<table>
<tr><td rowspan="6">Lesson Description/ Learner Characteristics</td><td>Lesson Title:
pGlo and Bacterial Art</td></tr>
<tr><td>Duration:
Day 1-55 minutes. Day 2 -15 minutes.</td></tr>
<tr><td>Discipline/Subject
Biology</td></tr>
<tr><td>Grade Level/Setting/Grouping:
10-11th grade.</td></tr>
<tr><td>Learner Characteristics:
N/A</td></tr>
<tr><td>Prior Learning experiences tied to lesson:
Pipetting, a possible review may be good. Sterile technique will be covered.
Students will be given a student sheet the day or weekend before the experiment. The first part of the sheet should be completed prior to starting the activity. The second half will contain assessment/review materials.</td></tr>
<tr><td rowspan="4">Goals, Objectives/ Standards, Essential Questions</td><td>Goals:
• To be able to understand plasmids, and how they can affect bacteria.
• To obtain a knowledge of sterile technique.
• To understand how this technique connects to biology and current research</td></tr>
<tr><td>Next Generation Science Standards:
HS-LS1-1. Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells</td></tr>
<tr><td>Objectives:
• Students will learn what a plasmid is and be able to see how introducing one can affect bacteria.
• Students will practice sterile technique and learn about cell culture.
• Students will also know about the transformation process and how this technique helps researchers.
•</td></tr>
<tr><td>Essential Questions:
• How do genes affect living things?
• What is a plasmid?
• Why do Bacteria have plasmids?
• Why might we want to give bacteria plasmids?</td></tr>
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Materials and Resources	*Vocab:* • Fluorescent Protein-Specific proteins that illuminate or cause a specific organism to glow. • pGlo-A protein extracted from jellyfish. One of the first used proteins in *in vitro* bacterial transformation. • Transformation (Bacterial)-A stable change of genetics due to an uptake of new DNA. • Cell Culture-A stable plate of cells (bacterial or other) used as a stock for future experimentation. •
Instructional Procedure	• Fluorescent Proteins in varying colors. (Roughly 1 color per student) • Inoculating loops. (5-7 per student) • Micropipettes (Varying sizes) • LB Agar and LB Amp plates. (One of each plate per student) • Competent bacterial cells. (1 plate per group, prepped ahead of time) • Transformation solution ($CaCl_2$, roughly 3 mL per group) • Microcentrifuge tubes (Only 1 is needed for each student 1.5 mL) • Ice (1 bucket of ice per group) • Hot Water Bath (1-2 for whole class) • LB Broth (3mL per group) • Incubator (Class wide) • Foam microcentrifuge tube holder • Black lights
	Launch/Warm-Up: • Students will share materials in partners, but will work to culture and transform their own plate of bacteria. • PowerPoint/discussion with information about the origins of transformation, plasmids and the infamous GFP protein should lay the foundation for this activity. Key points to hit would be: o What genes are and why they are important. o What a plasmid is and how do bacteria transfer them. o Why are plasmids important to bacteria? o What the transformation solution does for the process. o What the heat-shock technique does for the process. o Why is GFP important? o How does the protein allow growth on ampicillin plates and how does this help us show growth.
	Procedural Steps: 1. Students would be given a microcentrifuge tube that they would label with their initials and the date.

	2. Students would then pipette 250 microliters of the transformation solution into their tube with the use of a micropipette. i) This is a good time to go over again with the students what the $CaCl_2$ does for the transformation. 3. Next, students will get to practice sterile technique along with good culturing skills by grabbing a bacterial colony from the competent stock plate. They will place this colony into their tube and gently spin their loop in the tube to break up the colony, no chunks should remain. 4. With a new sterile loop, acquire some of the fluorescent protein onto the loop and place it into the microcentrifuge tube with the colony and the $CaCl_2$. ii) Be sure to discuss the protein. Where did it come from, why can it not enter the cell on its own? Maybe black light the protein and note some observations. iii) Some good examples of Fluorescent proteins are Green Fluorescent Protein, mCherry, mStrawberry, Cerulean, etc. Clontech has a wide variety of proteins. 5. Place the tubes on ice for 10 minutes. This is a good time to go over what we have done so far and what is happening in the tube. 6. After 10 minutes, take the tubes and place them in a 42 degree Celsius hot water bath for 45-50 seconds. Then place the tubes back onto ice for 2 more minutes. 7. After 2 minutes, remove the tube from the ice and add 250 microliters of LB Broth to the microcentrifuge tube. Allow the tube to sit for 10 minutes to incubate. i) LB Broth is made by either using purchased pre-mixed LB Broth and adding the amount on the container to de-ionized (Specially filtered) water, or mixing 10g of Tryptone, 10g of NaCl, and 5g of Yeast extract. Mix into water by vigorously stirring. 8. Once the incubation has finished, gently invert or flick the tube a bit. Ensure that each student has a LB agar and a LB amp plate. Using a sterile loop allow the students to transfer some of the liquid from the microcentrifuge tube onto each plate. The students should also try and use other classmates transformed bacteria (Ensure they use a fresh loop each time) to grow bacteria transformed with different colors. This will allow for different colors and designs (bacterial art!) on each students plate. 9. Place plates into an incubator overnight.
	Wrap-up/Closure: The next day allow the students to take their plates out of the

	incubator and look at them under the black light. Discuss why we used amp plates along with regular agar plates. Have students make observations of the different colors.
Assessment	• See attached Student Sheet.
Getting Started Activity	• https://www.teachengineering.org/view_activity.php?url=collection/uoh_/activities/uoh_genetic/uoh_genetic_lesson01_activity1.xml&std_open=true#!standards
Reflections	• I would like to thank Mrs. Amanda Grimes for who helped me with the idea for this lesson plan and for whose lesson this was adapted from.

Bacterial Art
Quick Guide and Student Sheet

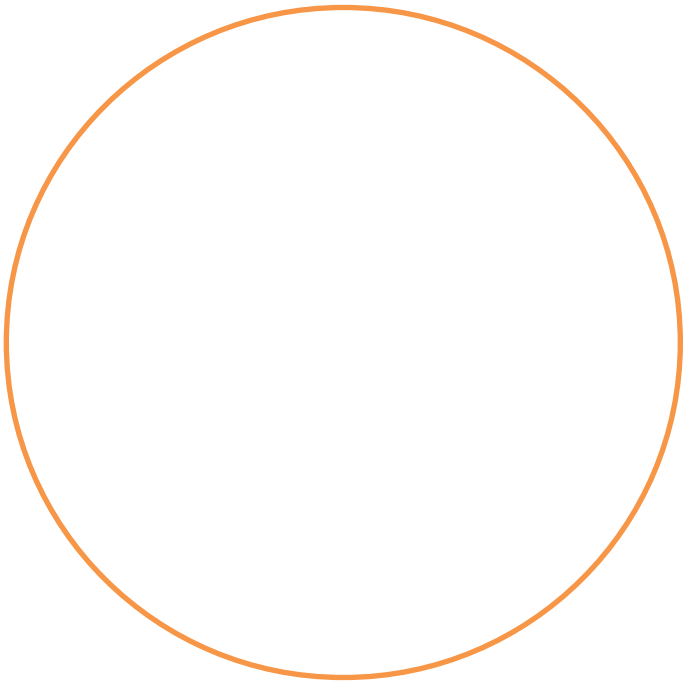
1. Please look through the procedures and create a Flow Chart of the steps using only drawings. One picture per step. Steps listed below.

10. Students would be given a microcentrifuge tube that they would label with their initials and the date.
11. Students would then pipette 250 microliters of the transformation solution into their tube with the use of a micropipette.
    i. This is a good time to go over again with the students what the $CaCl_2$ does for the transformation.
12. Next, students will get to practice sterile technique along with good culturing skills by grabbing a bacterial colony from the competent stock plate. They will place this colony into their tube and gently spin their loop in the tube to break up the colony, no chunks should remain.
13. With a new sterile loop, acquire some of the fluorescent protein onto the loop and place it into the microcentrifuge tube with the colony and the $CaCl_2$.
    i. Be sure to discuss the protein. Where did it come from, why can it not enter the cell on its own? Maybe black light the protein and note some observations.
    ii. Some good examples of Fluorescent proteins are Green Fluorescent Protein, mCherry, mStrawberry, Cerulean, etc. Clontech has a wide variety of proteins.
14. Place the tubes on ice for 10 minutes. This is a good time to go over what we have done so far and what is happening in the tube.
15. After 10 minutes, take the tubes and place them in a hot water bath for 45-50 seconds. Then place the tubes back onto ice for 2 more minutes.
16. After 2 minutes, remove the tube from the ice and add 250 microliters of LB Broth to the microcentrifuge tube. Allow the tube to sit for 10 minutes to incubate.

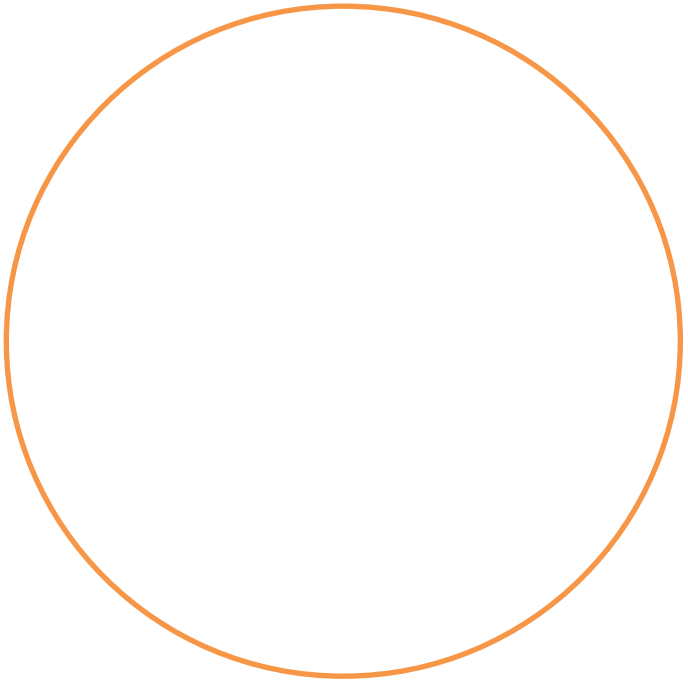
ii) LB Broth is made by either using purchased pre-mixed LB Broth and adding the amount on the container to de-ionized (Specially filtered) water, or mixing 10g of Tryptone, 10g of NaCl, and 5g of Yeast extract. Mix into water by vigorously stirring.

17. Once the incubation has finished, gently invert or flick the tube a bit. Ensure that each student has a LB agar and a LB amp plate. Using a sterile loop allow the students to transfer some of the liquid from the microcentrifuge tube onto each plate. The students should also try and use other classmates transformed bacteria (Ensure they use a fresh loop each time) to grow bacteria transformed with different colors. This will allow for different colors and designs (bacterial art!) on each students plate.
    a. Place plates into an incubator overnight

2. Once you have let your bacteria incubate, draw your results as seen under a black light.



LB Agar Plate



LB Amp Plate

3. Review questions.
    1. What are genes and how do they affect living things? Do humans have genes? Do bacteria?
    2. What is a plasmid? Do humans have plasmids? Why or why not?
    3. Why do Bacteria have plasmids?
    4. How does transformation help bacteria? Why might we as scientists want to transform bacteria? What real world applications could this have?