Recombinant DNA Kit

Teacher's Guide

Background:

Genetic recombination involves the production of new individuals which have one set of genes from one parent and a second set of genes from another parent. This is the basic way in which DNA (Deoxyribonucleic acid) is passed from parents to offspring by every living creature. Early civilizations learned to mate animals with desirable features to produce better stock for their purposes, but they didn't know what actually was happening on a cellular level. (They didn't even know what a cell was.) Only within the past century, and to a much greater extent within the past 20 years, has science allowed us to peek into the workings of the DNA that is responsible for those desirable traits.

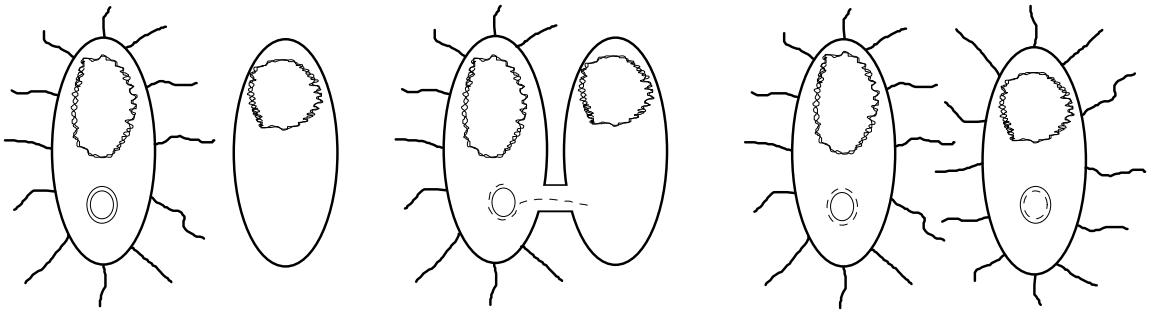
The momentous discovery by James Watson and Francis Crick of the structure of DNA in 1953 was the beginning of a new chapter in our understanding of the way genes control the biochemistry of the cell. However, it wasn't until the early 1970's that "new" organisms were created by manipulating DNA. This controlled movement of genes from one organism to another uses recombinant DNA technology, and is called genetic engineering. Specific traits can be conveyed to the recipient organism, allowing the expression of desirable characteristics or products. For instance, it is now possible to take human genes that control the production of insulin or human growth hormone and transfer them to a bacterial cell like *Escherichia coli* (*E. coli*) so that the cell will then produce the substance. When grown in a test tube, these bacteria will produce large amounts of the material as instructed by the implanted gene. Today, because of genetic engineering, human insulin and human growth hormone are both readily available to those who need it, like diabetics and children born with growth hormone deficiency.

Plants can be implanted with genes that are able to drastically alter their use. Potato plants have been engineered to produce potatoes containing plastic that can be harvested and used for manufacturing purposes. Research has greatly benefitted from recombinant DNA technology. For example, investigators hoping to determine whether organisms are controlled by a "biological clock" ran an experiment recently in which the gene for the production of the firefly enzyme (luciferase) was transplanted into bean plants. When a chemical (luciferin) was sprayed on the plants, which were kept in the dark all the time, the chemical glowed whenever the plants produced the luciferase enzyme. The researchers were able to determine by the varying amount of light produced (the glow turned on and off at set times) that plants do indeed have a biological clock, and they were also able to find the actual gene responsible for these timed patterns.

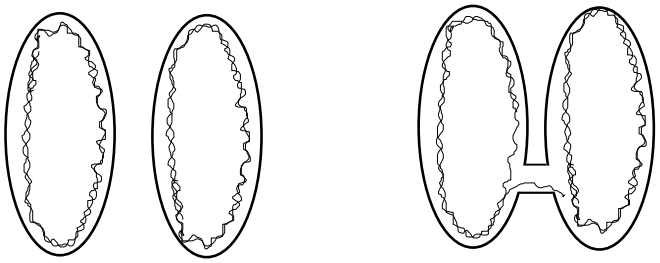
But will genetic engineering affect you directly? It will, even when you may not know about it. If you go to the grocery store and look in the tomato section , you may find a genetically engineered tomato which has had its "rot" gene removed. This extends the life of the tomato after it is picked, and allows it to stay on the vine longer than regular tomatoes (which sometimes have to be picked green in order to remain fresh until they are purchased). Food loss due to disease and pests can be reduced by genetically engineering plants to be resistant to both. We may be able to increase the food supply, and come to rely on recombinant DNA technology for our survival.

There are several different ways for scientists to get DNA into cells. Bacteria can be shocked by either electricity or chemicals in such a way that holes form in their cytoplasmic membrane. DNA can then pass through the holes, and become part of the cellular DNA. The cell is allowed to recover and will start to utilize the new DNA. Another way to insert DNA is to first place it into viruses. The viruses can then be used to infect the targeted cells with the DNA they are carrying. This tends to be the preferred method for plant or mammalian cells because they suffer less damage from viruses than they would from shock.

The idea of transferring DNA is nothing new. Bacteria have passed their genes on to other bacteria for millions of years using a form of sexual reproduction called conjugation. In conjugation, one bacterium forms a cytoplasmic bridge to another cell, and DNA is passed from the first bacterium (the donor) to the second bacterium (the recipient). This DNA can either be in the form of a piece of chromosomal DNA, or plasmid DNA. All bacteria have chromosomal DNA containing essential genetic information. Some of these bacteria also contain smaller, circular DNA called plasmids which are not part of the chromosomal DNA. Plasmids are often involved in producing resistance to antibiotics in bacteria and can easily be transferred by conjugation.



Exchange of plasmid DNA



Exchange of chromosomal DNA

In order to be resistant to an antibiotic, a bacterium needs to have the DNA that allows this resistance. The DNA tells the cell to make enzymes that disrupt the way the antibiotic works, and therefore confers resistance against that antibiotic to the bacterium. An antibiotic resistance gene will only work against a specific type of antibiotic. The gene responsible for tetracycline resistance will not also confer penicillin resistance. Antibiotic resistance genes may be carried in the chromosome, in the plasmid, or in both.

We can demonstrate the principle of genetic recombination through the process of conjugation using antibiotic resistant strains of the bacterium *E. coli*. For this project, you will use special strains of *E. coli*, which are the common bacteria found in your intestines and are therefore known as fecal coliforms. Although the strains that you will use are unlikely to survive and grow outside the artificial environment of the petri dish, precautions should be taken to prevent escape into the outside environment. These will include washing hands with soap, and washing the lab benches with 10% bleach before and after experiments.

Experiment Design:

This kit is designed to acquaint students on a fundamental level with genetic recombination through the process of conjugation between two strains of antibiotic resistant *E. coli*. Given two strains of *E. coli* which have separate types of antibiotic resistance, students will be able to determine that DNA can be transferred between bacteria, and will be able to determine whether plasmid or chromosomal DNA was transferred.

This will be accomplished by first having the students form hypotheses of what will happen when 2 antibiotic resistant *E. coli* strains are placed on growth media containing antibiotics. These hypotheses will then be checked by experimentation on the two cultures using antibiotic media. By comparing their hypothesized results with the experimental data, they should be able to match the experimental *E. coli* strains with the hypothesized *E. coli* strains.

In the second half of the experiment, the students will be given the locations of the resistance genes in each bacterial culture. By answering the questions on the student worksheet (or in a teacher-led class discussion), the students will realize that they cannot at that point yet determine which bacterium was the DNA donor, nor can they determine whether plasmid or chromosomal DNA was transferred. Only further experimentation will answer these two questions. On day 3 of the experiment, the students will evaluate their data and determine which strain was the DNA donor and what type of DNA was transferred.

Materials:

1. **Easygel** media bottles:
   6 units Plain Nutrient (no antibiotic added)
   6 units Streptomycin Nutrient
   6 units Ampicillin Nutrient
   6 units Naladixic Acid Nutrient
   6 units Streptomycin plus Ampicillin Nutrient
2. 30 **Easygel** pretreated petri dishes
3. 18 sterile swabs
4. 2 *E. coli* cultures
   E. coli 1 Resistant to streptomycin.
   E. coli 2 Resistant to ampicillin and naladixic acid.
5. Teacher's Guide
6. Student Worksheet (Make 1 copy for each student)

Not provided:
-Bleach, 100 mL beakers

Procedure for Teachers:

At least 1 hour prior to the class period (or the day before the experiment), the teacher should:

1) Prepare all 30 pretreated petri dishes (you must use the supplied pretreated petri dishes because regular petri dishes will not cause solidification) as follows using a permanent marker or wax pencil:
   Label the tops of 6 of the dishes with a P
   Label the tops of 6 of the dishes with an S
   Label the tops of 6 of the dishes with an A
   Label the tops of 6 of the dishes with an N
   Label the tops of 6 of the dishes with an S+A

2) Pour the **Easygel** from the appropriate bottles into the appropriate plates as follows:
   a. Remove the cap from the bottle, being careful not to touch the neck of the bottle with your hands.
   b. Holding the bottle in one hand, remove the top from the petri dish, and pour the liquid from the bottle into the bottom of the petri dish. Do not touch the inside of the petri dish with anything.
   c. Replace the top of the petri dish, and gently rock and swirl the petri dish so that the liquid covers the bottom.
   d. Repeat with remainder of the **Easygel** and pretreated petri dishes.
   e. Allow the dishes to sit undisturbed for at least 45 minutes while solidification takes place. Pre-poured **Easygel** plates may sit at room temperature until use.

<u>Day 1 of the experiment</u>:

1. Introduce the experiment with a lecture on genetic engineering/recombination.
2. Divide the class into 6 teams. Have the students develop and explain a hypothesis to answer the following question. (This may be assigned as homework to each student separately if desired.) The small superscript r in the strain types stands for resistant.

Given 2 strains of bacteria, strain A^rN^r *(r: resistant to the antibiotics ampicillin and naladixic acid), and strain (resistant to the antibiotic streptomycin), which strain will grow on each of the following media? (Assume the two strains are kept separate while growing so no conjugation occurs.): 1) medium containing no antibiotic 2) medium containing streptomycin and ampicillin 3) medium containing ampicillin 4) medium containing streptomycin 5) medium containing naladixic acid.*

The students should fill out Table 1 based on their hypotheses using a plus (+) for growth and a minus (-) for no growth:

Medium containing:	strain A^rN^r	strain S^r
no antibiotic		
ampicillin		
streptomycin		
naladixic acid		

Medium cont'g:	A^rN^r	S^r
no antibiotic	+	+
ampicillin	+	-
streptomycin	-	+
naladixic acid	+	-

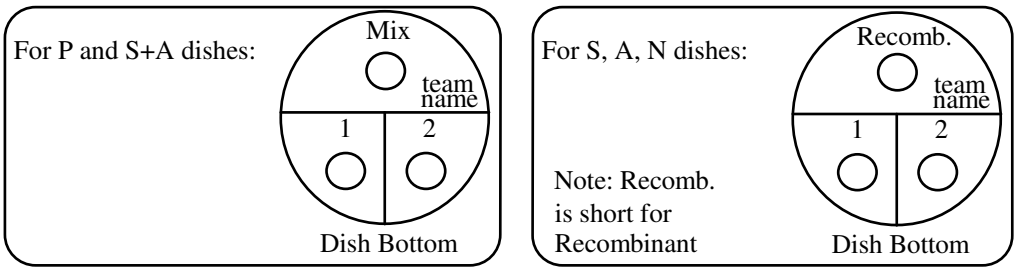
3. Wearing lab coats and gloves if available, teams should wash their work area with paper towels wet with 10% bleach (20 mL bleach in 180 mL H_2O). This cleans the area and helps prevent contamination. (Caution students not to get any bleach on their clothes.) It is important that aseptic technique be used all the way through this exercise.

4. Provide each team with the following

   5 pre-poured **Easygel** dishes:
   - 1 Plain (P)
   - 1 Streptomycin + Ampicillin (S+A)
   - 1 Ampicillin (A)
   - 1 Streptomycin (S)
   - 1 Naladixic Acid (N)

   2 Sterile Swabs

5. Have each team think of a short team name. Using a permanent marker or wax pencil, have students label their petri dishes as follows:



6. Place the *E. coli* cultures in separate areas of the room, so no mix-up occurs. Placing the culture bottles in a 100 mL beaker will reduce the likelihood of a spill. (Do not pour the culture into the beaker.) Have another small beaker containing 10% bleach nearby for swab disposal.

7. Instruct the teams to approach the culture stations one team at a time with their petri dishes in order to inoculate the dishes as follows:

   a. At the first station (*E. coli* 1) remove one sterile swab from its wrapper, touching only the end **opposite** the cotton swab tip.

   b. Dip the swab into the culture material marked *E. coli* 1. Remove excess liquid by pressing the swab against the inside of the culture tube. You will only need to get the swab wet with the culture once.

   c. First gently rub the swab in the circle in the area marked "1" on all petri dishes. When rubbing, **do not press hard**. On the petri dishes labeled P and S+A only, gently rub the swab in the circle in the area marked "mix". It is important that the "mix" section is inoculated last. Dispose of the swab by placing it in the beaker of 10% bleach.

   d. At the second station (*E. coli* 2) the students should remove a new sterile swab from its wrapper and moisten the swab in the culture as in step b. Gently rub the swab in the circle in the area marked "2" on all petri dishes. On the petri dishes labeled P and S+A only, gently rub the swab in the circle in the area marked "mix" so that both 1 & 2 are mixed together. It is important that the "mix" section is inoculated last. Dispose of the swab by placing it in the beaker of 10% bleach.

   e. Place the inoculated petri dishes in an incubator at 35˚C for 24 hours. (Alternatively, put the dishes in a warm place in the room and allow 48 hours before examining.)

   f. Students should again clean off their work areas with 10% bleach, and wash their hands with soap.

Day 2 of the experiment:

   g. After the appropriate incubation time, examine the dishes. In the appropriate space on Table 2 (shown on page 8), record your results using a plus (+) for growth or a minus (-) for no growth.

8. Draw this table on the board and have the students record their results.

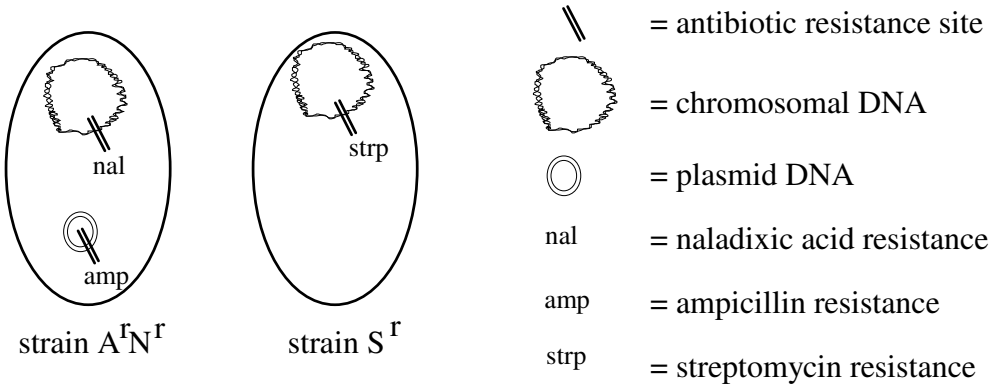
	Team Name			Team Name			Team Name			Team Name			Team Name			Team Name		
E. coli strain	1	2	mix	1	2	mix	1	2	mix	1	2	mix	1	2	mix	1	2	mix
plain (P)																		
streptomycin + ampicillin (S+A)																		
streptomycin (S)																		
ampicillin (A)																		
naladixic acid (N)																		

Do not place this small table on the board.

Expected results:

	E. coli 1	E. coli 2	Mix
Plain	+	+	+
Streptomycin + Ampicillin	-	-	+
Streptomycin	+	-	
Ampicillin	-	+	
Naladixic Acid	-	+	

9. While they are completing their tables, draw the following on the board:



10. Tell the class that the above drawing represents the two *E. coli* cultures that they used in the experiment. The teacher may either lead a class discussion to answer the following questions taken from the student worksheet, or assign the questions as homework.

Match the drawings to the 2 different *E. coli* cultures based on what you have discovered thus far.

***E. coli* 1=_____** **Strain S^r because it grew on the plate with streptomycin and therefore must have had a resistance gene to streptomycin.**

***E. coli* 2=_____** **Strain A^rN^r because it grew on the plate with naladixic acid and ampicillin and therefore must have had resistance genes to naladixic acid and ampicillin.**

Since only one of the strains can be a DNA donor, is there any way to tell which of the two strains was the DNA donor and which was the recipient? Is there any way to determine whether the DNA that was transferred was chromosomal DNA or plasmid DNA? Explain both of your answers.

No. If strain A^rN^r had donated its ampicillin plasmid to strain S^r, the resulting cell would have been resistant to streptomycin and ampicillin. If, on the other hand, strain S^r donated some of its chromosome to strain A^rN^r, the resulting cell would have been resistant to both streptomycin and ampicillin. In either case, the resulting cells are resistant to both streptomycin and ampicillin. Therefore, further experimentation is required in order to determine the DNA donor and DNA transfer type. The two possible resulting cells may be depicted as in these drawings:

1. If A^rN^r donated its ampicillin plasmid to S^r.

2. If S^r donated its streptomycin chromosome to A^rN^r.

11. Provide each team with 1 Sterile Swab.

12. Have each team:

    a. Wash their work area with 10% bleach.

    b. Open the new sterile swab and roll the swab in some of the bacterial growth from the "recomb." section of the S+A dish. Do not "load" the swab with lots of material. A little goes a long way. If too much is placed on the dish, it will be hard to see if new growth has occurred. (The teacher should supervise this aspect.)

c. Gently rub the swab in the circle in the "recomb." sections of the S, A and N petri dishes. Dispose of the swab in the 10% bleach disposal container.

d. Incubate the S, A and N petri dishes again.

e. Students should again clean off their work areas with 10% bleach, and wash their hands with soap.

Day 3 of the experiment:

13. Check the "recomb." sections for growth and record the results on Table 2.

Table 2

	E. coli 1	E. coli 2	Mix	Recomb.
Plain				
Streptomycin + Ampicillin				
Streptomycin				
Ampicillin				
Naladixic Acid				

Expected:

	E. coli 1	E. coli 2	Mix	Recomb
Plain	+	+	+	
Streptomycin + Ampicillin	-	-	+	
Streptomycin	+	-		+
Ampicillin	-	+		+
Naladixic Acid	-	+		-

Study Questions:

a. Which strain(s) of *E. coli* (1, 2 or recombinant) had antibiotic resistance to 2 antibiotics?
E. coli 2 and recombinant.

b. Why did the recombinant strain grow on the S+A dish when neither strain grew alone?
Conjugation between the two original strains allowed the gene for the resistance to ampicillin to be transferred to the streptomycin resistant cells.

c. Which strain was the DNA donor? How do you know?
E. coli 2. The new strain formed by the conjugation of strains 1 and 2 was able to grow on both streptomycin and ampicillin, but not on naladixic acid. If _E. coli_ 1 had been the donor, streptomycin resistance would have been transferred to _E. coli_ 2, and the new strain would have had resistance to all three antibiotics.

d. What type of DNA was transferred, chromosomal or plasmid? How do you know?
Plasmid DNA was transferred because the gene for ampicillin resistance was located on the plasmid.

e. A patient has a blood infection which has been determined to be *E. coli*. When a sample of the patient's blood is spread onto plain nutrient agar, the entire dish becomes covered with the bacterium after the dish is incubated. Another sample is placed on a new dish of nutrient agar, but before it is incubated, three antibiotic disks are placed on separate areas of the dish. One of the disks contains penicillin, the second contains ampicillin, and the third contains neomycin. After the dish has incubated overnight, there is a 15 cm bare area (devoid of bacterial growth) around the ampicillin, a 6 cm bare area around the ampicillin, and the third disk has no bare area around it. There is bacterial growth everywhere except in the bare areas. If you were this patient's doctor, how would you treat the infection? Why?
The best treatment for this specific instance would be to administer ampicillin. The antibiotic in the disk inhibited or killed the bacteria surrounding the disk, and therefore would inhibit growth of the bacteria in the patient. Second best would be the penicillin. The neomycin would probably not have any effect.

Disposal Notes:

After being placed in the bleach solution, the swabs may be washed off in tap water (to reduce the smell) and thrown in the trash. After the experiment is complete, plates and culture bottles may be sterilized by **one** of the following procedures:

a. autoclaved (sterilized) for 15 minutes at 121˚C (250˚F).
b. placed in an oven-proof bag (turkey bag) and heated in an oven at 300˚F for 45 minutes.
c. boiled for 45 minutes.

After the sanitation procedure is complete, the material may be safely thrown in the trash.