To close the yellow note, click once to select it and then click the box in the upper left corner.
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Transformation of Competent Cells with a Recombinant Plasmid

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INTRODUCTION

Description

This exercise demonstrates the use of competent *Escherichia coli (E. coli)* cells in the take-up of plasmids to cause their transformation. The strain used in this exercise is JM83; competent cells may be acquired from UC Davis, or they could be made competent in a previous exercise. These cells are made to take up pUC19, which contains two engineered genes, one for ampicillin resistance (ampicillin-resistance gene) and the other (β-galactosidase gene) to convert X-gal in nutrient agar to a blue color.

A sample of competent cells is made to take up pUC19 with the two genes. When *E. coli* cells with this insert are plated on nutrient agar with ampicillin and X-gal, the resulting colonies will be blue. Another sample is made to take up another pUC19 with a DNA insert (recombinant pUC19). The DNA is inserted in a restriction site located within the β-galactosidase gene. When *E. coli* cells with the recombinant pUC19 are plated on ampicillin nutrient agar with X-gal, the gene that would normally convert X-gal to a blue color is no longer functioning because the DNA insert disrupted the gene. These colonies will be white. (The ampicillin gene is not affected.)

This experiment demonstrates that DNA can be inserted into bacteria to change the properties of the cells. This exercise also demonstrates that colonies can be screened to determine which ones took up a plasmid and, of those that did, which ones contain the recombinant plasmid with the DNA insert.

Student Audience

This activity is easily done at the college level in an introductory biology course for biology majors. However, several local high school instructors use similar recombinant experiments in their biology courses.

Goals for the Activity

Students will

- learn sterile technique,
- understand the properties of DNA and genes as these properties apply to transformation,
- screen for those bacteria with inserted DNA for future experiments, and
- understand the importance of plasmids as a vector to transfer genes of interest.

Recommended Placement in the Curriculum

This exercise works well after the student has been introduced to the basic principles of DNA and is ready to learn how this information can be applied in biotechnology. The field of biotechnology and recombinant DNA can then be discussed.

STUDENT HANDOUT
Transformation of Competent Cells with a Recombinant Plasmid

Purpose

The purpose of this exercise is to

- gain a working knowledge of recombinant techniques,
- understand the principles of screening for a gene of interest,
- understand the function of genes and that genes are found on the DNA molecule, and
- transfer DNA to another organism and therefore change the phenotype of that organism.

Background

You have learned that genes are composed of DNA, which contains the code to determine the phenotype of an organism. In this way an organism can produce the necessary enzymes to carry out the specific functions that allow it to live and reproduce in its environment.

In this experiment we will be using *E. coli* because it is a fairly easy organism to grow and observe. The strain of *E. coli* being used is JM83. This strain has been made competent to take up a small circular DNA called a plasmid. This plasmid contains certain genes that give *E. coli* the ability to perform certain functions not present in its genome (library of genes). When *E. coli* has received this "new" set of genes, it is said to be transformed.

The plasmid being used is called pUC19. This plasmid has been engineered with two genes useful in transformation experiments. One gene (AP^r) confers resistance to the ampicillin antibiotic, and the other gene (β-galactosidase gene derivative) produces a product that can convert a colorless compound, X-gal, into a blue derivative that colors the bacterial colonies blue.

It is interesting to note that pUC19 has many sites within the β-galactosidase gene where DNA from another source can be inserted (multiple cloning sites, or MCS). However, if foreign DNA is inserted within this gene, the gene no longer has the ability to function properly. It is not able to produce a product that converts X-gal into a blue derivative, which in turn results in white colonies. The ampicillin resistance gene is not affected. Therefore, when we see white colonies growing on ampicillin media, we can assume that these bacteria have taken up the recombinant pUC19 (pUC19 with the DNA insert).

E. coli is sensitive to ampicillin and will not grow on nutrient plates in the presence of this antibiotic. *E. coli* with pUC19 is resistant to ampicillin and will grow on these plates. (This shows that DNA equals genes or traits.) *E. coli* with pUC19 will also be seen as blue colonies. Only the recombinants (those that took up pUC19 with a DNA insert) will be seen as white colonies. (Changing DNA changes the properties of the genes.) In this way we can distinguish between *E. coli* with the original wild-type pUC19 and *E. coli* with the recombinant pUC19, which contains the DNA insert. If we wanted to, we could pick up the recombinant *E. coli* to grow separately from the other colonies.

Scenario

Similar techniques are used to create a library of the genome of a particular organism. These techniques can also be used in agriculture and industry to create new organisms with specific adaptations and to create products for medical use.

Pre-Lab Questions

1. What color would this colony be on X-gal medium?

2. Would this same colony also have resistance to ampicillin? Why?

3. What would be a specific agricultural application of recombination?

4. What would be a specific industrial application of recombination?

Materials

Reagents

Per group

- 2, 0.5-mL tubes of competent *E. coli* (200 µL of JM83)
- 0.5-mL tube of pUC19 (10 µL of 100 µg/mL)
- 0.5-mL tube of recombinant pUC19 (10 µL of 100 µg/mL)
- nutrient agar plate
- 5 nutrient agar plates containing 100 µg/mL ampicillin (NA+Ap plates)
- tube of 2% X-gal in dimethylformamide (This solvent is necessary for X-gal.)
- tube of 100 mM IPTG in sterile water
- 95% ethanol in beaker (for sterilizing spreader bar)

Equipment and supplies

- water bath or temperature block at 43°C
- incubator for plate culture (may be done at room temperature, but will take two days)
- glass or stainless steel spreader bar
- P-20 micropipet and yellow tips
- P-200 micropipet and yellow tips
- P-1000 micropipet and blue tips
- 0.5-mL sterile tubes
- crushed ice in beakers
- Bunsen or propane burner
- waste container with 10% bleach solution
- gloves

Safety, Handling, and Disposal

It is your responsibility to specifically follow your institution's standard operating procedures (SOPs) and all local, state, and national guidelines on safe handling and storage of all chemicals and equipment you may use in this activity. This includes determining and using the appropriate personal protective equipment (e.g., goggles, gloves, apron). If you are at any time unsure about an SOP or other regulation, check with your instructor.

When dealing with biological materials, take particular precautions as called for by the kit manufacturer or supplier. Sterile technique must be practiced at all times to prevent contamination of the experiment and to prevent release of organisms and/or chemicals in the lab. Other students use the lab, and we do not want anything to leave the lab that could be dangerous to others or the environment. Wear gloves when doing this exercise.

Dispose of used reagents, gloves, and other materials as instructed by your teacher.

Procedure

The entire procedure must be done with sterile technique. Review and practice this technique before actually doing the lab experiment.

1. Pick up from the front counter:
   - nutrient agar plate
   - 5 nutrient agar plates with ampicillin
   - 2 small tubes of JM83 competent cells (JM83 200 μL)
   - 2 small empty sterile tubes
   - small tube labeled "pUC19 DNA"
   - small tube labeled "pUC19 Recombinant"
   - small tube of X-gal/IPTG mix (300 μL)
   - spreader
   - beaker of ethanol
   - beaker of ice

   KEEP ALL TUBES ON ICE!

2. Place 200 μL X-gal in a plastic tube. Add 50 μL IPTG to the tube and mix the two solutions.

3. Transfer 60 μL X-gal/IPTG mixture to the surface of one of the ampicillin plates. Dip a glass spreader in 95% ethanol, flame to sterilize, and let it cool for a few seconds by touching the agar surface. Use the spreader to distribute the X-gal/IPTG mixture evenly over the surface of the plate. Replace the lid on the plate and invert the plate. Repeat this process for three other plates of ampicillin. You should now have four plates of X-gal/IPTG and ampicillin. (Label them "**X-gal**.")

4. Label one of the tubes of competent cells "**no DNA**." Remove 100 μL of the cells from this tube and place in a sterile tube. Label this tube "**no DNA, Ap**." Return these tubes to the ice.

5. Label the second tube of competent cells "**pUC19**." Remove 100 μL of cells from this tube and place in a sterile tube labeled "**recombinant**." Return tubes to ice.

6. Place 2 μL pUC19 DNA in the tube of competent cells labeled "**pUC19**." Return the tubes to ice.

7. Place 2 μL recombinant plasmid DNA in the tube of competent cells labeled "**recombinant**." Return the competent cells to the ice. You will now have four tubes of competent cells:
   - no DNA
   - no DNA, Ap
   - pUC19
   - recombinant

   Allow these tubes to stand in the ice for 15–20 minutes. This step allows the DNA to stick to the surface of the competent cells.

8. To make the DNA enter the cells, place all four tubes in a 43°C water bath. Allow the tubes to incubate at 43°C for 60 seconds, then remove them to room temperature.

Next you'll transfer the JM83 cells to selective agar plates.

9. Transfer the contents (about 100 µL) of tube 1 ("**no DNA**") to the nutrient agar (NA) plate. Sterilize the spreader as in step 3 and use it to distribute the cells. Label the plate "**NA no DNA**."

10. Transfer the contents (about 100 µL) of tube 2 ("**no DNA, Ap**") to the nutrient agar/ampicillin plate (NA+Ap). Use the newly sterile spreader to distribute the cells. Label the plate "**NA+Ap no DNA**."

11. Transfer 10 µL of cells from tube 3 ("**pUC19**") to one X-gal plate (NA+Ap+X-gal/IPTG) and the rest of the cells (about 90 µL) to another X-gal plate. Use the sterile spreader to distribute the cells. Label the plates "**NA+Ap 10 µL pUC19**" and "**NA+Ap 90 µL pUC19**," respectively.

12. Transfer 10 µL of the cells from tube 4 ("recombinant") to one X-gal plate and the rest of the cells (about 90 µL) to another X-gal plate. Remember that each X-gal plate also had nutrient agar and ampicillin. Use the sterile spreader to distribute the cells. Label the plates "**NA+Ap 10 µL recombinant**" and "**NA+Ap 90 µL recombinant**," respectively.

13. Incubate the plates at 37°C for 12–18 hours to allow growth of colonies. (Your instructor will incubate these plates and ask you to read them during the next lab period).

14. Write down any questions you may have about this lab and bring them up in lecture or the following lab.

You should obtain the pattern shown in Table 1:

Table 1: Expected Results

	NA	NA+Ap	X-gal
no DNA	lawn	no growth	
10 µL pUC19			20– 100 blue colonies
90 µL pUC19			>1,000 blue colonies
10 µL recombinant			20– 100 white colonies
90 µL recombinant			>1,000 white colonies

The "no DNA" nutrient agar plate demonstrates that the cells are viable and can grow in the absence of ampicillin. The "no DNA" ampicillin plate demonstrates that the cells cannot grow in the presence of ampicillin because they do not contain the necessary DNA (gene) to allow them to do so.

Transformation with pUC19 confers the ability to grow in the presence of ampicillin and produce products to convert X-gal to produce blue colonies. The addition of an extra DNA fragment in the recombinant "pUC19 recombinant" does not affect the ability of the recombinant to be ampicillin resistant and therefore grow in ampicillin agar. However, the colonies remain white because, when the extra DNA fragment was inserted, it inactivated the X-gal gene. The bacteria were not able to use that gene, so no blue color resulted.

This exercise demonstrates two fundamental principles of recombinant DNA methods:

- DNA can be inserted into bacteria to change the properties of the cells (ampicillin-resistant colonies).
- DNA fragments can be rearranged to change the genetic properties of the DNA molecules. (Blue colonies caused by pUC19 are converted to white colonies when DNA fragments are inserted into pUC19 DNA.)

Post-Lab Questions

1. Do your plates follow the predicted growth pattern? Explain any variation from the predicted growth pattern.

2. Why was there no growth in the "no DNA" bacteria spread in the nutrient agar with ampicillin? What does this demonstrate?

3. Why were there colonies and not lawn in the plates with ampicillin?

4. If you should have obtained white colonies in the "recombinant" plate, what would account for some blue colonies?

5. Why was it necessary to re-flame the spreader between each inoculation?

Suggested Reading

Bloom, M.V.; Freyer, G.A.; Micklos, D.A. *Laboratory DNA Science: An Introduction to Recombinant DNA Techniques and Methods of Genome Analysis;* Benjamin/Cummings: Menlo Park, CA, 1996.

Micklos, D.A.; Freyer, G.A. *DNA Science: A First Course in Recombinant DNA Technology;* Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1990.

INSTRUCTOR NOTES
Transformation of Competent Cells with a Recombinant Plasmid

Time Required
About 90 minutes are required, plus the incubation time and readout at the next lab/class meeting.

Group Size
A group size of two to four students will work, although the ideal group size is three students (one to read instructions, one to perform manipulations, and one to record).

Materials
Reagents
If the author's instructions call for use of a commercial kit and specific materials provided in that kit, we strongly recommend that you use the recommended materials to attain the desired results. When using a commercial kit, read and follow the instructions provided by the kit manufacturer. The author's procedure provided in this activity is not necessarily intended to duplicate or reproduce the manufacturer's instructions. Rather, the procedure has been provided by the author as a summary of the general steps to follow.

Per group
- 2, 0.5-mL tubes of competent *E. coli* (200 µL JM83) in 0.5-mL tube
- 0.5-mL tube of pUC19 (10 µL 100 µg/mL)
- 0.5-mL tube of recombinant pUC19 (10 µL 100 µg/mL)
- nutrient agar plate
- 5 nutrient agar plates containing 100 µg/mL ampicillin (NA+Ap plates)
- tube of 2% X-gal in dimethylformamide (This solvent is necessary for X-gal.)
- tube of 100 mM IPTG in sterile water
- 95% ethanol in beaker (for sterilizing spreader bar)

Equipment and supplies
- water bath or temperature block at 43°C
- incubator for plate culture (may be done at room temperature, but will take two days)
- glass or stainless steel spreader bar
- P-20 micropipet and yellow tips
- P-200 micropipet and yellow tips
- P-1000 micropipet and blue tips
- 0.5-mL sterile tubes
- crushed ice in beakers
- Bunsen or propane burner
- waste container with 10% bleach solution
- gloves

Safety, Handling, and Disposal
As the instructor, you are expected to provide students with access to SOPs, MSDSs, and other resources they need to safely work in the laboratory while meeting all regulatory requirements. Before doing this activity or activities from other sources, you should regularly review special handling issues with students, allow time for questions, and then assess student understanding of these issues.

When dealing with biological materials, take particular precautions as called for by the kit manufacturer or supplier. Sterile technique must be practiced at all times to prevent contamination of the experiment and to prevent release of organisms and/or chemicals in the lab. Make sure students wear gloves when doing this exercise. Be careful to instruct students in the safe use of flammables such as 95% ethanol. Keep the open beaker of ethanol a safe distance away from the flame of the Bunsen burner (or propane unit) and out of the way so it does not get bumped over. Remove the beaker of ethanol immediately once the spreading procedure is accomplished.

Dispose of used reagents, gloves, and other material according to local ordinances.

Points to Cover in the Pre-Lab Discussion

Discuss the general concept behind recombination and bacteria including DNA, genes, plasmids, and antibiotics.

Mention the importance of sterile technique and give brief instructions on how to use sterile technique. Remind the students to read the procedures before (not just during) the actual experiment. Have them decide what responsibilities are to be assigned to each group member. Stress the importance of following the times for the heat/cold treatment for the take-up of the plasmid.

Instruct the students to remove gloves when leaving the laboratory for a break and replace gloves when returning. Review proper safety and disposal procedures in accordance with local ordinances.

Procedural Tips and Suggestions

This procedure specifies giving students competent cells in the laboratory to save time. However, if more time is available, the steps to make the cells competent could be included in the same lab (especially if it is a three-hour lab). Competent cells should be used within the week or stored at -70°C, otherwise they will slowly lose the ability to take up plasmid.

Ampicillin may be purchased separately and added to cooled agar before it is poured, or it may be added to the cell solution.

Purchasing plates is convenient and saves time but is more expensive.

An inexpensive pipet may be made using a 1-mL syringe and yellow plastic tips. The yellow tips must be cut with a razor blade near the base so that they fit snugly on the syringe tip. The gradations on the side of the syringe are usually in 0.01 mL (10 μL). These could be used to deliver cell mixtures or X-gal/IPTG mixes to the agar plates.

Plausible Answers to Questions

Pre-Lab

1. What color would this colony be on X-gal medium?
   White.

2. Would this same colony also have resistance to ampicillin? Why?
   Yes, because it contains the plasmid with the ampicillin resistance gene still intact.

3. What would be a specific agricultural application of recombination?
   One application would be making bacteria with genes to prevent strawberries from freezing. Another would be to use another vector and cause root cells in a plant to take up the gene for nitrogen fixation, therefore reducing the need for fertilizers.

4. What would be a specific industrial application of recombination?
   One application would be using E. coli *with a plasmid containing the human insulin gene to produce insulin for diabetic patients.*

Post-Lab

1. Do your plates follow the predicted growth pattern? Explain any variation from the predicted growth pattern.
   Student answers will vary.

2. Why was there no growth in the "no DNA" bacteria spread in the nutrient agar with ampicillin? What does this demonstrate?
   These bacteria did not have the plasmid with ampicillin resistance. This demonstrates that the original bacteria used in this experiment do not contain the gene for ampicillin resistance. This is a control.

3. Why were there colonies and not lawn in the plates with ampicillin?
   Not all of the bacteria were able to take up a plasmid. There is an efficiency quotient depending on the strain, method, and type of plasmid. Those that did not take up the plasmid could not grow in ampicillin agar. Each of the few that did take up the plasmid formed a colony.

4. If you should have obtained white colonies in the "recombinant" plate, what would account for some blue colonies?
   Not all of the plasmids actually took up the DNA insert in the procedure used to produce the recombinant plasmids (restriction/ligation with specific restriction enzymes and DNA). Some bacteria could have taken up those plasmids that did not have DNA insert and therefore had a ß-galactosidase gene that was not disrupted and would function normally to convert the X-gal/IPTG to a blue color.

5. Why was it necessary to re-flame the spreader between each inoculation?
   It was necessary to reflame the spreader to avoid contaminating a plate with the bacteria that was spread in the previous plate. If contamination occurred, there would be several "experiments" in the same plate, and the results would not be accurate.

Extensions and Variations

This lab could be taken a step further by having the students produce their own competent cells. Also, a colony could be picked off the plate and cultured, and the plasmid could be purified.

Electrophoresis could be done to analyze the fragment size of the plasmid with the DNA insert (white colony) compared to a plasmid from a colony that did not have the inserted DNA (blue colony).

References

Bloom, M.V.; Freyer, G.A.; Micklos, D.A. *Laboratory DNA Science: An Introduction to Recombinant DNA Techniques and Methods of Genome Analysis;* Benjamin/Cummings: Menlo Park, CA, 1996.

Brown, T.A. *Essential Molecular Biology: A Practical Approach,* Vol. 1; Oxford University: New York, 1991.

Micklos, D.A.; Freyer, G.A. *DNA Science: A First Course in Recombinant DNA Technology;* Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1990.