Scientists can insert genes into bacteria. The genes inserted in the Indo-Blu process (this lab) are on a circular piece of DNA called a **plasmid**. (The plasmid we use is called pBLU®.) The bacteria with the inserted genes are used as factories that produce large quantities of the gene and its product. The process of giving bacteria (or another organism) a new gene is called **transformation**. Once transformed the new DNA in the plasmid will be expressed by the bacteria. Expressed meaning the protein coded by the DNA will be produced.

Our transformation process uses the bacterium *Escherichia coli* (or *E. coli* for short) as the “factory” to produce the Indo-Blu dye. We insert the pBLU® plasmid, which contains a gene that produces the enzyme beta-galactosidase (β-gal for short). Indo-Blu is produced by the reaction between beta-galactosidase (the enzyme) and X-gal (the substrate). β-gal normally modifies lactose, the sugar in milk. X-gal chemically looks like lactose. β-gal splits a sugar group from X-gal, producing the blue dye, Indo-Blu.

Transformation is a rare event, so to make it easier to find transformants (transformed bacteria), a gene for ampicillin resistance was included on the pBLU® plasmid. Expression of this gene, which produces the enzyme beta-lactamase, allows transformed *E. coli* to grow in the presence of ampicillin, an antibiotic that usually prevents the growth of bacteria. The beta-lactamase enzyme dismantles ampicillin, rendering it non-functional. Hence, transformed bacteria will live in the presence of ampicillin, untransformed bacteria will not live.

### Research Question

How are genes transferred from one organism to another?

### Protocol

#### CAUTIONS

- Because we are adding DNA to living cells, all transfers should be made with sterile technique and sterile instruments. If you spill bacterial culture, notify your teacher immediately. Use gloves. Place waste in biohazard bag.

### Activity 1 – Transforming E.coli

#### Step 1 - Prepare tubes

1. Clean lab bench with 10% bleach. Wash your hands!
2. Obtain two sterile 1.5 ml microfuge tubes containing cold calcium chloride solution (CaCl2), 250 μl each.
   - Label one tube “C” (for control) and the other "pBLU.”
• Label both tubes with your team name.
• Place both tubes on ice.

3. Using a sterilized inoculating loop, gently scrape up a large, single bacteria colony from the stock plate.

4. Insert the inoculating loop into the tube labeled C and vigorously tap or twirl the loop against the side of the tube. Look closely to make sure the cell clump has come off.
   • Suspend the cells by pipetting repeatedly with a transfer pipette. After a few moments, hold the tube up to the light and check that no clumps of cells are visible.
   • Return the C tube to ice.
   • Place used pipettes in waste container.

5. Transfer another colony to the tube labeled pBLU just as you did for the C tube.
   • Suspend the cells in the pBLU tube with a clean pipette just as you did for the C tube.
   • Return the pBLU tube to ice. Both tubes should now be on ice.
   • Place used pipettes in waste container.

   Answer Stop questions #1-5 before continuing

Step 2 - Add plasmid to bacteria:
7. Using a 2-20 μl micropipet and a new sterile tip, add 10 μl pBLU® plasmid to the pBLU tube ONLY.
   • Mix the tube contents by tapping or flicking the tube with your fingers.
   • Return the pBLU tube to ice.

8. Using a new tip, add 10 μl of sterile distilled water to the C tube ONLY.
   • Mix by tapping the tube with your fingers.
   • Return the C tube to ice.

9. Wait 15 minutes. This gives time for the pBLU® plasmid to settle onto the surfaces of the bacteria.

   Answer Stop question #6 before continuing

Step 3 - Heat shock:
10. When the 15 minute waiting period is over, take the ice container with the pBLU and C tubes to the 42°C water bath (check the temperature).
11. Transfer the tubes into a floating tube rack.
12. Place the rack with the tubes in the water bath for a heat shock of exactly 90 seconds.

   IT IS ESSENTIAL THAT CELLS BE GIVEN A SHARP AND DISTINCT HEAT SHOCK

13. Immediately return tubes to ice for at least 2 minutes.
14. Add 250 μl of sterile Luria broth (LB) to each tube, using clean pipettes. Tap each tube with your finger to mix its contents.
15. Incubate the tubes for 15-30 min. at 37°C before plating. This allows the bacteria to recover and express the ampicillin resistance gene.
   • Dispose of designated materials in the appropriate places.
   • Leave equipment as you found it.
   • Check that your work station is in order.
   • Wipe down the table and your micropipettes with 10% bleach.
   • Wash your hands.
Activity 2 – Culturing E. coli

Step 4 - Plating bacteria

1. Clean lab bench with 10% bleach. Wash your hands.

2. Label plates:
   • Obtain your plates (Petri dishes).
   • Handle the plates carefully so that they remain sterile while you label them.
   • Using a permanent marker pen label the experimental plates with ‘pBlu’, your lab number, and class period on the bottom of:
     • 1 LB plate
     • 1 LB/amp/X-gal plate.
   • Label ‘Control’, your lab number, and class period on the bottom of:
     • 1 LB plate
     • 1 LB/amp/X-gal plate.

3. Control Plates
   • Resuspend the bacteria in the “C” tube by pipetting up and down gently.
   • Draw up 100 μl (1/10 ml) and drip it onto the center of the control LB plate.
   • Repeat the process (use a new pipette) to spread 100 μl of the control suspension on the control LB/amp/X-gal plate.
   • Sterilize a spreader, then evenly spread the cell suspension over the surface of the control LB plate. (This may remind you of frosting a cake or spreading peanut butter.)
   • Spread the suspension on the control LB/amp/Xgal plate.

4. pBLU Plates
   • Use the above procedure to spread 100 μl of the suspension from the pBLU tube on the pBLU LB plate and the pBLU LB/amp/x-gal plate.

Make Your Data Prediction in Table 1 NOW

Draw (colored pencils) your prediction and give the reasoning behind your prediction beneath the drawing

Step 5 - Incubation:

5. Allow 5 minutes for the plates’ surfaces to absorb the cell suspensions.
6. Place your four plates, upside-down, in the 37°C incubator.
7. Dispose of designated materials as directed, clean lab station, wash your hands.
8. Examine the plates and analyze them for bacterial growth and colony color after 24 hours of incubation. Record your results in Data Table 2.

Answer Stop question #7 before continuing
## Data

### Data Table 1 Transformation Predictions

<table>
<thead>
<tr>
<th>Control Plates</th>
<th>Experimental Treatment Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB</td>
<td>LB/Amp/Xgal</td>
</tr>
<tr>
<td>LB</td>
<td>LB/Amp/Xgal</td>
</tr>
<tr>
<td># of colonies =</td>
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<td># of colonies =</td>
<td># of colonies =</td>
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</tbody>
</table>

### Data Table 2 Transformation Observations

<table>
<thead>
<tr>
<th>Control Plates</th>
<th>Experimental Treatment Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB</td>
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</tr>
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</tbody>
</table>
Stop Questions

1) What is the purpose of the ‘C’ or control tubes? ________________________________
2) What is a plasmid? __________________________________________________________
3) What does beta-galactosidase normally break down? _____________________________
4) What is beta-galactosidase breaking down in this lab? ___________________________
5) What color is produced due to the action of beta-galactosidase?__________________
6) What makes the p-Blu plasmid different than the DNA already in the bacteria? __________

7) What does, “express the ampicillin resistance gene,” mean? (Use Wikipedia if you need a clue.)
______________________________________________________________________________

Analysis Questions

1. What is the evidence that cells are resistant (or not) to ampicillin?
_____________________________________________________________________________

2. What is the evidence that cells are producing the enzyme β-galactosidase?
_____________________________________________________________________________

3. Were the results as you predicted? _______ Explain.
_____________________________________________________________________________

4. Suppose the C tube cells grow on an LB/amp/X-gal plate.
   What could have gone wrong? ___________________________________
   Suggest a testable hypothesis to explain this result.
   The C (untransformed) bacteria grew on the LB/Amp/X-gal plates
   because ____________________________________________

5. What are three factors that might influence the success of this transformation procedure?
   1) _______________________________________________________________
   2) _______________________________________________________________
   3) _______________________________________________________________

6. Why would it be good for a bacterium (and bad for us) to maintain a plasmid that carries
   resistance to an antibiotic?
   _______________________________________________________________________

What if the antibiotic is not in that bacterium’s environment?
____________________________________________________________________________

Conclusion

You have just completed an exercise used daily in research labs around the world. By
recombining DNA from different organisms we can now create life forms that never existed on
this planet before. Is this a good thing to do? Write a persuasive essay stating your position.
Word process the essay and include the final product with this lab set. Address your essay to our
current congressional representative. Their name and address may be found at this link:
https://writerep.house.gov/writerep/welcome.shtml