



Genetic Counseling & GMO's

Essential Question(s):

What information can a genetic counselor provide to the USDA so they can make an informed decision on Genetically Modified Organisms?

Introduction:

This is a project-based unit of study that was created for tenth grade Biology students. Students will learn and practice Mendelian genetics. Students will develop expertise in using pedigrees, punnett squares, and karyotypes in the diagnosis of case histories. Also, students will study genetically modified crops throughout the lesson and debate the use of these crops in the world today.

Learning Outcome:

- Students will be able to explain the double-stranded, complementary nature of DNA through the DNA Project.
- Students will be able to explain how DNA and RNA code for proteins and determine traits through the Protein synthesis with words activity.
- Students will gain an understanding of how to use pedigrees, punnett squares, and karyotypes through the case study experience.
- Students will be able to explain what genetically modified foods are and where they come from through GMO research, questionnaire, and debate.
- Students will summarize how transgenic organisms are engineered to benefit society during the GMO debate.

Curriculum Alignment / Essential Standards Alignment:

Biology Standard Course of Study

- **OBio.3.1: Explain how traits are determined by the structure and function of DNA**
 - Bio.3.1.1 : Explain the double-stranded, complementary nature of DNA as related to its function in the cell
 - Bio.3.1.2: Explain how DNA and RNA code for proteins and determine traits.
 - Bio.3.1.3: Explain how mutations in DNA that result from interactions with the environment (i.e. radiation and chemicals) or new combinations in existing genes lead to changes in function and phenotype
- **OBio.3.2: Understand how the environment, and/or the interaction of alleles, influences the**
 - **expression of genetic traits.**

- Bio.3.2.2: Predict offspring ratios based on a variety of inheritance patterns (including: dominance, co-dominance, incomplete dominance, multiple alleles, and sex-linked traits)
- **OBio.3.3: Understand the application of DNA technology.**
 - Bio.3.3.1: Interpret how DNA is used for comparison and identification of organisms.
 - Bio.3.3.2: Summarize how transgenic organisms are engineered to benefit society.
 - Bio.3.3.3: Evaluate some of the ethical issues surrounding the use of DNA technology (including: cloning, genetically modified organisms, stem cell research, and Human Genome Project).

Classroom Time Required:

This lesson will take 17 consecutive days with 90 minute (block) class sessions.

Materials Needed:

- **Entry Documents**
 - Office of Admission
 - Genetic Counselor Position
 - USDA GMO Memo
- **Articles**
 - *A Baby, Please. Blond, Freckles -- Hold the Colic*, Feb 12, 2009, Wall Street Journal.
 - *Swarm troopers: Mutant armies waging war in the wild*, Sept 12, 2011, Magazine.
- **Hand outs**
 - Monohybrid Practice
(<http://www2.parkridge.k12.nj.us/JLiu/Worksheets/monohybrid-cross-worksheet.pdf>)
 - Dihybrid Practice
(<http://www.cbhs.k12.nf.ca/stephenwhalen/dihybridcross.pdf>)
 - GMO questionnaire
 - GMO worksheet
 - Gel Electrophoresis Webquest
(<http://www.learnnc.org/lp/media/uploads/2010/11/gel-electrophoresis-lab-sheet.pdf>)
 - Human Heredity Worksheet
 - Case Study Certification
 - Blood Type Worksheet
 - Case Study Interview
- **Case Studies**
 - "Frankenfoods? The Debate Over Genetically Modified Crops"
(http://sciencecases.lib.buffalo.edu/cs/collection/detail.asp?case_id=232&id=232)
 - "Do you really know what you are eating? A Case Study on Genetically Modified Foods"
(http://sciencecases.lib.buffalo.edu/cs/collection/detail.asp?case_id=290&id=290)

- "Golden Rice: An Intimate Debate Case"
(http://sciencecases.lib.buffalo.edu/cs/collection/detail.asp?case_id=279&id=279)
- **Activities**
 - The Genetics of Parenthood
(<http://www.accessexcellence.org/AE/AEPC/WWC/1994/parenthood.php>)
 - Strawberry DNA Extraction Lab
(<http://www.imb.uq.edu.au/download/large/strawberryDNAextraction.pdf>)
 - DNA Project & Rubric
 - Making a model of DNA
(<http://www.gov.mb.ca/conservation/sustain/dnadone.pdf>)
 - Protein Synthesis with Words (<http://www.learnnc.org/lp/pages/3300>)
 - Mutation Paper Lab (www.biologyjunction.com/mutation%20lab.doc)
 - **GMO Debate & Rubric**

Technology Resources:

- Computer access
- Internet
- Microsoft word or Open Office (word processing program)

Pre-activities:

- Students should be familiar with working within cooperative groups.

Activities:

- Day 1:
 - **Entry Event** Get students engaged and excited about the upcoming project. Introduce the lesson by having students read the following articles or ones that you find interesting:
 - *A Baby, Please. Blond, Freckles -- Hold the Colic*, Feb 12, 2009, Wall Street Journal.
 - *Swarm troopers: Mutant armies waging war in the wild*, Sept 12, 2011, Magazine.
 - After reading the articles, do an "agree/disagree" activity with students. Put an agree sign on one end of you classroom and a disagree sign at the other end of the classroom. Place statements like the ones that follow on the board or overhead and have students move to one end or the other and ask them to defend there choice. Students can not stay in the middle, they must make a choice. Sample statements:
 - It is ethical to choose the characteristics you would like displayed in your child.
 - Choosing traits for your child which are not based on medical necessity is okay.
 - The advantages of incorporating genetically modified organisms (GMOs) into the wild outweigh the disadvantages.

- The driving question for this unit is: What information can a genetic counselor provide to the USDA so they can make an informed decision on GMOs?
- Project Group Work: Entry Document = "Office of Admissions"
 - Ask students what they know & need to know to answer this question (list them on the board).
 - Possible things they need to know:
 - What are GMOs?
 - What is a genetic counselor 's job?
 - What is a pedigree?
 - What is a punnett square?
 - What is genetics?
 - What is DNA / RNA?
 - What is genetic engineering?
- The movie "GATTACA" could also be used as an entry event if you would like. At the beginning of the movie it talks about how the main characters mother and father selected what traits they wanted his brother to have.
- Day 2:
 - Workshop Topics: Introduce Mendelian Genetics; Monohybrid crosses
 - Activity: Monohybrid Practice (<http://www2.parkridge.k12.nj.us/JLiu/Worksheets/monohybrid-cross-worksheet.pdf>)
 - Project Group Work: Case Study "Frankenfoods? The Debate Over Genetically Modified Crops"
- Day 3:
 - Workshop Topics: Dihybrid Crosses
 - Activity: Dihybrid Practice (<http://www.cbhs.k12.nf.ca/stephenwhalen/dihybridcross.pdf>)
 - Project Group Work: Group discussion of Frankenfood Case Study
- Day 4:
 - Workshop Topics: Inheritance Patterns (codominance, incomplete dominance, multiple alleles)
 - Activity: The Genetics of Parenthood (<http://www.accessexcellence.org/AE/AEPC/WWC/1994/parenthood.php>)
 - Project Group Work: GMO research worksheet; Hand out GMO questionnaire (each student has to get 20 people to answer; students, teachers, family members)
- Day 5:
 - Activity: Finish the Genetics of Parenthood Activity
 - Project Group Work: Case Study "Do you really know what you are eating? A Case Study on Genetically Modified Foods"
- Day 6:
 - Activity: Presentation of power points created from "Do you really know what you are eating? A Case Study on Genetically Modified Foods" case study
 - Project Group Work: Compile GMO questionnaire data collected into a graph and turn in.
- Day 7:
 - Workshop Topics: DNA: Molecule of Heredity (Introduction plus DNA construction)
 - Activity: Assign DNA Project; Strawberry DNA Extraction Lab (<http://www.imb.uq.edu.au/download/large/strawberryDNAextraction.pdf>)

- Project Group Work: Making a model of DNA
(<http://www.gov.mb.ca/conservation/sustain/dnadone.pdf>)
- Day 8:
 - Workshop Topics: DNA to Protein (Steps of Protein Synthesis)
 - Activity: Learn NC Unit "Protein Synthesis with Words"
(<http://www.learnnc.org/lp/pages/3300>)
- Day 9:
 - Workshop Topics: Mutations (Point Mutations & Frameshift Mutations)
 - Project Group Work: Mutation Paper Lab
(www.biologyjunction.com/mutation%20lab.doc)
- Day 10:
 - Workshop Topics: Genetic Engineering (Karyotypes, Autosomes & Sex Chromosomes, Pedigrees)
 - Activity: Gel Electrophoresis Webquest; Assign Human Heredity Worksheet
 - Project Group Work: Entry Document = "Genetic Counselor Position"; Case Study Certification Worksheet
- Day 11:
 - Workshop Topics: Human Heredity (Blood types, Recessive & Dominant Diseases & Disorders)
 - Activity: Blood Type Worksheet; Hand in DNA Project & Paper
- Day 12:
 - Workshop Topics: Human Chromosomes (Nondisjunction, Sex-linked Diseases & Disorders)
 - Activity: Hand in Human Heredity Worksheet
 - Project Group Work: Case Study = Selecting the Perfect Baby: The Ethics of "Embryo Design"
- Day 13:
 - Project Group Work: Case Study Interview (you need people to help); turn in response after interview
- Day 14:
 - Activity: Case Study "Golden Rice: An Intimate Debate Case"
 - Project Group Work: Entry Document = "USDA GMO Memo"
- Day 15:
 - Activity: **Introduce GMO Debate protocol**
 - Project Group Work: Research side of GMO Debate
- Day 16:
 - Project Group Work: GMO Debate
- Day 17:
 - Activity: Reflection & project debrief

Assessment:

- Monohybrid Practice Worksheet
- Dihybrid Practice Worksheet
- "Frankenfoods? The Debate Over Genetically Modified Crops" Questions
- The Genetics of Parenthood
- GMO Worksheet
- GMO Questionnaire Data
- Powerpoint for "Do you really know what you are eating? A Case Study on Genetically Modified Foods"
- DNA Paper Model
- Human Heredity Worksheet

- Case Study Certification Worksheet
- DNA Project & Paper
- Selecting the Perfect Baby: The Ethics of “Embryo Design”
- Case Study Interview Results
- GMO Debate

Critical Vocabulary:

Alleles – one of a number of different forms of a gene

Dominant - a relationship between alleles of a gene, in which one allele masks the expression of another allele

Recessive - an allele that causes a phenotype that is only seen in a homozygous genotype and never in a heterozygous genotype

Heterozygous – an organism that has two different alleles for the same trait

Homozygous – an organism that has two identical alleles for a particular trait

Genotype – genetic makeup of an organism

Phenotype – physical characteristics of an organism

Karyotype – set of photographs of chromosomes grouped in order pairs

Pedigree – a graphic representation of a family tree

Sex-linked – genes located on the X or Y chromosome

Autosome – chromosome that is not a sex chromosome (number 1 -22)

Transcription – process in which part of the nucleotide sequence of DNA is copied into a complementary sequence in RNA

Translation – decoding of mRNA message into a polypeptide chain

Author Info:

Kirk Kennedy, is a secondary biology teacher at East Duplin High School, Beulaville, NC. He has his Master’s in Science Education and a National Board Certification in Adolescence & Young Adult science.

Kenanfellows.org



From Biology to Biofuels: Creating Diesel from Algae

Introduction:

In this lesson students will gain a deeper understanding of cell and plant biology through an investigation of a unicellular strain of salt water algae and its ability to produce enough lipids to be considered as a possible biofuel option that could replace gasoline or diesel. This investigation will involve having the students grow *Dunaliella salina* based on a current study underway at North Carolina State University that looks at the growth of that organism as a biofuel for jets. The students will create a photo-bioreactor made from water bottles while growing the common salt water alga. There are two options for the media the students will use to grow the algae in. The first option is to use the treated wastewater from your local treatment plant to show how growing this biofuel source could also help to further filter a local lake or river. The second option is to use a sterile high quality media that your chemistry classes can create using their knowledge of molar solutions. The students will observe the algae growth over two weeks and then dry the algae to see how well it burns.

This is the second lesson plan associated with the vertical alignment of your science department. I have used Biofuels as an example of a topic that could connect Earth Science, Biology, Chemistry, and AP Environmental Science together. The hope is to provide your students with a broad, relevant theme that shows them how each of the sciences are related to each other to create bridges between the traditional science islands that our students have experienced over the years. If the students are shown all of the ways they can build on their prior knowledge, then you can provide a deeper connection to the materials and topics that you present them with. Freshman Earth Science students will grow *Camelina Sativa* to get an understanding of why we are looking at biofuels to replace fossil fuels. Freshman/Sophomore Biology students will grow salt water algae to utilize their understanding of cell parts and plant cell function to learn why organisms make fatty acids that can be made into a biodiesel. Chemistry students will show their understanding of molar solutions by making the media that the Biology classes will use to grow the algae in. The Advanced Placement Environmental students can utilize all of the knowledge gained from these other classes to dive more deeply and determine which alternative fuel would be the best choice for North Carolina.

Learning Objectives:

- Students will gain a deeper understanding of the functions of the organelles in a cell through the production of lipids
- Students will discuss how the production of algae as a biofuel could reduce our reliance on foreign oil and neutralize the carbon emissions that gasoline is responsible for.
- Students will apply their knowledge of energy production through photosynthesis and aerobic respiration.

N.C. Essential Standards:

Bio.1.1 Understand the relationship between the structures and functions of cells and their organelles.

- Bio.1.1.1-Summarize the structure and function of organelles in eukaryotic cells (including the nucleus, plasma membrane, cell wall, mitochondria, vacuoles, chloroplasts, and ribosomes) and ways that these organelles interact with each other to perform the function of the cell.
- Bio.1.1.3-Explain how instructions in DNA lead to cell differentiation and result in cells specialized to perform specific functions in multicellular organisms.

Bio.1.2 Analyze the cell as a living system.

- Bio.1.2.2-Analyze how cells grow and reproduce in terms of interphase, mitosis and cytokinesis.
- Bio.1.2.3-Explain how specific cell adaptations help cells survive in particular environments (focus on unicellular organisms).

Bio.2.1 Analyze the interdependence of living organisms within their environments.

- Bio.2.1.1-Analyze the flow of energy and cycling of matter (water, carbon, nitrogen and oxygen) through ecosystems relating the significance of each to maintaining the health and sustainability of an ecosystem.

Bio.2.2 Understand the impact of human activities on the environment (one generation affects the next).

- Bio.2.2.1-Infer how human activities (including population growth, pollution, global warming, burning of fossil fuels, habitat destruction and introduction of nonnative species) may impact the environment.
- Bio.2.2.2-Explain how the use, protection and conservation of natural resources by humans impact the environment from one generation to the next.

Bio.4.1 Understand how biological molecules are essential to the survival of living organisms

- Bio.4.1.1-Compare the structures and functions of the major biological molecules (carbohydrates, proteins, lipids, and nucleic acids) as related to the survival of living organisms.

Bio 4.2 Analyze the relationships between biochemical processes and energy use in the cell.

- Bio.4.2.1-Analyze photosynthesis and cellular respiration in terms of how energy is stored, released, and transferred within and between these systems.
- Bio.4.2.2-Explain ways that organisms use released energy for maintaining homeostasis (active transport).

Time Required:

This lab can be completed in 10 days. The time spent on the first day depends on the option that you choose. Option 1 will take 20-30 minutes to set up the growing station and place the algae, salt, and Epsom salts in the water bottle. The second option will take approximately 45 minutes to set up the growing stations and fill the water bottles with algae using sterile technique. Recording daily observations on the checklist should not take more than 2-5 minutes per day. The best time to harvest using the lighting conditions I have suggested is between 8-10 days depending on whether the green color is still darkening. Harvesting occurs over two days with the first day taking between 45-60 minutes depending on how fine the filter is and how well it drains. Once dry, massing and burning the algae and the control take approximately 20 minutes on the second day.

Materials:

Per Group of Four:

Dunaliella Salina (can be ordered from most science catalogs for around \$7.30 per bottle.) One bottle is good for 30 students

500mL water bottle (Aquafina brand bottles work best with aquarium hood overhead lighting, Voss brand bottles worked best when light was placed horizontally on the side of the aquarium during lab tests)

Aquarium with light in hood or other fluorescent light source

Timer to plug light source into for 16 hour light/8 hour dark cycle

Glass stirring rods with holes in them

Digital balance

Option 1: Non-sterile option

Option 2: Sterile option (See Chemistry lab)

Treated Wastewater

Distilled Water (tap water is okay)

NaCl 14.612g (for 1 molar salt solution in 250mL)

Epsom Salts (MgSO₄) 0.3g

Hand crank pump for pipettes

10 ml pipette

2 Molar Sodium hydroxide

Filter paper or paper towels

Ring Stand

O-ring

Funnel

Large Beaker

Before You Begin:

Make sure the aquarium you choose is large enough to provide space between the water bottles to allow for the most light to come in, and place white paper around the tank to reflect light throughout. I recommend adding more light if possible to maximize your growth. If you have a construction class, talk to them about the possibility of creating a shelving unit. Our construction club students built a 6'10" mobile growing station with four 40Watt bulbs placed

underneath two shelves that are vertically spaced 3 feet apart. We painted the base of the shelves white to reflect the light, and the two levels allow us to grow plants for other labs as well as the algae at the same time. We put castors on the bottom of the station so that we can move it from class to class and even take it on the elevator.

Activity:

Pre Lab:

The first page of the Pre Lab attached to this curriculum is designed to be a review of plant cell organelles and their function. The second page is introducing the organism that the students will be growing for this lab, as well as focusing the student's attention on the specific functions of the algal cell that we can take advantage of for use as a biofuel.

Post Lab:

The Post-Lab is designed to incorporate Science Literacy into a Biology class. The student worksheet was created with the assistance of Sara Overby, our Wake County literacy Coach, to offer options for the teacher to differentiate between classes with each section on the worksheet requiring more in-depth understanding than the previous one. For example, an Honors Biology class could be assigned the post-lab as a homework assignment, and an Academic class could do part 1 or 2 on their own and then discuss with the class the answers to part 3. The article is a good overview of the potential of algae as a biofuel and looks at several different aspects associated with cost, outdoor growth versus growth in a photobioreactor, and oil production compared to other crops. The article is titled "Biodiesel from Microalgae" and was written by Yusuf Chisti at the Institute of Technology and Engineering at Massey University in New Zealand.

Click on the link for the science article used with the Post Lab:

www.tamu.edu/faculty/tpd8/BICH407/AlgaeBiodiesel.pdf

Growth Options:

This lab has two options for the creation of a media used in a photobioreactor for you to grow *Dunaliella Salina* in.

Option #1: This is the non-sterile option that requires the collection of water from a local Wastewater Treatment Plant. The students will use 500mL (16oz) water bottles filled with the treated wastewater to create a photobioreactor to grow algae in. The ultimate goal of any environmental solution that we create is to "close the system." If a system is "closed," then that means that everything you put into the system will stay in the system through the elimination of waste products with reduction, reuse, or recycling of all the materials used. Using treated wastewater to grow algae would be an excellent way to teach students how we could start to close our water cycle system. Algae would use the nutrients from the wastewater which would further filter the water before it is returned to our lakes and rivers. **Algae have been successfully grown without sterile technique being used at any stage of the creation of media or the growth process. Simply add the algae from the container to the water bottle using a 10mL pipette and the hand crank pump. You may want to look at the algae under a microscope when harvesting to see how much, if any, contamination occurred.**

Option #2: Give your Chemistry Teachers the lab entitled: High Quality Sterile Media Lab for Growing Algae. This option helps to vertically align your science classes by having the chemistry classes at your school apply their knowledge of molar solutions through the creation of a recognized high quality media that is used throughout the country for growing algae. The students will use 500mL (16oz) water bottles that have been sterilized in the Goggle Sterilizing Cabinet using Ultraviolet light. One of the major benefits of this option is that it can be used to teach your chemistry and biology class basic sterile technique, which university professors and biotech based corporations agree is a vital skill to learn for a prospective employee. This media is extremely well tested and is significantly more likely

to grow algae in greater volume than option 1 if made correctly. The Chemistry student's grade can be partially assessed through the successful growth of algae.

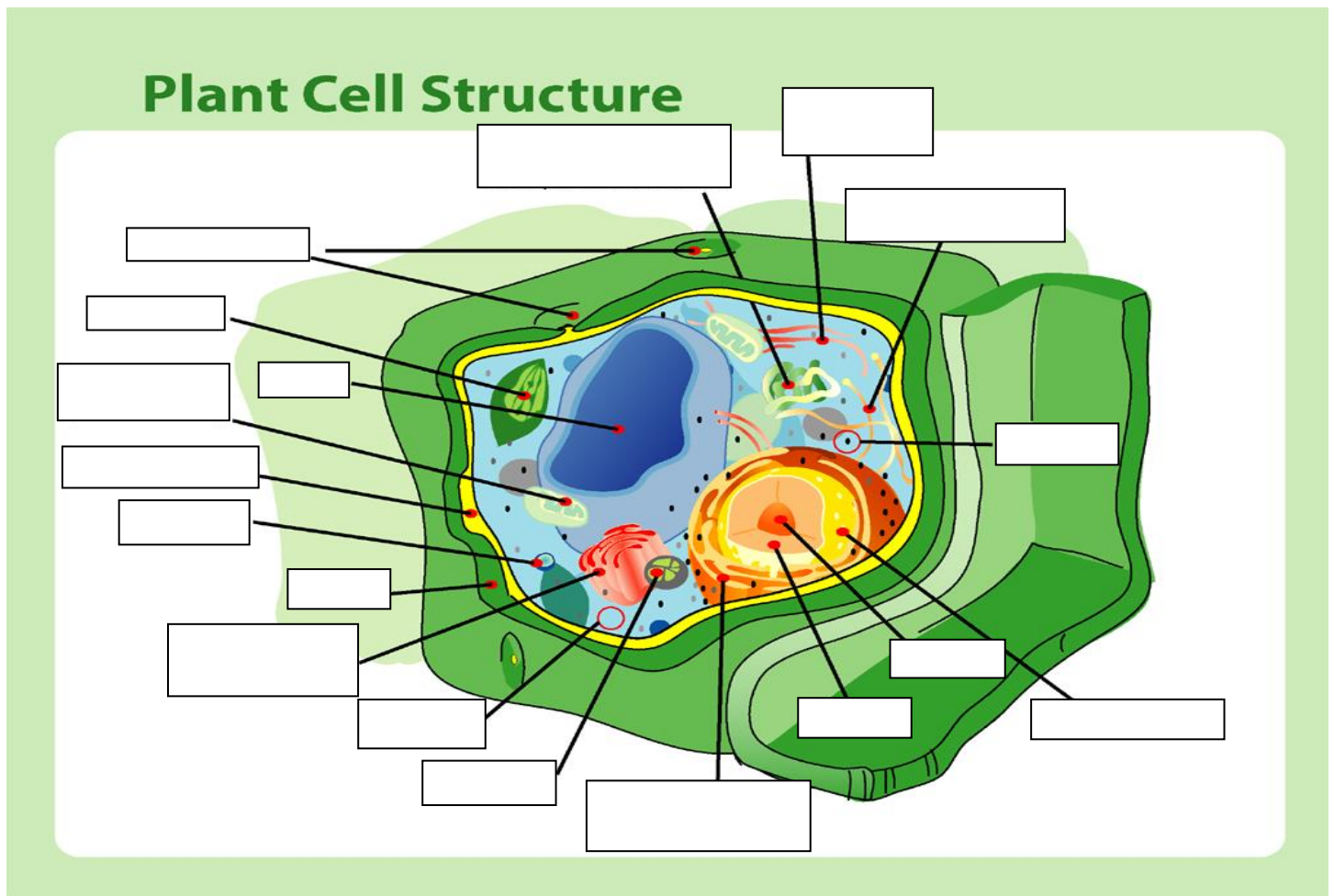
Additional Procedure Recommendation:

Take samples of the *Dunaliella Salina* during the 3rd, 6th, and 9th day of growth and make a slide of each sample to observe under a microscope the morphology, color, movement, and overall health of the algae. You can also have the students attempt to make a cell count, or contact either the plant biology department at a local university or a biotech company in the area to see if they can give you an accurate count. Making observations and taking pictures of the samples can help you to determine the best time to harvest the algae under the lighting conditions you set. You can make changes to the media if the algae seem to be struggling.

Because *D. salina* is a salt water alga, you can also teach students about osmotic pressure differences by adding a drop of fresh water to the slide. *D. salina* has a very thin and flexible cell membrane, so the addition of fresh water causes an osmotic imbalance allowing the fresh water in until the cell bursts. This is a major reason why *D. salina* is such a viable option as a biofuel because there is no need to spend millions of dollars on chemicals that break down cell walls to extract the lipids for conversion to biodiesel like what would be required for plants. When the alga bursts, the lipids float to the top and the starches sink to the bottom for a much simpler removal of either compound.

Pre Lab for From Biology to Biofuels: Creating Diesel from Algae

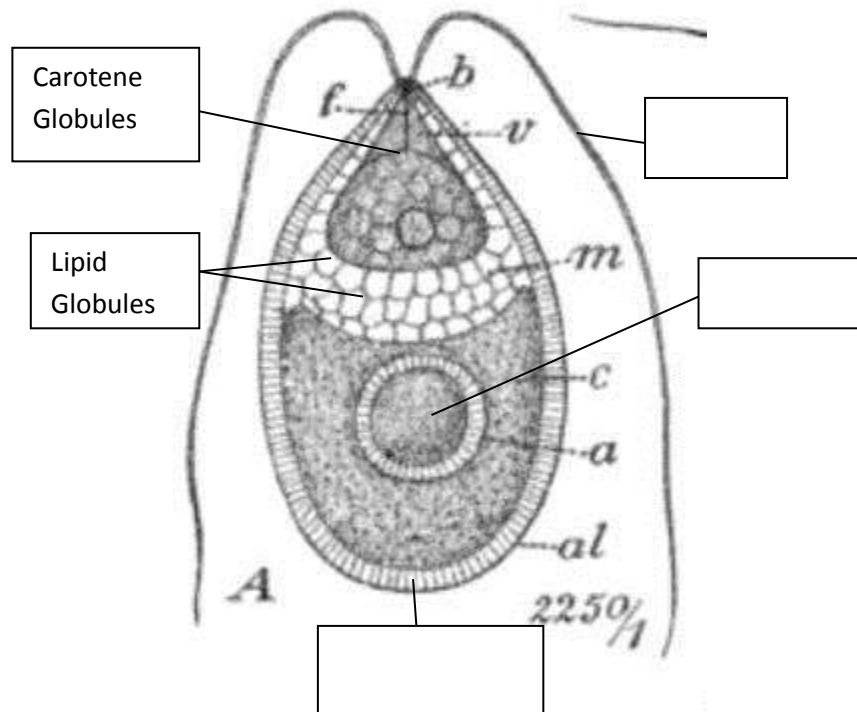
Part 1: Plant cell Review-Label the picture and fill out the chart below.



Picture from Wikimedia Commons

Compartment	Composition	Major Function	Analogy
Cell membrane (plasma membrane)	lipid, protein		
Cytoplasm (cytosol)	Mainly protein, small molecules, lipids		
Mitochondria	lipid, protein		
Chloroplasts	lipid, protein		
Nucleus	nucleic acid		
Ribosomes	Nucleic acid, protein		
Microtubules and Filaments	protein		
Golgi Apparatus	lipid, protein		
Endoplasmic Reticulum	lipid, protein		

Part 2: Introducing *Dunaliella Sativa*-Fill in the picture below and answer the following questions:



Picture adapted from Wikimedia Commons

This picture is of *Dunaliella Salina* (DOONA-lee-ella Sa-LEE-na), a salt water alga that is very high in β (Beta)-carotene and is commonly used as a feedstock for fish hatcheries. We use algae as a dietary supplement to help provide us with β -carotene as well since it can be converted to Vitamin A to help with our vision, or it can be converted to retinol after we digest it to help with growth and cell differentiation.

D. Salina is a single-celled organism with a thin cell wall that is very flexible. Today, *Dunaliella Salina* is being studied by the plant biology department at North Carolina State University as a possible source for a biofuel that could eventually replace conventional diesel. One reason for this is because of how D. Salina responds when placed under stressful conditions by creating more lipids than many other algae that can be converted into a biodiesel.

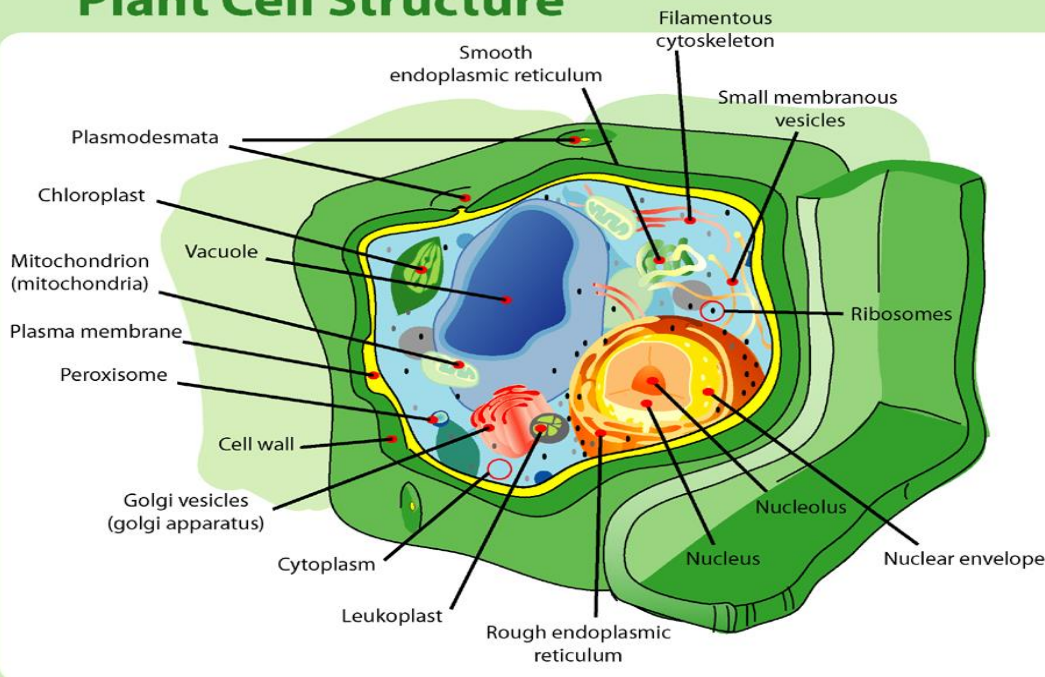
Answer the following questions based upon your prior knowledge of cells and the paragraph above.

1. What is β -carotene and how does it benefit the cell?
2. Why do you think D. Salina makes more lipids when responding to stress? What do the lipids do for this simple-celled organism?
3. What nutrients and other elements should be in the growing media that D. Salina would need to replicate and thrive?
4. D. Salina is a type of green algae that could be described as a halophilic eukaryote that creates high concentrations of glycerol to protect against osmotic pressure. What does all of that mean?
5. How do you think we could genetically modify D. Salina to cause it to produce even more lipids

Answers to Pre Lab for From Biology to Biofuels: Creating Diesel from Algae

Part 1: Plant Cell Review-Label the picture and fill in the chart below.

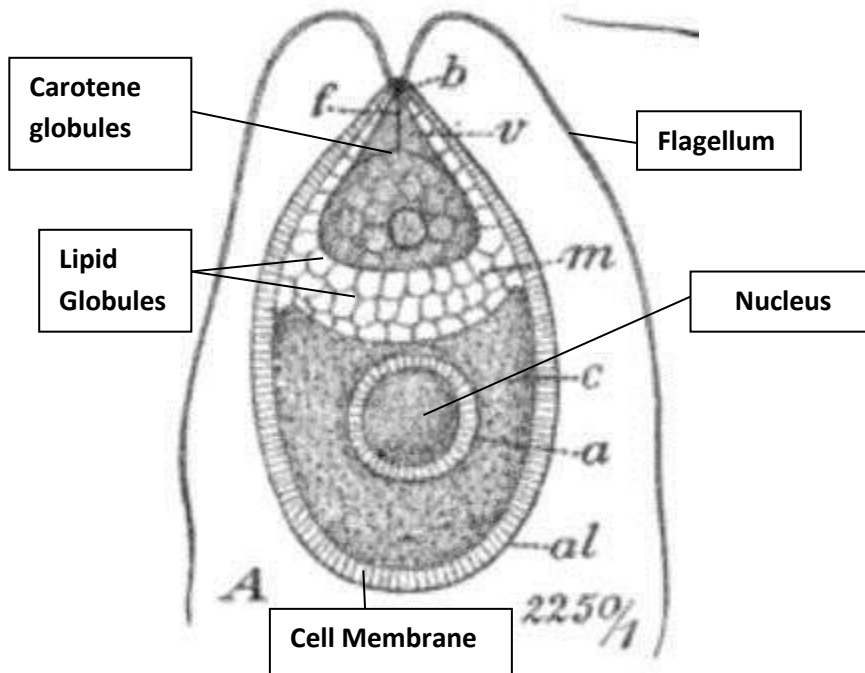
Plant Cell Structure



Picture from Wikimedia Commons

Compartment	Composition	Major Function	Analogy
Cell membrane (plasma membrane)	lipid, protein	Porous outer Boundary that regulates in/out of the cell	Security at the entrance of a gate into a facility
Cytoplasm (cytosol)	Mainly protein, small molecules lipids	Clear thick fluid that supports cell organelles	Factory minus the nucleus
Mitochondria	lipid, protein	Organelle that produces most of the cells energy in the form of ATP	Power plant
Chloroplasts	lipid, protein	Captures energy from sunlight. Uses energy to produce sugar (photosynthesis.)	Solar panels
Nucleus	nucleic acid	Control Center for all activity in the cell	Main office of factory
Ribosomes	Nucleic acid, protein	Produces Proteins	Small machine in factory
Microtubules and Filaments	protein	Function in the maintenance of cell-shape by bearing tension (filaments) and resisting compression (Microtubules)	Supports for the building
Golgi Apparatus	lipid, protein	Receives materials from endoplasmic reticulum, distributes materials	Shipping department
Endoplasmic Reticulum	lipid, protein	Carries proteins and lipids to various parts of the cell.	Conveyor belt

Answers for Part 2: Introducing *Dunaliella Saliva-Fill* in the picture below and answer the following questions.



Picture adapted from Wikimedia Commons

This picture is of *Dunaliella Salina* (DOONA-lee-ella Sa-LEE-na), a salt water alga that is very high in β (Beta)-carotene and is commonly used as a feedstock for fish hatcheries. We use algae as a dietary supplement to help provide us with β -carotene as well since it can be converted to Vitamin A to help with our vision, or it can be converted to retinol after we digest it to help with growth and cell differentiation. *D. Salina* is a single-celled organism with a thin cell wall that is very flexible. Today, *Dunaliella Salina* is being studied by the plant biology department at North Carolina State University as a possible source for a biofuel that could eventually replace conventional diesel. One reason for this is because of how *D. Salina* responds when placed under stressful conditions by creating more lipids than many other algae that can be converted into a biodiesel.

1. What is β -carotene and how does it benefit the cell? **Beta carotene is an orange pigment that the cell produces to protect itself from intense lighting conditions.**
2. Why do you think *D. Salina* makes more lipids when responding to stress? What do the lipids do for this simple-celled organism? **Lipids prevent water loss and form a protection against pathogens and other stresses from the environment. Lipids are also a major energy storage component of the cell, so the algae is storing energy in case it cannot make any more in the near future.**
3. What nutrients and other elements should be in the growing media that *D. Salina* would need to replicate and thrive? **The algae will need several metals such as potassium and magnesium, and nutrients like nitrogen and phosphorous to help with photosynthesis, growth, and support of the cell structures.**
4. *D. Salina* is a type of green algae that could be described as a halophilic eukaryote that creates high concentrations of glycerol to protect against osmotic pressure. What does all of that mean? **Halophilic means "salt-loving" and refers to a type of extremophile that survives under harsh conditions. A eukaryote is an organism with a well defined nucleus. Glycerol refers to the thick, colorless, odorless fluid that provides the backbone of the fatty acids**

the plant produces (mainly triglycerides) that is used to protect the cell against any unwanted inward flow of water through the cell membrane.

5. How do you think we could genetically modify *D. Salina* to cause it to produce even more lipids?
You could modify the algae to shut down the processes that limit lipid production, or you could have the algae think it is under full stress conditions all the time.

Procedure for adding algae with sterile technique to the water bottle photo bioreactor:

- 1.) See video of adding algae with sterile technique if you would like to see this procedure being performed.
- 2.) Spray closed container of algae, water bottles, and all necessary equipment (including hands) with 70% ethanol and place in sterile zone.
- 3.) Place all the water bottles and equipment nearby a Bunsen burner and then light the Bunsen burner to create a 10cm diameter sterile zone. **CAUTION: Do not spray ethanol around a lit Bunsen burner!**
- 4.) Loosen cap of water bottle but do not remove.
- 5.) Swirl the algae container so that each group is getting the same approximate amount of algae. Loosen the top of the algae without removing it.
- 6.) Open the top of the sterile pipette packaging where you will be attaching the pipette to the hand crank pump. **DO NOT TOUCH THE PIPETTE OR LAY IT DOWN.** Keep the pipette in the sterile field.
- 7.) Open algae container in the sterile field and use 10mL sterilized pipette and a hand crank pump pipette to withdraw 13mL of algae (max that 10mL pipette will hold).
- 8.) Take the cap off of the water bottle and release the algae into the bottle from the pipette. Hold the cap near the bottle in the sterile field with one hand while you release the algae into the bottle.
- 9.) Seal the bottle without touching inside the cap and gently swirl the algae to spread them throughout the medium.
- 10.) Place in aquarium and loosen the cap **WITHOUT REMOVING** to allow for venting of gas exchange.

Procedure for growing the algae:

- 1.) The timer for the fluorescent lighting should be set on a 16 hour light/8 hour dark schedule. Make sure that the light schedule is during daylight hours as well to take advantage of as much light as possible.
- 2.) Place the water bottles in an aquarium with a fluorescent hood light. If possible, place the lights on the back of the tank so that the light is not blocked by the caps on top. Feel free to experiment with different lighting options to see what light amounts grow the most algae. A typical aquarium starter kit should be sufficient for growing enough algae to harvest.
- 3.) Students should tighten cap, gently swirl, and then loosen the cap again daily in order to redistribute the algae and the medium.
- 4.) Record daily re-suspension and observations on the attached Algae Photo-bioreactor Daily Checklist. Observations should include color changes and settling or appearance changes from one day to the next.

Procedure for removing and drying the algae (Harvesting):

- 1.) Re-suspend the algae by swirling the algae in the bottle. Make any final observations and record them on your growing log.
- 2.) Remove the cap on the water bottle and add a drop at a time of Sodium hydroxide 2M solution with a plastic disposable pipette until the algae has flocculated into visible clumps. If you are using the treated wastewater, then 26-30 drops of 2M NaOH will be sufficient to make the algae flocs large enough to be caught by the paper towel filter. If you are using the high quality media, it will take 90 drops because the media is buffered. Place the algae aside and let it settle and clump as much as possible on the counter without re-suspending it.*
- 3.) Once the algae have flocculated it should be large enough for it to be filtered using a paper towel. Students can make a funnel shape while holding the paper towels over a large beaker, and simply pour the water through the paper towel slowly. Dip a clean paper towel into the filtered media below to be used as a control. If you would like to determine how much algae each group grew, then follow instructions 4-9 below. Otherwise you may skip to number 9.
- 4.) Set up a ring stand with an o-ring large enough to hold a funnel.
- 5.) Place a funnel in the o-ring and raise it high enough to place a jar below the funnel to catch the medium as it filters.
- 6.) Fold the round piece of filter paper in half and then half again to make it pie-shaped. Open an end of the filter to make a cone shape and place that inside of the funnel hanging from the o-ring.

- 7.) Very slowly pour the algae media into the filter cone.
- 8.) Dip a clean piece of filter paper into the filtered media that is in the jar below to be used as a comparison for mass. Let the algae and the control filter paper dry in an oven at 30-40 degrees Celsius for a few hours, or on the counter over a couple of days and then mass the filter paper again. Subtract the mass from the clean filter paper to the algae-coated filter to figure out the mass of algae.
- 9.) Once the algae coated paper and the control paper has dried completely, you are ready to burn. Using a fume hood or by taking the class outside, the teacher should place the control paper and the algae coated paper in separate glass jars. Light the paper with a long match or with a fireplace lighter. Have the students make observations of the appearance of the different papers before, during, and after burning. When the control paper is lit, you will notice that it burns extremely quickly and usually leaves a whole black piece behind. When the algae paper is burned, the flame is much slower and steadier and leaves behind a brittle, gray ash. This means that the algae coated paper burns much hotter than the control paper which suggests that the algae is producing lipids and starches that are maintaining and fueling the initial flame.

*** Flocculation is a necessary step in the large scale production of algae as a biofuel as well. Another reason why salt water algae shows so much promise as a biofuel option is because the salt water is higher in Magnesium ions which cause the algae to flocculate naturally once the pH rises to over 10. So a company would not have to spend money on a chemical additive when they are ready to harvest the algae. This could save millions of dollars in the production cost of algae as a biofuel.**

Post Lab for From Biology to Biofuels: Creating Diesel from Algae

Chisti, Yusuf. "Biodiesel from Microalgae." *Biotechnology Advances* 25.2007 (2007): 294-306. *ScienceDirect*. Web. 27 Feb. 2013.

Directions:

As you read, respond to these statements with True or False. Some of them have a definite T/F answer. Some of them expect you to make an evaluation of the information and record your reasoned opinion.

Level 1: Answer True or False. Provide the page/paragraph number from the text that supports your answer.

- ___ ___ 1. "Carbon neutral" fuels refer to fuels that have some level of carbon in them, such as natural gas, oil, or coal.
- ___ ___ 2. Biodiesel can be made from crops with high oil content, waste cooking oil, and microalgae, but oil crops offer the most sustainable productivity.
- ___ ___ 3. According to the chart, if the US wants to use canola as the major source of biofuel, we would need to set aside over half of America's farmland just for growing canola.
- ___ ___ 4. The best method of production for biodiesel from microalgae is the use of raceway ponds, because of their relatively low cost and high yields.
- ___ ___ 5. Producing microalgae for biodiesel will be expensive, but the costs will be significantly lowered if the refinery process is designed to use all the leftovers from making biodiesel and use them to make multiple other products.

Level 2: Answer True or False. Provide page/paragraph number from the text that best supports your answer.

- ___ ___ 1. The author of this article fails to present balanced evidence to support the claim that microalgae is the *only* source of renewable biodiesel that is capable of fulfilling the world's demand.
- ___ ___ 2. The author glosses over any factors that could keep microalgae production from being economically practical.
- ___ ___ 3. Europe would be more likely to accept the use of biodiesel from microalgae than the US would.
- ___ ___ 4. Tubes in a tubular photobioreactor are always placed in a North-South line so that they get the most sunlight they can in order to "cook the broth" that turns microalgae into biodiesel.
- ___ ___ 5. One of the main problems with raceway ponds is that the water tends to evaporate quickly and the algae can easily get contaminated because the ponds are open to the elements of nature.

Level 3: Answer True or False. Provide reasoning to support your answer.

- ___ ___ 1. Microalgae production is practical only in areas with reliable, consistent sunlight.

And Why?

- ___ ___ 2. In spite of the author's claim on page 301, column A, there are many relevant factors in choosing between raceways and photobioreactors for biodiesel production from microalgae.

And Why?

_____ 3. It will be fairly easy to convince businesses to go into the emerging microalgae biodiesel production industry.

And Why?

_____ 4. The author chose to leave out data that proves the practicality of creating biodiesel from oil-rich plants such as *Camelina sativa*, because she has a one-sided bias towards microalgae sources.

And Why?

_____ 5. It is likely that the price-per-barrel of petroleum-based gasoline will rise, or has already risen, to the point that bioalgae production is economically competitive in the US market.

And Why?

Answers to Post Lab for From Biology to Biofuels: Creating Diesel from Algae

Chisti, Yusuf. "Biodiesel from Microalgae." *Biotechnology Advances* 25.2007 (2007): 294-306. *ScienceDirect*. Web. 27 Feb. 2013.

Directions:

As you read, respond to these statements with True or False. Some of them have a definite T/F answer. Some of them expect you to make an evaluation of the information and record your reasoned opinion.

Level 1: Answer True or False. Provide the page/paragraph number from the text that supports your answer.

F-247/5 1. "Carbon neutral" fuels refer to fuels that have some level of carbon in them, such as natural gas, oil, or coal.

F-296/1 2. Biodiesel can be made from crops with high oil content, waste cooking oil, and microalgae, but oil crops offer the most sustainable productivity.

F-296/t1 3. According to the chart, if the US wants to use canola as the major source of biofuel, we would need to set aside over half of America's farmland just for growing canola.

F-298/2 4. The best method of production for biodiesel from microalgae is the use of raceway ponds, because of their relatively low cost and high yields.

T-302/2 5. Producing microalgae for biodiesel will be expensive, but the costs will be significantly lowered if the refinery process is designed to use all the leftovers from making biodiesel and use them to make multiple other products.

Level 2: Answer True or False. Provide page/paragraph number from the text that best supports your answer.

F-296/1 1. The author of this article fails to present balanced evidence to support the claim that microalgae is the *only* source of renewable biodiesel that is capable of fulfilling the world's demand.

F-301/3 2. The author glosses over any factors that could keep microalgae production from being economically practical.

F-301/2 3. Europe would be more likely to accept the use of biodiesel from microalgae than the US would.

T-298/6 4. Tubes in a tubular photobioreactor are always placed in a North-South line so that they get the most sunlight they can in order to "cook the broth" that turns microalgae into biodiesel.

T-298/1 5. One of the main problems with raceway ponds is that the water tends to evaporate quickly and the algae can easily get contaminated because the ponds are open to the elements of nature.

Level 3: Answer True or False. Provide reasoning to support your answer.

False 1. Microalgae production is practical only in areas with reliable, consistent sunlight.

And Why? **Artificial light can provide more consistent lighting conditions than sunlight, so photobioreactors may work better than raceways in that aspect.**

True 2. In spite of the author's claim on page 301, column A, there are many relevant factors in choosing between raceways and photobioreactors for biodiesel production from microalgae.

And Why? **It may cost less to use photobioreactors in the long run, but start-up costs and the amount of resources required to build a photobioreactor may push production back towards the raceways.**

False 3. It will be fairly easy to convince businesses to go into the emerging microalgae biodiesel production industry.

And Why? **Current fuel sources have the infrastructure in place to keep costs down even though they aren't sustainable. It will take quite a bit of marketing to encourage businesses to try microalgae.**

True 4. The author chose to leave out data that proves the practicality of creating biodiesel from oil-rich plants such as *Camelina sativa*, because he has a one-sided bias towards microalgae sources.

And Why? **The author chose to focus on the potential of algae rather than the practicality of oil-seed plants like *Camelina sativa*.**

True 5. It is likely that the price-per-barrel of petroleum-based gasoline will rise, or has already risen, to the point that bioalgae production is economically competitive in the US market.

And Why? Oil prices will continue to rise making it easier for grants and other funding to go into alternative fuels like biofuels from algae.

Algae Photo-BioReactor Daily Checklist

Names: _____ Date of setup: _____

Amount of Algae added to bioreactor: _____ Volume of Bioreactor: _____

DAY	RESUSPENDED (Y OR N)	COLOR	OBSERVATIONS	Initials
0				
1				
2				
3			*sample and observe microscope slide for color, movement, and overall health	
4				
5				
6			*sample and observe microscope slide for color, movement, and overall health	
7				
8				
9			*sample and observe microscope slide for color, movement, and overall health	
10				
11				
12				

13				
14				

Weight of filter paper _____

Weight of dried algae

Author Info:

Mark Townley is a 14-year National Board Certified Teacher and Kenan Fellow working as an AP Environmental/Earth Science teacher at Holly Springs High School in Wake County. He holds a degree in Geology from NC State University and is General Science Certified. In 2009, he was a finalist for the Wake County Teacher of the Year, and was named as the N.C. Outstanding Science Teacher of the Year for District 3 by the NC Science Teacher Association in 2011.

kenanfellows.org



From Crops to Shops: Where Does Food Come From?



Explore the food chain and educate the next generation of farmers, consumers and scientists.

Fast Facts	
Mentors	Jeff Bender, Warren County Farm Bureau and Gabe Cumming, Warren County Economic Development
Subjects	Science, math, social studies, ELA, technology, agriculture, business, counselor, librarians
Grade Levels	4-12
Location	Warren County
District Availability	Warren
Sponsor	Warren County Farm Bureau
Website	North Carolina Farm Bureau

You will work with representatives from various aspects of the agriculture and food processing industry to explore the critical role of agriculture in Warren County and worldwide. Partnered with industry experts, you'll discover how and why today's farmers are able to meet the food needs of growing populations locally and globally.

- Create resources that encourage students to analyze the intricate and foundational role of agriculture as it relates to food production and the corresponding societal and cultural implications.
- Develop videos and materials to highlight the varied careers available in agriculture related fields and expose your students to a variety of agriculture related topics.
- Guide students as they prepare videos or books targeted to a younger audience. Coordinate with other teachers to disseminate these resources to students across the county.

As Warren County's newest authority in agricultural science, you're charged with exposing students to the food supply chain, careers in agriculture, historical shifts in farming and the critical need for farming to feed the world population. You will play an active role in helping the Warren County students understand how agriculture has changed over the years and continues to transition, locally; yielding a more informed population.