Determining the Genetics of a Ca$H Cow MiniLab

Student's Guide

Cat# M3011

Version 081519

Table of Contents

Laboratory Safety

1. Wear lab coats, gloves, and eye protection as required by district protocol.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
4. Wash your hands thoroughly after handling biological materials and chemicals.

Objectives

In this MiniLab you will use gel electrophoresis to determine which cows and bull to purchase to breed to increase milk protein production in offspring to bring the farmer a higher return on his/her investment.

This will enable you to:

- Understand the basic structure of DNA and its role in genetic inheritance
- Comprehend how traits are passed from parent to offspring
- Learn about the existence of genetic polymorphisms
- Correlate genotype to phenotype
- Correctly use an adjustable volume micropipette
- Prepare, load, and run an agarose gel

Background

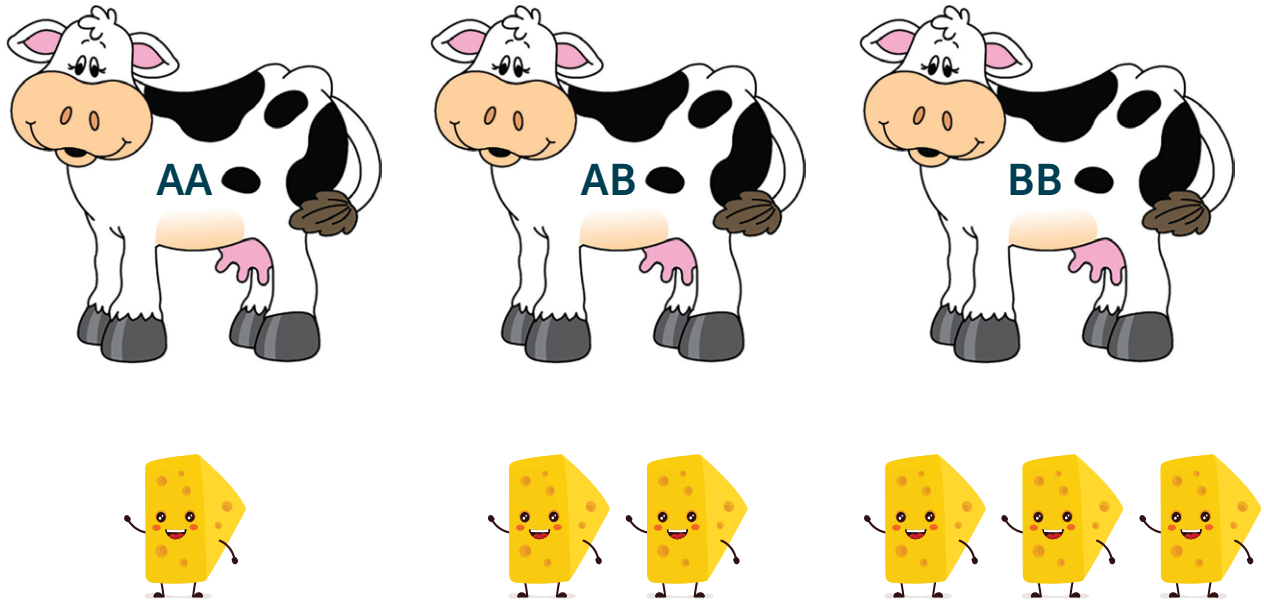
In the 1970's, Italian cheese makers were given a surprise when they discovered milk obtained from some nearby farms would not make cheese. Upon further investigation, it was found that the dairy cattle that produced the milk were sired by North American Holsteins. What was different about those cows?

In the cheese making process, the pH of milk is lowered to 4.6, which retards the growth of bacteria. Milk needs to form curds to be made into cheese, which are created when milk coagulates or precipitates.

There are 2 types of proteins in milk; whey and casein. Whey proteins remain in solution and do not cause milk to coagulate at a pH of 4.6, while casein proteins will precipitate out of milk at a pH of 4.6 because they contain phosphorus. Cheese makers want to have higher levels of caseins in milk to make better cheese. Scientists discovered that all casein proteins are not created equal – some precipitate better than others and will make even better cheese. Four forms of casein proteins have been identified in milk: alpha s1, alpha s2, beta, and kappa, and studies conducted in the early 1970s indicated that the gene that controls the production of K-casein has the greatest effect on quality and quantity of cheese production.

"The A-B-Es of Cheese"

There are 3 alleles of the K-casein gene: A, B and E. Since E allele carriers' milk does not coagulate, dairy farmers interested in selling milk to make cheese have no interest in cows with the E allele. The cattle they are interested in will have one of three genotypes: two copies of the A allele, denoted AA, or two copies of the B allele, BB (both of which are homozygous), or one copy of each, AB (heterozygous). The B allele causes a substantial increase of milk protein yield in the cows carrying it and results in a firmer and enhanced cheese. The genotype BB yields more protein than AB, which yields more than AA. Knowing this information, we can assume that the North American Holsteins which sired the dairy cattle that would not make cheese had some of those E alleles.



To ensure dairy farmers don't get the E or AA genotype as often, breeding programs have been implemented (the frequency of the K-casein B allele in dairy breeds is low [20%]). Selecting cattle with known genotypes for breeding can increase the frequency of the B allele. Implementation of such programs coupled with the desire to produce cattle with the B allele drives up the cost of cattle with that gene, so farmers looking to gain return on their investment desire cattle with the AB and BB alleles. Bull semen with that genotype may be used for artificial insemination (AI) technology because the AB or BB bull has a greater chance of passing on the gene to his offspring, making them more valuable to farmers.

Scenario

In this activity, a dairy farmer wants to take advantage of the premium pricing for milk that contains more K-casein protein that cheese-makers value. The farmer has enough money to buy one bull and two cows. The farmer also hopes to recover some of the initial cost by selling the bull's semen to other dairy farmers through an AI program.

This farmer is in North Carolina and is interested in increasing the protein content in the milk of the farm's dairy cattle to take advantage of the price premium from the cheese producers. The B allele causing an increase in milk protein that produces more of a better quality of cheese. This nets the farmer more cash.

You are to help the farmer decide in which cows to invest. The farmer can only afford to buy one bull and two cows for the herd. The farmer has located three cows in separate herds that show a history of producing milk with higher than normal protein content and two bulls whose daughters produce high protein milk. The farmer knows from experience that reading a pedigree is difficult and many times can give incomplete information about the actual genotype of the cattle for the particular trait of interest.

The farmer is ready to invest a great deal of money and wants to investigate the K-casein B gene allele in the cattle to be purchased. The farmer discovers that there is a new genetic test that can determine if the K-casein B allele is present in cattle. The farmer asks that DNA samples from the five animals being considered for purchase be sent to you for genetic analysis. Your group is assigned to do the analysis and write a report on the purchasing and breeding options for each of the five animals.

The DNA samples have been processed and turned over to your group for analysis. Assume that the farm's current stock does not have the K- casein B allele and that the cattle being tested are unrelated. A bull or cow with a genotype of AA is normal (no B allele). AB has one B allele. A genotype of BB has two B alleles. The BB genotype will produce more milk protein than the AB genotype, and the AB genotype will produce more milk protein than AA. Depending on the breed of cattle, there can be an increase of 3-12% in milk protein production when the B allele is present.

You will need to analyze the samples by separating them out using gel electrophoresis. Gel electrophoresis is a technique used in many areas of science to analyze the components of complex chemical mixtures. Mixtures of DNA, RNA, proteins, or dyes can be separated into their individual components based on molecular size and electrical charge using a separation matrix within an electric field.

The gel used in gel electrophoresis is a tangle of polymers forming a three-dimensional matrix with water-filled pores through which molecules migrate. A higher density of polymers creates smaller pores. Like the holes in a sieve or colander, the size of the pores must be the appropriate size for the molecules being separated. Gels can be made from different substances depending on the application. One of the most commonly used and effective materials is agarose, a polymer extracted from seaweed. Agarose gels are formed (or cast) by pouring molten (melted) agarose into a tray where it solidifies into the desired shape as it cools. A comb is placed while the agarose is molten and then removed after it solidifies to create wells where the samples are loaded.

After the gel solidifies it is placed in an electrically conductive buffer between parallel positive (⊕ anode) and negative (⊖ cathode) electrodes. A voltage is applied between the electrodes, creating a uniform electric field within the gel. Molecules in the wells begin to move under the influence of the electric field: positively charged molecules migrate toward the ⊖ cathode and negatively charged molecules migrate toward the ⊕ anode. DNA carries a net negative charge and will migrate toward the anode.

The speed of a molecule's movement in an electric field is determined by the strength of its electric charge relative to its molecular weight. This is quantified as the charge to mass ratio. Speed of movement within a gel is also influenced by the size of the molecule relative to the pores in the gel. The polymers in the gel are like an obstacle course: smaller molecules maneuver easily through the pores, traveling faster and farther than large, bulky molecules. However, a large molecule can move faster through a gel than a smaller molecule when the strength of its charge relative to its mass is significantly higher. Shape can also affect how a molecule moves through the gel. Long spaghetti-like molecules will move slower than compact molecules, which slip easily through the pores. Molecules of the same size, shape, and charge will move together and form a distinct band. If multiple types of molecules are present in the sample, they will separate from each other and each will form a distinct band.

Pre-Lab Questions

1. What are the two proteins found in milk?

2. Which protein is most economically important to dairy farmers that wish to see their milk to the cheese industry? Why?

3. Why is milk brought to a pH of 4.6 prior to the cheese making process?

4. What is an allele?

5. What genes are dairy farmers interested in and why?

6. Which specific alleles do farmers want their dairy cattle to carry?

7. How do genes pass from one generation to the next?

8. Why would farmers want to do genetic testing on cattle they wish to purchase?

9. Why would a farmer be interested in artificial insemination?

10. What are some characteristics or properties of DNA?

11. Which of these properties of DNA are of importance for gel electrophoresis to be effective for genetic analysis?

Part I: Electrophoresis

Materials

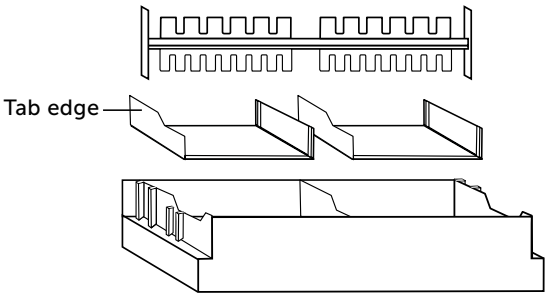
MiniOne® Casting System
1 MiniOne® Electrophoresis System
1 agarose gel cup (1.5%)
8 dye sample aliquots
135 mL of TBE running buffer, in conical flask or beaker
1 micropipette (2–20 µL) and 8 pipette tips

SAMPLE LOADING CHART:

Well	Sample Name	Volume
1	AA	10 µL
2	AB	10 µL
3	BB	10 µL
4		
5	M1	10 µL
6	M2	10 µL
7	F1	10 µL
8	F2	10 µL
9	F3	10 µL

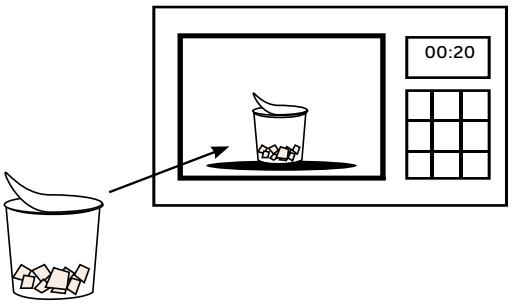
How to Cast a Gel

1. Place the MiniOne® Gel Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 9-well side facing down.



2. Partially peel the film off an agarose gel cup and microwave for 20 seconds. Allow to cool for 15 seconds. **DO NOT microwave more than 5 gel cups at a time.**

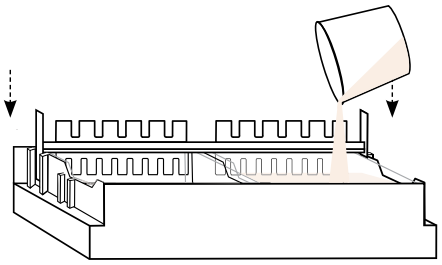
   ⚠**Safety requirement:** Adult supervision required if students are handling gel cups!



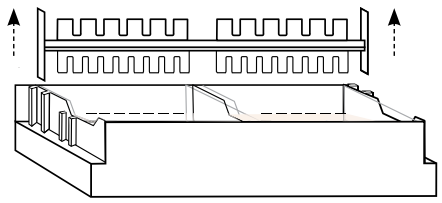
3. **One gel cup is for making one agarose gel!** Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque. **DO NOT disturb the gel until time is up.**

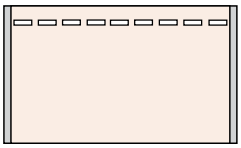
   OR

   If you are making your own gels, use approximately 11 mL of your molten agarose per gel tray.



4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from casting stand and wipe off any excess agarose from the bottom of the tray.

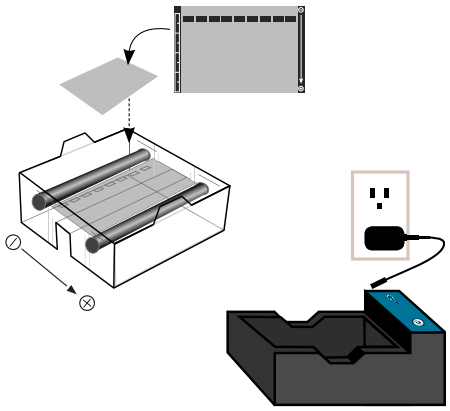




How to Load a Gel

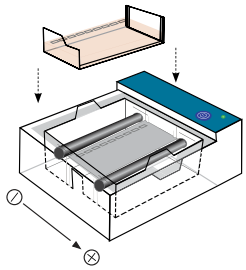
1. Ensure the **grey** viewing platform is in the tank if it is not already installed.

2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.



3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

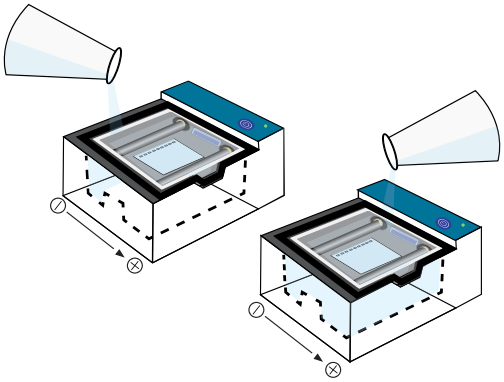
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.



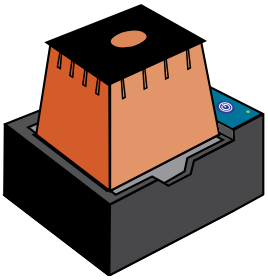
Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

5. Turn the low intensity blue LED on by pressing the button on the carriage.

6. Measure 135 mL of TBE running buffer and pour into **one side** of the tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the **other side** of the gel tank.

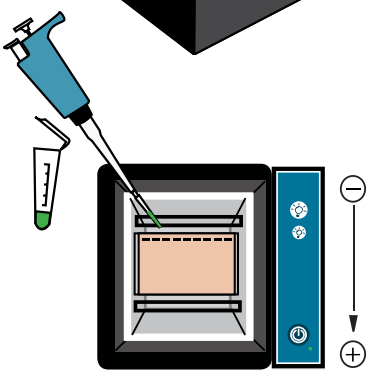


7. Place photo hood on the carriage.

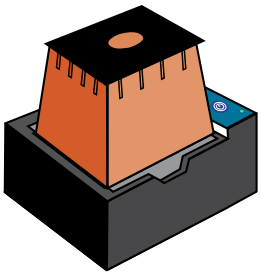


8. Press the power button which should now be a solid green light. If **green light is solid,** turn off the unit and proceed to loading gels.

9. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given on the Data Table (see Page 8).**



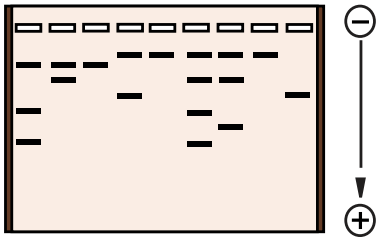
Run, Visualize and Capture Image



1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.

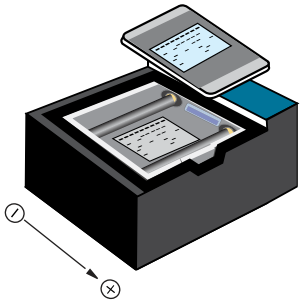
   The green power LED will not turn on if:

   - **The tank is not properly placed inside the carriage**
   - **There is no buffer in the tank**
   - **The buffer is too concentrated or too diluted**
   - **The photo hood is not on the carriage**
   - **There is too much or too little running buffer**
   - **The power supply is not plugged in. Check by turning on the blue LEDs**



2. Check the migration of the bands (~every five minutes).

3. Allow the gel to run **20 minutes** or until color dyes separation is sufficient. Keep in mind small molecules run faster so it's important to periodically check where your bands are. After your run is complete, turn off the power by pressing the button.



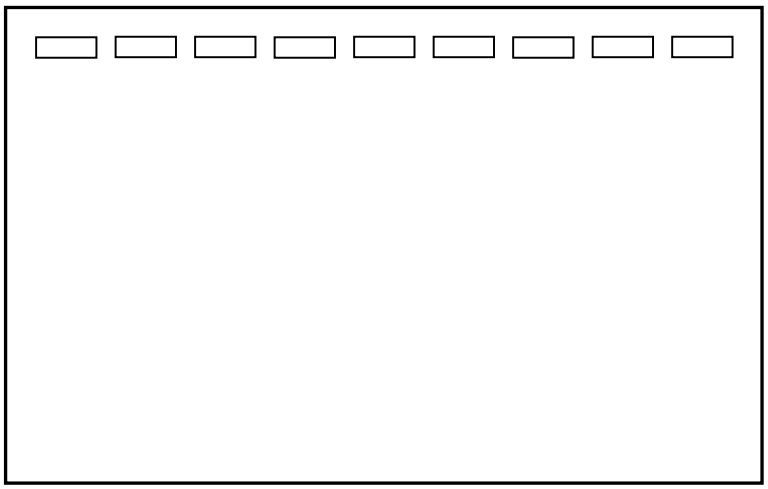
4. **Document your results.** At the end of the run, take photos following these steps: Remove the photo hood and **turn off** the blue LED light. Hold your cell phone or camera about three inches above the tank and take a picture of your gel.

Clean Up

1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne® Carriage. Remove the clear running tank from the carriage and remove the gel and tray from the running tank.
2. Pour the used running buffer down the drain or into a waste beaker. Throw the gel away. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.
3. Use a paper towel or kimwipe to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for cleanup and storage.

Part II: Results

What does your gel look like? Record images of the gel.



Lane 1: _____
Lane 2: _____
Lane 3: _____
Lane 4: _____
Lane 5: _____
Lane 6: _____
Lane 7: _____
Lane 8: _____
Lane 9: _____

Post-Lab Questions

1. What are the genotypes of each of the cattle?

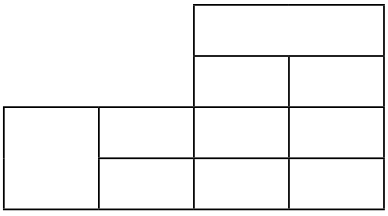
Identity	Genotype
M1	
M2	
F1	
F2	
F3	

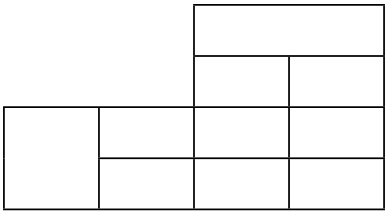
2. Which bull should the farmer purchase and why?

3. Which cow should the farmer purchase first and why?

4. What is the second cow the farmer should purchase?

5. Draw a Punnet Square of the bull/cow you would recommend to the farmer.





Appendix A - References and Recommended Reading

Zeller, Mike. "Marker Assisted Selection: Agarose Gel Analysis of the K-Casein B Allele." Office of Biotechnology. Iowa State University. Web. 3 Sept. 2015.

http://www.thebullvine.com/genetics/breeding-for-kappa-casein-to-increase-cheese-yield/

https://www.progressivedairy.com/news/industry-news/how-is-the-nations-milk-utilized

http://www.milkfacts.info/Milk%20Composition/Protein.htm

https://www.tandfonline.com/doi/full/10.1080/10942912.2016.1152480

Appendix B - Glossary

Term	Definition
Allele	One of two or more alternative forms of a gene.
Gel electrophoresis	A laboratory method used to separate mixtures of DNA according to molecular size.
Genotype	The genetic makeup of an individual, typically expressed in alphabetical letters.
Heterozygous	When an organism has two different alleles for a single gene.
Homozygous	When an organism has two of the same allele for a single gene.
Pedigree	The record of descent of an animal, showing it to be purebred.
Punnett square	A tool that shows all possible offspring genotypes in a test cross of parents with known genotypes.

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