Exploring STEAM with Transformation

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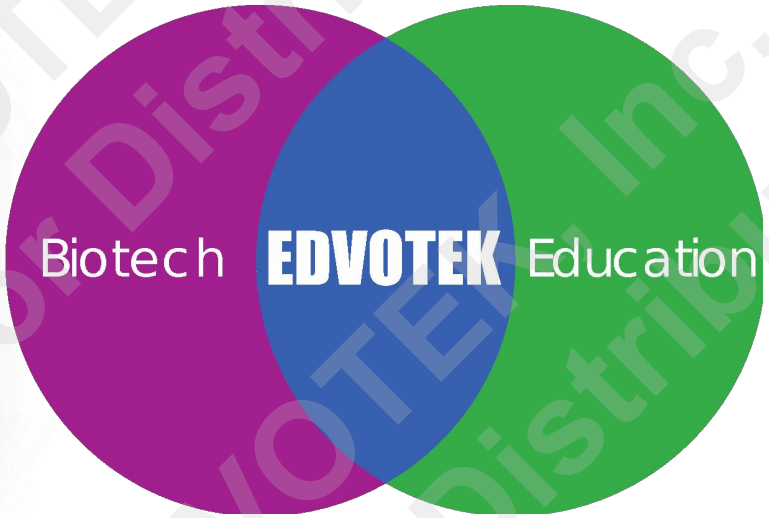

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What is STEAM, Anyway?

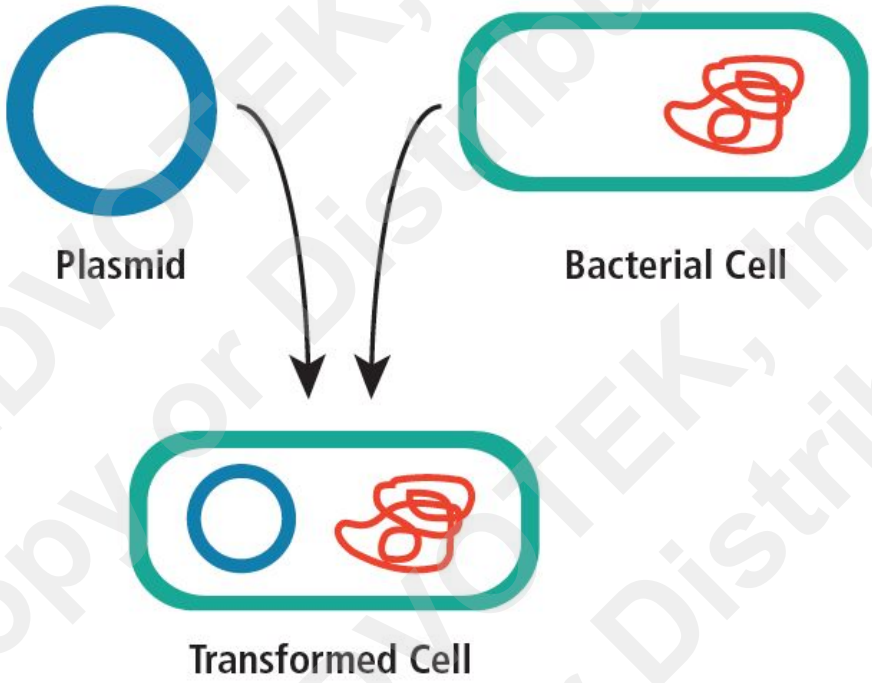
- STEAM integrates Science, Technology, Engineering, Art and Mathematics into one interdisciplinary unit.
- Students learn to make connections between STEAM concepts in the classroom and its practice in the real world.

Incorporating STEAM Into Your Curriculum

- Students will introduce a foreign plasmid into E.coli and select for a desired phenotype.
- Bacterial transformation experiments introduce students to a fundamental technique in research and industrial molecular biology labs.
- These labs provide an opportunity to discuss hot topics in biology, introduce or refine lab techniques, and fully explore STEAM concepts.

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.

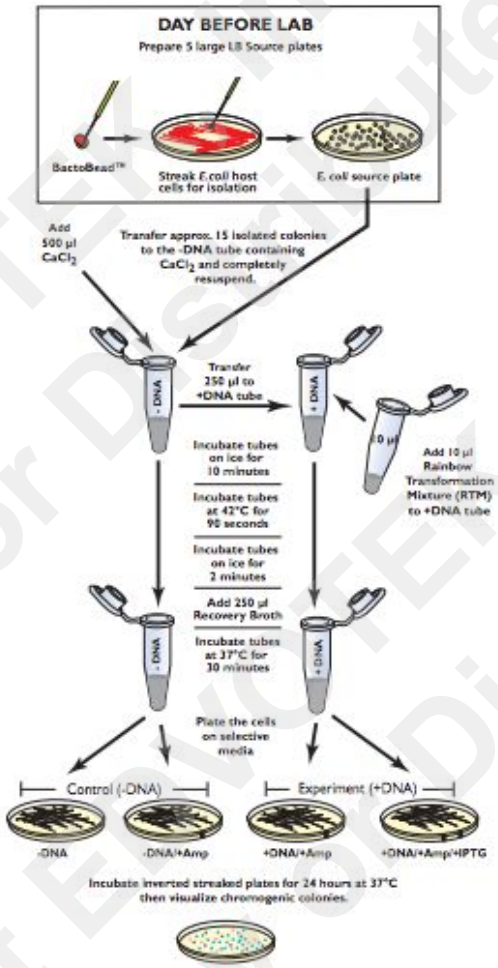


Recombinant DNA can be Transformed Into Bacteria

- 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

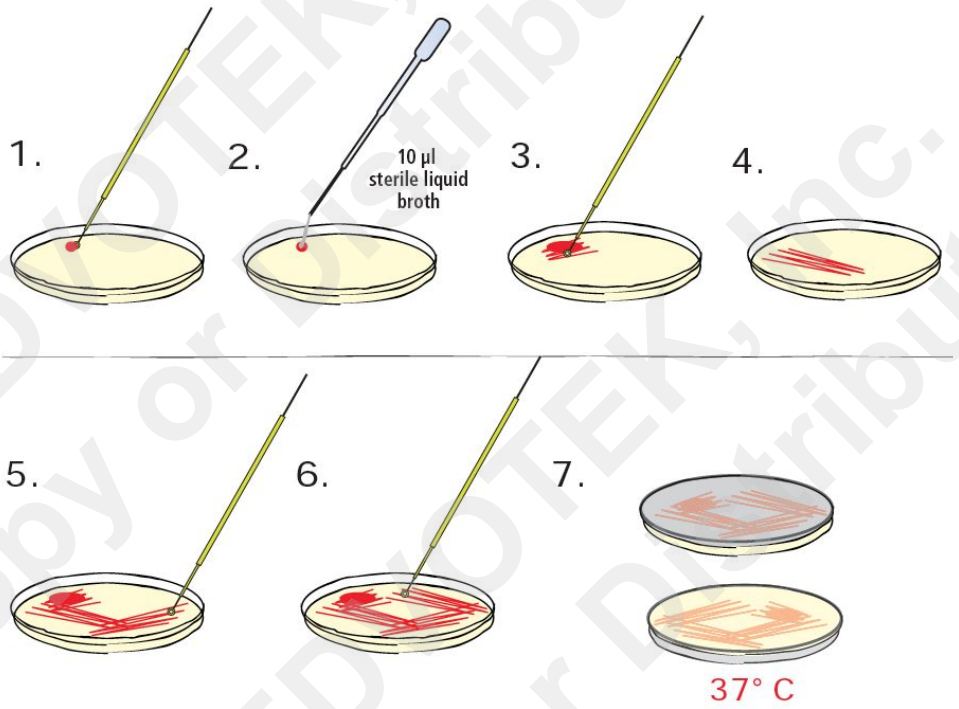


Trick #1: Proper preparation of the LB-Agar Plates

- 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.

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.

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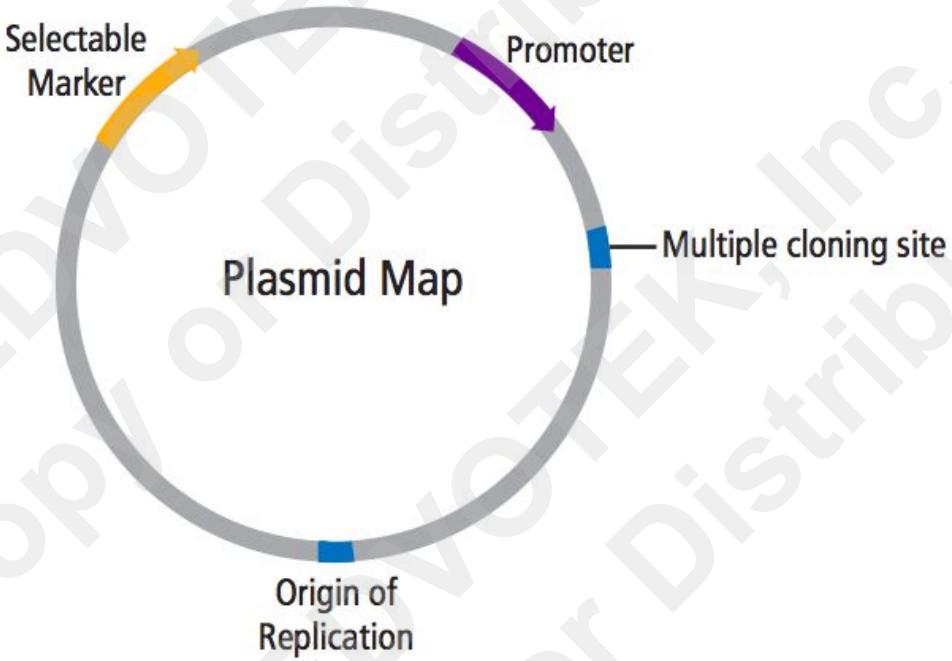
Trick #4: Add the Correct Amount of Plasmid DNA

- 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

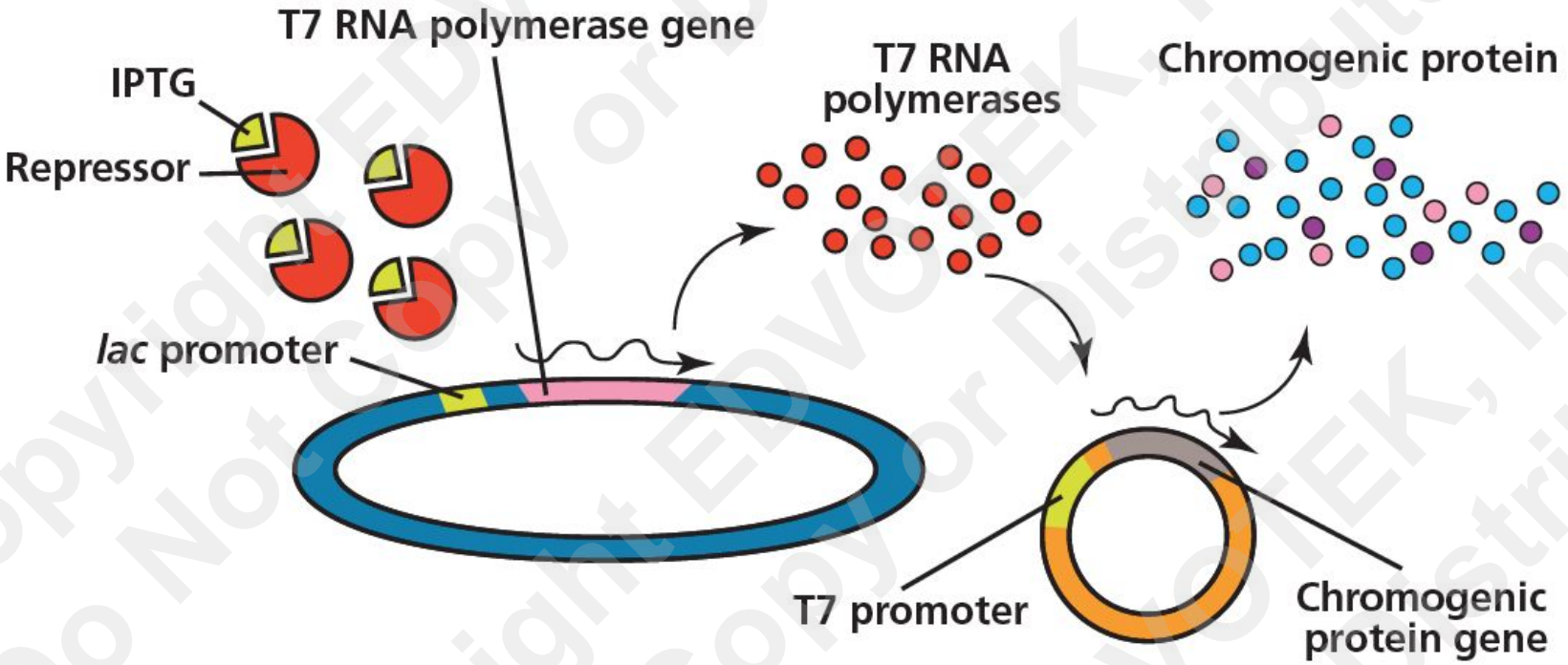
#S-44

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.



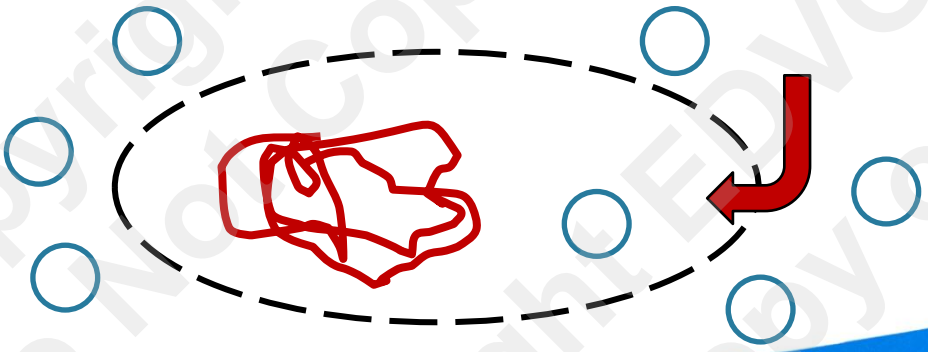
Expression of Recombinant Protein is Controlled by an Inducible Promoter



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.

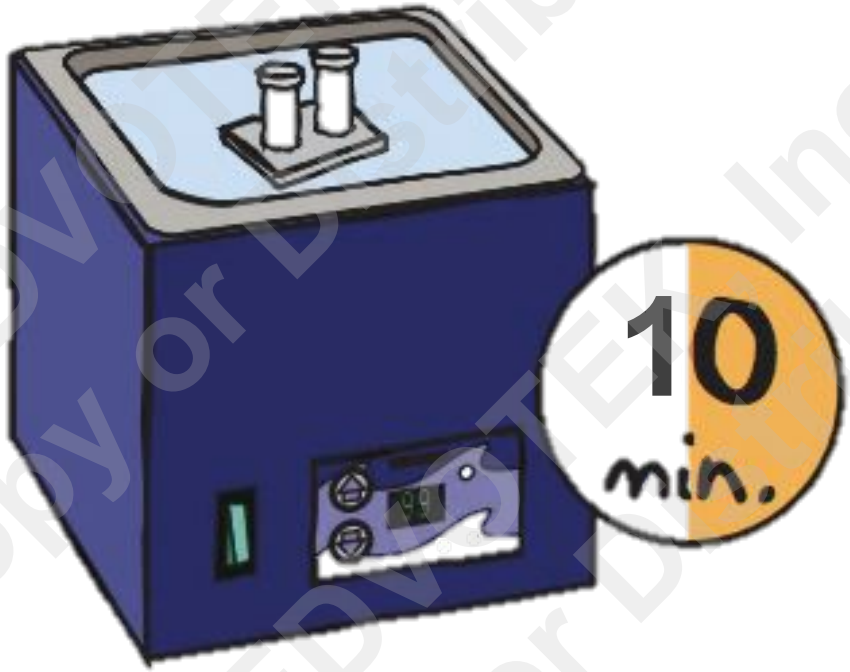


Trick #5: Time and Temperature of Heat Shock

- 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.

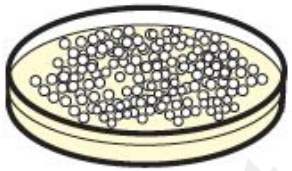
4. **INCUBATE** the cells for 10-30 minutes in a 37° C water bath. ***This recovery period allows cells to repair their cell walls and to express the antibiotic resistance gene.***

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

Plating the Cells on Selective Media

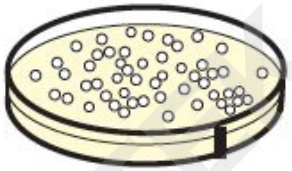
1. After the recovery period, **REMOVE** the tubes from the water bath.
2. Using a sterile 1 ml pipet, **TRANSFER** 250 µL recovered cells from the tube labeled "–DNA" to the middle of the –DNA plates.
3. Using a new sterile 1 ml pipet, **TRANSFER** 250 µL recovered cells from the tube labeled "+DNA" 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.

Results and Conclusions

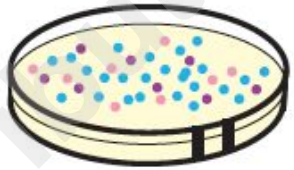


-DNA
plated with control cells
(no DNA)

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



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



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

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.

From Peas to PCR:
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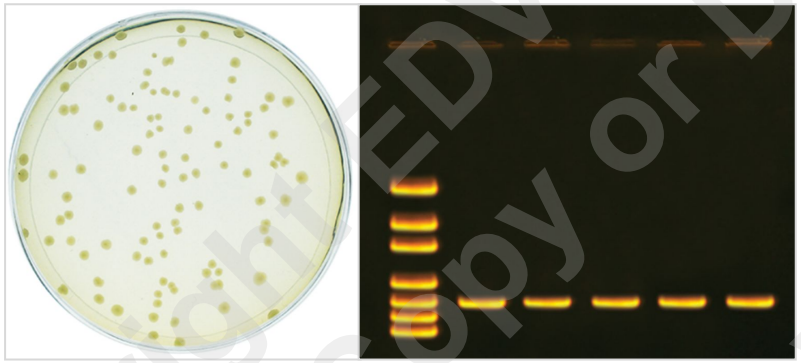

Synthetic Biology (#331)

Identification of GMOs using PCR (#962)

Exploring Biotechnology with GFP (#303)

Mistakes ~~Hurt!~~ Happen!

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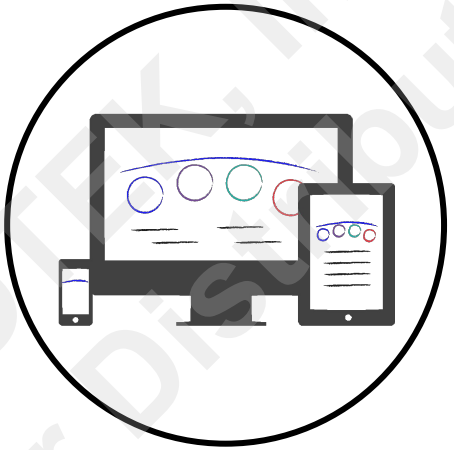

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