The Biotechnology Education Company®

EDVOTEK®

Revised and Updated

EDVO-Kit #

300

Blue/White Cloning of a DNA Fragment and Assay of β-Galactosidase

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment is to clone a DNA fragment in the pUC-linker and select colonies that have DNA inserts based on color selection. The experiment is divided into three modules which focus on (1) Ligation, (2) Transformation and selection, (3) Growth of transformants and β-galactosidase assay.

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Table of Contents

Experiment Components

Component Quantities:

Experiment # 300 is designed for 5 groups.

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

None of the experiment components are derived from human sources.

		Storage
Reagents for DNA Ligation		
L1	DNA Vector Linearized with *Eco* RI and DNA Fragments	-20°C Freezer
L2	Control Superhelical Plasmid	-20°C Freezer
L3	T4 DNA Ligase/ATP/Buffer Reaction Tube	Room Temp.
L4	TE Buffer, Sterile	Room Temp
Reagents for Transformation		
T1	Ampicillin	-20°C Freezer
T2	IPTG	-20°C Freezer
T3	X-Gal	-20°C Freezer
T4	Cell Reconstitution Buffer	-20°C Freezer
T5	$CaCl_2$	-20°C Freezer
T6	Sterile Water	-20°C Freezer
Reagents and Cells for Transformation		
•	Vial of Bacterial LyphoCells™ for Transformation	Refrigerator
•	Bottle Ready Pour Agar (sterile)	Refrigerator
•	Bottle Recovery Broth (sterile)	Refrigerator
Reagents β-Galactosidase Assay		
A1	Bottle LB Growth Medium	Room Temp.
A3	Sodium Phosphate Buffer	Room Temp.
A5	Stop Buffer (Na_2CO_3)	Room Temp.
Reagents β-Galactosidase Assay		
A2	Lysozyme	-20°C Freezer
A4	ONPG	-20°C Freezer

Disposable Supplies

- Microtest tubes (0.5ml)
- 1.5 ml microtest (microcentrifuge) tubes
- 1 ml pipets (sterile)
- 10 ml pipets (sterile)
- Petri plates (sterile, 60x15 mm)
- Inoculation loops (sterile)
- Toothpicks

Requirements

- Two waterbaths (37°C and 42°C)
- Microcentrifuge
- Table top clinical centrifuge or floor model centrifuge
- 37°C incubation oven
- Shaking incubator or shaking waterbath
- Automatic micropipets and sterile pipet tips
- Pipet pumps
- Balance
- Microwave or hot plate
- Spectrophotometer (Spectronic 20 or equivalent)
- Autoclave (optional)
- 10 - 125 ml sterile flasks with caps
- 80 - 13x100 mm test tubes
- 80 additional 1.5 ml microtest tubes
- Distilled or deionized water
- Ice

Technical Service Department

Mon - Fri
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Please have the following information ready:

- Experiment number and title
- Kit lot number on box or tube
- Literature version number (in lower right corner)
- Approximate purchase date

Blue/White Cloning of a DNA Fragment and Assay of β-Galactosidase

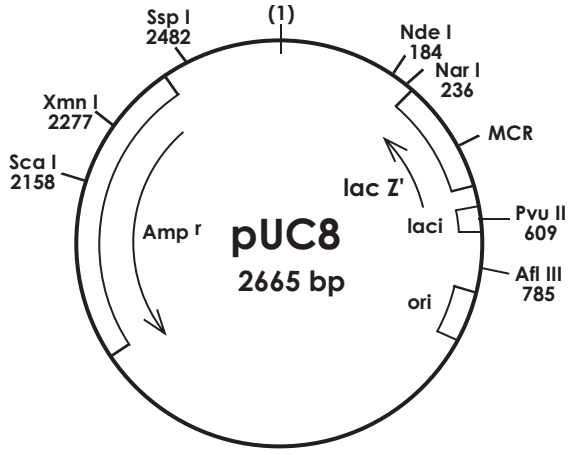
The majority of specialized recombinant DNA molecules used in biotechnology have been constructed by subcloning procedures. Recombinant molecules are vectors designed to meet specific needs in molecular biology research. For example, some vectors have high copy numbers and will produce large amounts of subcloned DNA inserts. Others have been designed to facilitate *in-vitro* transcription and super-expression of proteins *in-vivo*.

Subcloning involves the ligation of a previously cloned and purified DNA molecule into a vector. The resulting recombinant molecule is then introduced into the appropriate host cell where the cloned gene is expressed.

This experiment involves three experimental modules. They are: 1) the ligation of a DNA fragment in a plasmid vector; 2) Introduction of the recombinant DNA into *E. coli* cells by transformation; and 3) selection of ampicillin resistant transformants; selection and growth of Lac^+ and Lac^- colonies; assay of these colonies for β-galactosidase activity. As an optional activity, the recombinant plasmids may be extracted from cells, digested with restriction enzymes, and analyzed by agarose gel electrophoresis (materials not provided).

Plasmid Vector

pUC8 is a 2700 base pair plasmid that possesses a single recognition site for *Eco* RI endonuclease, which is located in a M13 mp derived polylinker in the Lac Z fragment. The polylinker region is approximately 30 base pairs in length and contains several unique restriction enzyme sites to facilitate the ligation of DNA in the vector.



pUC8 is present in multiple copies in host *E. coli* cells. The plasmid has been modified by genetic engineering to contain part of the lac Z gene which codes for β-galactosidase, an enzyme involved in galactoside metabolism.

An operon contains structural genes which carry information for the synthesis of proteins such as enzymes, and regulatory genes that control the expression of structural genes. The lac operon consists of structural and regulatory genes. The lac Z gene is a structural gene required for galactoside metabolism. The pUC8 plasmid carries the alpha fragment of the lac Z gene. The alpha fragment is the amino-terminus of the protein and is not functional by itself. The alpha fragment is denoted by lac Z'.

Blue/White Cloning of a DNA Fragment and Assay of β-Galactosidase

Background Information

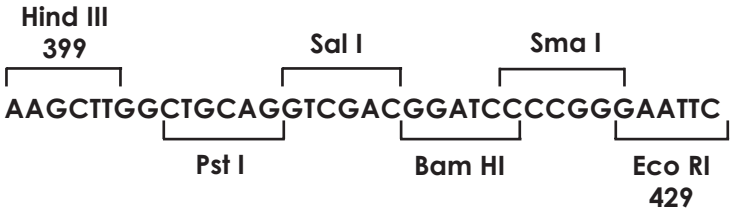
Typically, the *E. coli* host strain used for transformation is a mutant strain that has a deletion of the alpha fragment of lac Z. The *E. coli* chromosome contains the omega fragment, which is the carboxy-terminus of the protein. The omega fragment is also non-functional. When the alpha and omega fragments are expressed, they interact, which results in a functional β-galactosidase protein. This interaction is called alpha complementation. Alpha complementation was discovered by Ullman, Jacob, and Monod in 1967.

The lac operon is highly regulated by repressors and inducers. Repressors are constitutively produced at low levels by a bacterial cell and keep the operon "turned off". When inducers are present, such as isopropyl-β-D-thiogalactopyranoside (IPTG), the repressor protein binds to the inducer instead of the regulatory site in the DNA molecule. Transcription of the structural genes can then occur, followed by translation into a functional protein molecule.

The substrates for the β-galactosidase enzyme are galactosides, such as lactose. Lactose is hydrolyzed into galactose and glucose. Artificial galactosides, such as 5-Bromo-4-Chloro-3-Indolyl-beta-D-galactoside (X-Gal), are also substrates for β-galactosidase. When hydrolyzed, X-Gal will release a blue precipitate, hence pUC8-transformed *E. coli* colonies will be blue. Likewise, ONPG (orthonitrophenalgalactopyranoside) can be used as a colorimetric indicator for β-galactosidase activity. When hydrolyzed, it forms a yellow soluble product which can be quantified with a spectrophotometer.

The pUC8 plasmid has a Multiple Cloning Region (MCR) which is inserted into the lac Z' gene in a way that does not interfere with the lac Z function. The MCR or "polylinker" region is approximately 30 bases long and has several unique restriction enzyme sites which makes it versatile for molecular cloning. Foreign DNA can be inserted into the MCR, which interrupts the lac Z' gene and prevent the formation of a functional β-galactosidase protein. Such recombinants will appear as white colonies on selection plates.

MCR:
Multiple Cloning Region
(only one strand shown)

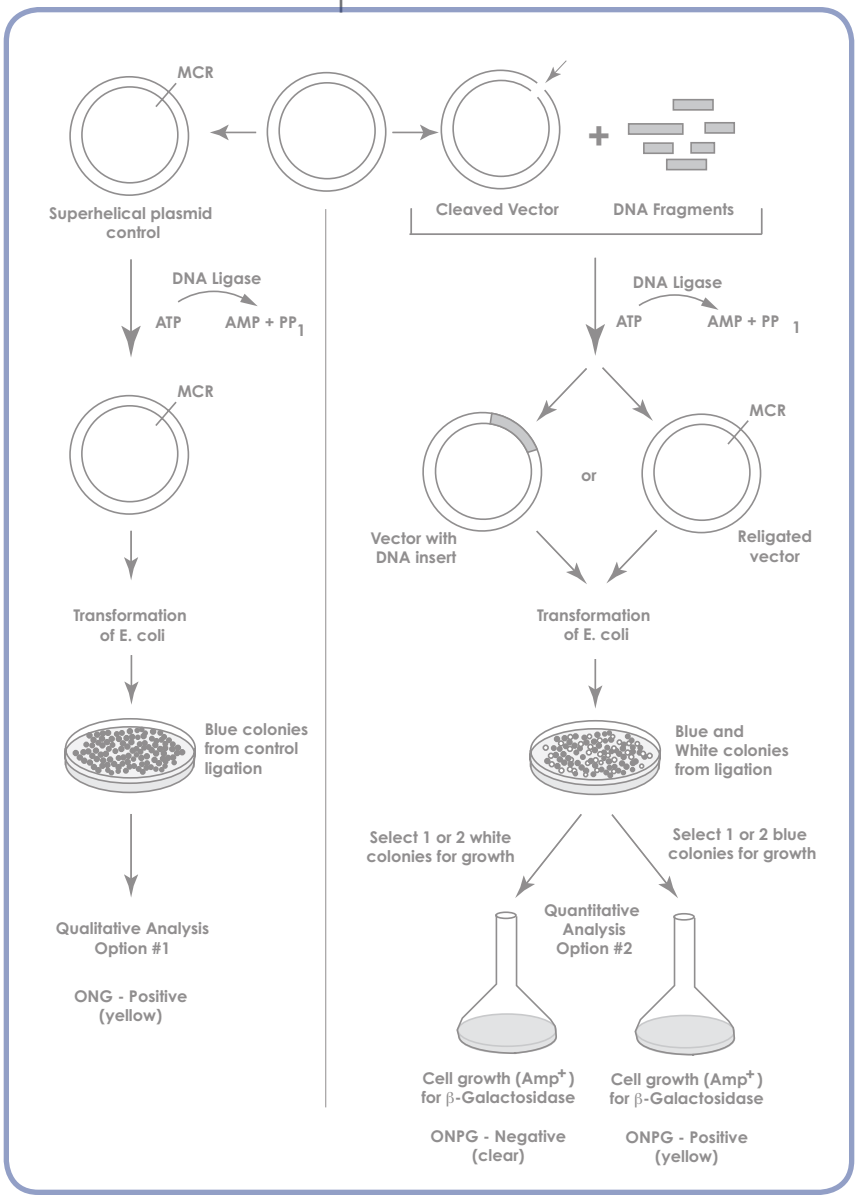


The plasmid also contains an ampicillin resistance gene which codes for β-lactamase. For this experiment, the plasmid has been linearized with *Eco* RI endonuclease to produce compatible termini for the subcloning experiment.

Blue/White Cloning of a DNA Fragment and Assay of β-Galactosidase

Ligation

The ligation of the fragments to the linearized vector will be accomplished by the addition of T4 DNA ligase to a reaction mixture of the cleaved vector and DNA fragments (see flow chart at left). The enzyme catalyzes the formation of phosphodiester bonds by the condensation of a 5' phosphate and 3' hydroxyl groups of adjacent nucleotides occurring at nicks or between cohesive or blunt termini of DNA. The DNA ligase is purified from T4 phage infected *E. coli*. It requires magnesium and ATP. Each phosphodiester bond formation results in the hydrolysis of ATP to AMP plus pyrophosphate. The catalytic efficiency of the enzyme is optimal at 37˚C. However, ligation of DNA fragments having cohesive termini is usually done at temperatures of 4˚C to 22˚C. Lower temperatures allow for annealing between complementary ends of DNA which is a prerequisite for the ligation of cohesive termini.



In the simplest case, ligation of a vector and the insert DNA result in a circular recombinant plasmid. Ligation of the DNA fragments would occur between the guanine 3' hydroxyl group and the adenine 5' phosphate in the *Eco* RI termini. However, the actual stoichiometry of

Blue/White Cloning of a DNA Fragment and Assay of β-Galactosidase

the vector and insert joined in the ligation reaction is a complex function based on the lengths and relative concentrations of the two DNA species. The concentration of enzyme and the ionic strength also have an effect. Due to the complimentarity of the *Eco* RI termini, the vector can undergo reclosure without an insert. At higher concentrations it can form concatamers, i.e., larger linear arrays consisting of repeating units of full length vector. Circularization and concatamer formation can also occur with the insert fragment. Alternative combinations and orientations between the vector and insert can also be envisioned. These multiple forms of DNA appear as complex banding patterns observed during electrophoresis of ligation reaction products.

Transformation of competent *E. coli* cells is very inefficient with linear DNA molecules. Therefore, production of circular molecules should be optimized. Furthermore, large recombinant molecules containing multiple arrays of vector and insert will not replicate efficiently and can complicate analysis. Linearized plasmid vectors are sometimes treated with alkaline phosphatase. This phosphomonoesterase removes the 5' phosphates at DNA termini, yielding a 5' hydroxyl group plus inorganic phosphate. Since ligase requires a 5' phosphate for phosphodiester bond formation, vector reclosure and concatamers are eliminated. In this case, the ligation of the insert in the DNA vector will produce nicks at the annealed junctions since only two instead of four phosphodiester bonds can be formed. The nicks are repaired in the transformed host. Concatamers of the insert can be reduced by lowering the concentration of the insert DNA. Increased yields of circular recombinant molecules can be obtained by adjusting the total DNA concentration and the molar ratio of vector to insert.

When the vector and insert contain the same cohesive termini, the orientation of the subcloned insert can vary between individual bacterial colonies that came from the same transformation experiment. This is due to the symmetrical nature of the termini and, statistically, one would expect to find a 50:50 occurrence of the two insert orientations if many colonies are analyzed. A single insert in the recombinant plasmid can be in either one of two directions relative to a fixed point in the vector.

Transformation

Competent cells were prepared from cultures of *E. coli* JM109. This strain does not have any natural antibiotic resistance or plasmids and lacks restriction enzymes. In addition, the strain does not produce the RecA protein which reduces the possibility of intracellular recombination events. All these features make *E. coli* JM109 an excellent host for cloning and subcloning experiments.

Blue/White Cloning of a DNA Fragment and Assay of β-Galactosidase

Transformation with the ligation reaction products performs several functions. Transformation acts as a purification step since it separates the complex mixture of ligation reaction products into individual bacterial colonies or eliminates some of them entirely. Linear vector and very large concatamers are not taken up well by competent *E. coli*. Supercoiled and relaxed circular DNA have the highest transformation efficiencies. Only small amounts of DNA, typically less than 10 nanograms, is required for transformation. In fact, transformation is inhibited by amounts of DNA exceeding 100 nanograms. Only 1 in 10,000 cells successfully incorporate the exogenous DNA. The uptake of two different molecules of DNA by the same cell during transformation occurs at a low frequency.

Transformation efficiency is defined by the number of transformants obtained per microgram of DNA. For example, if 10 nanograms of DNA was used for transformation in 1 ml of cells and one tenth (0.1 ml) was plated and produced 100 colonies on a selective agar medium, this would equate to1000 transformants per ml. Keeping in mind that each colony grew from one transformed cell, the efficiency would be 1000/0.01µg = 1 x 10^5. Transformation efficiencies of 10^5 to 10^6 are sufficient for most subcloning experiments. When the cloning of single copy genes from genomic DNA is done, the required efficiencies are 10^7 to 10^8.

Selection for Blue/White Colonies

Screening can often be tedious and time-consuming. Plasmid vectors usually contain antibiotic resistance genes that are used for the positive selection of bacteria containing the recombinant plasmid that contains the cloned DNA.

The aim of this experiment is to obtain two types of transformed bacterial colonies: blue and white in the presence of X-Gal and IPTG. The blue colonies contain "self" religated plasmids that do not have DNA inserts interrupting the lac Z gene. White colonies consist of bacteria that carry plasmids that have DNA insert fragments that interrupt the lac Z gene. The selection will be done on ampicillin containing medium.

β-galactosidase will be assayed from Lac^+ transformants (blue colonies that produce the active enzyme). A4-orthonitrophenalgalac-to-pyranoside (ONPG) will be used to assay for β-galactosidase. Upon catalysis, this substrate will form a yellow color. Lac^- (white colonies) will not hydrolyze ONPG and no yellow color will be observed.

Experiment Overview

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. Write a hypothesis that reflects the experiment and predict experimental outcomes.

EXPERIMENT OBJECTIVE:

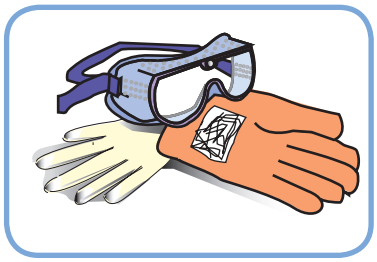
The objective of this experiment is to clone a DNA fragment in the pUC-linker and select colonies that have DNA inserts based on color selection. The experiment is divided into three modules which focus on the following:

I. Ligation of a DNA Insert in the Multiple Cloning Region (MCR) for pUC8 Vector

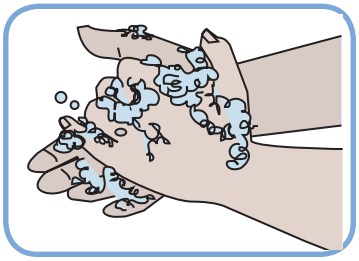
II. Transformation and Selection

III. Assay for β-galactosidase in Blue and White Colonies.

Laboratory Safety



1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment which is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.
4. The *E. coli* bacteria used in this experiment is not considered pathogenic. Although it is rarely associated with any illness in healthy individuals, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with bacteria.
5. Properly dispose materials after completing the experiment:
   A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.
   B. All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with bacteria should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:
   - Autoclave at 121° C for 20 minutes.
     Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.
   - Soak in 10% bleach solution.
     Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.

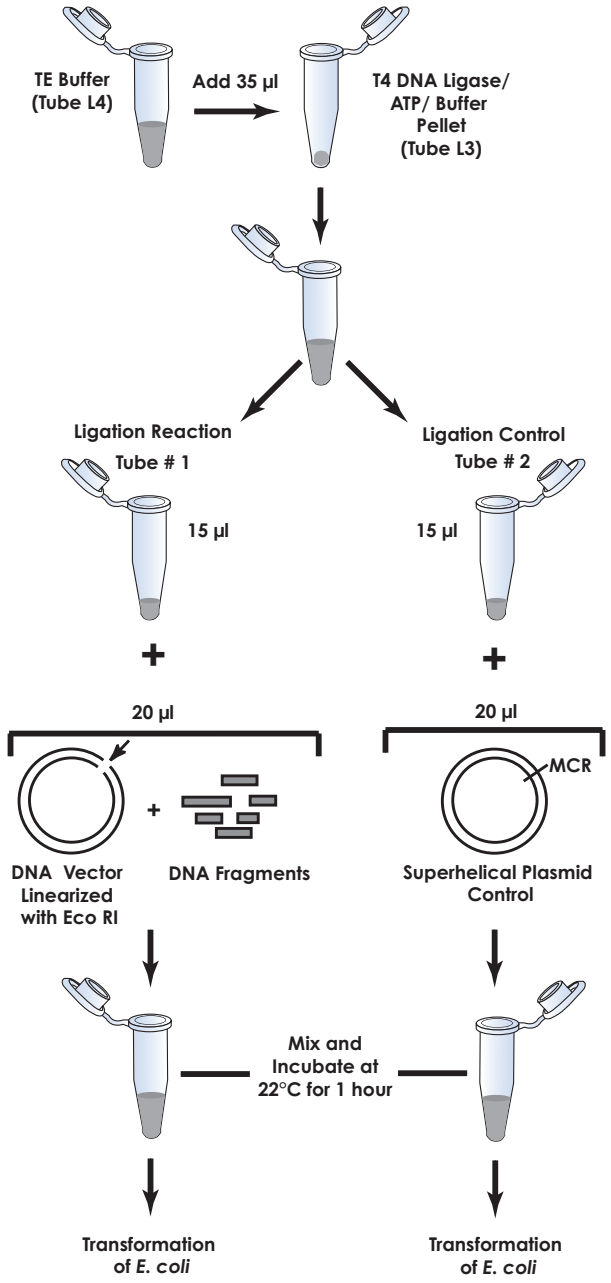


6. Wear gloves, and at the end of the experiment, wash hands thoroughly with soap and water.

The Experiment

MODULE I: Ligation of a DNA Insert in the MCR of pUC8 Vector

MODULE I OVERVIEW



1. Vortex the tube containing the T4 Ligase/ATP/Buffer pellet (L3). Tap it on the lab bench to collect the pellet in the bottom of the tube.

2. Add 35 µl sterile TE Buffer (L4) to the T4 Ligase/ATP Reaction Tube (L3). Allow it to hydrate for five minutes.

3. Carefully stir the mixture with a pipet tip and gently pipet the solution up and down to mix the buffer and ligase.

4. Briefly pulse the tube in a microcentrifuge to collect all of the solution at the bottom of the tube.

5. Label and initial two 1.5 ml microtest tubes:
   - "Ligation"
   - "Control"

6. Aliquot 15 µl of the hydrated T4 Ligase/ATP/Reaction Buffer to the tubes "Ligation" and "Control".

MODULE I: Ligation of a DNA Insert in the MCR of pUC8 Vector

7. To the tube "Ligation" add:

   L1 20 µl DNA Vector Linearized with *Eco* RI and DNA Fragments

   Mix by vortexing or tapping briefly.

8. To the tube "Control", add:

   L2 20 µl Control Superhelical Plasmid DNA

   Mix by vortexing or tapping briefly.

9. Pulse the tubes "Ligation" and "Control" in a microcentrifuge to collect all of the sample in the bottom of the tubes.

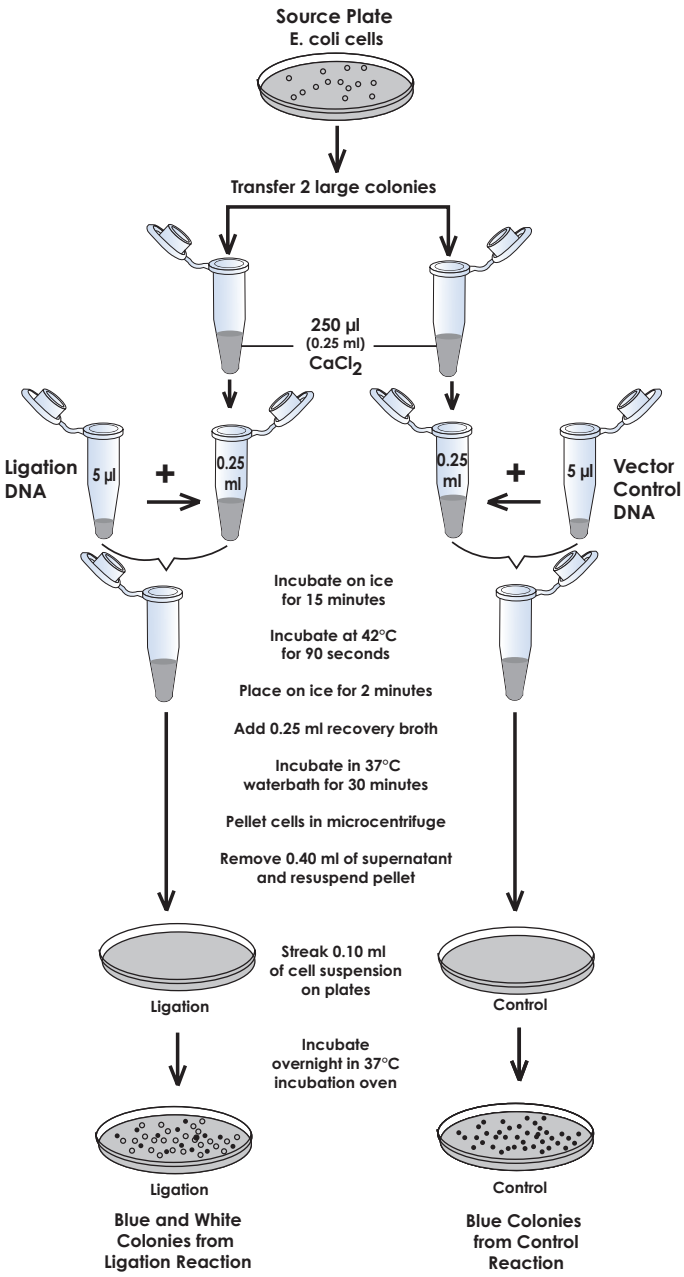
10. Incubate at room temperature (approximately 22°C) for 1 hour. Mix the tubes periodically by tapping or vortexing at 10 or 15 minute intervals.

Optional Stopping Point

Continue with the experiment, or freeze the Ligation and Control tubes until needed for Transformation in Module II.

MODULE II: Transformation and Selection

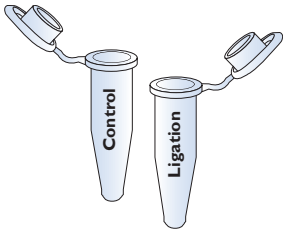
MODULE II OVERVIEW

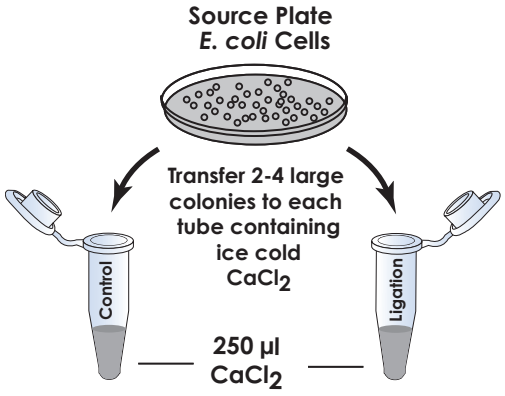


MODULE II: Transformation and Selection

SETTING UP THE TRANSFORMATION AND CONTROL EXPERIMENT

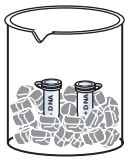
1. Label one microcentrifuge tube "ligation". This will be the transformation tube with ligation DNA.
2. Label a second microcentrifuge tube "Control ". This will be the experimental control with superhelical plasmid DNA.
3. Using a sterile 1 ml pipet, add 250 µl (0.25 ml) of ice cold $CaCl_2$ solution to each tube.





Avoid scraping up agar when transferring the cells from the source plate to the tubes with calcium chloride solution. It is important that the cells are resuspended in the calcium chloride solution and is not left on the toothpick or on the wall of the tube.

4. Pick colonies from the source plate of *E. coli* cells. To each of the test tubes labeled "ligation" and "control":
   - use a sterile toothpick to transfer 2 colonies (2-4 mm) from the source plate to the test tubes.
   - Between your fingers, twist the toothpick vigorously and up and down in the $CaCl_2$ solution to dislodge and emulsify the cells.
5. Suspend the cells in both tubes by tapping or vortexing.
6. Add reaction products from Module 1:
   - To the tube labeled "ligation" add 5 µl of the ligation reaction.
   - To the tube labeled "control" add 5 µl of the control reaction.
7. Incubate the two tubes on ice for 15 minutes.



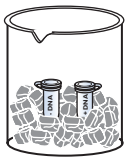
The Experiment

MODULE II: Transformation and Selection

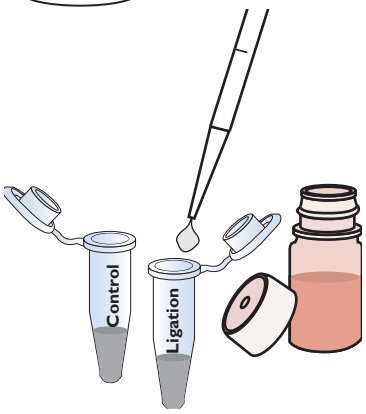
Quick Reference:

DNA and competent cells are combined in a suspension. After the cells have incubated with the DNA, growth medium (recovery broth) is added. Bacterial cells continue to grow through the recovery process, during which time the cell wall is repaired. Cells recover and begin to express the antibiotic resistance gene.

8. Place both tubes in a waterbath at 42°C for 90 seconds for the heat shock step. This facilitates the entry of DNA in bacterial cells.



9. Return both tubes immediately to the ice bucket and incubate for two minutes.



10. Using a sterile pipet, add 250 µl (0.25 ml) of Recovery Broth to each tube and mix.

    The Recovery Broth does not contain antibiotic.

11. Incubate the cells for 30 minutes in a 37°C waterbath for a recovery period.

    This allows the cells to recover and begin to express the antibiotic resistance genes.

12. After the recovery period, remove the tubes from the waterbath and place them in a microcentrifuge and spin for 5 minutes to pellet the cells.

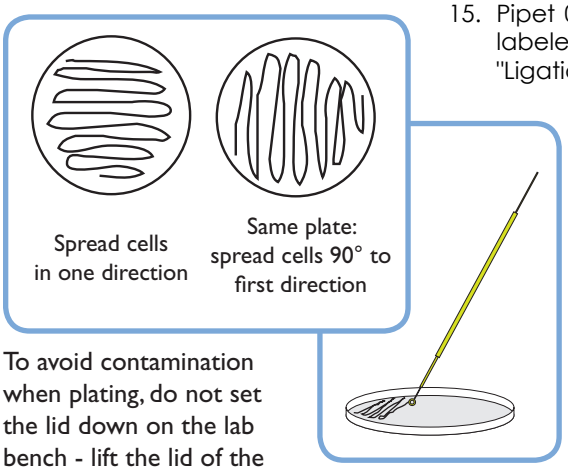
13. Remove and discard 0.40 ml of supernatant and resuspend pellet in remaining liquid.

MODULE II: Transformation and Selection

PLATING THE CELLS

14. Obtain two agar plates ("Ligation" and "Control") and label them with your initials or lab group number.

15. Pipet 0.1 ml of the recovered transformed cells in the tube labeled "Ligation" tube to the center of the agar plate labeled "Ligation".



16. Using a sterile loop, spread the cells evenly and thoroughly over the entire surface. Turn the plate 90° and thoroughly spread again.

17. With a fresh pipet, transfer 0.1 ml of recovered cells in the tube labeled "Control" to the middle of the agar plate labeled "Control".

18. Using a fresh loop, spread the cells over the entire surface of the plate as described.

19. Cover both plates and allow the liquid to be absorbed (approximately 15-20 minutes).

To avoid contamination when plating, do not set the lid down on the lab bench - lift the lid of the plate only enough to allow spreading. Be careful to avoid gouging the loop into the agar.

PREPARING PLATES FOR INCUBATION

20. Stack your group's set of plates on top of one another and tape them together.

21. Put your initials or group number on the taped set of plates.

20. Place the set of plates in a safe place where they will not be disturbed. The plates should be left in the upright position to allow the cell suspension to be absorbed by the agar for 15 - 20 minutes.

21. Place the plates in the **inverted** position (agar side on top) in a 37°C incubation oven for overnight incubation (15-20 hours).

If the cells have not been absorbed into the medium, it is best to incubate the plates upright. The plates are inverted to prevent condensation on the lid, which could drip onto the culture and may interfere with experimental results.

VIEWING PLATES AFTER INCUBATION

22. Proceed to analyzing your results.

23. After analyzing your results, save the plates to pick up colonies for inoculating liquid bacterial cultures. For other materials used in Module II, properly dispose contaminated materials.

MODULE II: Transformation and Selection

DETERMINATION OF TRANSFORMATION EFFICIENCY

Transformation efficiency is a quantitative determination of how many cells were transformed per 1 µg of plasmid DNA. In essence, it is an indicator of how well the transformation experiment worked.

You will calculate the transformation efficiency from the data you collect from your experiment.

1. Estimate the number of transformants (both white and blue colonies) on both plates. A convenient method to keep track of counted colonies is to mark the colony with a marking pen on the outside of the plate.

2. Calculate the transformation efficiencies for total transformants and for colonies that contain vectors with inserts (white colonies).

   The final recovery volume of the cells was 0.50 ml. Because the cells were centrifuged, the volume plated is 0.10 ml. The quantity of DNA used was approximately 25 ng.

   Determine the transformation efficiency using the formula:

$$\frac{\text{Number of transformants}}{\text{µg of DNA}} \times \frac{\text{final vol at recovery (ml)}}{\text{vol plated (ml)}} = \text{Number of transformants per µg}$$

Example: Assume you observed 40 colonies:

$$\frac{40 \text{ transformants}}{0.125 \text{ µg}} \times \frac{1.05 \text{ ml}}{0.25 \text{ ml}} = 1344 \ (1.3 \times 10^3) \text{ transformants per µg}$$

Quick Reference for Expt. 300:

25 ng of DNA is used.

The final volume at recovery is 0.50 ml.

The volume plated is 0.10 ml.

Optional Stopping Point

The plates may be wrapped and stored in the refrigerator for one week.

MODULE III: Assay for β-galactosidase in Blue and White Colonies

GROW LAC$^+$ AND LAC$^-$ CULTURES FOR ASSAY

Four to five hours before the lab, inoculate Lac$^+$ (blue colonies) and Lac$^-$ (white colonies) bacterial culture medium. Alternatively, follow instructions from your instructor.

1. Obtain 2 flasks (125 ml) that contain 25 ml each of sterile LB Growth Medium + Ampicillin.
2. Label one flask Lac$^+$ and the other Lac$^-$.
3. With a sterile inoculating loop, pick several (4 to 6) individual blue transformant colonies and inoculate the flask labeled Lac$^+$. Swirl the flask to suspend bacteria.
4. With a sterile inoculating loop, pick several (4 to 6) individual white transformant colonies and inoculate the flask labeled Lac$^-$. Swirl the flask to suspend bacteria.
5. Incubate cultures with shaking at 37°C for 4 to 5 hours.
6. Check the optical density (OD) at 600 nm. It should be 0.5 to 0.7 by placing 3 ml in a 13 mm x 100 mm glass tube or 1 ml in a cuvette and placing in a blanked spectrophotometer.

Shake loop in broth to allow bacteria to come off the loop and enter the broth.

For cell growth: Use leftover LB + AMP as a blank for OD_{600} absorbance readings.

For β-Galactosidase Assay: Use Distilled H_2O as a blank for OD_{420} and OD_{600} absorbance readings.

INDUCTION OF β-GALACTOSIDASE AND SAMPLING

1. Remove 3 ml from each of the flasks. Retain these samples as the zero timepoint for the β-galactosidase assay. Label the tubes Lac$^+$/ T-0 and Lac$^-$/ T-0. Place them on ice for the assay.
2. Read the OD_{600} and record.
3. To each of the remaining 22 ml of culture, add 22 µl IPTG as the inducer of β-galactosidase activity.
4. Return the cultures to the 37°C shaking incubator for 30 minutes.
5. After 30 minutes, remove 3 ml from each culture and place in a 13 x 100 mm tube. Label the tubes Lac$^+$/ T-1 and Lac$^-$/ T-1. Place them on ice.
6. Read the OD_{600} and record.
7. Return the remaining cultures (19 ml) to the 37°C shaking incubator.

MODULE III: Assay for β-galactosidase in Blue and White Colonies

Optional Steps 8 -10:

8. To obtain better results, after an additional 60 minutes, remove 3 ml from each culture and place in a 13 x 100 mm tube. Label the tubes Lac^+/ T-2 and Lac^-/ T-2. Place them on ice.
9. Read A_{600} and record.
10. Return cultures (16 ml) in flask to the 37°C shaking incubator.

β-GALACTOSIDASE ASSAY

1. Set up the assay tubes by placing six (6) 1.5 ml microcentrifuge tubes in a rack.
2. Label the 6 tubes:

Lac^+ / T0 min	Lac^- / T0 min
Lac^+ / T30 min	Lac^- / T30 min
Lac^+ / T90	Lac^- / T90 min

3. Transfer 2 ml of the cultures on ice from each sample into each respective assay tube.
4. Spin the cells for 5 minutes in a microcentrifuge to pellet the cells.
5. Decant and discard supernatant and save each pellet.
6. Resuspend the pellets in 500 µl phosphate buffer (A3).
7. Freeze the suspension until solid and immediately thaw.

   Cells can be frozen quickly in dry ice or by spreading tubes out (lay flat) in a -20°C freezer. Cells can be thawed at room temperature or by a brief incubation in the 42°C waterbath (just long enough to thaw).
8. Repeat the freezing and thawing a second time.
9. Add 100 µl of lysozyme to each tube and incubate at 37°C for 10 minutes.
10. Add 200 µl of ONPG to each assay tube.
11. Incubate fthe assay tubes or 15 minutes in a 42°C waterbath.
12. Add 0.1 ml of stop buffer (Na_2CO_3) to stop the reactions.

MODULE III: Assay for β-galactosidase in Blue and White Colonies

13. Centrifuge the tubes in a microcentrifuge for 1 minute to pellet the cells.

14. For each tube, transfer the clear supernatant to a clean tube or cuvette and label them appropriately.

15. Use distilled water as a blank. Read OD_{420} and OD_{550}.

16. If reading is higher than 0.8, dilute with distilled water and record the dilution factor.

The reading at 420 nm is the combined absorbance from O-nitrophenol and light scattering by particle materials such as cell debris. Absorbance at 550 nm corrects for light scattering with no contribution from the O-nitrophenol reaction. Light scattering at 420 nm is equal to ($-1.75 \times OD_{550}$).

17. Determine the units of enzyme activity. Units are defined as Miller units based on the equation which follows.

$$\text{Miller Units} = \frac{1000 \times [\, OD_{420} - 1.75 \times OD_{550} \,]}{T \times V \times OD_{600}}$$

where:
- OD_{420} and OD_{550} are read from the ONPG reaction
- OD_{600} is read from the cell culture optical density
- T is the time in minutes of the ONPG reaction
- V is the volume of the cell culture used in the ONPG reaction in mls

18. Disinfect all liquids, medium, plates, and plasticware that has been in contact with bacterial cells by soaking them in 10% bleach overnight or sterilize by autoclaving.

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Why does this cloning experiment yield both blue and white colonies?
2. Do all the white and blue colonies contain a plasmid?
3. Will the lysozyme used to lyse cells denature β-galactosidase?
4. Which restriction enzyme is best suited for cloning in pUC8?

Instructor's Guide

Notes to the Instructor:

IMPORTANT READ ME!

Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicilllin, ampicillin, kanamycin or tetracycine should not participate in this experiment.

ORGANIZING AND IMPLEMENTING THE EXPERIMENT

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students.

Prior to commencing this experiment, carefully check that you have all the necessary experiment components and required equipment. Check the lists of Components and Requirements on pages 3 and 4 to ensure that you have a complete inventory to perform the experiment.

The guidelines that are presented in this manual are based on five laboratory groups. The following are implementation guidelines, which can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are available at the EDVOTEK web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call 1-800-EDVOTEK for help from our knowledgeable technical staff.

This experiment has three modules:

I. Ligation of a DNA Insert in the Multiple Cloning Region (MCR) for pUC8 Vector

II. Transformation and Selection

III. Picking and Growth of Lac$^+$ and Lac$^-$ Transformants

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Please have the following information ready:

- Experiment number and title
- Kit lot number on box or tube
- Literature version number (in lower right corner)
- Approximate purchase date

Instructor's Guide

Notes to the Instructor:

NATIONAL CONTENT AND SKILL STANDARDS

By performing this experiment, students will develop skills necessary to do scientific inquiry, learn new techniques using several types of biotechnology equipment, and will learn standard procedures used in transformation. Analysis of the experiments will provide students the means to transform an abstract concept into a concrete explanation.

APPROXIMATE TIME REQUIREMENTS

1. After setting up the ligation, Module I requires a 1 hour incubation. The experiment can be temporarily stopped after the completion of Module I and later resumed. Experimental results will not be compromised if instructions are followed as noted under the heading "Optional Stopping Point" at the end of the module.

2. Module II includes a 30 minute incubation in a 37°C waterbath. There is also an overnight incubation of plates at 37°C before students can perform Module II and before proceeding to Module III.

3. Module III requires a 4-5 hour culture incubation to allow for growth of transformant colonies. This requires that the instructor or student(s) prepare the cultures for incubation (at 37°C with shaking) prior to induction of β-Glactosidase and sampling, followed by the enzyme assay.

LABORATORY NOTEBOOKS

It is highly recommended that students maintain a laboratory notebook to formulate hypotheses and to record experimental procedures and results.

- EDVOTEK Cat. # 1401, Laboratory DataBook is recommended.
- Guidelines for keeping a laboratory notebook is available at the EDVOTEK web site.

Pre-Lab Preparations - Module I

MODULE I - Ligation of DNA Fragments in pUC8

Enough reagents are provided to perform 5 ligation reactions. You may aliquot the reagents for each lab group as described in step 2.

Alternatively, students can share the stock tubes in a central location.

Note that sharing the tubes increases the risk of a spill or contamination.

1. Shortly before the lab begins, thaw and place on ice:

   L1 DNA vector linearized with *Eco* RI and DNA fragments

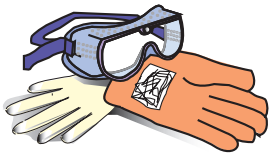
   L2 Control Superhelical Plasmid DNA

2. For each lab group, transfer the following volumes in separate, **ice cold** 0.5 ml microtest tubes that are appropriately labeled.

   - 25 µl L1, DNA vector linearized with *Eco* RI and DNA fragments
   - 25 µl L2, Control Superhelical Plasmid DNA
   - 50 µl L4, TE Buffer

3. Keep all of the tubes on ice.

Pre-Lab Preparations - Module II



Wear Hot Gloves and Goggles during all steps involving heating.

MODULE II - Introduction of DNA in *E. coli* Cells by Transformation and Selection of Transformants

Pour Agar Plates (prior to the Lab experiment)

For optimal results, prepare plates two days prior to plating and set aside the plates inverted at room temperature. If they are poured more than two days before use, they should be stored inverted in the refrigerator. Remove the plates from the refrigerator and store inverted for two days at room temperature before use.

Heat the ReadyPour™ Medium

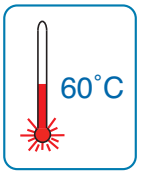
1. Thaw the X-Gal solution (T3) and Sterile Water (T6).
2. Add 0.75 ml (750 µl) Sterile Water (T6) to the tube containing Ampicillin (T1). Vortex or shake vigorously to dissolve the powder and place on ice.
3. Add 0.70 ml (700 µl) Sterile Water (T6) to the tube containing IPTG (T2). Vortex or shake vigorously to dissolve the powder and place on ice.
4. Equilibrate a water bath at 60°C for step 8 below.
5. Loosen, but **do not** remove, the cap on the ReadyPour medium bottle to allow for venting of steam during heating.

   Caution: Failure to loosen the cap prior to heating or microwaving may cause the ReadyPour medium bottle to break or explode.
6. Squeeze and vigorously shake the plastic bottle to break up the solid agar into chunks
7. Heat the bottle of ReadyPour medium by one of the methods outlined below. When completely melted, the amber-colored solution should appear free of small particles.

   A. Microwave method:
   - Heat the bottle on High for two 30 second intervals.
   - Using a hot glove, swirl and heat on High for an additional 25 seconds, or until all the medium is dissolved.
   - Using a hot glove, occasionally swirl to expedite melting.

   B. Hot plate or burner method:
   - Place the bottle in a beaker partially filled with water.
   - Heat the beaker to boiling over a hot plate or burner.
   - Using a hot glove, occasionally swirl to expedite melting.

Pre-Lab Preparations - Module II



8. Allow the melted ReadyPour medium to cool. Placing the bottle in a 60°C water bath will allow the agar to cool, while preventing it from prematurely solidifying.

   When the ReadyPour™ medium reaches approximately 60°C, the bottle will be warm to the touch but not burning hot.

9. While the ReadyPour medium is cooling, label a total of 15 petri plates. Label these plates on their bottom halves:

   5 plates: Source plates
   5 plates: Ligation
   5 plates: Control

After the ReadyPour medium has cooled:

10. Pour the 5 source plates 8 ml each (See Quick Reference: Pouring Agar Plates).

Add ampicillin, IPTG, and X-Gal to medium which has been cooled. Hot medium will cause rapid decomposition of ampicillin.

11. Add 0.30 ml of ampicillin (T1), 0.30 ml of IPTG (T2), and all of the X-Gal (T3) to the molten medium with sterile 1 ml pipets. Swirl the medium to mix. Return the remaining ampicillin and IPTG to the freezer for Module III.

12. Pour the remaining plates, 8 ml each. (See Quick Reference: Pouring Agar Plates.)

13. Let the agar cool and resolidify.

    If plates will be used within two days, store at room temperature, inverted without taping.

    If plates are prepared more than two days before use, rewrap in the plastic sleeve and store inverted in the refrigerator.

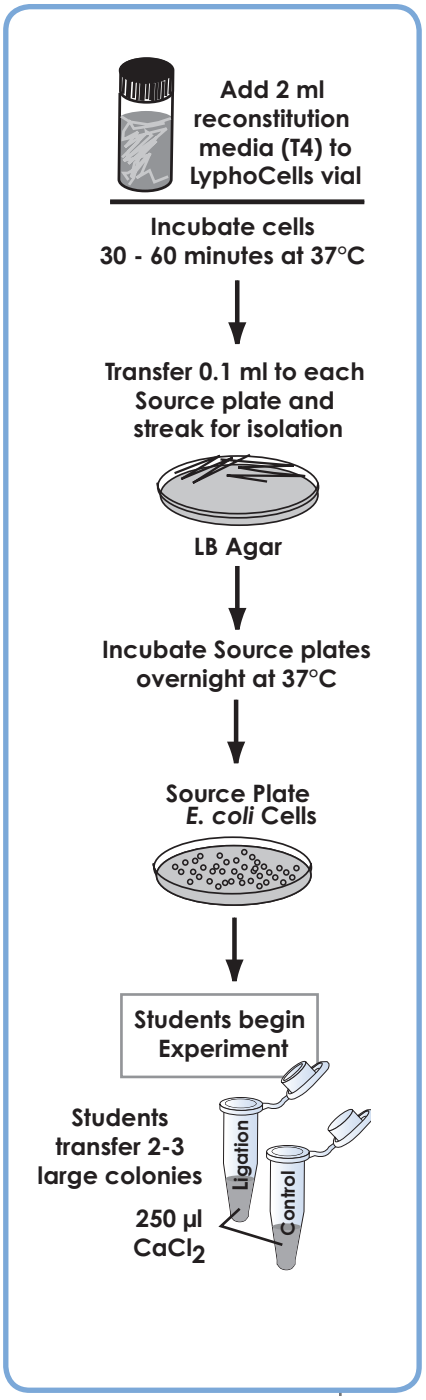
    Take plates out of the refrigerator and leave inverted at 37°C for several hours prior to use.

Quick Reference: Pouring Agar Plates

- Use a sterile 10 ml pipet with a pipet pump to transfer the designated volume of medium to each petri plate. Pipet carefully to avoid forming bubbles.
- Rock the petri plate back and forth to obtain full coverage.
- If the molten medium contains bubbles, they can be removed by passing a flame across the surface of the medium.
- Cover the petri plate and allow the medium to solidify.

Instructor's Guide

Pre-Lab Preparations - Module II



DAY BEFORE THE EXPERIMENT

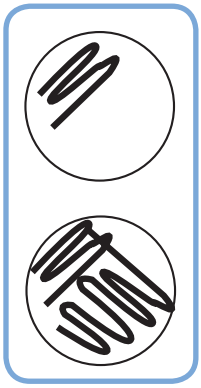
This experiment requires preparation of isolated *E.coli* host transformation colonies 16-24 hours before the laboratory experiment, so plan accordingly.

Important: Do not prepare source plates more than 24 hours before the experiment. Old source plates will compromise the success of the transformation experiment.

Preparation of *E. Coli* Cells

1. Use a 10 ml sterile pipet to add 2 ml cell reconstitution medium (T4) to the vial of LyphoCells.
2. Replace the rubber stopper and cap. Mix by gently inverting until the freeze dried plug is dissolved.
3. Incubate the vial of cells for 30 - 60 minutes in a 37°C incubation oven.

   Growth should be evident (Broth should be slightly turbid or cloudy). If growth is not evident, incubate for a longer period of time.
4. Use a sterile pipet to transfer 0.1 ml of cell suspension to a corner of each of the 100 mm petri plates.
5. Use a sterile loop to streak for isolated colonies by streaking through the drop of cells into a clean section of the plate. With a sterile loop, streak through the cells once or twice into another clean section of the plate.
6. Cover the plates and allow the cells to be absorbed by the medium.



7. Label the plates "*E. coli*", invert and incubate the plates overnight (16-24 hours) at 37°C in an incubation oven. If growth on plates is heavy (i.e. few or no isolated colonies), instruct students to touch the toothpick to a small amount of cells.

Pre-Lab Preparations - Module II

Other Preparations for Module II Transformation Experiment:

- **Day of the Lab**

1. Dispense 1 ml of $CaCl_2$ (T5) into microcentrifuge tubes labeled "$CaCl_2$" for each of the groups and place on ice.
2. Allow ample time for the equilibration of water baths and incubation ovens.
3. Assemble reagents and materials for 5 lab groups. Each group receives:
   - 1 Ligation plate
   - 1 Control plate
   - 1 *E. coli* source plate
   - 1 Ligation reaction from Module I
   - 1 Ligation control from Module I
   - 1 ml $CaCl_2$

Pre-Lab Preparations - Module III

Instructor's Guide

Qualitative β-galactosidase Reaction Analysis

Students can also use the plates from the transformation experiment. Pick an equal number of blue and white colonies (15-20 colonies each) and place them in two microcentrifuge tubes. Suspend in 500 µl of phosphate buffer and follow the protocol outlined on page 20 starting with step 5 in the β-galactosidase assay.

MODULE III - Assay for β-Galactosidase in Blue and White Colonies

1. Thaw the Ampicillin (T1). Prepare growth medium by adding 0.4 ml of ampicillin to LB growth medium (A1).
2. Obtain 10 sterile (autoclaved) 125 ml flasks and aliquot 25 ml of growth medium + ampicillin into each.
3. Arrange for students to inoculate assay cultures 4 to 5 hours before the lab.

   Alternatively, the instructor may inoculate the cultures. Cultures may be grown to early exponential phase (OD_{540} = 0.3 to 0.5) and can be stored on ice for up to 4 hours.
4. Add all of the Sodium phosphate buffer (A3) to 27 ml of distilled water. Aliquot 5.5 ml for each group into covered tubes.
5. Aliquot 1.5 ml of stop buffer (A5) into covered tubes for each group.
6. Dissolve the ONPG (A4-orthonitrophenalgalactopyranoside) in 20 ml distilled water (the ONPG may be difficult to get into solution). Aliquot 3 ml into covered tubes and store on ice. The final concentration is 4 mg/ml of ONPG.
7. Thaw the IPTG (T2) and aliquot 60 µl into 5 microtest tubes.
8. Dissolve the lysozyme (A2) in 10 ml distilled water and dispense 1.5 ml into 5 tubes labeled "lysozyme". Store on ice.
9. Prepare a 42°C waterbath for the latter part of Module III.

Please refer to the kit insert for the Answers to Study Questions

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Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Ampicillin

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 07/01/03

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Ampicillin CAS# 7177-48-2	No data			

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Slightly soluble

Appearance and Odor: Odorless, white crystaline powder

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		N.D.	N.D.

Extinguishing Media: Dry chemical, carbon dioxide, water spray or regular foam

Special Fire Fighting Procedures: Move container from fire area if possible. Do not scatter spilled material with water streams.

Unusual Fire and Explosion Hazards: Avoid breathing vapors.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Incompatibles
	Stable	X	

Incompatibility: Strong oxidizers

Hazardous Decomposition or Byproducts: Toxic oxides of carbon, nitrogen and sulfur

Hazardous Polymerization			Conditions to Avoid
	May Occur		Incompaticles
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Sensitizers may result in allergic reaction

Carcinogenicity: No data NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Repeated exposure may result in sensitization and possible anaphylactic shock.

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Ingestion: Allergic symptoms.
Eyes/Skin: Flush with water Inhalation: Move to fresh air

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear suitable protective clothing. Sweep up and place in suitable container for later disposal. Do not flush spilled material down sink.

Waste Disposal Method: Observe all federal, state, and local regulations

Precautions to be Taken in Handling and Storing: Keep away from incompatible substances

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation				
	Local Exhaust	Yes	Special	None
	Mechanical (General)	No	Other	None

Protective Gloves: Yes Eye Protection: Splash or dust proof

Other Protective Clothing or Equipment: Eye wash

Work/Hygienic Practices: Wear protective clothing and equipment to prevent contact.

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Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Solvent for Induction of Competency

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

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Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 05-25-05

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
None	---------- Not established ----------			

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Clear liquid

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		No data	No data

Extinguishing Media: Use water spray, alcohol foam, dry chemical, or carbon dioxide

Special Fire Fighting Procedures: Wear protective equipment and SCBA with full facepiece. Move container from fire area if possible.

Unusual Fire and Explosion Hazards: Vapors may flow along surfaces and flash back.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Avoid incompatibles
	Stable	X	

Incompatibility: Strong oxdizers

Hazardous Decomposition or Byproducts: Sulfur dioxide, mercaptans, carbon monoxide, carbon dioxide, formaldehyde

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Inhalation/Ingestion: Nausea and vomiting
Skin/eye contact: Rapid absorption causing irritation

Carcinogenicity: None identified NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Irritation

Medical Conditions Generally Aggravated by Exposure: Skin disorders

Emergency First Aid Procedures: Ingestion: Call medical help, do not induce vomiting Inhalation: remove to fresh air
Skin/eye contact: Flush w/ water

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear protective clothing. Take up with sand or other absorbant and place in container Dispose of properly.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid contact

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): SCBA

Ventilation				
	Local Exhaust	Yes	Special	None
	Mechanical (General)	Yes	Other	None

Protective Gloves: Butyl rubber gloves Eye Protection: Safety goggles

Other Protective Clothing or Equipment: Uniform or apron

Work/Hygienic Practices: Avoid contact

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Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): IPTG

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 07/01/03

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Not applicable				

Section III - Physical/Chemical Characteristics

Boiling Point	None	Specific Gravity (H_20 = 1)	Unknown
Vapor Pressure (mm Hg.)	None	Melting Point	109-110C
Vapor Density (AIR = 1)	None	Evaporation Rate (Butyl Acetate = 1)	None

Solubility in Water: Moderate

Appearance and Odor: White crystals/slight odor thiophenol

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
Unknown			

Extinguishing Media: Water, carbon dioxide, or dry chemical

Special Fire Fighting Procedures: None

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: Strong oxdizing agents

Hazardous Decomposition or Byproducts: Carbon dioxide and sulfur dioxide

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes; Skin? Not studied; Ingestion? Not studied

Health Hazards (Acute and Chronic): Toxcity has not been studied

Carcinogenicity: Unknown; NTP? No data; IARC Monographs? No data; OSHA Regulation? No data

Signs and Symptoms of Exposure: Unknown: avoid dust

Medical Conditions Generally Aggravated by Exposure: Unknown

Emergency First Aid Procedures: External: flush with water Internal: Induce vomiting, consult physician

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Cover and sweep up with inert carrier

Waste Disposal Method: Dissolve in a combustible solvent and burn in a chemical incinerator with afterburner and scrubber, or sweep up and return inoriginal container.

Precautions to be Taken in Handling and Storing: Avoid dust store cool

Other Precautions: Information CAS #367-93-1

Section VIII - Control Measures

Respiratory Protection (Specify Type): Filter mask

Ventilation				
	Local Exhaust	Yes	Special	None
	Mechanical (General)	Yes	Other	None

Protective Gloves: Rubber or vinyl

Eye Protection: Face mask or goggles

Other Protective Clothing or Equipment: Lab apron

Work/Hygienic Practices: Avoid dust or contact with skin

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Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): ONPG

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 08-25-2005

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
CAS # 369-07-3				

Section III - Physical/Chemical Characteristics

Boiling Point	**No data**	Specific Gravity (H_20 = 1)	**No data**
Vapor Pressure (mm Hg.)	**No data**	Melting Point	**195°C**
Vapor Density (AIR = 1)	**No data**	Evaporation Rate (Butyl Acetate = 1)	**No data**

Solubility in Water:

Appearance and Odor: **White to off-white powder**

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL

Extinguishing Media: **Water spray, CO2, Dry chemical powder or appropriate foam**

Special Fire Fighting Procedures: **Wear SCBA and protective clothing to prevent contact with skin and eyes**

Unusual Fire and Explosion Hazards: **Emits toxic fumes under fire conditions.**

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	

Incompatibility: **Strong oxidizing agents**

Hazardous Decomposition or Byproducts: **Toxic fumes of Carbon Monoxide, Carbon Dioxide and Nitrogen Oxide**

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? **Yes**; Skin? **Yes**; Ingestion? **Yes**

Health Hazards (Acute and Chronic): **May be harmful by inhalation, ingestion, or skin absorption. May cause eye and skin irritation**

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

To our knowledge, chemical, physical,toxicological properties havent been thoroughly investigated

Signs and Symptoms of Exposure:

Medical Conditions Generally Aggravated by Exposure:

Emergency First Aid Procedures: **Flush with copious amounts to area of contact w/ H20 for at least 15 minutes . Remove to fresh air & remove contaminated clothing**

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: **Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Ventilate area and wash spill site after pickup.**

Waste Disposal Method: **Dissolve or mix the material w/ a combustible solvent and burn in a chemical incinerator equipped with afterburner and scrubber**

Precautions to be Taken in Handling and Storing: **Observe all federal, state and local laws.**

Other Precautions: **Keep tightly closed, refrigerate, avoid prolonged or repeated exposure, avoid inhalation**

Section VIII - Control Measures

Respiratory Protection (Specify Type): **NIOSH/MSHA approved respirator**

Ventilation				
	Local Exhaust		Special	
	Mechanical (General)	**Mechanical exhaust**	Other	

Protective Gloves: **Heavy rubber gloves**

Eye Protection: **Chem. Safety goggles**

Other Protective Clothing or Equipment:

Work/Hygienic Practices: **Wash thoroughly after handling**

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): X-Gal in solvent

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 02/08/05

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)

This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.

Section III - Physical/Chemical Characteristics

Boiling Point at 760mm Hg	189C	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.) 20C	0.46	Melting Point	18C
Vapor Density (AIR = 1)	2.7	Evaporation Rate (Butyl Acetate = 1)	N/A

Solubility in Water: Complete (100%)

Appearance and Odor: Colorless liquid, faint odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
(closed cup) 88C (192F)		3%	43%

Extinguishing Media: Water spray, carbon dioxide, dry chemical, ordinary foam

Special Fire Fighting Procedures: Wear SCBA with full facepiece operated in positive pressure mode. If possible, move container from fire area

Unusual Fire and Explosion Hazards: Vapors may flow along surfaces to distant ignition sources and flashback. Closed containers exposed to heat may explode. Contact w/ strong oxidizers may cause fire.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Heat, flame, other sources of ignition
	Stable	X	

Incompatibility: Strong oxidizing agents, active halogen compounds, alkali metals

Hazardous Decomposition or Byproducts: Oxides of sulfur, mercaptane, active halogen compounds, alkali metals

Hazardous Polymerization			Conditions to Avoid
	May Occur		None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes; Skin? Yes; Ingestion? Yes

Health Hazards (Acute and Chronic): Chronic affects: kidney damage, liver damage

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No	No	No

Signs and Symptoms of Exposure: Skin/eye irritation, headache, nausea, vomiting, dizziness

Medical Conditions Generally Aggravated by Exposure: Skin disorders

Emergency First Aid Procedures: Flush skin/eyes with large amounts of water. If ingested do not induce vomiting.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Shut off ignition sources. Wear protective clothing. Use water spray to reduce vapors. Take up with sand or other noncombustible absorbent material and dispose of properly

Waste Disposal Method: Dispose in accordance with all applicable federal, state, and local environmental regulations

Precautions to be Taken in Handling and Storing: Store in cool, dry, well ventilated flammable liquid storage area or cabinet, store above 20C

Other Precautions: Product may solidify at room temperature

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator w/ organic vapor cartridge.

Ventilation			Special	
	Local Exhaust	Yes	Special	None
	Mechanical (General)	Yes	Other	None

Protective Gloves: Butyl rubber gloves

Eye Protection: Splash Proof Safety goggles

Other Protective Clothing or Equipment: Uniform or apron

Work/Hygienic Practices: Avoid contact with skin/eyes Keep container tightly closed.