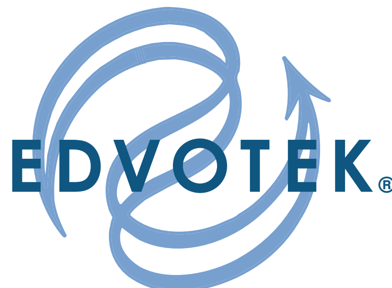
Revised and Updated

The Biotechnology Education Company®



EDVO-Kit #

201

Transformation of *E. coli* with pBR322

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

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

No IPTG used in this experiment.

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

	Page
Experiment Components	4
Experiment Requirements	5
Background Information	6
Experiment Procedures	
Experiment Overview	8
Laboratory Safety	9
Experimental Procedures	
Protocol A (Conventional)	10
Protocol B (AP Biology)	15
Experiment Results and Analysis	
Experiment Questions	20
Determination of Transformation Efficiency	22
Study Questions	23
Instructor's Guidelines	
Notes to the Instructor	25
Pre-Lab Preparations	
Pour Agar Plates	27
Protocol A	30
Protocol B	32
Protocol Hint and Optional Activity	34
Experiment Results and Analysis	
Experiment Questions	34
Idealized Schematic of Results	36
Study Questions and Answers	37
Material Safety Data Sheets	38

Important READ ME!

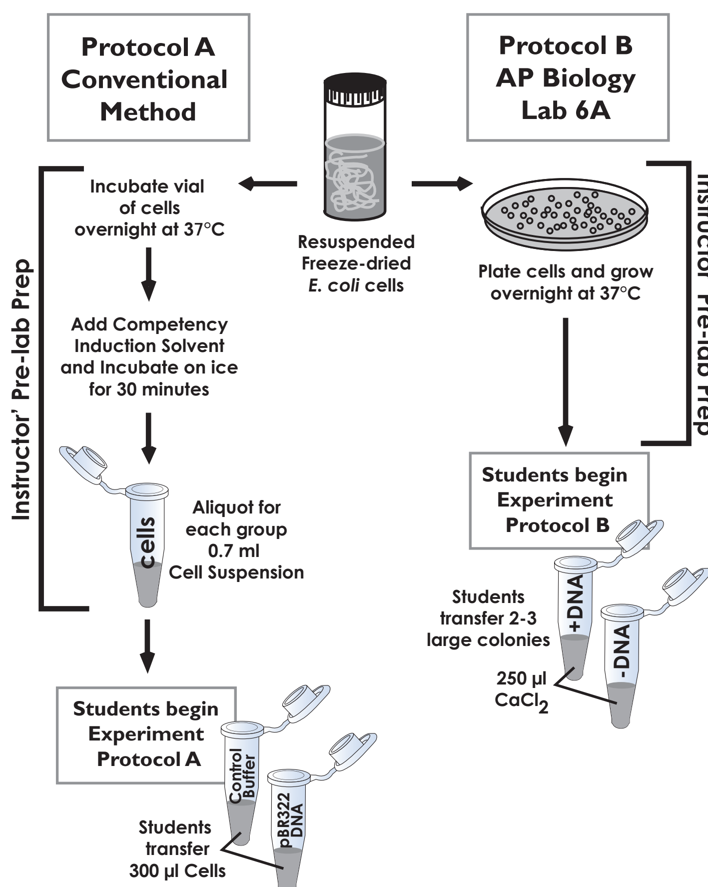
Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicilllin, ampicillin, kanamycin or tetracycine should not participate in this experiment.

This transformation experiment can be conducted by two different methods.

Protocol A: Conventional Method Using EDVOTEK LyphoCells™

Protocol B: Alternate Method (1997) for Advanced Placement Biology, Lab 6A

There are subtle differences between the two protocols provided with this experiment. The major difference between the two options are illustrated in the figure at right.



After choosing a protocol option, perform all pre-lab and experimental procedures consistently in accordance with instructions for the chosen protocol.

Experiment Components

Component Quantities:

Experiment # 201 is designed for 10 groups (2-4 students per group).

Storage: Store components A-G in the refrigerator.

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 medium
F Solvent for induction of competency (not used in Protocol B)
G $CaCl_2$ (not used in Protocol A)

Storage: Store components listed below at Room temperature

- Bottle ReadyPour™ Luria Broth Agar, sterile (also referred to as ReadyPour medium)
- Bottle Luria Broth Medium for Recovery, sterile (also referred to as Luria Recovery Broth)
- Petri plates, small
- Petri plates, large
- Plastic microtipped transfer pipets
- Wrapped 10 ml pipet (sterile)
- Toothpicks (sterile)
- inoculating loops (sterile)
- Microtest tubes with attached lids

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.

IMPORTANT READ ME!

In this experiment, antibiotics are used for the selection of transformed bacteria. Students who have or suspect to have allergies to penicilllin, ampicillin or tetracycine should not participate in this experiment.

Requirements

- Automatic Micropipet (5-50 µl) and tips
- Two Water baths* (37°C and 42°C)
- Thermometer
- Incubation Oven
- Pipet pumps or bulbs
- Marking pens
- Bunsen burner, hot plate or microwave oven
- 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 needs to be held at 42°C.

Online Ordering
now available

Visit our web site for information about EDVOTEK's complete line of experiments for biotechnology and biology education.

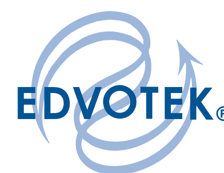
Technical Service Department

Mon - Fri
9:00 am to 6:00 pm ET

FAX: (301) 340-0582
web: www.edvotek.com
email: edvotek@aol.com

Please have the following information:

- The experiment number and title
- Kit Lot number on box or tube
- The literature version number (in lower right corner)
- Approximate purchase date



Background Information

Bacterial Transformation

Bacterial transformation is of central importance in molecular biology. It allows for the propagation of recombinant DNA molecules that are constructed *in vitro* or found in nature. 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.

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

Specific example:

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

Figure 1:
Bacterial Transformation Efficiency Calculation

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 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* which does not enter a stage of natural competency. *E. coli* can be artificially induced to enter competency upon treatment with the chloride salts of cations, such as calcium, magnesium and rubidium. Sudden cycles of heat and cold help to bring about competency. Metal ions and temperature changes affect the structure and permeability of the cell wall 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 with care.

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 higher concentrations of DNA inhibit the transformation process. Typically, 10 nanograms (0.01 microgram) of DNA is used to transform cells that are in a final volume of 1 ml. After transformation, the cell suspension is plated on agar medium in the presence of the antibiotic (coded by the transforming plasmid). This allows the selection of transformed cells since the presence of antibiotics will inhibit the growth of cells that had not acquired the antibiotic resistance gene.

After the incubation of plates with the transformed cells, colonies will appear on the plate. Keeping in mind that each colony originally

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved EVT 091102K

Bacterial Transformation

grew from **one** transformed cell, the transformation efficiency can be calculated. For example, if 100 colonies are present, the transformation efficiency can be calculated as outlined in Figure 1. In research laboratories, bacterial transformation efficiencies generally range from 1 x 10^6 to 1 x 10^8 cells per microgram of DNA. There are procedures which can produce cells having transformation efficiencies approaching 10^{10}. Transformation is never 100%; in a typical experiment, 1 in 10,000 cells successfully incorporate the transforming plasmid. However, there is such a large number of bacterial host cells in a sample (typically 1 x 10^9) and only a small fraction of cells need to be transformed to obtain colonies on a plate. Transformation efficiency can be demonstrated by plating equal volumes (0.1 ml) of recovered cells on selective (containing antibiotic) and nonselective bacterial growth agar medium. The nonselective medium, which does not contain the antibiotic, will have a large number of cells known as a "lawn". Bacterial agar plates will be covered heavily with untransformed cells, forming a "lawn" in contrast to individual colonies.

Plasmid DNAs are extrachromosomal, double-stranded circular molecules that are found in various strains of bacteria. Many plasmids contain genes that code for antibiotic resistance. *E. coli* plasmid pBR322 consists of 4,362 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 beta-lactamase. This enzyme is secreted by transformed bacterial cells and destroys the ampicillin in the surrounding agar medium. Subsequently, cells that were not transformed are able to undergo limited growth in the zones that have been cleared of ampicillin. Colonies consisting of untransformed cells typically form small colonies that are called satellites since they only appear around the larger transformed colonies. Larger plating volumes of cells and longer incubation times increase the amount of satellite colonies.

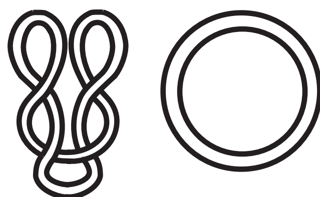


Figure 2: Supercoiled and circular forms of plasmid DNAs

In bacterial cells, plasmids are present primarily in supercoiled forms. In that conformation, the two strands of DNA are condensed entangled structures compared to the relaxed (non-supercoiled) DNA (Figure 2). Competent *E. coli* cells are sensitive to the conformation of DNA and are selective to the supercoiled plasmid form during transformation.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved

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 develop an understanding of bacterial transformation by plasmid DNA. This experiment enables the students to observe an acquired phenotypic trait exhibited by transformed bacterial cells.

BRIEF DESCRIPTION OF EXPERIMENT:

In this experiment, you will 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 medium containing ampicillin. The transformation efficiency will then be estimated.

This transformation experiment can be conducted by two different methods.

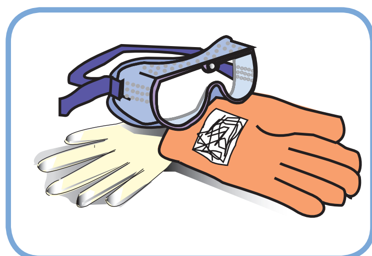
Protocol A: Conventional Method Using EDVOTEK LyphoCells™

Protocol B: Alternate Method (1997) for Advanced Placement Biology, Lab 6A

Two protocols are provided with this experiment. There are subtle differences throughout the two experiment protocols. Please make sure you are following the appropriate protocol as determined by your instructor.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved EVT 091102K

Laboratory Safety



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.

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.

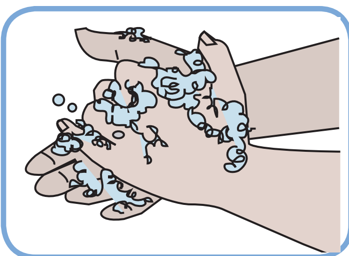
4. The *E. coli* bacteria used in this experiment is not considered pathogenic. Although it is rarely associated with any illness in healthy individuals, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with bacteria.

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.

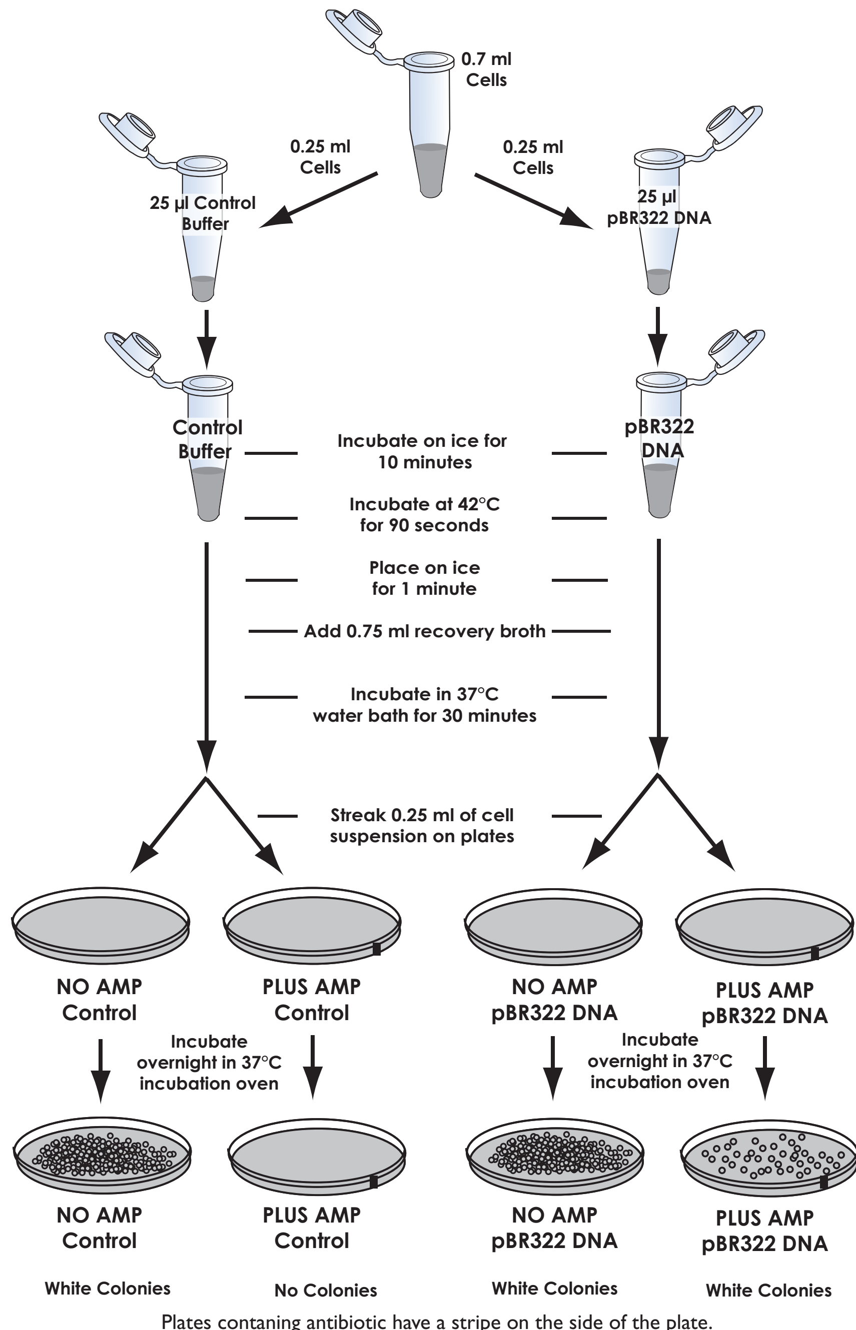


   - Soak in 10% bleach solution.
     Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.

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

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved

Protocol A - Conventional Method Using EDVOTEK Lyphocells™

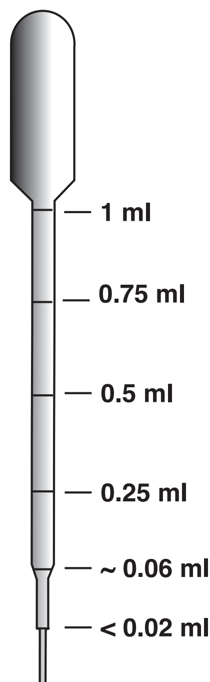


Plates contaning antibiotic have a stripe on the side of the plate.

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 20 minutes, invert the plates for overnight incubation at 37°C.

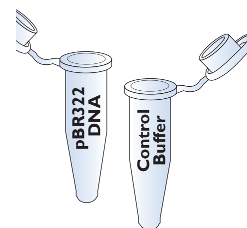
Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved EVT 091102K

Protocol A - Conventional Method Using EDVOTEK Lyphocells™



Guide for Sterile Calibrated Transfer Pipet

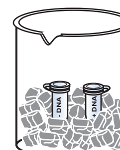
SETTING UP THE TRANSFORMATION AND CONTROL EXPERIMENT



1. Put your initials or group number on the tubes labeled "pBR322 DNA" (contains 25 µl of plasmid DNA) and "Control Buffer" (contains 25 µl of buffer) . Place them back on ice.

2. Set up the Control:
   - Using a sterile transfer pipet, transfer 0.25 ml (250 µl) of the cell suspension 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.

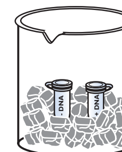
3. Set up the transformation:
   - Using the same pipet from Step 2, transfer 0.25 ml (250 µl) of the cell suspension to the tube "pBR322 DNA".
   - Cap the tube; mix by tapping. Put the tube back on ice.



4. Incubate the cells prepared in steps 1 - 3 on ice for **10 minutes**.

5. Place both tubes in a waterbath at **42°C for 90 seconds**.

   This heat shock step facilitates the entry of DNA in bacterial cells.



6. Return both tubes to incubate on ice for for **1 minute**.

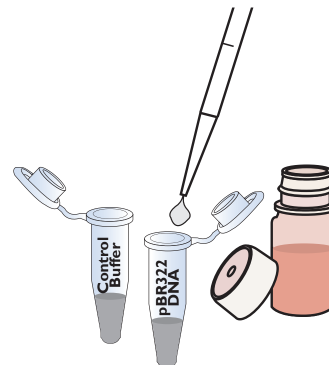
Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved

Protocol A - Conventional Method Using EDVOTEK Lyphocells™

7. Add **0.75 ml** of the Recovery Broth to the tube "Control Buffer".

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

8. Add **0.75 ml** of the Recovery Broth to the tube "pBR322 DNA".



Quick Reference:

DNA and competent cells are combined in a 0.25 ml 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.

37°C

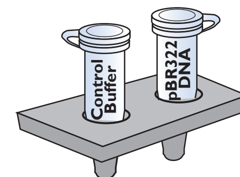
9. Incubate the closed tubes in a 37°C water bath for **30 minutes** for a recovery period.

10. While the tubes are incubating, label 4 agar plates as indicated below. Write on the bottom or side of the petri plate.

    - Label one unstriped plate: NO AMP Control
    - Label one unstriped plate: NO AMP pBR322 DNA

    - Label one striped plate: PLUS AMP Control
    - Label one striped plate: PLUS AMP pBR322 DNA

    - Put your initials or group number on all the plates.



11. After the recovery period, remove the tubes from the water bath and place them on the lab bench. Proceed to plating the cells for incubation.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved

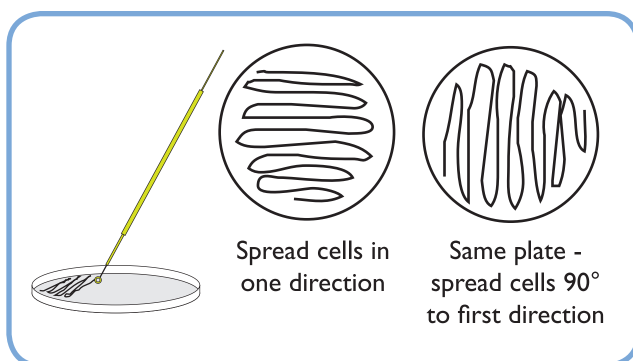
Protocol A - Conventional Method Using EDVOTEK Lyphocells™

PLATING THE CELLS

Plating cells from the tube labeled "Control":

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



Spread cells in one direction

Same plate - spread cells 90° to first direction

13. Spread the cells over tne entire plate with a sterile inoculating loop.

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

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.

Plating cells from the tube labeled "pBR322 DNA"

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

- 0.25 ml to the plate labeled NO AMP pBR322 DNA
- 0.25 ml to the plate labeled PLUS AMP pBR322 DNA

16. Spread the cells with a sterile inoculating loop.

17. Cover the plate and allow the liquid to be absorbed (approximately 15-20 minutes).

Reminder: Follow proper procedures for disposal of contaminated materials.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved

Protocol A - Conventional Method Using EDVOTEK Lyphocells™

PREPARING PLATES FOR INCUBATION

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

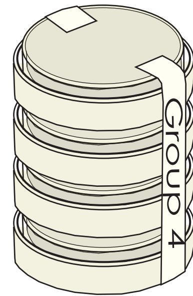
19. Put your initials or group number on the taped set of plates.

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.

20. Place the set of plates in a safe place designated by your instructor. 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 bacterial 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 interfere with experimental results.



Reminder: Follow proper procedures for disposal of contaminated materials.

VIEWING PLATES AFTER INCUBATION

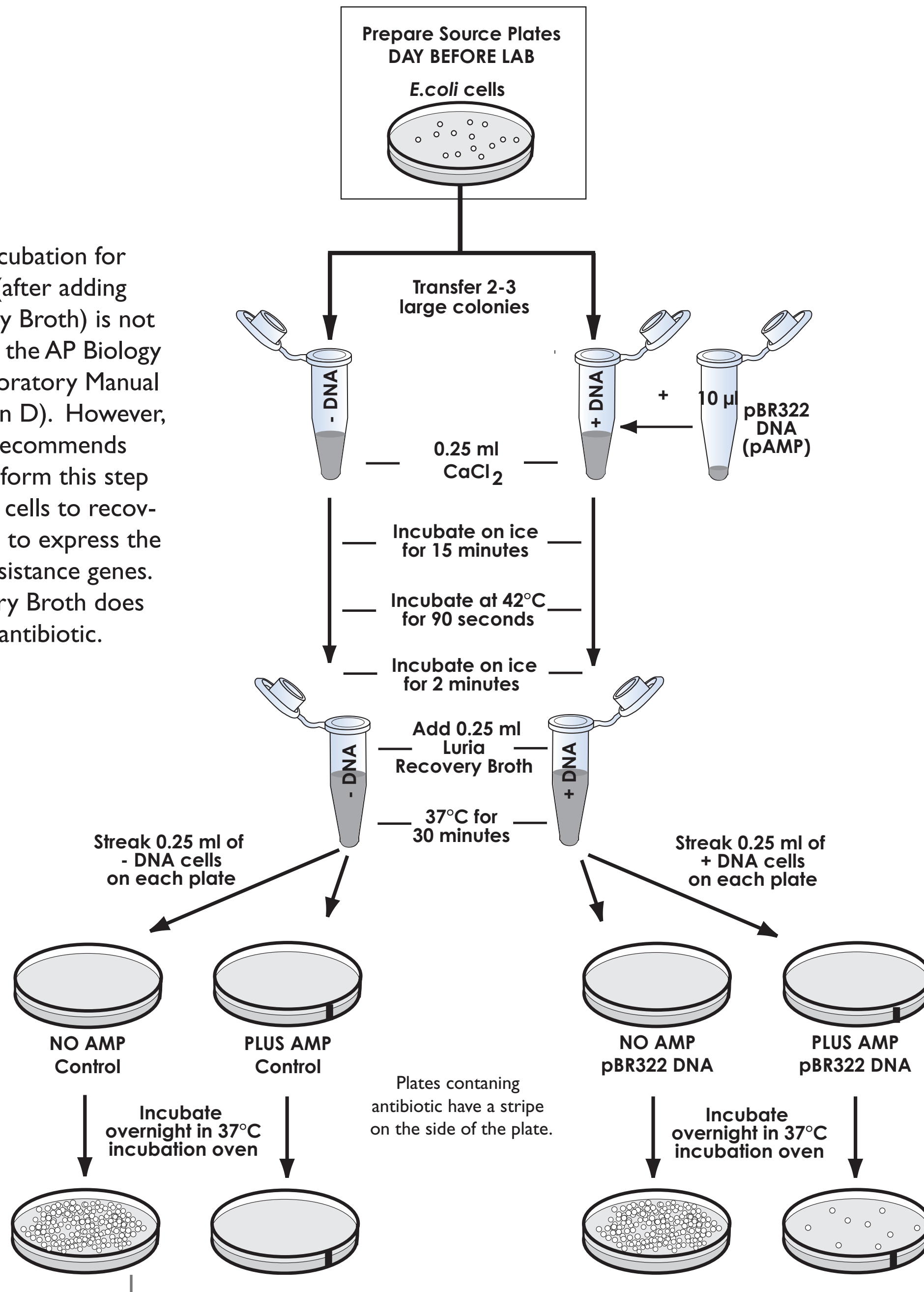
22. Proceed to analyzing your results.

23. After analyzing your results, follow proper procedures for disposal of contaminated materials.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved

Protocol B - Advanced Placement 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.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved EVT 091102K

Protocol B - Advanced Placement Biology Lab 6A

The Experiment

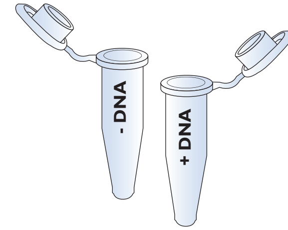
Reminder

"+" Contains plasmid DNA

"-" Does not contain plasmid DNA

SETTING UP THE TRANSFORMATION AND CONTROL EXPERIMENT

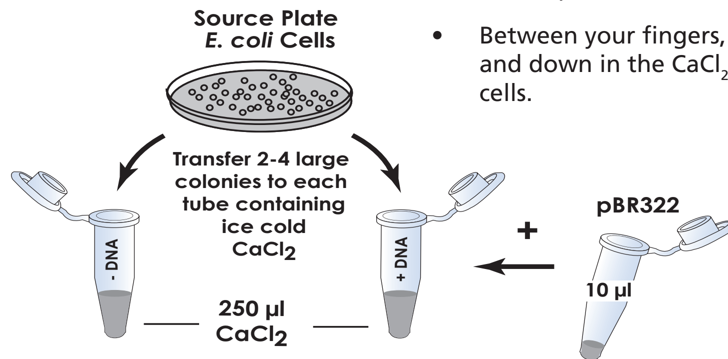
1. Label one microcentrifuge tube " + DNA ". This will be the transformation tube with plasmid DNA.



2. Label a second microcentrifuge tube " - DNA ". 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 $CaCl_2$ solution to each tube.

4. Pick colonies from the source plate of E. coli cells to each of the test tubes labeled " + DNA " and " - DNA ":
   - 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.



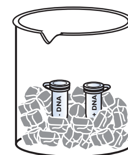
Avoid scraping up agar when transferring the cells from the source plate to the tubes with calcium chloride solution. It is important that the cells are resuspended in the calcium chloride solution and is not left on the toothpick or on the wall of the tube.

5. Suspend the cells in both tubes by tapping or vortexing.

6. Add 10 µl of pBR322 DNA to the tube labeled " + DNA ".

   This plasmid contains the ampicillin resistance gene, often referred to as pAMP.

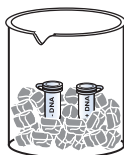
7. Incubate the two tubes on ice for **15 minutes**.



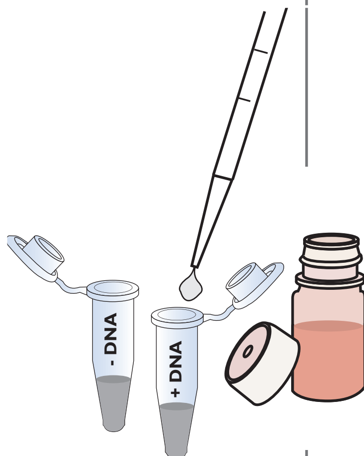
Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved

Protocol B - Advanced Placement Biology Lab 6A

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.

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

* 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.

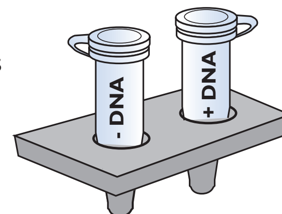
12. While the tubes are incubating, label 4 agar plates as indicated below. Write on the bottom or side of the petri plate.

- Label one unstriped plate: NO AMP Control
- Label one unstriped plate: NO AMP pBR322 DNA

- Label one striped plate: PLUS AMP Control
- Label one striped plate: PLUS AMP pBR322 DNA

- Put your initials or group number on all the plates.

13. After the recovery period, remove the tubes from the water bath and place them on the lab bench. Proceed to plating the cells for incubation.



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved

Protocol B - Advanced Placement Biology Lab 6A

The Experiment

Reminder

"+" Contains plasmid DNA

"-" Does not contain plasmid DNA

PLATING THE CELLS

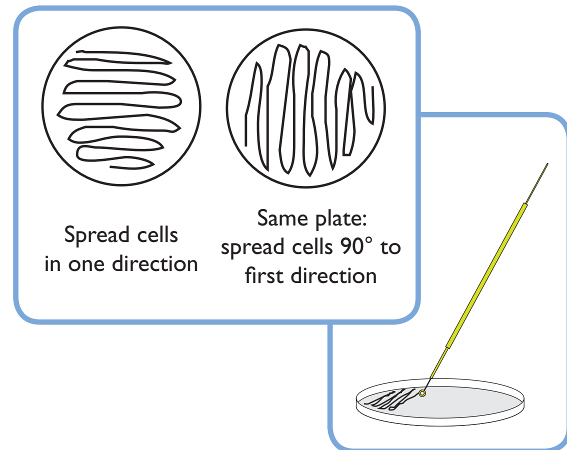
Plating cells from the tube labeled " - DNA "

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

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

15. Spread the cells over the entire plate with a sterile inoculating loop.

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.



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

Plating cells from the tube labeled "+ DNA"

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

- 0.25 ml to the plate labeled NO AMP pBR322 DNA
- 0.25 ml to the plate labeled PLUS AMP pBR322 DNA

18. Spread the cells with a sterile inoculating loop.

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

.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved

Protocol B - Advanced Placement Biology Lab 6A

PREPARING PLATES FOR INCUBATION

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

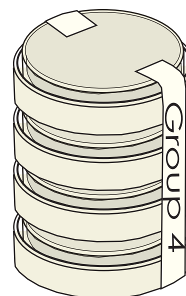
19. Put your initials or group number on the taped set of plates.

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.

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



Reminder: Follow proper procedures for disposal of contaminated materials.

VIEWING PLATES AFTER INCUBATION

22. Proceed to analyzing your results.

23. After analyzing your results, follow proper procedures for disposal of contaminated materials.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1987, 88, 89, 90, 93, 94, 95, 97, 98, 99, 2005, 2009 EDVOTEK, Inc., all rights reserved