KANSAS CORN STEM

Kansas Corn: Chopped Using CRISPR-Cas9

qrco.de/chopped

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Updated 2023

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

KANSAS CORN COMMISSION

Kansas Corn: Chopped
Grade Level: High School

Overview

What are the newest techniques in the creation of new hybrid plants? For decades we have relied upon artificial selection, plant breeding, and genetic manipulation using genes from other organisms to impart beneficial traits. The latter has been the center of many controversy's and misunderstandings around Genetically Modified Organisms. But what if we could improve crops by only utilizing the naturally occurring genes inside the crops already? Gene editing is a relatively new technology that allows just that, the direct editing of the genome already in the crops themselves. The ability to change the DNA in an organism has enabled corn geneticists to turn undesirable traits off without adding genes. A perfect example of this improvement would be a type of corn called Waxy Corn which has a higher percentage amylopectin than other varieties. This amylopectin is then milled into "corn starch" and utilized extensively in the food industry. This form of Waxy corn was originally developed through selective breeding, crossing varieties with a gene mutation to turn off the production of a particular protein consequently causing the corn to produce MORE amylopectin per corn kernel. Due to the nature of selective breeding this trait has been limited to the varieties with direct lineage to the originally mutated strains. However, through using the CRISPR-Cas9 system geneticists can turn off that same gene in other high yielding corn varieties to produce new sources of Waxy Corn. This allows us to combine multiple positive traits in one variety, maximizing yield AND increasing amylopectin at the same time. Using the "Chopped" MiniPCR lab, students will use this system to cut DNA using the enzyme and two different guide RNA molecules and the CRISPR nuclease. A gel electrophoresis is used to determine the size of the fragments produced.

Kansas College and Career Ready Standards

Science

- **HS-LS1-1.** Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.
- **HS-LS2-7.** Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.
- **HS-LS3-1.** Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- **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) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

Learning Objectives

- Understand the structure of DNA and its role in genetic inheritance.
- Comprehend how DNA encodes traits that are passed across generations.
- Understand the components of the CRISPR-Cas system.
- Understand that CRISPR can be used to edit specific genes in living organisms.
- Understand that editing genes can induce positive traits in crops.

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Materials

For class

- Kansas Corn: Genetic Cut and Paste PowerPoint presentation (available at kansascornstem.com)
- 37 degree C heat source, e.g., thermocycler, incubator, or water bath
- micropipette (20-200 mL) for dispensing reagents
- Distilled Water

For each student group:

Prelab

- CRISPR model handout (p 40-42 MiniPCR Chopped)
- Scissors
- Transparent tape or copy of digital Chopped Prelab (google doc)

Lab

- Electrophoresis Gel box
- micropipette (2-20 mL)
- micropipette tips (at least 24/group)
- gel cup
- buffer
- Disposable laboratory gloves
- Protective eyewear
- Permanent marker
- Cup to dispose of tips
- Crushed ice

Safety Considerations

Keep lab area clean and free of unnecessary equipment. Students should wear protective eyewear and gloves throughout the lab.

Procedures for Instruction

Length of Time for Preparation: 30 minutes to thaw reagents and dilute the Cas9 enzyme.
Length of Time for Classroom Teaching: Two 45-50 minute class periods or one 90 minute class period.

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Preparation Procedure/Instructions

- Located on page 7-10 of "Chopped" MiniPCR Teacher's Guide.

Background Information

CRISPR-Cas systems are naturally found in bacteria and other prokaryotes as a form of protection. These systems defend these simple organisms from bacteriophage, viruses that attack bacteria, by identifying sequences of viral DNA and cutting these strands before the bacteria can utilize this viral DNA to create protein which effectively prevents the virus from reproducing. This action is very similar to restriction enzymes that perform a similar function. Restriction Enzymes cut any DNA that matches their short individual sequences. We often utilize these naturally occurring restriction enzymes to cut DNA sequences at targeted locations in the lab but we have been limited by the sequences found in nature. CRISPR-Cas systems are different in a very important way. They use guide RNA to find specific viral DNA sequences. Honing in like a sniper on a target. This allows the same nuclease enzyme to target different sequences and adapt more quickly to changes in the virus. In 2012 scientists programmed CRISPR RNA to target DNA sequences they wanted to change. This approach enables scientists to cut genes that they want to disable or even cut a specific gene out while pasting in a different gene to replace it. Effectively turning on, turning off, or replacing genes at the discretion of the desired traits. This ability to edit genes has many potential applications in treating diseases such as sickle cell disease, cystic fibrosis, and types of cancer. This technology also shows promise in the development of crops. Waxy Corn has a different starch composition that is desirable for certain cooking and industrial applications. This difference is due to mutations disabling a gene called Waxy1. These Waxy1 alleles have arisen naturally as well as being induced using chemicals and radiation. Using CRISPR scientists precisely deleted the waxy1 gene in high yielding corn lines. This process produced waxy hybrids much more quickly than conventional methods and the yields were dramatically higher. This gene editing tool has enormous potential in the medical field as well as the agricultural and industrial setting and has the potential to confer a public perception benefit. Currently, food products containing gene edited crops do not require genetically modified labeling eliminating some controversy surrounding such varieties.

More information located on page 12-15 of "Chopped" MiniPCR Teacher's Guide.

CRISPR Webinars and Other Resources

Available for download at https://www.minipcr.com/products/minipcr-learning-labs/crispr/

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Classroom Discussion

In the prelab activity, located on pages 17-20, the DNA sequence that will be edited is written out in A, G, T, and C format in a google document. Students will look for the target sequence for each of the guide RNA strands and determine the location where the Cas-9 enzyme will cut the strand. The length of the fragments produced can then be predicted and compared with the fragment lengths found in the gel during the lab analysis. There are two options for this activity depending on preference and technology available. Yeast and anaerobic respiration

- Pre-Lab Paper Model- Located on pages 40-42. The paper model involves cutting the sequence from paper and taping it into one piece. The entire sequence isn't written out but the number of bases left out in the strands are labeled. Students search for the complementary sequence in the parts that are written out. Once the location of the cut is found they can count the typed bases in each fragment and add the number counted to the number given that was not typed. This number will be the predicted fragment length.
- Digital Prelab- available as a google document. Can be found in the PowerPoint presentation or at https://links.minipcr.com/chopped_prelab This version of the prelab is quicker and requires less counting than the paper model but does require a device that can open and edit a google doc. Students can open and edit a copy of the google doc by clicking the link above. This version uses the character count and search capability to find the target sequence and count the letters representing bases in the fragment before and after the Cas-9 edit. This activity can be performed together as a class using a projector. There are detailed instructions and demonstrations in the PowerPoint available at (kansascornstem.com).
- Prelab questions located on pages 27-29 of Chopped Students Guide.

Procedure for Lab

Follow lab guide 21-26 miniPCR Chopped

Teacher Tips

- Page 44-54 of Instructors Guide includes sections on differentiation, trouble shooting, expected results, etc. this will assist you as you conduct the lab.

Lab Analysis

- Did the fragments on the gel match the prediction?

Reflection and Conclusion

Pages 34-36 miniPCR Chopped Students Guide

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Science and Agriculture Careers

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

Sources

- https://bitesizebio.com/47927/history-crispr/
- https://bioengineeringcommunity.nature.com/posts/61316-using-crispr-to-develop-superior-corn-hybrids

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.

Laboratory guide

Gloves and protective eyewear should be worn for the entirety of this experiment.

Keep the Cas9 Nuclease on ice at all times.

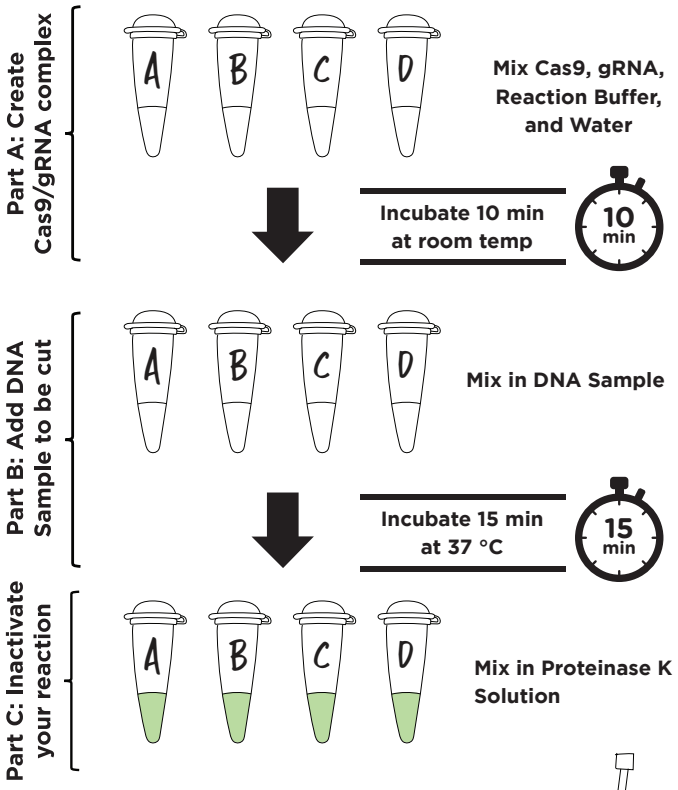
A. Create Cas9/gRNA complex

Follow the steps below to mix the Cas9 Nuclease and the gRNA to allow the Cas9/gRNA complex to form.

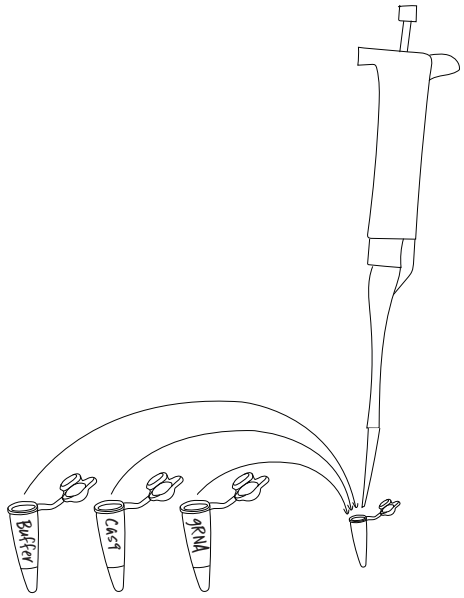
1. **Label four plastic tubes**
   - Use a fine-tipped permanent marker to label the tubes **A-D**.

2. **Follow the table below to add reagents to each of your tubes**
   - Use a micropipette to add each of the reagents.
   - After adding a reagent to a tube, check it off in the table below so you know you have already added it!
   - Remember to change tips at each step!
   - The total volume in each tube should be 15 µl after adding all reagents.

Overview of experiment workflow



	Tube A	Tube B	Tube C	Tube D
Condition	**Control 1:** No Cas9 No gRNA	**Control 2:** Cas9 only	**gRNA1:** Cas9 + gRNA1	**gRNA2:** Cas9 + gRNA2
Nuclease-Free Water	10 µl	5 µl	-	-
Reaction Buffer	5 µl	5 µl	5 µl	5 µl
Cas9 Nuclease	-	5 µl	5 µl	5 µl
gRNA1	-	-	5 µl	-
gRNA2	-	-	-	5 µl
TOTAL VOLUME	**15 µl**	**15 µl**	**15 µl**	**15 µl**



Use a micropipette to add each of the reagents. Remember to change tips at each step!

3. **Thoroughly mix the reagents by pipetting up and down 10 times, then cap the tubes**
   - If you have a vortexer, you can mix the reagents by vortexing for 5 seconds.

4. **Make sure all the liquid volume collects at the bottom of the tube**
   - If necessary, spin the tubes briefly using a microcentrifuge or shake down with a flick of the wrist.

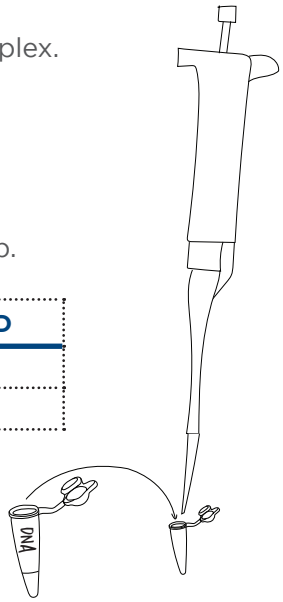
5. **Incubate the tubes at room temperature for 10 minutes**
   - Incubate the tubes in a tube rack or lying flat on the lab bench or table.

B. Add DNA sample

Next, follow the steps below to add the DNA Sample to the Cas9/gRNA complex.

6. **Add 5 µl of DNA Sample to each of the tubes**
   - Use a micropipette to add the DNA Sample.
   - Remember to change tips at each step!
   - The total volume in each tube should be 20 µl after completing this step.

	Tube A	Tube B	Tube C	Tube D
DNA Sample	5 µl	5 µl	5 µl	5 µl
TOTAL VOLUME	**20 µl**	**20 µl**	**20 µl**	**20 µl**

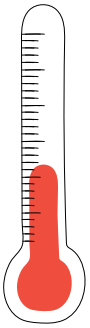


7. **Thoroughly mix the reagents by pipetting up and down 10 times, then cap the tubes**
   - If you have a vortexer, you can mix the reagents by vortexing for 5 seconds.

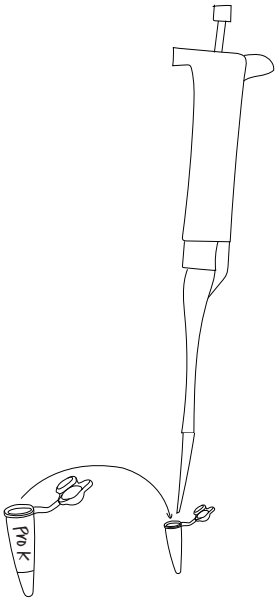
Use a micropipette to add each of the reagents. Remember to change tips at each step!

8. **Make sure all the liquid volume collects at the bottom of the tube**
   - If necessary, spin the tubes briefly using a microcentrifuge or shake down with a flick of the wrist.

9. **Incubate the tubes at 37 °C for 15 minutes**
   - Use a miniPCR® in heat block mode, a 37 °C incubator, or other 37 °C heat source.



C. Inactivate Cas9 enzyme with Proteinase K



Proteinase K breaks down proteins and is used here to remove the Cas9/gRNA complex from the DNA prior to running your sample on an agarose gel.

Note: The Proteinase K Solution is green because it also contains gel loading dye for running the sample on the gel; this will save the step of adding loading dye later.

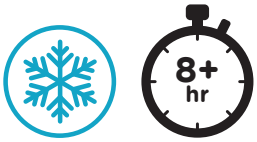
10. Add 4 µl Proteinase K Solution to each of the tubes

- Use a micropipette to add the Proteinase K Solution.
- Remember to change tips at each step!
- The total volume in each tube should be 24 µl after completing this step. Your reactions should now have a dark green appearance instead of clear.

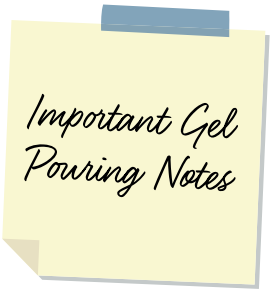
	Tube A	Tube B	Tube C	Tube D
Proteinase K Solution	4 µl	4 µl	4 µl	4 µl
TOTAL VOLUME	**24 µl**	**24 µl**	**24 µl**	**24 µl**

11. Ask your instructor whether you will be stopping here for the day or continuing with gel electrophoresis in the same class period

Option A: Stop here for the day	**Option B: Run your samples on the gel in the same class period**
• Store your samples in the freezer. • In the next class period, samples can be thawed and used immediately for gel electrophoresis. • Note: If freezing your samples overnight, there is no need to incubate tubes at 37 °C after adding proteinase K as indicated on the right column, Option B.	• Incubate your samples at 37 °C for at least 10 minutes before running your gel. • Longer incubation times (up to 20 minutes) may result in clearer gel results.



Gel electrophoresis: Pouring gels (before or during class period)



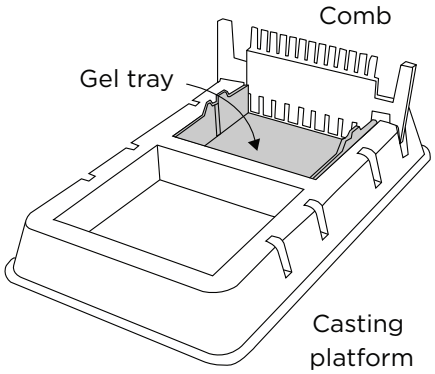
This lab uses 1% agarose gels. You will need four lanes per group, plus one lane for the ladder. If groups are sharing gels, a single lane for ladder is sufficient.

Gels can be prepared up to three days ahead of time and stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

These instructions are designed for use with the blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

1. **Prepare 1X TBE buffer (to be completed by teacher in advance)**
   - TBE buffer is often provided as liquid concentrate or powder.
   - Follow manufacturer's instructions to prepare 1X TBE buffer solution.

2. **Prepare a clean and dry casting platform with a gel tray and comb**
   - Place the clear gel tray in the white casting platform.
   - Place a well-forming comb at the top of the gel tray.



3. **Prepare a 1% agarose solution using the method indicated by your instructor**

IMPORTANT NOTE: There are several ways to prepare agarose gels

- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.
- The video demonstrates making a 2% gel as an example. Use the volumes specified in the written instructions for making a 1% gel to prepare gels for this lab.

www.minipcr.com/agarose-gel/

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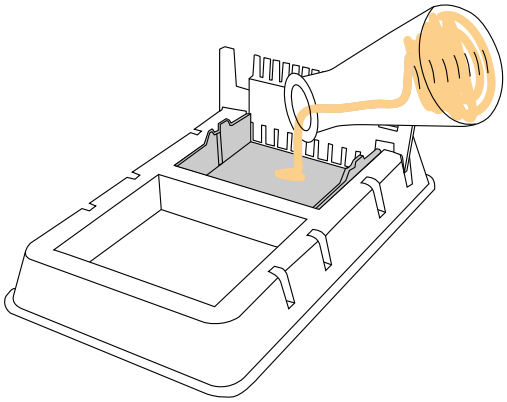
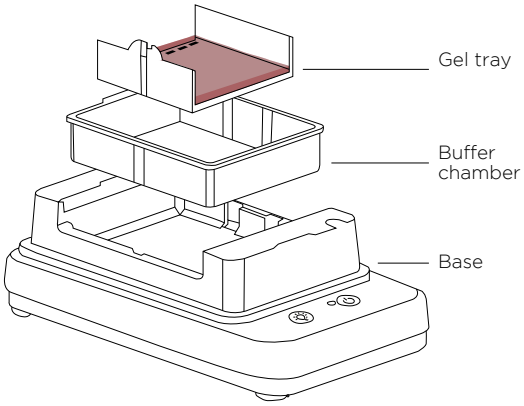
4. **Pour the agarose solution into the prepared casting platform with a gel tray and comb**
    - The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).

5. **Allow gel to solidify completely and remove the comb by pulling firmly upwards**
    - Gels will typically be ready in about 10 minutes.
    - Gel is ready when cool and firm to the touch.

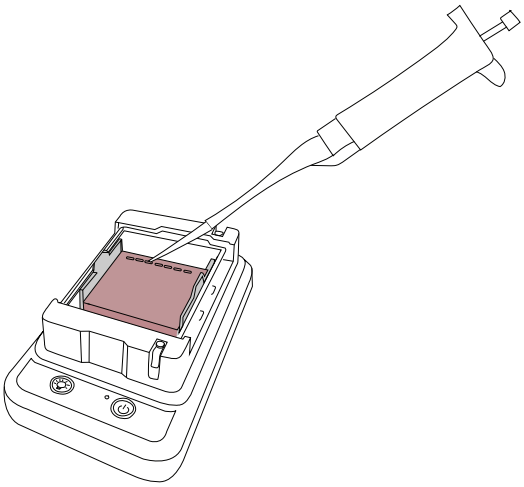


Gel electrophoresis: Running the gel

These instructions are designed for use with blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.



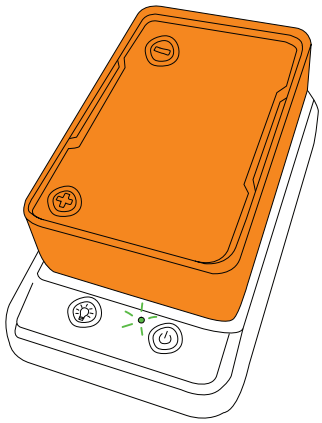
1. **Place the gel tray containing your gel in the buffer chamber**
    - Ensure that the clear buffer chamber is inside the blueGel™ electrophoresis system.
    - The wells of the gel should be on the same side as the negative electrode, away from the power button.



2. **Add 30 ml of 1X TBE electrophoresis buffer**
    - The buffer should just cover the gel and fill the wells.
    - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).

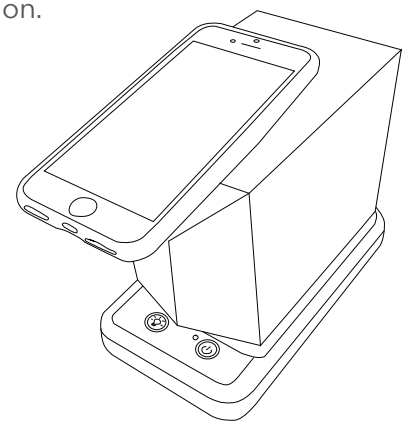
3. **Load samples onto the gel in the following sequence**
    - **Lane 1:** 10 µl Fast DNA Ladder 3
    - **Lane 2:** 15 µl of Tube A
    - **Lane 3:** 15 µl of Tube B
    - **Lane 4:** 15 µl of Tube C
    - **Lane 5:** 15 µl of Tube D

4. **Place the orange cover on the blueGel™ electrophoresis system**
    - To prevent fogging, make sure that ClearView™ spray has been evenly applied to the inside of the orange cover.
    - Match the positive and negative electrode signs on the orange lid with the corresponding positive and negative signs on the blue base.
    - The electrodes of the lid should be aligned with the metal leads on the base.
    - The orange lid should sit flush with the blue base using little force.



5. **Press the "Run" button**
    - Check that the green light beside the power button remains illuminated.

6. **Conduct electrophoresis for 20-30 minutes**
    - The colored dye should progress to about half the length of the gel.
    - Longer electrophoresis times will result in better size resolution.

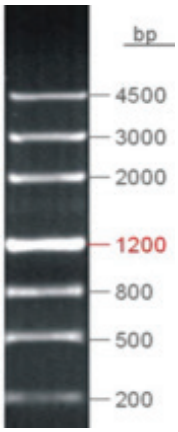


Gel electrophoresis – Visualizing results

1. **Press the "light bulb" button to turn on the blueGel™ transilluminator**
    - For best viewing, dim lights or use Fold-a-View™ photo documentation hood with a smartphone camera.
    - Gels may be viewed at the end of the run or periodically throughout the run.
    - If the image appears hazy, wipe off the inside of the orange cover and reapply ClearView™ spray.

2. **Ensure that there is sufficient DNA band resolution**
    - Run the gel longer if needed to increase resolution.

3. **Document your results**
    - Place Fold-a-View™ photo documentation hood on the blueGel™ electrophoresis system to take a picture with a smartphone or other digital camera.
    - Compare the bands from the DNA samples to the ladder to obtain size estimates.



Fast DNA Ladder 3.