



The Biotechnology  
Education Company®

**201**  
**Transformation** EDVO-Kit #  
**of *E. coli* with**  
**Plasmid pBR322**

**Storage:**  
Store entire experiment in the refrigerator.

**Experiment Objective:**

The objective of this experiment module is to develop an understanding of the biological process of bacterial transformation by plasmid DNA. This experiment enables the students to observe an acquired phenotypic trait exhibited by transformed bacterial cells.

**1-800-EDVOTEK • (301) 251-5990 • 24-hour FAX: (301) 340-0582**  
**<http://www.edvotek.com> • email: [edvotek@aol.com](mailto:edvotek@aol.com)**

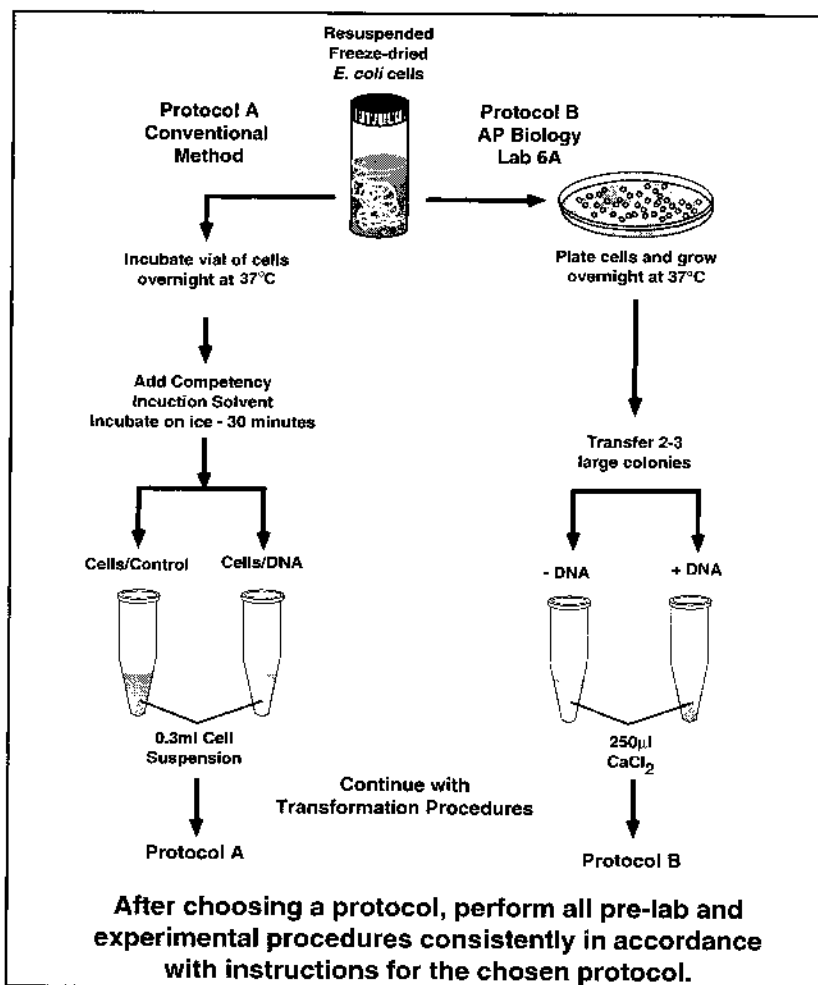
All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

## Important READ ME!

This transformation experiment can be conducted by two different methods.

- Protocol A: Conventional Method Using EDVOTEK LyphoCells™  
 Protocol B: Revised Method (1997) for Advanced Placement Biology, Lab 6A

Two protocols are provided with this experiment. There are subtle differences throughout the experiment, but the major difference between the two methods are illustrated in the figure below.



## Major Section Headings

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## Experiment Components

This experiment is designed for 10 lab groups.

**Storage:**

Store entire experiment  
in the refrigerator

This experiment is a  
simulation. None of the  
experiment components are  
derived from human  
sources.

**Contents**

- A Transformation LyphoCells™ (DO NOT FREEZE)
  - B Supercoiled pBR322 DNA
  - C Control Buffer (no DNA) (not used in Protocol B)
  - D Ampicillin
  - E Cell reconstitution media
  - F Solvent for induction of competency (not used in Protocol B)
  - G CaCl<sub>2</sub> (not used in Protocol A)
- 
- 1 Bottle ReadyPour™ Luria Broth Agar Base, sterile
  - 1 Bottle Recovery Broth, sterile
  - 40 Petri plates, small
  - 5 Petri plates, large
  - 40 Plastic microtipped transfer pipets (sterile)
  - 3 Wrapped 10 ml pipet (sterile)
  - 20 Toothpicks (sterile)
  - 20 Inoculating loops (sterile)
  - 50 Microtest tubes with attached lids

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## Requirements

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- Automatic Micropipet (5-50  $\mu$ l) and tips (required for PreLab prep)
- Two Water baths\* (set at 37°C and 42°C)
- Incubation Oven (37°C)
- Bunsen burner, hot plate or microwave oven
- Marking pens
- Hot gloves
- Ice

\* If a second water bath is not available, water can be heated to 42°C in a beaker. The cells will require this temperature for only a few minutes. Alternatively, 42°C water can be put in a small styrofoam container with a cover. The temperature will hold for 10 to 15 minutes.

## BACKGROUND INFORMATION

## Bacterial Transformation

Bacterial transformation is a phenomenon of central importance in molecular biology. It allows for the propagation, genetic expression and isolation of recombinant DNA molecules that have been constructed *in vitro* as well as natural DNA molecules. Transformation is also of historical importance since it led to the discovery by Avery, in the late 1940's, that DNA was the genetic material.

The transformation process involves the uptake of exogenous DNA by the cell which results in a newly acquired genetic trait that is stable and heritable. Bacterial cells must be in a particular physiological state before they can be transformed. This state is referred to as competency. Competency can occur naturally in certain species of *Haemophilus* and *Bacillus* when the levels of nutrients and oxygen are low. Competent *Haemophilus* express a membrane associated transport complex which binds and transfers certain DNA molecules from the medium into the cell where they are incorporated and their genes are expressed. In nature, the source of the external DNA is from other cells that have died and lysed.

Much of the current research and experimentation in molecular biology involves the transformation of *E. coli*. However, this organism does not enter a stage of natural competency. *E. coli* can be artificially induced to enter competency

when they are treated with the chloride salts of the metal cations calcium, magnesium and rubidium. In addition, sudden cycles of heat and cold help to bring about competency. The metal ions and temperature changes affect the structure and permeability of the cell wall and membrane so that DNA molecules can pass through. The reasons why this occurs are still unknown. Competent *E. coli* cells are fragile and must be treated carefully.

The amount of cells transformed per 1 microgram of DNA is called the transformation efficiency. In practice, much smaller amounts of DNA are used (5 to 100 nanograms) since too much DNA inhibits the transformation process. For example, 10 nanograms (0.01 microgram) of DNA was used to transform cells that were in a final volume of 1 ml after recovery. Assume 0.1 ml of these cells were plated on agar media that would allow only the cells that acquired the DNA to grow. This procedure is called selection. After incubation, 100 colonies were found on the plate. Keeping in mind that each colony originally grew from

## Bacterial Transformation Efficiency Calculation

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

## Specific example:

$$\frac{100 \text{ transformants}}{0.01 \mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml}} = \frac{100,000 (1 \times 10^5)}{\text{transformants per } \mu\text{g}}$$

Figure 1

## BACKGROUND INFORMATION

Background Information,  
continued

one transformed cell, the transformation efficiency in this case is outlined in Figure 1. In research laboratories, transformation efficiencies generally range from  $1 \times 10^4$  to  $1 \times 10^7$  cells per microgram of DNA. There are special procedures which can produce cells having transformation efficiencies approaching  $10^{10}$ . Transformation is never 100% efficient. Approximately 1 in every 10,000 cells successfully incorporates the DNA in preparations having average competency. However, there is such a large number of cells in a sample (typically  $1 \times 10^9$ ) that only a small fraction needs to be transformed to obtain colonies on a plate. These ideas can be demonstrated by plating the same volume of recovered cells on selective and nonselective agar media. The nonselective media will have many more growing cells. In this case, all the untransformed cells also survive. The bacterial agar plates will be covered heavily with untransformed cells, forming a "lawn", in contrast to individual colonies.

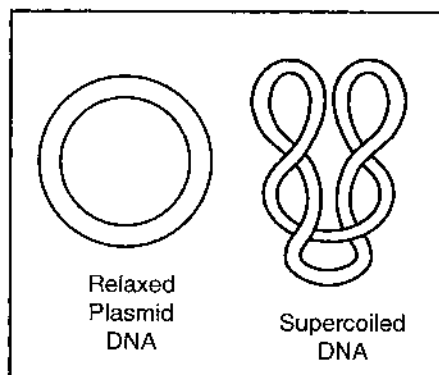


Figure 2

Plasmid DNAs are extrachromosomal, double-stranded circular molecules that are found in different strains of bacteria. Many plasmids contain genes that code for antibiotic resistance. The *E. coli* plasmid pBR322 consists of 4362 base pairs and contains antibiotic resistance genes for ampicillin (amp) and tetracycline (tet). Ampicillin is a derivative of penicillin and inhibits bacterial growth by interfering with the synthesis of cell walls. The product of the ampicillin resistance gene is the enzyme beta-lactamase. This enzyme is secreted by the cell and it destroys the ampicillin in the surrounding media. Cells that were not transformed are able to undergo limited growth in the zones that have been cleared of ampicillin. Colonies consisting of these untransformed cells are called satellites since they only appear around the larger colonies of transformed cells. Larger plating volumes of cells and longer incubation times increase the amount of satellite colonies.

Plasmids naturally exist as supercoiled molecules. The two strands of DNA in the supercoiled molecule are wound and folded around each other in a way that produces a condensed, entangled structure when compared to relaxed (non-supercoiled) DNA (Figure 2). Competent *E. coli* are sensitive to the conformation of the DNA they will accept. Supercoiled DNA gives the highest transformation efficiencies.

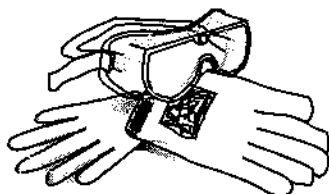
The objective of this laboratory is to transform a strain of competent *E. coli* which has no antibiotic resistance with supercoiled pBR322 DNA. The cells will be selected for the presence of plasmid by plating them on agar media containing ampicillin. The transformation efficiency will then be estimated.

## EXPERIMENTAL PROCEDURES

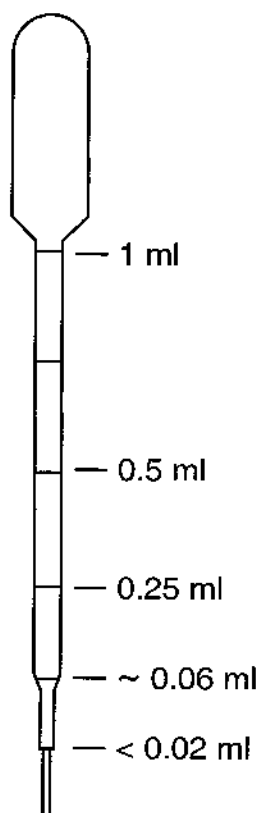
## EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of the biological process of bacterial transformation by plasmid DNA. This experiment enables the students to observe an acquired phenotypic trait exhibited by transformed bacterial cells.

## LABORATORY SAFETY:



Gloves and goggles should always be worn.




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. At the end of the experiment:
  - A. Wipe down the lab bench with a 10% bleach solution, disinfectant or soapy water.
  - 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 media 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.
  - C. Always wash hands thoroughly with soap and water after handling contaminated materials.

# EXPERIMENTAL PROCEDURES

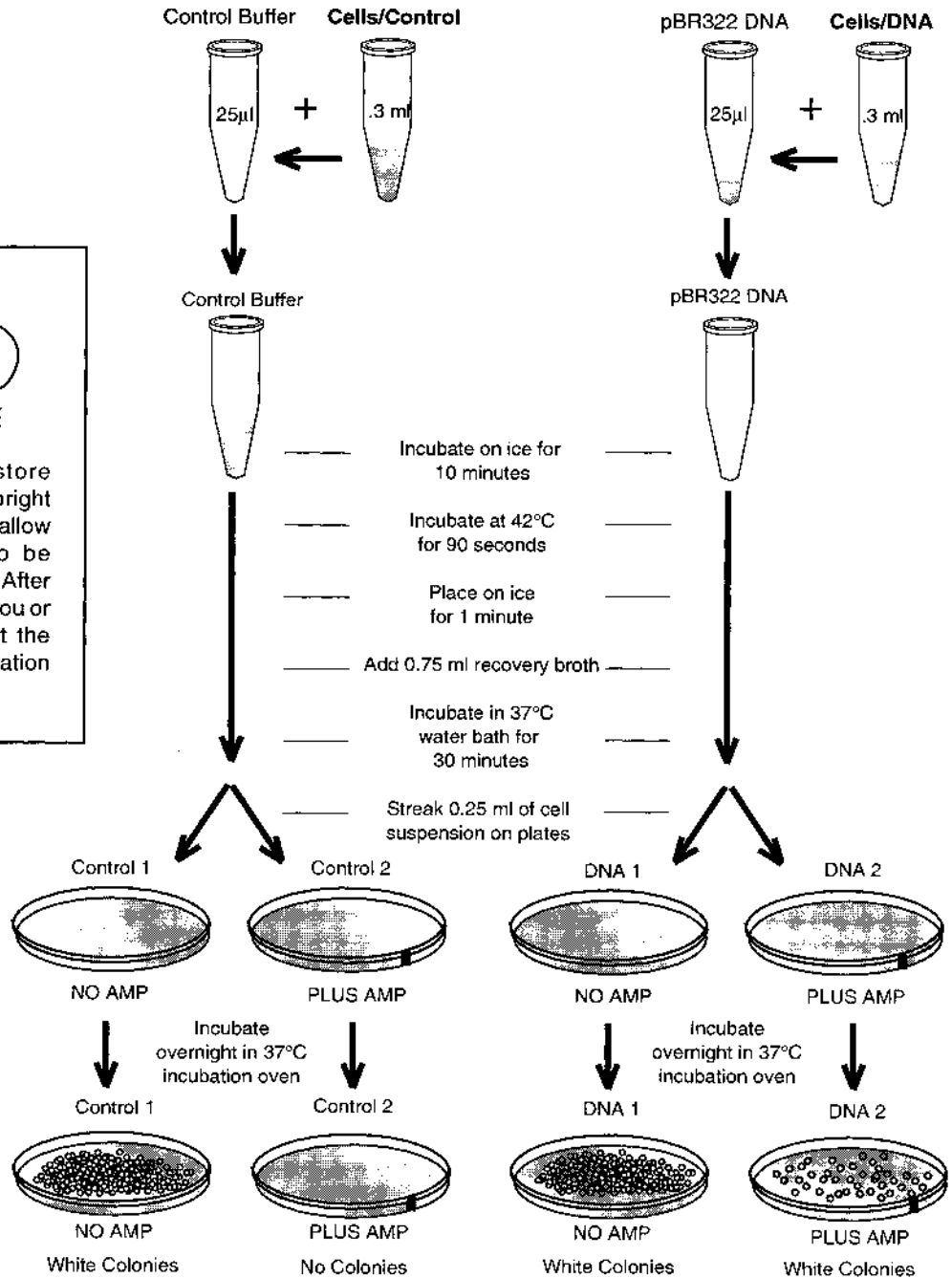
## Protocol A/Option 1 - Conventional Method Using EDVOTEK Lymphocells™

### TRANSFORMATION EXPERIMENT FLOW CHART

**Useful Hint!**

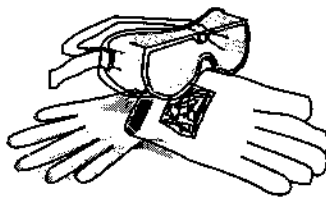


For optimal results, store covered plates in the upright position after streaking to allow the cell suspension to be absorbed by the agar. After approximately one hour, you or your instructor will invert the plates for overnight incubation at 37°C.



## EXPERIMENTAL PROCEDURES

## Protocol A/Option 1 - Conventional Method Using EDVOTEK Lyphocells™



WEAR SAFETY GOGGLES  
AND GLOVES

**Quick Reference:**

DNA and competent cells are combined in a 0.3 ml suspension. After the cells have incubated with the DNA, growth media (recovery broth) is added. Cells continue to grow through the recovery process, during which time the cells repair their membranes, grow and express the genes of the newly acquired DNA.

**Remember!**

Add the recovery broth with a sterile 1 ml pipet. **Avoid touching the cells with the pipet.**

## SETTING UP THE TRANSFORMATION AND CONTROL EXPERIMENT

- Put your initials or group number on the tubes labeled "pBR322 DNA" and "Control Buffer". Place them back on ice.
- Set up the Control:
  - Using a sterile transfer pipet, transfer all the cell suspension (0.3 ml) in the tube "Cells/Control" to the tube "Control Buffer". Carefully place the pipet back into the wrapper.
  - Cap the tube; mix by tapping. Put the tube back on ice.
- Set up the transformation:
  - Using a the same pipet from Step 2, transfer all the cell suspension (0.3 ml) in the tube "Cells/DNA" to the tube "pBR322 DNA".
  - Cap the tube; mix by tapping. Put the tube back on ice.

## Procedure for Transformation

- Incubate the cells prepared in steps 1 - 3 on ice for 10 minutes.
- Place the cells in 42°C waterbath for 90 seconds.
- Place the tubes back on ice for 1 minute.
- Add 0.75 ml of the Recovery Broth to the tube "Control Buffer".
- Add 0.75 ml of the Recovery Broth to the tube "pBR322 DNA".
- Incubate the closed tubes in a 37°C water bath for 30 minutes for a recovery period.
- While the tubes are incubating, label 4 agar plates as follows:
  - Label the two unstriped plates: Control 1 NO AMP  
DNA 1 NO AMP
  - Label the two striped plates: Control 2 PLUS AMP  
DNA 2 PLUS AMP
  - Put your initials or group number on all the plates.
- After the recovery period, remove the tubes from the water bath and place them on the lab bench.

## EXPERIMENTAL PROCEDURES

Protocol A - Experimental  
Procedures, continued

## PLATING AND SELECTION OF BACTERIAL CELLS

## Plating cells from the tube labeled "Control":

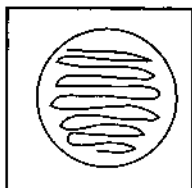


Figure 3:  
Spread cells  
in one direction

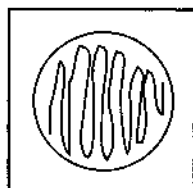


Figure 4:  
Same plate:  
spread cells 90°  
to first direction

12. Use a fresh, sterile 1 ml pipet to transfer recovered cells from the tube "Control Buffer" to the middle of the following plates:

- 0.25 ml to the plate labeled Control 1 NO AMP
- 0.25 ml to the plate labeled Control 2 PLUS AMP

13. Spread the cells with a sterile inoculating loop. (See Figures 3 & 4)

14. Cover both control plates and allow the liquid to be absorbed.

## Plating cells from the tube labeled "DNA"

15. Use a fresh, sterile 1 ml pipet to transfer recovered cells from the tube "pBR322 DNA" to the middle of the following plates:

- 0.25 ml to the plate labeled DNA 1 NO AMP
- 0.25 ml to the plate labeled DNA 2 PLUS AMP

16. Spread the cells with a sterile inoculating loop. (See Figures 3 & 4)

17. Cover the plate and allow the liquid to be absorbed.

## Preparing Plates for Incubation

18. Stack your group's set of plates on top of one another and tape them together. Put your initials or group number on the taped set of plates.

The plates should be left in the upright position to allow the cell suspension to be absorbed by the agar.

19. Place the set of plates in a safe place designated by your instructor.

After the cell suspension is absorbed by the agar for approximately 1 - 2 hours, you or your instructor will place the plates in the **inverted** position (agar side on top) in a 37°C bacterial incubation oven for overnight incubation (15-20 hours). The plates are inverted to prevent condensation on the lid, which could drip onto the culture and interfere with experimental results.

## Useful Hint!

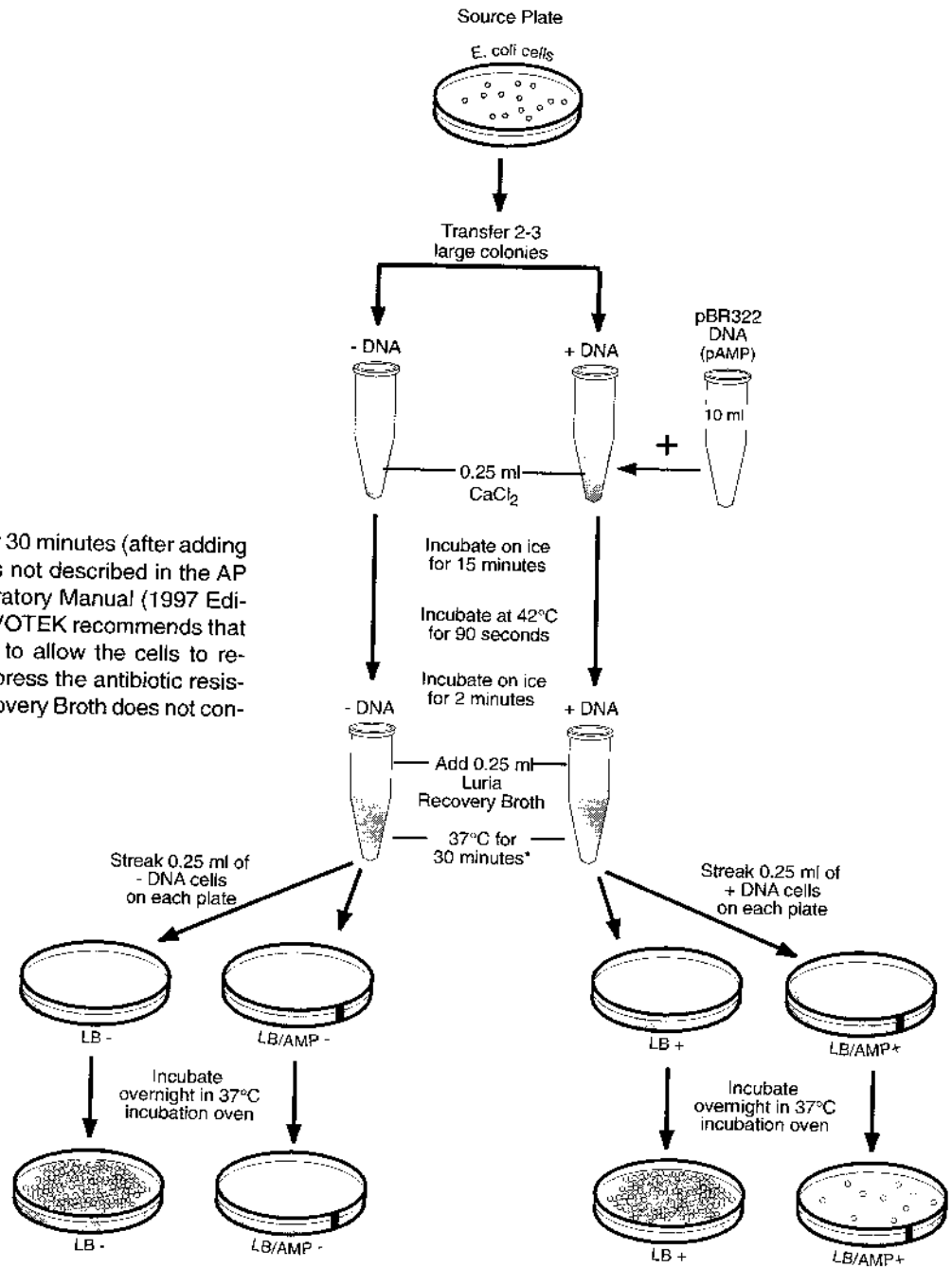


If you do not have an incubation oven, the plates can be left at room temperature. Colonies of transformed cells should appear between 24 - 48 hours.

EXPERIMENTAL PROCEDURES

Protocol B / Option 2 - Revised (1997) AP Biology Lab 6A

TRANSFORMATION EXPERIMENT FLOW CHART



\* The 37°C incubation for 30 minutes (after adding the Recovery Broth) is not described in the AP Biology Student Laboratory Manual (1997 Edition D). However, EDVOTEK recommends that you perform this step to allow the cells to recover and begin to express the antibiotic resistance genes. The Recovery Broth does not contain antibiotic.

## EXPERIMENTAL PROCEDURES

**Protocol B / Option 2 - Revised (1997) Advanced Placement Biology Lab 6A**

1. Label one microcentrifuge tube "+". This will be the transformation tube with plasmid DNA.
2. Label a second microcentrifuge tube "-". This will be the experimental control tube without plasmid DNA.
3. Using a sterile 1 ml pipet, add 250 µl (0.25 ml) of ice cold CaCl<sub>2</sub> solution to each tube.
4. As is done in research laboratories, pick colonies from the source plate of *E. coli* cells. To each of the test tubes labeled "+ " and "- ":
  - 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 in the CaCl<sub>2</sub> solution to dislodge the cells.
5. Suspend the cells in both tubes by tapping or vortexing.
6. To the tube labeled "+ " add 10 µl pBR322 DNA. This is a plasmid that contains the ampicillin resistance gene, often referred to as pAMP.
7. Incubate the two tubes on ice for 15 minutes.
8. Place both tubes 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 of Recovery Broth to each tube and mix.
- 11.\* Incubate the cells for 30 minutes in a 37°C waterbath for a recovery period.
12. While the tubes are incubating, label 4 agar plates as follows:
  - Label the two unstriped plates: LB -  
LB +
  - Label the two striped plates: LB / AMP -  
LB / AMP +
  - Put your initials or group number on all the plates.

\* This step (#11) is not described in the AP Biology Student Laboratory Manual (1997 Edition D). However, EDVOTEK recommends that you perform this step to allow the cells to recover and begin to express the antibiotic resistance genes. The Recovery Broth does not contain antibiotic.

## EXPERIMENTAL PROCEDURES

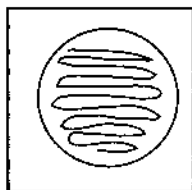
Protocol B - Experimental  
Procedures, continued

Figure 3:  
Spread cells  
in one direction

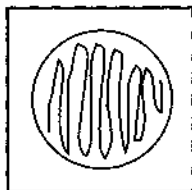


Figure 4:  
Same plate:  
spread cells 90°  
to first direction

13. After the recovery period, remove the tubes from the water bath and place them on the lab bench.

**Plating cells from the tube labeled " - ":**

14. Use a sterile 1 ml pipet to transfer recovered cells from the tube labeled " - " to the middle of the following plates:

- 0.12 - 0.25 ml to the plate labeled LB -
- 0.12 - 0.25 ml to the plate labeled LB / AMP -

15. Spread the cells with a sterile inoculating loop. (See Figures 3 & 4)
16. Cover both plates and allow the liquid to be absorbed.

**Plating cells from the tube labeled "+"**

17. Use a sterile 1 ml pipet to transfer recovered cells from the tube labeled "+" to the middle of the following plates:

- 0.12 - 0.25 ml to the plate labeled LB +
- 0.12 - 0.25 ml to the plate labeled LB / AMP +

18. Spread the cells with a sterile inoculating loop. (See Figures 3 & 4)
19. Cover the plate and allow the liquid to be absorbed.

**Preparing Plates for Incubation**

20. Stack your group's set of plates on top of one another and tape them together. Put your initials or group number on the taped set of plates.

The plates should be left in the upright position to allow the cell suspension to be absorbed by the agar.

21. Place the set of plates in a safe place designated by your instructor.

After the cell suspension is absorbed by the agar for approximately 1 - 2 hours, you or your instructor will place the plates in the **inverted** position (agar side on top) in a 37°C bacterial incubation oven for overnight incubation (15-20 hours). The plates are inverted to prevent condensation on the lid, which could drip onto the culture and interfere with experimental results.

**Useful Hint!**

If you do not have an incubation oven, the plates can be left at room temperature. Colonies of transformed cells should appear between 24 - 48 hours.

**Determination of Transformation Efficiency****Quick Reference for  
Protocol A:**

In this experiment, 125 ng  
(0.125 mg) of DNA is used.

The final volume at recovery is  
1.05 ml. The volume plated is  
0.25 ml.

**Quick Reference for  
Protocol B:**

In this experiment, 50 ng (0.05 µg)  
of DNA is used.

The final volume at recovery is  
0.50 ml. The volume plated is  
0.25 ml.

- Count the number of colonies on the plate with ampicillin labeled:

Protocol A: DNA 2 PLUS AMP

Protocol B: LB / AMP +

A convenient method to keep track of counted colonies is to mark the colony with a marking pen on the outside of the plate.

- Determine the transformation efficiency using the formula:

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

For example, assume you observed 40 colonies using Protocol A:

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

**Study Questions**

- Exogenous DNA does not passively enter *E. coli* cells that are not competent. What treatment do cells require to be competent?
- The recovery broth used in this experiment did not contain ampicillin. Why?
- The antibiotics, kanamycin and tetracycline, interfere with translation by binding to the ribosomes. Competent *E. coli* cells can be transformed with plasmids encoding resistance to these drugs. When the cells are plated on agar media containing kanamycin or tetracycline, satellite colonies are not found. Increasing incubation times and the amount of cells plated do not give rise to satellites in this case. Why?