From Fossils to Phylogenies Part 1: Mass Spectrometry

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Dinosaur Vocabulary Words	Chemistry Vocabulary Words	Biology Vocabulary Words
Fossilization	Mass spectrometry	Central Dogma of biology
T. rex	Mass-to-charge ratio	Proteins
Mastodon	Ion	Peptide
Hadrosaur	Relative abundance	Protein
	Spectrum	Collagen

Background:

Imagine it is time for your lunch break. You take your sandwich outside and you sit down to enjoy your lunch with a beautiful view of Montana's Rocky Mountains. As you look up, you see what appears to be a bone sticking out of the side of a rock wall. That bone just so happens to be part of one of the best-preserved *Tyrannosaurus rex* fossils ever found. If you are Bob Harmon, a field crew chief of the Museum of the Rockies, that is exactly what happened. In the year 2000 Bob Harmon discovered a 68 million-year-old fossil, which is now named "B-Rex" after him.

Figure 1: Tyrannosaurus rex fossil

Tyrannosaurus rex lived 65 to 70 million years ago, in what is now the western parts of the United States. They were among the last of the large dinosaurs that lived on Earth. In certain rare cases, dinosaur bones were trapped in the Earth and were preserved until the present day, through a process called fossilization. Much of what we know about dinosaurs comes from the scientific study of the shape, appearance, composition, and location of fossils. Dinosaurs' bodies were made up of the same general types of biological building blocks seen in all animals, such as tissues, cells, and proteins. However, since fossilization involves the replacement of dinosaur bone tissues with minerals over millions of years, the bone's biological material has long since degraded. Therefore, fossils usually do not provide any molecular information about dinosaur proteins (i.e., they don't equip us to answer questions like "what *kinds* of proteins are in this fossil"). However, in the last decade scientists have been able to isolate dinosaur proteins from some remarkably well-preserved dinosaur fossils, which will finally enable dinosaurs to be studied at the *molecular level*.

You and your team members are being called in to work with paleontologist Dr. Mary Schweitzer, in order to extract protein material from the "B-rex" fossil. You will determine what type of proteins it contains, and use it to learn more about how dinosaurs and present-day animals fit together in the evolutionary tree of life. It

is your job obtain a protein sequence from the B-rex fossil to compare to the protein sequences from other present-day animals using bioinformatics tools (specialized computer programs for analyzing biological data), which you will learn about more about later.

To analyze the fossil sample, you will use mass spectrometry (MS), a standard technique in analytical chemistry. Protein mass spectrometry (MS) (Fig. 2) is a technique in which chemical molecules are ionized and the resulting ions are sorted based on their mass-to-charge ratio (Fig 2). Using MS and specialized computer programs, scientists can take a protein mixture of unknown composition and identify the types of proteins in it. The whole process is analogous to how fingerprints can identify individuals: when a crime lab is provided with a fingerprint from a crime scene, they run it through a large computer database of fingerprints from known individuals, in order to find a matching result. Similarly, in MS, once you have the spectrum of an unknown protein you can use it to search a database of spectra of *known proteins* in order to identify

Figure 2: This is a picture of a Mass Spectrometer. It takes small samples and determines the molecular composition.

the unknown protein. In today's activity, you will learn a bit about how this process works by identifying protein sequences from the fossilized bones of a *T. rex*, a Hadrosaur, and a mastodon (an elephant-like species that lived hundreds of thousands of years ago).
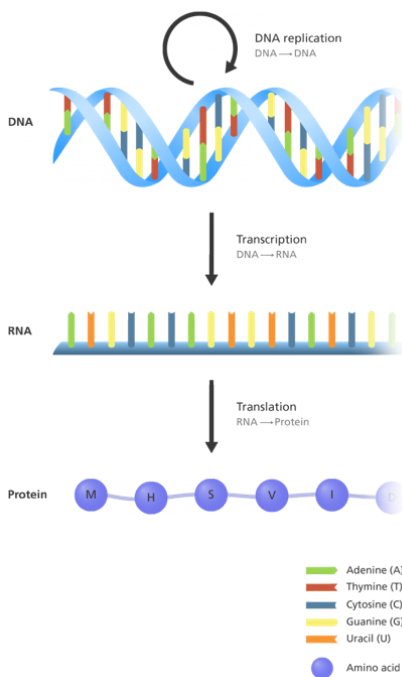

Figure 3. The central dogma is the first step is transcription of DNA into RNA. The second step is translating the RNA into proteins.

This is a two-part activity. In the first part, you will learn about how MS "fingerprints" protein samples based on ion charges and masses. In the next step, you will move up to the cellular level where proteins are made and used.

A bit about proteins and where they come from: The process by which proteins are made in cells is known as the Central Dogma of biology (fig 3). It is a two-step process involving DNA, RNA, and amino acids, which are the building blocks of proteins. DNA carries all of the genetic information for an organism. In order for the DNA to be decoded and utilized in the cell, it must be transcribed into RNA, and then translated into an amino acid chain (sometimes referred to as a peptide). Once the amino acid chain folds into its final shape (not shown in the figure), it is called a protein. Since you will be analyzing the protein content of a bone fossil, it is most likely that you will identify collagen proteins. Collagen proteins are sturdy and flexible in order to support our bones, and they make up 90-95% of the organic matter in bones.

Comparing DNA sequences across species is a powerful technique that scientists use to study the evolution of organisms. Sequences from specific DNA regions can be lined up with the same sequence from other organisms, in order to determine where mutations have occurred over time (Fig 4). This can be used to learn which animals have the same mutations, and how they evolved from each other. Although the B-rex fossil did not yield any DNA fragments, it did yield protein fragments. Since a protein is made up of a chain of amino acids (which has a corresponding letter sequence, like G for glycine, L for leucine, P for proline, and R for arginine (see Table 1)), it can be compared to other species' protein sequences in the same way as DNA. In the activity below, you will draw and then analyze the mass spectrum of an unknown protein fragment from the B-rex fossil. In effect, you will be doing the work of the mass spectrometer to fingerprint a peptide, and then you will be replicating by hand the exact search procedure that is today performed (much more efficiently!) by computers to match the protein spectrum to the spectrum from a previously studied peptide. Scientists use this procedure to identify the proteins that are present in a biological sample.

|	
Human	ATGAACGCATGC
Chimp.	ATGCACGCATGC
Gorilla	ATGCATGCATGC
Mouse	ATGCATGCATGC
Ancestor	ATGCATGCACGC
Horse	ATGCATGCACGC

Figure 4. This is an example of DNA sequences from multiple species lined up together. Species more closely related. This is how molecular biology can help determine evolutionary relationships.

Learning Objectives

After completing this activity, you should be able to:

- Describe what protein mass spectrometry is
- Read and analyze protein mass spectra
- Describe a biological application of identifying and sequencing proteins from sample of unknown composition

Materials

- A bag with Legos clusters, **please do NOT disassemble any of the Legos.**
- Transparent paper with a blank spectrum
- 12 mass spectra from known peptides – this will represent the "database" of spectra that you will search against (in real life, this database would contain spectra from hundreds of thousands of peptides!).

Amino Acid	3-Letters	1-Letter
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Procedure

You and Dr. Mary Schweitzer have collected a sample from the femur bone of the "B-rex" fossil. To understand in detail *how* the MS determines the molecular composition of a sample, you can read the supplementary document, "Spectrometry in a Suitcase". However, it is not necessary to understand for this activity. What is necessary is understanding the results after it has analyzed the sample and produced a spectrum.
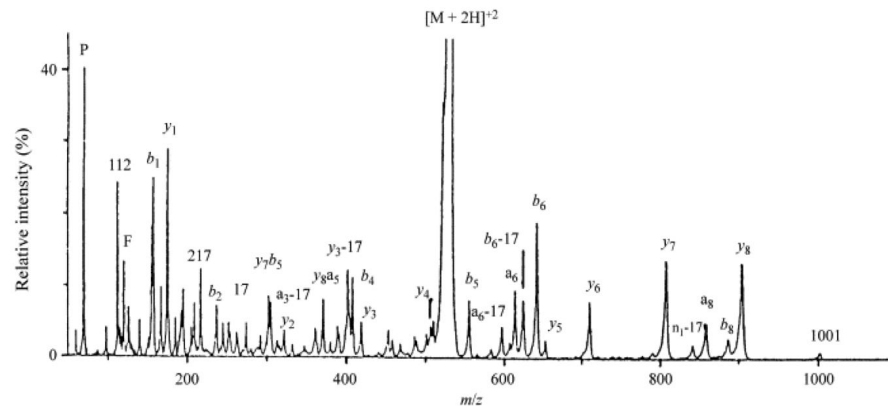
The graph above is what your results will look like; this is a <u>spectrum</u> of a peptide. The main parts of this graph that you need to understand are: the relative intensities, the m/z ratio, and what the peaks represent.

An <u>ion</u> is a molecule that has lost or gained an electron, changing its charge. Each peak corresponds to a different ion resulting from breaking up (or fragmenting) the peptide; the taller the peak, the more of that fragment ion is found in the sample. Therefore, the vertical axis (<u>relative intensity</u>, also referred to as relative abundance) indicates **how much** of each ion is present in the sample. For example, the tallest peak on the graph is $[M + 2H]^{+2}$, which means that $[M+2H]^{+2}$ is the most abundant ion present. The <u>mass-to-charge ratio (m/z)</u> of an ion is indicated by its **position** on the horizontal axis of the spectrum. To summarize:

- Peaks are specific ions resulting from fragmentation of the peptide
- The height of the peak (vertical axis) is **how much** of that ion is present
- The peak's location on the horizontal axis indicates its **mass-to-charge ratio** (so by definition, each peak in the spectrum has a unique mass-to-charge ratio).

You will be creating a spectrum based on the information contained in Lego clusters that will represent the ions recorded by the MS, for a specific (but unknown) peptide from the "B-rex" sample. You will be provided with a bag with 15 different clusters of Legos. When you remove them from the bag, please <u>do not disassemble the Legos (</u>if you disassemble them the rest of the activity will not work). Each cluster of Legos represents one ion, and thus, one peak on the spectra from the "B-rex" sample. In order to draw the peptide's spectrum, it is your job to decode what the Legos represent, based on the following rules:

The Lego cluster's....	Corresponds to the peak's….
Height (how many Legos **tall**)	Position on the horizontal axis (mass-to-charge ratio, or m/Z) 1 Lego brick = 100 m/Z (so, a cluster with three bricks would be 300 m/Z)
Color of the "small brick" on the cluster	The cluster's precise position on the horizontal axis (Clusters will only have a small, differently colored brick when used when two or more clusters have the same height in Lego bricks). Add the following to your peak's m/Z ratio, based on the color of the cluster's small Lego brick: ● black: 00 ● gray: 30 ● green 50 brown: 80
Width (size of the Lego)	Height on the spectrum (i.e., abundance) Use the following list to convert between Lego brick size and peak height on the vertical axis: ● 2x1 Lego: <15 ● 2x2 Lego: 15 - 35 ● 2x3 Lego: 35-60 ● 2x4 Lego: >60

On the next page, you will find examples of what a peak on your spectrum would look like based on the properties of a Lego cluster.

The final result from the mass spectrometer is the completed spectrum that you draw. This spectrum represents the ions in a peptide from the *T. rex* fossilized bone. In reality, this unknown spectrum would be used to search through a large database of mass spectra of known peptides, in order to find the closest match to a known peptide. To mimic this process, you will be provided with sheets of paper containing 12 known spectra that are already identified to specific peptide amino acid sequences. Take your drawn spectra on the transparency and line up the axes with each known spectra provided. The closest match to your spectra will tell you the exact sequence of amino acids from the peptide. In the next activity, you will learn how to use bioinformatics tools to identify what peptide this sequence belongs to.

Examples of Lego Clusters:

- A cluster that is 5 Legos tall, all size 2x4, and an additional black Lego attached on top it.
  - Since it is 5 Legos tall with a black additional Lego added, its mass-to-charge (horizontal axis) is 500, with a relative abundance over 60. You would draw the peak like this:
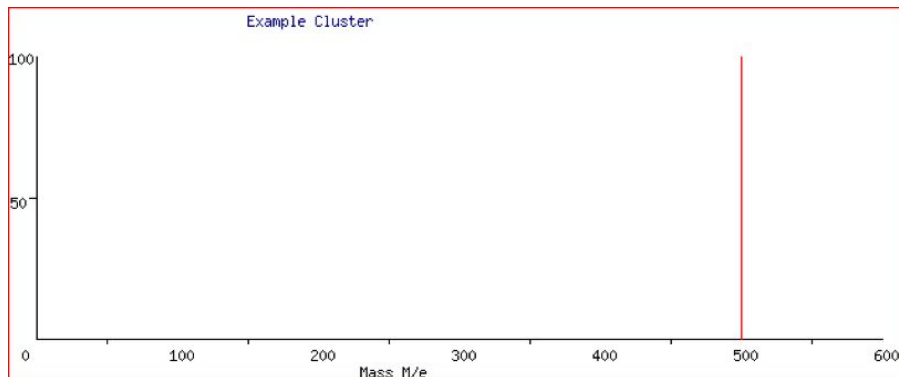
- A cluster that is 3 Legos tall, all size 2x3, and an additional grey Lego on top.
  - Since it is 3 Legos tall with a grey additional Lego, its mass-to-charge (horizontal axis) is 330, with a relative abundance between 35-60. You would draw the peak like this:
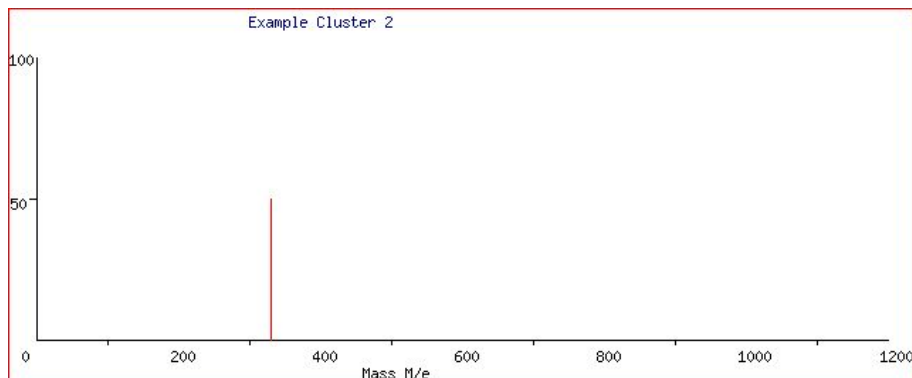
- A cluster that is 7 Legos tall, all size 2x1, and an additional green Lego on top.
  - Since it is 7 Legos tall with a green additional Lego, its mass-to-charge (horizontal axis) is 750, with a relative abundance less than 15. You would draw the peak like this:
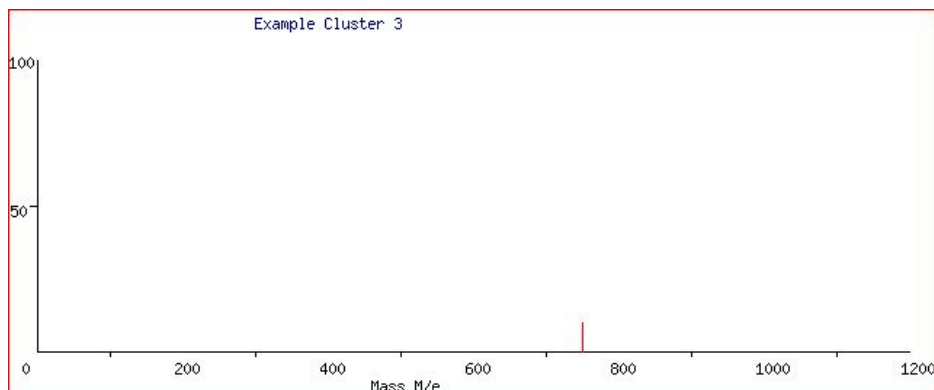
Analyzing Results

1. What is the amino acid sequence of the peptide from the B-rex fossil?

2. Would MS be a good method for determining protein amino acid sequences in live animals?

3. What is the difference between a protein and a peptide?

Evaluating Results

1. What are any other real-world applications of mass spectrometry that you can think of?

2. Why might it be beneficial to know the order of the amino acids in a peptide sequence, rather than just the name of the protein (like "isoform 3X collagen") from which the unknown peptide was derived? (*Figure 4)*

3. If you had to compare your spectrum against a stack of 200,000 spectra, how long do you think that process would take? Do you think a modern computer is likely to be faster? (Think about other types of searches you perform using computers, such as Google searches or Spotlight searches).

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USING BLAST TO IDENTIFY PROTEINS THAT ARE EVOLUTIONARILY RELATED ACROSS SPECIES

HOW CAN BIOINFORMATICS BE USED AS A TOOL TO DETERMINE EVOLUTIONARY RELATIONSHPS AND TO BETTER UNDERSTAND PROTEIN HERITAGE?

Background

Between 1990–2003, scientists working on an international research project known as the Human Genome Project were able to identify and map the ~20,000 genes that define a human being. As you learned in Activity 2, a gene's DNA sequence is the template that dictates – according to a three-letter code – the sequence of amino acids out of which a specific protein is made. Amino acids have their own code as well, seeing as there are 20 amino acids and 64 codes. Protein-coding genes are an important class of molecular "building blocks" for the human body. In addition to human genes, scientists have also sequenced the genes of hundreds of other species across the tree of life. These gene sequences are freely available for anyone in the world–including you–to access via a web browser and examine.

How are gene sequences useful for science? First, mapping DNA sequences to locate specific genes allows scientists to align the genes across species (for example, a pair of human and mouse genes). These genes might be "similar" in that they evolved from the same common ancestral gene. We call the two genes in such a pair orthologs. Figure 1 is an example of orthologs.
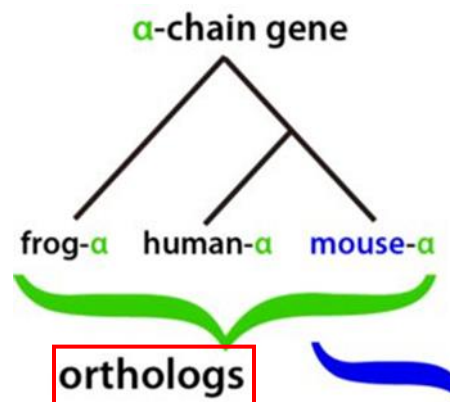

Often, genes that are orthologs will have similar functions in their respective species, so scientists can learn about the function of a human gene by studying that gene's ortholog in another species, such as in a fruit fly or a mouse. Second, comparing related genes among two or more species can provide insight into the species' evolutionary relationships, more than comparing the

species' physical appearance or characteristics. Finally, knowing the sequences and locations of human genes helps enable scientists to investigate how variation in a gene's sequence across humans leads to variation in human traits: eye color, hair color, height, or risk of various health conditions.

Suppose you are a scientist who has identified a fruit fly gene that, when the gene is disrupted in fruit fly embryos, results in a developmental abnormality. You would likely want to know: does that gene have an ortholog in humans? If so, do mutations in that human gene cause disease, and how do the fruit fly gene and human gene differ in terms of DNA or amino acid sequence? In theory, you could answer these questions by comparing paper printouts of the sequences of each of the ~20,000 human protein-coding genes to a printout of the sequence of your fruit fly gene, in order to find a potential ortholog that would have a close sequence match. But this process, if carried out by hand, would take many years. Fortunately, computers can carry out the same search in seconds or minutes. The software program that would be used for such a search is but one example of a broad class of bioinformatics software tools and computational methods. More precisely, bioinformatics is a field of science that blends biology, computer science, statistics, and mathematics in the systematic analysis of biological data and information. Using bioinformatics tools, entire genomes can be quickly compared to detect genetic similarities and differences. An extremely powerful and versatile bioinformatics tool is the Basic Local Alignment Search Tool (BLAST). Using BLAST, you can input a DNA or amino acid sequence and search entire genomic libraries for identical or similar known sequences.

In this activity, you will use BLAST to analyze amino acid sequences from several extinct species, determine what proteins they come from, and find the proteins' orthologs in modern-day animal species. You will then use the information from your BLAST analysis to create a phylogenetic tree. A phylogenetic tree is a diagram that depicts the evolutionary relatedness of species or groups of closely related species. Figure 2 is a simple phylogenetic tree.
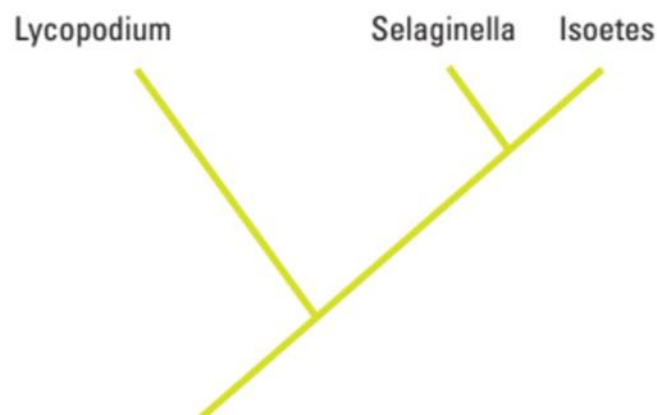
Note that the phylogenetic tree is shaped like a tree, with the endpoints of each branch representing a specific group of organisms. The closer the two groups are located to each other, the more recently they shared a common ancestor. For example, Selaginella (spikemoss) and Isoetes (quillwort) share a more recent common ancestor than the common ancestor that is shared by all three organisms.

Figure 3 includes additional details, such as the evolution of particular physical structures called derived characteristics. Note that the placement of the derived characteristics corresponds to when (in a general, not a specific, sense) that character evolved; every species above the character label possesses that structure. For example, tigers and gorillas have hair, but lampreys, sharks, salamanders, and lizards are not hairy.
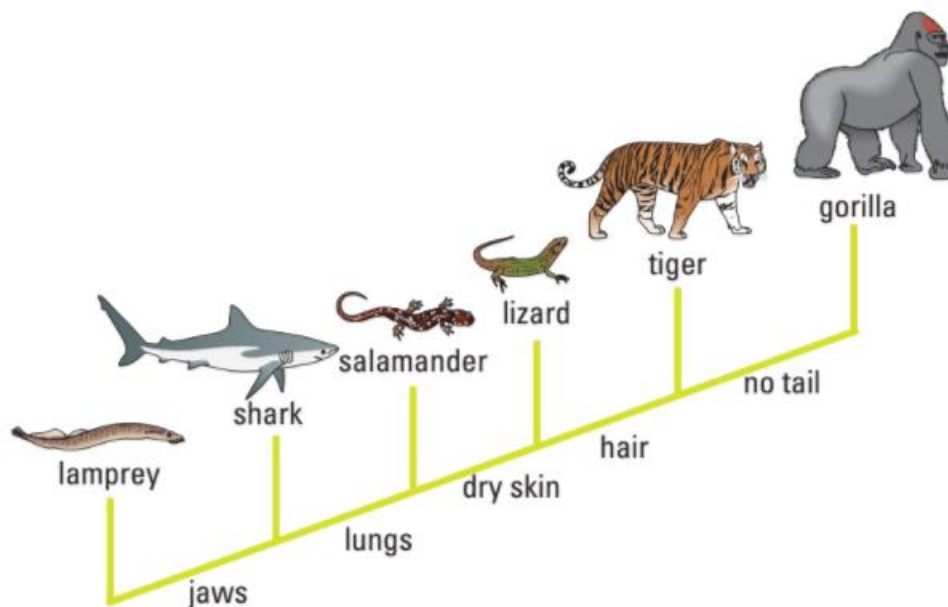
The phylogenetic tree above can be used to answer several questions. Which organisms have lungs? What three structures do all lizards possess? According to the tree, which structure — dry skin or hair — evolved first?

Historically, physical characteristics were used for deciphering the evolutionary relationships among species; however, today scientists rely heavily on gene sequence information as well. Chimpanzees and humans share 95%+ of their DNA, which would place them closer together on a phylogenetic tree. Humans and fruit flies share approximately 60% of their genes, which would place them farther apart on a phylogenetic tree.

Can you draw a phylogenetic tree that depicts the evolutionary relationship among humans, chimpanzees, fruit flies, and mosses?

Learning Objectives

- Understand how phylogenetic trees depict evolutionary relationships
- Understand how the bioinformatics tool BLAST enables the identification of evolutionarily related proteins in different species (orthologs)
- Be able to critically analyze the results form a BLAST analysis to assess consistency with the current phylogenetic tree for various animal species groups.
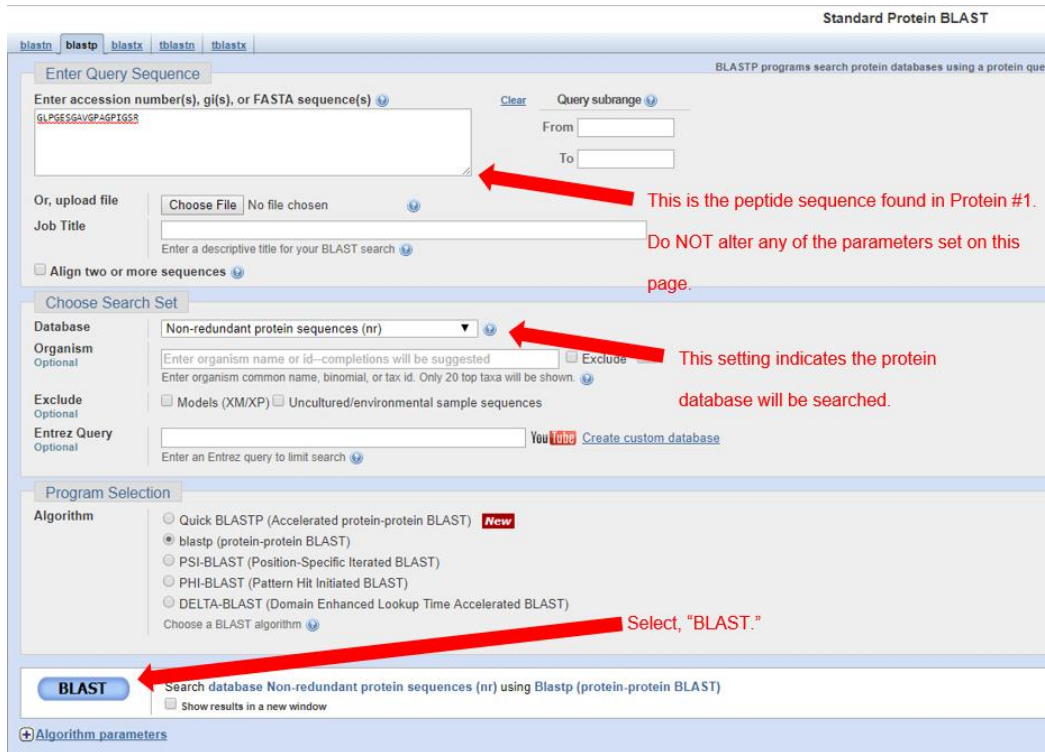
Procedure

You are a member of a scientific team that has discovered three unusually well-preserved fossilized bone specimens from an extinct mastodon species (*Mammut americanum*) and two dinosaur species: *Tyrannosaurus rex* and the hadrosaur *Brachylophosaurus canadensis.* Upon careful examination of the fossil, small amounts of *soft tissue* have been discovered, which is unusual because normally soft tissue does not survive over this time-scale. From the soft tissue in the bone specimen, your team was able to extract amino acid sequences of several protein fragments–*the first time an actual dinosaur protein fragment has ever been sequenced!* Your task is to use BLAST to compare these amino acid sequences to protein sequences from other species. Then, use the results from the BLAST analysis to determine where these extinct species branch off from the evolutionary tree (Figure 4) in relation to modern animals like birds, crocodiles, and mammals.
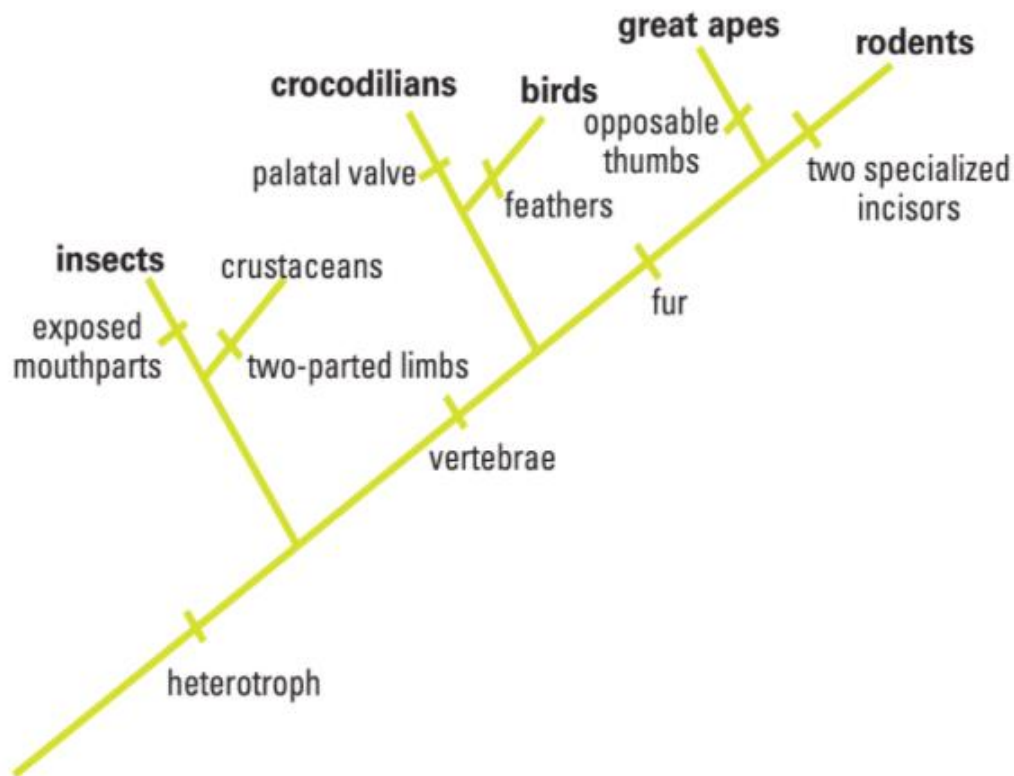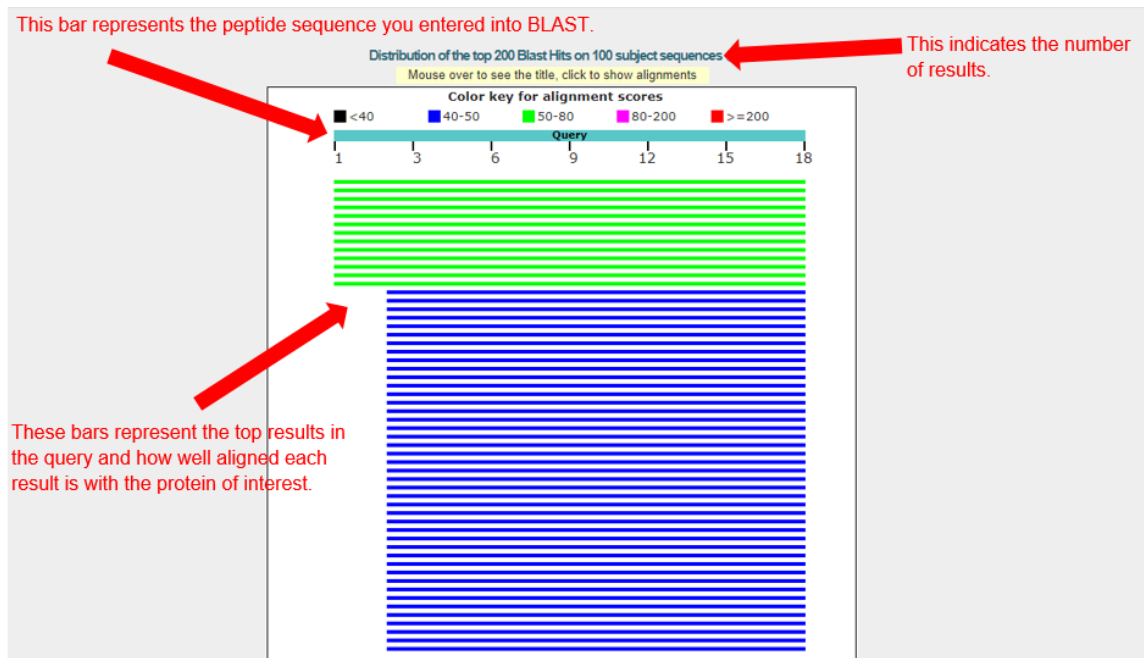
I) Step 1: Form an initial hypothesis about where the mastodon and the two dinosaur species belong on the phylogenetic tree (Figure 4) based on what you know about the physical characteristics of mastodons and dinosaurs. Mark the locations as "branches" from the tree on Figure 4.

II) Step 2: Locate and download the "BLAST Protein Fragments" file for the *T. rex* bone specimen.

III) Step 3: Insert the gene sequence into BLAST by doing the following:

    a) Use your web browser to access the BLAST homepage: https://blast.ncbi.nlm.nih.gov/Blast.cgi

    b) Click on "Protein BLAST" from the menu at the bottom of the page

c) Under "Enter Query Sequence," paste the first amino acid sequence from "BLAST Protein Fragments."

d) A screen will appear with the parameters for your query already configured. NOTE: Do not alter any of the parameters. Scroll down the page and click on the "BLAST" button at the bottom left.

e) After collecting and analyzing all the data for that particular amino acid sequence (see instructions below), repeat this procedure for the other two amino acid sequences (mastodon and hadrosaur).

IV) Step 4: The results page has two sections. The first section is a graphical display of the matching sequences.

This bar represents the peptide sequence you entered into BLAST.

This indicates the number of results.

These bars represent the top results in the query and how well aligned each result is with the protein of interest.

Scroll down to the section titled "Sequences producing significant alignments." The species in the list that appears below this section are those with sequences identical to or most similar to the protein of interest. The most similar sequences are listed first, and as you move down the list, the sequences become less similar to your protein of interest. Each matching protein sequence is annotated with a description on the left. Based on scanning the descriptions in the table, what type of protein did your amino acid sequence come from? Do a Wikipedia search for this protein name. Does it make sense that this type of protein would be found in a bone sample?

The E value is the likelihood that a match occurred purely by chance. The lower the E value, the better the match.

This is the protein and species name that matches the peptide of interest. Phenotype is sometimes identified as well.

Click the reference number for a specific sequence to learn more about that sequence.

If you click on a particular species listed, you'll get a full report that includes the classification of the species, the research journal in which the protein was first reported, and the sequences of bases that appear to align with your protein of interest.

GenPept ▾   Under the BLAST table is a full report of each result.   Send to: ▾

RecName: Full=Collagen alpha-2(I) chain; AltName: Full=Alpha-2 type I collagen

UniProtKB/Swiss-Prot: P0C2W4.1
Identical Proteins   FASTA   Graphics

Go to: ☑

```
LOCUS       CO1A2_TYREX            18 aa            linear   VRT 05-OCT-2016
DEFINITION  RecName: Full=Collagen alpha-2(I) chain; AltName: Full=Alpha-2 type
            I collagen.
ACCESSION   P0C2W4
VERSION     P0C2W4.1
DBSOURCE    UniProtKB: locus CO1A2_TYREX, accession P0C2W4;
            class: standard.
            created: May 1, 2007.
            sequence updated: May 1, 2007.
            annotation updated: Oct 5, 2016.

            xrefs (non-sequence databases): PRIDE:P0C2W4, GO:0005581,
            GO:0005578
KEYWORDS    Collagen; Direct protein sequencing; Extinct organism protein;
            Extracellular matrix; Repeat; Secreted.
SOURCE      Tyrannosaurus rex
  ORGANISM  Tyrannosaurus rex
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Archelosauria; Archosauria; Dinosauria; Saurischia; Theropoda;
            Coelurosauria; Tyrannosauridae; Tyrannosaurus.
REFERENCE   1  (residues 1 to 18)
  AUTHORS   Asara,J.M., Schweitzer,M.H., Freimark,L.M., Phillips,M. and
            Cantley,L.C.
  TITLE     Protein sequences from mastodon and Tyrannosaurus rex revealed by
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This indicates the protein sequence and the type of molecule it is.

This identifies the species the protein sequence originated from.

This shows who discovered the protein sequence.

Analyzing Results

Recall that species with common ancestry will share similar genes. The more similar genes two species have in common, the more recent their common ancestor and the closer the two species will be located on a phylogenetic tree.

As you collect information from BLAST for each of the protein files, you should be thinking about your original hypothesis and whether the data support or cause you to reject your original placement of the fossil species on the phylogenetic tree.

For each BLAST query, consider the following:

- The higher the alignment score, the closer the alignment (the more similar the fossil protein and its matching protein from the database).
- The lower the E value, the less likely the alignment score this high occurred "by chance".
- Sequences with E values less than 10^{-4} (depicted as 1e-04 in the BLAST results table) can be considered highly likely to be evolutionarily related, i.e., orthologs.

1. What is the likely protein that your fossil-derived amino acid sequence came from?
2. What species in the BLAST result has the most similar amino acid sequence to your fossil-derived amino acid sequence?
3. Where is that species located on the Figure 4 phylogenetic tree?

4. How similar is that amino acid sequence to your fossil-derived amino acid sequence?
5. What species has the next most similar amino acid sequence to your fossil-derived amino acid sequence?

Based on what you have learned from the sequence analysis and what you know from the structure, decide where the fossil specimens (*M. americanum*, *T. rex*, or *B. canadensis*) belong on the phylogenetic tree for modern-day animals. If necessary, redraw the phylogenetic tree you created before.

Evaluating Results

Compare and discuss your phylogenetic tree with your classmates. Does everyone agree with the placement of the fossil specimens? If not, for which species is there disagreement?

On the main page of BLAST, under "Specialized searches," click on the link "SmartBLAST." What phylogenetic trees do you see when you put in different collagen sequences for the BLAST search? How does the lack of other sequenced species impact the proper analysis of the protein data used in this lab?

What other data could be collected from the fossil specimens to more convincingly determine their species' locations in the evolutionary tree?

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From Fossils to Phylogenies Part 3: How Dinosaurs Fit into the Evolutionary Tree of Life

Written by: Baylee Goodwin, Dane Besser, and Stephen A. Ramsey

Vocabulary Words	
Phylogenetics	Most Recent Common Ancestor (MRCA)
Taxa	Descendants
Node	Sister Clades
Speciation event	Outgroup

Background

During the Mass Spectrometry and BLAST activities, you were given amino acid sequences that had been recovered from a fossilized bone specimen of a *Tyrannosaurus rex* (as well as sequences from a Hadrosaur and a Mastodon). You learned how to input the *T. rex* amino acid sequence into BLAST to identify which present-day animals are most closely related to the *T. rex*. In this activity, you will learn how to use a computer to analyze related amino acid sequences from a variety of animals to gain insight on their evolutionary relationships.

Phylogenetics is the study of evolutionary relationships among a set of species, or a set of groups of animals. Groups of animals that can be studied using phylogenetic methods include taxa (plural of taxon), where a taxon is a group of organisms that share similar characteristics, like "plants" or "animals". Evolutionary relationships can be visualized using a phylogenetic tree (Figs. 1 and 3). The root of the tree is the start of the evolutionary lineage being depicted. In
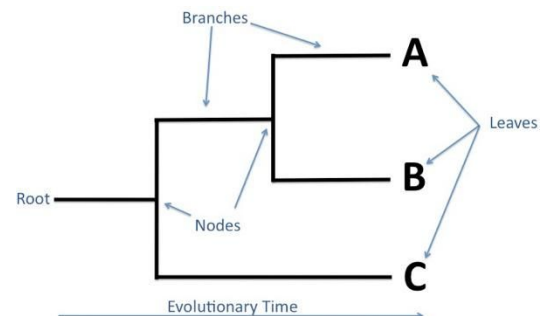

Figure 1: This is an example of a phylogenetic tree with labels of important characteristics.

the tree depicted in Fig. 1, as you move from the left to the right, you are moving forward in time. As time passes, you can see that nodes (which represent ancestral species) diverge in two directions. Where the lines

Figure 2: This is an example of DNA sequences from multiple species lined up together. Species share mutations that other do not have are more closely related. This is how can help determine evolutionary relationships.

of the tree terminate on the right-hand side are called "leaves"; these represent a species or taxa, i.e., the descendants of the tree. A split in the tree depicts a speciation event. A speciation event is when an ancestral group of animals (a node) separates and evolves into two brand new, different groups of animals, which may be nodes or leaves. To be precise, a node represents the most recent common ancestor (MRCA) of the groups or species that branch off from the node. For example, in Figure 3, leaves A and B are taxa that diverged from a node (MRCA) found at the node that joins A and B branches. Phylogenetic trees are a useful way to depict how animals are related to one another. In Figure 3, animals from groups A and B are more closely related to each other than they are to

animals in group C. Therefore, A and B would be called <u>sister clades</u>; since A is the most closely related group to B, and B is the most closely related group to A in the tree. Because Group C is the most distantly related to the other groups in the tree, it is given a special name; it is referred to as the tree's <u>outgroup</u>.

There are two main methods for determining the evolutionary relatedness of a set of taxa: morphology and molecular data. Morphology refers to the physical features of animals (e.g., shape, weight, color, anatomic structure, etc.). Molecular data refers to DNA or amino acid sequences of the organism's genes. In general, molecular phylogenetic methods are thought to be more accurate than morphology since two distantly related organisms can have similar physical characteristics that arise from distinct mutations.

When mutations arise in the DNA sequence of an organism, they can result in changes to the translated amino acid sequence of a protein. For example, the original DNA sequence in a small portion of a gene might have read ATAAGT, but after the mutation it reads ATAACT (i.e., a G was replaced with a C). This changes the amino acid in the sequence from a leucine codon (one of the twenty types of amino acids found in eukaryotes) to a stop codon (a three-letter DNA sequence that signals the end of the protein that is encoded by the gene), which results in the cell making a shortened protein whose function may substantially differ from the original full-length protein. When a mutation is present in an organism's cell, it can be passed on from the organism to its offspring, which is how animals evolve on a molecular scale.
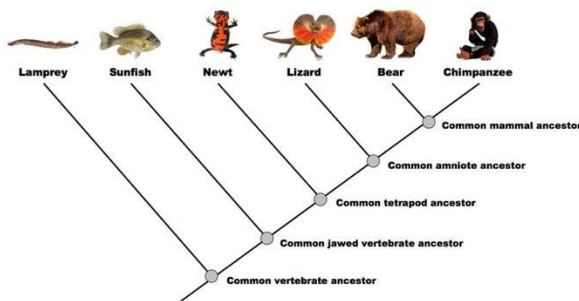

Figure : This is an example of a basic phylogenetic tree. It highlights where the most recent common ancestors (MRCA) are found on the tree, and which animal groups evolved from the ancestor.

The genetic differences between two species, such as a bird species and a lizard species, represent the accumulation of billions of mutations over many millions of years. The differences in the DNA (or, as we will study today, protein) sequences among a set of representative species can be used to determine how the species are related. Molecular phylogenetics is based on a fundamental assumption that more closely related organisms will have more similar protein sequences, and the more distantly related organisms will have more dissimilar protein sequences.

To create a phylogenetic tree, the first step is to obtain protein sequence data from a set of animal species that we want to compare. We will be searching for the "alpha-2 type 1 collagen" protein sequence since that is what the scientists were able to extract from the fossilized femur bone of the *T. rex*. Collagen is relatively well-conserved across species, which is why it is a good choice for using amino acid sequences to build a phylogenetic tree. When a protein is "well-conserved", it means that the protein is found in multiple species that are distantly related; collagen is a well-conserved protein found in all animals with true bone. In order to find the collagen sequence, you will conduct a search in an online database called GenBank. The alpha-2 type 1 collagen protein sequences have already been collected for you for most of the animals. However you still need to collect the appropriate amino acid sequence for the *T. rex*.

The animal species for which you will be building a phylogenetic tree are: chicken, rainbow trout, human, dog, cattle, a toxodon species (*Toxodon platensis*), a mastodon species (*Mammut americanus*), a salamander species (*Ambystoma mexicanum*), a frog species (*Xenopus tropicalis*), and *T. rex*. (Note: these species were selected for this activity because they have alpha-2 type 1 collagen protein sequences available in the GenBank database). These animals will allow you to analyze where the *T. rex* fits in the phylogenetic tree relative to birds, mammals, amphibians, and fish, which are four out of the five major vertebrate taxonomic groups (no alpha-2 type 1 collagen sequences were available for any reptile species). It also allows you to see how other extinct animals like mastodon and toxodon relate to present-day animals.

Learning Objectives

- Understand how amino acid sequences can be compared using a computer program in order to reconstruct a phylogenetic tree
- Learn how to obtain protein or peptide sequence data in the correct formatting
- Understand how to interpret a phylogenetic tree

Procedure

Obtain *T. rex* amino acid sequence data:

1. Use your web browser to go to the following site: http://www.ncbi.nlm.nih.gov

(NCBI stands for the National Center for Biotechnology Information, which is a branch of the National Library of Medicine that hosts the GenBank database). The NCBI website is free for the public to access, and it contains libraries of genomic, genetic, and biomedical data. We will be using it to access protein sequences in GenBank. GenBank contains the sequences of many genes and their protein products, for hundreds of thousands of different species.

2. In the search bar at the top of the web page, type in "collagen type I alpha 2 T rex" or "α2t1 collagen T rex" and select the protein database from the drop-down menu. Do not include the quotations in the search (The "type I" is a capital I, not the number 1).
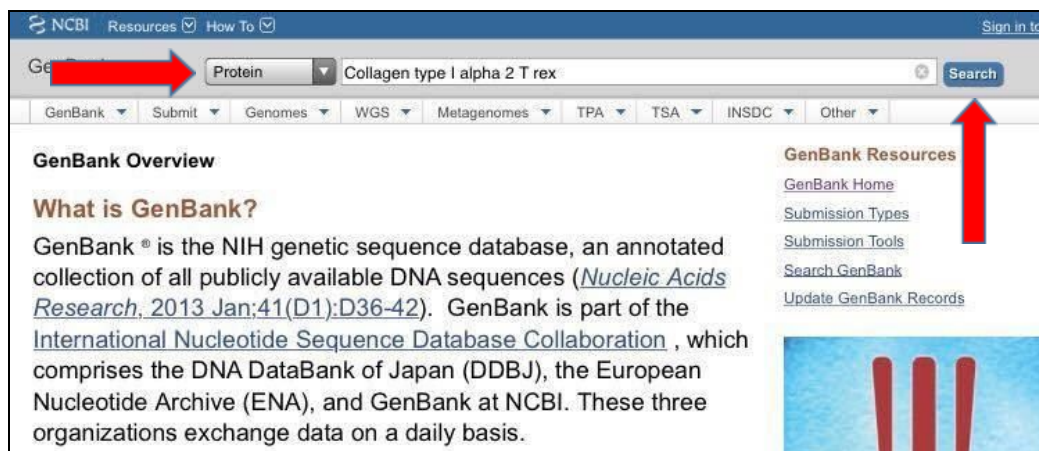
3. Click on the blue Search button.
4. It will provide a list with the top results relevant to your search. 3 to 4 items should be present under the search, so be sure to click on the result that says alpha-**2**(I) chain, **not** alpha-1(I).

The results display every known protein sequence that matches with the key words "alpha 2", "type 1", and "*T. rex*". It will show results that do not precisely match your search, so be sure to fully read the names of the results. If you were to broaden your search to "alpha 2 collagen" it will result in hundreds of matches, rather than only three or four.

5. Once you select the correct result, it will open up a detailed results page (pictured below). In order to make sure that you have selected the correct result, look at the column on the left hand side of the page. The fourth heading down should say "source organism", and the organism should be *Tyrannosaurus rex*. If that is not correct, hit the back button and retype the search query exactly as shown in Step 3. Once you reach the correct Protein record page, click on the FASTA button underneath the protein's name in bold black writing.

GenBank should then display a FASTA record page, like this:

"FASTA" (an abbreviation for "Fast-All") is the simple text-based file format that is often used to transmit DNA or amino acid sequences from one computer program to another. In a FASTA file, the DNA nucleotides or protein amino acids are represented by individual letter codes. The FASTA file format begins with a ">" (greater than) character followed by a description, which is then followed by lines of sequence data.

6. On the FASTA record page, select and **copy all of the text from the ">" all the way to the end of the amino sequence.**

7. Paste this sequence into the phylogeny building program in order to include the *T. rex* in your phylogenetic tree.

Constructing a phylogenetic tree using MAB:

1. With your web browser, open the following web page: http://www.phylogeny.fr/alacarte.cgi

This page is the MAB (Methods and Algorithms for Bioinformatics) Phylogeny Analysis tool, which you will use to generate a phylogenetic tree. (Note: most of the MAB website is in French, but the form that you will use to run the Phylogeny Analysis tool is in English.)

2. This link will open up directly to "A la carte" mode. Under "Workflow Settings" insert a name for your analysis.

3. Scroll to the bottom of the page and select "create workflow". Do <u>not</u> change any of the settings; they are already set to the correct options for creating your phylogenetic tree.

4. MAB should open up to a new browser tab: "Data and Settings". This tab gives you the option to upload your file or paste the sequence. Copy and paste the *T. rex* sequence into the big text box below titled "Input Data" in the MAB browser window. You need to change the description to "T-rex". **Delete** the text "<u>P0C2W4.1 RecName: Full=Collagen alpha-2(I) chain; AltName: Full=Alpha-2 type I collagen</u>" and **replace** it with "T-rex". (Be sure to leave the ">" character, otherwise MAB will not recognize the format. This step is important, because now instead of the tree reading the full protein name, it will read the name of the animal.)

"A la Carte" Mode

Alignment MUSCLE ⇒ Curation Gblocks ⇒ Phylogeny PhyML ⇒ Tree Rendering TreeDyn

Alignment

1. Overview | 2. Data & Settings | 3. Alignment | 4. Curation | 5. Phylogeny | 6. Tree Rendering

Input Data

Upload your set of sequences in FASTA, EMBL or NEXUS format from a file:
Browse...   No file selected.
Or paste it here (load example of sequences)

Clear

"A la Carte" Mode

Alignment MUSCLE ⇒ Curation Gblocks ⇒ Phylogeny PhyML ⇒ Tree Rendering TreeDyn

1. Overview | 2. Data & Settings | 3. Alignment | 4. Curation | 5. Phylogeny | 6. Tree Rendering

Input Data

Upload your set of sequences in FASTA, EMBL or NEXUS format from a file:
Browse...   No file selected.
Or paste it here (load example of sequences)

>t-rex
GLPGESGAVGPAGPIGSR

>Human
MLSFVDTRTLLLLAVTLCLATCQSLQEETVRKGPAGDRGPRGERGRPPGPPGRDGEDGPTGPPGPPGPPGP
PGLGGNFAAQYDGKGVGLGPGPMGLMGPRGPPGAAGAPGPQGFQGPAGEPGEFGQTGPAGARGPAGPPGK
AGEDGHPGKPGRPGERGVVGPQGARGFPGTPGLPGFKGIRGHNGLDGLKGQPGAPGVKGEPCAPGENGTP
GQTGARGLPGERGRVGAPGPAGARGSDGSVGPVGPAGPIGSAGPPGFPGAPGPKGEIGAVGNAGPAGPAG
PRGEVGLPGLSGPVGPPGNPGANGLTGAKGAAGLPGVAGAPGLPGPRGIPGPVGAAGATGARGLVGEPGP
AGSKGESGNKGEPGSAGPQGPPGPSGEEGKRGPNGEAGSAGPPGPPGLRGSPGSRGLPGADGRAGVMGPP
GSRGASGPAGVRGPNGDAGRPGEPGLMGPRGLPGSPGNIGPAGKKEGPVGLPGIDGRPGPIGPAGARGEPG
NIGFPGPKGPTGDPGKNGDKGHAGLAGARGAPGPDGNNGAQGPPGPQGVQGGKGEQGPPGPPGFQGLPGP
SGPAGEVGKPGERGLHGEFGLPGPAGPRGERGPPGESGAAGPTGPIGSRGPSGPPGPDGNKGEPGVVGAV
GTAGPSGPSGLPGERGAAGIPGGKGEKGEPGLRGEIGNPGRDGARGAPGAVGAPGPAGATGDRGEAGAAG
PAGPAGPRGSPGERGEVGPAGPNGFAGPAGAAGQPGAKGERGAKGPKGENGVVGPTGPVGAAGPAGPNGP
PGPAGSRGDGGPPGMTGFDGAAGRTGPPGPSGISGPPGPPGPGPACKEGLRGPRGDGQPVGRTGEVGAVGPP

Maximum number of sequences is 200 for proteins and 200 for nucleic acids.
Maximum length of sequences is 2000 for proteins and 6000 for nucleic acids.

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5. Now you will need to paste the sequences for all of the other animals being compared in your phylogenetic tree. Scroll down to the end of this PDF and copy everything under the heading "Collagen Sequence Data (Copy and paste everything below, including the ">"):". Paste all of the sequences into the textbox below the *T. rex* sequence.

6. Scroll down to the bottom and enter your email address if you wish to be emailed your tree. If not, select "submit". Do not change any settings before hitting submit.

After clicking the Submit button, MAB will display a brief animation of a phylogenetic tree. During this time, MAB is aligning the sequences and then comparing them.[1]

7. MAB Phylogeny Analysis may take anywhere from 1 to 5 minutes to construct the phylogenetic tree. Once it loads, it should look like this:

"A la Carte" Mode

Alignment MUSCLE ⇒ Curation Gblocks ⇒ Phylogeny PhyML ⇒ Tree Rendering TreeDyn

1. Overview | 2. Data & Settings | 3. Alignment | 4. Curation | 5. Phylogeny | 6. Tree Rendering

Tree Rendering results

- Salamander
- Frog
- 0.76 Human
- Mastodon
- Cattle
- 0.8
- 0.83 t-rex
- Chicken
- 0.93
- Toxodon
- Dog
- Trout

0.3

Figure 1: Phylogenetic tree
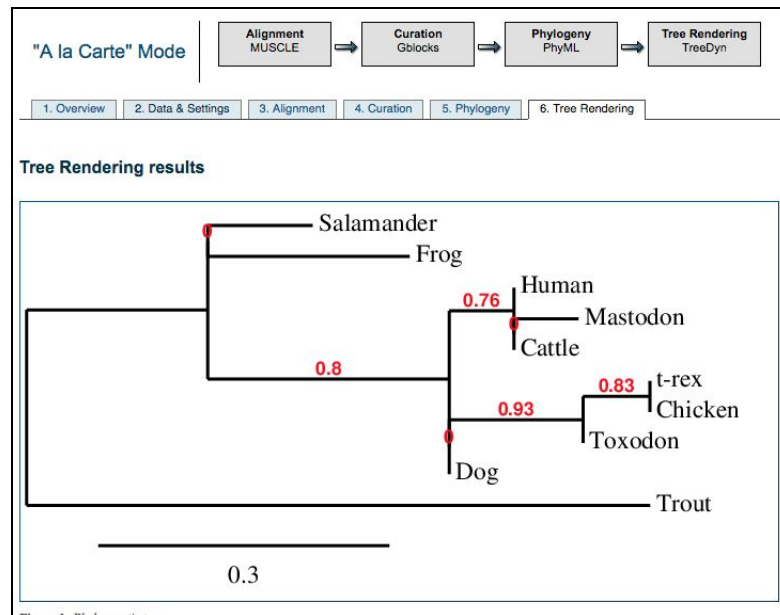
[1] This program uses a common method for aligning the sequences, called MUSCLE (Multiple Sequence Comparison by Log-Expectation). This step is important because it uses an algorithm to align each peptide sequence in order to accurately predict where mutations occurred that signal how the animals evolved. If the sequences are not aligned they cannot be used to generate a phylogenetic tree.

8. Scroll down to the "Tree Styles" section toward the bottom of the web page: Click on the button for "Cladogram" (in this context, "cladogram" is telling MAB to show a phylogenetic tree without scaling the length of tree branches based on degree of dissimilarity).

Tree style:
- ◉ Phylogram
- ○ Cladogram (ignore branch lengths)
- ○ Radial (by Drawtree)
- ○ Radial (by TreeDyn)
- ○ Circular
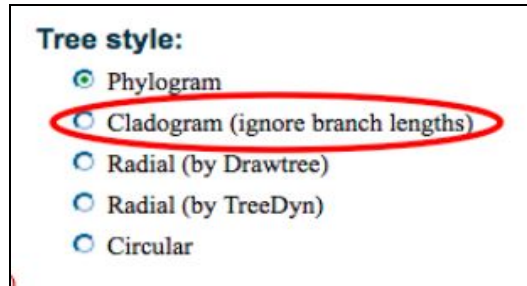
This will make it easier to read and understand the evolutionary relationships. The tree should now look like this:
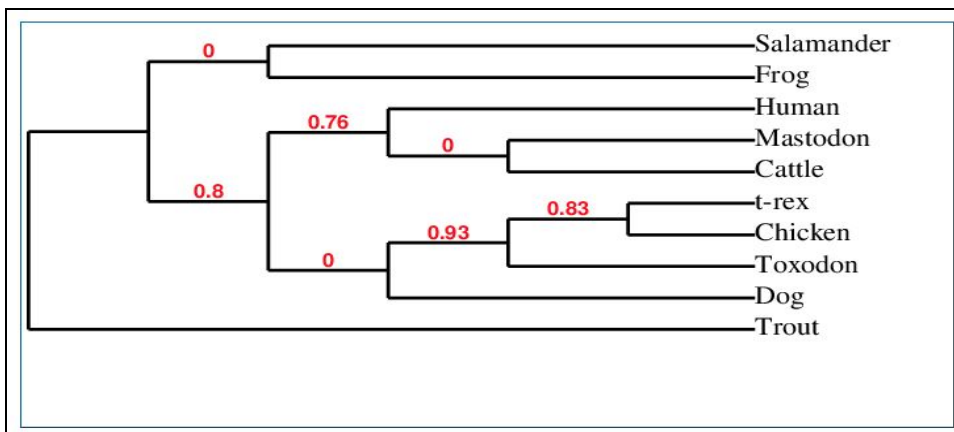
9. The final setting that needs to be adjusted is under "Display:". Change the setting from "Branch support values" to "none".

Display:
- ◉ Branch support values ○ Branch lengths ○ None   color: red
- ☐ Display branch support values in %
- ☑ Display legend
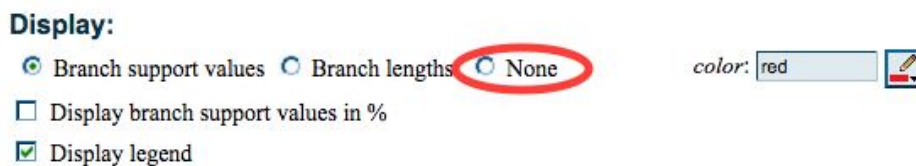
10. You now have your finished phylogenetic tree. It should look like this:
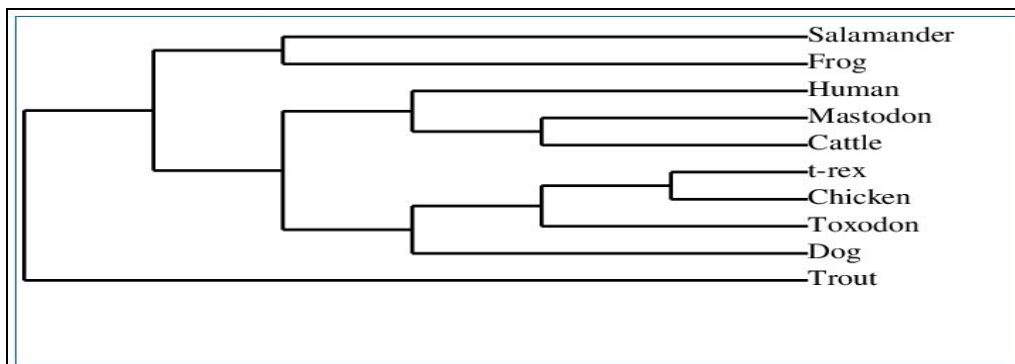
11. If you want to save your tree, you can click on the "PNG" or "PDF" links underneath the tree:

==> Download the tree: PNG - PDF - SVG - TGF (Treedyn format) - Newick - Text

Analyzing results

1. Which of the species that you analyzed is the *T. rex* most closely related to? Does this match with the BLAST results from Session 2?

2. Which pairs of animal species are "sister species"? (i.e., which animals are most closely related?)

3. What species is the "out-group" (i.e., the least related to the rest of the species) in this phylogenetic tree?

4. Can you find anything puzzling with the relationships depicted in this phylogenetic tree? (hint, look at dog). Do you suppose this might reflect the fact that only a very short amino acid sequence from a single gene was analyzed?

Evaluating results

1. Why is it important to understand evolutionary relationships among animals?

2. Why is it important to learn more about extinct animals?

3. Why is it important for scientists to publish their findings, such as genetic sequences, in public databases?

4. What other questions could these same techniques be used to answer?

Collagen Sequence Data (Copy and paste everything below, including the ">"):

>Human

MLSFVDTRTLLLLAVTLCLATCQSLQEETVRKGPAGDRGPRGERGPPGPPGRDGEDGPTGPPGPPGPPG
PPGLGGNFAAQYDGKGVGLGPGPMGLMGPRGPPGAAGAPGPQGFQGPAGEPGEPGQTGPAGARGPA
GPPGKAGEDGHPGKPGRPGERGVVGPQGARGFPGTPGLPGFKGIRGHNGLDGLKGQPGAPGVKGEPG
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SRGPSGPPGPDGNKGEPGVVGAVGTAGPSGPSGLPGERGAAGIPGGKGEKGEPGLRGEIGNPGRDGA
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GHKGERGYPGNIGPVGAAGAPGPHGPVGPAGKHGNRGETGPSGPVGPAGAVGPRGPSGPQGIRGDKG

EPGEKGPRGLPGLKGHNGLQGLPGIAGHHGDQGAPGSVGPAGPRGPAGPSGPAGKDGRTGHPGTVGP

AGIRGPQGHQGPAGPPGPPGPPGPPGVSGGGYDFGYDGDFYRADQPRSAPSLRPKDYEVDATLKSLNN

QIETLLTPEGSRKNPARTCRDLRLSHPEWSSGYYWIDPNQGCTMDAIKVYCDFSTGETCIRAQPENIPAK

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TGNLKKAVILQGSNDVELVAEGNSRFTYTVLVDGCSKKTNEWGKTIIEYKTNKPSRLPFLDIAPLDIGGA

DQEFFVDIGPVCFK

>Chicken

MLSFVDTRILLLLAVTSYLATSQHLFQASAGRKGPRGDKGPQGERGPPGPPGRDGEDGPPGPPGPPGPP

GLGGNFAAQYDPSKAADFGPGPMGLMGPRGPPGASGPPGPPGFQGVPGEPGEPGQTGPQGPRGPPGP

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ARGLVGEPGPAGAKGESGNKGEPGAAGPPGPPGPSGEEGKRGSNGEPGSAGPPGPAGLRGVPGSRGL

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KGERGAPGNPGPSGALGAPGPHGQVGPSGKPGNRGDPGPVGPVGPAGAFGPRGLAGPQGPRGEKGEP

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TPEGSKKNPARTCRDLRLSHPEWSSGFYWIDPNQGCTADAIRAYC

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RTNKPSRLPILDIAPLDIGGADQEFGLHIGPVCFK

>Trout

MLSFVDNRILLLLAVTSLLASCQSGGLKGPRGAKGPRGDRGPQGPNGRDGKAGLPGIAGPPGPPGLGG

NFAAQFDGGKGSDPGPGPMGLMGSRGPNGPPGAPGPQGFTGHAGEPGEPGQTGSIGARGPTGSAGKP

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YMDGENGNLKKAVLLQGSNDVELRAEGNSRFTFNVLEDGCTRHTGQWSKTVIEYRTNKPSRLPILDIAP
LDIGEADQEFGLDIGPVCFK

>Dog
MLSFVDTRTLLLLAVTSCLATCQSLQEATARKGPTGDRGPRGERGPPGPPGRDGDDGIPGPPGPPGPPG
PPGLGGNFAAQYDGKGVGLGPGPMGLMGPRGPPGASGAPGPQGFQGPAGEPGEPGQTGPAGARGPPG
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>Cattle
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>Frog
MLSFVDLRSVLLLAVTLYLVTCQEVRRGPRGDKGPPGEQGPPGIPGRDGEDGLPGLPGPPGVPGLGGNF
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GHPGKDGSRGPRGDSGPVGRPGEQGQHGPVGLAGDKGPSGEAGPAGPPGAAGPSGVLGARGILGLP
GTRGERGLPGGPGSNGEPGPSGLAGSSGPRGPPGSVGSPGPVGHSGEAGRDGHPGNDGPPGRDGLP
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>Toxodon
GPMGIMGPRGPPGASGAPGPAGEPGEPGQTGPAGARGPPGPPGKAGEDGHPGKPGRPGERGVVGPQG
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>Mastodon
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>Salamander

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ANGENGSPGQAGARGLPGERGRVGGAGPGARGSDGSAGPSGPAGPIGSAGAPGLPGAPGAKGEIGSA
GNNGPSGPAGSRGDPGLPGSVGPVGPAGNPGSNGVSGAKGAAGLPGVGGAPGLPGPRGIPGPQGASG
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NSIAYMDGETGNLKKAVLLQGSNDVELRAEGNSRFTFSVLEDSCTKHTGEWGRTVMEYRTNKPSRLPIL
DIAPMDIGGAEQEFRVDIGPVCFK

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CASE STUDY: SHOULD DINOSAURS BE "CLONED" FROM ANCIENT DNA?

WHAT ARE THE ETHICAL CONSIDERATIONS FOR AND AGAINST THE CLONING OF DINOSAURS INTO TODAY'S WORLD?

[Adapted by Dane Besser, Baylee Goodwin, and Stephen A. Ramsey from an activity written by Constance M. Soja and Deborah Huerta]

Background

NOTE: This activity describes a <u>hypothetical scenario</u> *in which the technology has been developed that will enable the cloning of dinosaurs from ancient DNA samples. In the process of introducing the fictional scenario for the activity, some relevant non-fiction background material will be provided in round text boxes.*

You've been asked to participate in a Presidential blue-ribbon commission that will consider whether dinosaurs should be cloned from ancient DNA and brought back to life. Your commission will make a recommendation for or against dinosaur cloning that will be considered, and then ultimately decided on, by a panel of High Court judges in a court proceeding. The commission's team includes experts with various backgrounds and interests, to ensure that diverse points-of-view are considered in the decision-making process. The question of whether dinosaurs *should* be reintroduced is particularly urgent because scientists at multiple sites around the world are currently refining the laboratory techniques that make dinosaur cloning possible.

Dinosaurs were the dominant form of animal life on land for more than 100 million years. Dinosaurs lived on all continents in a wide variety of environments from the poles to the tropics. Many scientists believe that during the Mesozoic era (which began 252 million years ago) mammals were unable to dominate life on land due to the presence of dinosaurs. Only during the breakup of Pangea and after dinosaurs became extinct, did mammals undergo an evolutionary variation and growth to occupy many of the ecological roles left vacant as a result of the dinosaurs' mass extinction at the end of the Mesozoic era (which scientists refer to as the "K-T" extinction event).

Sources of intact dinosaur DNA have been identified at several sites around the world. Recent advances in molecular biology now enable scientists to extract the fossilized DNA from dinosaur remains, purify it, concentrate or amplify it, and replicate it before implanting the dinosaur DNA into

donor eggs from closely related species. In theory, this provides the opportunity to undo the mass extinction of dinosaurs and return them to the Earth's ecosystems.

Some researchers have suggested that humans are causing a new mass extinction. Many scientists now believe that an extinction event began as recently as 50,000 years ago when humans as hunters began a worldwide devastation of large-bodied animals (i.e. mammoths, giant sloths, etc.). Scientists from every continent have expressed their growing concerns that this mass extinction event continues to accelerate today, rivaling the K-T mass extinction in the scope and intensity of species extinctions around the globe.

With new cloning techniques, humans now have the opportunity to reverse the decline of global biodiversity and reinstate to Earth members of global ecosystems that existed here only a short time ago, geologically speaking. Your team's opinion will help determine the ultimate fate of the dinosaurs. Should they remain extinct forever? Or should they be brought back, and if so, in what numbers? Your job is to carefully evaluate the situation and prepare a report with recommendations. Your report will be considered by the judges on the High Court, who will make a final decision. Information about scientific research on cloning has been made available to you, including some discussion about human cloning. But remember, this is a case about whether *dinosaurs*, not humans, should be cloned. The President thanks you for your participation in an historic case that will have global ramifications.

Learning Objectives

This activity will provide students with an opportunity to:

- Employ scientific facts in an argument regarding a globally-impacting decision
- Predict and consider the long-term consequences of this decision
- Consider the decision from multiple stakeholders' points of view

Procedure

Part I

Your team must issue a recommendation on the fate of the dinosaurs before a world audience anxious to know your decision. Before you come to any conclusions, however, you will need some background information about the science of cloning; genetic engineering of ancient DNA; how to develop a dinosaur embryo and successfully raise it to adulthood; animal husbandry issues related to supporting a living, adult dinosaur under present-day ecological conditions; safety issues; ethical issues, etc. Each of you will serve either as a judge or represent a particular specialty on one of two teams: one team will argue in support of dinosaur cloning and the other will argue against dinosaur cloning.

Here's a review of how it will work and your responsibilities:

ROLE ASSIGNMENTS

High Court Judges

The judges are responsible for making final decision after hearing from two teams of specialists

Two Teams- One For and the Other against Dinosaur Cloning

Each team includes people with the following six roles (five specialist roles and a "citizen" role, who will represent a non-specialist point of view):

Investors   Ethicists

Paleontologists   Veterinarians

Geneticists   Citizens

<u>Individual Specialist and Team Responsibilities</u>

Each team member will be assigned one of the six roles, and her/his responsibility is to represent the point of view of her/his assigned role in arguing for/against (depending on the team assignment) dinosaur cloning. Each team also includes one person who will serve as the team's leader. The team leader will be the primary spokesperson in the court proceedings before the High Court.

Below, you will be provided with readings that will give some starter ideas for an approach to take and clues about how a person with your specialty might think. Be sure to read over carefully Parts I and II of the case and the discussion questions, as well as the material available via the web links and in the cloning e-folder (see the "Cloning e-folder" subsection at the end of this document). If you wish, you may also choose to use your school's library resources for your research. Each of you individually will be responsible for preparing a one page report representing your position using your own words and citing any references that you used. In your report, you should cite facts to support your arguments. It is okay to confer with your partners and teammates (not the judges), but write your report on your own.

You and your partners should prepare to present your case in verbal arguments before the judges using whatever means you decide upon—but keep in mind that each team will have no more than 30 minutes to present its entire case. Also, please note that the "team leader" on each team will not give a one-minute presentation, but rather will be responsible for answering about a minute of questions from the judges, answering one question from the opposing team's "team leader," and posing one question to the opposing team's "team leader."

Judges' Responsibilities

A judge's job is to serve as objective, thoughtful, and reliable decision-makers. Judges should not engage in conversations with members of either team before testimony is given. We suggest that the judges conduct their research together, reading carefully Parts I and II of the case and the discussion questions, the judges' page, web links and the cloning e-folder, library resources, and/or your textbooks. Each judge is individually responsible for preparing a half-page report indicating his/her position (for or against dinosaur cloning) before hearing the oral arguments in class. It is okay to confer with your fellow judges, but please complete your write-up on your own using your own words and citing any references that you used.

Before the court session, judges should designate someone as "Chief Judge" and should have predetermined how to call upon the specialists in an orderly, organized, and fair fashion, allowing each team an equal amount of time (e.g., 30 minutes) to plead its case for or against dinosaur cloning. All judges should be prepared to pose questions to the team leader for each specialist group, and the Chief Judge should make sure that the team leaders question each other after each specialist group has given its testimony. Judges will have a chance to confer with each other briefly after hearing all of the oral arguments and so will have the opportunity to change their positions in response to particularly persuasive argument. By the end of class, the judges will announce their decision (made by majority vote among the judges) about whether or not to allow dinosaur cloning.

Part II

Take a look at the cloning diagrams in the cloning e-folder that help to explain general cloning procedures in mammals.

(A bit of background for the diagrams: in 1996, "Dolly", a sheep, was the first animal to be cloned from the cells of an adult, living animal). The diagrams reveal that three animals are generally involved in cloning one individual. An egg cell (which scientists call an <u>ovum</u>) is donated by animal 1 but the cell's nucleus is removed, after which the cell is referred to as <u>enucleated</u>. The nucleus from a body cell of animal 2 (the animal to be cloned) is transferred into the enucleated cell of animal 1, typically after jolts of electricity are used to open the egg cell's pores and allow nuclear transfer to occur. Once nucleated, the genes from animal 2 direct the egg cell from animal 1 to grow and develop. After cell differentiation takes place, animal 1's egg cell, which now contains animal 2's DNA, is implanted into the uterus of animal 3, which (if successful) will give birth to a nearly genetically identical clone of animal 2 (why "nearly"? That is because removing the nucleus from animal 1 egg cell does not remove all of its DNA, as there is still a tiny amount of DNA in the egg cell's mitochondrion).

How would such a cloning procedure work for dinosaurs? Presumably animal 1 would be an animal closely related to dinosaurs, such as a bird or crocodile, from which an egg cell would be obtained. "Animal 2" would be the dinosaur, whose DNA would need to be extracted from a fossil. Animal 3 would be the surrogate mother, once again either a bird or crocodile.

Questions that you will want to consider: Let us assume that dinosaur "cloning" is possible using fossil DNA. What would it take to raise a juvenile dinosaur to adulthood and to maintain a captive breeding program for dinosaurs? What kinds of environments and foods would be right for the dinosaurs? Could cloned dinosaurs be susceptible to disease from present-day microbes? Could dinosaurs be used to save some endangered species from extinction? Conversely, would cloned dinosaurs be expected to cause some species to become extinct? What ethical questions should be considered about the rights of humans and of non-human species?

As a team member, your first assignment is to prepare a one-page report–based on research that you will carry out using materials provided with this Activity and Internet information resources hyperlinked from this Activity. Your report serves two purposes: it will aid the judges in their decision, and it will help guide the oral arguments that your team will make before the High Court. Below, we provide material to help with your research and with playing your assigned role on your team. In the section Questions for Background Research, we provide questions (organized roughly by the chronological stage in a hypothetical project to clone dinosaurs) that we recommend you try to answer by consulting information resources. In the section Role Assignments Documents we provide documents that will provide you some insight into the point-of-view of your assigned role on your team. You'll want to carefully read the document for your specific team role assignment (e.g., "veterinarian") and for your specific team assignment ("for" or "against" cloning).

Questions for Background Research

Phase 1- The DNA Hunters

- What are the sources and approximate ages of ancient DNA in the geological record?
- What are the major problems associated with ancient DNA?
- How common or rare is dinosaur DNA in the ancient record?

Phase 2- Hello, Dolly!

- Once fossil DNA is extracted, what steps would be required to synthesize enough DNA for cloning a dinosaur?
- Once enough DNA is acquired, what problems or challenges would be associated with developing a dinosaur embryo?

Phase 3- Bringing up Baby

- What factors will play a role in successfully raising a dinosaur from embryo (created from ancient DNA) to adulthood?

Phase 4- Dinosaur Husbandry I: Habits and Habitats

- Under what kinds of environmental conditions would adult dinosaurs thrive?
- How might environmental conditions vary by dinosaur species?

Phase 5- Dinosaur Husbandry II: Care and Condition

- How would the dietary needs of herbivorous dinosaurs be satisfied with post-Mesozoic food sources?
- How would the dietary needs of carnivorous dinosaurs be satisfied with post-Mesozoic food sources?
- What precautions might be taken to safeguard dinosaurs from deadly viruses or diseases of the Cenozoic era?
- What kind of care would dinosaurs require throughout their adult lives?
- What would be required to ensure that enough genetic diversity is maintained in the dinosaurs to avoid inbreeding and to prevent a disease or virus from wiping out the entire population of cloned dinosaurs?

Phase 6- Safety, Ethics, and Animal Rights

- What steps would need to be taken care to protect the dinosaurs from humans and humans from the dinosaurs?
- What ethical and animal rights issues are raised by dinosaur cloning?
- In your opinion (no matter what your teammates think), do you think humans should try to recreate a living dinosaur- why or why not?

Thank you and good luck! The world is waiting to hear the court's final decision!

ROLE ASSIGNMENT DOCUMENTS

Date: September 1996
To: Karelis Securities, Inc.
From: Roslin Institute, Scotland
Re: "Dolly"

Here are the sketches you wanted. We're experimenting with another
clone, but this is the basic technique. "Dolly" is doing fine; she's just
about to be weaned from her surrogate mother.

sheep 1--donates egg cell

nucleus is removed

enucleated cell

sheep 2--donates cell nucleus

enucleated cell
(discard)

nuclear transfer
of sheep 2's
nucleus into sheep
1's enucleated cell

differentiated cell is
implanted into uterus
of sheep 3. This
"surrogate mom"
gave birth to "Dolly,"
a clone of sheep 2
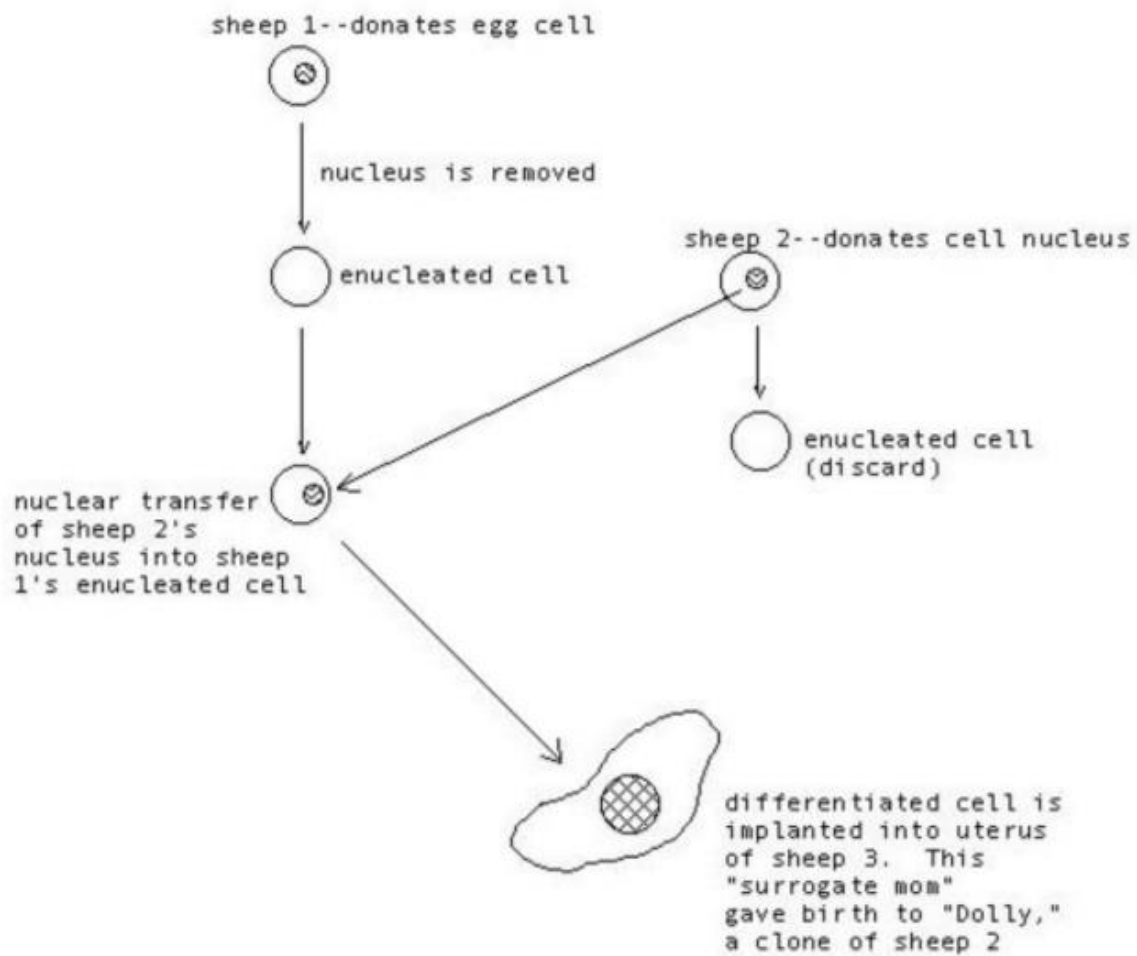
Date: June 2019
To: Karelis Securities, Inc.
From: Sayonara Institute, Japan
Re: Woolly mammoth

Here are the sketches you wanted. We're sad the baby died, but we're working
on another clone experimenting with this same basic technique. You can see where
you would substitute dinosaur DNA for the mammoth DNA, etc. Best wishes for
a successful project!

elephant 1--donates egg cell

nucleus is removed

enucleated cell

DNA donated from
frozen mammoth cell

enucleated cell
(discard or use
for another purpose)

nuclear transfer
of mammoth's
nucleus into elephant
1's enucleated cell

differentiated cell is
implanted into uterus
of elephant 2. This
"surrogate mom" gave
birth to the mammoth
"clone" after 22 months
gestation
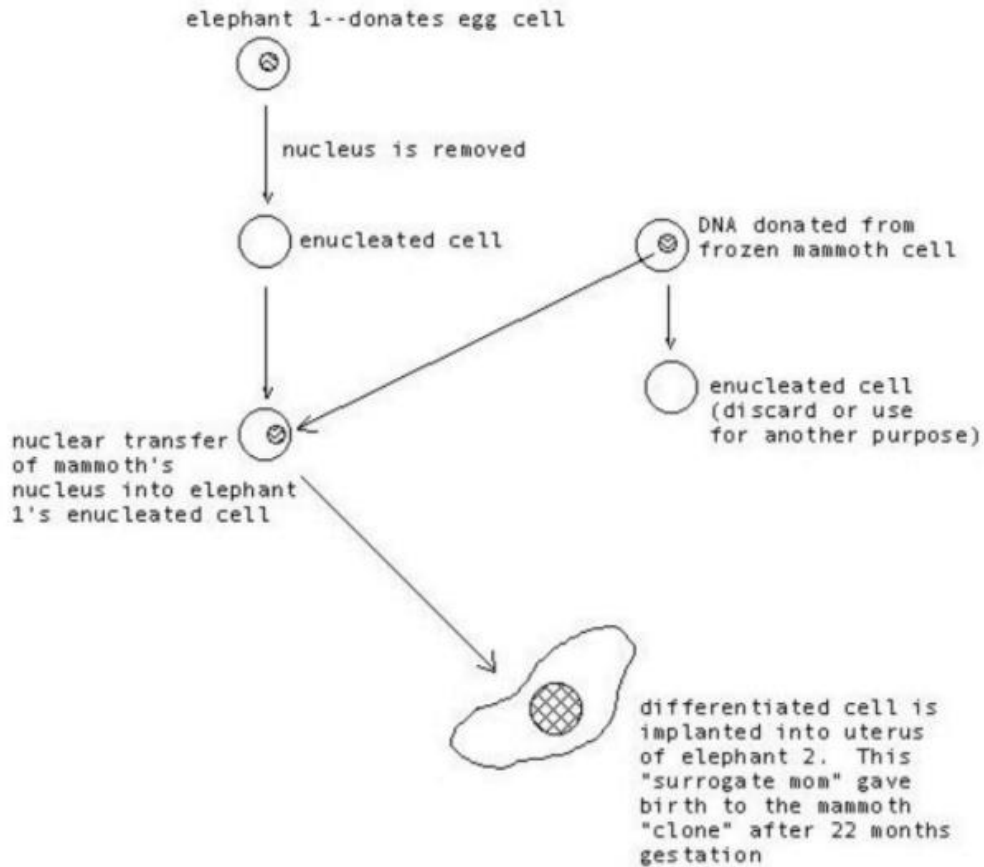
To: Esteemed Judges of the High Court

From: Supreme Court Justice Goodwin

Re: Dinosaur Cloning

I've been informed that you are going to be reviewing a case brought before your court on whether extinct forms of life (i.e., dinosaurs) should be cloned from ancient DNA. As this is the first such attempt at cloning dinosaurs, I would remind you that your decision carries great importance. I would also warn you that special interests are attempting to influence the case on both sides. It is your duty as judges to investigate the scientific and ethical aspects of the matter thoroughly so that you are sure of the evidence and arguments presented in court. I would urge you to review the various facts and theories of cloning, evolutionary principles, and dinosaurs. After you have completed your research, I recommend that you prepare questions to pose to both sides of the case before you. I have placed a large group of specialists on hand to advise you in your decision. Feel free to call on them to explain a fact or point out a discrepancy in the lower court's argument. I will be watching this case carefully, and expect YOU to reach the final decision. Good luck!

C. M. Goodwin,

Supreme Court Justice

To: Investor

From: Karelis Securities, Inc.

Re: Investments

I don't think I need to remind you how important this decision is for the future of this company. Karelis Securities has been a leader in cloning research since 1990 when we underwrote some of the initial research that led to the cloning of the sheep "Dolly" in the U.K. in 1996. Let's not forget that in 1997, The Lost World (sequel to the movie Jurassic Park) brought in a staggering $1 million per hour on opening week-end, and the T. rex dinosaur named "Sue" was auctioned for a record $8.4 million. I think it's clear that we cannot afford to miss this opportunity to create the ultimate theme park--the REAL Jurassic Park! Everyone loves dinosaurs so here's our chance to make a fortune. Who cares if the dinosaurs are artificially reproduced from hybridization with birds? This will be the ultimate fantasy. Instead of trying to build a time machine for travel into the past, we can bring the past to the present. We've just located a game park in Louisiana for sale--the initial investment needed to refurbish the park is incidental when compared to the millions of dollars it would take to locate, study, and prepare a new site. All of the infrastructure, buildings, roads, pens, and landscaping are intact. As for the dinosaurs and human safety, let's remind ourselves that humans are used to dealing with far more complex and dangerous life forms that evolved long after dinosaurs went extinct--we'll just put in a series of strategic fences to keep the dinosaurs in and humans out. I hear the paleontologists may be arguing against cloning by citing Gould's ideas about chance as an important process in evolution, using that as a scare tactic to conjure up visions of dinosaurs evolving into bizarre, truly frightening life forms in the future. Make sure you mention ideas about the dinosaur-bird link as a counter-attack and all the ways that dinosaurs can benefit humankind. I have compiled a short list of resources you might find useful. I don't care what you argue so long as you convince those judges to allow dinosaur cloning.

I'll be waiting for your report,

C. B. Karelis, CEO Karelis Securities

To: Investor

From: L&R Financing

Re: Dinosaur cloning

It would be an understatement to mention how much the judges' decision next week will affect our future--and yours. L&R Financing funded the building of DinoAdventures Theme Park in Wyoming several years ago. As you know, we've invested millions of dollars in the design of lifesize, robotic dinosaurs that will be guided by advanced computer technologies as they roam through a recreated Mesozoic landscape and engage in all sorts of real-live activities. Our engineers have been working with a team of geologists and biologists to make this the most compelling theme park of our age and one that can be duplicated at many other sites around the world. Should dinosaurs be brought back to life through cloning, we might as well close up shop now because robotic dinosaurs will never stand a chance against living, breathing dinosaurs in the public's eye. Your job is to convince the judges to veto dinosaur cloning. I have spoken to several specialists on evolution issues as well as animal rights activists and they all agree you could make a good case. You might try to argue that the planet will be unsafe and dangerous if dinosaurs are brought back to life--consider mentioning Phil Currie's latest discoveries or give them a first-hand look at one of our T. rex robotics! Point out that evolution cannot be controlled, not even by us. According to Gould, chance plays such an important role in evolution that using the Earth's past as a "future forecast" is foolish. Point out the enormous costs of producing, raising, and maintaining a captive breeding program of dinosaurs. I really don't care what you argue as long as you win this case. Dinosaurs must not be cloned! I've had my assistant type up a list of resources that might help you prepare your report.

Remember, we're counting on you!

Cassandra Moulton III

Managing Director, L&R Financing

PALEONTOLOGIST FOR CLONING

Since you were a kid, you've been absolutely fascinated with dinosaurs. By age 5, you knew all the names of the saurischians and ornithischians and pointed out with glee every time someone mistakenly referred to Apatosaurus as Brontosaurus. (You must have seen the Jurassic Park movie a dozen times!). Your favorite dinosaur is Suchomimus, first described in 1998 by Paul Sereno based on his discoveries in northwest Africa. Since graduating from Fullam in 2003 (you got an A+ in Geo 115), you've become an expert on the detection and extraction of dinosaur DNA. The Ph.D. dissertation you completed a few years ago at a prestigious university on that very topic has placed you at the forefront of research on dinosaur cloning. Your research shows that there are more sites of potential DNA fossilized in dinosaur bones and blood proteins than most scientists realize, particularly bone beds like those in Montana where thousands of hadrosaurs were asphyxiated suddenly by ashfall during a volcanic eruption. The time is ripe for cloning dinosaurs-- imagine the research possibilities! Here would be the chance to view evolution first hand and to observe the locomotory styles, physiology, and reproductive behaviors of dinosaurs that scientists have debated for centuries. Who could turn down the opportunity to glimpse into the Earth's past and to undo the damage caused by the asteroid 66 million years ago? If you think about it, we (humans) aren't even supposed to be here--many scientists believe that if an asteroid hadn't wiped out the dinosaurs they'd still be the ruling forms of life in terrestrial environments. The best plan is for you to support dinosaur cloning and try to win a research grant to study the clones. Fame, fortune, and a pet dino might surely follow!

Since you were a kid, you've been absolutely fascinated with dinosaurs. By age five, you knew all the names of the saurischians and ornithischians and pointed out with glee as often as possible that birds are really "feathered dinosaurs." (You must have read the Jurassic Park book a dozen times!). Your favorite dinosaur sites are in Argentina, where hundreds of sauropod eggs and some embryonic dinosaurs were discovered at the end of the last century. As much as you would love to see, hear, smell, and touch a living dinosaur, you realize that we are at a profound crossroads in the history of our planet if the judges allow cloning of extinct forms of life to proceed. Scientists are still debating if dinosaur DNA is fossilized intact or if it has survived in good enough shape to be used in cloning experiments. But it's only a matter of time before the technology will be developed that can replicate an entire genome from scraps of fossil DNA. It's no longer a question of technology but rather a question of what's right. The Mesozoic world of the dinosaurs no longer exists--many of the dinosaurs' cohort species, including multituberculate mammals, archaic crocodiles, Archaeopteryx, pterosaurs, as well as early species of cycads and even primitive angiosperms, went extinct millions of years ago. Even Pangea and the climatic conditions that prevailed on Earth during the "Age of Dinosaurs" no longer exist! It would be unfair to the dinosaurs to bring them back into a world that no longer has a place for them. Their time has come and gone. You've joined with a prestigious group of fellow scientists to urge the judges to ban dinosaur cloning.

Robin Forster, Columbia Ph.D., vertebrate paleontologist

and signatures of other Scientists Against Cloning (SAC):

Xenia Krasnikova, Moscow Ph.D., conservation biologist

Jim Starr, Harvard Ph.D., pathologist

R.J. Browne, Stanford Ph.D., paleobotanist

+100 other names

New cloning techniques have made what was once believed impossible now possible. These new technologies allow for the extraction and purification of minute amounts of fossilized DNA, which is then activated, amplified, and replicated before being used for in vitro fertilization. Just last year a Japanese scientist cloned the first living mammoth by extracting the nucleus from the cell of a frozen (Pleistocene) mammoth, injecting it into an elephant's enucleated cell, and then implanting the viable embryo into an Asian elephant. Even though the baby mammoth only lived for a few days and was the clone of an animal that died out only a few thousand years ago, this represents a real step forward in cloning dinosaurs. What a fantastic opportunity—to be in on the ground level of a major scientific discovery that builds on the technologies already benefitting many humans worldwide, especially infertile couples who want to have children. There would be little to fear in bringing dinosaurs back from extinction--no "monsters" would evolve because genetic manipulations would carefully limit evolution. Also in your testimony next week it will be important to point out that cloning dinosaurs could help to develop new drugs to fight human diseases. The technological advances stemming from research on cloned dinosaurs could also potentially improve food production around the world with genetically engineered plants that could save the thousands of people who die each year from starvation. One of your colleagues has also proposed producing genetically engineered plants as benign alternatives to our dwindling fossil fuel resources. You plan to urge the judges to approve the cloning of dinosaurs because of the many potential benefits to society.

New advances in genetic engineering are on the cusp of bringing extinct species back to life, but nobody explains how difficult, risky, and expensive this is--especially given the high percentage of failed attempts before a successful live birth is achieved. For example, some molecular biologists estimate that one out of every 1000 attempts will result in a fully formed, live dinosaur hatchling, and then there's the challenge of preventing high rates of infant mortality. Problems with verifying it's really dinosaur DNA and changes in DNA over the past 66 million years can't be ignored, either--you're concerned about the possibility of creating a "Frankenstein"-like hybrid that will be out of control and beyond the limits of nature and natural selection in the Darwinian sense. It still isn't clear how a dinosaur clone would be created--for example, would the clone be a bird-dinosaur or crocodile-dinosaur hybrid? Or would the "clone" be just a chicken walking around with some dinosaur DNA as part of its genetic make-up? After considerable expense, it's still unknown if the hybrid would be fertile or sterile and which dinosaur would be resurrected--T. rex perhaps? Which dinosaur-related species would provide the donor eggs, and which species would be the surrogate mothers? Now is the time for scientists and society to acknowledge that it is justifiable to use new techniques and scientific advances to solve today's problems but wrong to add new problems. You plan to explain to the court that dinosaur cloning is an improper use of scientific technology that shows little regard for the animals being brought back into a world unprepared to receive them. Is it really desirable to clone dinosaurs with the express purpose of making them into living drug factories for pharmaceutical companies? If dinosaurs are cloned, what's next--cloned trilobites? Cloned ichthyosaurs? You even heard mention of a report that someone wants to search for frozen sperm in the mummified Ice Man, Ötzi, and clone him 5000 years after his death in the Italian Alps! It was a mistake to attempt the cloning of the mammoth last year, and cloning even older forms of life would only create more problems. You hope to convince the judges that we have absolutely no right to play God!

What a fantastic opportunity!--to be in on the ground level of a major scientific discovery that builds on the technologies already benefiting many humans worldwide, especially infertile couples who want to have children. As the geneticists have pointed out, there would be little to fear in bringing dinosaurs back from extinction--no "monsters" would evolve because genetic manipulations would carefully limit evolution. In your testimony next week, it will be important to research all of the various ways that cloning dinosaurs could be beneficial to humans. Could the cloned dinos help to develop new drugs to fight human diseases or be used for organ transplants, tissue regeneration in burn victims, or bone grafts? Could technological advances stemming from research on cloned dinosaurs also potentially improve food production around the world with genetically engineered plants that could save the thousands of people who die each year from starvation? Would advances in cloning research enable us to produce genetically engineered plants as benign alternatives to our dwindling fossil fuel resources? Forget the arguments that this goes against nature--the fact of the matter is that in reality we already select which natural processes to manipulate for the benefit of humankind. You'll provide specific examples to the judges so that they fully appreciate the extent to which humans have been manipulating nature since the advent of agriculture 10,000 years ago and more recently with the accelerated development of bioengineered plants, medicines, and selectively bred livestock. It's important to acknowledge that scientific and technological breakthroughs aren't achieved without some risks. If cloning dinosaurs could yield tremendous insights into how and why certain manipulations of cellular material are successful, as an ethicist you need to thoughtfully assess if the benefits outweigh the risks. The judges may also wish to have clear assurances that the dinosaurs will be managed under carefully monitored, humane conditions. It's time to quit demonizing science--you plan to urge the judges to approve the cloning of dinosaurs because of the many potential benefits to society.

You are gravely concerned that we are at a profound crossroads in the history of our planet if the judges allow cloning of extinct forms of life to proceed. Your geneticist colleagues assure you that it's only a matter of time before the technology will be developed that can replicate an entire genome from scraps of fossil DNA. It's no longer a question of technology but rather a question of what's right. This will be your opportunity to ask some probing questions--should scientists and society acknowledge that it is justifiable to use new techniques and scientific advances to solve today's problems but wrong to introduce new hazards through uncontrolled ecological experiments? You'll need to explain to the court why dinosaur cloning is an improper use of scientific technology, which from an ethical standpoint shows little regard for the animals being brought back into the modern world. Is it relevant that the Mesozoic world of the dinosaurs no longer exists--for that reason alone is it unfair to resurrect the dinosaurs? Has their time really come and gone? Is it really desirable to clone dinosaurs with the express purpose of making them into living drug factories for pharmaceutical companies? If dinosaurs are cloned, what's next--cloned trilobites? Cloned ichthyosaurs? You even heard mention of a report that someone wants to search for frozen sperm in the mummified Ice Man, Ötzi, and clone him 5000 years after his death in the Italian Alps! You'll have to convince the judges that it was a mistake to attempt the cloning of the mammoth last year and that cloning even older forms of life would only create more problems. You'll need to investigate if a rush for profits and slow action on the part of governments to establish regulations for safety oversight will promote unethical behaviors, including mistreatment of these complex, intelligent, social animals and possible environmental damage caused by doctored genes spreading out of control. You've joined with a prestigious group of fellow scientists to urge the judges to ban dinosaur cloning. From an ethical standpoint there's no good basis or rational reason for cloning dinosaurs--we have absolutely no right to play God!

Robin Forster, Columbia PhD, vertebrate paleontologist

and signatures of other Scientists Against Cloning (SAC): Xenia Krasnikova, Moscow PhD, conservation biologist Jim Starr, Harvard PhD, pathologist R.J. Browne, Stanford PhD, paleobotanist +100 other names

As the chief veterinarian at game parks in western North America, Africa, and Australia, you oversaw the care and feeding of reptiles, including the Komodo "dragon," as well as large herds of mammals in nature preserves in Kenya. The chance to manage the first group of cloned dinosaurs is a job too exciting to pass up. You plan to tell the judges that years of experience in animal husbandry in wild and domesticated stock lead you to believe that the management of dinosaurs is not an insurmountable problem. You'll note that during the Mesozoic, dinosaurs had co-evolved with a diversity of plants, including early angiosperms. Dinosaurs demonstrated over millions of years a considerable adaptability to new food sources throughout the Mesozoic. As ecologic generalists, you predict that they will adjust well to the wide assortment of grains and grasses that modern mammals depend on to fuel their active lives. You've already been involved in an experimental program in Tanzania where vaccinations of lions and cheetahs successfully boosted their immune systems and prevented the further spread of deadly viruses, which have culled many populations of African felids. Similar techniques could be applied to dinosaurs so that their Mesozoic immune systems would be able to tolerate Cenozoic diseases. Daily maintenance and care of dinosaurs would ensure their survival under carefully monitored conditions by well-trained staff. Furthermore, as a pathologist interested in the origins of diseases that still plague humankind, you see a real benefit in being able to investigate the factors associated with arthritis and syphilis, diseases that also affected dinosaurs. Finally, you will urge the judges to allow dinosaur cloning by pointing out that cloned dinosaurs could also benefit humans by serving as a source for bone grafts and possibly even organ and tissue transplants.

As an experienced pathologist who specializes in large-bodied animals, you have considerable discomfort about the monumental efforts, expense, and uncertainty involved in the care, maintenance, and management of cloned dinosaurs. Anyone who knows anything about modern ecosystems appreciates that boundaries are diffuse and that ecological "osmosis" takes place across invisible or non-existent borders. In other words, captive animals are not completely protected from outside influences and vice versa. Dinosaurs would probably need to be fed with genetically altered plants from which the deadliest toxins have been removed. Angiosperms have experienced enormous evolution in the last 60 million years, and dinosaurs would not have adaptations to aid in the digestion of plants they never encountered in the Mesozoic. Didn't somebody once propose that dinosaurs became extinct after suffering severe digestive disorders shortly after the evolution of the first angiosperms? Modern viruses could wreak havoc on the immune systems of the dinosaurs as well; even new experiments to boost the immune systems of endangered species have not been able to save all members afflicted with a deadly virus. You're also worried that Mesozoic diseases that died out with the dinosaurs could be reintroduced into the modern world. Cloning dinosaurs could possibly recreate a dangerous pathogen and contaminate other animals in nearby habitats. Mosquitoes and other insects are known vectors that transfer diseases among species. You're fearful that many birds and crocodiles, already threatened with extinction in many parts of the world, might suffer even greater losses as evolutionary relatives of the dinosaurs that are susceptible to the same diseases. Finally, you plan to end your testimony with an image that the judges will be unable to forget of a five-ton Triceratops with meter-long horns charging towards a bus filled with tourists... Dinosaur cloning is better left alone!

What's the big deal? Nature's already full of clones--your doctor once told you that you and your twin brother are a form of cloning and there's nothing particularly bizarre or frightening about the two of you! Doesn't it come down to the Nature vs. Nurture debate--that individuals are shaped both by their genetic heritage and by the environment? You don't really understand all the stuff about how a clone is actually developed, but if the scientists are telling us that dinosaurs can be genetically engineered with careful manipulations of their DNA and that they will exist under controlled environmental conditions, what's the big scare? What could be cooler than visiting an outdoor theme park and watching dinosaurs do their thing. They've got to be the biggest, baddest beasts that ever walked the Earth. Science fiction just doesn't do it anymore; this will be the REAL thing. Everyone seems to be worried about science unleashing uncontrollable, violent forces into society, but humans are used to dealing with all kinds of violence, like inner-city gangs, earthquakes, killer viruses, etc. Also haven't the newspapers been reporting all the benefits we derive from genetic engineering, like medicines, better foods, organ transplant research, etc.? Scientific research on cloning isn't necessarily a bad thing, we just have to be careful what kind of cloning is done. Dinosaurs obviously weren't the brightest critters, otherwise they wouldn't have gone extinct as evolutionary failures, right? So here's a second chance for them to reinhabit planet Earth while providing some enjoyment and benefit to humankind. Sounds like a pretty fair deal!

What's the big deal? Who are we trying to kid? Here we are talking about bringing back the largest animals that ever walked the planet, including T. rex. Doesn't planet Earth already have enough problems—human overpopulation, famine, disease, poverty, pollution, species extinctions, you name it. We can't even feed all the starving people in the world, never mind a bunch of cloned dinosaurs. What kind of world will we be leaving our children if in addition to everything else they have to deal with extinct forms of life brought back from the past? Didn't Newsweek say that 60 million years separates the dinosaur age from the human age? Think of all the changes that must have taken place in that amount of time. It doesn't seem like a good idea to fool around with nature on that scale. So they tell us the dinosaurs would be caged in zoos and big animal parks. What about the reports from Florida last year of lions and tigers escaping from that zoo? It's only a matter of time before some smart dinosaur finds a way to sneak out of the zoo, and then imagine what would happen. There must be some mad scientists somewhere out to control the world. You plan to say loudly and clearly in court next week that under no circumstances should dinosaur cloning be allowed!

Information Resources

Magazine and Journal Articles:

"Cloning the Woolly Mammoth." Richard Stone. *Discover*, vol. 20, April 1999, p. 56-63.

"Ancient DNA." Svante Pääbo. *Scientific American*, vol. 269, November 1993, p. 86-92.

"Ancient DNA." George Poinar, Jr. *American Scientist*, vol. 87, September-October 1999, p. 446-457.

"Dino DNA: The Hunt and the Hype." Virginia Morell. *Science*, vol. 261, 9 July 1993, p. 160-162.

"The Use of Ancient DNA in Paleontological Studies." Lori M. and Zvi Kelman. *Journal of Vertebrate Paleontology*, vol. 19, March 1999, p. 8-20.

"The Real Jurassic Park." Mary Schweitzer and Tracy Staedter. *Earth*, vol. 6, June 1997, p. 54-57.

"Will the Dinosaurs Rise Again?" Mary H. Schweitzer and Raul J. Cano. In *DinoFest* (edited by Gary D. Rosenberg and Donald L. Wolberg), 1994, p. 309-326.

"DNA Sequence from Cretaceous Period Bone Fragments." Scott R. Woodward et al. *Science*, vol. 266, 18 November 1994, p. 1229-1232.

"Detecting Dinosaur DNA." [various authors.] *Science*, vol. 268, 26 May 1995, p. 1191-1194.

"Dino Hunter." Josh Fischman. *Discover*, vol 19, May 1999, p. 72-78.

"Is Science Dangerous?" Lewis Wolpert. *Nature*, vol. 398, 25 March 1999, p. 281-282.

Cloning E-Folder:

Memo dated September 1996

Memo dated June 2019

Internet Sites:

Recreating Dinosaurs: Fact or Fiction?

http://www.nhm.ac.uk/about-us/page-not-found.html

http://www.gplatt.demon.co.uk/amberdna.htm

http://unmuseum.mus.pa.us/dnadino.htm

https://www.scientificamerican.com/askexpert/biology/biology1.html

https://www.newscientist.com/nsplus/insight/rexfiles/backfrom

http://www.dinosauria.com/

http://dinosaurs.eb.com/dinosaurs/index2.html

Cloning Info

https://www.scientificamerican.com/explorations/030397clone/030397beards.html

https://www.scientificamerican.com/1998/1298issue/1298wilmut.html

https://www.newscientist.com/nsplus/insight/clone/clonelinks

http://library.thinkquest.org/24355/data/debatenav.html

http://powayusd.sdcoe.k12.ca.us/dolly/resources.htm

Books:

The Science of Jurassic Park and The Lost World or, How to Build a Dinosaur. Rob DeSalle and David Lindley. 1997. BasicBooks.

The Quest for Life in Amber. George O. Poinar. 1994. Addison Wesley Longman, Inc.

The Second Creation: Dolly and the Age of Biological Control. Ian Wilmut, Keith Campbell, and Colin Tudge. 2000. Farrar Straus & Giroux.

Clone: The Road to Dolly and the Path Ahead. Gina Kolata. 1998. W. Morrow & Co.

Remaking Eden: Cloning and Beyond in a Brave New World. Lee Silver. 1997. Avon Books.

Biology. N.A. Campbell. 1987. Benjamin-Cummings Publishing Co.