I-LEARN TEACHING RESOURCES

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Toward Genetic Engineering: Teaching Transformation Using the pGLO Plasmid in High School Classrooms

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***Abstract*:** In this lesson plan, students will learn about genetic engineering and perform the same transformation protocol used by scientists on a daily basis around the world. Genetic engineering is a technique used for direct manipulation, alteration, or modification of genes or genomes of an organism to manipulate the phenotypes. The use of genetically modified organisms (GMOs) has emerged everywhere as a mainstay from agriculture to pharmaceuticals. Through this lesson, students will transform *Escherichia coli* with pGLO plasmid, which give a brilliant fluorescent green glow under UV light. Students will also learn the central dogma of molecular biology: DNA → RNA → Protein → Trait (Green Fluorescence), and the basics of gene regulation. This hands-on module is designed for 9th through 12th grade students. To adjust the difficulty level of the modules, some aspects can be modified or removed based on the grade. This module includes an instructional slide show, assessment options, and links to additional resources.

Included in this lesson: Lab worksheet, multiple-choice questions and answer key

Supporting File: PowerPoint presentation

Lesson Description

- Grade Level: Grade 9-12

Estimated Time for Completing Activity

- Two 45-minute class periods and one 20-minute class period, or one 90-minute block with one 20-minute class period.

Learning Outcomes

Students will be able to:

- Define bacterial transformation
- Gain hands-on-experience of genetic engineering; from handling plasmids to performing heat shock and plating the transformants.
- Identify various genetic engineering terms such as inducer, promoter, and cell competence.
- Examine gene expression using an inducer (trigger) based on the outcomes of the plates.

South Dakota State 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. (SEP: 6; DCI: LS1.A; CCC: Structure/Function)
- **HS-LS1-2:** Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms. (SEP: 2; DCI: LS1.A; CCC: Systems)
- **HS-LS3-1:** Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring. (SEP: 1; DCI: LS1.A, LS3.A; CCC: Cause/Effect)
- **MS-LS3-1:** Develop and use a model to describe why structural changes to genes (mutations) located on chromosomes may affect proteins and may result in harmful, beneficial, or neutral effects to the structure and function of the organism. (SEP:2; DCI: LS3.A, LS3.B; CCC: Structure/Function)
- **MS-LS1-2:** Develop and use a model to describe the function of a cell as a whole and ways parts of cells contribute to the function. (SEP: 2; DCI: LS1.A; CCC: Structure/Function)

Standards link: https://doe.sd.gov/contentstandards/documents/sdSciStnd.pdf

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National Standards

Next Generation Science Standards (NGSS: MS-LS1-2, MS-LS3-1, HS-LS1-1; www.nextgenscience.org; definitions on the last page)

- **MS-LS1-2**: Develop and use a model to describe the function of a cell as a whole and ways the parts cells contribute to the function**.**
- **MS-LS3-1**: Develop and use a model to describe why structural changes to genes (mutations) located on chromosomes may affect proteins and may result in harmful, beneficial, or neutral effects to the structure and function of the organism.
- **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.

Pre-requisites

- A general level of pipetting skill
- An understanding of plasmids and how they function in cells
- An understanding of central dogma or how genotype can affect phenotype
- An understanding of the structure of the plasma membrane and DNA

Video Tutorial on Pipetting

- https://www.youtube.com/watch?v=QGX490kuKjg

Acquiring lab kits:

There are two options for you when acquiring materials to do this lab.

- SDSU-Butzin Lab Bacterial Transformation Kit – Email: *nicholas.butzin@gmail.com*
- BioRad Bacterial Transformation Kit (Purchase link: Click here)

The protocol in this lesson is written for using materials acquired via SDSU-Butzin Lab. If you decide to purchase the BioRad Kit, a protocol will be provided with the kit. The worksheets provided at the end of this lesson are compatible with each version of the lab. The purchase link of some materials which will not come with SDSU-Butzin Lab Bacterial Transformation Kit are given below:

Pre-lab Materials:

- Computer with Transformation PowerPoint
- Pre-lab worksheet
- Lab protocol (1 per lab group)
- Data analysis worksheet

Lab Materials:

- Ice and Ice bucket (or Styrofoam cup)
- Water Bath (preheated to 42℃)
- 37℃ incubator
- Micropipette and tips (or plastic pipettes or inoculation loops)
    - Purchase link: 1. Click here 2. Click here
- Nitrile gloves, one pair per student
    - Purchase link: Click here
- Microcentrifuge tubes (2mL)
    - Purchase link: Click here
- Tube rack
    - Purchase link: Click here
- Plasmid (pGLO)
- Competent cells (*E. coli* DH5alphaZ1)
- SOC media
    - Purchase link: Click here
- Appropriate antibiotic (Ampicillin)
    - Purchase link: Click here
- Inducer (Arabinose)
    - Purchase link: Click here
- Petri plate
    - Purchase link: Click here
- LB Agar powder
    - Purchase link: Click here

- Spreader or sterile beads
    - Purchase link: Click here
- UV light
    - Purchase link: Click here
- Timer
- Laboratory tape
- Sharpie
- Foam floater
    - Purchase link: Click here

Glossary of Terms

- **Activator**: A DNA-binding transcription metabolite that positively modulates an allosteric enzyme or regulates one or more genes by increasing the rate of transcription
- **Antibiotic**: A type of antimicrobial substance active against bacteria
- **Cloning**: Several different processes that produce individuals with identical or virtually identical DNA, either naturally or artificially
- **Competent cells/Cell competence**: Cell competence refers to a cell's ability to take up foreign (extracellular) DNA from its surrounding environment. Artificial or induced competent cells are cells researchers have made competent through electrical (electroporation) or chemical manipulation
- **Green fluorescent protein (GFP)**: Protein that glows green when exposed to ultraviolet light
- **Inducer**: A molecule that regulates gene expression by either disabling repressors or binding to activators
- **Origin of replication**: A particular sequence in a genome/plasmid at which replication is initiated
- **Plasmid**: A small, extrachromosomal DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently
- **Promoter**: A sequence of DNA to which RNA polymerase and transcription factors bind to initiate transcription of mRNA (can be "induced" by inducer)
- **Restriction enzyme**: An enzyme that cleaves DNA into fragments at or near specific recognition sites known as restriction sites

- **Transformation:** In molecular biology and genetics, transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane(s)

Helpful Links and References

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- https://www.youtube.com/watch?v=h9BFBJJl3pg
- Cloning Slideshow.pptx

Background

Transformation is the uptake of exogenous genetic material from a cell's surroundings. First, a plasmid with our desired gene is made by using restriction enzymes to cut the gene and plasmid. The gene and the plasmid fragments are pasted together using an enzyme, the DNA ligase. Competent cells, cells which can readily uptake DNA from their surroundings, are mixed with the plasmid to allow the plasmid to attach to the membrane of the cell. Heat shock is given to the cells in order to facilitate the entry of the plasmid

into the cell. The cells are then placed in ice to close the pores that are created due to the heat shock, subsequently the cells will be added in SOC media which gives them the nutrients needed to recover and start gene expression. Finally, these cells are put on plates and observed for growth in the following day to determine if the transformation was successful.

In this experiment, students will visualize the process of transformation by inserting a pGLO plasmid in one sample and no plasmid in another sample and observe their results on a variety of plates. These plates include LB agar only, LB + Amp, and LB + Amp + Ara plates. The pGLO plasmid contains a selective marker gene (beta-lactamase gene), a regulatory gene (*araC*), and a green florescent gene (GFP) under the *pBAD* promoter. Beta-lactamase gene will degrade ampicillin, thus only the transformed bacteria (having pGLO plasmid) will survive on all plates. But, non-transformed cells will not grow on LB + Amp and LB + Amp + Ara plate, since they do not have the beta-lactamase gene necessary for survival on Ampicillin containing plates.

In bacteria, groups of related genes are often clustered together and transcribed into RNA from a single promoter, these clusters of genes are called operons. For example, three genes that encode the digestive enzymes (*araB, araA,* and *araD*) involved in metabolizing the simple sugar arabinose are clustered together in araBAD operon, and all depend on initiation of transcription from a single promoter, *pBAD*. Transcription requires the simultaneous presence of RNA polymerase, a DNA-binding protein called AraC, and arabinose. When arabinose is absent, the AraC protein binds to the DNA at the binding site for RNA polymerase, preventing transcription of the digestive enzymes. When arabinose is present, it interacts with AraC, causing AraC to change shape, allowing RNA polymerase to bind the promoter; araB, araA, and araD are then expressed and can do their job to break down arabinose until the arabinose runs out.

The pGLO plasmid contains both the promoter (*pBAD*) and *araC* gene, but *araB, araA,* and *araD* have been replaced by the single gene that codes for GFP, which serves as a reporter gene. In the presence of arabinose, the AraC protein promotes the binding of RNA polymerase to the promoter, which causes transcription of the GFP gene into messenger RNA (mRNA), followed by the translation of this mRNA into GFP. This process is called gene expression. As they produce more and more protein, the cells expressing GFP fluoresce a brilliant green. In the absence of arabinose, however, AraC no longer facilitates the binding of RNA polymerase, and the GFP gene is not expressed, and bacterial colonies have a wild-type (natural) phenotype — white colonies with no fluorescence. Thus, the only plate with arabinose (LB + Amp + Ara) will cause green fluorescence under UV light.

This is an excellent example of the central dogma of molecular biology in action: DNA → RNA → protein → trait.

Procedure:

The attached PowerPoint slides have notes for guidance through the lesson.

Cloning Slideshow.pptx

Day 1: Introduction and Media preparation

- **Introduction: Genetic Engineering**
  - Define genetic engineering (slide 2)
  - Partner/Group Discussion on GMOs

    1. Prior to teaching on the widespread use of GMOs, give students 1 minute to share their own understanding and views on the subject
    2. Have groups share their conclusions or something they learned from their partner with the class
    3. Provide commonplace examples of GMOs (slide 3) (such as use in agriculture or in the production of insulin) as well as more specified uses (such as addressing using genetically modified mosquitoes *Aedes Aegypti* to reduce vector-born viral diseases such as dengue)

       Discussion Topic 1: How has your perception/understanding of GMOs changed after our short discussion of them today? (i.e., what are they, are their applications essential, or what are some ethical questions you could pose concerning their use?)

  - **Instruction:** Provide an overview of the development of a plasmid as well as the procedure used in genetic cloning (Slides 4-40)
    - Clarify the meaning of cloning in a genetic context (slides 4-5)
    - Lead by having student pairs define the word "cloning" on their own
    - When students have provided their definitions, differentiate between how the term "cloning" is used in everyday life and how it is used in genetics
    - Walk through the steps used in developing the plasmid used for cloning (slides 6-17)

1. Identify a gene for the protein – genes code for proteins which ultimately confer the phenotype of an organism. In our case, we will be using the green fluorescence protein first isolated from *Aequorea victoria.*
    - *Extension – 2008 Nobel Prize in Chemistry*
2. Put the gene into bacteria through plasmid – plasmids are made by digesting the target gene sequence and the plasmid with the same restriction enzyme and creating complimentary sticky ends. Ligase can then create the recombinant DNA.
    - *Extension – operon drawing*
3. Grow lots of bacteria – Once the plasmid has been transformed into bacteria, the bacteria need time to heal their membrane and produce the b-lactamase protein. SOC media is nutrient-rich bacterial growth medium used to grow *E. coli* that result in higher transformation efficiencies of plasmids than other common media such as LB
4. The transformed bacteria will begin to transcribe and translate the desired gene present on the plasmid.
5. Purify the protein – This is *not* a step that is taken in this protocol. However, in many cases of genetic engineering, the resulting gene product is isolated and potentially used for a purpose. An example of this would be purifying insulin from modified *E. coli* for subsequent distribution to patients.

- Discuss transformation of the pGLO plasmid (slides 18-40)
    - The plasmid contains the selective marker gene (beta-lactamase gene), a regulatory gene (*araC*), and a green florescent gene (GFP) under the *pBAD* promoter. Beta-lactamase protein degrades beta-lactam antibiotics (such as Ampicillin) and this gene helps to select only the transformed bacteria, because non-transformed bacteria will die in Ampicillin plate. AraC protein regulates GFP gene expression. In the presence of arabinose, the AraC protein promotes the binding of RNA polymerase to the promoter, which causes transcription of the GFP gene into messenger RNA (mRNA), followed by the translation of this mRNA into GFP. As they produce more and more protein, the cells expressing GFP fluoresce a brilliant green. In the absence of arabinose, however, AraC no longer facilitates the binding of RNA polymerase, and the GFP gene is not expressed, and bacterial colonies have a wild-type (natural) phenotype — white colonies with no fluorescence.

- In the presence of arabinose, the AraC protein promotes the binding of RNA polymerase to the promoter, which causes transcription of the GFP gene into messenger RNA (mRNA), followed by the translation of this mRNA into GFP. This process is called gene expression
- The plasma membrane is negatively charged due to the negative charge on the phosphate heads of the phospholipids. DNA also possesses a net negative charge due to the phosphates in the phosphodiester linkages of the DNA backbone. To overcome this, the cells used in this protocol are termed "competent" cells and will be pre-washed with cold $CaCl_2$ solution prior to the start of the lab, to prepare the cells to take up the plasmid DNA.
- Heat shock for 90s is used to create pores in the plasma membrane. The 90s time limit should be followed strictly for this step, otherwise the cells could die. During this time, the plasmid is able to enter the cells through pores in the membrane.
- Following heat shock, the cells are put on ice for 2 minutes to close the pores in the plasma membrane.
- The cells are then incubated in SOC media.
- The cells will then be plated onto various plates and incubated at 37°C for 24 hours before visualizing.
  - - pGLO will be plated onto LB and LB + Ampicillin
  - +pGLO will be plated onto LB + Ampicillin & LB + Ampicillin + Arabinose
  - Students should make predictions prior to visualizing their actual results. They will justify their predictions based on whether or not the cells were transformed and their predictions about how the construction of the pGLO plasmid would affect the phenotype of successfully transformed cells in different environments.

- **Media preparation: Prepare LB plates for next experiment**

  - Add appropriate amount of LB Agar powder in a microwave safe bottle, add appropriate amount of water, mix it, loosen the cap, put it into microwave for few minutes and bring it to boil for 3 times.
  - Bring the LB agar temperature into ~55-60˚C, add appropriate amount of Antibiotic (Ampicillin, final concentration: 100 ug/mL) and Inducer (Arabinose, final concentration: 1%) if needed, mix and pour the plates. (https://www.youtube.com/watch?v=aQWQRN7QL84)

    - LB plate – 1 per group

- LB with ampicillin plate – 2 per group
- LB with ampicillin and arabinose plate – 1 per group

- **Experimental Procedure Safety:**
  - Wear heat resistant gloves when handling hot LB Agar media.
  - Clean the workbench before and after pouring plates.

Assessment Day-1:

- Diagnostic: Predictions of plates at the end of the pre-lab
- Formative: Circulate during discussion and facilitate class discussion

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Day 2: Perform Transformation Lab Protocol

- **Teacher to Prepare beforehand:**

***** DIRECTIONS IF USING SHIPMENT FROM SDSU (BUTZIN LAB)**

- Lab Day preparation
  - Turn on water bath (42 °C)
  - Get crushed ice & cooler
  - Have pGLO plasmid (70 ng/uL) and competent cells (*E.coli* Dh5αZ1 strain) from fridge readily accessible (provided from Butzin lab)
  - Put at teacher lab station
  - 1 loop/group near the plasmid vial
- Prepare lab trays for student groups
  - Sharpie
  - Paper towels
  - Masking tape
  - Styrofoam cup (to have ice bath at their lab stations)
  - 1 foam floater
  - 1 tube – 1mL SOC media

- 2 micro-centrifuged tube
  - 1 tube will be used by students to combine competent cells and the plasmid for the "+pGLO" sample
  - 1 tube will be used by students for the "-pGLO" sample
- Students will need access to ***either:***
  - Sterile loops (2 per group) or 2 pipets and tips

*****DIRECTIONS IF USING BIORAD KIT**

- Rehydration & Incubation of *E. coli* bacteria (2 days prior to lab)
  - Add 250 µl of LB broth to *E. coli* vial with sterile pipet
  - Recap and shake gently
  - Incubate at 37 °C for 8-24 hours
- Prepare starter plates (1 day prior to lab)
  - Get *E. coli* from incubator
  - Dip sterile loop into *E. coli* vial
  - Streak two LB plates for use by entire class (used to make competent cells)
- Prepare pGLO plasmid (1 day prior to lab)
  - Add 250 µl of Transformation solution ($CaCl_2$) to the pGLO plasmid vial
  - Store in Fridge overnight
- Lab Day preparation
  - Turn on water bath (42 °C)
  - Get crushed ice & cooler
  - Have plasmid from fridge readily accessible
    - Put at teacher lab station
    - 1 loop/group near the plasmid vial
  - Prepare lab trays for student groups
    - Sharpie
    - Paper towels
    - Masking tape
    - 1 foam floater (provided by BioRad)
    - Yellow tube – 1mL LB broth
    - 1 Empty orange tube & 1 empty green tubes

- 1 tube will be used by students to combine competent cells and the plasmid for the "+pGLO" sample
- 1 tube will be used by students for the "-pGLO" sample

- Students will need access to ***either:***
  - Sterile loops (2 per group) or 2 pipets and tips

- **Introduction – Protocol Walkthrough (slides 41-51)**
  - Provide a brief overview of steps (2-3 minutes)
  - Host a brief discussion about students' predictions for each plate
- **Experimental Procedure Safety:**
  - Wear gloves when handling samples.
  - Clean the workbench before and after use.
  - Properly dispose of all reagents upon completion.
- **Protocol (starting with competent cells):**

1. Label two 2mL microcentrifuge tubes, one with +pGLO and one with -pGLO.
2. Place these tubes in ice bucket for 5 minutes.
3. Thaw competent cells in your hand (NO glove) and place in ice for 10 minutes.
4. Transfer 100ul (0.1mL) of the competent cells into each of the chilled 2mL microcentrifuge tubes (+pGLO and -pGLO) using micropipette.
5. Add 2ul of plasmid into +pGLO tube.
   a. When adding plasmid into tube swirl the pipet 2-3 times in the liquid to mix the solution.
   b. Add 2ul of sterile water into -pGLO tube

[**Note:** If 1-10 ul pipette & tips are not available, immerse a sterile loop into the pGLO plasmid solution. There should be a film across the loop that looks like the soap film across a ring in preparation to blow bubbles. Immerse the loop into the +pGLO tube containing the competent cells, and mix.]

7. Incubate both +pGLO and -pGLO tubes on ice for 10 minutes.

8. Heat shock both tubes at 42℃ for **exactly 90 seconds**.

[**Note:** No more or less than 90 s and do not shake the tubes. More than 90s will result to decrease in the transformation efficiency.]

9. Immediately return the tubes to the ice (1-2 min is fine) for 90 seconds.

10. Add 400 ul (0.4 mL) of SOC media to both +pGLO and -pGLO tubes.

11. Incubate at 37°C shaker (225-250rpm) for 10 minutes.

[**Note:** Incubation time can be changed from10 min - 1hour based on the class schedule, increase of incubation time will increase the transformation efficiency]

12. While tubes are in the shaker, label your 4 plates.

i. Group Member Initials, Date, Class Period, +pGLO, LB/Amp

ii. Group Member Initals, Date, Class Period, +pGLO, LB/Amp/Ara

iii. Group Member Initials, Date, Class Period, -pGLO, LB/Amp

iv. Group Member Initals, Date, Class Period, -pGLO, LB

13. Pipet 200 ul (0.2 mL) of -pGLO onto each plate labeled with -pGLO and spread.

14. Pipet 200 ul (0.2 mL) of +pGLO onto each plate labeled with +pGLO and spread.

15. Place in 37°C incubator overnight.

16. Next day observe the plates and note down the result. Observe all plates under UV Light to see the florescent color.

Assessment for Day-2:

1. Diagnostic: Pre-lab prediction table
2. Formative: Monitor student discussion for rationalization of the lab procedure; monitor students' abilities to follow the procedure and ask questions to redirect.

Day 3: Lab Analysis

Review: Have students look at their prediction tables and discuss why they expected certain results.

- **Day 3 Activities**

1. Retrieve plates from the incubator and observe colony growth for each plate.

2. Utilize a UV light source to visualize all of the plates.

3. It is essential that students to compare their predictions to their results and use scientific reasoning to justify their findings.

4. Complete lab analysis

Discussion Questions:

- Based on your observations, was transformation accomplished?
- Why did or why didn't you observe growth on certain plates?
- What evidence has led you to these conclusions?
- Do your observations match with your predictions?

Assessment for Day 3:

- Formative Assessment – Pre-lab and Post lab Discussions
- Diagnostic Assessment – Post lab worksheet

Lab Worksheet

Table 1. Pre-Experiment Prediction of Growth. A good scientist predicts (hypothesizes) the result of an experiment based on available data even when little data is available. Predict your results and justify your prediction.

Cell	LB	LB + Amp	LB + Amp + Ara
Competent cell (-pGLO)			
Transformed cell (+pGLO)			

Justify your predictions:

Table 2. Experiment Observations Table. Describe your results and how they are similar or different from what you predicted before the experiment. A good scientist accepts the results of an experiment regardless of prior predictions.

Cell	LB	LB + Amp	LB + Amp + Ara
Competent cell (-pGLO)			
Transformed cell (+pGLO)			

Compare your observed results to your predicted results:

Post-Lab Questions

1. What is bacterial transformation?

2. Is transformation only observed in bacteria?

3. Arabinose is an inducer for a gene that encodes GFP protein. How does this fact cause a difference in the colonies found on the +pGLO plate with LB + Amp + Ara compared to the other +pGLO plate with LB + Amp?

4. What results do you expect to see if we forget to add Ampicillin into the LB Agar plate media?

Multiple choice questions

1) What is the advantage of using $CaCl_2$ solution to make competent cells in the transformation experiment?

   a) Helps the cell membrane to prepare for heat shock
   b) Charges the cell membrane to allow the plasmid to adhere with the cell
   c) Helps the cell to recover after heat shock
   d) Opens pores in the cell membrane

2) What organisms are capable of transformation?

   a) Bacteria
   b) Eukaryotes
   c) Archaea
   d) All of the above

3) What will happen if we do not add Arabinose into the plate media?

   a) The transformed colonies will not fluoresce
   b) No colonies will grow on the plate
   c) The transformed colonies will fluoresce
   d) There will be limited growth on the plate

4) What is the function of the SOC media?

a) To allow the plasmid to enter the cell
b) Provide cells the nutrients needed to recover and grow after heat shock
c) To open pores in the cell membrane
d) It is not necessary to add; it just makes it easier to plate

Answer Key

Post Lab Questions

1. What is bacterial transformation?

The uptake of foreign genetic material by a cell.

2. Is transformation only observed in bacteria?

No, other organisms such as archaea and eukaryotes can also uptake foreign genetic material.

3. Arabinose is an inducer for a gene that encodes GFP protein. How does this fact cause a difference in the colonies found on the +pGLO plate with LB + Amp + Ara compared to the other +pGLO plate with LB + Amp?

The colonies on the plate with Arabinose will appear green under UV light due to the GFP production being induced by the Arabinose present. On the plate without Arabinose, there will still be growth but the colonies will not fluoresce since no Arabinose is present to induce the production of GFP.

4. What results do you expect to see if we forget to add Ampicillin into the LB agar plate media?

Bacterial lawn will be observed on all over the plates, since there will be no selection for transformed colonies. Thus all bacteria (transformed or non-transformed) will grow on the plate.

Multiple choice questions

1) What is the advantage of using $CaCl_2$ solution to make competent cells in the transformation experiment?

a) Helps the cell membrane to prepare for heat shock

b) Charges the cell membrane to allow the plasmid to adhere with the cell

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