

# Kansas Corn: Feeding the World – DNA to the Rescue



#### Overview

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 responsible for abiding appropriate legal standards and better professional practices 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.

Since man first started raising crops, we have guested for stable varieties with high yield, no matter what the conditions may be. Our early ancestors developed many techniques such as cross breeding to develop some of our earliest crops. Humans have been seeking out plants that produced high yield fruit and grains, and nurturing specific varieties through selective breeding, until we developed the very crops we know today. Corn was once a small plant, which is today considered a weed, teosinte. Teosinte had very few grains on each fruiting body, and all grains had very hard outer seed cases that would protect the nutrition to be found inside. Through humans selecting varieties from the wild and cross breeding them by hand, we developed the many maize varieties we have built our civilization on. Today, we face an ever increasing challenge of producing more and more food for a growing human population. We must go back to our roots and continue to develop varieties, just as our ancestors before us, to meet this demand. Through the expanded biological understandings of genetics and new techniques of genetic modification we have to create varieties that increase yield, decrease herbicide and pesticide use, and ultimately grow more food. In this exercise students will learn more about the origins of plant varieties, and how we continue to develop new varieties by utilizing new technologies.

#### Kansas College and Career Ready Standards Addressed:

#### 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.



#### Kansas College and Career Ready Standards Addressed (Continued):

#### Science (Continued)

- **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.

#### 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. (LA)
- 2. Connect biology, chemistry, and biochemistry to agriscience. (S)

#### **Learning Objectives:**

- To demonstrate the universality of DNA and its expression
- To explore the concept of phenotype expression in organisms
- Understand how to use phenotypic expression of DNA to screen for a gene of interest and the importance of marker genes
- Investigate how horizontal gene transfer is a mechanism by which genetic variation is increased in organisms
- Explore the relationship between environmental factors and gene expression
- To investigate the connection between the regulation of gene expression and observed differences between individuals in a population of organisms
- Learn the importance of sterile techniques and how to properly and safely handle bacteria and the decontamination process

#### **Materials Needed:**

#### **Materials for Plasmid Modeling:**

- Paper Plasmid Modeling (pg. \$1-\$2, or available online at kscorn.com)
- Plasmid Modeling Technology-Based (pg. S3-S4, or available online at kscorn.com)



#### Materials Needed (Continued):

#### Materials for pGLO Transformation Lab (Class):

- pGLO Quick Guide (pg. S5-S6, or available online at kscorn.com)
- pGLO Student Sheet (pg. S7-S8, or available online at kscorn.com)
- pGLO Lab Kit:
  - Microtubes
  - Floating foam micro tube racks
  - Disposable sterile pipettes
  - Disposable sterile inoculating loops
  - Sterile disposable plates
  - LB powder
  - Ampicillin
  - Arabinose sugar
  - DNA plasmid
  - E. coli
- Spray bottles containing 10% bleach solution
- Disposal/waste containers with 10% bleach solution
- Water bath
- Incubator (if available)
- Parafilm
- UV Light

#### Materials for Each Lab Group:

- Disposable gloves
- Safety goggles
- 2 different colored micro tubes
- 1 floating micro tube rack
- 6 disposable sterile pipettes
- 1 package of sterile loops
- Foam cup with crushed ice
- 4 prepared plates (1 LB, 2 LB & Amp, 1 LB, Amp & Ara)
- Fine tipped Sharpie
- Waste container with 10% bleach solution

#### Materials for Assessments:

NSTA Lab Report Rubric (pg. T20, or available online at kscorn.com)

**Safety Considerations:** The E. coli strain used in this lab is non-pathogenic, meaning it will not directly cause illness in humans, but it is important to teach the students good sterile technique and safe disposal of bacteria or anything that could be contaminated.



#### Safety Considerations (Continued):

- Gloves and safety glasses are to be worn at all times during this experiment.
- Be cognizant of all surfaces, wiping down the surfaces with the 10% bleach solution before the lab to prevent growing anything you aren't intending to grow.
- Use a 10% bleach solution to wipe down the benches at the end of the experiment as well.
- Disposal beakers with a 10% bleach solution to place all contaminated or used loops or pipettes should be provided to students in a convenient location at each lab station.
- Wash hands well with warm soap and water before leaving lab.

**To dispose of contaminated material:** Immerse all disposable pipettes, tubes, and loops that have come in contact with bacteria in 10% bleach solution for at least 20 minutes before draining, rinsing, and disposing of in the trash.

Immediately after use of the pipette or loop, place it directly into the waste beaker. Remind them that these items are designed to be used one time only.

When you are finished with the lab, collect all petri dishes, open, and immerse in a 10% bleach solution to kill all bacteria. This can be easily accomplished by flooding all the plates with a few ml of the bleach solution. Allow materials to stand in bleach solution for 20 minutes or more. Drain excess solution, seal materials in a plastic bag, a large zip lock bag works well to contain any liquid that may seep from the plates. Place all bags containing the materials and double bag in trash bags then simply place in the trash for final disposal.

#### **Procedures for Instruction**

#### **Length of Time for Preparation:** 3-5 days

Day 1: Pour plates

Day 2: Cure plates (room temp.)

Day 3: Inoculate starter plates with E. coli and incubate

Day 4: Complete transformation; begin incubation (overnight in incubator, or 2 days on lab table)

Day 5: Review lab results

#### **Length of Time for Classroom Teaching:** 2-3 days

Day 1: Complete transformation; begin incubation (overnight in incubator, or 2 days on lab table)

Day 2: Review lab results



### Part 1: Activating Prior Knowledge and Anticipatory Discussion (30-45 minutes)

**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 Problem of the Past: How do we grow enough food to be able to stay in one place and support our growing population?"

- Write the problem above on the board.
- Put students in groups of 2-3 to discuss and (potentially) research how humans solved the problem of the past.
- Additional Guiding Questions:
  - What food sources should we try and grow for ourselves?
  - How do we make sure our crops will survive to harvest?
  - How do we make sure our crops will produce enough food?

#### Solutions from the Past: The Human-Guided Evolution of Corn

- Have students research the plant teosinte and its modern day relative corn.
- Create a Venn Diagram showing the similarities and difference between the two crops.
- Note: As an alternative activity, have students create a trait time line, showing how
  the plant itself changed overtime from what teosinte was in the past to corn we know
  today. Paying close attention to what specific traits changed over time (ex: number of
  kernels, protective outer layer on seed...).

"Today's Problem: The Food and Agriculture organization of the United Nations estimates that in order to support the world population we will need to produce 70% more food than current levels now by the year 2050. How can we do this?"

- In the same groups, discuss the new question above do you see any crossovers?
- Additional Guiding Questions:
  - How can we increase the yield of our crops?
  - How can we decrease the amount of pesticide used in agriculture?
  - How can we produce more food with the same amount of land?

#### **Potential Crossover Points:**

- Domestication of plants and animals leads to more food security.
- More food security leads to the ability to have larger populations in one fixed area.
- Intentional production of new lines of plants and animals for desirable traits through cross breeding and artificial selection.



### Kansas Corn: Feeding the World – DNA to the Rescue

**Grade Level: High School** 

#### Part 1: Activating Prior Knowledge and Anticipatory Discussion

Classroom Discussion (Continued): Explain to the class that domestication of plants and animals has always been about trying to meet the needs of humans based on our available resources. This is how civilization as we know it developed. We have used domestication methods of breeding and hybridization in the past with great success. Now we are growing our population more and more, so the demand for food is larger. These traditional methods of breeding are no longer able to provide the large amount of food needed to support our civilization. However, biotechnology allows us to do the same things we did with traditional breeding techniques, with higher success rates and more direct trait selection.

**NOTES:** 



#### Part 2: Development and Use of Plasmids

(45-60 minutes)

**Background Information:** What is a plasmid? A plasmid is a small circular piece of DNA, which is often passed between bacteria. This allows genetic traits to move from one bacteria to another. A great example of this is the growing number of bacterial infections that are developing antibiotic resistance. The DNA that codes for this antibiotic resistance can sometimes be found on plasmids; once this plasmid has been developed it is passed from bacteria to bacteria taking with it the ability to resist certain antibiotics. The plasmid DNA is incorporated into the new bacterial DNA and all of the future offspring of that one bacteria (which is an asexual producing organism) now possess the gene for this antibiotic resistance.

Scientists have used these plasmids to intentionally transmit genes that code for specific traits from one host organism, where the advantageous genes have been found, into a new organism (this process is called "transformation"). This new organism is now transgenic. Transgenic organisms have genes from the new host organism and their own genes. This process has been a great tool for humanity. We have used it to create crops that contain genes that will protect themselves from pest, resist herbicides, and survive in areas where the environment is not hospitable to traditional crop varieties.

The process of making plasmids involves multiple steps:

**Step 1:** Find a gene that codes for the trait you want to add to the new crop variety from a host organism.

**Step 2:** Find a restriction enzyme that will cut that gene from the host organism's DNA and will cut the plasmid you have chosen to insert the gene into.

**Step 3:** Join both the source DNA with the desired trait with the plasmid DNA.

You then have to introduce the plasmid into the crop's DNA and carefully select the cells where the new gene has been successfully inserted into the genome. This step can be difficult to do, because of this most scientist also include marker genes into their plasmid that allows scientists to easily determine if the transformation process occurred in the cells. One such marker gene will cause bacterial cells that have been successfully transformed to glow under ultra violet light.

For student activities, utilize the Paper Plasmid Modeling handout (pg. \$1-\$2, or available online at kscorn.com) and the Plasmid Modeling Technology-Based handout (pg. \$3-\$4, or available online at kscorn.com).





### **Paper Plasmid Modeling**

#1	•	#2		#3	3	#4	1	#5	5	<b>P</b> :	1	P2	2	P3	3	PΔ	1	RE	3	RE	7
T	Α	Т	Α	<b>T</b>	<b>A</b>	C	G	Α	Т	G	C	Т	Α	G	С	Т	Α	С	G	C	G
G	C	Т	Α	A	<b>  T</b>	<b>T</b>	A	G	C	С	G	Т	Α	С	G	С	G	C	G	T  <i> </i>	4
G	C	С	G	C	G	<b>T</b>	A	Α	Т	С	G	Α	Т	Α	Т	G	C	т	Α	C	G
G	C	Т	Α	A	<b>  7</b>	<b>T</b>	A	Α	Т	С	G	Α	Т	Α	Т	Α	Т	Α	Т	G	C
С	G	Т	Α	<b>T</b>	A	<b>T</b>	A	Т	Α	Α	Т	С	G	G	C	Α	Т	G	C	A	<u> </u>
С	G	Α	Т	A	<b>  T</b>	A	<b>T</b>	Т	Α	G	C	С	G	G	C	С	G	G	C	G (	С
T	Α	Α	Т	A	<b>T</b>	A	<b>T</b>	Т	Α	Α	Т	Т	Α	Т	Α	G	C	RE	4		
Α	Т	G	C	C	G	A	<b>T</b>	С	G	G	C	Α	Т	Т	Α	С	G	Т	Α	RE	8
G	C	Т	Α	G	C	<b>T</b>	A	G	C	Т	Α	G	C	Α	Т	С	G	c	G	G (	C
G	C	С	G	<b>T</b>	A	G	C	Α	Т	Т	Α	G	С	Т	Α			т	Α	G	С
С	G	Α	Т	C	G	<b>T</b>	A	Α	Т	Т	Α	Α	Т	Α	Т	RI	Ξ1	Α	Т	G	С
Α	Т	Α	Т	<b>T</b>	A	A	<b>T</b>	С	G	С	G	G	C	С	G	С	G	G	C	C	G
С	G	G	C	C	G	A	<b>T</b>	G	C	Т	Α	G	C	Т	Α	$\overline{c}$	G	Α	Т	C	G
Α	Т	С	G	<b>T</b>	A	<b>T</b>	A	G	C	Т	Α	G	С	Т	Α	Т	Α	RE	5	C	G
G	C	Α	Т	<b>T</b>	A	A	<b>T</b>	G	C	Α	Т	С	G	Α	Т	G	<u>C</u>	G	C		
G	C	G	C	C	G	<b>T</b>	A	G	C	Α	Т	С	G	Α	Т	G	C	G	C	RE	9
G	C	G	C	G	C	<b>T</b>	A	С	G	G	C	С	G	G	C			c	G	<u>A</u>	Γ
С	G	Т	Α	<b>T</b>	A	С	G	С	G	G	C	Т	Α	С	G	RI	<b>2</b>	С	G	A	Γ
С	G	Т	Α	C	G	С	G	С	G	Т	Α	G	C	С	G	T	Α	RE	6		<u>G</u>
С	G	С	G	A	<b>T</b>	Т	Α	Т	Α	C	G	G	C	G	C	T	Α	C	G	G	С
G	C	G	C	<b>T</b>	A	С	G	Α	Т	Т	Α	Т	Α	Т	Α	С	G	╗	Α		
G	C	Α	Т	G	C	С	G	G	C	С	G	G	<b>C</b>	Α	Т	G	С	Т	Α		
Α	Т	Α	Т	<b>T</b>	A	Т	Α	G	C	G	C	G	C	G	C	Α	Τ	A	Т		
G	C	G	C	G	C	Т	Α	Α	Т	Α	Т	G	C	G	C	Α	Т	A	<u>T</u>		
Α	Т	G	С	<b>C</b>	G	Α	Т	С	G	G	С	G	<b> </b>	Т	Α			G	С		



#### Paper Plasmid Modeling (Continued)

- 1. Cut out each column.
- 2. Tape together P1, P2, P3, P4, in order, the top of P2 onto the bottom of P1 and continue, until all the strips are taped together.
- 3. Now tape the end of P4 to the beginning of P1 to form one continuous loop, creating your plasmid DNA sequence.
- 4. Cut out and tape the other sequences labeled #1, #2, #3, #4, #5. Tape these together in numerical order as before but **do no**t tape into a circle. This represents your DNA sequence with the desired gene. The desired gene sequence is indicated by the **bold text** and horizontal line between the base pairs.
- 5. Cut out the restriction enzyme cards, represented by RE1... Cut out all 9 individually.
- 6. The goal is to determine what restriction enzyme will work to cut both the Desired gene and the plasmid. In order to insert the gene into the plasmid you will want to cut the plasmid once, but the desired gene twice.
  - The trick is you don't want to cut into the gene as it might cause a mutation and change the trait you are trying to convey.
  - You also cannot cut into the part of the plasmid that causes the DNA to be expressed. This is represented in the plasmid DNA the same way, **bold text** and a vertical line.
- 7. Compare the restriction enzymes to the plasmid DNA, mark on the sequences where each enzyme would cut the sequence. **Do not** cut the sequence now.
  - Make sure you show the "sticky ends" when you mark on the plasmid DNA
  - You may find it helpful to use a different colored pencil for restriction enzyme to help you keep track of each location.
  - Some restriction enzymes may not cut the plasmid. Anything that doesn't cut the sequence, can be thrown out.
  - We are looking for enzymes that cut the sequences on both side of the desired gene as closely as possible.
- 8. Compare the restriction enzymes left to the source DNA, mark on the sequences where each enzyme would cut the sequence. **Do not** cut the sequence now.
  - Make sure you show the "sticky ends" when you mark on the plasmid DNA.
  - We are looking for enzymes that cut the sequences on both side of the desired gene as closely as possible.
  - Anything that does not cut the plasmid and the source DNA can be thrown out.
- 9. You should be down to one successful enzyme! Use this enzyme to cut both the plasmid and the source DNA.
  - Tape the source gene into the plasmid matching up the sticky ends.
- 10. Congratulations you have successfully engineered your plasmid!



#### Plasmid Modeling: Technology-Based Version

Problem: How do you get a gene with a desirable trait into another organism?

DNA Sequence with Desired Gene:

TGGGCCTAGGCACAGGGCCCGGAGATTCTTAAGTCAAGCAGGTTCGAAGG**TACATAACGTCTCTTC-GCTGTGCCTTTAAATGTAATATT**CCTCCTTAAGAATTTCGAACGGGGCCCTAGGACC

Bold text – Representing the Desired Gene – This area cannot be cut!

#### <u>Plasmid Sequence:</u>

GCCCAGAGTTCTTAAGGTCTCGAGTTAACCTAGGAGGGCCCTGGTG**GGGG**CAAGGTTATACTTAAGCCGTAGGTTCGAACGCC

#### Bold text – Representing the Plasmid Replication Sequence – This area cannot be cut!

Restriction	Plasmid Cuts	DNA Cuts	Distance from Gene – Front	Distance from Gene – Back
Enzyme				
Possibilities				
1: CCTGG				
2: TTCGAA				
3: CCTAGG				
4: TCTAGA				
5: GGCC				
6: CTTAAG				
7:CTCGAG				
8: GGGCCC				
9: AAGCG				

To fill in the table type "Control F" and then type the enzyme sequence into the Navigation Dialog box. This lets Microsoft Word work as a Genome Analyzer, by highlighting the areas in the sequences where the specific sequences coded for the restriction enzyme sequences are located.

Remember if the highlight is in the **bold text** area it does not count as a "cut" because you cannot cut in those areas.

Which restriction enzyme do you recommend to use in this case? Remember the goal is to cut the DNA sequence as closely as possible to the desired gene without cutting into the gene sequence itself.



### Plasmid Modeling: Technology-Based Version (Continued)

What is the sequence for the resulting plasmid DNA?
There is one thing this model does not show in regards to restriction enzymes. A restriction enzyme cuts the DNA at a certain location through both strands of DNA. This model only shows you the sequence on one side of the helix, not both antiparallel strands. Restriction enzymes work on both strands simultaneously. They cut both horizontally through the back bone of the helix, and they also cut vertically through the hydrogen bonds, which hold together the base pairs. Ultimately, leaving one side of the helix slightly longer than the other side. This is what we call "sticky ends". If it is cutting the plasmid, the restriction enzyme leaves a tail of exposed bases on the plasmid DNA that must match up perfectly with the opposing tail from the cut on the DNA with the desired gene. Creating two "sticky ends", as opposed to blunt ends, is of major importance. What would happen if the restriction enzyme were to create "blunt ends" instead of sticky ones?

#### Part 3: Bacterial Transformation

(Pre-Lab: 20-30 minutes and Lab: 45-60 minutes)

Complete the Bacterial Transformation Lab as outlined with the instructions from the Bio-Rad Laboratories, Inc. Transformation Kit – Quick Guide. Below, on (pg. S5-S6, you will find the pGLO Quick Guide for students (also available online at kscorn.com). This visual reference should be utilized in the pre-lab with students.

**Procedure for Pre-lab:** Each student should have a printed copy of the pGLO Quick Guide.

- Have each student highlight the actions taken during the lab, go through the entire guide and look for verbs.
- Once you have isolated the actions you must take, rewrite the instructions next to the picture of each step. Use abbreviations and as few words as possible. Write these in a different color (red, bright blue, pink....) next to each picture.
- Pay special attention to times and temperatures listed on each step. The times required for the heat shock are especially important.
- Count up the number of sterile pipettes and loops you will need in total for the lab. Write a large "P" next to each step that requires the use of a new sterile pipette, write a large "L" next to each step that requires the use of a new sterile loop. If you are using multiple loops or pipettes you can use a short hand notation, "2xP" or "2xL", using the total number needed in place of the "2" when necessary.

**Procedure for Lab:** Follow the instructions on the pGLO Quick Guide that you have highlighted. It is important to make sure all students have done the pre-lab before you complete the actual lab, as there are time sensitive elements that must be followed correctly for the best results. Refer to the pGLO Quick Guide handout from Bio-Rad for lab instructions.

#### Teacher Tips:

• Visit kscorn.com to view the following video to help with the lab: Plate Prep Video.

**NOTES:** 

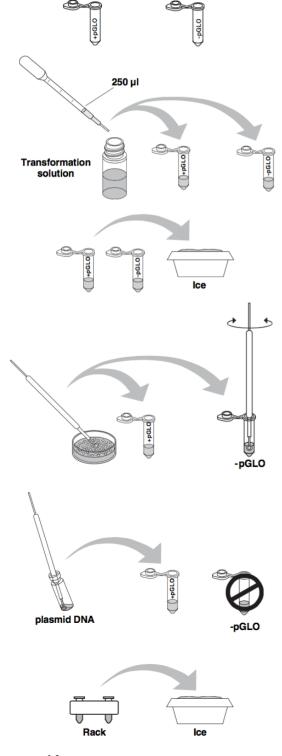




#### pGLO Quick Guide

#### **Transformation Kit—Quick Guide**

- Label one closed micro test tube +pGLO and another -pGLO. Label both tubes with your group's name. Place them in the foam tube rack.
- 2. Open the tubes and using a sterile transfer pipet, transfer 250 µl of transformation solution (CaC1<sub>2</sub>).
- 3. Place the tubes on ice.
- 4. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Place the tube back in the tube rack in the ice. Using a new sterile loop, repeat for the -pGLO tube.
- 5. Examine the pGLO plasmid DNA solution with the UV lamp. Note your observations. Immerse a new sterile loop into the plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles. Mix the loopful into the cell suspension of the +pGLO tube. Close the tube and return it to the rack on ice. Also close the -pGLO tube. Do not add plasmid DNA to the -pGLO tube. Why not?
- Incubate the tubes on ice for 10 minutes. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.



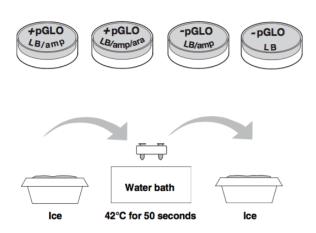


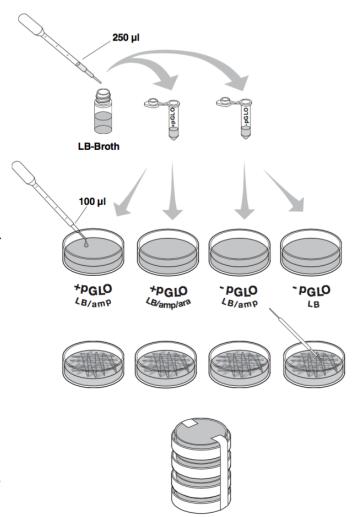




#### pGLO Quick Guide (Continued)

- While the tubes are sitting on ice, label your four agar plates on the bottom (not the lid) as follows:
   Label one LB/amp plate: +pGLO;
   Label the LB/amp/ara plate: +pGLO;
   Label the other LB/amp plate: -pGLO;
   Label the LB plate: -pGLO.
- 8. Heat shock. Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42 °C, for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. When the 50 seconds are done, place both tubes back on ice. For the best transformation results, the change from the ice (0°C) to 42°C and then back to the ice must be rapid. Incubate tubes on ice for 2 minutes.
- 9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipet, add 250 µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the other tube. Incubate the tubes for 10 minutes at room temperature.
- Tap the closed tubes with your finger to mix. Using a new sterile pipet for each tube, pipet 100 μl of the transformation and control suspensions onto the appropriate plates.
- 11. Use a new sterile loop for each plate. Spread the suspensions evenly around the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface.
- 12. Stack up your plates and tape them together. Put your group name and class period on the bottom of the stack and place the stack upside down in the 37°C incubator until the next day.









### pGLO Student Sheet

Name:	
nullie.	

		Prediction	Reason	Results: with Explanation!
- pGLO	LB LB Amp			ROSSIS. WITH EXPIGITIONS
+ pGLO	LB Amp Ara			



### pGLO Student Sheet (Continued)

#### **Analysis Questions:**

1. How did the introduction of the plasmid change the bacteria?
2. How can you be sure that the bacteria were transformed with the particular gene?
3. How could you prove that the transformation process occurred if the GFP gene was not included in the plasmid?
4. How does this lab connect to the production of transgenic crop varieties?

**Reflection and Conclusion:** Refer to the pGLO Student Sheet (pg. S7-S8, or available online at kscorn.com) for post-lab activity.

After the incubation of the transformed plates, you will have 4 observations to make. There are guiding assessment questions to answer on the following document as well as a place to record data. This can be printed or can be sketched into a science journal.

Do not forget to use this as an opportunity to expand student ideas about how we would then use these genes. In corn, one of the first transgenic varieties contained a gene from the bacteria, Bacillus thuringiensis (Bt), which causes the plant to create a toxin which stops insects from eating the plant and killed any insect that ingested the plant. This allowed the newly transformed plant to be less susceptible to damage from insects; thus increasing yield overall and decreasing the use of additional pesticides. As an extension activity, pose the following questions:

- Is this the only type of gene we could insert?
- If you were a scientist, what other genes would you be looking for to help develop new varieties of crops?

#### Assessments:

**Plasmid Modeling:** Final plasmid can be checked for accuracy. This is easily accomplished if done via the technology-based version. The paper version can be visually inspected as well. The Paper Plasmid Modeling handout can be found on pg. \$1-\$2 (or available online at kscorn.com). The Plasmid Modeling Technology-Based handout can be found on pg. \$3-\$4 (or available online at kscorn.com).

**Bacterial Transformation Lab:** In order for students to achieve transformation it is critical that they work through the lab using the precise steps and times outlined in the pGLO Quick Guide (pg. S5-S6, or available online at kscorn.com). If they have completed the lab correctly, one plate and one plate only should have glowing bacteria, the + LB/Amp/ Ara. If their results do not show this, allow students the opportunity to analyze where their mistake may have taken place and determine what they would do differently if they could complete the procedure again.

**Overall Assessment:** Written or oral assessments on how and why this transformation lab is considered genetic modification is a fantastic way to assess the overall understanding of the students' knowledge. This can be done via a presentation or through a paper explaining each step in the process, the creation of plasmids, the transformation process, and how it relates to the overall goals of food stability. While performing assessments, refer to NSTA's Lab Report Rubric (pg. T 20, or available online at kscorn.com).



**Science and Agriculture Careers:** Almost all new seed varieties being created today have some level of biotechnology involved in their development. From plant tissue culturing to genomic analysis and alteration, all rely on the primary skills of sterile technique and basic understanding of the genetic code. 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.

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

#### Sources:

- Ohio Corn and Wheat curriculum http://ohiocorneducation.org/
- pGLO Quick Guide Courtesy of Bio-Rad Laboratories, Inc., © 2018
- NSTA Lab Report Rubric http://www.static.nsta.org
- 70% more food by 2050 http://www.fao.org/news/story/en/item/35571/icode/
- Teosinte to corn weedtowonder.org/
- The Evolution of Corn http://learn.genetics.utah.edu/content/selection/corn/
- Corgi's to Corn; graphic of the history of GM http://sitn.hms.harvard.edu/flash/2015/ from-corgis-to-corn-a-brief-look-at-the-long-history-of-gmo-technology/
- How to make transgenic plants http://www.unc.edu/depts/our/hhmi/hhmi-ft\_ learning\_modules/plantmodule/transgenicplants.html
- NSTA Minimum Safety Practices and Regulations for Demonstrations, Experiments, and Workshops – http://static.nsta.org/pdfs/MinimumSafetyPracticesAndRegulations.pdf

#### **NOTES:**





### Lab Report Rubric

Lab report rubric.		

Name:		Lab:						
	4	3	2	1	0			
Introduction	Background information is researched and cited. Hypothesis is stated in "Ifthen" format and explained.	Background information is researched and cited. Hypothesis is stated but not explained and not in "Ifthen"	Background information is vague or brief. Hypothesis is stated but not explained and not in "Ifthen" format.	Background is vague or brief, hypothesis is vague, or background or hypothesis is missing.	No introduction is presented.			
Materials and methods	Materials and amounts are identified. Steps are easy to follow and in paragraph form.	Materials are mentioned but without amounts. Steps are easy to follow and in paragraph form.	Materials are mentioned but without amounts. Steps are vague but in paragraph form.	Doesn't provide enough information to represent an experimental procedure.	No materials or methods described.			
Data	Data is complete and relevant. Tables are easy to read and units are provided. Graphs are labeled and show trends. Questions are answered completely and correctly.	One component of data incomplete:TablesGraphsQuestions	Two components of data incomplete or one missing:TablesGraphsQuestions	Data is brief and missing significant pieces of information.	No data reported.			
Conclusion	Conclusion summarizes experiment, cites data, addresses hypothesis, and cites sources of error.	One component of conclusion missing: SummaryDataHypothesis _Errors	Two components of conclusion missing: SummaryDataHypothesis _Errors	Conclusion is brief and is missing significant pieces of information.	No conclusion present.			
Report quality	Report is well organized and cohesive and contains no mechanical errors.  Presentation seems polished.	Report is well organized and cohesive but contains some spelling or grammatical errors.	Report is somewhat organized with some spelling or grammatical errors.	Report contains many errors.	No attention to detail evident.			