

# CSI AFRICA: TRACKING IVORY POACHERS

## DATA ACTIVITY

### INTRODUCTION

The excitement that many people feel when gazing at majestic African elephants is matched by their despair when they realize that these animals are disappearing, in large part because of illegal poaching for elephant tusks. As described in the annotated paper “Genetic assignment of large seizures of elephant ivory reveals Africa’s major poaching hotspots,” Dr. Samuel Wasser and colleagues describe how they use genetic and other data to determine the geographical location of seized elephant tusks to support law enforcement officials who seek to bring criminals to justice and prevent future poaching. In this activity, you will analyze data from the scientists’ research to identify the type of elephants slaughtered in a poaching event and to support claims about the location of the slain elephants.

### MATERIALS

- 1 copy of “DNA Profiling—How It Works” (optional)
- 1 copy of “Locating the Source of Ivory—Procedure” (optional)
- 1 copy of the “Map of Africa” handout
- 1 copy of the “Allele Frequency Data for Three Different Regions” handout

### PROCEDURE

It is your first month on the job for an international police force dedicated to protecting wildlife and holding people responsible for illegal poaching. You are excited to put your scientific skills to work. As part of your first case, your boss gives you an elephant tusk from a recent seizure. Your task is to determine if the tusk came from a forest elephant or a savanna elephant.

You carefully extract DNA from the tusk, following the lab protocols. Next, you use the polymerase chain reaction (PCR) to determine the genotype of the tusk for one of the microsatellite DNA loci used by other elephant researchers. The name of your microsatellite locus is **FH 127**.

1. While you are waiting for the results, you realize that you may need a reminder on the technique of DNA profiling. If you are unfamiliar or just want a reminder of the process, please read “DNA Profiling—How It Works.”
2. Not everyone who will later learn about your findings is a geneticist or scientist. To help people with different backgrounds, including the police force, understand your results, you decide to define some important terms in your own words.

a. Genotype:

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b. Microsatellite:

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c. Allele:

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d. DNA locus (plural “loci”):

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e. Allele frequency:

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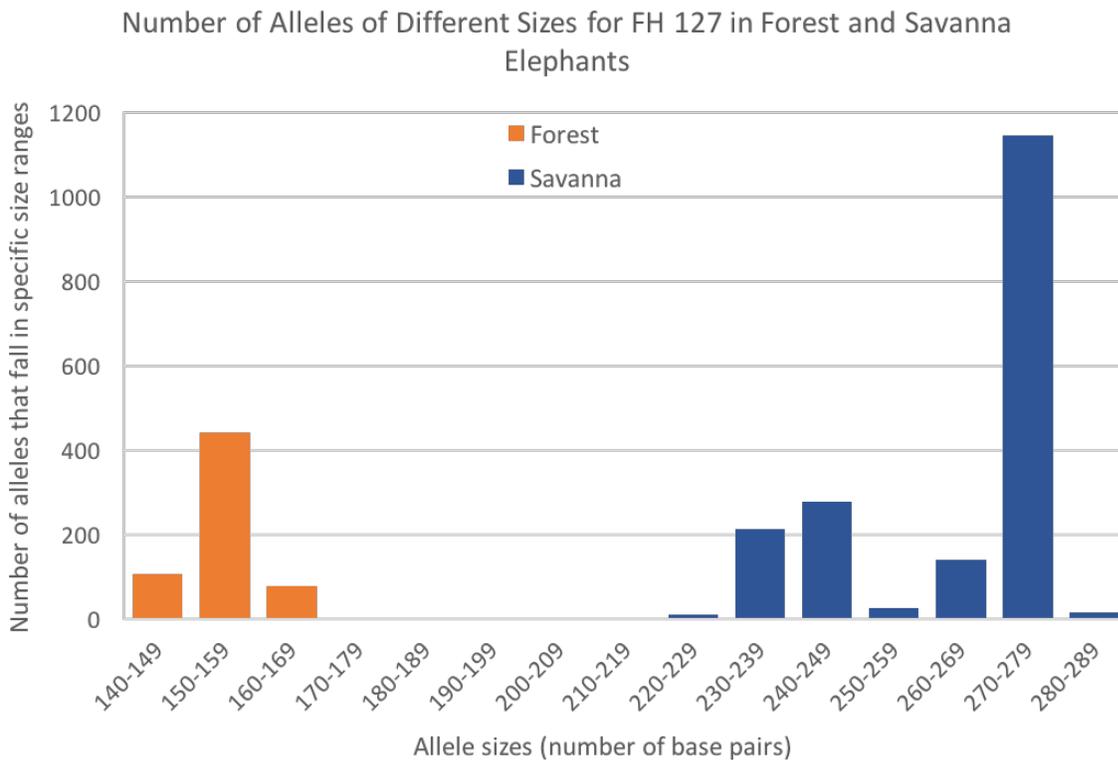
**Good news! Your PCR amplification worked.** You determine that your ivory sample has one allele that is 145 base pairs (bp) in size, and a second allele that is 147 bp.

Wasser and colleagues developed a database of elephant alleles for 16 microsatellite loci sorted by location and type of elephant (forest or savanna). For the FH 127 locus, they included 626 alleles from forest elephants and 1826 alleles from savanna elephants. The graph in Figure 1 below summarizes how many alleles there were with a specific number of alleles for the two different elephant types (forest and savanna).

3. Compare the data from your PCR amplification with the data in Figure 1. What type of elephant was the source of your tusk? Provide evidence to support your claim.

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**Figure 1.** Summary of the allele sizes found in two different types of elephants in the reference populations studied by Wasser and his colleagues (2015).

Impressed with your efforts, your team now asks you to help with a criminal case. Before you begin, your boss asks you to review the procedure used to link seized ivory samples to a particular location.

4. Read “Locating the Source of Ivory – Procedure” and ask your boss (teacher) any questions you still have.

**The details of the criminal case:** Three different suspects (A, B, and C) have been linked to recent killings of elephants. Table 1 shows where each suspect is located. The police eventually seized the ivory from the poaching event but they are not sure which site was the source of the seized ivory.

**Table 1.** Locations of the three suspects in the case.

Suspect	Latitude (degrees)	Longitude (degrees)
A	4.0	29.5
B	8.3	-9.3
C	1.8	12.7

5. Your team wants to start building a reference map for the elephants that were the source of the ivory. In step 2 you already identified which type of elephant the ivory came from. Table 2 lists the sizes of alleles (as number of bp) at FH 127 for elephants from different locations and shows the precise geographical location (in latitude and longitude degrees) for the site at which each reference sample was collected.
- a. Using Figure 1 above, decide which of the samples in Table 2 are from forest and which are from savanna elephants. Enter your answers into the table.

**Table 2.** Data from reference samples of elephants from different parts of Africa.

Location #	Genotype from one elephant at the location for FH 127 (bp)	Latitude (degrees)	Longitude (degrees)	Forest or Savanna Elephant?
3	249, 269	-3.2	35.6	
7	239, 243	-0.4	36.6	
8	249, 249	8.1	14.0	
10	249, 271	5.5	36.3	
11	149, 151	6.3	-3.0	
13	151, 157	5.0	9.5	
15	145, 145	2.8	16.4	
19	237, 237	11.3	14.8	
30	239, 263	-12.9	33.5	
44	151, 153	9.7	-12.1	
46	219, 237	11.1	19.5	
47	145, 149	-2.0	9.9	
50	159, 161	8.7	1.1	
51	151, 151	-1.2	21.0	
53	277, 277	-1.6	30.7	
57	145, 151	7.5	20.2	
62	145, 153	-1.5	29.5	
72	239, 239	-8.6	30.4	
89	151, 157	4.5	24.7	
102	153, 157	0.6	15.6	
113	237, 271	-12.6	30.2	

- b. Plot each sample **for only the type of elephant you identified in Step 3 (forest or savanna)** on the handout “Map of Africa” using the latitude and longitude values. You now have a reference map showing the distribution of forest or savanna elephants. This reference map will help you link the genotypes of elephant tusks to a particular location.

Your team now wants to use allele frequencies for the seized ivory samples to determine if one of the three suspects is more likely to have committed the crime than the others.

6. Your co-worker already calculated the allele frequencies for the **FH 67** locus. She divided the number of times an allele of a certain size (# bp) appeared in the seized ivory sample by the total number of alleles found. To get the percentage she multiplied the result with 100. Use the same approach to calculate the allele frequencies for locus **FH 39**. Further details are in the “Locating the Source of Ivory— Procedure.”

**Table 3.** Allele frequencies for FH 67 calculated by your colleague.

Allele size (# bp)	Number of times allele appeared in seized ivory	Allele frequency in seized ivory (in %)
83	0	$0/10 \times 100 = 0\%$
85	0	$0/10 \times 100 = 0\%$
87	0	$0/10 \times 100 = 0\%$
89	2	$2/10 \times 100 = 20\%$
91	1	$1/10 \times 100 = 10\%$
93	4	$4/10 \times 100 = 40\%$
95	2	$2/10 \times 100 = 20\%$
97	0	$0/10 \times 100 = 0\%$
99	1	$1/10 \times 100 = 10\%$
101	0	$0/10 \times 100 = 0\%$
103	0	$0/10 \times 100 = 0\%$
105	0	$0/10 \times 100 = 0\%$
107	0	$0/10 \times 100 = 0\%$
<b>Total # alleles</b>	10	----

**Table 4.** Data for FH 39. Calculate the allele frequencies for this population.

Allele size (# bp)	Number of times allele appeared in seized ivory	Allele frequency in seized ivory (in %)
229	0	
231	1	
233	0	
235	0	
237	3	
239	3	
241	1	
243	0	
245	1	
247	0	
249	1	
<b>Total # alleles</b>	10	----

Your next task is to examine the allele frequency data for three reference populations, each near the spot where one of the suspects was caught slaughtering elephants.

7. Use the data from Step 6 and the allele frequency data on the “Allele Frequency Data for Three Different Regions” handout to make a claim about which suspect is most likely to be involved in poaching the elephants that were the source of the seized ivory.

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8. What makes you confident that you have helped identify the correct suspect as the ivory poacher?

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9. What evidence would make you more confident in pinpointing the geographic location of the seized ivory?

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10. How does the location of your seized tusks compare with the “hotspots” for elephant poaching described by Wasser and colleagues in their paper?

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**REFERENCE**

Wasser, S. K., Brown, L., Mailand, C., Mondol, S., Clark, W., Laurie, C., and Weir, B. S. (2015). Genetic assignment of large seizures of elephant ivory reveals Africa’s major poaching hotspots. *Science*, 349, 84-87.  
Data samples taken from Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.435p4>.

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## DNA PROFILING—HOW IT WORKS

DNA profiling (also known as DNA fingerprinting) is used to identify individuals based on differences, or **variations**, in DNA.

Some regions of the DNA in your cells' chromosomes are very different (variable) compared to the same region in other people. There can even be differences between your two copies of the same region. One type of highly variable sequence is called a **microsatellite** region. An example of a microsatellite is the 38-**base-pair (bp)** fragment below. (DNA is double stranded, but here only one strand is shown.)

-AAGTCTACTACTACTACTACTACTACTACTACTACGAATCA-

All microsatellites contain a repeat unit of a few bases, or **nucleotides**. In this case, the repeat unit is CTA. There are 9 repeats of the CTA sequence. An individual can have two versions, or **alleles**, of a microsatellite sequence, one from each parent—for example, one allele with 9 CTA units and one with five CTA units. Repeat units like these are also known as “short-tandem repeats” or “STRs.” Units of two to 13 nucleotides can be repeated up to several hundred times. There may be many different alleles among individuals in a population, with each allele having a different number of repeat units.

An individual's **DNA profile** consists of microsatellites from several locations, or loci, throughout the genome. A DNA profile can be visualized as a pattern of bands on an agarose gel after **electrophoresis**, with each repeat unit (or STR) yielding one or two bands for one individual. Because STRs are so highly variable, the likelihood of two individuals possessing the exact same DNA profile is low. If you sample enough loci, the pattern of bands in an individual's DNA profile can be considered unique and can be used to identify individuals, much like a fingerprint.

DNA profiling starts with isolating DNA from an organism's cells. A single sample does not provide enough DNA to analyze, so scientists use a technique called the **polymerase chain reaction (PCR)** to make billions of copies of (or **amplify**) certain regions of an individual's DNA. The end product is a DNA sample that contains billions of copies of individual STR fragments.

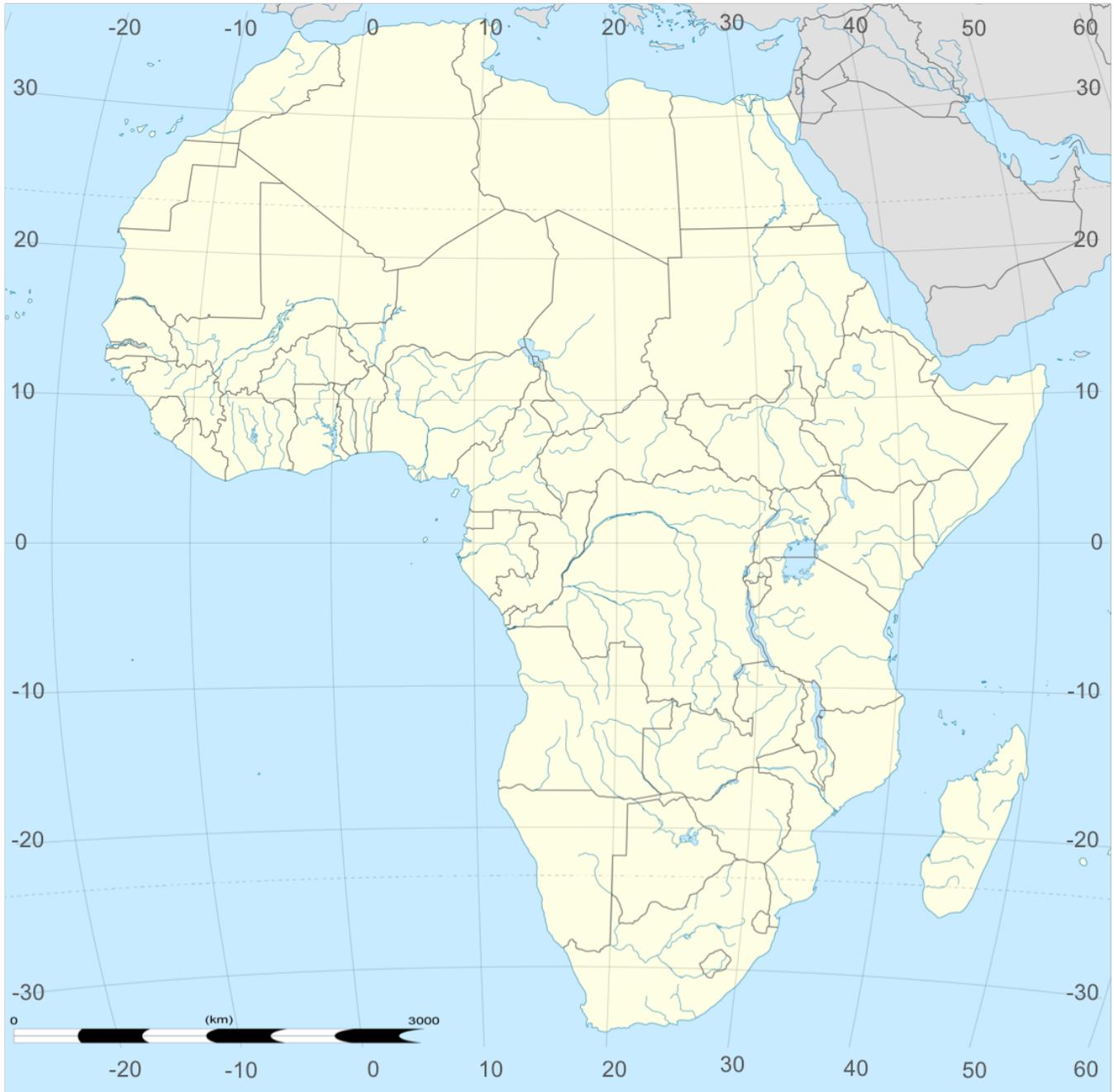
After PCR, DNA samples from each individual are placed in their own **wells** on an **agarose** gel. An electrical current is then applied to the gel, causing the negatively charged DNA to move toward the positively charged electrode. The longer the DNA fragment, the more slowly it moves through the gel. By the end of the “run,” the fragments are separated out; the longest ones will be closest to the starting point and the shortest ones will be nearest the far end. A sample of DNA containing fragments of known lengths (a **DNA ladder**) is used as a reference for determining the STR fragment sizes in each sample.

## LOCATING THE SOURCE OF IVORY – PROCEDURE

Employees, remember that our job is to help track down criminals involved in poaching. To do that and to help us decide where to send law enforcement officials to prevent poaching, we need to know the geographical origin of the tusks. The following steps help us accomplish this task:

1. Isolate DNA from a representative portion of ivory samples. For example, we might isolate DNA from ten out of 50 seized tusks.
2. Determine the genotypes (which two alleles are present) for each individual ivory sample using many microsatellite loci.
3. Count the **number of times a particular allele** shows up in all the ivory samples in total. Use the following formula to determine the frequency of an allele in the population.
  - a. Frequency of allele  $X = \frac{\text{\# of times allele } X \text{ occurs}}{\text{total \# alleles studied}}$ .  
Example: If you are studying the FH 127 locus, you may have five alleles that are each 151 bp in size out of 20 total alleles. The frequency of the 151 bp allele =  $\frac{5}{20} = 0.25$ .
  - b. You can calculate the percentage of the allele in the population by multiplying the frequency with 100.  
Example: The percentage of the 151 bp allele above is  $0.25 \times 100 = 25\%$ .
4. Repeat steps 2-3 the for every marker in the study to get a genetic profile of the seized ivory samples.
5. Examine a reference map of elephants which shows the genetic profile for different elephant populations and the geographic location of these populations.
6. To map the ivory's likely origin, look for the closest match between the genetic profile of the seized ivory samples and all the reference elephant population profiles.

## MAP OF AFRICA



*Courtesy of Eric Gaba/Wikimedia Commons*

## ALLELE FREQUENCY DATA FOR THREE DIFFERENT REGIONS

**Table 5.** Allele frequency data for marker FH 67 from three different regions.

Allele size (# bp)	Location of Suspect A (% of the alleles for this region at this site)	Location of Suspect B (% of the alleles for this region at this site)	Location of Suspect C (% of the alleles for this region at this site)
83	0	0	0
85	0	0	2
87	4	0	2
89	13	20	20
91	26	0	5
93	24	20	37
95	13	60	15
97	11	20	2
99	9	0	10
101	0	0	5
103	0	0	0
105	0	0	0
107	0	0	2

**Table 6.** Allele frequency data for marker FH 39 from three different regions.

Allele size (# bp)	Location of Suspect A (% of the alleles for this region at this site)	Location of Suspect B (% of the alleles for this region at this site)	Location of Suspect C (% of the alleles for this region at this site)
229	4	0	0
231	4	10	13
233	17	0	4
235	8	20	2
237	21	60	30
239	19	10	32
241	0	0	4
243	2	0	2
245	4	0	6
247	21	0	2
249	0	0	4