

## Lab 2 - Illustrating Evolutionary Relationships Between Organisms: Emperor Penguins and Phylogenetic Trees

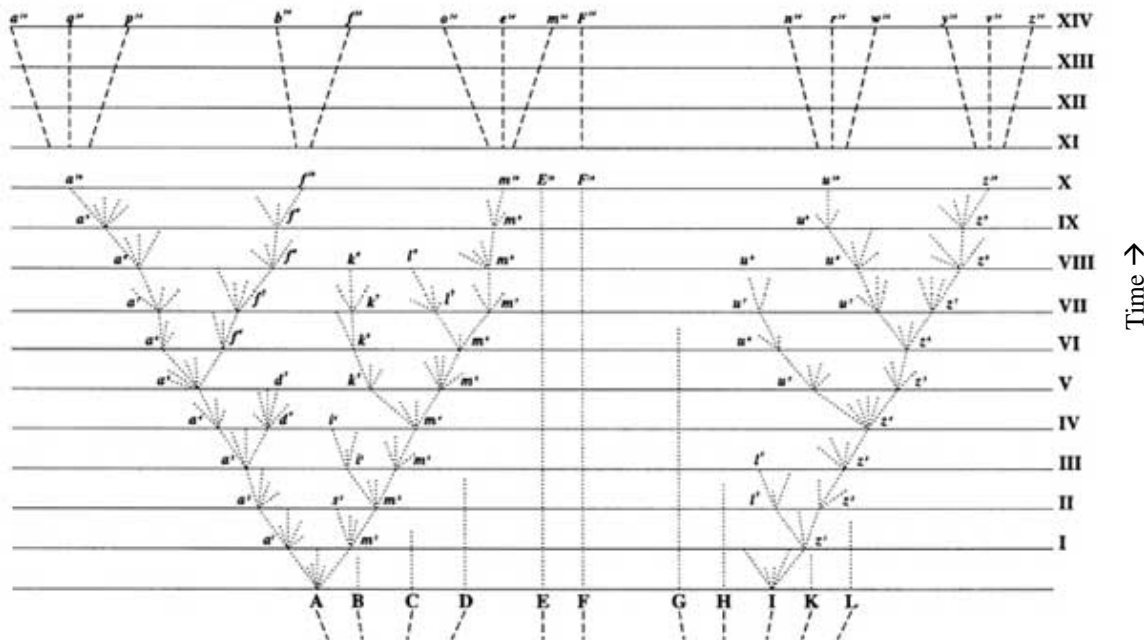
### Pre-Lab Reference Reading:

Review pp. 542-556 and pp. 722-737 in *Life* by Sadava et al., 8<sup>th</sup> edition, 2006.

### I. Introduction

The ability to think broadly about the living world is rooted in an understanding of life's diversity. However, the large number of ways in which various organisms differ from one another can often mask their similarities. The *diversity* and *unity* of living organisms would seem to be contradictory concepts. Recall, though, that these were the major themes in Darwin's great work, in which he asked and answered the following question. Since life is so diverse, are there any unifying themes at all? By looking backwards in time, Darwin showed that the answer to this question is a resounding "Yes!"

Darwin proposed both a pattern invoking common descent (as the basis for the unity of life) and a mechanism to explain how the relationships between species became fragmented over time (resulting in diversity). In addition, Darwin himself is responsible for the metaphor that is used today to illustrate this shared descent of living organisms: the Tree of Life (TOL). In fact, the diagram shown below was the *only* figure from his book *On the Origin of Species by Natural Selection*.



“...great tree of life...with its ever-branching and beautiful ramifications...”

(C. Darwin, 1859).

The **ATOL (Assembling the Tree of Life)** Project represents a collective effort of biologists working on different organisms to understand how the diversity of life fits together. Resolving evolutionary relationships that underlie the TOL is unquestionably one of the most important problems in biology today, for two major reasons.

- Because it is relevant to *all* organisms, with the tree potentially encompassing the 1.75 million living species characterized to date and an estimated tens of millions more to be discovered (not to mention the even larger number of species that existed and are now extinct).
- There are major applied consequences of understanding the evolutionary relationships between organisms with respect to human health and the environment. These include the origins and dynamics of disease-causing microbes, or the interactions between species that constitute a rich and self-sustaining ecosystem.

Reconstructing an evolutionary tree (i.e., a *phylogeny*) is a powerful way to answer questions about the evolutionary relatedness of living organisms. In addition, modern tools of molecular biology are greatly advancing our analytical power, particularly in the use of DNA and protein sequence data to investigate the similarity/divergence between species.

## II. Overview of Activity

Millions of people around the world have been captivated by the 2005 documentary movie *The March of the Penguins*. In this movie, viewers learn about the complex reproductive behaviors of Emperor penguins, which include a 70-mile march (by walking or belly gliding) to an inland breeding ground. (See Appendix for life cycle.) Strong selective pressures have likely been in force to establish reproductive behaviors that differ so greatly between Emperor penguins and other birds, thereby reinforcing the evolutionary independence and integrity of related species.

A number of interesting biological questions come to mind while watching *The March of the Penguins*. For example,

- 1) What is the closest living relative of Emperor penguins?
- 2) Are all penguins living today the descendants of the same common ancestor?
- 3) If so, to which taxonomic group of birds are penguins most closely related?
- 4) What would the common ancestor of penguins and their closest bird relatives have looked like?
- 5) What is the evolutionary relationship between penguins (and birds in general) to other classes of vertebrates?

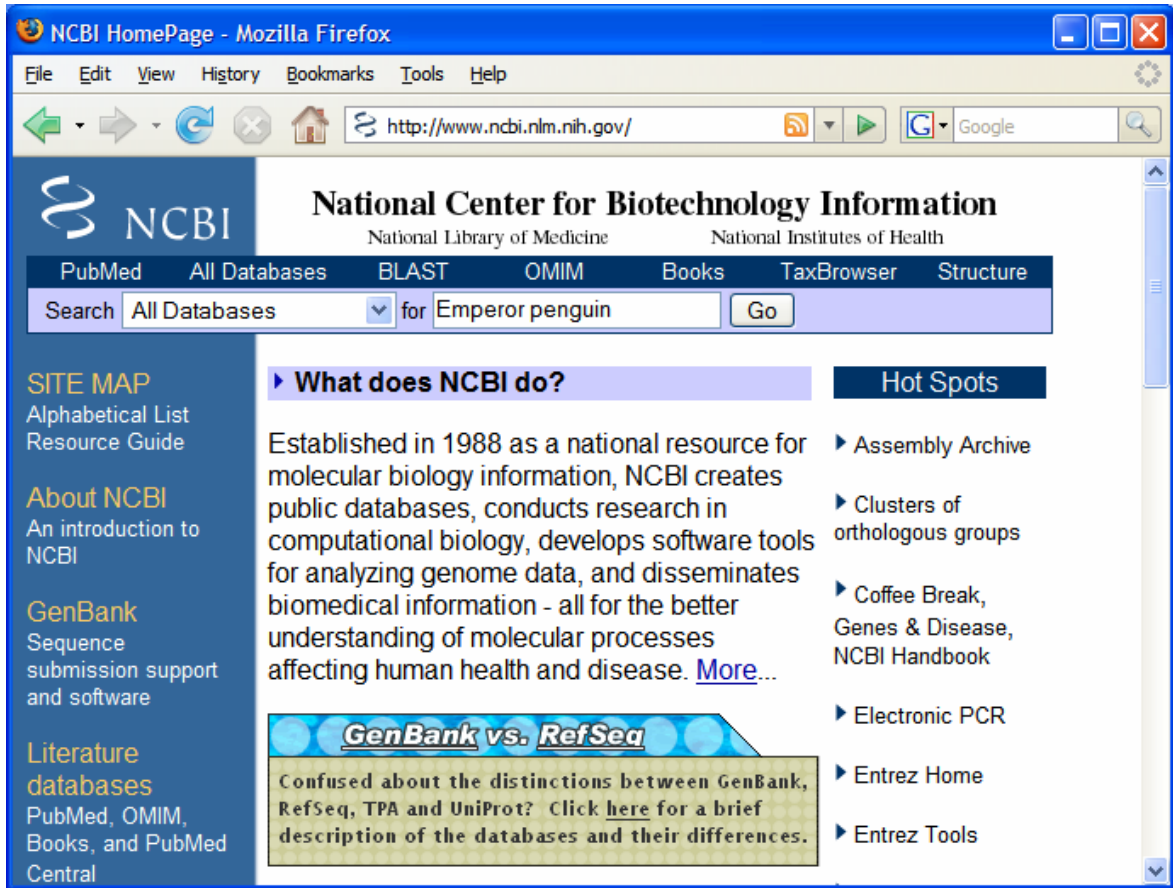
To address these and other questions, students will use the National Center for Biotechnology Information (NCBI) Website to reconstruct a phylogeny, which will illustrate the evolutionary relationships between Emperor penguins and other living organisms. Students will use gene sequence databases to do this, and DNA sequence comparisons will be done “behind the scenes” using NCBI’s BLAST search engine. BLAST stands for **B**asic **L**ocal **A**lignment **S**earch **T**ool, and “the program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches” (<http://www.ncbi.nlm.nih.gov/BLAST/>).

A comparison of DNA or protein sequences from different species can reveal evolutionary relationships. This assumption is based on the idea that the nucleotide or amino acid sequence of a particular gene or the protein it encodes, respectively, mutates at a similar rate in different individuals. However, students should remember that the rate of mutation alone does not determine how fast a DNA sequence will change. The effects of mutation on survival and reproductive success (*natural selection*), as well as the generation time of the organism, also play important roles in the overall rate of genetic change in a population.


To sum up, the overall goal of this lab is for students to examine the evidence held within genetic sequences to determine how living organisms are related to one another and to confirm that they share common ancestors. You will do so by using BLAST to measure the degree of gene sequence similarity between various types of organisms. Each pair of students should go through the exercises below with Emperor penguins, using the worksheet on pp. 17-21 to record your answers to questions (in bold) asked in the text. **The completed worksheet is due at the beginning of lab next week.**

### III. Procedure

1. Go to the NCBI Home Page at <http://www.ncbi.nlm.nih.gov/>.

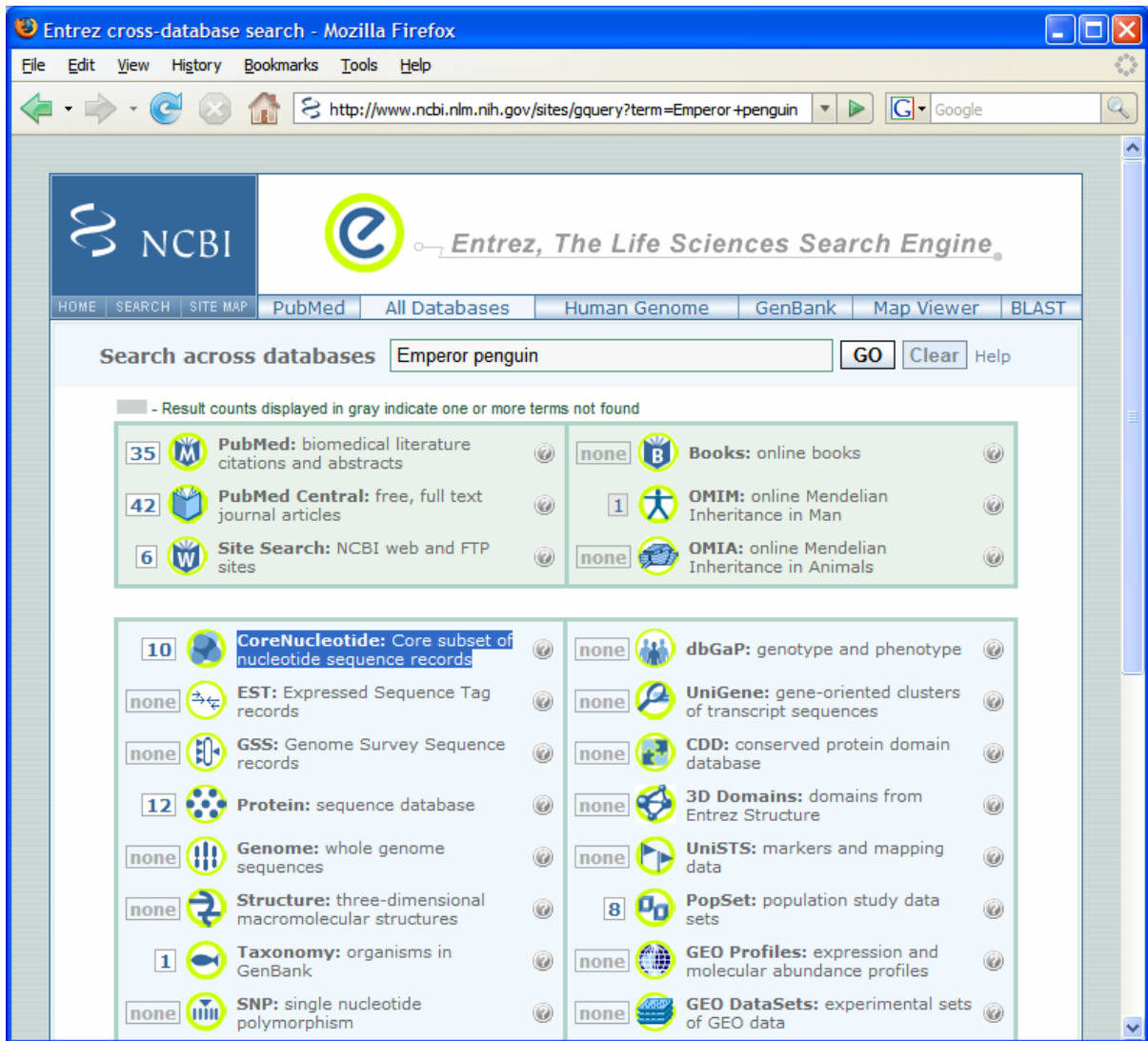


This page contains links to dozens of different databases and other resources.

Every page that is linked to the main NCBI page has a small  icon somewhere in the upper-left corner of the screen. Clicking on this icon will immediately return the computer to the NCBI home page.

2. We will build a phylogenetic tree by using BLAST to compare the degree of sequence similarity in a gene between different individuals. Generally, the more divergent gene sequences are, the more time and evolutionary distance separate the two species.
3. To begin our analysis, we would ideally select a gene that is present within the genome of all the organisms that we wish to compare. Think about the shared characteristics of different groups of organisms and the gene products that may contribute to these characteristics. **What types of genes would you expect to be present in all organisms? In all eukaryotes? In all plants (but not in animals or fungi)? In all animals (but not in plants or fungi)? In all vertebrates (but not in invertebrates)?**

- To identify possible gene candidates for the Emperor penguin analysis, type “Emperor penguin” in the “for” search box, and click on the “Go” button (as above).
- A screen with all the NCBI-managed databases will appear next (partial screen shot below). Any database that contains Emperor penguin entries will have a number (representing the number of different entries in that database) in the small box to the left of the database name.



- Click on the “10” in the box to the left of the “CoreNucleotide” database (highlighted in blue above).
- The DNA sequence search results are listed on the next screen (partial screen shot on next page). Think about your answers to Question 3 above. **Which of the ten gene entries, if selected, would enable you to build the most inclusive tree (i.e. containing the greatest number of organisms)? Why? Which entry would result in the least inclusive tree? Why?**

Emperor penguin - CoreNucleotide Results - Mozilla Firefox

http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&cmd=search&term=Emperor%20

NCBI Nucleotide

Search CoreNucleotide for Emperor penguin

Found 10 nucleotide sequences. CoreNucleotide [10]

Display Summary Show 20 Sort by Send to

All: 10 Bacteria: 0 RefSeq: 0 mRNA: 0

Items 1 - 10 of 10 One page.

1: [DQ137185](#) Reports Links  
Aptenodytes forsteri voucher EG3 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial gi|75353879|gb|DQ137185.1|[75353879]

2: [DQ137246](#) Reports Links  
Aptenodytes forsteri voucher EG3 recombination activating protein (RAG-1) gene, partial cds gi|75863953|gb|DQ137246.1|[75863953]

3: [DQ137225](#) Reports Links  
Aptenodytes forsteri voucher EG3 cytochrome b (cytb) gene, partial cds; mitochondrial gi|75354632|gb|DQ137225.1|[75354632]

4: [DQ137187](#) Reports Links  
Aptenodytes forsteri voucher EG3 12S ribosomal RNA gene, partial sequence; mitochondrial gi|75354097|gb|DQ137187.1|[75354097]

8. We will build our first tree using a mitochondrial cytochrome gene. Scroll down to Entry 3 for the cytochrome b gene and click on the accession number [DQ137225](#) (highlighted in yellow above) to find out more about this gene.
9. The next screen (partial screenshot on the next page) contains a lot of useful information. First, note that the scientific name of Emperor penguins is *Aptenodytes forsteri*. Second, the classification hierarchy of Emperor penguins is listed below the ORGANISM name. Third, reference information for the paper in which this gene sequence was first published is next, followed by specific sequence information for the gene. Note that we will build our tree using a partial gene sequence of 1008 nucleotide base pairs (bp), which codes for a polypeptide chain of 336 amino acids.

NCBI Sequence Viewer v2.0 - Mozilla Firefox

http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucore&id

NCBI Nucleotide

Search CoreNucleotide for [ ] Go Clear

Display GenBank Show 5 Send to Hide:  sequence  all but gene, CDS and mRNA features

Range: from begin to end  Reverse complemented strand Features: + Refresh

1: [DQ137225](#). Reports [Aptenodytes forsteri](#) [gi:75354632] Links

Features Sequence

LOCUS DQ137225 1008 bp DNA linear VRT 29-DEC-2005

DEFINITION [Aptenodytes forsteri](#) voucher EG3 cytochrome b (cytb) gene, partial cds; mitochondrial.

ACCESSION DQ137225

VERSION DQ137225.1 GI:75354632

KEYWORDS .

SOURCE mitochondrion [Aptenodytes forsteri](#) (emperor penguin)

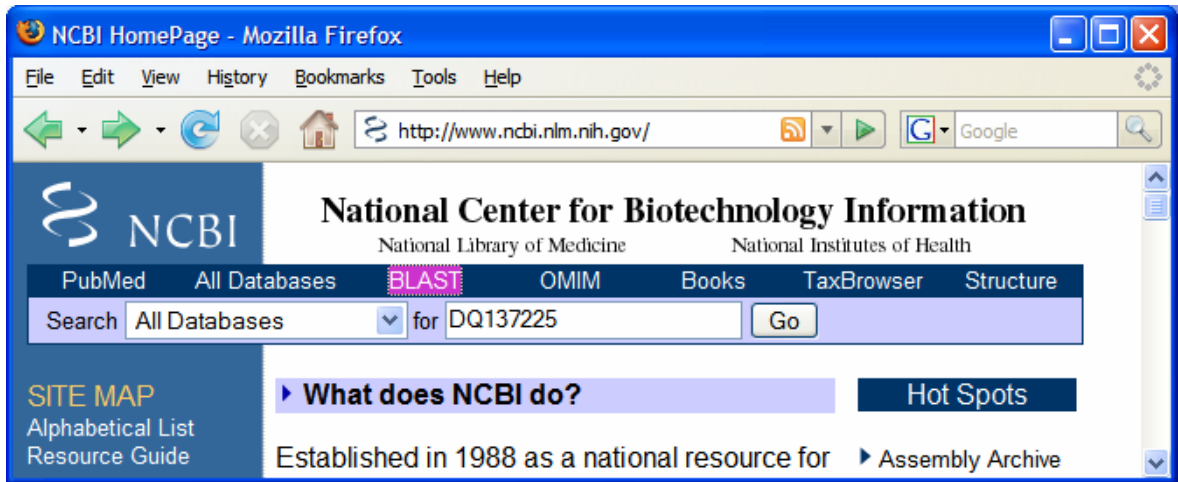
ORGANISM [Aptenodytes forsteri](#)  
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;  
 Archosauria; Dinosauria; Saurischia; Theropoda; Coelurosauria;  
 Aves; Neognathae; Sphenisciformes; Spheniscidae; Aptenodytes.

REFERENCE 1 (bases 1 to 1008)  
 AUTHORS Baker,A.J., Pereira,S.L., Haddrath,O.P. and Edge,K.-A.  
 TITLE Multiple gene evidence for expansion of extant penguins out of Antarctica due to global cooling  
 JOURNAL Proc. Biol. Sci. 273 (1582), 11-17 (2006)

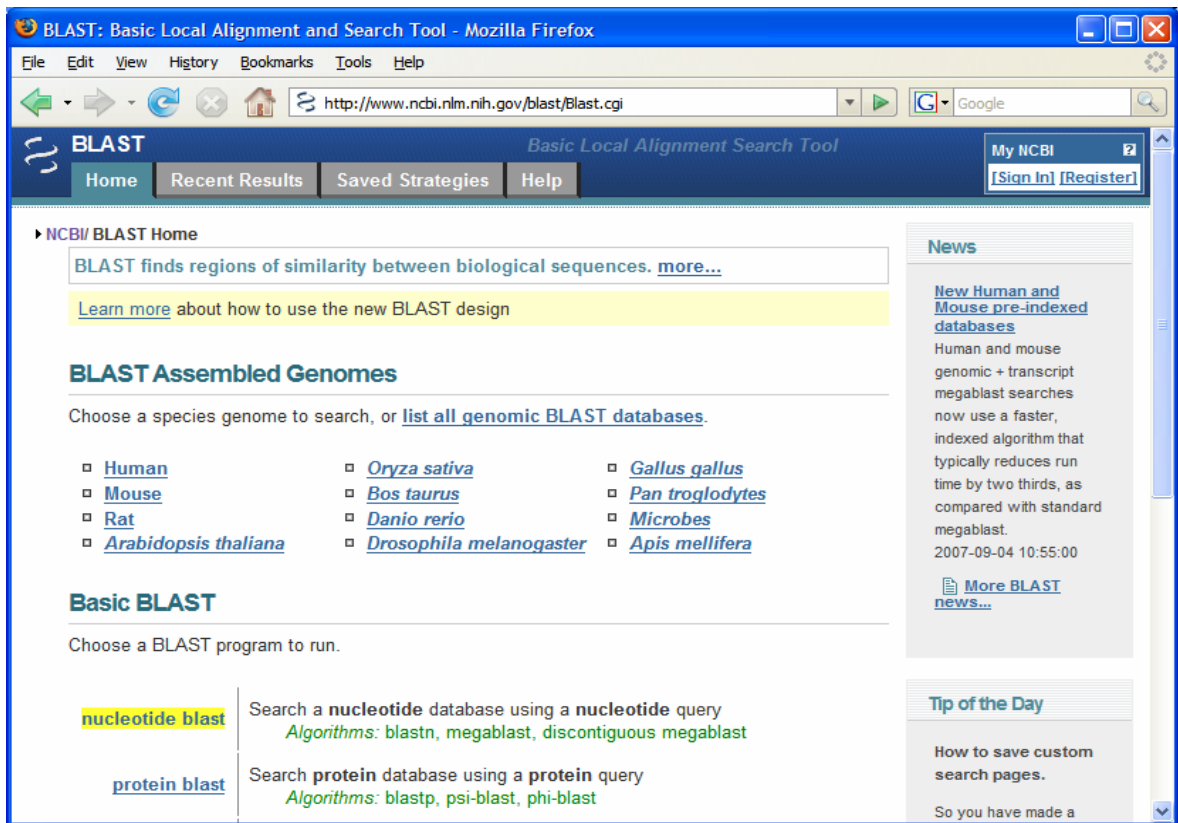
REFERENCE 2 (bases 1 to 1008)  
 AUTHORS Baker,A.J., Pereira,S.L., Haddrath,O.P. and Edge,K.-A.  
 TITLE Direct Submission  
 JOURNAL Submitted (19-JUL-2005) Natural History, Royal Ontario Museum, 100 Queen's Park, Toronto, ON M5S 2C6, Canada

10. If you click on the [Aptenodytes forsteri](#) link in the above screen, a new window opens that shows a more detailed taxonomic classification of that species. Also, a box will appear with the taxonomic rank (if applicable) of each entry in the lineage as one “mouses over” the entry. **What is the class to which all birds belong?**
11. Based on their morphological and behavioral characteristics, penguins have been grouped into a single family. **What is the name of this family?** Click on the family name, to see a taxonomic listing of the six genera of penguin species. **Note: Print out this page or jot down the six genus names, as you will need this list later in the exercise.**
12. Standard hierarchies such as this are based largely upon physical, easy-to-see characteristics. Phylogenies based on molecular sequence data often are consistent with the taxonomic classification system. However, as you will see shortly, there can be surprises with the molecular data. Thus, factors *other* than common descent must be responsible for why some organisms look very similar to one another.

13. To build our tree, we will now use BLAST to compare the cytochrome b gene from Emperor penguins to the same gene in a large number of other organisms. Click on the NCBI icon to go back to the NCBI homepage.



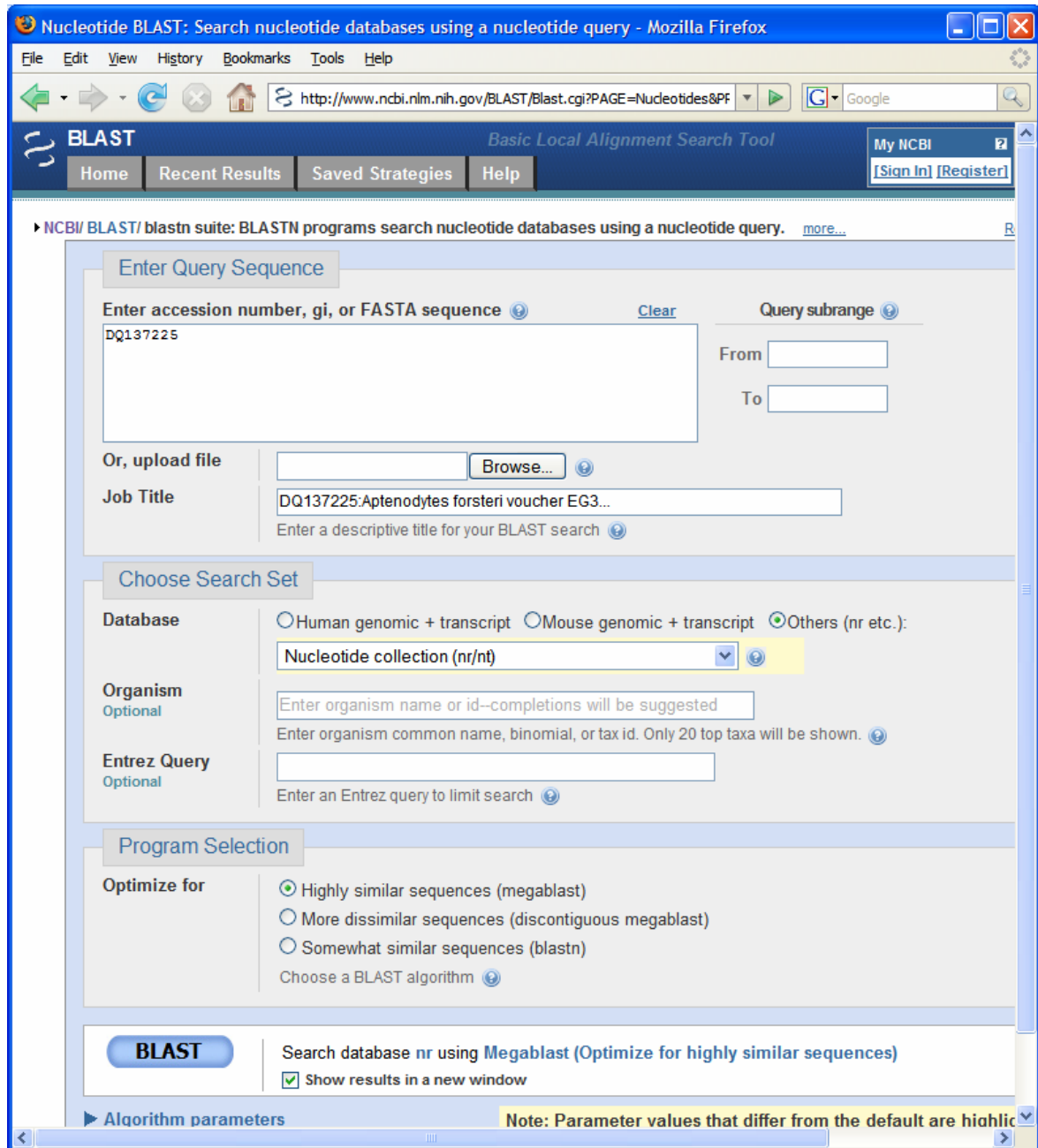
14. Type or paste the accession number for entry #2 ([DQ137225](#)) in the “for” search box and click on “BLAST” in the menu bar (purple highlight above). A screen that gives BLAST search options appears next, part of which is shown below.



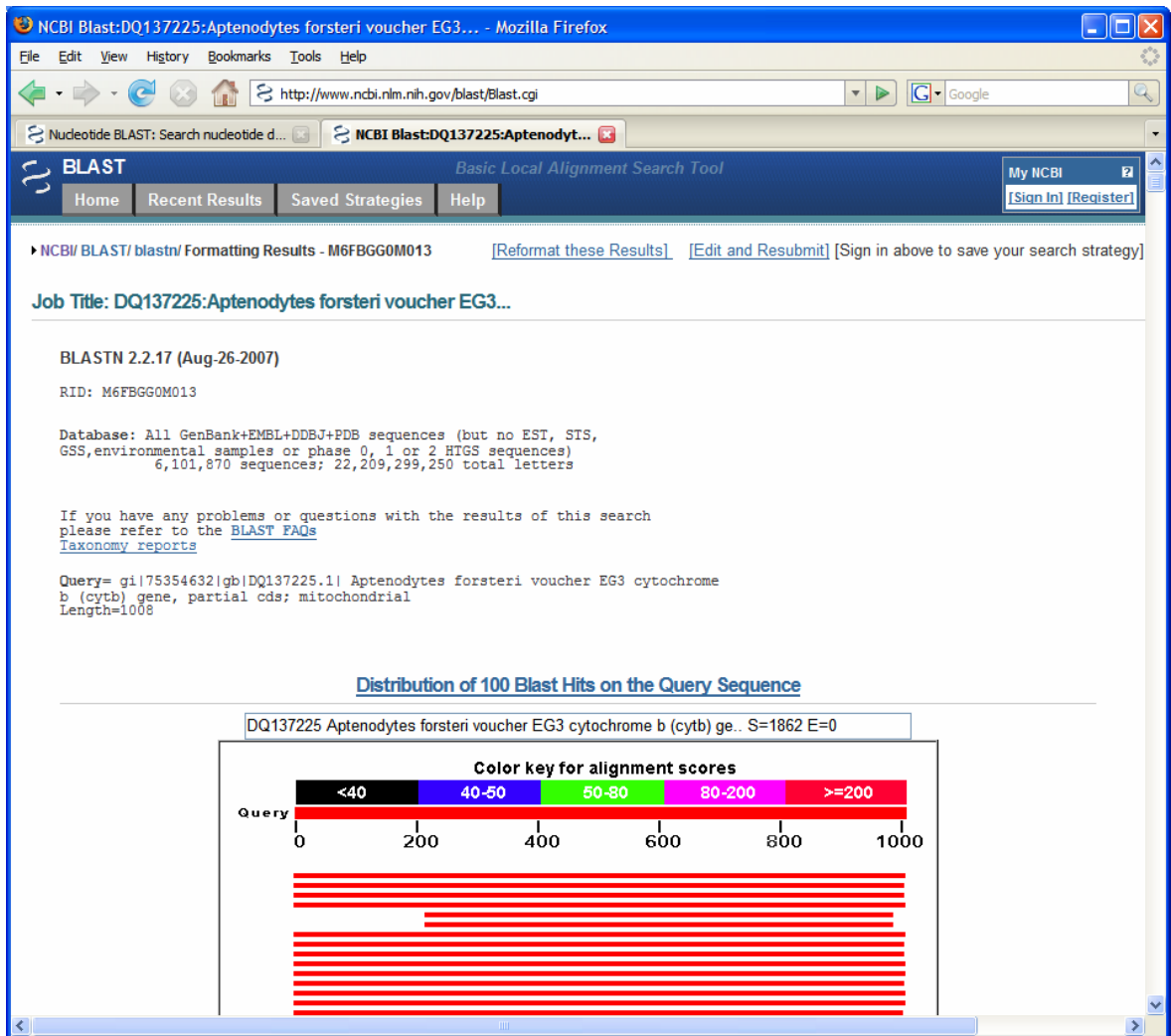
15. Click on the “nucleotide blast” link following **Choose a BLAST program to run** in the Basic BLAST window (yellow highlight above).



16. In the BLASTN window that next appears (screenshot below), type the DQ137225 accession number in the **Enter Query Sequence** box and select “Others (nr etc.):” next to **Database** in the **Choose Search Set** box. Note that **Optimize for “Highly similar sequences (megablast)”** is the default feature. Now, check the “Show results in new window” box, click on the **BLAST** button and prepare to wait!



17. The next window will tell you whether or not your request has been successfully submitted, and, if so, approximately how much time you will have to wait to get your results. The results will automatically appear in this same window once the BLAST search is completed.



18. The BLAST results (partial screenshot above) show the 100 best sequence matches (e.g., BLAST Hits) to the cytochrome b gene from the Emperor penguin. The results are in graphical form first, with the extent of sequence overlap shown as red bars. If you mouse over the first red bar, you will see that it is our query entry (e.g., the cytochrome b gene of *Aptenodytes forsteri*).
19. Now scroll down the screen to below the graphical output, to examine the table with the species names and BLAST scores of these 100 hits (see next partial screen shot). The species most closely-related to Emperor penguins are at the top of the table and have the highest scores.

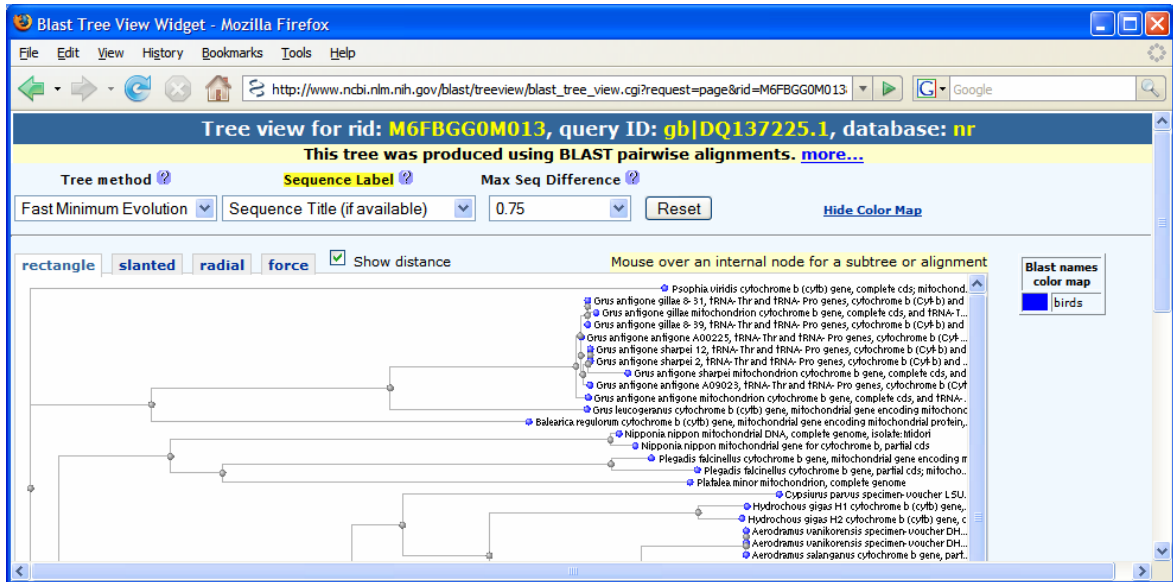
Distance tree of results **NEW**

Legend for links to other resources: **U** UniGene **E** GEO **G** Gene **S** Structure **M** Map Viewer

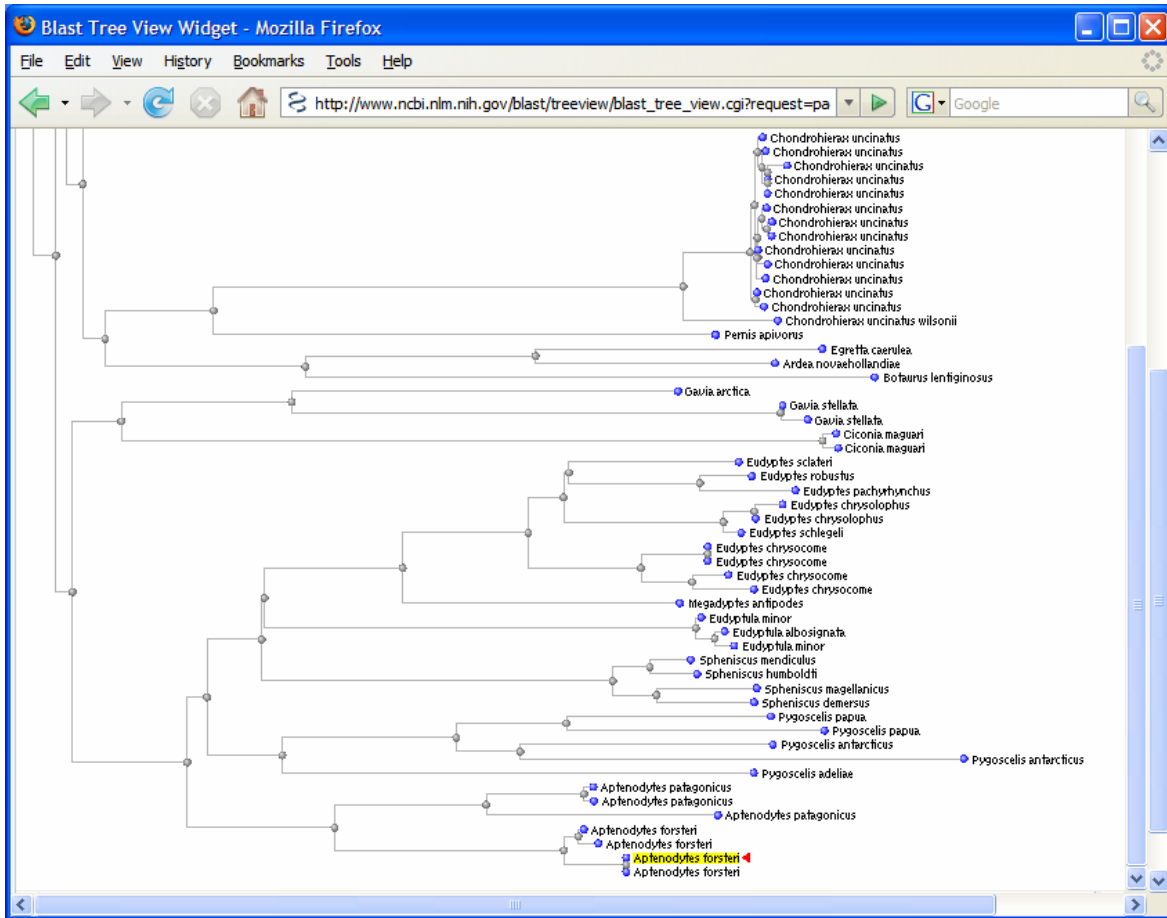
Sequences producing significant alignments:  
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
<a href="#">DQ137225.1</a>	Aptenodytes forsteri voucher EG3 cytochrome b (cytb) gei	<a href="#">1862</a>	1862	100%	0.0	100%
<a href="#">DQ137226.1</a>	Aptenodytes patagonicus voucher KI2 cytochrome b (cytb)	<a href="#">1559</a>	1559	99%	0.0	94%
<a href="#">AY139623.1</a>	Aptenodytes patagonicus cytochrome b gene, complete cd	<a href="#">1548</a>	1548	99%	0.0	94%
<a href="#">AF076044.1</a>	Aptenodytes patagonicus cytochrome b (cytb) gene, mitoc	<a href="#">1413</a>	1413	100%	0.0	92%
<a href="#">AF336603.1</a>	Aptenodytes forsteri haplotype APFD2 cytochrome b (cytb)	<a href="#">1369</a>	1369	76%	0.0	98%
<a href="#">AF336607.1</a>	Aptenodytes forsteri haplotype APFO1 cytochrome b (cytb)	<a href="#">1358</a>	1358	76%	0.0	98%
<a href="#">DQ137219.1</a>	Spheniscus mendiculus voucher GalPenF cytochrome b (cy)	<a href="#">1279</a>	1279	99%	0.0	99%
<a href="#">DQ137214.1</a>	Eudiptula minor voucher CIB6 cytochrome b (cytb) gene,	<a href="#">1277</a>	1277	99%	0.0	99%
<a href="#">DQ137213.1</a>	Eudiptula albusigneta voucher WFed cytochrome b (cytb),	<a href="#">1277</a>	1277	99%	0.0	99%
<a href="#">DQ137222.1</a>	Pygocelia pupa voucher CPB1 cytochrome b (cytb) gene	<a href="#">1275</a>	1275	100%	0.0	99%

20. Note that some species appear multiple times in the above list (due to multiple sequence database entries for the same gene and organism). Thus, the number of unique species in our data set is actually less than 100 species.
21. Now for the fun part! We will have BLAST reconstruct a phylogenetic tree to more easily see the evolutionary relationships between these 100 gene sequences (and the species of organisms from which the genes were sequenced!). To do so, simply click on the “[Distance tree of results \*\*NEW\*\*](#)” link, which is between the graph and the table.
22. The top part of the resultant phylogeny is on the next page (you will have to use the arrow buttons to scroll down to see the entire tree). The entry for the gene from our query species (*Aptenodytes forsteri*) is highlighted in yellow near the bottom of the window.
23. In a molecular phylogeny, the closer two particular entries are among the branches of the tree, the more similar their DNA sequences are and, we assume, the shorter the evolutionary distance between the species from which the genes were obtained. Conversely, two species with a greater distance between their branches have less similar DNA sequences and are assumed to be less closely-related.

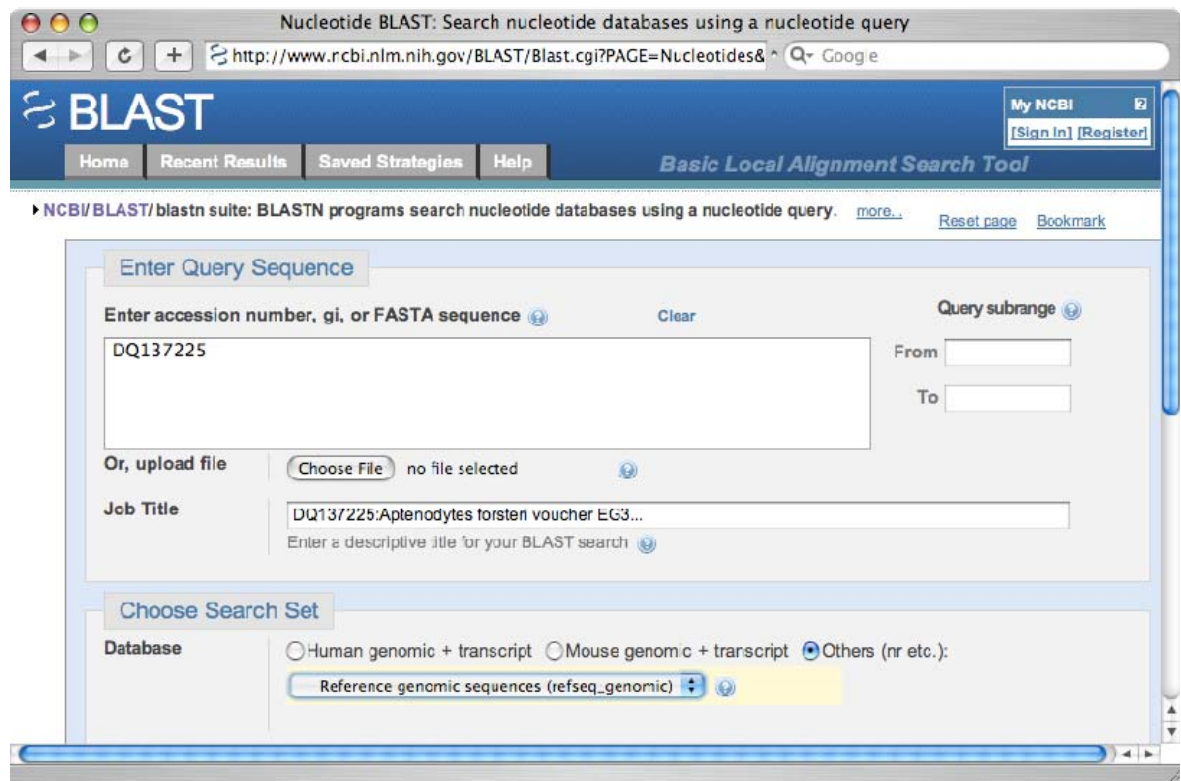


24. By default, the Sequence Title of each of the 100 hits is indicated on the above tree. This view is interesting, since it shows which gene sequences were used to build the tree, and you should spend a few minutes looking at the tree in this format.
25. For the rest of this exercise, you can simplify the tree to see just the species names by selecting “Taxonomic Name (if available)” in the drop-down menu under “Sequence Label” (highlighted in the screen shot above).
26. At this point, you are probably wondering what all the different animals are. By selecting “BLAST Name (if available)” in the same drop-down menu, you can find out what general class of organism each entry is. **To which class of organisms do all the members of this phylogeny belong?** (*Note: one hint that they are all in the same class is that only “birds” appears in the BLAST names color map legend and each entry on the tree is preceded by the same blue circle.*)
27. On the next page is a screen shot of the bottom half of the tree with “Taxonomic Names” selected. Using this tree you can now answer the first question on p. 2 of this exercise. **What are the names (scientific and common) of the closest living relative of Emperor penguins?** *Note: make sure that you use the live Web site and not the printed screenshot to answer this and the following questions, as the tree may have changed since the lab manual was printed!*
28. **Does this tree support the standard NCBI taxonomy that you looked at earlier, which indicates that all penguins living today are in the same family (and thus share the same most-recent common ancestor)? Why or why not?**
29. The standard NCBI taxonomy does not indicate the evolutionary relatedness amongst the different genera of penguins. **What does your BLAST tree tell you about the evolutionary relationship of the genus *Aptenodytes* to the other penguin genera? Of the non-*Aptenodytes* penguin genera to each other?**



30. From the BLAST tree, what are the scientific and common names of the closest *non-penguin* relative(s) of the Emperor penguins? What do you think the common ancestor of Emperor penguins and the closest bird relative(s) would have looked like? (To obtain more information about the species and the families to which they belong, start by opening the Taxonomy Browser window of NCBI and typing the species name into the Search box. There are also many Web sites with detailed information, photographs, etc. of most living organisms, as well as reference bird atlases on the front table to flip through.)
31. Currently, there are conflicting interpretations of sequence data from the bird lineages in the scientific literature. Links to two such recently published papers are posted in the Laboratory Material area on Blackboard. See Figure 2 in the Van Tuinen et al., 2001 paper and Figs. 1 & 2 in the Watanabe et al., 2006 paper. **What type of birds does each paper suggest are the closest living relatives to penguins? Does your BLAST tree agree with either of these trees? If not, which of the three trees do you think makes the most sense? Why?**
32. To answer question 5 on page 2, you will need to build another tree that includes more distantly-related species. To do this, return to the BLAST Home page, open a new “nucleotide BLAST” window, type the DQ137225 accession number (for the cytochrome b gene of *A. forsteri*) into the **Enter Query Sequence** box, and select

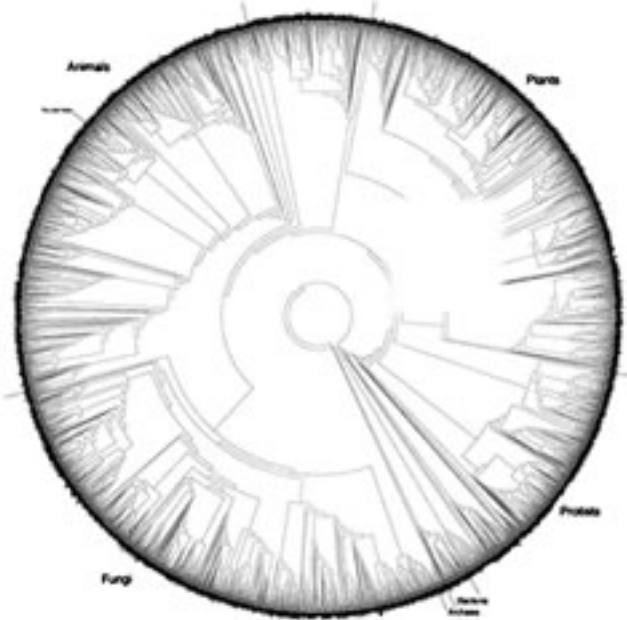
the button next to the “Others (nr etc.):” database. This time, however, select the “Reference genomic sequences (refseq\_genomic)” database using the drop-down menu under the line of database selections.



33. Refseq refers to sequences that have been double checked and verified by a person at NCBI. Since there are fewer of these sequences in the database, this tree will include more distantly-related organisms (e.g., non-birds). Next, scroll down to the **Program Selection** box in the same window and next to **Optimize for**, select “More dissimilar sequences (discontiguous megablast).” Finally, click on **Algorithm parameters**, scroll down to the **General Parameters** box and select “250” next to **Max target sequences**. Now, BLAST away!
34. Repeat steps #17-26 above to analyze the tree created with this search. Once again, you will have to scroll down to find the query species *A. forsteri*. Examine the differences between this tree and the first phylogeny. **What classes of organisms are represented in the second tree? Is there anything in common about all the organisms in the second phylogeny? Is there anything surprising about the taxonomic relationships in the second tree?**
35. **What do you think the common ancestor of all the animals in this tree looked like? Where do you think this common ancestor lived, how did it move, etc.? Be specific!**

#### IV. Extension Exercises - The Circular Tree of Life

1. The Circular Tree of Life (*Science* 300:1692-1697, 2003) is an alternative depiction of sequence relationships between extant species. This tree was created by David Hillis, Derrick Zwickl and Robin Gutell at the University of Texas, and their description follows.
2. *“About this Tree: This tree is from an analysis of small subunit rRNA sequences sampled from about 3,000 species from throughout the Tree of Life. The species were chosen based on their availability, but we attempted to include most of the major groups, sampled very roughly in proportion to the number of known species in each group (although many groups remain over- or under-represented). The number of species represented is approximately the square-root of the number of species thought to exist on Earth (i.e., three thousand out of an estimated nine million species), or about 0.18% of the 1.7 million species that have been formally described and named.”*



3. The circular format of this phylogeny has some great advantages. First, one can easily visualize the relationships between thousands of species. Also, this format suppresses hierarchical thinking (such as the placement of humans at the top or far right of a tree), while at the same time emphasizing that all living species trace back to a common ancestor.
4. You can download the tree directly by going to the following link:  
<http://www.zo.utexas.edu/faculty/antisense/Download.html>
5. Using the Circular Tree of Life
  - a. **How is time represented in this tree? Where would extinct species lie on this tree? Where on the map is the last common ancestor of all living organisms?**
  - b. Using a meter stick and the wall poster of the Circular Tree, measure the distance to the center of the tree from the perimeter. **What is this distance (in cm)?**
  - c. To see if a particular species is on the Circular Tree of Life, search (under the Edit tab in the menu bar) the pdf of this diagram, a link to which is posted on

Blackboard. **Note:** you will need to search for the scientific names of species of interest. You can instead randomly pick species from the perimeter of the tree.

- d. Once you have confirmed that a species is on the Circular Tree (and approximately which quadrant of the circle's circumference the species is in), examine the poster on display in lab to find the exact location of each species.
- e. Carefully mark each species with a sticky arrow on the wall poster.
- f. **Which two species are you comparing? What is the distance (in cm) to the common ancestor of these two species from the perimeter? What percent is this distance relative to the distance to the center (which you measured for part b)?**
- g. **How many branch points are there from the common ancestor to each of the two species? If the number of branch points is different for the two, what is one possible explanation for this?**
- h. **Now, keep one of your two species the same but compare it to a third species. What is the distance in cm to the common ancestor of this new pair of species from the perimeter? What percent is this distance relative to the distance to the center (which you measured for part b)? Why is this distance different than the distance you measured for part (f)?**
- i. **Where on the map is the common ancestor of animals, plants and fungi? What might the common ancestor of these three groups of organisms have looked like?**



**Student Worksheet for Penguin Phylogeny**

**Names** \_\_\_\_\_

**Question III.3.** What types of genes would you expect to be present in all organisms?

In all eukaryotes?

In all plants (but not in animals or fungi)?

In all animals (but not in plants or fungi)?

In all vertebrates (but not in invertebrates)?

**Question III.7** Which of the ten gene entries, if selected, would enable you to build the most inclusive tree (i.e. containing the greatest number of organisms)? Why?

Which entry would result in the least inclusive tree? Why?

**Question III.10** What is the *class* to which all birds belong?

**Question III.11** What is the scientific name of the *family* to which all penguins belong?

**Question III.26** To which class of organisms do all the members of this phylogeny belong?

**Question III.27** What are the names (scientific and common) of the closest living relative of Emperor penguins?

**Question III.28** Does this tree support the standard NCBI taxonomy that you looked at earlier, which indicates that all penguins living today are in the same family (and thus share the same, most-recent common ancestor)?

Why or why not?

**Question III.29** What does your BLAST tree tell you about the evolutionary relationship of the genus *Aptenodytes* to the other penguin genera?

Of the non-*Aptenodytes* penguin genera to each other?

**Question III.30** From the BLAST tree, what are the scientific and common names of the closest *non-penguin* relative(s) of the Emperor penguins?

What do you think the common ancestor of Emperor penguins and the closest bird relative(s) would have looked like?

**Question III.31** What type of birds does each paper suggest are the closest living relatives to penguins?

Does your BLAST tree agree with either of these trees?

If not, which of the three trees do you think makes the most sense? Why?

**Question III.33** What classes of organisms are represented in the second tree?

Is there anything in common about all the organisms in the second phylogeny?

Is there anything surprising about the taxonomic relationships in the second tree?

**Question III.34** What do you think the common ancestor of all the animals in this tree looked like? Where do you think this common ancestor lived, how did it move, etc.? *Be specific!*

**Question IV.5**

- a. How is time represented in this tree?

Where would extinct species lie on this tree?

Where on the map is the last common ancestor of all living organisms?

- b. What is this distance (in cm) to the center of the tree from the perimeter?

- f. Which two species are you comparing?

What is the distance (in cm) to the common ancestor of these two species from the perimeter?

What percent is this distance relative to the distance to the center (which you measured for part b)?

- g. How many branch points are there from the common ancestor to each of the two species?

If the number of branch points is different for the two, what is one possible explanation for this?

- h. Now, keep one of your two species the same but compare it to a third species. What is the distance in cm to the common ancestor of this new pair of species from the perimeter?

What percent is this distance relative to the distance to the center (which you measured for part b)?

Why is this distance different than the distance you measured for part (f)?

- i. Where on the map is the common ancestor of animals, plants and fungi?

What might the common ancestor of these three groups of organisms have looked like?

## **Appendix: Emperor Penguin Life Cycle**

(as described in the 2005 documentary *The March of the Penguins*)

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1. March (summer over): both sexes walk to inland breeding area (up to 70 miles away)
2. By May, courting and mating over. Days cold and dark - huddle together to stay warm
3. Eggs laid early June: transferred from Mom to Dad
4. Moms return to sea to feed (more ice/ longer journey), and Dads continue incubating eggs for > 2 months (go >125 days without food by end)
5. July:
  - Moms walk back to breeding ground
  - Chicks start hatching
  - Dads have a little food in throat pouch that can sustain chicks for 1-2 days
6. Mothers back:
  - Recognize mates by song
  - Chicks passed to Moms, regurgitate food for chick
  - Chick sings to Dad so he can recognize chick later
  - Dads walk back to sea
  - Chicks with Moms, chicks walking within a few days
7. Late August:
  - Moms leave chicks to feed in sea
  - Fathers return, recognize chicks by song, regurgitate food for chicks
8. Over next 2-3 months, parents take turns going to/from sea for food for themselves and chicks (shorter distances as ice melts & cracks w/ warmer summer temperatures); sometimes whole family together at breeding ground
9. November: Females and males have spent 9 mos. as a couple, now part ways & return to sea until following March (when will likely mate w/ someone else)
10. A few weeks later, chicks into the sea (which is very close by); live at sea for *four years*, then start at #1!