Designed for the Classroom
SINCE 1987

Background Information

Excerpts from EDVO-Kit 920

A cell's DNA contains a wealth of valuable information. This molecule is made of long chains of four nucleotides and is often called life's "instruction manual" because it allows organisms to create the proteins needed to grow and live. DNA's structure also allows this essential information to be passed down from mother to daughter cells and from parents to offspring. Locked within these sequences of nucleotides is also the story of how DNA itself has changed over multiple generations.

Evolutionary biologists and molecular ecologists study these patterns of change to reconstruct the past. For example, by analyzing DNA changes scientists have been able to reconstruct how life evolved on Earth and the evolutionary relationships between different species. Phylogeographers, scientists who specialize in the intersection of evolution, ecology, and biogeography, have similarly analyzed DNA changes to discover the migration routes of our own ancestors and how ancient communities responded to past ice ages. How do phylogeographers make the leap from DNA sequences to a species history? How do species histories help us solve current-day problems? And what does this all have to do with two orphaned lion cubs? Read on to discover.

PHYLOGEOGRAPHY – WHEN MAPS AND TREES COMBINE

In phylogeography, information about differences in the DNA of individuals (i.e. a species' genetic variation), the geographic distribution of this variation, and the evolutionary relationships between different DNA sequences are analyzed together in order to detect genetic signals of past events. Phylogeography studies are diverse – some involve tens of thousands of sampled individuals and look at DNA changes

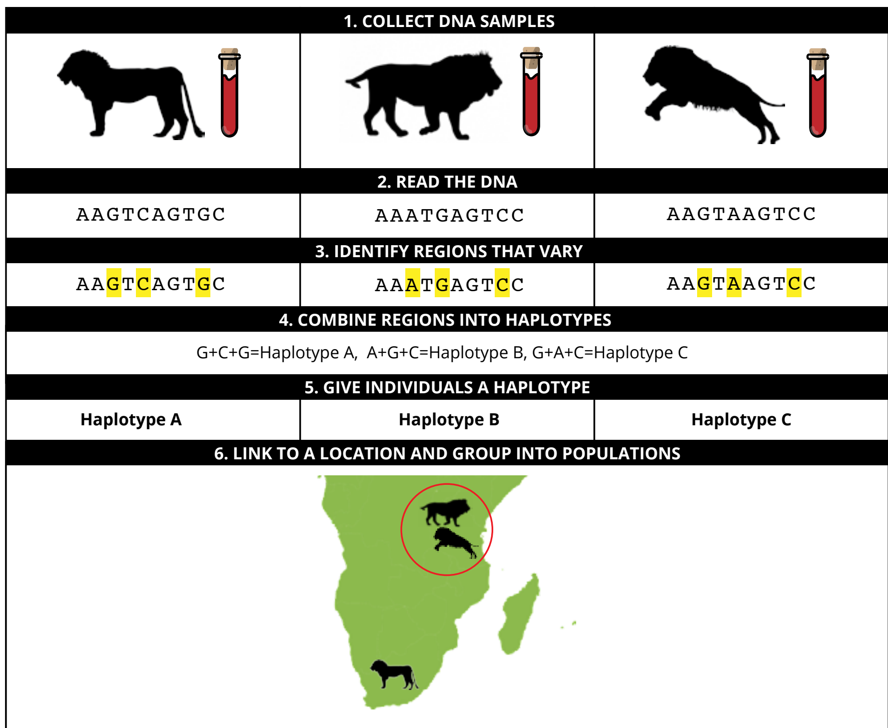

Figure 1: Key steps in a phylogeography study.

at every nucleotide within a genome, while others are based on much smaller data sets. Moreover, the exact mathematical methods used to analyze the data differ and in some cases, are even kept as tightly guarded company secrets. However, the broad process is consistent and outlined in Figure 1.

Scientists begin by collecting DNA samples from many individuals throughout the species' current range - and sometimes from fossils or museum samples. Using biotechnologies like sequencing or restriction enzyme analysis, they then identify regions of DNA that are variable and that are likely inherited from a single parent (Box 1). These regions are combined, scored, and then categorized as different haplotypes often using letters. Next, each sampled individual is assigned to a haplotype based on his or her genetic profile. This haplotype is then linked back to a location based on where the sample was collected or where the individual was born. Researchers then group samples that are geographically close to each other into populations. Finally, these results are analyzed using a combination of population genetic and phylogenetics.

BOX 1: Haplotypes, Just Like Your Mother/Father

Haplotypes are unique combinations of DNA polymorphisms that are inherited together and are usually named using letters. While most of an individual's genome is created by recombination genetic reshuffling activities like meiosis and chromosomal crossover that create a unique genetic mixture of both parent's DNA - there are DNA regions that are inherited together from a single parent. DNA that meets this criterion includes that found in mitochondria plasmids, plant chloroplasts plasmids, and (in humans) the Y chromosome. In some cases, genomic DNA – genetic material that occurs in paired chromosomes and undergoes recombination - can still be used like a haplotype provided that it is subdivided into subunits of very closely spaced or "linked" regions. Because they are inherited relatively unchanged from parent to child haplotypes are much easier to trace back in time! In many cases, phylogeographers will construct several histories based on different haplotype-types (i.e. a mtDNA haplotype and a Y chromosome haplotype) as each tells a slightly different history that both complements and double-checks the other histories.

Population genetics is a subfield of genetics that deals with genetic differences within and between populations. In phylogeography, scientists pay particular attention to which haplotypes are unique to a single population and which are shared by many populations as well as the number of haplotypes observed in each population. This information is described using mathematically based measures like effective population size, expected heterozygosity and F-statics. It is also visually summarized by pie graphs of each population's haplotypes added to a map of each population's location (Figure 2). Together, population genetic statistics and haplotype maps describe the genetic patterns of a species or the "geography" part of its phylogeography.

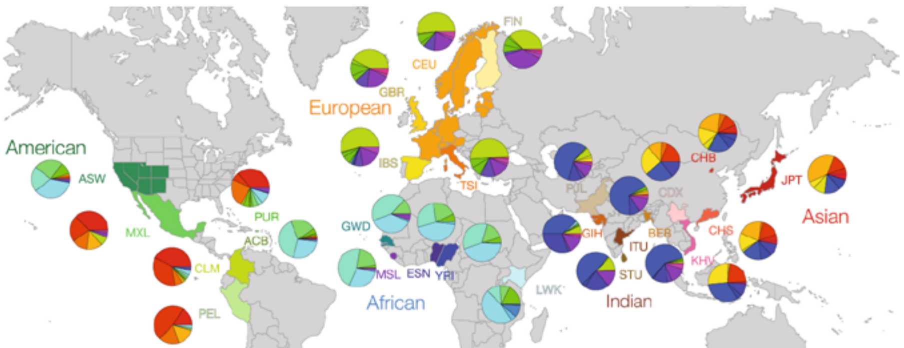

Figure 2: An example genetic map - human mtDNA haplotypes.

In order to predict what events created the observed genetic patterns, scientists next reconstruct the evolutionary history of all the different haplotypes using phylogenies – the "phylo" part of phylogeography. Phylogenies describe patterns of ancestry. Often phylogenies are displayed as trees or networks where whatever is being compared – family members, species, or in this case unique haplotypes – are on the tips of each branch (Figure 3). Common ancestors are represented by the intersection between two branches, which are known as a node. Haplotypes that are several nodes away from one another are more distantly related than those that are only separated by a single node. Similarly, haplotypes that are connected by short branches are more closely related than those connected by longer branches. The tip of each branch is labeled with the haplotype's name and in some cases an additional pie chart showing all the populations where the haplotype can be found.

By combining data from phylogenies and population genetics scientists can reveal key events in the history of a species. More specifically phylogeographical studies can help scientists determine when/where members colonized new areas (expansion events), when/ where populations went through a sudden decline in size (bottleneck events), when/where populations divided and became isolated from each other (vicariance events), and when/where members from two different populations met (admixture events). In addition, once a species' phylogeography has been created, the genetic information can be organized and shared in a database called a GRDB (Genotype Reference Database) that can be used to predict the geographic origins of an individual.

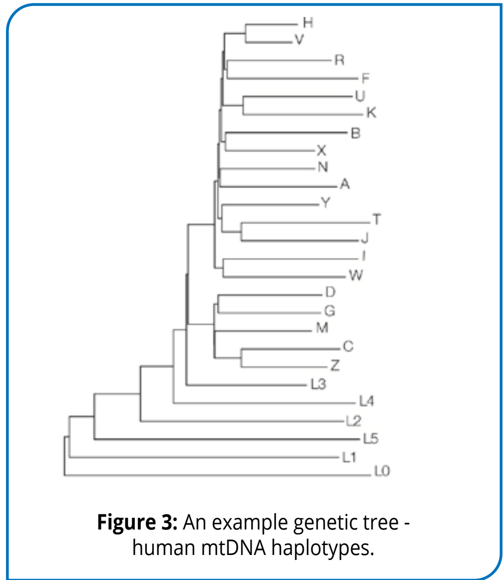

Figure 3: An example genetic tree - human mtDNA haplotypes.

APPLICATIONS - PAST HISTORIES, FUTURE CHALLENGES, CURRENT DECISIONS

Phylogeography studies are helping conservation biologists preserve current diversity. By knowing the geography of a species' genetics, scientists can identify hot spots (areas of high or unique genetic diversity) that should be protected. Scientists tasked with helping threatened species also use these studies to subdivide these species into biologically based management units (mu) or evolutionarily significant units (esu). Finally, phylogeography is being used to decide where best to reintroduce individuals that were removed from their habitat, either because of illegal wildlife selling or habitat destruction (Box 2).

Box 2: You Want Me To Live Where?

Introducing animals back to their native habitats has clear advantages – it's an enriching environment for the animal, helps maintain the diversity of the species, and minimizes animal care costs. However, it's also very difficult especially for more complex animals (primates, large cats, elephants, dolphins, etc.). These animals don't have necessary survival skills, are too trusting of humans, and are susceptible to local disease. To overcome these challenges, reintroduction is now being carried out in two stages. First, captured animals are introduced to a semi-wild preserve where they are encouraged to relearn key skills. Then, offspring from these 'rehabilitated' animals are released as a group back into the wild where they help each other adapt to the harder conditions. In addition, scientists identify the likely geographic origin of captured animals and release them nearby to maximize the chance that the reintroduced animals will be genetically adapted to the environment.

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RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

Several methods exist that allow scientists to read DNA and visually see areas that are variable within a population or a species. One popular method is restriction fragment length polymorphisms (RFLP) analysis. This technique allows scientists to visualize small nucleotide changes in the DNA of different samples as difference in the number and lengths of bands produced when extracted DNA samples are digested by restriction enzymes and then separated by agarose gel electrophoresis.

Restriction enzymes are proteins that cut DNA molecules at very specific nucleotide sequences known as recognition sites. For example, the popular restriction enzyme EcoRI cuts DNA whenever the sequence of nucleotides is GAATTC. A sample digested with EcoRI will produce several fragments of DNA of different sizes depending on how many times this recognition site is present in the DNA and where these recognition sites are located (Figure 4). Because the presence or absence of a fragment is directly related to a change in the nucleotide sequence, RFLP patterns contain both diversity and evolutionary information.

After a sample has been digested by a restriction enzyme the number of fragments produced by this digestion and the size of these fragments can be determined using agarose gel electrophoresis. First samples of DNA are loaded into wells within an agarose gel. The gel is then placed in an electrophoresis chamber containing a buffer solution and electrodes. Next a direct current is applied from a power source. Since DNA is negatively charged in the neutral buffer it migrates through the gel towards the positive electrode. During this process, the agarose gel - which consists of microscopic pores - acts as a molecular sieve and separates the different DNA fragments according to their size. Shorter fragments migrate quickly through the gel and will travel farther down the gel during the experiment than longer fragments that move more slowly through the gel.

Following electrophoresis the RFLPs are "scored" by recording all the fragment lengths generated by all the samples in a study and then by identifying whether each individual has (0) or lacks (1) that particular fragment. This creates long sequences of 0s and 1s which are then used to determine the number of unique haplotypes and the haplotype of each individual. In phylogeography, multiple enzymes are often used to increase the number of data points in a study. This is important as larger datasets create more reliable and more descriptive phylogenies and haplotype maps as well as more precise predictions about ancestry and historical events.

In this experiment, you are a conservation biologist who is working with two adolescent lion cubs the were rescued from a private house. Based on their age, you've decided to reintroduce them into the wild (or wildlife sanctuary) rather than to a zoo. Using their genetic profiles and information about lion phylogeography, you will identify the best sanctuary for them. In Modules I and II you will run gel electrophoresis to determine the lions' restriction fragment length polymorphisms (RFLP), and in Module III you will determine the haplotype of each lion. Using this information, you'll determine where to reintroduce each animal so that they're optimally matched to their environment.

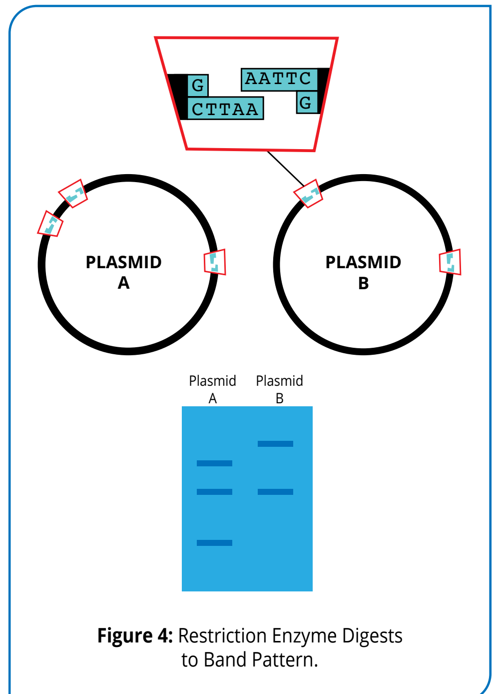
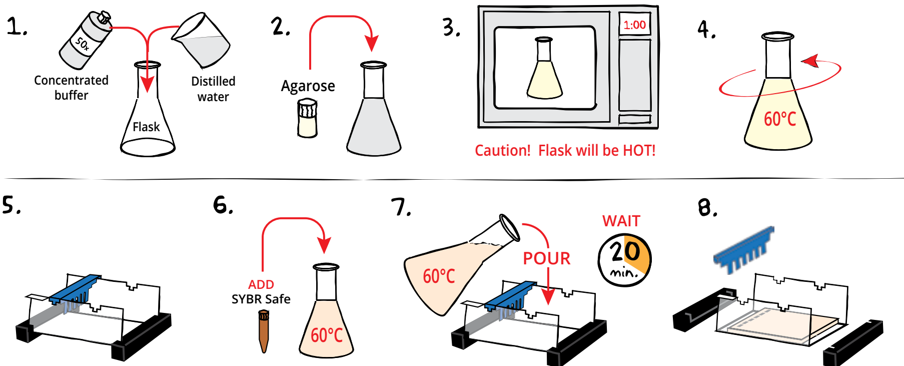


Figure 4: Restriction Enzyme Digests to Band Pattern.

Agarose Gel Electrophoresis

Conduct agarose gel electrophoresis to determine the lions' restriction fragment length polymorphisms (RFLP). This will be used to determine the lions' haplotypes for reintroduction to the wild.



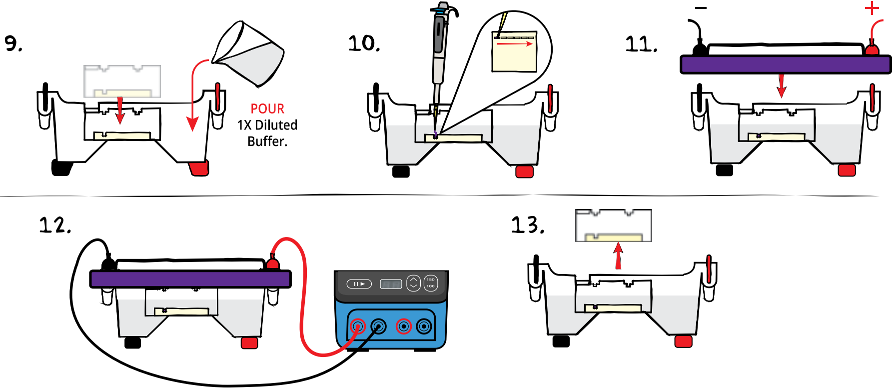
1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 mL flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60°C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. Before casting the gel, **ADD** ***diluted* SYBR® Safe** to the molten agarose and swirl to mix (see Table A).
7. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
8. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — Individual 0.8% UltraSpec-Agarose™ with SYBR® Stain

Size of Gel Casting tray	Concentrated Buffer (50x) +	Distilled Water +	Amt of Agarose =	TOTAL Volume	Diluted SYBR® (Step 6)
7 x 7 cm	0.6 mL	29.4 mL	0.23 g	30 mL	30 µL
10 x 7 cm*	1.0 mL	49.0 mL	0.39 g	50 mL	50 µL
14 x 7 cm	1.2 mL	58.8 mL	0.46 g	60 mL	60 µL

** Recommended gel volume for the EDGE™ Integrated Electrophoresis System.*

Agarose Gel Electrophoresis, continued



9. **PLACE** the gel (on the tray) into the electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
10. **LOAD** the entire sample (35 µL) into the well in the order indicated by Table 1.
11. **CHECK** that the gel is properly oriented, then **PLACE** the safety cover onto the chamber. Remember, the DNA samples will migrate toward the positive (red) electrode.
12. **CONNECT** the leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
13. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

PROCEED TO VISUALIZATION.

Table 1: Gel Loading

Lane	Tube	Sample
1	Tube A	Standard DNA Marker
2	Tube B	Lion A's mtDNA cut with Enzyme 1
3	Tube C	Lion A's mtDNA cut with Enzyme 2
4	Tube D	Lion B's mtDNA cut with Enzyme 1
5	Tube E	Lion B's mtDNA cut with Enzyme 2

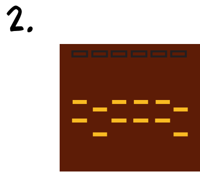
Table B: 1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

Table C: Time and Voltage Guidelines (0.8% Agarose Gel)

	Electrophoresis Model: EDGE™	M12 & M36
Volts	Min/Max (minutes)	Min/Max (minutes)
150	10/20	20/35
125	N/A	30/45
100	15/25	40/60

Visualizating the SYBR® Gel



1. **SLIDE** the gel off the casting tray onto the viewing surface of the transilluminator and turn the unit on. **ADJUST** the brightness to the desired level to maximize band visualization. DNA should appear as bright green bands on a dark background.

2. **PHOTOGRAPH** the results.

3. **REMOVE** and **DISPOSE** of the gel and **CLEAN** the transilluminator surfaces with distilled water.

Related Experiment:

Safari Family Reunion

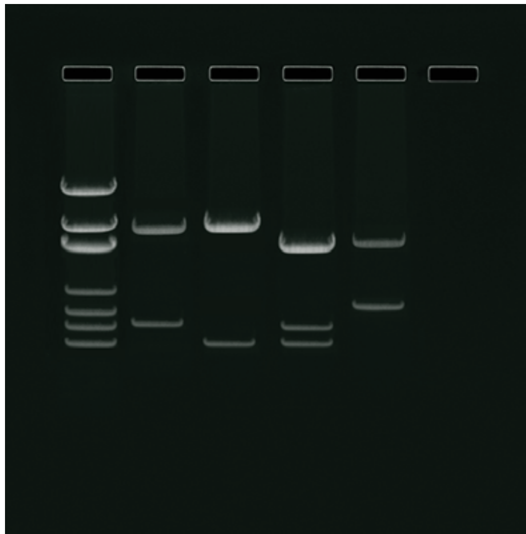
For 8 Gels/8 Lab Groups.

In this lab, students will perform electrophoresis on the DNA samples of two lions in order to return them to wildlife sanctuaries close to their ancestral home.

- Learn how DNA is used to discover an individual's ancestry.
- Perform DNA electrophoresis and RFLP analysis.
- Analyze phylogenetic tree and haplotype maps.
- Explore how conservation biologists use genetic data.

Cat. #920 **Safari Family Reunion with FlashBlue™ Stain**
Cat. #920-S **Safari Family Reunion with SYBR® Safe Stain**

Electrophoresis Results and Analysis



Sample	Number of Fragments	Size of each Fragment
Standard DNA Marker	7	6751, 3652, 2827, 1568, 1118, 825, 630
Lion A's mtDNA cut with Enzyme 1	2	3440, 840
Lion A's mtDNA cut with Enzyme 2	2	3650, 630
Lion B's mtDNA cut with Enzyme 1	3	2830, 820, 630
Lion B's mtDNA cut with Enzyme 2	2	3000, 1280

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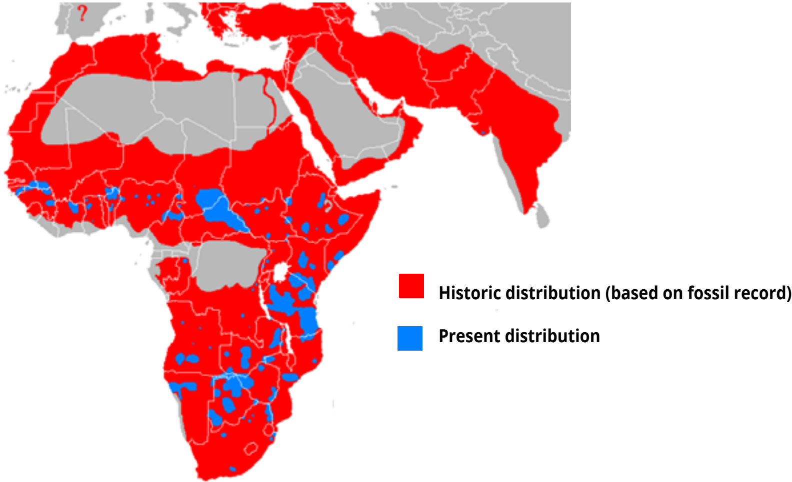
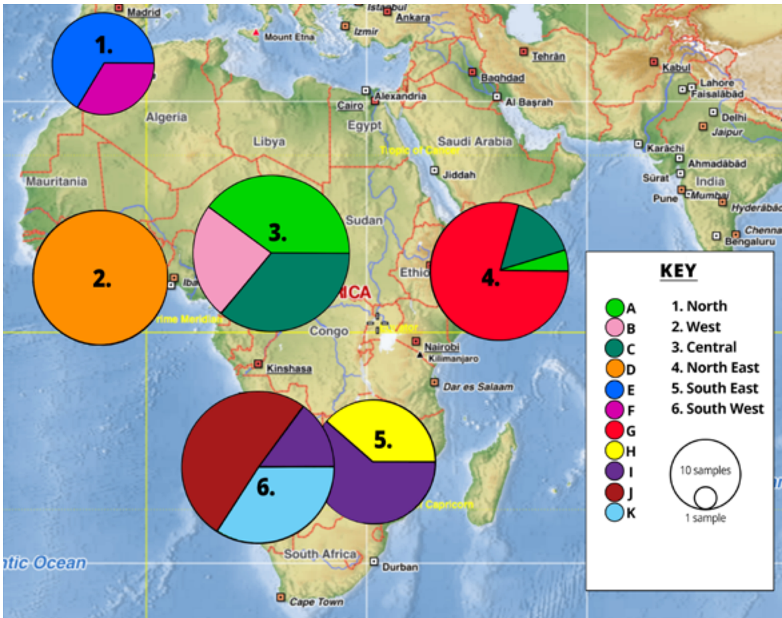
Haplotype Assignment

FAMILIARIZE yourself with *Panthera leo*'s phylogeography by reviewing its current and historical range, the geographic distribution of eleven major mtDNAa haplotypes, and the phylogeny of these haplotypes.

CURRENT AND HISTORICAL RANGE



THE GEOGRAPHIC DISTRIBUTION OF ELEVEN MAJOR MTDNA HAPLOTYPES



Haplotype Assignment, continued

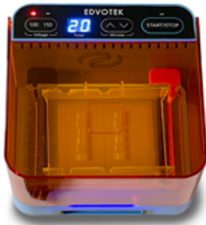
1. Use your electrophoresis results and the table (below) to **DETERMINE** the haplotype of both captive individuals.

Lion A Haplotype: ________ Lion B Haplotype: ________

	Enzyme 1 Fragments	Enzyme 2 Fragments
Haplotype A	2010, 920, 840, 510	2000, 1280, 650, 350
Haplotype B	1300, 1220, 920, 840	2000, 1780, 500
Haplotype C	1920, 920, 840, 600	2000, 1500, 780
Haplotype D	3440, 840	3650, 630
Haplotype E	2400, 840, 540, 500	2000, 1280, 1000
Haplotype F	2400, 840 ,700, 340	2000, 1280, 850, 150
Haplotype G	2830, 820, 630	3180, 1100
Haplotype H	2830, 1450	2540, 1300, 440
Haplotype I	2830, 1450	2540, 920, 820
Haplotype J	2830, 820, 630	3000, 1280
Haplotype K	2830, 820 ,630	3000, 980, 300

2. Based on this information where would you relocate lion cub A so that it was best adapted to the local environment? What do you know about the population of lions currently living in this location? Where would you relocate lion cub B? What do you know about the population at this location?

Related Products



EDGE™ Integrated Electrophoresis System
Runs one 10 x 7 cm gel
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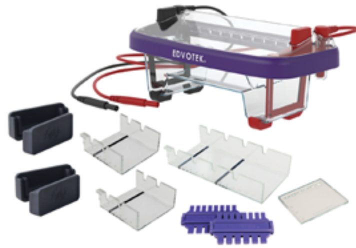

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