Student Lab Guide

Lactose Intolerance PCR Experiment

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Scientific Background: The Story of Lactose Tolerance

Can you enjoy a bowl of cereal or a scoop of ice cream without a second thought? Or does dairy leave you feeling bloated and uncomfortable? These everyday experiences are actually a window into a massive switch in your DNA.

The Biology of Milk

Milk contains a sugar molecule called lactose. To turn this sugar into energy, your body uses the enzyme lactase (produced in the small intestine) to break it down into two simpler sugars: glucose and galactose.

The instructions for building this enzyme are found in a gene called LCT. In most humans, the LCT gene is highly active during infancy but naturally switches off after childhood. When this happens, lactose is no longer broken down efficiently, leading to lactose intolerance. Because of this, lactose intolerance is actually the global norm for adults, especially in populations from East Asia, the Americas, and many parts of Africa.

The Evolutionary "Switch"

So, why can some adults still digest milk? The secret isn't actually inside the LCT gene itself. Instead, it is located in a nearby stretch of DNA inside a neighboring gene called MCM6.

Think of this region inside the MCM6 gene as a switch or a regulatory control center for LCT. While this switch usually turns the LCT gene off as we grow up, a single letter change in the DNA code (called a Single Nucleotide Polymorphism or SNP) can occur in this regulatory region.

In people with certain versions of this SNP, the regulatory region continues to stimulate the LCT gene throughout adulthood. As a result, lactase production continues for life, allowing lactose to be digested. This is a fascinating example of recent human evolution. In populations with a long history of dairy farming, such as Northern Europeans and some East African groups, there was strong natural selection for this genetic switch to stay on.

A Global Perspective on Genetics

It is important to remember that human evolution happened independently in different parts of the world. Because of this, different populations have different lactase tolerance SNPs, and no single genetic marker explains lactose tolerance worldwide. In this lab, you will explore the specific SNP (rs4988235) that acts as the switch for many people, helping us understand how our history is written in our genes.

Key Terms to Know

Genotype: Your specific genetic makeup; the "code" you carry in your DNA.

Phenotype: Your observable traits, such as whether you are lactose tolerant or intolerant.

SNP (Single Nucleotide Polymorphism): A single-letter change at a specific position in the DNA sequence.

Homozygous: Having two identical versions (alleles) of a gene.

Heterozygous: Having two different versions (alleles) of a gene.

Experimental Plan

- You will be **performing** a basic human DNA extraction from your own cheek cells using a saline mouthwash sample to release the cells; centrifuging to concentrate the cells; and heating to break open the cells and release DNA.
- You will be **learning** the principles of Polymerase Chain Reaction (PCR) and how to apply it to detect tiny genetic differences (Single Nucleotide Polymorphisms or SNPs), by using a PCR technique called *Tetra-Primer ARMS-PCR*.
- You will be **discovering** the concept of how one gene (MCM6) acts as a switch, controlling the expression of another gene (LCT).
- You will be **interpreting** the results of gel electrophoresis to confidently determine a person's genotype (homozygous dominant, homozygous recessive, or heterozygous) for the MCM6 gene variation.

The Three Stages of the Lab

#	Stage	Goal	What you will do
1	**DNA Extraction**	Isolate your DNA	You will collect cheek cells using a saline rinse. You will then use a centrifuge to gather the cells and heat to release the DNA.
2	**PCR**	Copy your target gene	You will use your extracted DNA as a template. The thermocycler will make millions of copies of the MCM6 gene region that controls lactose digestion.
3	**Gel Analysis**	Visualize your results	You will load your samples into an agarose gel. By comparing your DNA bands to a known ladder, you will determine your genotype.

Protocol 1: DNA Extraction Using a Saline Mouthwash

What are we trying to achieve in this part of the experiment?

The goal of today's experiment is to extract a DNA sample from cheek cells in your saliva using a 0.9% saline solution. The saline solution is isotonic (of the same salt concentration as your cells), which helps protect your cells during collection.

Note: *This protocol should take approximately* ***20 minutes*** *to complete.*

Before You Start

Important: For best results, make sure it's been at least 30 minutes since you last ate food, drank liquids (other than water), or brushed your teeth with toothpaste. If you don't, these substances might carry through into your DNA extraction and PCR, and make DNA amplification more difficult.

Step-by-Step Protocol

Step 1. Label your sample tubes

- ☐ Prepare and label a 1.5 mL microcentrifuge tube with a permanent marker (e.g., your initials and the date).

Step 2. Get a buccal cell sample by rinsing your mouth with 0.9% saline solution.

- ☐ Measure approximately 10 mL of 0.9% saline (salt water) into a plastic cup or shot glass and pour it into your mouth.
- ☐ Rinse your inner cheeks vigorously for 60 seconds, swishing the water from side to side and rubbing your cheeks against your teeth to dislodge cheek cells.
- ☐ Spit the saline solution back into the plastic cup or shot glass.

Step 3. Transfer your sample into the microcentrifuge tube

- ☐ Use the transfer pipette to transfer your saliva sample from the glass into the labeled 1.5 mL microcentrifuge tube, filling it up to the 1 mL mark.

Step 4. Centrifuge your sample to form a cell pellet

- ☐ Place the tube with your saliva sample into the centrifuge.

Important: Make sure that the centrifuge is balanced, using another tube of equal weight (e.g., a water-filled balancing tube) if necessary.

- ☐ Close the lid and run the centrifuge at 4k xG for 120 seconds (or ~2,700 xG for 5 min on slower centrifuges).
- ☐ Once finished, check the tube for a small white pellet (cheek cells) at the bottom. The remaining liquid (supernatant) should be clear.

Step 5. Recover the pellet

Note: Check that the pellet is firmly attached to the bottom. If loose, centrifuge again.

- ☐ Carefully pour the supernatant away into a waste container, avoiding the pellet.
- ☐ Use a transfer pipette to remove any remaining liquid without disrupting the pellet.

Step 6. Wash the pellet

- ☐ Add 1 mL of 0.9% saline solution to the tube with a clean transfer pipette.
- ☐ Close the tube and break up the pellet by flicking the tube.

Step 7. Recover the washed cells

- ☐ Centrifuge the sample tube again to reform a pellet (4k ×G for 90 seconds, or ~2,700 xG for 5 min on slower centrifuges).
- ☐ Carefully remove as much liquid as you can by pouring and/or pipetting into a waste container.

Step 8. Resuspend the washed cells

- ☐ Transfer approximately 100 µL of fresh 0.9% saline into the sample tube (you can estimate this volume).
- ☐ Close the tube and resuspend the pellet by flicking the bottom of the tube until the cells are entirely dispersed into the solution.

Step 9. Transfer the sample into a PCR tube for extraction

- ☐ Set your micropipette to 20 µL.
- ☐ Transfer the cell mixture from the 1.5 mL tube into a new 0.2 mL PCR tube, pipetting 20 µL five times to make 100 µL (or use a 200 µL pipette for a single transfer).

Step 10. Label the PCR Tube

- ☐ Click the lid closed and label the side of the PCR tube to identify the sample. Do not label the lid, as the heated lid might remove the ink.

Step 11. Heat the sample

- ☐ Place your PCR tube in the thermocycler block.
- ☐ Set the thermocycler to heat the sample at 95°C for 10 minutes to boil the cells and release the DNA.

Step 12. Mix the sample

- ☐ Carefully remove the PCR tube (the block will be hot).
- ☐ Flick the PCR tube for 5–10 seconds to mix the sample.

Step 13. Clean up the sample

- ☐ Use the PCR tube adapter inside a normal microcentrifuge tube to fit the PCR tube into the centrifuge.

Important: make sure the centrifuge is balanced, using another tube of equal weight (e.g., a water-filled balancing tube) if necessary.

- ☐ Run the centrifuge (4,000 ×G for 120 seconds, or ~2.7k ×G for 5 min for slower centrifuges).

ℹ️ After centrifugation, the DNA is in the clear supernatant liquid, and the cell debris is at the bottom.

Step 14. Transfer the clean sample to a new tube

- ☐ Transfer **40 µL** of the clear supernatant into a new, clean 0.2 mL PCR tube using a micropipette set to 20 µL (two transfers). Be careful to avoid the cell debris.
- ☐ Label the new tube containing the **DNA template sample**.
- ☐ If not proceeding immediately to PCR, store the template sample in the freezer at around -20°C.

Protocol 2: Set Up the PCR

What are we trying to achieve in this part of the experiment?

In this step, you will use PCR to amplify part of the MCM6 gene. The sequence of gene fragment we are copying includes the C/T mutation that contributes to lactose intolerance. This C/T mutation is also known as a *single nucleotide polymorphism* (SNP) with the code rs4988235.

The experiment uses a method called Tetra-Primer ARMS PCR, which uses four primers. Two primers copy the whole fragment (these are called the outer primers), while two primers located within this fragment are specific to either the C or T mutation of the SNP rs4988235 (these are called the inner primers).

Before You Start

To set up the PCR, you will need to add four components to a PCR tube in specific volumes:

- PCR Grade Water
- 5x HOT FIREPol® Master Mix
- Lactose intolerance primer mix
- Your DNA template.

The total volume for your PCR reaction will be 20 µL in an empty PCR tube.

Step-by-Step Protocol

Step 1: Label your PCR tube

- ☐ Label or number your PCR tube on the side and top with a fine permanent marker.

Step 2: Add PCR Grade water

- ☐ Set your micropipette to 12 µL.
- ☐ Using a fresh tip, transfer 12 µL of PCR Grade Water into the PCR tube. Discard the tip.

Step 3: Add Master Mix

- ☐ Set your micropipette to 4 µL.
- ☐ Using a fresh tip, transfer 4 µL of the 5x PCR master mix into the PCR tube. Discard the tip.

Step 4: Add Primer Mix

- ☐ Set your micropipette to 2 µL.
- ☐ Using a fresh tip, transfer 2 µL of the primer mix (Lactose Intolerance PCR primers) into the PCR tube. Discard the tip.

Step 5: **Add DNA Template**

- ☐ Set your micropipette to 2 µL.
- ☐ Using a fresh tip, transfer 2 µL of your Template Sample into the Reaction Tube. Discard the tip, and close the tube securely.
- ☐ Mix the contents by gently flicking the tube or briefly centrifuging. The tube is now ready to be placed in the thermocycler.

Step 6: Run the PCR program

- ☐ Place your PCR tube in the thermocycler block.
- ☐ Check that the PCR program has been set to the following program:
    - Initial Denaturation: 15 mins at 94°C (hot start PCR)
    - 35 Cycles of:
        - Denaturation: 30 seconds at 94°C
        - Annealing: 30 seconds at 65°C
        - Extension: 30 seconds at 72°C
    - Final Extension: 120 seconds at 72°C
- ☐ Start the PCR program.

Note: *The thermocycler will now run for* ***between 1.5 and 2 hrs*** *(depending on thermocycler model).*

- ☐ When finished, store the PCR product in a fridge or freezer, or use it immediately for gel electrophoresis.

Protocol 3: Load and Run an Agarose Electrophoresis Gel

What are we trying to achieve in this part of the experiment?

The goal of this part of the experiment is to take the DNA fragments amplified in the PCR step, physically separate them by size, and then visualize them using a fluorescent DNA stain on an agarose electrophoresis gel.

Loading the amplified DNA into the gel wells and applying an electric current (electrophoresis) causes the fragments to migrate. Larger fragments move more slowly than smaller fragments through the gel's matrix, separating the original mixture into discrete bands based on their size.

To determine the exact size of these fragments, a 100 bp DNA ladder (a set of fragments with known sizes) is run in a parallel lane. The separated DNA is visualized by exposing the stained gel to blue light, causing the DNA bands to fluoresce.

To interpret your results, you can compare a photograph of your results with the example results on the next page.

Step-by-Step Protocol

Loading and running an agarose gel

You will now load your DNA samples and the DNA ladder into the gel wells.

Step 1: Loading the DNA Ladder

- ☐ Set your micropipette to 5 µL.
- ☐ Using a fresh pipette tip, draw up the 100 bp DNA Ladder and carefully load it into the first well (usually Well 1). Discard the tip.

Step 2: Loading the PCR Result

- ☐ Set your micropipette to 5 µL.
- ☐ Using a fresh pipette tip, load 5 µL of your PCR Result into the next empty well (Well 2). Discard the tip.

Step 3: Running the gel

- ☐ Gently close the gel box and connect the leads to the power supply.
- ☐ Run the gel for 30-45 minutes at 50 V - 80 V (depending on your gel tank model).

Step 4: Turn off the power supply

- ☐ Once the run is complete, disconnect the gel box. Your gel is now ready for visualization.

Visualizing and Analysing Results

Step 5: Agarose gel results visualization

- ☐ Place the gel box or agarose gel slab on the blue light transilluminator (Bento Lab or equivalent system).
- ☐ Place the Gel Imaging Hood, or the transilluminator orange filter, over the gel to filter out the blue light.
- ☐ Turn the blue light transilluminator on to visualize the DNA bands, which will fluoresce due to the DNA stain bound to the DNA.
- ☐ Take a photograph for documentation.

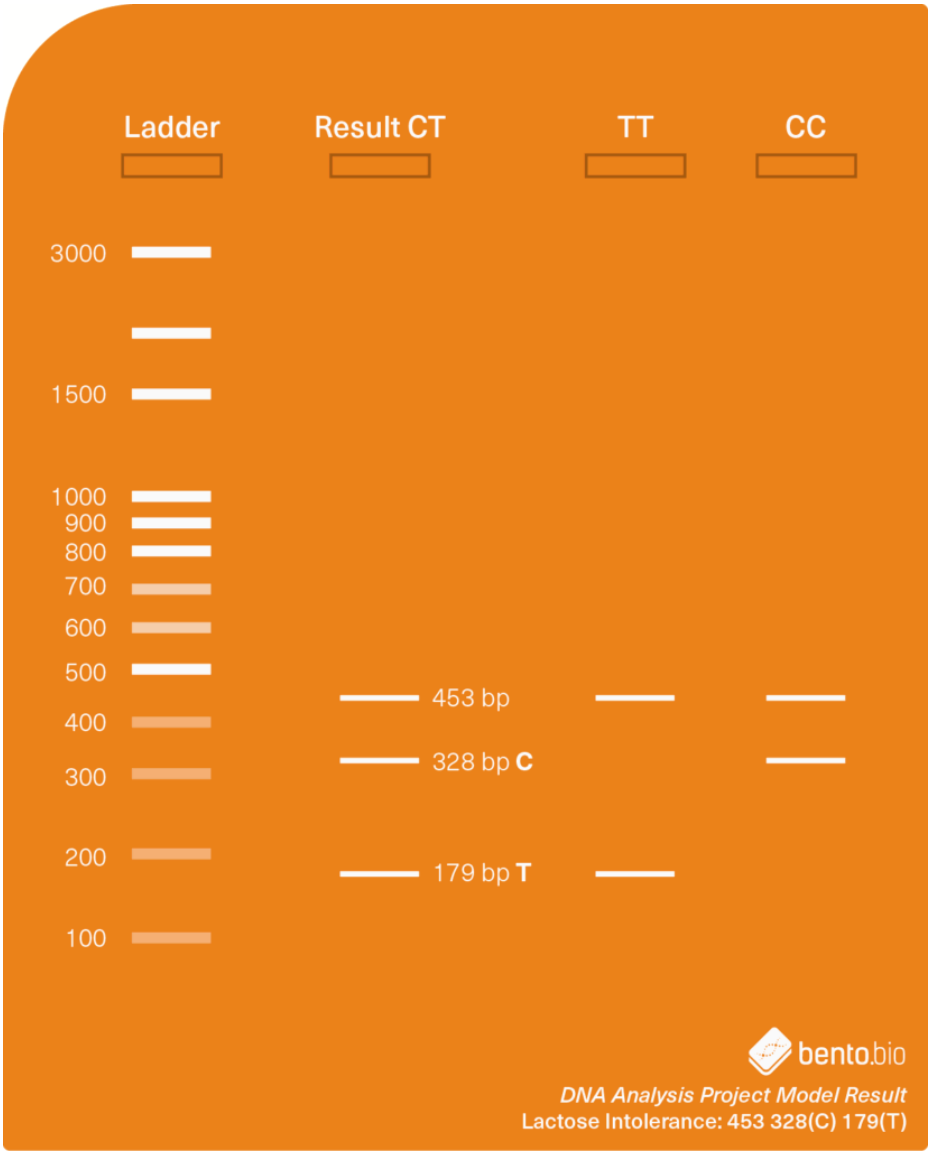
Step 6: Agarose gel results interpretation

- ☐ Compare the bands in your sample lanes to the expected results for each genotype in the table below and the model gel results on the next page.

Note: The Control band (453 bp) should be present in all positive PCR results. The presence of the other two bands determines the genotype according to the table below.

Sample Lane	Genotype (Alleles)	Expected Bands (base pairs)	Lactose Status
TT	Homozygous dominant	Control (453 bp), T allele (179 bp)	Likely Lactose Tolerant
CT	Heterozygous	Control (453 bp), C allele (328 bp), T allele (179 bp)	Likely Lactose Tolerant (T is dominant)
CC	Homozygous recessive	Control (453 bp), C allele (328 bp)	Likely Lactose Intolerant as an adult

- ☐ After documentation, dispose of the agarose gel in the regular trash.



Model agarose gel results for the Lactose Intolerance PCR assay