of Reading’s National Centre for Biotechnology Education. Your teacher has a list of references.
About this Activity
The simplest demonstrations of restriction enzymes, electrophoresis, and restriction analysis use pre-cut Bacteriophage lambda DNA. Bacteriophage lambda is a viral parasite of bacteria with a relatively short sequence of 48,502 base pairs comprising about 45 genes. The lambda DNA sequence and recognition sites for many restriction enzymes are known. This activity uses both uncut lambda DNA, and lambda DNA pre-cut with EcoRI and HindIII restriction enzymes. The DNA can be purchased in kits from Wards Biology (catalog # 88-W-8503), Fotodyne (SafeKit 102), Edvotek (Kit 112), or Carolina Biological Supply (catalog # D8-21-1149). All four accomplish the same goals for about the same price. Instructions below reference Carolina Biological Supply’s Restriction Enzyme Cleavage of DNA Kit, but these instructions are useful for any of the kits.

In this activity, students cast a gel, add the uncut and pre-cut DNA to the wells, apply an electric current to the gel, stain and destain, sketch or photograph the resulting pattern of fragments, and clearly label the lanes. Afterward, they compare the gel with a restriction map of lambda DNA and analyze the fragment pattern.

Objectives
- explain the process of restriction analysis, including the function of restriction enzymes, the mechanism of electrophoresis, and the meaning of the pattern of stained fragments.
- interpret a stained gel using a restriction map
- identify specific DNA fragments

Relevant Skills Standards
- perform gel electrophoresis.

Time Required
Two class periods.

Background Reading
Do this after the class has read Lesson 2, Cells and DNA, and Lesson 3, Recombinant DNA. Students should also have a copy of the student worksheet that comes with the kit, and read the whole lab procedure the night before the lab.

Materials/ Equipment
The materials and equipment listed are for the 6-station Restriction Enzyme Cleavage of DNA Kit, Carolina Biological Supply Catalog #D8-21-1149.
- a sink with running water
- 3–6 power outlets (Carolina Biological mini-gel systems use one for every two gel boxes)
- a 60° C. water bath
- one 6-station Restriction Enzyme Cleavage of DNA Kit containing cut and uncut DNA, agarose for casting gels, buffer, disposable micropipettes, stain, and staining trays. Any comparable kit may also be used.
- six electrophoresis chambers, gel trays, with three power supplies (Carolina Biological Supply catalog #D8-21-3650). This mini-gel electrophoresis system provides one power supply and two electrophoresis chambers. Three of these would be required for six lab stations.
Suppliers
Carolina Biological Supply – 1-800-334-5551, www.carolina.com
Edvotek – 1-301-251-5990, www.edvotek.com
Fotodyne – 1-414-369-7000, www.fotodyne.com

Teacher/Student Preparation
Students can help to prepare the lab stations and walk through the activity on the day before the lab. Prior to Day 1, the class will need to:

1. Mix the TBE buffer (the buffer is stable for weeks after making it).
2. Prepare 1% agarose solution. There are two options for preparation:
   a. Prepare agarose in one large batch, make 50 ml portions for lab stations, and place the portions in foil covered flasks in a 55-60½ C. water bath.
   b. Prepare the agarose and cast the gels on the day before the lab, and store the cast gels in TBE buffer for use the next day.
3. Loading the wells requires a steady hand and a little practice. The wells are very small, and the amount of DNA placed in the well is minute. Therefore, some teachers cast practice gels with Jell-O and have students practice loading the gels.
4. Just prior to distributing the DNA vials, concentrate the very small amounts of DNA in them either by spinning in the centrifuge (preferred) or by striking sharply against a bench top.
5. On Day 1, place at each lab station:
   a. one electrophoresis chamber
   b. one power supply (possibly shared with another station)
   c. one gel casting tray, about 16 inches of masking tape, and gel casting comb
   d. 250 ml Tris-Borate-EDTA (TBE) buffer
   e. 50 ml of hot 1% agarose to be used to cast the gel (.5 g agarose, 50 ml TBE buffer) at 60½ C or warmer in a water bath. If gels were precast, distribute the gels on trays in buffer.
   f. 3 plastic transfer pipettes
   g. a small test tube rack for the DNA sample vials
   h. 1 vial lambda DNA
   i. 1 vial lambda DNA EcoRI digest
   j. 1 vial lambda DNA HindIII digest
   k. disposable micropipettes
   l. stain

The gels will run overnight, and on Day 2, students will stain and destain them. Depending on
the kit purchased, staining procedures vary, and in some cases stain will be needed on day 1. At least one sink with running water will be necessary. The class will also need at least one light box (several would be better). The lab stations will need:

- stain
- a water tight plastic bag
- graph paper and a ruler

Procedure

Divide the class into teams of about 3–4 students or as many as needed to fill 6 lab stations. If your class is small, students can work in pairs. Walk students through the steps before they begin.

Answer Key for Student Questions

1. How many fragments are produced when both EcoRI and HindIII are used at once to cut the same DNA? Use the restriction maps or lists of fragment sizes for both EcoRI and HindIII to count the fragments that would be produced. If you have only a list of fragment sizes, draw two parallel restriction maps, one for each enzyme.

**ANSWER:** Students would draw lines between the two parallel maps to produce thirteen fragments. (see diagram below)

2. What fragment sizes are produced when both EcoRI and HindIII are used at once to cut the same DNA? Use the restriction maps or lists of fragment sizes for both EcoRI and HindIII to find out.

**ANSWER:** In base pairs, the fragments would be 21,226; 1,904; 2,027; 947; 1,375; 4,268; 5,151; 564; 125; 1,581; 4,973; 831; 3,530.

3. Often, a dye which is equivalent to a 2,000 base pair DNA fragment is placed in a well before the gel is run. Electrophoresis is halted when the dye reaches the far end of the gel. If this dye were used to time electrophoresis of a HindIII restriction digest, which fragments would definitely not be seen on the gel?

**ANSWER:** The 564 and 125 fragments would be off the end of the gel and not appear. The 2,207 and 2,322 would be crowded together just behind the dye band, and would probably not appear as separate bands.

Related Activities

The Paper Plasmids activity in Recombinant DNA and Biotechnology is an excellent demonstration of recombinant DNA in a bacterium.

Teachers who want to go further can purchase excellent inexpensive kits that let students go through all of the steps. Advanced kits such as Carolina Biological’s E-Z GeneSplicer DNA Recombination and Transformation Kit allow students to transform bacteria with recombinant plasmids and confirm the transformation.

Conducting more complex activities requires microcentrifuges, micropipettes, gel boxes, power supplies, buffers, stains, and other necessities of molecular biology. Reference books such as DNA Science by Micklos and Freyer contain complete instructions. Equipment for a typical classroom may cost from $5,000 - $10,000, so some states have established equipment loan systems for classrooms that cannot afford it.
About this Activity

In this activity, you will use gel electrophoresis to group and sort DNA fragments that have been cut with the restriction enzymes EcoRI and HindIII. The DNA is from Bacteriophage lambda, a virus which exists inside of bacteria. This virus is a simple and extensively studied organism. Your kit contains a map of lambda DNA which shows restriction sites for the two enzymes.

You will cast the gel, add uncut and pre-cut DNA to the wells, apply an electric current to the gel, stain and destain the gel, sketch or photograph the resulting pattern of fragments, and clearly label the lanes. Afterward, you will compare the gel with a restriction map of lambda DNA and analyze the fragment pattern using log graph paper.

Materials Day 1

- 50 ml of hot 1% agarose in a water bath.
- one electrophoresis chamber
- one electrophoresis power supply (possibly shared with another station)
- gel casting tray, masking tape, and gel casting comb
- 250 ml Tris-Borate-EDTA (TBE) buffer
- 3 plastic transfer pipettes
- a small test tube rack for the DNA sample vials
- 1 vial lambda DNA
- 1 vial lambda DNA EcoRI digest

Materials Day 2

- stain
- logarithmic (base 10) graph paper and a ruler
- plastic bag

Directions for Both Days

Follow the directions for the kit you have purchased. The activity in all of the kits asks you to examine your stained gel over a light box and compare it with an ideal shown in your instructions.

The kits also give you a list of the fragment lengths (in base pairs) for the fragments cut by the two enzymes. The DNA fragments migrate through the gel at rates inversely proportional to the log of their molecular weight. Be sure to keep a careful record of which DNA you load into which well. You will record the amount of time the gel has run, and you should have a ruler and a sheet of logarithmic graph paper. With these, you can match the bands on your gels with the fragment lengths listed under each enzyme. In your lab notebook, label each band with its size in kilobase pairs (thousands of base pairs or kbp). For example, label the 9,416 base pair HindIII fragment is 9.416 kbp.
Thought/Reflection Questions to Answer in the Lab Notebook

1. How many fragments are produced when both EcoRI and HindIII are used at once to cut the same DNA? Use the restriction maps or lists of fragment sizes for both EcoRI and HindIII to count the fragments that would be produced. If you have only a list of fragment sizes, draw two parallel restriction maps, one for each enzyme.

2. What fragment sizes are produced when both EcoRI and HindIII are used at once to cut the same DNA? Use the restriction maps or lists of fragment sizes for both EcoRI and HindIII to find out.

3. Often, a dye equivalent to a 2,000 base pair DNA fragment is placed in a well when the gel is run. Electrophoresis is halted when the dye reaches the far end of the gel. If this dye were used to time electrophoresis of a HindIII restriction digest, which fragments would definitely not be seen on the gel?

Ideas for Experiments and Further Research

1. What might you do to better resolve the bands in the gel?

2. Viruses mutate frequently, slightly changing their DNA. Can you design an experiment on paper that would use restriction enzymes (as many as you like) and electrophoresis to search for mutated viruses?