Kansas Corn:
Protein Production Jackpot

This lab is made possible with the support and content contributions of the Kansas Corn Commission.

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Kansas Corn: Protein Production Jackpot
Grade Level: High School

Overview
One major accomplishment in the corn industry during this century has been the development of Bt Corn. This variety of corn is genetically modified with a gene that produces a highly selective toxin, designed to control for European Corn Borers. How did scientists know this toxin was indeed being produced by the altered corn variety? Scientists take samples of the new plants and purify the protein they are trying to produce in order to confirm their overall results and determine whether they were successful in achieving their overall goals. Purifying proteins from cells is a multi-step process and it is dependent, in part, on the properties of the protein themselves. During this lab students will purify the Green Florescent Protein (GFP), which was previously transformed into a strain of E. coli bacteria during the previous lab, Bacterial Transformation, which is Part 3 of Kansas Corn: Feeding the World – DNA to the Rescue (available online at kscorn.com). They then break down the genetically altered cells to extract the newly created proteins from their freshly grown bacteria cultures.

Kansas College and Career Ready Standards
Science

- **HS-LS1-1.** Construct and 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.
- **HS-LS3-1.** Ask questions to clarify relationship about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- **HS-LS3-2.** Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) variable errors occurring during replication, and/or (3) mutations caused by environmental factors.
- **HS-LS3-3.** Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.
- **HS-ESS3-4.** Evaluate or refine a technological solution that reduces impacts of human activities on natural systems.
- **HS-LS4-4.** Construct and explanation based on evidence for how natural selection leads to adaptation of a population.
- **HS-ETS1-1.** Analyze a major global challenge to specify qualitative and quantitative criteria and constraints for solutions that account for societal needs and wants.
- **HS-ETS1-2.** Design a solution to a complex real-world problem by breaking it down into smaller more manageable problems.

Language Arts

- **RST.11-12.1.** Synthesize information from a range of sources (e.g., texts experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.
- **WHST.9-12.1.** Write arguments focused on discipline-specific content.

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Math

- **MP.2.** Reason abstractly and qualitatively.
- **HSS-IC.B.6.** Evaluate reports based on data.

Ag Competencies: 018.Agriscience in Our World

- **1.** Define and relate agriscience to agriculture, agribusiness, and renewable natural resources.
- **2.** Connect biology, chemistry, and biochemistry to agriscience.
- **3.** Analyze how the world population affects today's agriculture industry.
- **4.** Identify significant historical developments in agriscience.
- **5.** Examine important research achievements in agrisciences and future research implications.
- **6.** Compare and contrast the methods of agriculture used in the local, county, state, nation, and world.
- **7.** Describe the importance of agriculture products in everyday life.
- **31.** Agricultural Issues
  - **3.** Research a current agriculture issue.

Learning Objectives

- To understand the importance of gene modifications.
- To understand the process of cloning bacteria.
- Understand the process of hydrophobic chromatography.
- Understand the importance of genes and proteins.
- To understand the importance of a sterile workstation and learn how to safely handle potentially dangerous materials.

Materials

- *Protein Production Jackpot* PowerPoint (available online at www.kscorn.com)
- GFP Procedure – Quick Guide (pg. S1-S4, or available online at www.kscorn.com)
- GFP Purification Kit
  - Items provided in kit:
    - Ampicillin
    - Arabinose
    - LB broth capsule
    - Inoculation loops (10 loops per pack)
    - Sterile pipettes (individually wrapped)
    - Microcentrifuge tubes (2.0 ml)
    - Sterile culture tubes (15 ml)
    - Collection tubes (5 ml)
    - TE buffer
    - Lysozyme

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Materials (continued)

- Binding buffer
- Column equilibration buffer
- Column wash buffer
- HIC chromatography columns
- Column end caps

- Items not included in kit:
  - Transformation plates
  - UV lamp (long wavelength)
  - Centrifuge
  - Microwave oven
  - Erlenmeyer Flask (250 ml)
  - Graduated cylinder (100 ml)
  - Distilled water
  - Beaker of water for rinsing pipettes
  - Marking pens
  - Refrigerator/freezer
  - Microcentrifuge tube racks
  - Incubation oven

Safety Considerations

- The E coli strain used in this lab is not a pathogenic organism. It has been genetically modified to prevent its growth unless grown on an enriched medium. However, handling of the E. coli strain requires the use of standard Microbiological Practices. Work surfaces should be decontaminated once a day and after a spill of viable material. All contaminated liquid or solids wastes are decontaminated before disposal. All persons must wash their hands after they handle material containing bacteria and before exiting the lab. Do not eat or drink in the lab. Protective eyewear and gloves are strongly recommended.
- Anything that comes into contact with the bacteria should be placed into a 10% bleach solution for at least 20 min for proper sterilization if an autoclave is not available. Once sterilized any plates that are used can be double bagged and treated as normal trash. Safety glasses are recommended when using bleach solutions.
- Ampicillin may cause allergic reactions or irritation to the eyes, skin, respiratory systems. In case of contact, rinse area immediately with plenty of water. Ampicillin is a member of the penicillin family and those with penicillin allergies should avoid contact with ampicillin.
- UV radiation can cause damage to eyes and skin. Short-wave UV is more damaging than long-wave UV light.

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Procedures for Instruction

Length of Time for Preparation: 1 day
Day 1: Prep culture medium/culture tubes (approx. 15 minutes). Set up microcentrifuge tubes, test tubes and buffers.
Note: Set up can also be done before each day (work station prep takes approximately 5 min).

Length of Time for Classroom Teaching: 4-5 days
Day 1: Inoculating Cell Cultures
Day 2: Bacterial Concentration and Lysis
Day 3: Removing Bacterial Debris
Day 4: Protein Chromatography

Preparation Procedure/Instructions

- Preparation for Day 1: Make LB broth with arabinose and ampicillin. This can be done by boiling 55 ml of distilled water and adding the LB capsule provided in your lab kit. While the capsule is dissolving rehydrate the arabinose and the ampicillin. After the capsule is completely dissolved and cooled add 0.5 ml of the arabinose and 0.5 ml of the ampicillin. Once mixed, place 2 ml of the mixture into the 16 culture tubes. Cap each and place in the refrigerator until needed for lab day. (Note: This mixture can be prepared up to two weeks in advance, but it is best if prepared 12-24 hours in advance.)
- Preparation for Day 2: Students will be transferring liquid cultures into microcentrifuge tubes. You will need a tube for each group, a centrifuge, the TE buffer, the lysozyme, and a UV light for the lab. There is no special set up required.
- Preparation for Day 3: You will need to have the columns ready for students to prepare, as well as the equilibration buffer, and the binding buffer that will be used on the pellet. No other special set up required.
- Preparation for Day 4: 3 test tubes per group will be needed for the lab day. Each time the solution in the chromatography column reaches the top of the medium a different buffer will be added. Students will need the UV light after each step and observations will be made between adding each new buffer.

Background Information

One major accomplishment in the corn industry during this century has been the development of Bt Corn. This variety of corn is genetically modified with a gene that produces a highly selective toxin designed to control for European Corn Borers. In this case, the donor organism is a naturally occurring soil bacterium, Bacillus thuringiensis, hence the abbreviation Bt Corn. The toxin produced, Bt delta endotoxin, is very specific in its targeting of the Lepidoptera larvae. In this stage of development, Lepidoptera larvae create the most damage to growing crops. Once the larvae ingest the protein, Bt delta endotoxin, it binds to the organism's gut and the

larvae stop eating, which prevents further damage. It also decreases growing populations of the European Corn borer by causing the larvae to die. This is due to the release of normal gut bacteria from the organism into the body, which causes septicemia and eventual death. The increase in death rate and decrease in birth rate of the organisms ultimately leads to overall population control, all by simply adding one protein into the corn genome. The need for the widespread spraying of pesticides for this pest is eliminated, and it leads to an increase in yield due to less crop destruction by the pests themselves.

How did scientists know these effects would occur? How did they know there were sufficient levels of the protein being produced in the newly genetically altered corn crops? Multiple tests conducted over many years must be performed to confirm and purify the intended protein. Purifying proteins from cells is a multiple-step process and is dependent, in part, on the properties of the protein themselves.

In this lab students will be extracting and purifying the GFP protein they transformed into E. coli cells in *the Bacterial Transformation lab*, which is Part 3 of the lab previously performed, *Kansas Corn: Feeding the World – DNA to the Rescue* (available on www.kscorn.com). Students begin by using one colony of transformed bacteria from each condition of the *Bacteria Transformation* lab. The colonies are added to nutrients to grow, then incubated. This produces many exact copies of one genetically unique organism. After 24-48 hours, students will put the bacteria through many physical and chemical processes to release the produced protein GFP by breaking down the bacteria cell walls and removing bacterial debris from the sample. The remaining proteins are then purified by going through a chromatography column treated with different buffers to retain the GFP protein and rinse away any other proteins present. The final rinse in the process releases the GFP proteins into a collection tube.

Classroom Discussion

Introduce the topic and assess students for prior understanding. Let students discuss their ideas, and guide the discussion without telling them if they are right or wrong. The questions below can be used to help facilitate small group discussions of two to four people or as post-lab review.

Day 1: Genetic Transformation Review and Inoculation of Cell Cultures

- Review proteins:
  - What are they? What are some examples that can be found in our bodies? What is the relationship between genes and proteins?
- Describe cloning
- Perform lab for Day 1: *Inoculation of Bacterial Cultures*
- Review what bacterial colonies are
- What would happen if we took a colony grown on the LB/AMP/Ara plate and streaked it onto a LB/AMP plate?

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Day 2: Bacterial Concentration and Lysis

- What is the function of the following items on the bacterial colony?
  - Centrifuge
  - Lysozyme
  - Freezer
- Why do the cells outer walls rupture when frozen?
- What was the purpose of rupturing (lysing) the bacteria?

Day 3: Removing Bacterial Debris

- Describe the pellet and the supernatant? What kind of information does this tell you? Hint: Use the UV light.
- Why is the pellet no longer needed?
- Describe the hydrophobic interaction chromatography and its purpose in this lab.

Day 4: Protein Chromatography

- Predict what will happen in the chromatography column when each buffer is added (3 different predictions, with one for each buffer).
  - Finish lab and write down results
- Compare your predictions with your observations for each buffer.
- Explain the roles or functions of each buffer (use the name of each buffer to relate to its function).
- Were you successful in isolating and purifying GFP from the cloned bacterial cells? What evidence supports your answer?

Procedure for Lab

Refer to the GFP Procedure – Quick Guide handout from Bio-Rad Labratories, Inc. (pg. S1-S4, or available online at kscorn.com).

Teachers Tips

- Please visit kscorn.com for videos to help with the lab.
- Day 1 Tips: After students have transferred bacteria to the culture tubes, if you do not have a shaker, allow two days for growth of bacteria. You will still get a lot of bacterial growth for the later days of the lab.
- Day 2 Tips: There is some down time while waiting on the centrifuge to finish. While students are waiting, this is a good time to review why the lysozyme is being added and what purpose freezing the bacteria has.

- Day 3 Tips: Use this day to continue discussions on gene transfer. Make sure students transfer the supernatant immediately after the centrifuge is complete so the pellet does not contaminate the supernatant or clog the chromatography column.
- Day 4 Tips: Patience is key today. It can take 5-10 minutes for the buffer to pass through the chromatography column.

Lab Analysis

For analysis during the lab, use the black light pen that was provided in the kit. You should see a light green line in the chromatography column that will be located towards the top. This will remain until the last step of adding solution into the chromatography column where you will see the green fluorescent line move through the column and eventually into the final test tube. The green fluorescent line is the protein produced by the E. coli that was made in the following lab: *Bacterial Transformation*, which is Part 3 of *Kansas Corn: Feeding the World – DNA to the Rescue* (available online at www.kscorn.com).

Reflection and Conclusion

Review chromatography and how we used it in this lab. What purpose did each buffer serve when running the chromatography?

- Equilibration buffer: Equilibrate the chromatography column
- Binding buffer: Supernatant with the GFP will have the same salt concentration as the equilibrated column. The higher salt concentrations allow for more of the hydrophobic regions of the protein to be exposed, giving them a better chance to interact and bind with the hydrophobic regions of the column.
- Wash buffer: Washes weakly associated proteins from the column, leaving behind proteins that are strongly hydrophobic, and in this case leaves our GFP.
- Elution buffer: Causes hydrophilic regions in the GFP to become exposed, as higher water concentration means the GFP now has a higher affinity to the buffer and will move through the column.

Why is it important to purify proteins in this manner? Do we do anything like this today? Reviewing some of the questions in the background can be done here. How did scientists know that there were sufficient levels of the protein being produced in the newly genetically altered corn crops?

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Critical Takeaways:
Creating new varieties of any crop through genetic modification is a complex multiple-step process. All along the way there are many checks and balances to ensure that products are safe and that the results follow the original intent of the program. Millions of dollars, and sometimes decades of work, can go into a single variety of a genetically modified crop. We are not playing gene-roulette and randomly selecting genes to add into these varieties. The cost and time associated with that type of approach to genetic modification would be a huge obstacle to the development of any new variety. Students must understand that the selection of donor genes depends on the needs of the industry as a whole. Genes that convey drought tolerance are much more critical in arid regions of the world than genes that provide resistance to types of fungi. Careful analysis of the genes occurs to ensure the genes selected fit not only the organism but also produce the appropriate traits in the crop for specific regions. A central message to students must include the careful analysis of not only the host organism but the selected donor gene as well.

We see this cautious approach being used in the multitude of tests newly created varieties must pass before they reach the wide spread market. As a part of these tests, scientists would analyze what new proteins are being created by the new variety. This method of protein purification can be utilized to ensure not only what protein is being produced but also that the appropriate levels of protein are present in the new varieties. After the extraction, purification, and collection of these new proteins, additional tests would then be undertaken to ensure the products safety years before it would be available for use in the open market.

Science and Agriculture Careers
The production of Bt Corn was a great accomplishment and the trait has been expanded to other lines of crops as well. However, this agricultural innovation has created one downfall. By introducing this trait into crops produced in large-scale agriculture we have artificially increased its prevalence in the environment. This has caused the evolutionary process of natural selection to speed up in the case of pests. The effectiveness of this trait was so great that most pest populations remaining are resistant to toxins such as those found in Bt corn. As a result, new strategies and lines of defense must be discovered in order to continue to protect our crops from destruction from these newly evolved pests. For this we will need highly skilled individuals with an eye for detail and a passion for laboratory investigation. Several different careers in agriculture are fundamental in the development of new varieties of crops: botanists, horticulturalists, biochemists, biological engineers, climatologist, ecologists, food scientists, geneticist, microbiologists, plant pathologist, and an army of lab technicians are all involved in the development of each and every variety.

To learn more about agriculture careers visit agexplorer.com. You can also find career profiles at kscorn.com.

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Sources

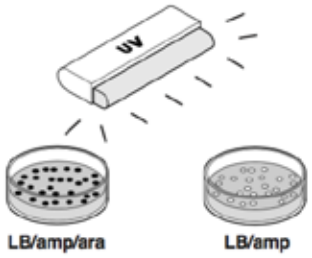
- pGLO Quick Guide - Courtesy of Bio-Rad Laboratories, Inc., © 2018
- Bt Corn: What is it and How does it work? https://entomology.ca.uky.edu/ef130

Any educator electing to perform demonstrations is expected to follow *NSTA Minimum Safety Practices and Regulations for Demonstrations, Experiments, and Workshops*, which are available at http://static.nsta.org/pdfs/MinimumSafetyPracticesAndRegulations.pdf, as well as all school policies and rules and all state and federal laws, regulations, codes and professional standards. Educators are under a duty of care to make laboratories and demonstrations in and out of the classroom as safe as possible. If in doubt, do not perform the demonstrations.

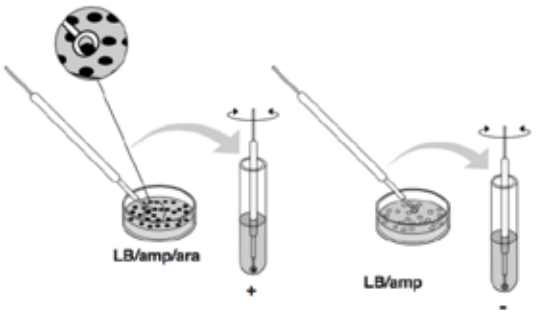
GFP Purification—Quick Guide

Lesson 2 Inoculation
Growing Cell Cultures

1. Remove the transformation plates from the incubator and examine using the UV light. Identify several green colonies that are not touching other colonies on the LB/amp/ara plate. Identify several white colonies on the LB/amp plate.



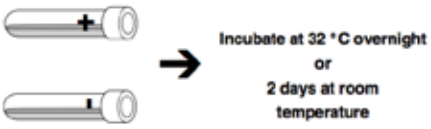
2. Obtain two culture tubes containing the growth media LB/amp/ara. Label one "+" and one "-". Using a sterile loop, lightly touch the loop to a green colony and immerse it in the "+" tube. Using a new sterile loop, repeat for a white colony and immerse it in the "-" tube (it is very important to pick only a single colony). Spin the loop between your index finger and thumb to disperse the entire colony.



3. Cap the tubes and place them in the shaking incubator or on the shaking platform and culture overnight at 32 °C or 2 days at room temperature.

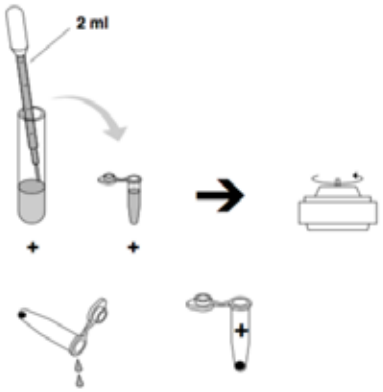
   or

   Cap the tubes and shake vigorously by hand. Place in the incubator horizontally at 32 °C for 24–48 hours. Remove and shake by hand periodically when possible.



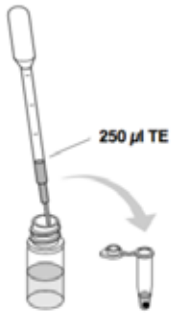
Lesson 3 Purification Phase 1
Bacterial Concentration

1. Label one microtube "+" with your name and class period. Remove your liquid cultures from the shaker and observe with the UV light. Note any color differences between the two cultures. Using a new pipette, transfer 2 ml of "+" liquid culture into the "+" microtube. Spin the microtube for 5 minutes in the centrifuge at maximum speed. The pipette used in this step can be repeatedly rinsed in a beaker of water and used for all following steps of this laboratory period.

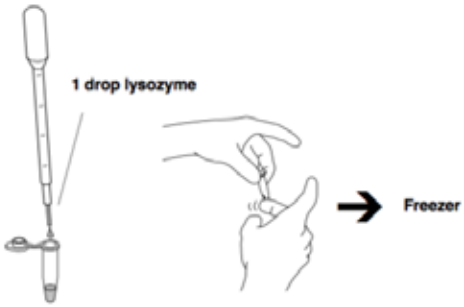


2. Pour out the supernatant and observe the pellet under UV light.

3. Using a rinsed pipette, add 250 µl of TE solution to the tube. Resuspend the pellet thoroughly by rapidly pipetting up and down several times.



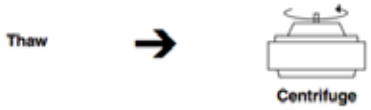
4. Using a rinsed pipette, add 1 drop of lysozyme to the resuspended bacterial pellet to initiate enzymatic digestion of the bacterial cell wall. Mix the contents gently by flicking the tube. Observe the tube under the UV light.



5. Place the microtube in the freezer until the next laboratory period. The freezing causes the bacteria to rupture completely.

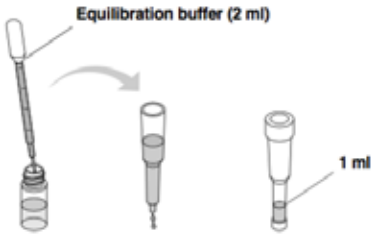
Lesson 4 Purification Phase 2
Bacterial Lysis

1. Remove the microtube from the freezer and thaw using hand warmth. Place the tube in the centrifuge and pellet the insoluble bacterial debris by spinning for 10 minutes at maximum speed.

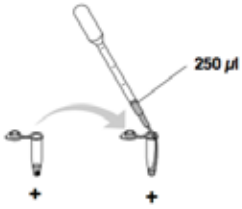


2. While your tube is spinning, prepare the chromatography column. Remove the cap and snap off the bottom from the prefilled HIC column. Allow all of the liquid buffer to drain from the column (~3–5 minutes).

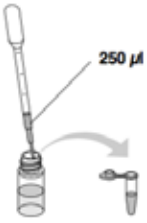
3. Prepare the column by adding 2 ml of Equilibration Buffer to the top of the column. This is done by adding two 1 ml aliquots with a rinsed pipette. Drain the buffer to the 1 ml mark on the column. Cap the top and bottom and store the column at room temperature until the next laboratory period.



4. After the 10 minute spin, immediately remove your tube from the centrifuge. Examine the tube with the UV light. Using a new pipette, transfer 250 µl of the "+" supernatant into a new microtube labeled "+". Again, rinse the pipette well for the rest of the steps of this lab period.



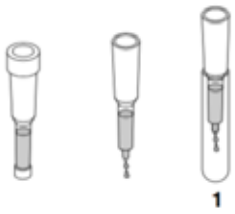
5. Using a well rinsed pipette, transfer 250 µl of binding buffer to the "+" supernatant. Place the tube in the refrigerator until the next laboratory period.



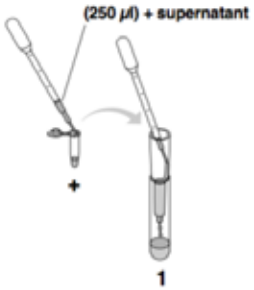
Lesson 5 Purification Phase 3

Protein Chromatography

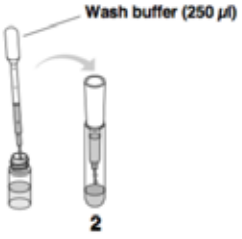
1. Label 3 collection tubes 1–3 and place the tubes in the foam rack or in a rack supplied in your laboratory. Remove the caps from the top and bottom of the column and place the column in collection tube 1. When the last of the buffer has reached the surface of the HIC matrix proceed to the next step below.



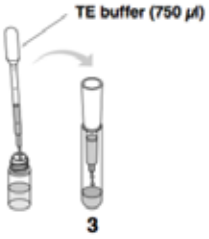
2. Using a new pipette, carefully and gently load 250 µl of the "+" supernatant onto the top of the column. Hold the pipette tip against the side of the column wall, just above the upper surface of the matrix and let the supernatant drip down the side of the column wall. Examine the column using a UV light. Note your observations. After it stops dripping transfer the column to collection tube 2.



3. Using the rinsed pipette, add 250 µl of wash buffer and let the entire volume flow into the column. Examine the column using the UV light. Note your observations. After the column stops dripping, transfer it to tube 3.



4. Using the rinsed pipette, add 750 µl of TE Buffer and let the entire volume flow into the column. Examine the column using the UV light. Note your observations.



5. Examine all three collection tubes and note any differences in color between the tubes. Parafilm or Saran Wrap the tubes and place in the refrigerator until the next laboratory period.