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Terrific Transformations

PLTW SUMMIT

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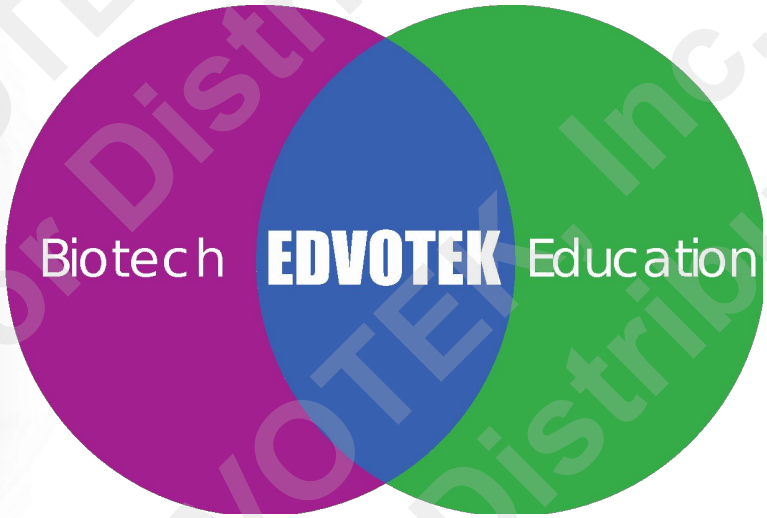
The **Biotechnology Education** Company



Celebrating 30 years of science education!

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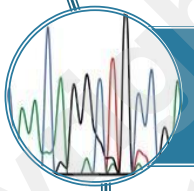
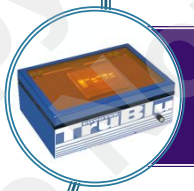
Experiments



Reagents



Equipment

Resources

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Edvotek PLTW Experiments

Kit	Description
118-PLTW → 418	Hypercholesterolemia Kit
225-PLTW → 425	DNA Detectives Kit
235-PLTW → 435	DNA Microarrays
268-PLTW → 468	Mystery Infections Kit
303 - PLTW → 403	Exploring Biotechnology with GFP
339 - PLTW → 439	Sequencing The Human Genome Kit
990-PLTW → 490	Morphology of Cancer Cells Kit
301	Construction and Cloning of a DNA Recombinant
330	PCR Amplification of DNA
491 (1.1.4)	Forensic Blood Typing
401 (4.2.3)	Under the Sea
485	Preparation of Metaphase Chromosome Spread
430	DNA Fingerprinting

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Edvotek Equipment

Cat #	Description
581	MV10 Electrophoresis Apparatus
5010	TetraSource™ 300 Digital Electrophoresis PS
557	TruBlu™ Blue Light Transillumiantor
589 / 591 / 592-1	Edvotek Variable Micropipets, 0.5-10 µl, 2-20 µl, 10-100 µl, 100-1000 µl
546	Bacterial Incubator
539	Water Bath

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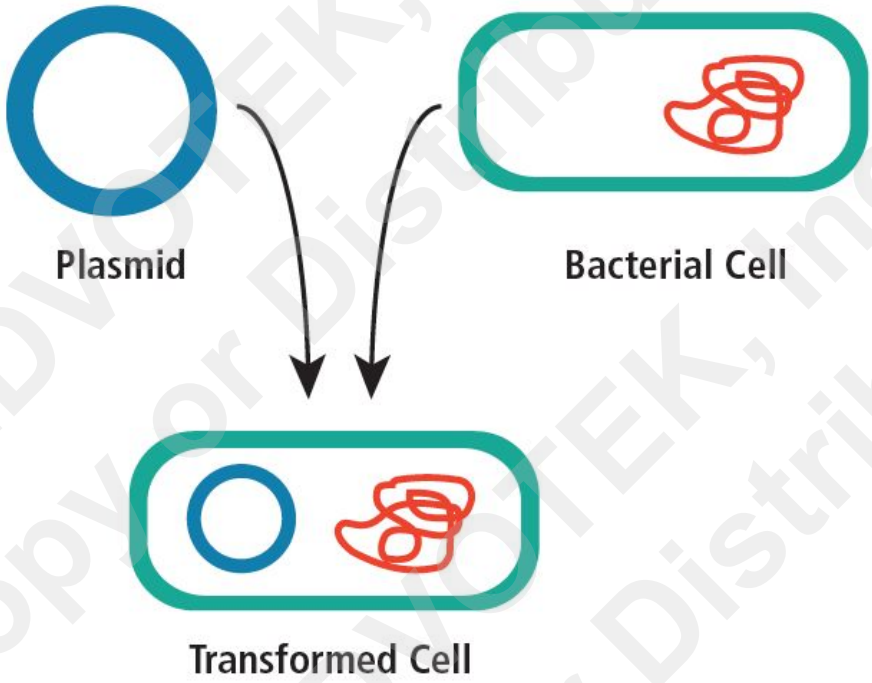
What is bacterial transformation?

- In nature, some bacteria can acquire exogenous DNA from the surrounding environment in a process called transformation.
- Small circular DNA molecules called plasmids allow bacteria to exchange beneficial genes.
  - antibiotic resistance
  - virulence factors
  - nitrogen fixation
- The newly acquired genetic information is both stable and heritable.

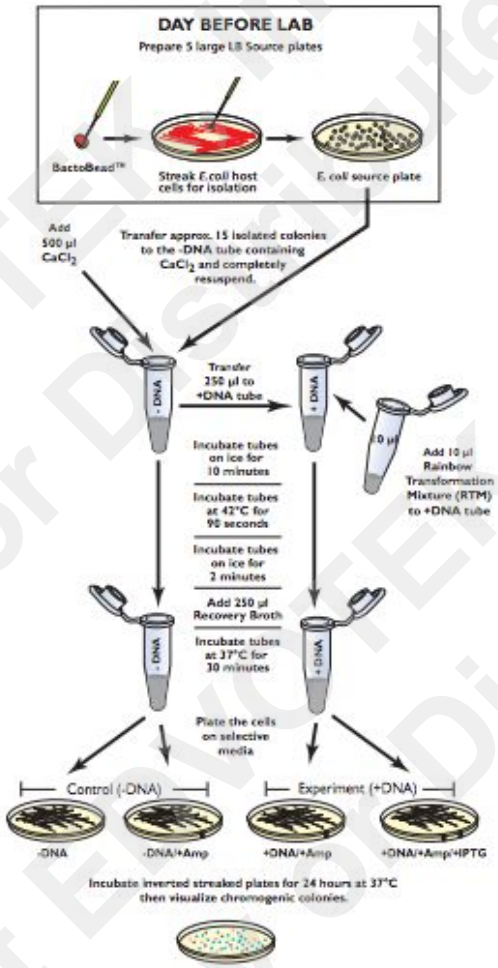


Recombinant DNA can be Transformed Into Bacteria

- In the laboratory, we can induce bacteria to take up DNA from the environment.
- The cells are treated with calcium chloride to make them "competent" for transformation.
- DNA is added to the treated cells, and the suspension is "heat shocked".
- The cells become living factories to produce a useful product.

Transformation Tricks and Tips

- Trick #1: Proper preparation of the Agar Plates.
- Trick #2: Prepare the source plate 16-18 hours before transformation
- Trick #3: Keep everything cold!
- Trick #4: Add the Correct Amount of Plasmid DNA
- Trick #5: Time and Temperature of Heat Shock
- Trick #6: Let the cells recover

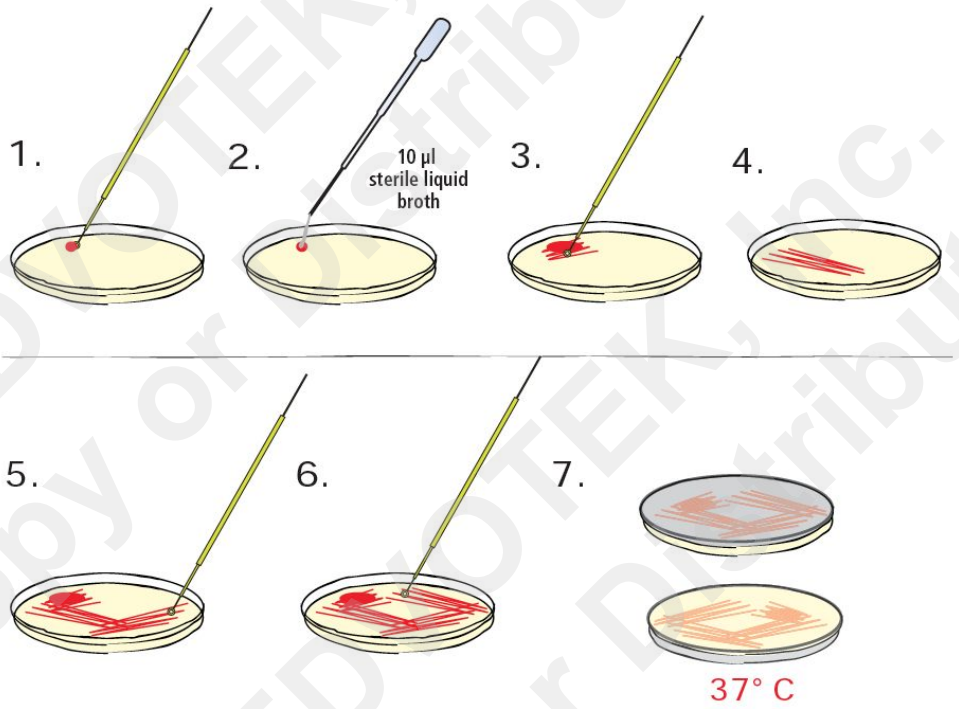


Trick #1: Proper preparation of the LB-Agar Plates

- These petri plates contain a solid nutrient agar that we use to culture microorganisms.
- Reagents like IPTG and Ampicillin are heat sensitive, so be sure to cool the media to 60°C before adding.
- Prepare plates 2-7 days before performing the experiment.
  - Leave at RT for 12-24 hours after pouring

Trick #2: Prepare the source plate **16-18 hours before** transformation

- Prepare the *E. coli* source plate with a great quadrant streak.
  - Remove one *E. coli* BactoBead™ from the vial
  - Place bead on LB agar plate
  - Add a drop of sterile broth to dissolve the BactoBead™
  - Use the loop to spread the cells on the plate
- Incubate the source plate at 37°C for 16-18 hours.



Trick #3: Keep everything cold!

- Pre-chill the reagents before starting the lab.
- Resuspend the cells by pipetting up and down until no clumps are visible.
  - If the cells are not completely resuspended, the plasmid will not come into contact with most of the bacterial cells.
- Keep the cells cold during the resuspension. This ensures the cells are at the correct temperature before the heat shock.
  - Hold the top of the tube to prevent heat transfer.

Preparing Competent Bacteria for Transformation

1. **LABEL** one tube with "+DNA" and a second with "-DNA".
2. **TRANSFER** 500 µL ice-cold $CaCl_2$ solution into the "-DNA" tube using a sterile one mL pipet. **PLACE** tube on ice.
3. Using a sterile loop, **TRANSFER** 5 well-isolated colonies from the *E. coli* source plate to the "-DNA" tube.
4. **TWIST** the loop between your fingers to free the cells. **RESUSPEND** the bacterial cells in the $CaCl_2$ 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 plasmid DNA to the tube labeled "+DNA".
7. **INCUBATE** the tubes on ice for 10 min.

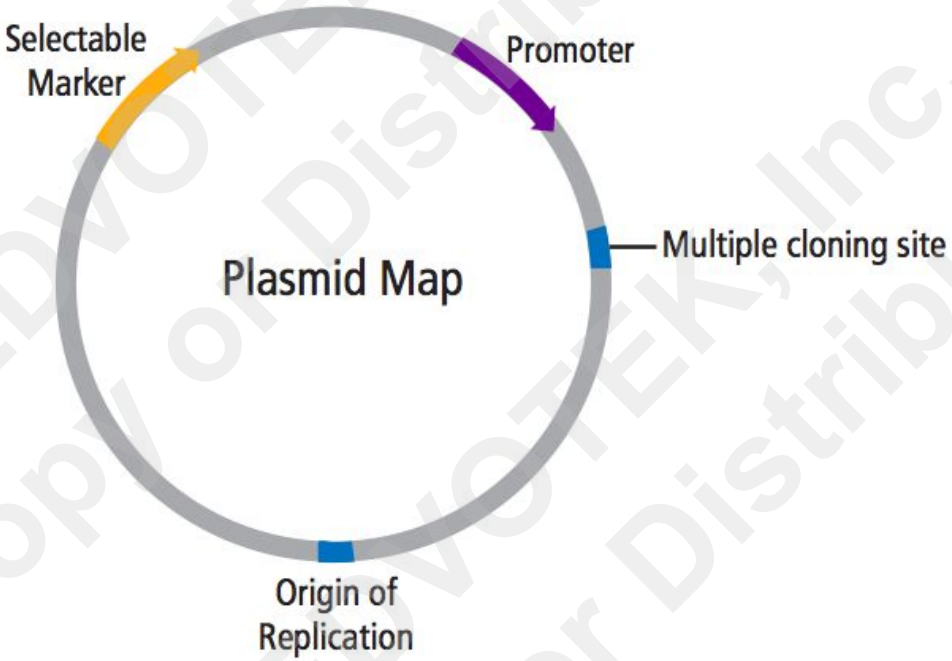
Trick #4: Add the Correct Amount of Plasmid DNA

- Adding too much or too little plasmid DNA will reduce the transformation efficiency.
- Pipetting error can affect your student's results.
- Make sure your students know how to accurately pipet before the experiment by practicing

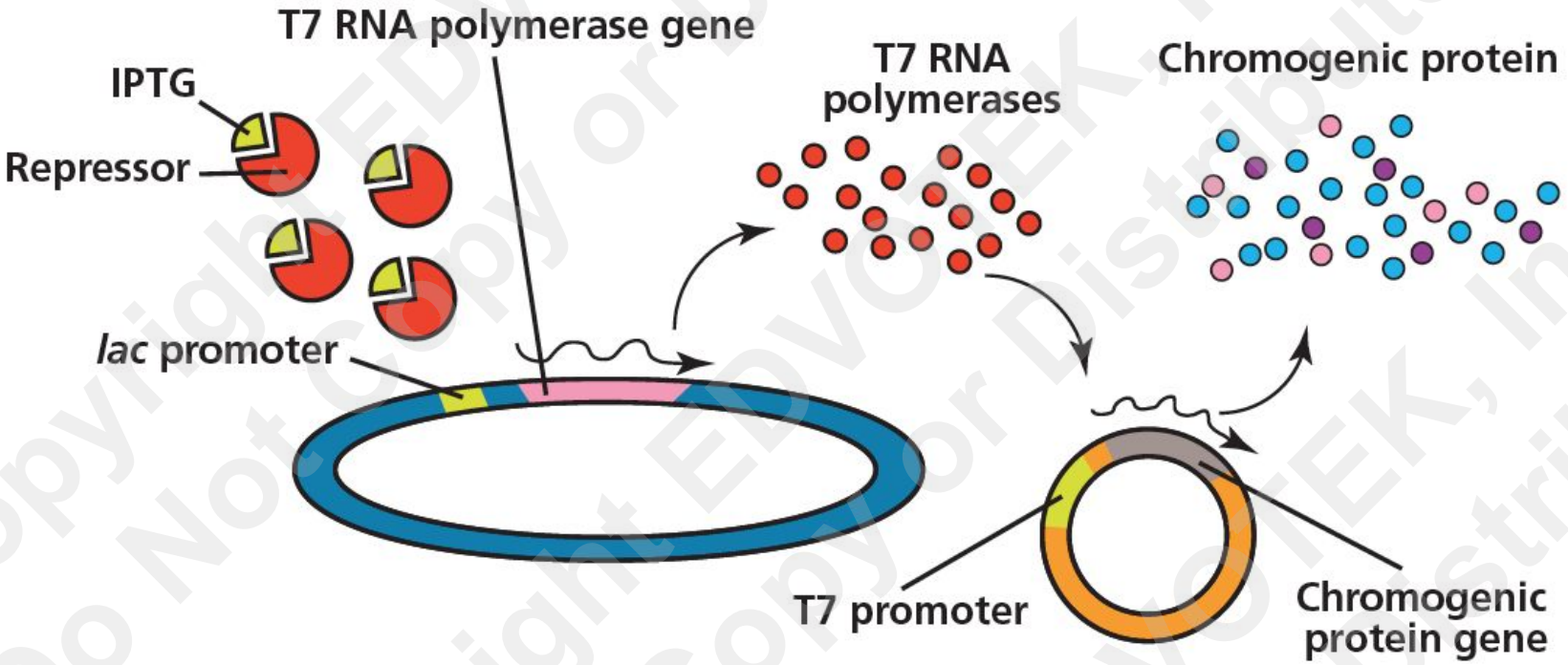
***PLTW version coming soon!**

Recombinant Plasmids are used for Transformation

- Origin of Replication: a DNA sequence from which bacteria can initiate the copying of the plasmid.
- Promoter: a DNA sequence that recruits RNA polymerase to the gene.
- Multiple Cloning Site: a short DNA sequence that contains many unique restriction enzyme sites.
- Selectable marker: a gene that allows researchers to identify cells that have been successfully transformed.



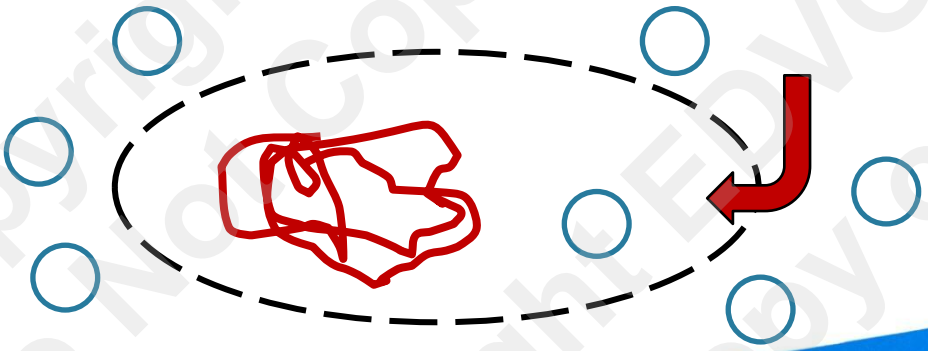
Expression of Recombinant Protein is Controlled by an Inducible Promoter



Inducible promoters allow precise control because expression of the gene will only "turn on" in the presence of a small molecule like arabinose, tetracycline, or IPTG.

Transformation of Bacteria by Heat Shock

- After DNA is added to the cells, the suspension is "heat shocked" — or moved quickly between widely different temperatures.
- The combination of chemical ions and the rapid change in temperature alters the permeability of the cell wall and membrane, allowing DNA molecules to enter the cell.

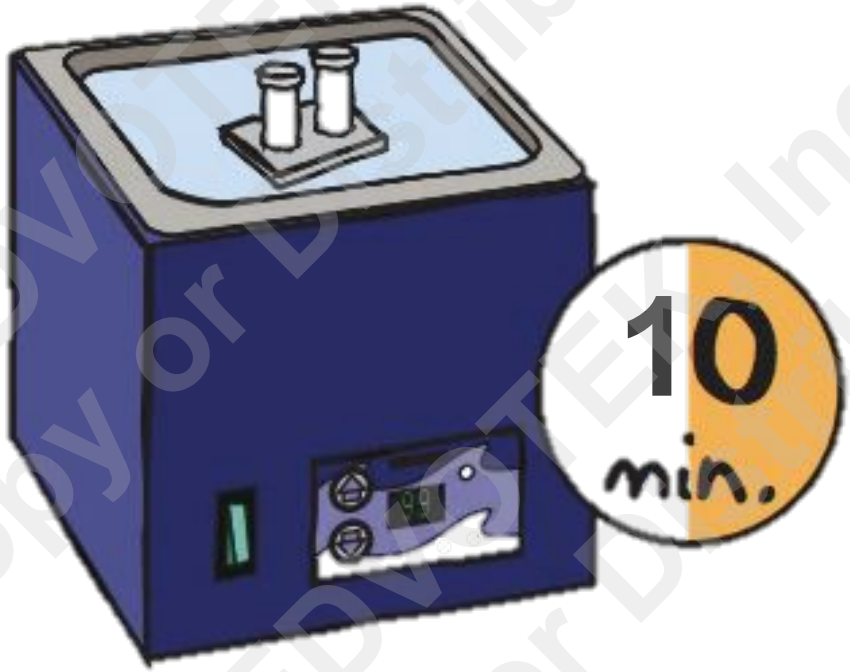


Trick #5: Time and Temperature of Heat Shock

- For best results, be sure to keep the cell suspension ice-cold before and after the heat shock.
- Use a thermometer to confirm that the water bath reaches 42°C.
- Make sure the cells are only heat shocked for 45 seconds.

Trick #6: Let the cells recover

- During this incubation, the bacteria repair their cell walls and express the antibiotic resistance gene.
- If the cells are plated too soon, they may not be able to grow on the selective media.
- After 20-30 minutes of recovery, the bacteria will double, meaning that many of the "transformants" are produced by cell division.



Transformation of Bacteria by Heat Shock

1. **PLACE** the transformation tubes in a water bath at 42° C for 45 seconds. ***This heat shock step facilitates the entry of DNA by E. coli.***

2. Immediately **RETURN** the tubes to the ice bucket and **INCUBATE** for two min.

3. **TRANSFER** 250µL of Luria Recovery Broth to the tubes using a sterile one mL pipet. Gently **MIX** by flicking the tube.

4. **INCUBATE** the cells for 10-30 minutes in a 37° C water bath. ***This recovery period allows cells to repair their cell walls and to express the antibiotic resistance gene.***

5. While the cells are recovering, **LABEL** the bottom of four agar plates as specified in the protocol.

Plating the Cells on Selective Media

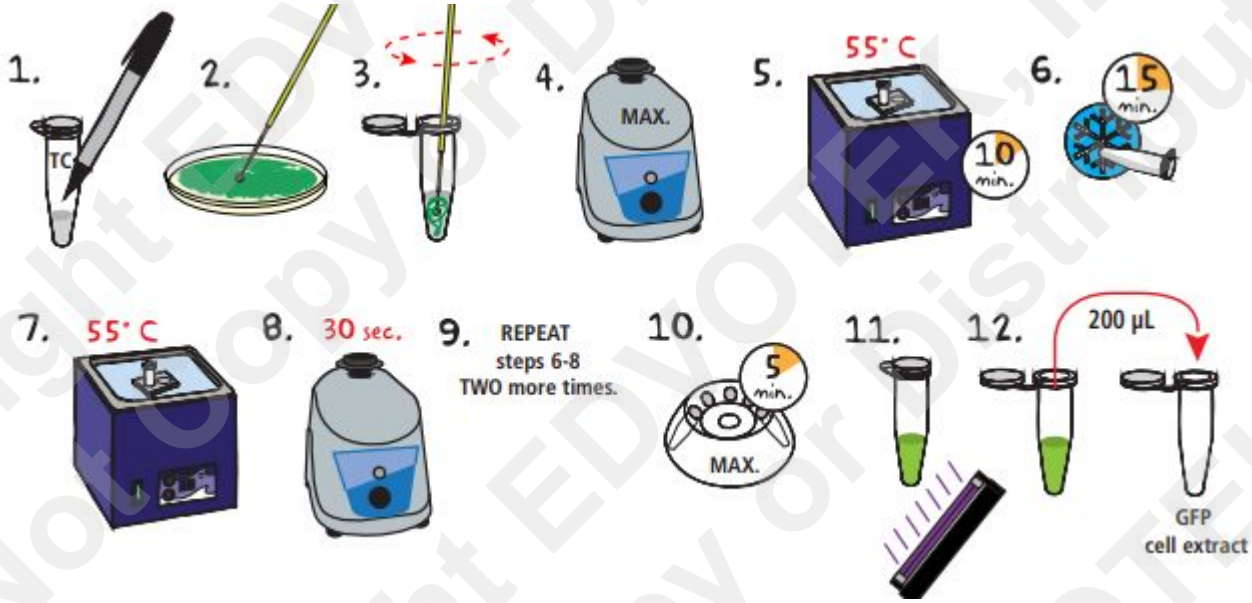
1. After the recovery period, **REMOVE** the tubes from the water bath.
2. **TRANSFER** 250 µL recovered cells from the tube labeled "–DNA" to the middle of the –DNA plates.
3. Using a new sterile 1 ml pipet, **TRANSFER** 250 µL recovered cells from the tube labeled "+DNA" to the middle of the +DNA plates.
4. **SPREAD** the cells over the plate using a sterile loop. **COVER** the plates.
5. **WAIT** five minutes for the cells to be absorbed by the agar.
6. **STACK** the plates on top of one another and TAPE them together.
7. **PLACE** the plates in a 37° C incubator for 16-18 hours.
8. **VISUALIZE** the transformation and control plates as per protocol.

-DNA
plated with
non-transformed cells
(no DNA)

Result: No fluorescent cells visible. White colonies. Will likely look like a smeared layer of cells (lawn).

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

303-PLTW Other Tips & Tricks

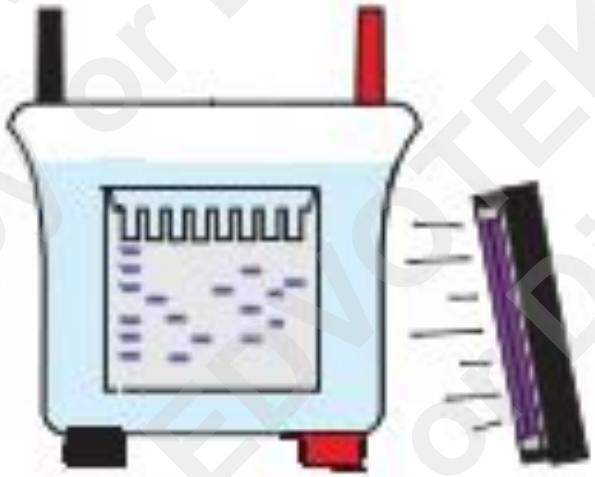


- Place a beaker with Isopropanol or 70% Ethanol in your freezer the hour before and float the samples in it
- Incubate at 55 for the entire 10 minutes

303-PLTW Other Tips & Tricks

- Packed column can be stored overnight

303-PLTW Other Tips & Tricks



- The protein denaturing solution will be blue
- Keep track of the loading dye as the bands migrate
- Watch ladder

303-PLTW Resources

Edvotek Kit #303 - Pre-lab Preparations - Student...

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Edvotek Kit #303 - Student Module II - Isolation of GFP

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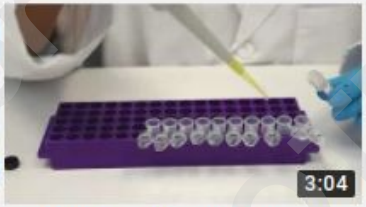

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Edvotek Kit #303 - Student Module I - Transformation...

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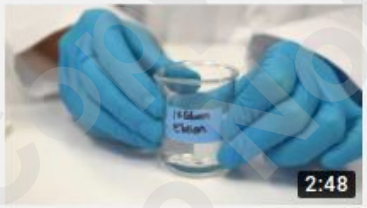

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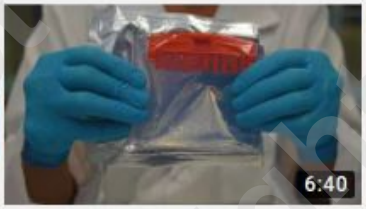

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Resources

QUICK GUIDES

- Quick Guide - Using Technology to Create a DNA Standard Curve
- Quick Guide - Agarose Gel Electrophoresis
- Quick Guide - Culturing Microorganisms
- Quick Guide - Micropipeting Basics
- Quick Guide - Polymerase Chain Reaction
- Quick Guide - Protein Electrophoresis
- Quick Guide - Transformation
- Quick Guide - Quick Plants™
- Quick Guide - Visualizing DNA
- Quick Guide - SYBR® Safe Stain
- Quick Guide - Transfer Pipets
- Quick Guide - Biotechnology Basics: Outfitting Your Biotech Classroom
- Quick Guide - Biotechnology Basics: Innovations In Your Classroom

PLASMID MAPS

- Plasmid Map - pEdvo301 (Kit 301)
- Plasmid Map - pFluoroGren (Kits 223, 303, 305)
- Plasmid Map - pFluoroGreen and pFluoroBlue (Kit 222)
- Plasmid Map - pGAL (Kit 221)

TROUBLESHOOTING GUIDES

- Troubleshooting Guide - DNA Extraction & Human PCR
- Troubleshooting Guide - PCR & Electrophoresis
- Troubleshooting Guide - Transformation
- Troubleshooting Guide - Protein
- Troubleshooting Guide - *C. elegans*

LESSON PLANS

- Lesson Plan - Left at the Scene of the Crime! An Introduction to Forensic Science
- Lesson Plan - Outbreak! Learning About Zika Virus Transmission and Testing
- Lesson Plan - Exploring DNA
- Lesson Plan - Modeling DNA Amplification by Polymerase Chain Reaction (PCR)

Genetic Engineering and Transformation

- Transformation of *E. coli* with Blue and Green Fluorescent Proteins
- Transformation of *E. coli* with pGAL™
- Rainbow Transformation
- Construction and Cloning of a DNA Recombinant

Adding Art to your Transformation experiment

- Incorporating art or design into your transformation experiment can be as simple or involved as you would like to make it.
- Bacteria can become “living paint” for student art projects.
- Extension activity, Kit #226, can work with any transformation experiment.

Adding Art to your Transformation experiment

1. Scrape bacteria off of the transformation plate and resuspend in 1 ml of LB or Recovery Broth.

2. Using a loop, toothpick, or paintbrush, spread the resuspended bacteria onto a fresh LB/Amp/IPTG plate.

3. Invert plates and place into a 37˚C incubator overnight.

Edvotek PLTW Experiments

Kit	Description
118-PLTW	Hypercholesterolemia Kit
225-PLTW	
235-PLTW	
268-PLTW	
301-PLTW	
303 - PLTW	
339 - PLTW	
990-PLTW	
116	
330	
345	
951	
953	
956	Bioremediation by Oil-eating Bacteria Kit

Mistakes ~~Hurt!~~ Happen!

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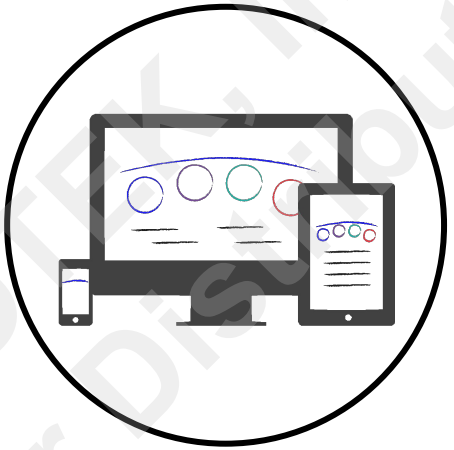

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