KANSAS CORN STEM

Kansas Corn: Yield by Design, Hybrid Seeds

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KANSAS CORN COMMISSION

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Grade Level: High School

Overview
Throughout history, the human race has been molding and bending our flora and fauna to meet the needs of our existence. This includes anything from cultivation of crops, irrigation of arid lands, all the way to developing new varieties of crops through artificial selection. Even today, the process continues. Meeting the needs of people has led to many advancements in technology and practices. One such advancement was the development of crop hybrids. In 1926, a Des Moines, Iowa, company released the first corn hybrid under the name "Pioneer Hi-Breds." These new varieties were developed by cross breeding two plants of inbred lines to create a plant that had increased vigor and, ultimately, increased yield. Today, we are still reaching for the same goal, increased production to meet the demands of our society. Yet, we have a whole new set of tools at our disposal to help us reach this goal. Combining traditional breeding techniques with genetic engineering practices has allowed companies to produce new varieties of corn that are more resilient in harsh environmental conditions, such as drought, weed and insect infestations. But how do you tell these newly developed varieties from their counter parts? Seeing two plants side by side will often not provide enough evidence to distinguish one variety from another. So, how can we distinguish varieties? Genetics, the basis of the differences between all living things, lies within the delicate strands of DNA that are found in most of all living cells. By comparing the DNA of different plants, we can highlight the difference in DNA that will ultimately lead to differences in traits that can, under the right conditions, lead to increased yields. In this lab, we will analyze DNA already harvested from different plants to determine which plant holds a particular trait using the techniques of restriction enzyme digestion and gel electrophoresis.

Kansas College and Career Ready Standards
Science

- **HS-LS4-2.** Construct an explanation based on evidence that the process of evolution primarily results from four factors...(2) the heritable genetic variation of individuals in a species due to mutation and sexual reproduction.
- **HS-LS3-2.** Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- **HS-LS3-3.** Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

Learning Objectives
- Students will analyze DNA using gel electrophoresis technique.
- Students will use restriction enzymes to cut model sections of DNA.
- Students will model restriction enzyme digestion and gel electrophoresis using paper based techniques.
- Students will gain real world lab experiences utilizing pipettes and electrophoresis equipment.

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Materials
- Kansas Corn: Yield by Design, Hybrid Seeds PowerPoint (available at www.kansascornstem.com)
- Copies of Restriction Enzymes and Electrophoresis and DNA Sequences (pg. T10-11, or available online at www.kansascornstem.com)
- Restriction Enzymes and Electrophoresis Graph (pg. S1-2, or available online at www.kansascornstem.com)
- Copies of Hybrid Electrophoresis Lab Analysis Worksheet (pg. S3, or available online at www.kansascornstem.com)
- Colored pencils, markers or highlighters
- Scissors
- Minione Crime Scene Genetics Kit
- Minione Electrophoresis Lab Station, including electrophoresis apparatus, variable volume micropipette (2-20 uL) and micropipette tips
- Solo cup labeled as trash for disposing of used tips
- 100 mL graduated cylinder
- 250 uL Erlymyer flask
- Prepared TBE buffer (provided in lab kit)
- Aliquoted samples of DNA

Safety Considerations
As with all labs, make sure your lab area is clean and free of debris and other unnecessary equipment. Ensure the lab table is dry before plugging in any electrical equipment, and clean any spills immediately. When removing gel, make sure you unplug the apparatus from the outlet before you take it out of the tank.

Procedures for Instruction
Length of Time for Preparation: 30-45 minutes
Length of Time for Classroom Teaching: 60 minutes

Preparation Procedure
- Prep will include pipetting out samples into individual micro centrifuge tubes for each lab group.
- Mixing electrophoresis buffer as per the directions provided.
- Obtain a microwave and place it in an easily accessible location in the lab area.

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Background Information
A hybrid, as a biological term, often refers to an organism of two different parents who exhibit different traits. This term had been so popularized in the agriculture community by its use in variety names that it has become synonymous with variety in some situations. To be clear, a crop variety is characterized as a classification group that is below the level of species and subspecies and that includes certain phenotypic and consequently genetic characteristics. When using the word hybrids to describe new varieties of plants, confusion can be created. Most varieties today are created through a combination of traditional breeding techniques as well as genetic engineering practices. All new varieties must go through a rigorous process of development and testing prior to the process of applying for deregulation status, which allows the variety to be sold in both the United States and abroad. This process can take upward of 12 to 14 years and is the culmination of the efforts of thousands of individuals.

A fair understanding of DNA as the molecule of heredity is necessary for students to comprehend this lab. Understanding the basic structure of DNA as well as the processes that create proteins are essential to understanding how different DNA can cause different traits to be seen and expressed in organisms. Since most of the traits that are included in new varieties do not carry distinct visual phenotypic differences, students must be able to understand the underlying differences in DNA before they can appreciate the subtle differences in the varieties of crops.

Classroom Discussion
Macro vs. Micro Differences

- Post the picture of a man, for 10 seconds, tell students to take a good look at the man because you will have to identify him later. You can take notes to help you remember.
    - Remove the picture. Ask: What kinds of notes did you take?
        - Allow students to respond, possible responses might include: eye color, hair color, facial hair vs. no facial hair, skin color, etc.
    - Show the array of pictures. Which one of these is the same many of you saw before? Write your number down.
- Post the picture of a corn plant for 10 seconds. Tell students to take a good look at the plant, you will have to identify it later. You can take notes to help you remember.
    - Remove the picture. Ask: What kids of notes did you take?
        - Allow students to respond, possible responses might include: height of plant, number of leaves, color of plant, name of plant, etc.
    - Show the array of plant pictures. Which one of these is the same as the one you said before? Write down your number.

- Review answers. The man was easy to spot. You have visual clues that you have been trained to spot your entire life! The plant might not be so easy to spot. We see a corn plant and just see a corn plant. It's hard for us to visually distinguish plants of different varieties from each other. They visually look the same.
- What caused the man's features to be the way they are?
    - Hopefully students' responses will be DNA!
- What cause the plant's features to be the way they are?
    - Hopefully, again, the same answer will be DNA.
- So, how do I tell if they are the same or not? I can test a man's DNA to compare his traits to another person. This is how we catch some criminals!
- Can I also test a plant's DNA to compare to other plants?

Activity: Comparing DNA...

- We know that DNA is made up of four basic nitrogen bases, and the order those nitrogen bases are put together determines our traits. How can we compare those bases? Letter by letter would take forever! What's a faster way to do it? Restriction enzymes!
- Give each student a sequence of DNA, a pair of scissors, and the first restriction enzyme.
    - There are four different sequences of DNA. Working in groups, have each member of the group use a different sequence.
- Tell the students to cut their sequence right after they see the matching code on their DNA. Everyone's DNA is different so yours' will not be the same as your neighbors'!
- Have the students hold up their cut or not cut DNA. Are the fragments the same size?
- Why are the fragments different? The letters were at different places or weren't there at all. What if we keep doing this with more restriction enzymes? We will end up with multiple fragments based on where they would cut your sequence!
- Use the other three restriction enzymes to cut your sequences.
- Now go find someone with the same DNA as you.
    - Match up with members of the other lab groups. How can we be sure that their DNA is the same? Look at the size of the DNA fragments.
    - Count up the number of base pairs in each fragment. Write that number on the back. Compare with your matching DNA. You have the same sized fragments because you had the same DNA.

*** More activities will be completed during the lab process, there is a 15-20-minute window where the process of electrophoresis is explained. This can be done before or during the lab, whichever is your preference as a teacher. ***

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Procedure for Lab

- Peel up one corner of the gel cup. Microwave for 20 seconds.
- Be aware of the steam coming out of the cup, pick up the cup and swirl it several times, making sure that all the solid agar has liquefied.
- Let the cup cool slightly. Continuing to swirl the cup will help with even cooling.
- Set up the gel tray by inserting the clear gel tray into the gel mold. Place the six-sided comb into the top position of the mold.
- Pour in the warm gel. Each gel cup will create one tray of gel. To fill both sides of the gel mold, you will need two cups. This would be enough for two lab groups.
- Set the gels aside to cool and solidify. The gel takes a slightly cloudy appearance once set. You should be able to touch the gel and feel a resistance. Do not press too hard or you will put a divot in your gel.
- Measure out approximately 150 mL of TBE buffer. Note: buffer may precipitate out. Make sure you are stirring the buffer well before use. All precipitate may not dissolve, but this should not affect results.
- Once the gel has solidified, remove it from the mold. Pull the comb straight up to reveal the wells. Then, pull up on the clear plastic gel tray, keeping the gel on top of the gel plastic gel tray. Wipe any excess liquid off the bottom of the gel tray and the sides. Be careful when handling the gel. The gel tray only has sides, no front or back, so the gel may slide off the front if you are not holding the gel parallel to the ground.
- Place the gel into the electrophoresis tank, match up wells with the lines on the black plate on the bottom of the tank. The well should be oriented at the negative side of the tank, as is indicated by the negative sign (-) at the top of the tank. The gel will only slide into the tank in one way. It should slide all the way to the bottom and rest on top of the black plate. The gel must be on the gel tray and not touching the black electrodes on either side of the tank.
- Pour the TBE buffer into the tank. Pour in one side of the tank only. This will force any air from under the gel out so that no bubbles are present. 150 uL should just barely cover the gel in the tank. If the gel slightly floats up, simply press down on the gel until it is resting on the black plate once again.
- Place the tank into the electrophoresis power supply. Make sure that the gold rivets are touching the electrodes in the tank. The tank should slide in easily, do not force a tank in. It is designed to slide in only one direction.
- Once the gel is in the tank, plug in the power supply and turn on the black light. The larger lightbulb button will provide sufficient light to see the wells.
- Pipette in the samples, 10 uL in each well, making sure to change pipette tips as you move from one sample to another. Make sure to keep the pipette plunger depressed as you remove the pipette tip from the gel. Otherwise, you will draw the sample back up into the pipette instead of leaving it in the gel.

- Once all samples are loaded, depress the smaller lightbulb button, and place the orange viewing hood on top of the tank (this will only work in one direction). Then, press the power button light. The light indicator should turn green to indicate the apparatus is working. You should also be able to see bubbles developing on the electrodes in the tank. Do not remove the viewing cover until you are finished with the lab.
- Check on the gel every 5 minutes for a total of 15-20 minutes. Within the first 5 minutes, you should be able to see the bands migrating down toward the positive electrode in the tank. Continue to watch and check the gel every 5 minutes until 20 minutes have elapsed.
- Once 20 minutes has elapsed, a photo can be taken of the gel using the viewing hood. The hole in the top of the viewing hood is at the perfect focal length for student devices. The photo will also include a ruler for assistance in analysis later either by hand or software analysis tools, such as Logger Pro, can be utilized if desired. Pictures should be taken to preserve the results. As time passes, the bands will disperse in the gel, and results will fade.
- Once good pictures have been taken, your lab is complete. TBE buffer can be reused; however, sufficient concentrate has been provided for new buffer every time the lab is run. Gels can be disposed of in the trash. Make sure students remove the clear plastic gel trays before they throw out the gels.

During wait time: Finish Restriction Enzyme and Gel Electrophoresis Activity.

- You will be modeling the process of electrophoresis in this kinesthetic activity.
- Divide your students into groups, one group of 6, a few groups of 3 to 4, and a few groups of 2 to 1.
- Make a "lane" or line for your students to walk down with a few obstacles. Three to five chairs work well, depending on the space you have available. Place the chairs directly in the "lane" or path you want the students to take.
- Have the groups take turns trying to get from one end of the lane to the other in a very short amount of time. The trick is you cannot lose members of your group. They must all stay together. You only make it as far as your last member. Give each group 5 seconds to walk down the lane. Time can be adjusted to fit the number of students.
- Mark how far each group got with a piece of tape or meter stick, indicating the number of group members on it.
- You will find that the groups with the smallest number of individuals will make it farther down the lane. There is less to move, literally one person vs. five or six.
- Explain to your students that the same thing happens in electrophoresis. The larger the DNA fragment, the slower it is moving down the lane.
- The gel is represented by the chairs in their path. Everyone had the same number of chairs to navigate around. The gel is the same no matter what lane we are talking about.
- The larger the fragment, the short the distance it can travel from the wells.
- The smaller the fragment, the farther the distance it can travel from the wells.

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Now, have students finish their modeling activity by filling out the Restriction Enzyme and Electrophoresis Graph of their own DNA fragments from the Restriction Enzyme Digestion earlier. Make sure that students are using a writing utensil that is bright and easy to see on their graph. Afterward, students can answer the questions at the bottom of the graph sheet on a separate piece of paper or simply on the back of their graph.

Teacher Resources

Students should have experience with pipetting before you attempt this lab. Proper technique is critical due to the small amounts of samples being used in each well. If the samples are released outside of the well, no bands will be produced for that particular sample. Remind students about the functionality of both the hard and soft stop for the pipette will help prevent accidental release. It is also possible for students to puncture the gel and deposit their samples underneath the gel. This, too, will not produce any results. Stead hands and patience are critical for the best results. For a lesson on pipetting, check out www.kansascornstem.com.

Lab Analysis and Conclusion

See Hybrid Electrophoresis Lab Analysis Sheet for student questions:

- Photograph and visually analyze the gel.
- Have students take a picture of the completed gel through the window on the top of the UV hood. Compare the bands seen in the completed gel. Recall that the sequence of DNA we are looking for is for one particular gene.
- Have students complete the questions on the Lab Analysis Worksheet. At this point, students can upload their pictures into analysis software, such as Logger Pro, and apply measurements and base pair lengths to each particular band, if you wish to go that deep.

Science and Agriculture Careers

Understanding the basics of genomic analysis is the key to truly understanding the vast diversity of seed varieties being developed on an ongoing basis. Since the vast majority of hybrid plants cannot be distinguished from their non-hybrid peers, it is critically important for students to understand the differences between the two. Primarily, that this difference is not visual but genetic. Analysis, such as this, takes basic laboratory skills and an understanding of laboratory equipment that can be found across the world. Several different careers in agriculture are fundamental in the development of new varieties of crops. Botanists, horticulturalists, biochemists, biological engineers, climatologist, ecologists, food scientists, geneticists, microbiologist, plant pathologists, and an army of lab technicians are all involved in the development of every new variety that hits the market. Without their valuable hard work and dedication, we would not be able to provide for the growing demands we see in agriculture.

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Note
To learn more about agriculture careers, visit www.agexplorer.com. You can also find career profiles at www.kansascornstem.com.

Sources

- Daniel Herschlag. RNA Chaperones and the RNA Folding Problem. J. Biol. Chem. 1995 270: 20871-. doi:10.1074/jbc.270.36.20871
- Castiglioni, P., Warner, D., Bensen, R. J., Anstrom, D. C., Harrison, J., Stoecker, M., Abad, M., Kumar, G., Salvador, S., D'Ordine, R., Navarro, S., Back, S., Fernandes, M., Targolli, J., Dasgupta, S., Bonin, C., Luethy, M. H., ... Heard, J. E. (2008). Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. Plant physiology, 147(2), 446-55.

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.

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TEACHERS KEY

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Restriction Enzymes and Electrophoresis:

- Each restriction enzyme has been color coded so that it is visually easier for you to see. Make sure you provide students with the all black sequences.
- Each sequence is 100 BPs long. Put students in groups of 4 initially, give each group member a different number.
- Have students "apply" all four restriction enzymes. That is, search their sequence for the corresponding sequence in each restriction enzyme, mark the sequence with the appropriate colored pencil, and then cut after the restriction enzyme.
- Have students graph each member of the groups of RNA.

RE1: CGTAA RE2: TAGCG RE3: AAGTA RE4: GGCAT

#1 ATGCTACGCTATCGCTATCCCAAGTATAGCTTAAACGGCTATAGCGATGCTACGCTATCGCTATCCCGCTAAAAGCTACTAGGCTACTAGCGCTAGCTAG

1. 3 bands
2. 20, 26, 54 BPs long
3. 2 REs: RE 3 and RE 2

#2 CGTGGCATGCGCGTACGTAACGTAGCTGATGCTACGCTATCGCTATCCCGCTAAAATCCTAGCAAGTATAGCTTAAACGGCTATAGCGGCTACTAGGCTA

1. 5 bands
2. 8, 11, 12, 20, 48 BPs long
3. All 4 REs

#3 GTAGCTGATGCTACGCTATCGCTATCCCGCTAAAATCCTAGCTTAAACGGCTACGTGGCATGCGCGTACGTAAGCTACTAGGCTACTAGCGCTAGCTAGT

1. 3 bands
2. 12, 27, 61 BPs long
3. 2 REs: RE 1 and RE 4

#4 GGCTAAAGTATAGCTTAAACGGCTATAGCGTAGCTTAAACGGCTACGTGGCATGCGCGTACGTAATAAAATCCTAGCTTAAACTAAAATCCTAGCATTAA

1. 5 bands
2. 10, 12, 20, 23, 35 BPs long
3. All 4 REs

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Student Sequences. Make as many copies as necessary for each group to have one complete set.

#1 ATGCTACGCTATCGCTATCCCAAGTATAGCTTAAACGGCTATAGCGATGCTACGCTATCGCTATCCCGCTAAAAGCTACTAGGCTACTAGCGCTAGCTAG
#2 CGTGGCATGCGCGTACGTAACGTAGCTGATGCTACGCTATCGCTATCCCGCTAAAATCCTAGCAAGTATAGCTTAAACGGCTATAGCGGCTACTAGGCTA
#3 GTAGCTGATGCTACGCTATCGCTATCCCGCTAAAATCCTAGCTTAAACGGCTACGTGGCATGCGCGTACGTAAGCTACTAGGCTACTAGCGCTAGCTAGT
#4 GGCTAAAGTATAGCTTAAACGGCTATAGCGTAGCTTAAACGGCTACGTGGCATGCGCGTACGTAATAAAATCCTAGCTTAAACTAAAATCCTAGCATTAA

#1 ATGCTACGCTATCGCTATCCCAAGTATAGCTTAAACGGCTATAGCGATGCTACGCTATCGCTATCCCGCTAAAAGCTACTAGGCTACTAGCGCTAGCTAG
#2 CGTGGCATGCGCGTACGTAACGTAGCTGATGCTACGCTATCGCTATCCCGCTAAAATCCTAGCAAGTATAGCTTAAACGGCTATAGCGGCTACTAGGCTA
#3 GTAGCTGATGCTACGCTATCGCTATCCCGCTAAAATCCTAGCTTAAACGGCTACGTGGCATGCGCGTACGTAAGCTACTAGGCTACTAGCGCTAGCTAGT
#4 GGCTAAAGTATAGCTTAAACGGCTATAGCGTAGCTTAAACGGCTACGTGGCATGCGCGTACGTAATAAAATCCTAGCTTAAACTAAAATCCTAGCATTAA

#1 ATGCTACGCTATCGCTATCCCAAGTATAGCTTAAACGGCTATAGCGATGCTACGCTATCGCTATCCCGCTAAAAGCTACTAGGCTACTAGCGCTAGCTAG
#2 CGTGGCATGCGCGTACGTAACGTAGCTGATGCTACGCTATCGCTATCCCGCTAAAATCCTAGCAAGTATAGCTTAAACGGCTATAGCGGCTACTAGGCTA
#3 GTAGCTGATGCTACGCTATCGCTATCCCGCTAAAATCCTAGCTTAAACGGCTACGTGGCATGCGCGTACGTAAGCTACTAGGCTACTAGCGCTAGCTAGT
#4 GGCTAAAGTATAGCTTAAACGGCTATAGCGTAGCTTAAACGGCTACGTGGCATGCGCGTACGTAATAAAATCCTAGCTTAAACTAAAATCCTAGCATTAA

#1 ATGCTACGCTATCGCTATCCCAAGTATAGCTTAAACGGCTATAGCGATGCTACGCTATCGCTATCCCGCTAAAAGCTACTAGGCTACTAGCGCTAGCTAG
#2 CGTGGCATGCGCGTACGTAACGTAGCTGATGCTACGCTATCGCTATCCCGCTAAAATCCTAGCAAGTATAGCTTAAACGGCTATAGCGGCTACTAGGCTA
#3 GTAGCTGATGCTACGCTATCGCTATCCCGCTAAAATCCTAGCTTAAACGGCTACGTGGCATGCGCGTACGTAAGCTACTAGGCTACTAGCGCTAGCTAGT
#4 GGCTAAAGTATAGCTTAAACGGCTATAGCGTAGCTTAAACGGCTACGTGGCATGCGCGTACGTAATAAAATCCTAGCTTAAACTAAAATCCTAGCATTAA

Restriction Enzyme and Electrophoresis Graph

Graph each DNA sample by marking a line with a highlighter or marker for each base pair piece you have.

- Ex: one piece of DNA is 51 base pairs (letters) long. Mark on the line between 50 and 52.
- For even numbers, draw a line in the middle of the box. For odd numbers, mark your line on the line dividing the boxes.

Wells (- negative electrode)

Base Pair	Sample 1	Sample 2	Sample 3	Sample 4
66				
64				
62				
60				
56				
54				
52				
50				
48				
46				
44				
42				
40				
38				
36				
34				
32				
30				
28				
26				
24				
22				
20				
18				
16				
14				
12				
10				
8				
6				

Positive Electrode (+)

Analysis Questions

1. Does every restriction enzyme cut every piece of DNA? How can you be sure?

2. Does having an equal number of bands mean that organisms share DNA? Why or why not?

3. What is the relationship between the band size and the distance the DNA travels down the gel?

4. Can different restriction enzymes produce bands of the same size in different samples?

5. Does using one restriction enzyme provide enough evidence when comparing DNA sequences to positively determine if two samples come from the same organisms? Why or why not?

Hybrid Electrophoresis Lab Analysis

Paste a copy of your gel picture here.

1. Fill in the table below with the number of bands that you see in each lane.

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6

2. Estimate how many restriction enzymes it would take to create the number of fragments seen.

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6

3. Which sample has the shortest fragment of DNA? How do you know?

4. Which sample has the longest fragment of DNA? How do you know?

5. Which two samples share the same DNA sequence for the sampled trait? How do you know?

6. Can you determine whether or not organisms share the same forms of genes by visual appearance alone? Why or why not?

7. Genetic engineering often times involves adding one gene from species A into a different organism Species B. How is it possible to take DNA from one organism and have it function in a very different organism?