

### Lion Phylogeny: Finding connections

Review the DNA samples below. The DNA listed is a segment of a single gene for *cytochrome b*, a critical protein found in all organisms. What do you notice about the samples?

Cameroon	TTCTCCA <b>T</b> TCTTCTAATCCTAATACCCATCTCAGGCATTAT <b>T</b> GAAAACCGCCTCCTCAAAT
Tsavo	TTCTCCA <b>T</b> TCTTCTAATCCTAATACCCATCTCAGGCATTAT <b>T</b> GAAA <b>T</b> CGCCTCCTCAAAT
Fannie Roberts	TTCTCCA <b>T</b> TCTTCTAATCCTAATACCCATCTCAGGCATTAT <b>C</b> GAAAACCGCCTCCTCAAAT
Sabi Sands	TTCTCCA <b>C</b> TCTTCTAATCCTAATACCCATCTCAGGCATTAT <b>T</b> GAAAACCGCCTCCTGAAAT
Umfolozi	TTCTCCA <b>T</b> TCTTCTAATCCTAATACCCATCTCAGGCATTAT <b>C</b> GAAAACCGCCTCCTCAAAT
Zimbabwe	TTCTCCA <b>T</b> TCTTCTAATCCTAATACCCATCTCAGGCATTAT <b>C</b> GAAAACCGCCTCCTCAAAT
Zambia	TTCTCCA <b>T</b> TCTTCTAATCCTAATACCCATCTCAGGCATTAT <b>C</b> GAAAACCGCCTCCTCAAAT
Kalahari	TTCTCCA <b>T</b> TCTTCTAATCCTAATACCCATCTCAGGCATTAT <b>C</b> GAAAACCGCCTCCTCAAAT
Botswana	TTCTCCA <b>C</b> TCTTCTAATCCTAATACCCATCTCAGGCATTAT <b>T</b> GAAAACCGCCTCCTCAAAT
Etosha	TTCTCCA <b>C</b> TCTTCTAATCCTAATACCCATCTCAGGCATTAT <b>T</b> GAAAACCGCCTCCTCAAAT

1. Look at the DNA samples from Cytochrome b shown above. What do you notice about the sequences for each population?
  
2. Lion populations are found all over Sub-Saharan Africa. How do the sequences above reinforce or contradict the idea that populations of lions who are genetically similar live in the same geographic area?
  
3. Based only on the DNA, which populations do you think live in closer proximity to each other? What do you expect to see when comparing genes of lions that live closer together?
  
4. With the previous activity, multiple genes were used to create the bar microsatellite data. Now that we are zooming in on one gene, does the data support yesterday's conclusions? Cite specific evidence to support your answers.
  
5. We will now use the DNA sequences to analyze the DNA in order to find which lion populations are most closely related. We will construct a picture, called a phylogenetic tree. Use the directions that follow to construct your tree.

Name \_\_\_\_\_ Date \_\_\_\_\_ Period \_\_\_\_\_

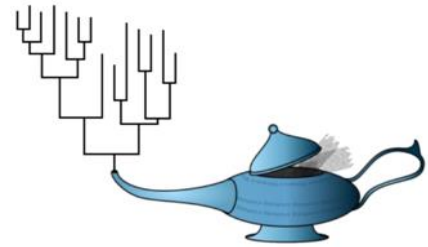
You will now copy and paste actual DNA sequences from the different lion populations into a program that will generate a phylogenetic tree, a diagram that helps illustrate the evolution of different groups of lions.

Follow this link to get the Lion DNA DATA: <http://tinyurl.com/LionDNADATA>

Go to the phylogenetic tree maker website at <http://phylogeny.lirmm.fr/phylo.cgi/index.cgi>

- Click on the "One Click" option.
- You will copy all of the DNA sequences into the large window on the website. (See below)
- IMPORTANT! Be sure there are NO spaces before the first sequence or after the last sequence. Delete only those spaces and not the spaces between the sequences of the different lion populations.

**Phylogeny.fr**  
Robust Phylogenetic Analysis For The Non-Specialist



● **Phylogeny analysis**

- **"One Click"**  
Paste your set of sequences and let the software make decisions on your behalf (Each step is optimized for your data).
- **"Advanced"**  
Manually set parameters for the various steps.
- **"A la Carte"**  
Create your own phylogeny workflow using more programs available.

- Click Submit.
- You do NOT need to enter your email to get your results. They will simply show on the screen once the analysis is complete. This usually takes up to a minute or two.

- Your results will look something like this:

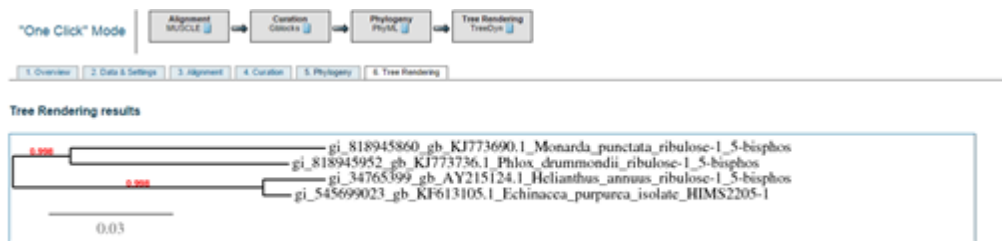


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).

- Now you need to manipulate the tree so that it is easier to analyze.
  - Use the screenshot below to check and uncheck the appropriate boxes as shown.
  - Click on Reroot using mid-point rooting.
  - Under “Display,” unclick Branch annotation.
  - To make the branches easier to view, click “Ignore branch length” under “Display.”

Dynamic Tree Edition

<input checked="" type="checkbox"/> Color	<input checked="" type="checkbox"/> leaf using color <input type="text" value="blue"/>	<input checked="" type="checkbox"/> Reset to original tree	<input checked="" type="checkbox"/> Reroot using <b>mid-point</b> rooting
	<input checked="" type="checkbox"/> branch and assign the group name <input type="text"/>	<input checked="" type="checkbox"/> Reroot (outgroup)	<input checked="" type="checkbox"/> Flip subtree
<input checked="" type="checkbox"/> Add annotations using color <input type="text" value="red"/>		<input checked="" type="checkbox"/> Swap subtrees	<input checked="" type="checkbox"/> Change leaf name

Display:

<input type="checkbox"/> Branch annotation:	<input checked="" type="radio"/> Branch support values	<input type="radio"/> Branch length values	Use color: <input type="text" value="red"/>
<input checked="" type="checkbox"/> Legend at position	<input type="text" value="25"/>	<input type="text" value="120"/>	<input type="button" value="Update"/> Use color: <input type="text" value="dimgray"/>

Ignore branch length

Leaves font:

Tree conformation:  Rectangular  Radial

Image size:  Small  Medium  Large  Extra large

- You can download and save the photo or you can simply right-mouse click on it and print a copy for each of your group members. This should be included with your assignment.

**Phylogeny results**

Compare your tree with the sample tree provided here. Pay particular attention to pairs of populations who appear to be closely related on this tree as well as your own tree.

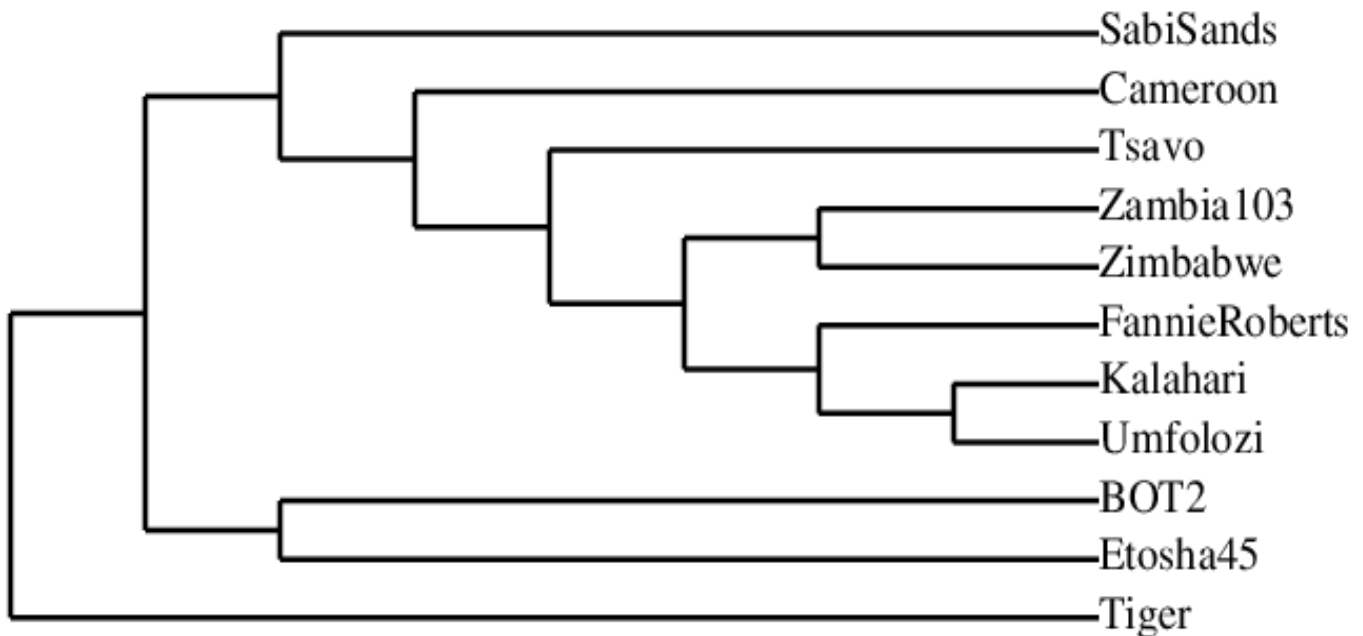


Figure 1: Phylogenetic tree.

