Show Me the Moo-ney!
MiniLab
Student Guide

Cat# M3022

Version 122324

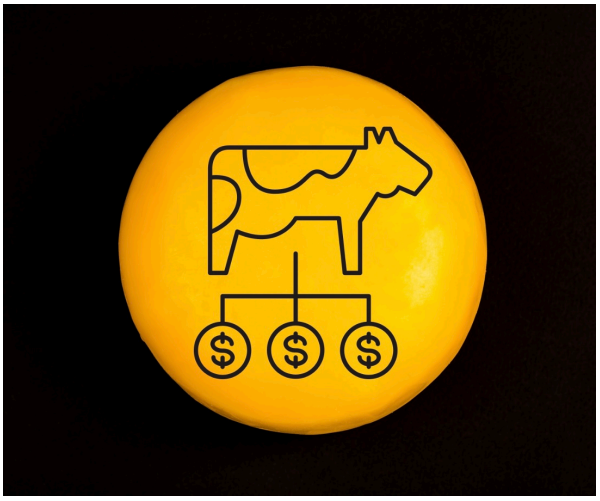


Table of Contents

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.

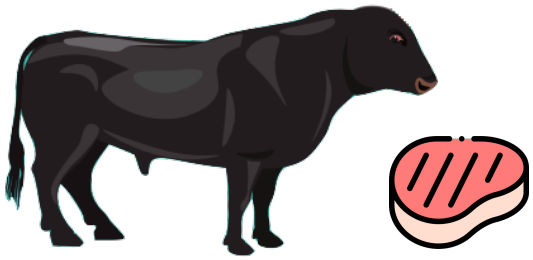
Background

In the 1970s, Italian cheese makers were puzzled when they discovered that milk obtained from some nearby farms would not make cheese. The cheese makers were puzzled and investigated the milk itself, including its color, thickness, smell, and also looked into breeds of the cows on the nearby farms. That is when they discovered that the dairy cattle that produced the non-cheese making milk were sired by North American Holsteins. What was different about those cows?

There are actually several different breeds of dairy cows and farmers decide what breed of dairy cow to purchase based on their production goals, climate and environment, feed availability and costs, health and disease resistance, market demand, breed characteristic, and the farmer's personal preference and experience. Depending on the goals and market demands, the farmer selects the cattle breeds that are most suitable for their production. The milk a farmer produces has many different uses, including milk for drinking, cheese, butter, yogurt, whey-protein-infused fitness beverages, and many other dairy foods. According to the Department of Agriculture (USDA), the per capita consumption of dairy products reached 661 pounds per person in 2023, an increase of 7 pounds per person over the previous year and matching the all-time record set in 2021 (U.S. Department of Agriculture, 2024). The International Dairy Foods Association (IDFA) found that butter and cheese consumption each surpassed all previous records, reaching all-time highs of 6.5 pounds (butter) and 42.3 pounds (cheese) per person, respectively, in 2023 (International Dairy Foods Association, 2023)

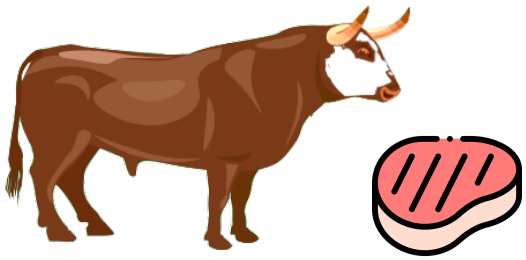
If a farmer aims to maximize milk yield per cow, they might prefer breeds like Holsteins or Holstein crosses, which are known for their high milk production. If they prioritize milk quality or specific cheese characteristics, they might opt for breeds like Jerseys or Guernseys known for their rich and flavorful milk. Furthermore, certain breeds may be better suited to specific climates or environmental conditions. For example, some breeds are more heat-tolerant or cold-hardy than others, so farmers in different regions may choose breeds that are well-adapted to their local climate. Some breeds may also have genetic traits that make them more resistant to certain diseases or health issues prevalent in the region. Farmers may also prioritize breeds known for their overall health and resilience to common ailments.

Additionally, if there's a high demand for certain types of cheese or dairy products associated with a particular breed, farmers may choose to raise that breed to cater to market preferences and potentially fetch higher prices. Each breed has its own unique characteristics beyond milk production, such as temperament, ease of management, and longevity. Farmers may consider these factors when choosing a breed that aligns with their management style and farm objectives. Finally, a farmers' personal preferences and experiences with specific breeds can also play a significant role. Some farmers may have experience with a particular breed and prefer to stick with what they know works well for their operation.



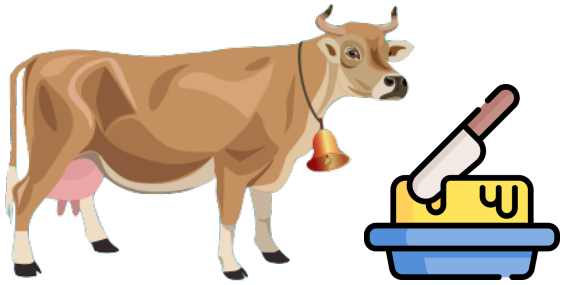
Angus

Known as Aberdeen Angus around the world the breed arose in Scotland. Solid black or red, Angus is valued for adaptability, health and marbled meat



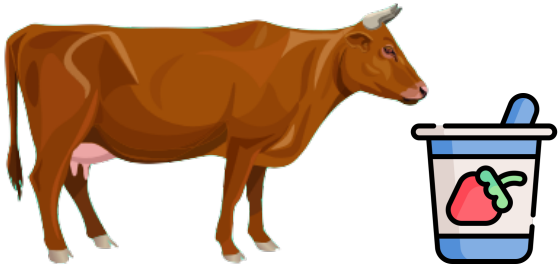
Hereford

From England, Hereford is an ancient breed. Red in color, they are fast-growing cattle with good beef quality



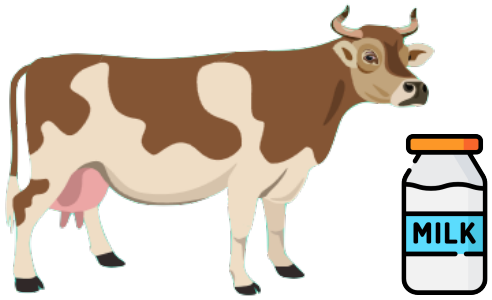
Jersey

From an island in the British Channel, Jerseys are smaller than Holsteins and produce denser milk that is high in butterfat.



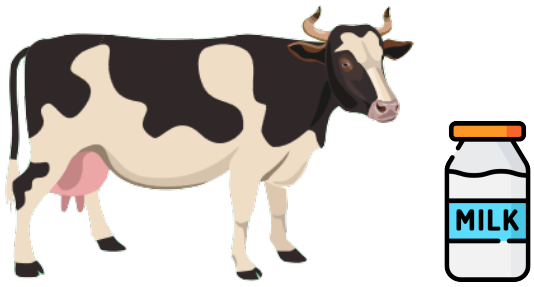
Red Poll

The milk from the Red Poll cow is quite good quality according to most owners of the breed. They yield an average of 5,000 liters per year, with butterfat content of 4.2% and protein level of 3.5%



Guernsey

The Guernsey cow is often referred to as the "Royal Breed" because their milk is almost golden.



Holstein

Recognizable by their black and white markings, Holsteins are known as a dairy cow and have the world's highest milk product.

Fig 1 - Cattle Breeds - Modified from Image: https://www.mydraw.com/templates-infographics-cattle-breeds-infographic

The Chemistry of Milk

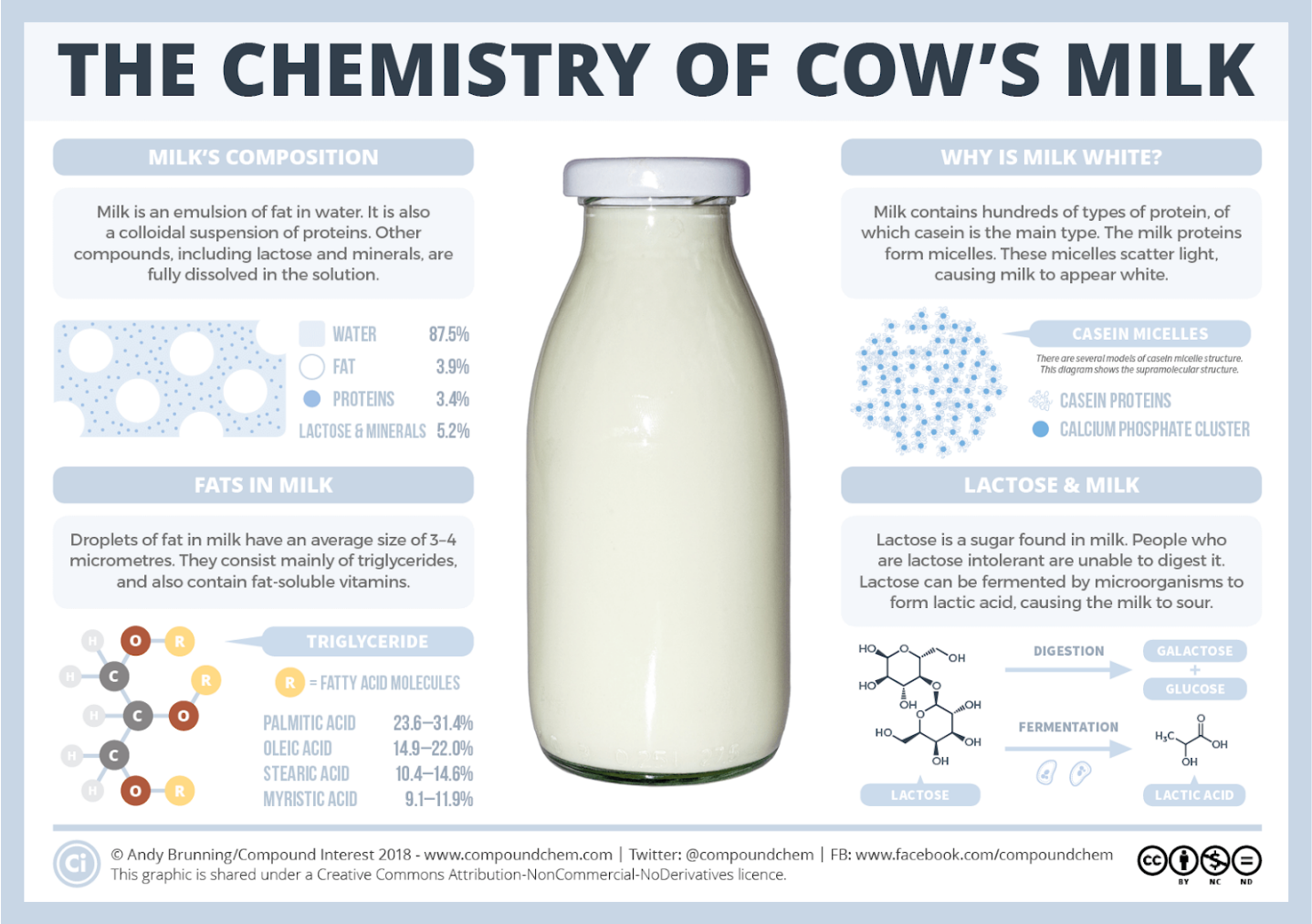


Fig 2 - Milk is primarily composed of water, mixed with a variety of proteins, fats and lactose plus minerals.

Proteins in Milk

There are 2 types of proteins in milk; whey (alpha-lactalbumin and beta-lactoglobulin) and casein. Whey makes up about 20% of the protein in cow's milk, while casein proteins, which are the primary proteins found in milk, make up the remaining 80%. When milk is processed to make cheese or yogurt, the liquid that separates from the curds is whey, which contains the whey proteins. Whey protein is a rich source of essential amino acids, like leucine, isoleucine, and valine, which are crucial for muscle repair and growth.

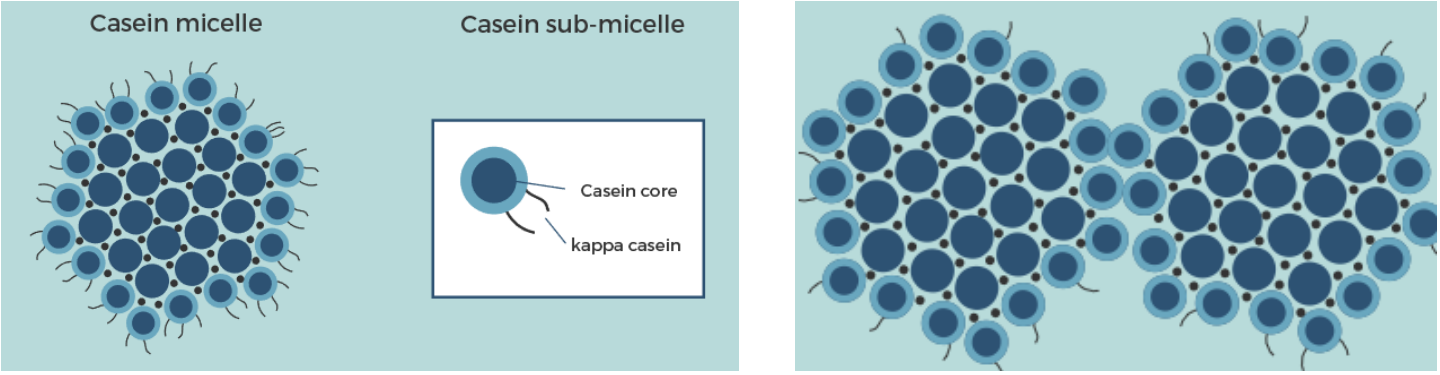


Figure 3 - Left image - casein micelle and sub-micelle. Right image - casein micelles that have coagulated or "clotted"
Image courtesy of https://www.cheesescience.org/milk.html

Casein proteins are unique because of their structure and how they interact with calcium and other minerals, making them vital for bone health and an excellent dietary protein source. Casein is not a single protein but a group of related phosphoproteins that include alpha casein (α-casein), beta casein (β-casein), and kappa casein (k-casein). Casein proteins in milk exist in the form of micelles, which are tiny spherical structures composed of individual casein molecules bound together. These micelles give milk its white appearance and help keep the protein suspended in liquid form. Casein molecules contain both hydrophilic (water-attracting) and hydrophobic (water-repelling) regions within their structure which allows the casein micelles to interact with water molecules while also repelling them, maintaining the stability of the micelles in milk.

How is cheese made?

The cheese making process starts with heating the milk in order to kill any microorganisms that may be present that could cause the milk to spoil, in a process known as pasteurization. After the milk has been heat-treated, it is then cooled and bacterial cultures are added to the milk to begin the fermentation process. These bacterial cultures are specifically selected for their ability to ferment lactose and produce desirable flavors and textures in cheese. The fermentation process lowers the pH of the milk by converting lactose (milk sugar) into lactic acid. When the pH of the milk decreases, the casein proteins undergo a conformational change and aggregate (clump) together, forming a network that traps fat, protein, calcium and water molecules. The fermentation and aging process then starts to develop the flavor and texture of the cheese by breaking down proteins and lipids in the milk. Different strains of ripening bacteria are used depending on the type of cheese being produced. Some examples include Propionibacterium, used to make Swiss and Emmental cheeses, and Penicillium molds used to make blue cheeses.

The lowered pH of the milk is crucial because it helps create a more conducive environment for the coagulation of casein and the formation of solid curds. To further speed up the coagulation process, rennet is added to the milk. Rennet is a complex mixture of enzymes, primarily

chymosin (also known as rennin). In nature, chymosin is primarily found in the stomachs of ruminant animals, such as calves, lambs, and goats, where it helps in the digestion of their mother's milk. Chymosin's main role is to coagulate milk, allowing the animal to more effectively digest the milk proteins. By curdling the milk, chymosin slows down the passage of milk through the stomach, allowing for better absorption of nutrients.

In cheesemaking, chymosin is used to coagulate the milk and separate it into solid curds and liquid whey, a critical step in cheese production. The liquid portion of the milk, called whey, contains the water-soluble components, such as whey proteins, lactose, and soluble minerals. After the curds form, the whey is drained off, leaving the solid curds to be further processed into cheese. The curds can then be cut, cooked, and pressed to expel more whey, resulting in a firmer cheese of a desired texture.

Figure 4 - Curds and Whey Image credit: https://www.wikihow.com/Make-Curds-and-Whey

Cheese makers require higher levels of casein in milk to make better quality cheese. Scientists discovered that not all casein proteins are created equal – some coagulate better than others and will make even firmer cheese. Four forms of casein proteins have been identified in cattle milk: Alpha-S1 (αS1-casein), Alpha-S2 (αS2-casein), beta (β-casein), and kappa (k-casein). Studies conducted in the early 1970s indicated that the expression of the k-casein gene has the greatest effect on quality and quantity of cheese production.

Kappa casein protein is coded for by the CSN3 gene, which has been mapped on chromosome 6 (6q31) in cattle. The k-casein gene comprises a 13 kb sequence divided into 5 exons. To date, nine alleles of the k-casein gene (CSN3) have been identified in cattle populations (A, B, C, E, F, G, H, I, and J). These alleles can have different effects on milk yield, milk protein content, and cheese-making properties, making them of interest in dairy cattle breeding programs. The 3 main alleles (different variations of a gene) of the k-casein gene are: A, B and E.

Variants	Amino acid positions						
	10	97	104	135	136	148	155
A	Arg	Arg	Ser	Thr	Thr	Asp	Ser
B					Ile	Ala	
C		His			Ile	Ala	
E							Gly
F	His						
G		Cys					
H				Ile			
I			Ala				
J					Ile	Ala	Arg

Bovine kappa casein variants.

Figure 5 - showing Bovine kappa casein variants. (Yahyaoui, M, 2001)

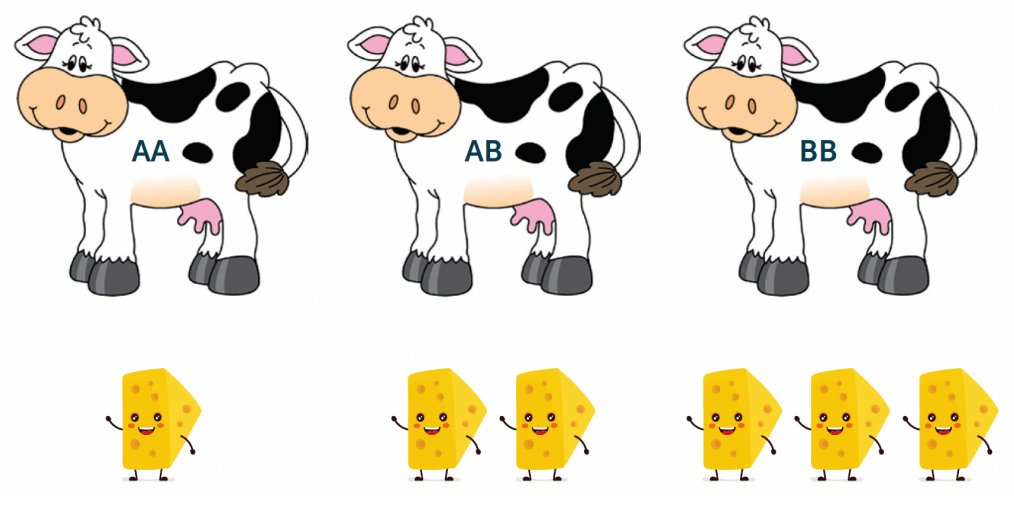
As mentioned above, the k-casein gene is located on an autosome, meaning it is not sex-linked. Therefore, both male and female cattle can inherit and pass on the gene equally. The alleles A, B, and E exhibit co-dominance. This means that when an animal inherits two different alleles (for example, A and B), both alleles are expressed in the phenotype. The milk from an animal with an AB genotype will have characteristics influenced by both the A and B alleles. Similarly, an animal with an AE or BE genotype will express traits associated with both alleles.

Researchers found that milk from cattle that carry the EE genotype of the k-casein gene does not coagulate, and thus dairy farmers interested in selling milk to make cheese have no interest in cows with the E allele. The A allele is often considered the “standard” or wild-type variant of the k-casein gene. The B allele is associated with improved cheese-making properties because it tends to produce milk with better curdling characteristics and higher protein content.

The A and B alleles are the result of two point mutations found in exon 4 of the k-casein (CSN3) gene, amino acid positions 136 and 148 (see figure 5 above) (Yahyaoui, M, 2001). The A allele has amino acids Thr (ACC) and Asp (GAT) at positions 136 and 148, respectively, while the B allele has

amino acids Ile (ATC) and Ala (GCT) at the same positions. Cattle that carry the B allele produce milk with higher levels of k-casein, leading to a substantial increase of milk protein yield and shorter coagulation time, which results in cheese with improved texture and firmness (Yahyaoui, M, 2001).

The dairy farmers that supply milk to cheese makers are interested in cattle that carry either the A or B variants of the CSN3 gene. Therefore, the cattle that the farmers are interested in will have one of three genotypes: AA, AB or BB. The genotype BB yields more protein than AB, which yields more than AA. The cheese yield from cows with genotype BB is 10% higher when compared with AA cows.



Breeding programs have been implemented that allow farmers to select for preferred genotypes to increase the frequency of the B allele. The frequency of the B allele for k-casein in various breeds of cattle varies, with about 10% of North American Holsteins with the BB genotype, and North American Jerseys possessing a significantly higher percentage, at approximately 20% (The Bullvine, 2017). 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 a return on their investment desire cattle with the AB and BB alleles. Bull semen with that genotype may be used for artificial insemination 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.

K-Casein Protein Structure

K-casein is a glycoprotein that plays a role in stabilizing micelles. The k-casein protein consists of two main parts, the hydrophobic N-terminal domain and the hydrophilic C-terminal domain. The hydrophobic N-terminal domain interacts with the other casein proteins (α and β) within the micelle while the hydrophilic C-terminal domain extends outward from the micelle and helps maintain the solubility of the micelle by attracting water molecules, thus preventing aggregation, as no one wants to drink lumpy milk!

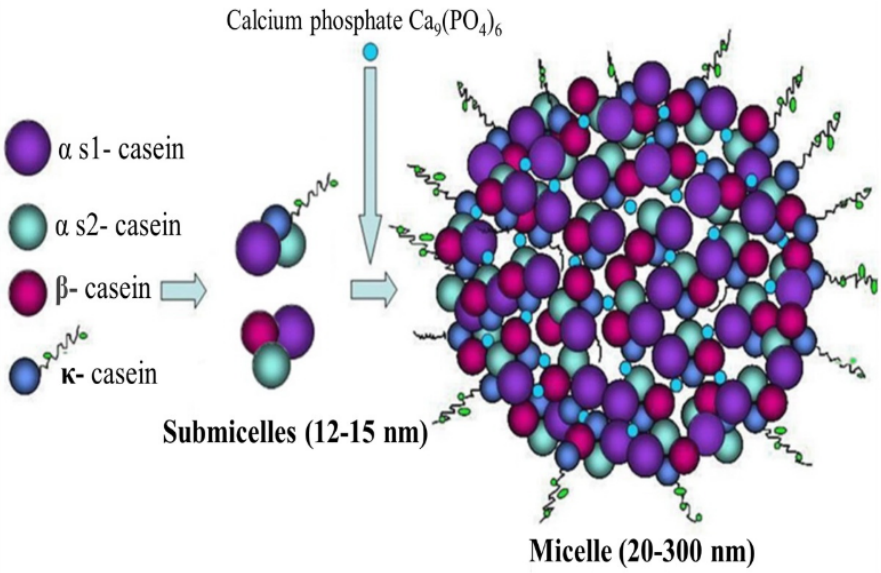


Figure 6 - Schematic representation of the structure of casein submicelles and casein micelles, composed of submicelles held together by calcium phosphate. (Petrova, 2022)
Image credit: https://romj.org/2022-0209

In the cheesemaking process, the enzyme chymosin (or rennin) is added to the milk to promote coagulation. Remember that the casein proteins in milk form structures known as micelles and k-casein prevents the micelles from clumping together, keeping the milk in a liquid state. Chymosin specifically targets and cleaves the k-casein at a particular peptide bond, breaking the k-casein into two fragments: para-kappa-casein (which remains with the micelle) and glycomacropeptide (which becomes soluble and is released into the liquid whey). The cleavage of k-casein by chymosin reduces the micelle's stability, leading to curd formation (milk coagulation), which is essential in cheese-making.

As mentioned previously, the A and B alleles of the k-casein gene are the result of two point mutations at locations 136 and 148 on exon 4 of the CSN3 gene (Yahyaoui, M, 2001). What is the effect of each of these amino acid changes on the k-casein protein? In position 136, threonine in the A allele has been replaced with isoleucine in the B allele. Threonine is a polar amino acid, meaning it can interact with water molecules, while isoleucine is hydrophobic (water-repelling). This change makes the B variant slightly less hydrophilic in this region, which reduces its solubility in the milk serum and the way it interacts with other casein molecules. Meanwhile, in position 148, aspartic Acid in the A allele has been replaced with alanine in the B allele. Aspartic acid is negatively charged and polar, while alanine is non-polar and hydrophobic. This substitution reduces the overall negative charge in the B variant of k-casein, making the protein less repulsive to other proteins in the micelle. This change can lead to closer packing of the micelles and more rapid aggregation when the protein is cleaved by chymosin.

Therefore, the A allele produces a k-casein protein that is more hydrophilic and has slightly more electrostatic repulsion between casein micelles. This leads to slower curd formation because the micelles are more stabilized in solution. This is why dairy breeding programs often select for the B allele to enhance cheese-making properties of milk, leading to better yields and higher quality cheeses.

Pre-Lab Questions

1. What are some factors that influence a farmer's decision on what breed of dairy cow to purchase?

2. What is pasteurization?

3. What two ingredients are added to milk in the cheesemaking process?

4. What are the two types of proteins found in milk?

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

6. What are the four forms of casein protein?

7. What is an allele?

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

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

10. What is the difference between the A and B alleles?

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

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

13. Knowing what you now know about the k-casein gene, what would you predict is the genotype of the North American Holsteins which sired the dairy cattle that would not make cheese?

Scenario

A farmer in North Carolina is interested in increasing the protein content in the milk of the dairy cattle on their farm in order to take advantage of the price premium paid by cheese producers. The farmer would like to increase the number of cattle carrying the B allele that has shown an increase in milk protein that produces higher quality cheese which nets more profits.

Your job is to help the farmer decide which cows to invest in in order to increase his profit margin. 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 content in 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. Furthermore, conventional methods for selective breeding are time-consuming and expensive.

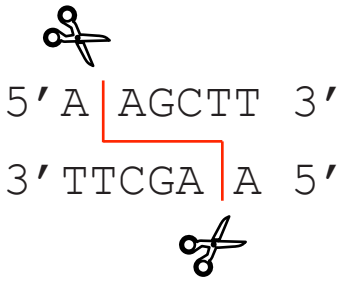
The farmer is ready to invest a great deal of money and wants to investigate the genotypes of the k-casein gene in the cattle to be purchased. A new genetic test that can determine the genotype of the k-casein gene is available in cattle and the farmer is excited about the possibility of knowing the milk production traits of the cattle even before phenotypes are expressed and independent of age and gender.

The farmer asks that DNA samples from the five animals being considered for purchase be sent to you for genetic analysis. Your laboratory is assigned to do the analysis and write a report on the purchasing and breeding options for each of the five animals.

The farmer obtained hair samples from the cattle by gently pulling the hairs from the neck or tail switches of the cattle, and submitted them to your lab for processing. Upon receiving the hair samples at the lab, your group extracted DNA from the hair samples using alkaline lysis protocol, which disrupts the cell and nuclear membranes to release the nuclear DNA into the solution. The DNA samples are then purified and prepared to be amplified in a polymerase chain reaction (PCR). (See Appendix B).

Briefly, in the PCR reaction, millions of copies of DNA are made of a portion of the k-casein gene. A PCR reaction is made up of a combination of the extracted DNA, forward and reverse primers that are specific for the portion of DNA of interest (in this case, the k-casein gene), PCR master mix containing free nucleotides and buffer, and Taq polymerase.The Taq polymerase is a DNA polymerase that was isolated from a species of bacteria that thrives in the higher temperatures, such as those found in the hot springs at Yellowstone National Park, called *Thermus aquaticus*. In this scenario the following primers were used to amplify a 935 bp fragment of the k-casein gene Forward 5'-AGCGCTGTGAGAAAGATG-3' and Reverse 5'- GTGCAACAACACTGGTAT-3'. This PCR reaction is carried out in a thermal cycler which has the ability to cycle through various temperatures required for the PCR reaction to take place.

The resulting PCR product is then digested using HindIII restriction enzyme (obtained from the bacteria *Haemophilus influenzae*), which recognizes and cuts the sequence A^AGCTT.



In this process of restriction fragment length polymorphism (RFLP), the B allele which has an amino acid alanine (GCT) at position 148 will be cleaved (or cut) by the HindIII restriction enzyme to produce two smaller fragments at 520 bp and 415 bp. Meanwhile, in the A allele, position 148 codes of the amino acid aspartic acid (GAT), and does not contain the recognition site for HindIII, and thus remains intact and uncut.

Therefore, to easily visualize the results of the RFLP experiment, scientists run an agarose gel electrophoresis of the digested PCR product. If a single band is visible that is approximately 935 bp, it can be concluded that the cattle has the genotype AA. If two bands are seen on the agarose gel, one at 520 bp and another at 415 bp, it can be concluded that the cattle has the genotype BB. And if there are three bands on the agarose gel that are 935 bp, 520 bp, and 415 bp, it can then be concluded that the cattle is heterozygous for both the A and B allele, making its genotype AB.

Genotype	Fragment Length(s) after HindIII digestion (bp)
AA	935
AB	935 + 520 + 415
BB	520 + 415

In this MiniLab, you have been provided the PCR products of 5 cattle that have undergone the PCR and RFLP process. Your role is to determine the genotypes of the cattle (2 males and 3 females) and make a recommendation to the farmer on which cattle to purchase in order to increase the frequency of the B allele within his dairy cattle.

Assume that the farmer's current stock does not have the k-casein B allele and that the cattle being tested are unrelated. Remember that a bull or cow that has the BB genotype will produce more milk protein than the AB genotype, and the AB genotype will produce more milk protein than the AA genotype.

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. (See Appendix A)

Part I: Electrophoresis

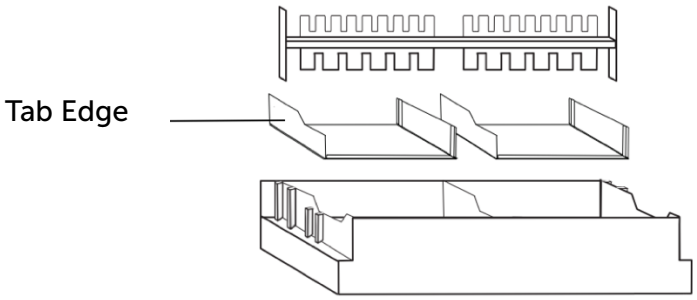
Materials

1 Minione® Casting System
1 MiniOne® Electrophoresis System
1 agarose GreenGel™ cup (1 %)
6 DNA sample aliquots
135 mL of running buffer
1 micropipette (2-20µL)
6 pipette tips

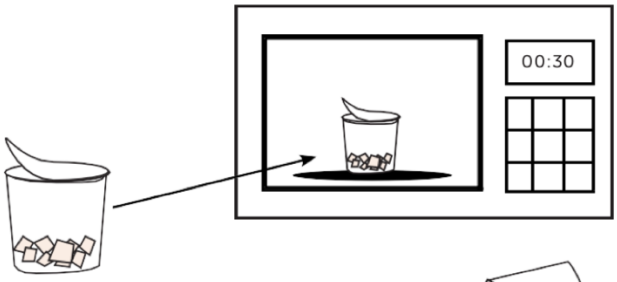
Lane #	Sample Name	Volume
1	MiniOne® Marker (2000, 1000, 500, 300, 100 bp)	10 µL
2	M1 - Bull 1	10 µL
3	M2 - Bull 2	10 µL
4	F1 - Cow 1	10 µL
5	F2 - Cow 2	10 µL
6	F3 - Cow 3	10 µL

How to Cast a Gel

1. Place the MiniOne® 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 6-well side facing down.

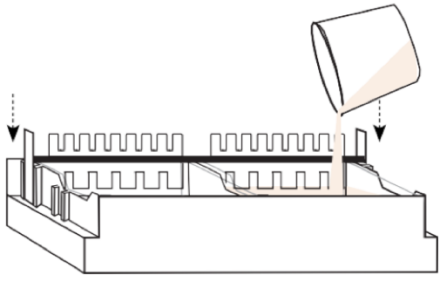


2. **Partially** peel the film off a GreenGel™ cup and microwave for 25-30 seconds. Allow to cool for 15 seconds. DO NOT microwave more than 5 gel cups at a time.



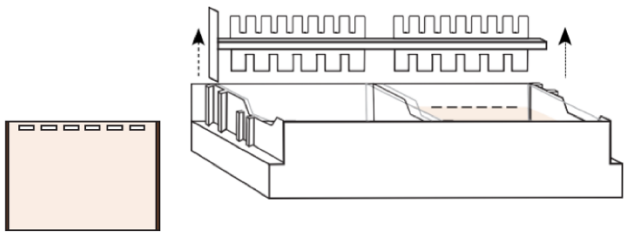
Safety requirement: Adult supervision required if students are handling gel cups!

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.



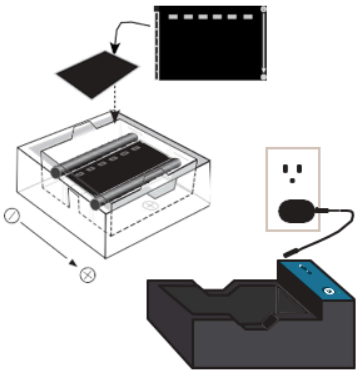
DO NOT disturb the gel until time is up.

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

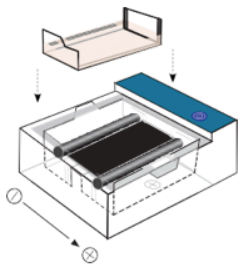
1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



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

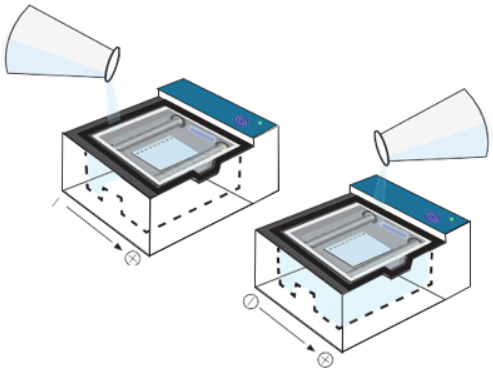
3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

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.



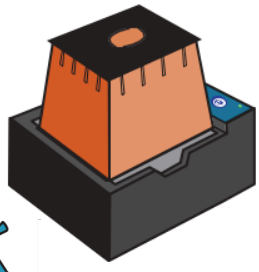
Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

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



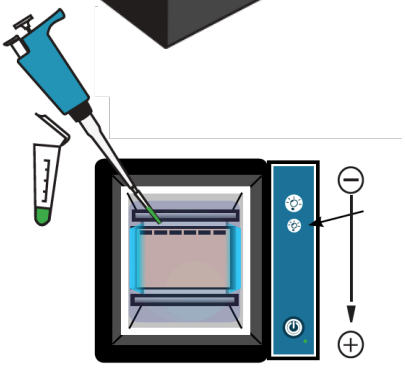
6. Measure 135 mL of TAE running buffer and pour into **one side** of the gel tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the **other side of the gel tank.**

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.

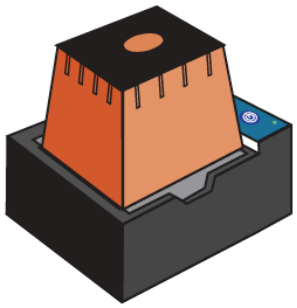


10. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

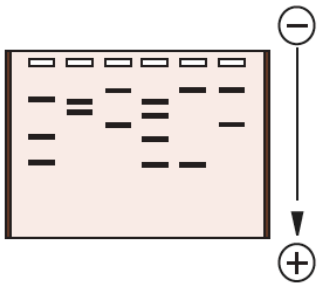
Run, Visualize and Capture Image

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 diluted.**
   - **The photo hood is not on the carriage.**
   - **There is too little running buffer.**
   - **The power supply is not plugged in. Check by turning on the blue LEDs.**
   - **If the green power LED is blinking, please refer to the troubleshooting steps in the MiniOne Electrophoresis Instruction Manual**



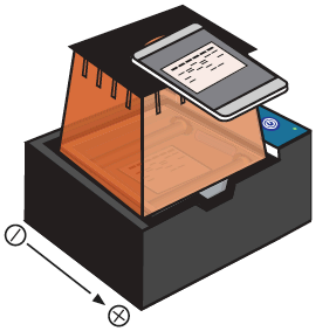
2. Have students periodically check the migration of the bands (~every five minutes).



3. Allow the gel to run **25 minutes** or until DNA separation is sufficient. Keep in mind small DNA samples 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. Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.

4. Document your results.

   Wipe off the condensation from the inside of the hood with a soft cloth if necessary, then place the hood back on the carriage. **Turn on** the high intensity light. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. **DO NOT** zoom in as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device.)



5. Clean up. Follow teacher's instructions on disposal and clean up.

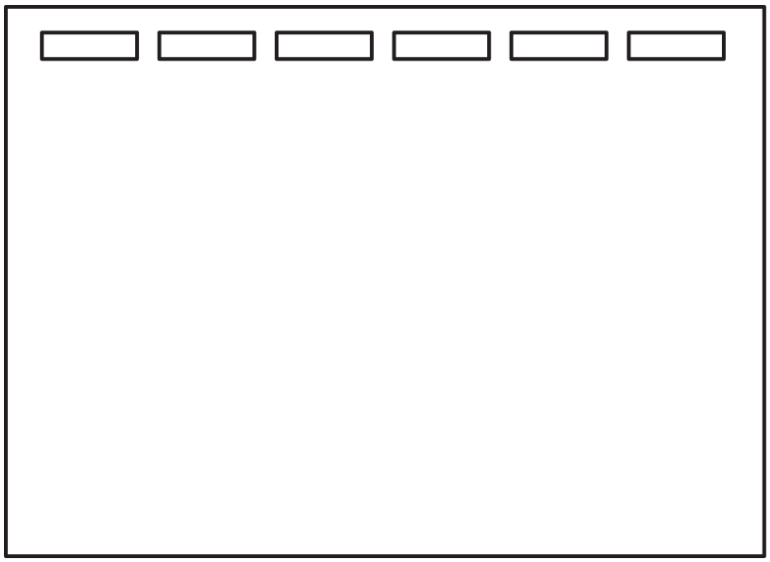
Clean Up

Note: All reagents in this lab can be disposed of as non-hazardous waste.

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 AND SAVE THE GEL TRAYS. 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 clean up and storage.

Part II: Results

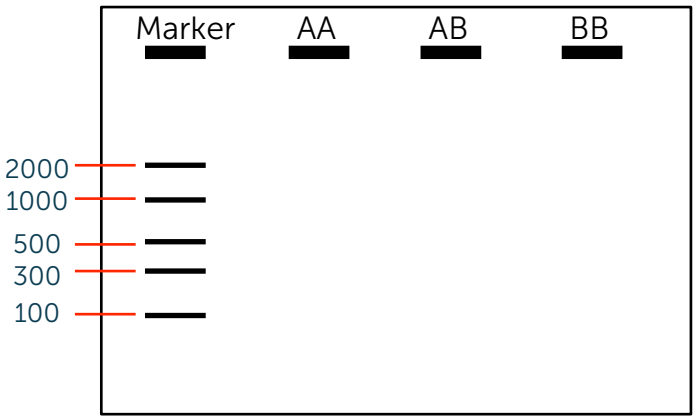
What does your gel look like? Record images of the gel in the gel below along with the resulting fragment sizes.



Lane 1: _____
Lane 2: _____
Lane 3: _____
Lane 4: _____
Lane 5: _____
Lane 6: _____

Post-Lab Questions

1. Predict what the bands on an agarose gel would look like after gel electrophoresis for each genotype, AA, BB, and AB.



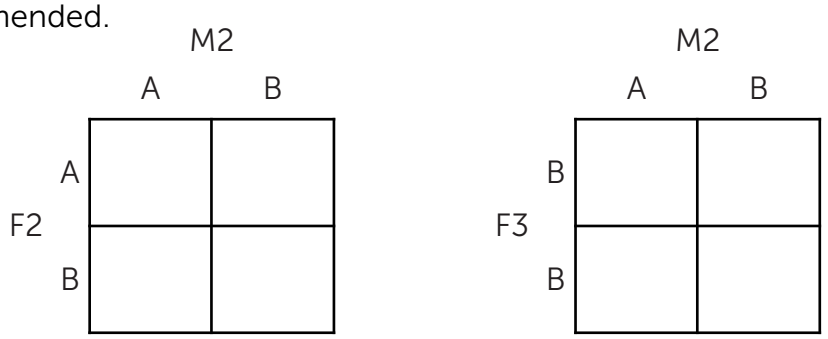
2. What are the genotypes of each of the cattle?

3. Which bull should the farmer purchase and why?

4. Which cows should the farmer purchase first and why?

5. Why would you not advise the farmer to purchase the F1 cow?

6. Draw a Punnett Square to demonstrate to the farmer the results of mating the bull with the cows you recommended.



7. If the farmer found out that he could only purchase one bull and one cow, which cow would you suggest that the farmer purchase to achieve the highest return on investment and why?

8. What characteristics would the milk of the offspring of M1 and F1 have?

9. Should the farmer breed the first generation offspring to the original bull and cows that he purchased? Why or why not?

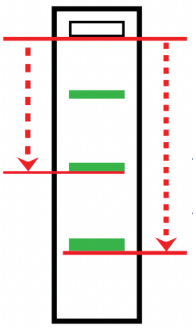
Extension Activity - Calculate the DNA Fragment Sizes

You examined the patterns in your gel to predict the genotypes of the bulls and cow. In your background reading you learned the size of the PCR product of the κ-casein gene is 935 bp. That product was then incubated with the restriction enzyme HindIII. There are 2 alleles of this gene. The B-allele has the restriction site HindIII recognizes. The PCR product of that allele was cut into 2 pieces, 520 bp and 415 bp. The A-allele is not cut by the restriction enzyme and will be 935 bp. You need to verify the sizes of the bands in your gel to provide evidence to support your conclusions about your prediction. In order to provide evidence to support your reasoning you will need to construct a standard curve using your Molecular Marker. We know the sizes of the bands of the MiniOne® Marker. The band closest to the well is 2,000 bp, followed by 1,000, 500, 300, and 100. To construct your standard curve:

1. Open the image you took of your gel.

2. Use the ruler along the side of the gel to measure the distance of the known bands in the MiniOne® Marker in Lane 1.

3. Measure from the positive edge of the well to the positive edge of the band.



Measure from the positive edge of the wall to the positive edge of the band.

4. Record the known fragment sizes in basepairs (bp) and the distance the band traveled, measured in centimeters (cm) in the data table below.

Size (bp)	Migration Distance (cm)

Now consider the sample data below:

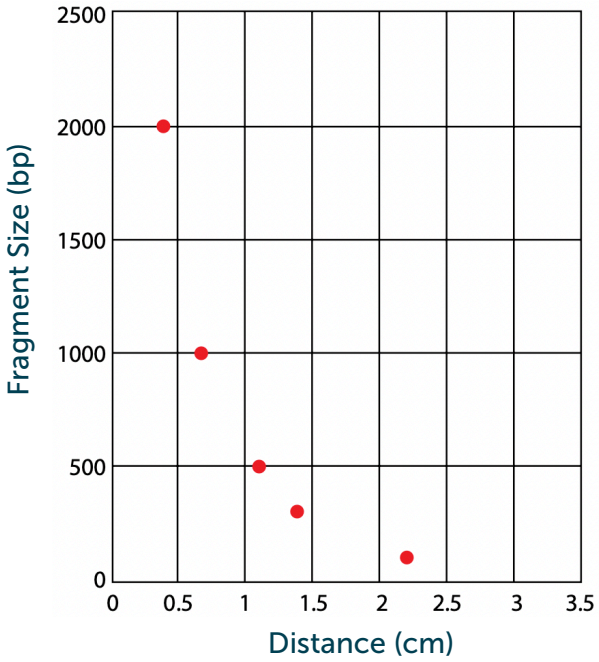
Sample Data

Size (bp)	Migration Distance (cm)
2000	0.4
1000	0.7
500	1.15
300	1.4
100	2.2

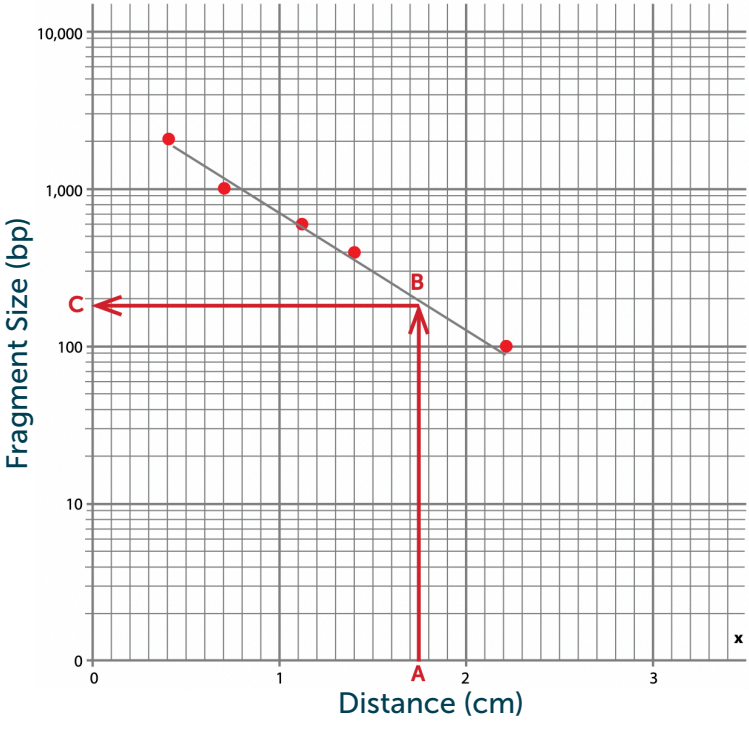
5. You will use Log Y graph paper to construct your standard curve. Why?

On Log Y graph paper, the X-axis is linear and the Y-axis is logarithmic. If you plot the data on linear graph paper for both the X- and Y-axis your graph would look something like this:

Sample Graph:



Predicting unknown band sizes would be difficult using this graph. When you see a pattern of data with that shape on linear graph paper it may be a hint it follows a logarithmic relationship. You can transform the data using Log Y graph paper. The transformation occurs because the ticks on the log axis are not evenly spaced out. They get closer together to represent the logarithm of the numbers (notice spacing for the Y-axis).



6. Use Log-Y paper to graph your data. Use the graph to estimate the molecular sizes of the unknowns. Move along the x-axis to find the distance your unknown travelled (point A). Draw a line vertical from point A to where it intersects the standard curve (point B). Draw a line horizontal from point B to where it intersects the y-axis (point C). This is the estimated fragment size for your unknown. Record the size in the "Estimated Size" Column.

Sample	Distance migrated (cm)	Estimated Size (bp)
M1		
M2		
F1		
F2		
F3		

7. Provide a summary paragraph outlining your prediction (claim), how the data you collected from your gel supports your claim, and how calculations from your standard curve provides evidence for your reasoning.

Appendix A - What is 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 has to 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.

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 or sizes of molecules are present in the sample, they will separate from each other and each will form a distinct band.

Appendix B - Polymerase Chain Reaction

Polymerase chain reaction (PCR) addresses two major challenges in molecular biology producing usable quantities of DNA for analysis and isolating a specific region of the genome. First, the DNA that scientists want to analyze is often collected in extremely small quantities (for example, a single drop of blood from a crime scene). To have usable quantities of DNA we must make many copies using the original DNA as a template. Second, among the whole genome we must find only the part we want to analyze, whether it is a gene that causes a disease or a sequence that can help identify a species. This is no small matter since the human genome is over 3 billion base pairs (bp) long and we are often interested in regions less than 300 bp long.

The History of PCR

As with many ideas in biotechnology, nature provides us with most of the tools we need. Every time a cell divides it makes two replicates of its entire genome with the help of specialized enzymes. The phenomenon of complementary base pairing should give you a hint as to how a specific region can be targeted. In the late 1970s Frederick Sanger developed a method for copying DNA in vitro that used short pieces of DNA, called primers to initiate replication by a DNA polymerase, similar to the RNA primers in your model of cellular DNA replication. An artificial primer used in the Sanger method has a sequence that allows it to bind at only one location in the target DNA sequence.

Using only one DNA primer, the Sanger method can only produce one copy of the target at a time. In 1983 Kary Mullis proposed a modification to this method where a second primer is used to initiate replication using the first copy as a template. Recall that DNA has an antiparallel structure where one strand runs in the opposite direction relative to the second strand. To use the first copy as a target for replication, the second primer that Mullis added needed to initiate replication in the opposite direction, thus one primer is called the forward primer and the other is called the reverse. With the original sequence and its copy serving as templates, two copies are produced instead of one. Further, these two copies can each be used as templates in a second round of copying, which produces four copies. After each round, or cycle, the number of copies has doubled. Over multiple cycles, billions of copies of the DNA sequence between the two primers are generated. This method is called Polymerase Chain Reaction (PCR) – *polymerase* because of the enzyme that is used to copy DNA and *chain reaction* because the products of one cycle serve as templates for the next round of copying. This rapid and efficient technology for generating usable quantities of DNA won Mullis the Nobel Prize in Chemistry. The first application of PCR was a test for sickle cell anemia.

How PCR Works

Instead of trying to recreate the cell's intricate biochemical machinery in a test tube, scientists rely on heat to control the various steps of the PCR reaction. For DNA to be copied the nucleotide bases must be exposed, which means double stranded DNA must be separated into single strands. Just as heat applied to an ice cube weakens the hydrogen bonds between water molecules and causes a phase transition to liquid water, heat applied to double-stranded DNA weakens hydrogen bonds between bases causing separation of the strands into single-stranded

DNA. As with ice, this is sometimes called melting, but is commonly referred to as denaturation. In a PCR cycle, denaturation is performed at 90-98°C for 5-30 seconds. This temperature is just below the boiling point of water.

Once the template DNA has been separated into single strands, the temperature is lowered to encourage primers to bind to their target sequences. Just as in the water analogy, lower temperature favors the formation of hydrogen bonds between molecules, in this case the primer and the template DNA. This step, called annealing, is typically between 45 and 65°C for 5-30 seconds. The ideal temperature and duration of the annealing step depends on the sequences of the primers being used. It must be low enough that hydrogen bonds are able to form between the primer and specific complementary sequence, but not so low that nonspecific, or random, binding between primer and template occurs.

DNA polymerase binds to the primer-template complex and begins to add nucleotides (dNTPs) onto the 3' end of the primer. This step, called extension, results in a new copy attached to the template as double-stranded DNA. The duration of the extension step depends on the length of the DNA segment being copied and can be anywhere from 5 seconds to 5 minutes.

At this point you may have noticed a problem – DNA polymerase, which the entire PCR process relies on, is a protein enzyme that needs a very specific three-dimensional structure to copy DNA. Before getting to the extension step, the entire PCR mixture, including the polymerase, has already been through the denaturation step where the reaction was heated almost to boiling. Anyone who has cracked an egg into a frying pan will know what high heat does to proteins!

In the early days of PCR, fresh polymerase was added to the reaction tube every cycle to replace the polymerase that had been destroyed by the denaturation step. Having to open the tube and add new enzyme for up to 30 cycles was expensive, inefficient, and increased the chances of contaminating the reaction. The innovation that would remove these obstacles came from an unusual source – Yellowstone National Park.

In the 1970s, scientists had isolated a species of bacteria called *Thermus aquaticus* from a hot spring in Yellowstone. *T. aquaticus* thrives at 75 - 80°C and can survive much higher temperatures. Its enzymes are similarly heat-tolerant. E. coli, the original source for the polymerase used in PCR, thrives at 37.5°C, the same temperature as the gut of mammals.

Since the biochemistry of DNA is similar across the tree of life, polymerase from *T. aquaticus* (called Taq Polymerase) can replace E. coli polymerase in PCR, with the modification that the extension step is performed at 70-75°C.

This innovation led to PCR becoming the ubiquitous, inexpensive, and efficient tool that it is today. Reactions can be set up once, sealed inside a tube, and placed in an automated thermal cycler. The thermal cycler (or PCR machine) is a specialized instrument that accurately and rapidly changes the temperature of the tube between denaturation, annealing, and extension. After 20-40 cycles of these three temperatures, billions of copies of the desired DNA product can be produced. The amplified DNA can be analyzed with gel electrophoresis at the end of the experiment or detected as they are being formed using more advanced equipment.

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