miniOne®
MINILABS

# What's in the Trunk? An Elephant Conservation Expedition

## Student Guide for STR Genotyping Analysis

Cat# M3016TAE

Version 122023

Based on the original activity developed by

Fred Hutch Cancer Center

**Science Education Partnership**

# 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.

# Lab Overview

## Background information

Elephants are a keystone species, meaning they have a disproportionately large effect on the environment relative to their abundance. Also known as "ecosystem engineers", elephants play a critical role in shaping the savanna landscape. During the dry season they use their tusks to dig up dry riverbeds and create waterholes for many other animals. They uproot trees and shrubs, helping to maintain the savannah and preventing it from becoming a forest. If the elephants are removed, or their populations are greatly reduced, the ecosystem starts to unravel.

As a K-selected species, animals like elephants are prone to extinction because of their low fecundity. They take a long time to reach reproductive maturity; most females will give birth for the first time between 14 and 15 years old. A female will only produce a calf about once every five years throughout her reproductive years. Therefore, if something causes a sharp decline in the population it is difficult for it to recover. Because the calf is so dependent on the mother, calf survival is dependent on the mother surviving.

Elephant populations across Africa are in sharp decline due to poaching for the illegal ivory trade. Tusks are a pair of elongated teeth that continue to grow throughout its life. They use them to strip bark from trees and dig watering holes. Tusks are essential to males, who use them to fight other males for access to females; therefore there is strong selective pressure for tusks and few males are born without them. Typically, 2-4 % of females are born with tusks.

Dr. Samuel Wasser from the University of Washington is sounding the alarm about the the shrinking elephant population. His team uses DNA analysis of confiscated ivory to pinpoint the population the ivory came from. This will allow them to target law enforcement and conservation efforts to reduce poaching.

## Short Tandem Repeat (STR) Analysis

Once the dung samples have been analyzed in the lab, different single tandem repeats (STRs) in the genome are identified as helpful in distinguishing populations. The unique allele frequencies of a population across many STRs act as a genetic ID tag which is then stored in a database. In this analysis students will first identify the fragment length and then use an STR table to determine the number of repeats in each of the fragments. The STR database should be used to look up the repeat numbers for elephant population identification. These genotypes will be represented by the number of repeats of the STR region , for example STR 1 (104,144) means STR 1 has 104 repeats on allele 1 and 144 on allele 2, and STR 2 (2, 327) has 2 repeats on allele 1 and 327 on allele 2.

By using an established database to analyze the DNA of seized ivory, authorities can pinpoint which population of elephants it came from, giving insight into the location of the poachers and trends in their behavior.

# Pre-Lab Questions

Assignment: Watch the Video: CSI Wildlife and answer the following:

1. Describe what is happening to the elephant population.

2. Explain the main cause for the decline in the elephant population

3. What is the goal of the research team led by Dr. Sam Wasser?

4. Although the Convention on Trade in Endangered Species (CITES) banned ivory trade in 1989, illegal ivory poaching continues at an alarming rate. Researchers have been creating DNA profiles by sampling dung from elephant populations across Africa. The goal is to try to match illegally poached ivory to the population in which it originated. The figure shows locations where elephants were likely to have been poached between 2006 and 2014.
   https://www.biointeractive.org/classroom-resources/using-genetic-evidence-identify-ivory-pocing-hotspots

   a. Do the maps reveal any patterns in the origin of the seized ivory? What do you conclude from these patterns?

   b. Explain why is it important to collect dung samples from elephant populations in different parks and reserves when preparing a DNA database?

Congratulations! You have been hired as a Wildlife Crime Scene Investigator! Your research team will analyze 40 DNA samples from a large shipment of ivory that was confiscated from a port in Hong Kong. Your mission is to determine the elephant population the ivory came from to track down the poachers.

# Part I: Electrophoresis

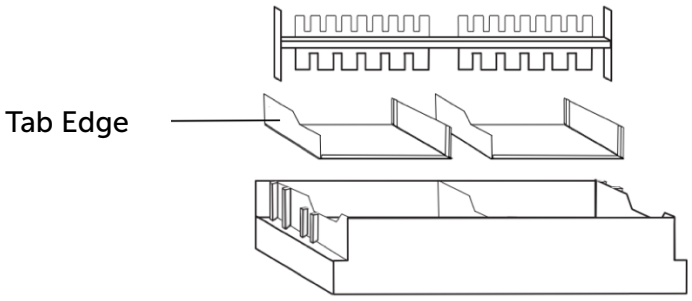
## Materials

1 Minione® Casting System
1 MiniOne® Electrophoresis System
1 agarose GreenGel™ cup (1 %)
4 numbered DNA samples
1 MiniOne Universal Marker
TAE running buffer (135 mL)
1 micropipette (2-20µL)
5 pipette tips

| Lane # | Sample Name | Volume |
|---|---|---|
| 1 | MiniOne Universal Marker | 10 µL |
| 2 | Sample | 10 µL |
| 3 | Sample | 10 µL |
| 4 | Sample | 10 µL |
| 5 | Sample | 10 µL |
| 6 | Empty | 10 µL |

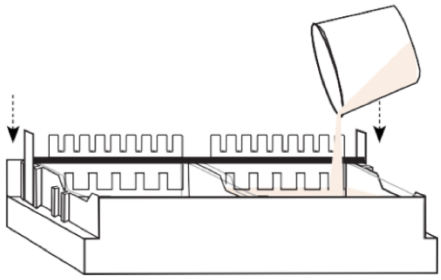
## How to Cast a Gel

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.



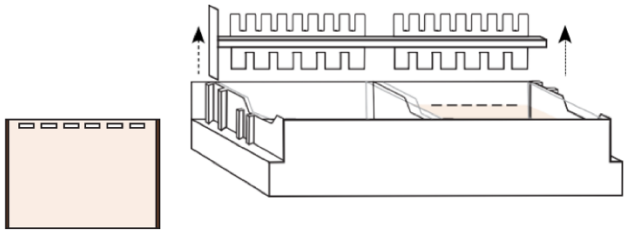

2. **Partially** peel the film off a GreenGel™ cup and microwave for 25-30 seconds. Allow to cool for 15 seconds. DO NOT microwave more than 5 gel cups at a time.


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.



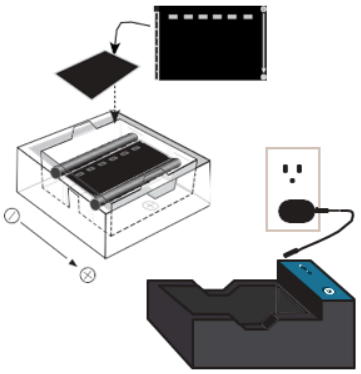
**DO NOT disturb the gel until time is up.**

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

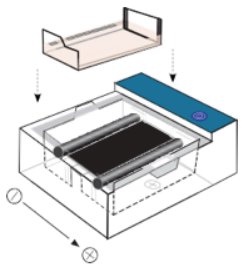
1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



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.

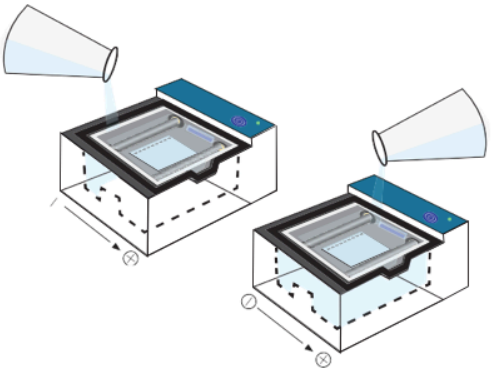
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.



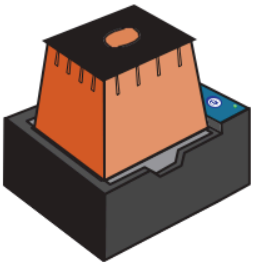
Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

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.**

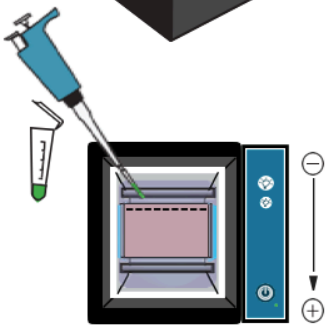


7. Place photo hood on the carriage.



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.



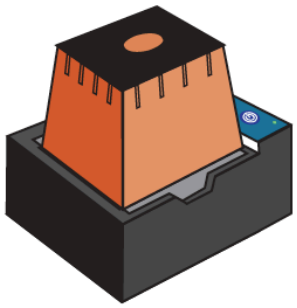
10. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

## Run, Visualize and Capture Image

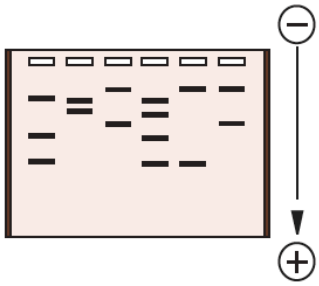
1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.

   **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**



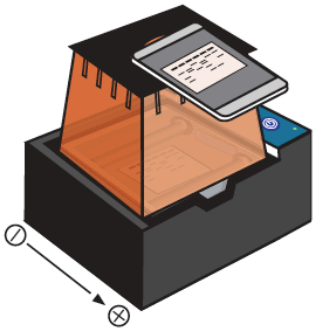
2. Have students periodically check the migration of the bands (~every five minutes).



3. Allow the gel to run **20 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.

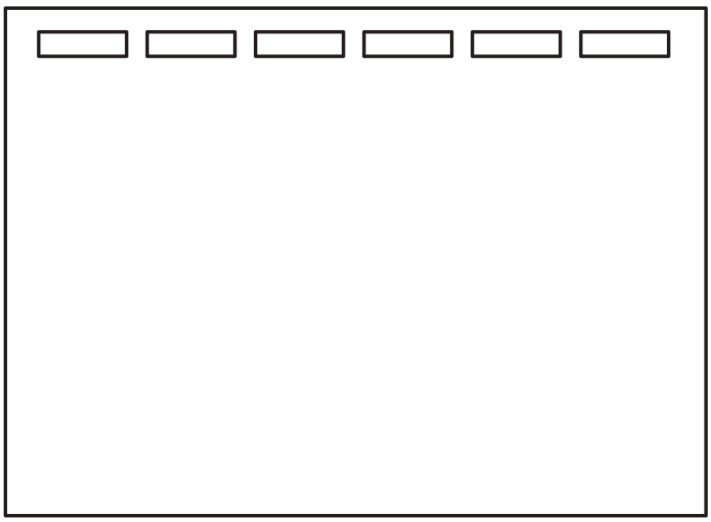
## Clean Up

**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



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

# Part III STR Analysis Worksheet

1. **Estimate fragment sizes:** Using the MiniOne Universal Marker as a reference, estimate the sizes of the bands of your samples. The MiniOne marker has fragment sizes of 10k, 6k, 3k, 2k, 1k, 800, 600, 400, and 200 base pairs (bp). Write your estimated fragment sizes below for each of your samples.

| Step | Sample number | Sample # _____ | Sample # _____ | Sample # _____ | Sample # _____ |
|---|---|---|---|---|---|
| 1 | Fragment size 1 | | | | |
| | Fragment size 2 | | | | |
| | Fragment size 3 | | | | |
| | Fragment size 4 | | | | |
| 2 | Forest or Savannah Elephant? | | | | |
| 3 | STR 1 genotype | | | | |
| | STR 2 genotype | | | | |
| 4 | Elephant Population ID | | | | |

2. **Forest or Savannah Elephant? -** If the largest band is larger than 3000 bp then the elephant is a Savannah Elephant. If the largest band is less than 3000 bp, then the elephant is a Forest Elephant. Write down the type of elephant for each of your samples.
3. **STR determination -** Use the *Short Tandem Repeat* tables on page 10 to find the number of repeats of the STR sequence in each band of a sample. Look for the fragment length that most closely matches the estimated length of band, then record in your worksheet the number of repeats from the corresponding column labelled "# of repeats". The genotype is written as the number of repeats on each allele for the 2 different STR's, for example (963,4) for STR 1 corresponds to the 6795 bp band and 2000 bp band for STR 1, and (327,2) corresponds to 1498 and 198 bp's for STR 2 respectively.
4. **Population Identification -** Using the Forest or Savannah classification of your first sample, find the correct database to use on page 11. Look for the STR that most closely matches the estimated length of bands to those found under the STR1 and STR2 columns for the first sample. The elephant population ID found in the leftmost column of your matches. Write this down on the worksheet. Continue to look for the fragment size matches for the other samples tested.
5. **Tally -** Take a tally of which elephant populations your and your classmates were able to identify to determine the hotspot. Collect the information on the Classroom Data Table.

# Part III STR Analysis: Short Tandem Repeat Tables

The Short Tandem Repeats (STR) Database contains the number of repeats found for STR1 and STR2. You will use the fragment sizes of your samples to look up the number of repeats in each band. Figure 1 represents the bands found in the MiniOne Universal Marker lane after running electrophoresis and indicates whether to use STR1 or STR2 depending on the fragment size.

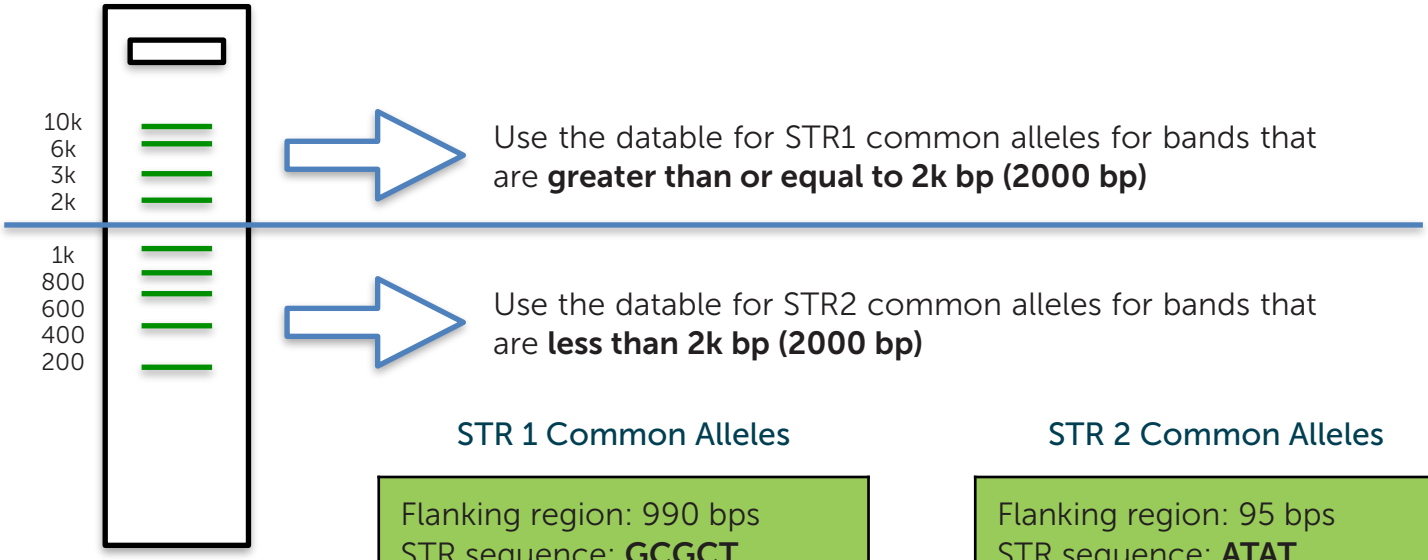



**Fig 1. MiniOne Universal Marker band profile and sizes**

### STR 1 Common Alleles

| Flanking region: 990 bps<br>STR sequence: **GCGCT** | |
|---|---|
| # of Repeats | Fragment length including flanking regions (bps) |
| 963 | 6795 |
| 842 | 6190 |
| 737 | 5664 |
| 448 | 4220 |
| 208 | 3020 |
| 144 | 2700 |
| 104 | 2500 |
| 4 | 2000 |

### STR 2 Common Alleles

| Flanking region: 95 bps<br>STR sequence: **ATAT** | |
|---|---|
| # of Repeats | Fragment length including flanking regions (bps) |
| 327 | 1498 |
| 265 | 1250 |
| 202 | 998 |
| 78 | 502 |
| 39 | 346 |
| 2 | 198 |

# Part III STR Analysis: Database Identification of the Population the Ivory Sample Came From

Elephant dung samples taken from different parks and reserves across Africa are used to create population databases. By analyzing the DNA from many dung samples, scientists can create databases of elephant populations based on the unique pattern of DNA bands found in their genome. Dung samples were retrieved within 50 km of the coordinates listed below.

## Forest Elephant Database

| Population ID | Park/Reserve | Country | Coordinates• | STR1 | STR2 |
|---|---|---|---|---|---|
| CongoOda | Parc National d'Odazala-Koloua | Republic of Congo (Congo) | 0.873379, 14.911781 | 104, 4 | 39, 39 |
| DRCSalongaN | Parc National De La Salonga-Nord | Democratic Republic of Congo | -1.843995, 21.899092 | 144, 144 | 202, 327 |
| DRCSalonaS | Parc National De La Salonga-Sud | Democratic Republic of Congo | -2.509554, 21.451127 | 4, 4 | 202, 2 |
| DRCSankuru | Sankuru Nature Reserve | Democratic Republic of Congo | -2.142413, 24.002863 | 104, 4 | 78, 39 |
| GabonLope | Lope National Park | Gabon | -0.632631, 11.599303 | 104, 144 | 2, 327 |

## Savannah Elephant Database

| Population ID | Park/Reserve | Country | Coordinates• | STR1 | STR2 |
|---|---|---|---|---|---|
| TanSerengeti | Serengeti National Park | Tanzania | -2.278820, 34.607081 | 842, 448 | 39, 39 |
| TanNgoron | Ngorongoro Conservation Area | Tanzania | -2.948512, 35.738113 | 842, 842 | 265, 265 |
| TanRuaha | Ruaha National Park / Rungwa Game Reserve | Tanzania | -7.425413, 34.729586 | 737, 208 | 327, 78 |
| TanUsung | Usungu Game Reserve | Tanzania | -7.665523, 34.182096 | 737, 144 | 202, 202 |
| KenyaTsavo | Tsavo East/West National Park | Kenya | -2.850899, 38.740338 | 737, 737 | 39, 78 |
| KenyaNamun | Namunyak Wildlife Conservation Trust | Kenya | 1.350921, 37.527241 | 842, 144 | 2, 202 |
| SSBoma | Boma National Park | South Sudan | 5.862643, 34.087840 | 737, 4 | 78, 2 |
| BotChobe | Chobe National Park | Botswana | -18.834440, 24.099010 | 842, 104 | 265, 39 |
| ZimHwange | Hwange National Park | Zimbabwe | -18.852646, 26.498348 | 963, 208 | 327, 327 |

# Classroom Data Table

| Sample # | Fragment 1 Length (BP) | Fragment 2 Length (BP) | Fragment 3 Length (BP) | Fragment 4 Length (BP) | Genotype STR 1 | Genotype STR 2 | Population |
|---|---|---|---|---|---|---|---|
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | | | | | | | |
| 5 | | | | | | | |
| 6 | | | | | | | |
| 7 | | | | | | | |
| 8 | | | | | | | |
| 9 | | | | | | | |
| 10 | | | | | | | |
| 11 | | | | | | | |
| 12 | | | | | | | |
| 13 | | | | | | | |
| 14 | | | | | | | |
| 15 | | | | | | | |
| 16 | | | | | | | |
| 17 | | | | | | | |
| 18 | | | | | | | |
| 19 | | | | | | | |
| 20 | | | | | | | |

# Classroom Data Table

| Sample # | Fragment 1 Length (BP) | Fragment 2 Length (BP) | Fragment 3 Length (BP) | Fragment 4 Length (BP) | Genotype STR 1 | Genotype STR 2 | Population |
|---|---|---|---|---|---|---|---|
| 21 | | | | | | | |
| 22 | | | | | | | |
| 23 | | | | | | | |
| 24 | | | | | | | |
| 25 | | | | | | | |
| 26 | | | | | | | |
| 27 | | | | | | | |
| 28 | | | | | | | |
| 29 | | | | | | | |
| 30 | | | | | | | |
| 31 | | | | | | | |
| 32 | | | | | | | |
| 33 | | | | | | | |
| 34 | | | | | | | |
| 35 | | | | | | | |
| 36 | | | | | | | |
| 37 | | | | | | | |
| 38 | | | | | | | |
| 39 | | | | | | | |
| 40 | | | | | | | |

# Post-Lab Questions

1. Explain how DNA analysis can be used to reduce African Elephant poaching.

2. Where is the location of a major poaching hotspot. Support your claim with evidence..

3. Explain why reducing elephant poaching is critical to the stability and resilience of the Savannah ecosystem.

4. What did you do if your fragments or STR matches were not exact to what was in the database? How can you still use the information to determine the population ID of the elephant that the tusks came from?

5. Do your samples have the same number of bands? If not why do you think that is?

# 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 - Recommended Reading

HHMI Biointeractive-CSI Wildlife-How DNA Profiling Works
https://media.hhmi.org/biointeractive/click/elephants/dna/one-how-dna-profiling-works.html

DNA electrophoresis sample loading: Video from Kirkwood Community College showing proper technique and some common mistakes:
https://www.youtube.com/watch?v=tTj8p05jAFM

Ivory poaching and the rapid evolution of tusklessness in African elephants
https://www.science.org/doi/10.1126/science.abe7389

# Appendix C - 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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