WORKSHOP

Color Your Classroom: Engaging Students with Bacteria and Bio-Art

EDVOTEK®

Designed for the Classroom

SINCE 1987

Introduction

Transforming bacteria with vibrant colored proteins is an unforgettable way to teach the central dogma of molecular biology. Take it further by creating bio-art with your students! In this workshop, we'll share transformation tips, explore microbial creativity, and even award our favorite designs!

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Online Resources

We've created **FREE Quick Guide manuals**, for you to download from our website:
www.edvotek.com/guides-lesson-plans

We also have many **Instructional Videos** on Youtube that show step-by-step procedures:
www.youtube.com/EdvotekInc

We hope you take advantage of these resources
and enjoy teaching and learning with EDVOTEK®!

Background Information

DNA CAN BE TRANSFERRED BETWEEN BACTERIA

In nature, DNA is transferred between bacteria using two main methods— transformation and conjugation. In transformation, a bacterium takes up exogenous DNA from the surrounding environment (Figure 1). In contrast, conjugation relies upon direct contact between two bacterial cells. A piece of DNA is copied in one cell (the donor) and then is transferred into the other (recipient) cell. In both cases, the bacteria have acquired new genetic information that is both stable and heritable.

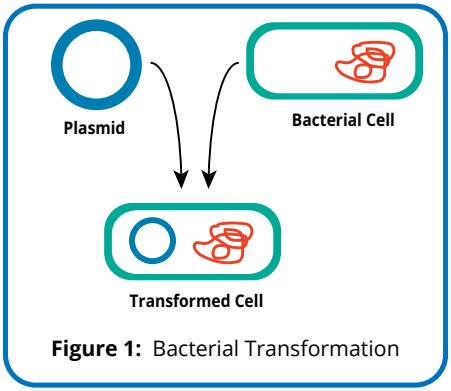


Figure 1: Bacterial Transformation

Frederick Griffith first discovered transformation in 1928 when he observed that living cultures of a normally non-pathogenic strain of *Streptococcus pneumonia* were able to kill mice, but only after being mixed with a heat-killed pathogenic strain. Because the non-pathogenic strain had been "transformed" into a pathogenic strain, he named this transfer of virulence "transformation". In 1944, Oswald Avery and his colleagues purified DNA, RNA and protein from a virulent strain of *S. pneumonia* to determine which was responsible for transformation. Each component was mixed each with a non-pathogenic strain of bacteria. Only those recipient cells exposed to DNA became pathogenic. These transformation experiments not only revealed how this virulence is transferred but also led to the recognition of DNA as the genetic material.

The exact mode of transformation can differ between bacteria species. For example, *Haemophilus influenzae* uses membrane-bound vesicles to capture double-stranded DNA from the environment. In contrast, *S. pneumoniae* expresses competency factors that allow the cells to take in single-stranded DNA molecules. In the laboratory, scientists can induce cells—even those that are not naturally competent—to take up DNA and become transformed. To accomplish this, DNA is added to the cells in the presence of specific chemicals (like calcium, rubidium, or magnesium chloride), and the suspension is "heat shocked"—moved quickly between widely different temperatures. It is believed that a combination of chemical ions and the rapid change in temperature alters the permeability of the cell wall and membrane, allowing the DNA molecules to enter the cell. Today, many molecular biologists use transformation of *Escherichia coli* in their experiments, even though it is not normally capable of transforming in nature.

GENETIC ENGINEERING USING RECOMBINANT DNA TECHNOLOGY

Many bacteria possess extra, non-essential genes on small circular pieces of double-stranded DNA in addition to their chromosomal DNA. These pieces of DNA, called plasmids, allow bacteria to exchange beneficial genes. For example, the gene that codes for ß-lactamase, an enzyme that provides antibiotic resistance, can be carried between bacteria on plasmids. Transformed cells secrete ß-lactamase into the surrounding medium, where it degrades the antibiotic ampicillin, which inhibits cell growth by interfering with cell wall synthesis. Thus, bacteria expressing this gene can grow in the presence of ampicillin. Furthermore, small "satellite" colonies of untransformed cells may also grow around transformed colonies because they are indirectly protected by ß-lactamase activity.

Recombinant DNA technology has allowed scientists to link genes from different sources to bacterial plasmids (Figure 2). These specialized plasmids, called vectors, contain the following features:

1. Origin of Replication: a DNA sequence from which bacteria can initiate the copying of the plasmid.

2. Multiple Cloning Site: a short DNA sequence that contains many unique restriction enzyme sites and allows scientists to control the introduction of specific genes into the plasmid.

3. Promoter: a DNA sequence that is typically located just before ("upstream" of) the coding sequence of a gene. The promoter recruits RNA polymerase to the beginning of the gene sequence, where it can begin transcription.

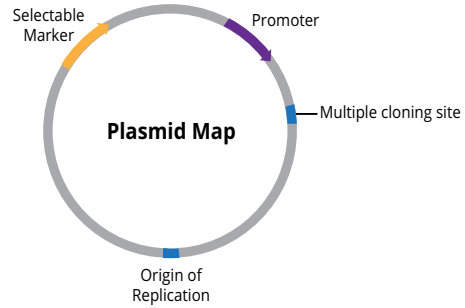


Figure 2: Plasmid Features

4. Selectable marker: a gene that codes for resistance to a specific antibiotic (usually ampicillin, kanamycin or tetracycline). When using selective media, only cells containing the marker should grow into colonies, which allows researchers to easily identify cells that have been successfully transformed.

TRANSFORMATION EFFICIENCY

In practice, transformation is highly inefficient—only one in every 10,000 cells successfully incorporates the plasmid DNA. However, because many cells are used in a transformation experiment (about 1 x 10^9 cells), only a small number of cells must be transformed to achieve a positive outcome. If bacteria are transformed with a plasmid containing a selectable marker and plated on both selective and nonselective agar medium, we will observe very different results. Nonselective agar plates will allow both transformed and untransformed bacteria to grow, forming a bacterial "lawn". In contrast, on the selective agar plate, only transformed cells expressing the marker will grow, resulting in recovery of isolated colonies.

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

Specific example:

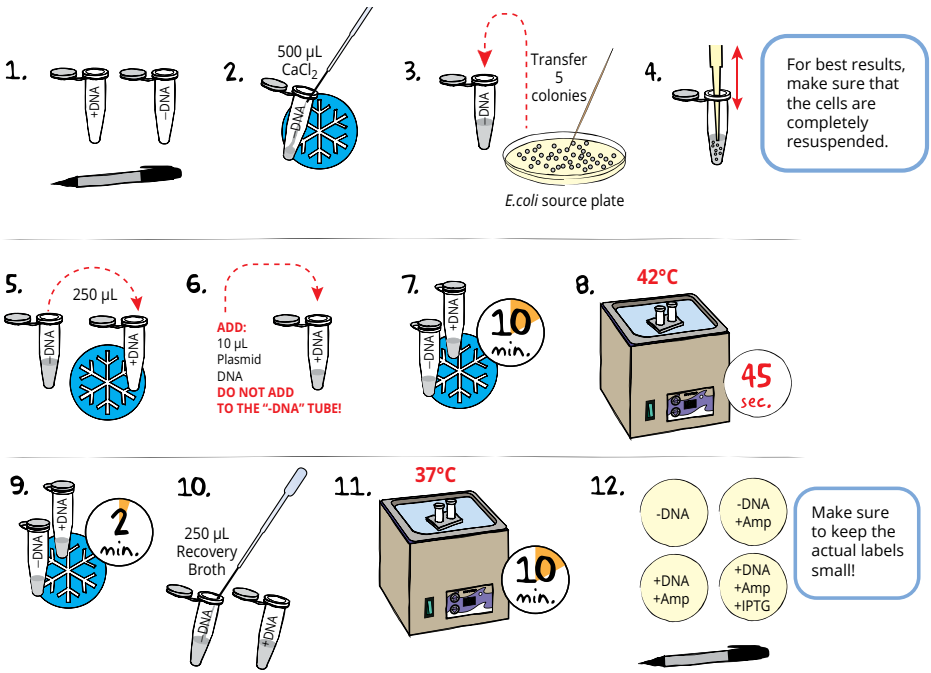
$$\frac{100 \text{ transformants}}{0.01\ \mu\text{g}} \times \frac{1\text{ mL}}{0.1\text{ mL}} = 100{,}000\ (1 \times 10^5) \text{ transformants per } \mu\text{g}$$

Figure 3:
Bacterial Transformation Efficiency Calculation

Because each colony originates from a single transformed cell, we can calculate the transformation efficiency, or the number of cells transformed per microgram (µg) of plasmid DNA (outlined in Figure 3). For example, if 10 nanograms (0.01 µg) of plasmid were used to transform one milliliter (mL) of cells, and plating 0.1 mL of this mixture (100 microliters, or 100 µL) gives rise to 100 colonies, then there must have been 1,000 bacteria in the one mL mixture. Dividing 1,000 transformants by 0.01 µg DNA means that the transformation efficiency would be 1 X 10^5 cells transformed per µg plasmid DNA. Transformation efficiency generally ranges from 1 x 10^5 to 1 x 10^8 cells transformed per µg plasmid.

Experiment Procedure

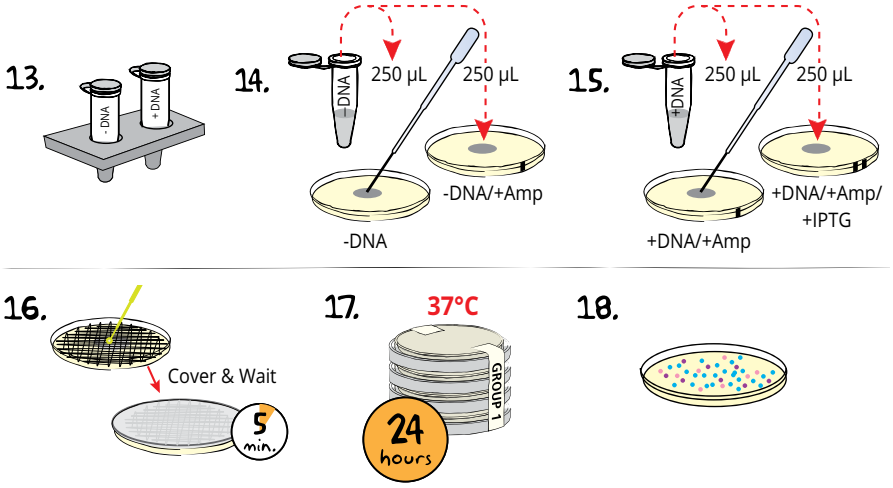
Transformation of *E. coli* with Blue, Pink, Purple Chromogenic Proteins



1. **LABEL** one microcentrifuge tube with "+DNA" and a second microcentrifuge tube with "-DNA".
2. **TRANSFER** 500 µL ice-cold $CaCl_2$ solution into the "– DNA" tube using a sterile 1 mL pipet.
3. Using a toothpick, **TRANSFER** 5 well-isolated colonies (each colony should be approx. 1-1.5 mm in size) from the *E. coli* source plate to the "-DNA" tube.
4. **RESUSPEND** the bacterial cells in the $CaCl_2$ solution by pipetting up and down until no clumps of cells are visible and the cell suspension looks cloudy.
5. **TRANSFER** 250 µL of the cell suspension to the tube labeled "+ DNA". **PLACE** tubes on ice.
6. **ADD** 10 µL of the Plasmid DNA to the tube labeled "+ DNA".
   DO NOT add the plasmid to the "-DNA" tube.
7. Gently **MIX** the samples by flicking the tubes. **INCUBATE** the tubes on ice for 10 minutes.
8. **PLACE** the transformation tubes in a 42°C water bath for 45 seconds.
9. Immediately **RETURN** the tubes to the ice bucket and **INCUBATE** for two minutes.
10. **TRANSFER** 250 µL of Recovery Broth to each tube using a sterile 1 mL pipet. Gently **MIX** by flicking the tube.
11. **INCUBATE** the cells for 10 minutes in a 37°C water bath.
12. While the cells are recovering, **LABEL** the bottom of four agar plates as indicated below.
    - **-DNA** *(plate with no stripe)*
    - **-DNA/+Amp** *(plate with one stripe)*
    - **+DNA/+Amp** *(plate with one stripe)*
      +DNA/+Amp/+IPTG *(plate with two stripes)*

Experiment Procedure, continued

Transformation of *E. coli* with Blue, Pink, Purple Chromogenic Proteins



13. After the recovery period, **REMOVE** the tubes from the water bath and place them on the lab bench.
14. Using a sterile 1 mL pipet, **TRANSFER** 250 µL recovered cells from the tube labeled " –DNA " to the middle of the -DNA and -DNA/+Amp plates.
15. Using a new sterile 1 mL pipet, **TRANSFER** 250 µL recovered cells from the tube labeled " +DNA " to the middle of the +DNA/+Amp and +DNA/+Amp/+IPTG plates.
16. **SPREAD** the cells over the entire plate using an inoculating loop. Use one sterile loop to spread both -DNA samples. Change to a fresh loop before spreading the +DNA samples. Make sure the cells have been spread over the entire surface of the plates. **COVER** the plates and **WAIT** five minutes for the cell suspension to be absorbed by the agar.
17. **STACK** the plates on top of one another and **TAPE** them together. **LABEL** the plates with your initials or group number. **PLACE** the plates in the inverted position (agar side on top) in a 37°C bacterial incubation oven for overnight incubation (24 hours). If you do not have an incubator, colonies will form at room temperature in approximately 24 - 48 hours.
18. **VISUALIZE** the transformation and control plates and **RECORD** the following:
    - The number of colonies on the plate.
    - The color of the bacteria.

Experiment Summary:

E. coli from the source plate are resuspended in an ice-cold $CaCl_2$ solution. Plasmid DNA is added to half of the cells before they are "heat shocked" in a 42°C water bath. The heat shock step facilitates the entry of DNA into the bacterial cells. Recovery Broth is added to the cell suspension, and the bacteria are allowed to recover for 10 minutes at 37°C. This recovery period allows the bacteria to repair their cell walls and to express the antibiotic resistance gene. Lastly, the transformed *E. coli* are plated on LB plates and allowed to grow at 37°C overnight.

NOTE for Step 17:
It may take longer for the cells to absorb into the medium. Do not invert plates if cells have not completely been absorbed.

Experimental Results and Analysis

DATA COLLECTION

1. Observe the results you obtained on your transformation and control plates.

   Control Plates: (-) DNA
   - -DNA
   - -DNA/+Amp

   Transformation Plates: (+) DNA
   - +DNA/+Amp
   - +DNA/+Amp/+IPTG

2 Draw and describe what you observe. For each of the plates, record the following:

   - How much bacterial growth do you observe? Determine a count.
   - What color are the bacteria?
   - Why do different members of your class have different transformation efficiencies?
   - If you did not get any results, what factors could be attributed to this fact?

DETERMINATION OF TRANSFORMATION EFFICIENCY

Transformation efficiency is a quantitative determination of the number of cells transformed per 1 µg of plasmid DNA. In essence, it is an indicator of the success of the transformation experiment.

You will calculate the transformation efficiency using the data collected from your experiment.

1. Count the number of colonies on the plate that is labeled: +DNA/+Amp/+IPTG

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

2. Determine the transformation efficiency using the following formula:

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

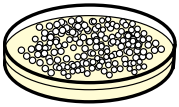
EXAMPLE:
Assume you observed 40 colonies:

$$\frac{40 \text{ transformants}}{0.05 \text{ µg}} \times \frac{0.5 \text{ mL}}{0.25 \text{ mL}} = 1600 \ (1.6 \times 10^3) \text{ transformants per µg}$$

QUICK REFERENCE:

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

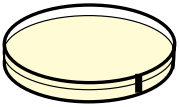
Experiment Results and Analysis



-DNA plated with control cells (no DNA)

Result: No colored cells visible. White colonies. May look like a smeared layer of cells.

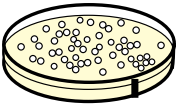
Demonstrates: Host bacterial cells are viable in the absence of ampicillin.



-DNA/+Amp plated with control cells (no DNA)

Result: No colonies visible.

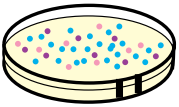
Demonstrates: Untransformed cells are sensitive to ampicillin.



+DNA/+Amp plated with transformed cells (Rainbow Transformation Mixture)

Result: White colonies.

Demonstrates: Cells are resistant to Ampicillin when transformed with the mixture of three plasmids (pChromoBlue, pChromoPink, pChromoPurple). The colored proteins are not produced in the absence of IPTG.



+DNA/+Amp/+IPTG plated with transformed cells (Rainbow Transformation Mixture)

Result: Individual pink, blue, and purple colonies.

Demonstrates: Cells are resistant to Ampicillin when transformed with the mixture of three plasmids (pChromoBlue, pChromoPink, pChromoPurple). Production of chromogenic protein is turned on in the presence of IPTG.

Incorporating STEAM into Your Transformation Experiment

Art, design, and innovation are key components to a balanced science education. Incorporating Art into your STEM curriculum can be a fun way to engage students and build a truly integrated curriculum. In this simple STEAM experiment the bacteria previously transformed by your students will become living paint for their artwork.

All You Need:

- ReadyPour™ Luria Broth Agar Base with Ampicillin *Cat. #616*
- Large Petri Plates *Cat. #643*
- IPTG *Cat. #613*
- Luria Broth Media *Cat. #611*
- Sterile Loops *Cat. #667*

Brief Experimental Procedure

Each student or student group will share the transformed bacterial plate.

1. Using a sterile loop, scrape bacteria off of the transformation plate and resuspend in 1 mL of LB or Recovery Broth in a snaptop tube.
2. Using the same loop, spread the resuspended bacteria onto a fresh LB/Amp/IPTG plate.

NOTE: Encourage students to sketch their design before streaking onto their plates.

3. Place the plates in an inverted position in a 37°C incubator overnight before viewing the results.

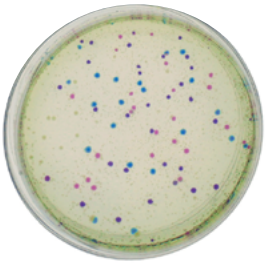
Transformation Troubleshooting

PROBLEM:	CAUSE:	ANSWER:
Poor cell growth on source plate	Incubation time too short	Continue to incubate source plate at 37ºC for a total of 16-20 hours.
	Antibiotic added to source plate	When pouring plates, be sure to add antibiotics & additives at the correct step.
	Incorrect incubation temperature	Use a thermometer to check incubator temperature. Adjust temp. to 37°C if necessary.
Satellite colonies seen on transformation plate	Incorrect concentration of antibiotics in plates	Ensure the correct concentration of antibiotic was added to plates - Make sure ReadyPour is cooled to 60°C before adding antibiotic.
	Antibiotic is degraded	Make sure ReadyPour is cooled to 60°C before adding antibiotic.
	Plates were incubated too long	Incubate the plates overnight at 37°C (24 hours).
Colonies appeared smeary on transformation plate	Plates containing transformants were inverted too soon	Allow cell suspension to fully absorbed into the medium before inverting plates.
	Experimental plates too moist	After pouring plates, allow them dry overnight at room temp. Alternatively, warm plates at 37°C for 30 min. before plating cells
No colonies seen on transformation plates	Plasmid DNA not added to transformation mix	Ensure plasmid DNA was added to transformation tube.
		Make sure that pipets are used properly. If using micropipets, make sure students practice using pipets
	Incorrect host cells used for transformation	Confirm that correct bacterial strain was used for transformation
	Cells were not properly heat shocked	Ensure that temp. was 42°C & heat shock step took place for no more than 90 seconds.
	Incorrect antibiotics	Be certain that the correct antibiotic was used.
	Cells not well resuspended in $CaCl_2$	Completely resuspend the cells in the $CaCl_2$, leaving no cell clumps (vortex or mix vigorously to fully resuspend cells). Cell suspension should be cloudy.
Low transformation efficiency	Not enough cells used for transformation	Pick more colonies from source plate (1-2 colonies @ 1-2 mm width per 500µl $CaCl_2$)
	Source plates were incubated for more than 20 hours	Important that source cells grow no longer than 20 hrs. Refrigerate plates after 20 hrs if necessary. Do not use source plates that have been incubated longer than 24 hours, refrigerated or not).
	Experimental plates too old	Prepare transformation plate and use shortly after preparation
	Cells not well resuspended in $CaCl_2$	Completely resuspend the cells in the $CaCl_2$, leaving no cell clumps (vortex or mix vigorously to fully resuspend cells). Cell suspension should be cloudy.
	$CaCl_2$ solution not cold enough	Pre-chill $CaCl_2$ before adding cells to the $CaCl_2$
	Cell solution not cold enough	Extend incubation of celll suspension on ice 10-15 min. (should not exceed 30 min. total). This increases the transformation efficiency.
	Too much or too little plasmid DNA added to cell suspension	Ensure that correct volume of plasmid was added to the transformation tube. If using micropipets, make sure students practice using pipets.
	Cells were not properly heat shocked	Ensure that temperature was 42°C and that heat shock step took place for no more than 90 seconds.
	Antibiotics were degraded prior to pouring plates	Make sure ReadyPour is cooled to 60°C before adding antibiotic.
	Incorrect concentration of antibiotics in plates	Ensure that the correct concentration of antibiotic was used

Workshop Products

Cat. #224

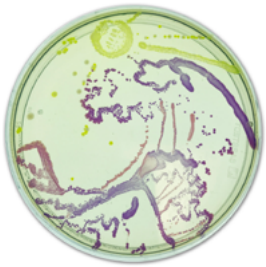
Rainbow Transformation



For 10 groups. Transformation is of central importance in molecular cloning since it allows for the selection, propagation, expression and purification of a gene. Positive selection for cells containing plasmid DNA is accomplished by antibiotic growth selection. In this experiment, your students will transform bacteria with a new set of rainbow color plasmids that transform non-pathogenic bacterial cells into bright, colorful cells.

Cat. #228

Agar Art: Creating Masterpieces with Microbes



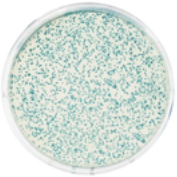
For 25 students. At the intersection of art, science, and technology is Bio-Art, the creation of works of art using living matter. One common way to create Bio-Art uses bacteria transformed with DNA codes for brightly-colored proteins. In this experiment, students create "Bio-Paints" using bacterial culture techniques. The color-producing microbes are painted onto an agar plate canvas. After incubation will inspect, admire, and hopefully share your living art.

Details for all these products and MORE can be found on our website!

Related Products

Cat. #221

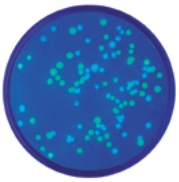
Transformation of *E.coli* with pGAL™ (Blue Colony)



For 10 groups. In this experiment, your students can see a blue color change in transformed cells due to the switching on of a gene. The pGAL™ plasmid gives them a blue color due to the production of the ß-galactosidase protein by the lacZ gene. IPTG is not required in this experiment since pGAL™ contains the complete lacZ gene.

Cat. #222

Transformation of *E.coli* with Blue and Green Fluorescent Proteins



For 10 groups. Green Fluorescent Protein (GFP), which is responsible for bioluminescence in the jellyfish *Aequorea victoria*, is used extensively in all areas of science. Many organisms have been transformed with the GFP gene. It has proven to be so useful that scientists have mutated it to produce Blue Fluorescent Protein (BFP). In this simple experiment, your students will transform bacteria either with GFP, BFP or both!

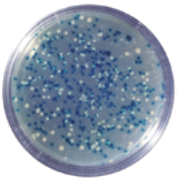
Cat. #223-AP08

Transformation of *E.coli* with Green Fluorescent Proteins

For 10 groups. Transformed cells take up a plasmid containing the GFP gene. The GFP gene was isolated from the jellyfish *Aequorea victoria*. Transformed colonies expressing the GFP protein are visibly green in normal light but will fluoresce brightly when exposed to long wave UV light.

Cat. #300

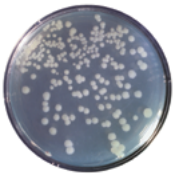
Blue/White Cloning of a DNA Fragment & Assay of ß-galactosidase



For 5 groups. When DNA is subcloned in the pUC polylinker region, ß-galactosidase production is interrupted, resulting in the inability of cells to hydrolyze X-Gal. This results in the production of white colonies amongst a background of blue colonies. This experiment provides a DNA fragment, linearized plasmid, and T4 DNA Ligase. Following the ligation to synthesize the recombinant plasmid, competent E.coli cells are transformed and the number of recombinant antibiotic resistant white and blue colonies are counted. ß-galactosidase activity is assayed from blue and white bacterial cells. This experiment can be broken down into three modules: ligation, transformation, and assay of ß-galactosidase.

Cat. #301

Construction & Cloning of a DNA Recombinant



For 5 Plasmid Constructs & Analyses. Cloning is frequently performed to study gene structure, function, and to enhance gene expression. This experiment is divided into five modules. Clones are constructed by ligation of a vector and a fragment insert. The constructs are then transformed into competent cells and the cells are grown and selected for resistance. Plasmid DNA is then isolated from the transformants, cleaved with restriction enzymes, and analyzed by agarose gel electrophoresis. Recommended for college level courses.

Related Products

Edvotek® 3.0 L Digital Water Bath

This classic Edvotek® water bath has been updated & improved yet again - 60% larger than its predecessor and now has a built-in timer! The water bath still includes a digital temperature control and low-water sensor to prevent burn-outs. The stainless steel chamber is corrosion resistant and temperature-controlled from ambient to 99°C while using the included cover.
Cat# 539

EDVOTEK® 12 L Digital Waterbath

The all-new Edvotek® 12 L water bath has a built-in timer, digital temperature control, and low-water sensor to prevent burn-outs. The stainless steel chamber is corrosion resistant and temperature controlled from ambient to 99°C while using the included covers. The unit also includes a convenient hose that makes it easy to drain and clean.
Cat# 538

Incubation Oven

This economical bacterial incubator features digital temperature control with a range from 5°C above ambient to 60°C. Ideal for growing bacteria on agar plates at 37°C or for Southern and Western Blot analysis at 60°C. Includes two adjustable/removable shelves for increased capacity. Accepts bottles and flasks up to 2 L.
• Internal Dimensions: 23.5 x 29 cm
• External Dimensions: 26 x 23.5 x 32.5 cm
Cat# 546

Fixed Volume MiniPipet™

40 µL MiniPipet™
Cat. # 588

EDVOTEK® Variable Micropipette

5-50 µL Micropipette
Cat. # 590

Details for all these products and MORE can be found online!

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