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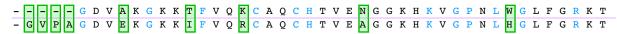
Cytochrome C Comparison Lab

PURPOSE: To compare the relatedness between organisms by examining the amino acid sequence in the protein, Cytochrome C.

BACKGROUND: Genes are made of DNA and are inherited from parent to offspring. Some DNA sequences code for mRNA which, in turn, codes for the amino acid sequence of proteins. Cytochrome C is a protein involved in using energy in the cell. Cytochrome C is found in most, if not all, known eukaryotes. Over time, random mutations in the DNA sequence occur. As a result, the amino acid sequence of Cytochrome C also changes. Cells without usable Cytochrome C are unlikely to survive.

METHOD:

- **A.** Compare the amino acid sequence of Cytochrome C in various organisms.
 - 1. Mark the amino acids which are different Use the example to show you how.



Note that some of the amino acids are always the same in all species, these have been shaded blue or light gray.

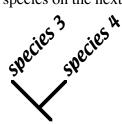
2. Count and record the total number of differences.



- 3. Note: Be sure to study the entire molecule. Each protein sequence has 103-112 amino acids; the sequence extends onto two (2) lines.
- 4. Share your data with the rest of the class and complete Table 1.
- **B**. Make a branching tree, or cladogram using the data in Table 1.
 - 1. The two most closely related species have the fewest differences in amino acid sequence. Place the two most closely related species on the two shortest branches of the tree.



2. Place the next two closest species on the next shortest branches.



- 3. Place the species which is the next closest on the next longest branch. Continue until all the species have been placed.
- **C**. Answer the *questions*.

AMINO ACID SEQUENCES IN CYTOCHROME-C PROTEINS FROM DIFFERENT SPECIES

		1.0	20 30	40 50
Amino Acid Number>	1 2 3 4 5 6 7 8	10 9 0 1 2 3 4 5 6	8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5	
Human, Chimpanzee.		- G D V E K G K K	FIMKCSOCHTVEKGGKHI	
Rhesus monkey		- G D V E K G K K	FIMKCSOCHTVEKGGKHI	TGPNLHGLFGRKTGOAPGYSYTAA
Horse		- G D V E K G K K	F V O K C A O C H T V E K G G K H I	~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Donkey		- G D V E K G K K	F V O K C A O C H T V E K G G K H I	~
Common zebra		- G D V E K G K K	F V O K C A O C H T V E K G G K H I	
Pig, Cow, Sheep		- G D V E K G K K	F V O K C A O C H T V E K G G K H I	
Dog		- G D V E K G K K	F V Q K C A Q C H T V E K G G K H F	~
Gray whale		- G D V E K G K K	F V O K C A O C H T V E K G G K H I	
Rabbit		- G D V E K G K K	F V O K C A O C H T V E K G G K H I	
Kangaroo		- G D V E K G K K	F V O K C A O C H T V E K G G K H I	
Chicken, Turkey		- G D I E K G K K		TGPNLHGLFGRKTGOAEGFSYTDA
Penguin		- G D I E K G K K	F V Q K C S O C H T V E K G G K H I	TGPNLHGIFGRKTGÕAEGFSYTDA
Pekin duck		- G D V E K G K K	F V O K C S O C H T V E K G G K H I	
Snapping turtle		- G D V E K G K K	F V Q K C A Q C H T V E K G G K H I	TGPNLNGLIGRKTGOAEGFSYTEA
Rattlesnake		- G D V E K G K K	F S M K C G T C H T V E E G G K H I	
Bullfrog		- G D V E K G K K	F V O K C A O C H T C E K G G K H I	~
Tuna		- G D V A K G K K	F V Q K C A Q C H T V E N G G K H F	
Screwworm fly	GVP			V G P N L H G L F G R K T G O A A G F A Y T N A
Silkworm moth		A G N A E N G K K	F V Q R C A Q C H T V E A G G K H I	~
Tomato horn "worm"	G V P A		F V O R C A O C H T V E A G G K H I	
Wheat	ASFSEAPI	,,, -,	F K T K C A O C H T V D A G A G H I	~
Rice	ASFSEAPI		~	Q G P N L N G L F G R Q S G T T P G Y S Y S T A
Baker's yeast (Fungus)	TEFK			V G P N L H G I F G R H S G O A O G Y S Y T D A
Candida yeast(Fungus)				V G P N L H G I F S R H S G Q A Q G Y S Y T D A
Neurospora (Fungus)				I G P A L H G L F G R K T G S V D G Y A Y T D A
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60				7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2
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Amino Acid Number-> 0 1 Human, Chimpanzee. N K Rhesus monkey N K Horse N K Donkey N K Common zebra N K Pig, Cow, Sheep N K Gray whale N K Rabbit N K Rabbit N K Rabbit N K Rabbit N K Raparoo N K Rabpit N K Raparoo N K Rabpit N K Rabpit N K Rabpit N K Rabbit N K Romagaroo N K Refin duck N K Penguin N K Penguin N K Screwworm fly N K Screwworm fly N K Screwworm moth N K Tomato horn "worm" N K Wheat N K	N K G I I W G I N K G I T W K I N K G I T W K I N K G I T W K I N K G I T W G	0 1 2 3 4 5 6 7 E D T L M E Y L E E D T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L F E Y L E D D T L F E Y L E D D T L F E Y L E D D T L F E Y L E	9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 1 2 3 4 5 6 7 8 9 0 1 1 2 3 4 5 6 7 8 9 0 1 1 2 3 4 5 6 7 8 8 9 0 1 1 2 3 4 5 6 7 8 7 8 8 9 0 1 1 2 3 4 5 6 7 8 7 8 8 9 0 1 1 8 8 8 7 8 9 8 8 8 9 1 1 8 8 8 9 1 8 8 9 1 8 9 1 8 8 9 1 8 8 9 1 8 8 9 1 8 9 1 8 8 9 1 8 8 9 1 8 8 9 1 8 8 9 1 8 9 1 8 8 9 1 8 8 9 1	7
Amino Acid Number-> 0 1 Human, Chimpanzee. N K Rhesus monkey N K Horse N K Donkey N K Common zebra N K Pig, Cow, Sheep N K Gray whale N K Rabbit N K Rabbit N K Rabbit N K Raparoo N K Chicken, Turkey N K Penguin N K Pekin duck N K Snapping turtle N K Rattlesnake N K Rattlesnake N K Screwworm fly N K Screwworm fly N K Tuna N K Tomato horn "worm" N K Tomato horn "worm" N K Rice N K	N K G I I W G I N K G I T W K I N K G I T W K I N K G I T W K I N K G I T W G	0 1 2 3 4 5 6 7 D T L M E Y L E D T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E D T L M E Y L E E D T L M E Y L E E D T L M E Y L E E D T L M E Y L E E D T L M E Y L E E D T L M E Y L E E D T L M E Y L E E D T L M E Y L E E D T L M E Y L E E D T L M E Y L E E D T L M E Y L E E D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L M E Y L E D D T L F E Y L E D D T L F E Y L E D D T L F E Y L E	9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 1 2 3 4 5 6 7 8 9 0 1 1 2 3 4 5 6 7 8 9 8 8 8 9 0 1 1 2 3 4 5 6 7 8 9 8 8 8 9 0 1 1 2 3 4 5 6 7 8 9 8 8 8 9 1 1 5 7 8 9 1 7 8 8 9 1 7 8 9 1 8 8 9 1 7 8 9 1 8 9	7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 AMINO ACID SYMBOLS
Amino Acid Number-> 0 1 Human, Chimpanzee. N K Rhesus monkey. N K Horse N K Donkey N K Common zebra N K Pig, Cow, Sheep. N K Gray whale. N K Rabbit. N K Rabbit. N K Rabbit. N K Raparoo. N K Chicken, Turkey. N K Penguin. N K Pekin duck. N K Snapping turtle. N K Rattlesnake. N K Bullfrog. N K Screwworm fly. N K Silkworm moth. N K Tomato horn "worm" N K Wheat. N K Rice N K Baker's yeast. N I	N K G I I W G I N K G I T W K I N K G I T W K I N K G I T W K I N K G I T W G	0 1 2 3 4 5 6 7 7 E D T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E D T L F E Y L E D D T L F E Y L E D D T L F E Y L E D D T L F E Y L E D D T L F E Y L E D D T L F E Y L E D D T L F E Y L E E N T L Y D Y L L E N N M S E Y L T	9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 1 2 3 4 5 6 7 8 8 9 0 1 1 2 3 4 5 6 7 8 8 9 0 1 1 2 3 4 5 6 7 8 8 9 0 1 1 2 3 3 4 5 6 7 8 8 9 0 1 1 2 3 3 4 5 6 7 8 8 9 0 1 1 2 3 3 4 5 6 7 8 9 8 8 8 9 1 1 2 1 2 3 3 4 5 6 7 8 9 8 8 8 9 1 1 2 1 2 3 3 4 5 6 7 8 9 8 8 8 9 1 1 2 1 2 3 3 4 5 6 7 8 9 8 8 8 9 1 1 2 1 2 3 3 4 5 6 7 8 9 8 8 9 1 1 2 1 2 3 3 4 5 6 7 8 9 8 9 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1	7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 AMINO ACID SYMBOLS
Amino Acid Number-> 0 1 Human, Chimpanzee. N K Rhesus monkey N K Horse N K Donkey N K Common zebra N K Dog N K Gray whale N K Gray whale N K Kangaroo N K Chicken, Turkey N K Penguin N K Penguin N K Peskin duck N K Snapping turtle N K Snapping turtle N K Stattlesnake N K Bullfrog N K Screwworm fly N K Screwworm fly N K Tomato horn "worm" N K Wheat N K Baker's yeast N I Candida yeast N K	N K G I I W G I N K G I T W K I N K G I T W K I N K G I T W K I N K G I T W G	0 1 2 3 4 5 6 7 E D T L M E Y L E E D T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E E T L M E Y L E E D T L M E Y L E E N T L Y D Y L L E N N M S E Y L T E N N M S E Y L	9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 1 2 3 4 5 6 7 8 9 0 1 1 2 3 4 5 6 7 8 9 0 1 1 2 3 3 4 5 6 7 8 8 9 0 1 1 2 3 3 4 5 6 7 8 9 8 8 8 9 0 1 1 2 3 3 4 5 6 7 8 9 8 8 8 9 1 1 1 1 1 1 1 1 1 1 1 1 1 1	7

Symbols in light blue or gray represent amino acids which show NO differences in any organism on the list, so you can ignore them.

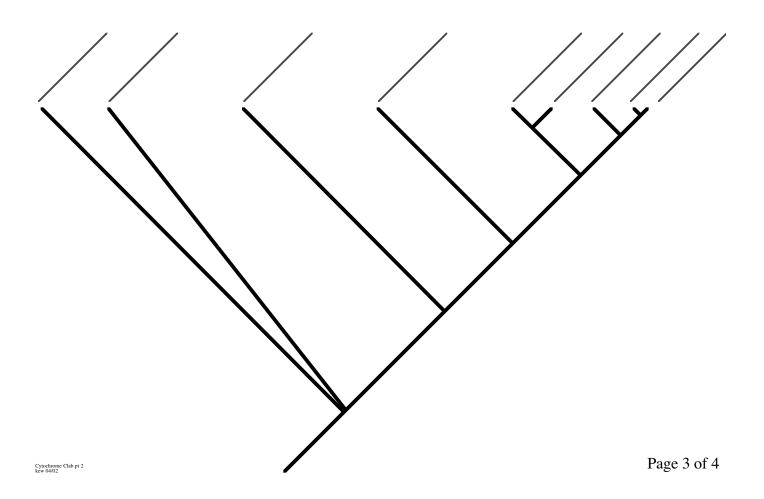
(Adapted from Strahler, Arthur, Science & Earth History, 1987, p. 348)

Name	
Name	

Table 1: Differences between amino acid sequences in Cytochrome C protein for nine species.

	horse	donkey	whale	chicken	penguin	snake	moth	yeast	wheat
horse	0	·						· ·	
donkey		0							
whale			0						
chicken				0					
penguin					0				
snake						0			
moth							0		
yeast								0	
wheat									0

Cladogram, or Branching Tree, Based on Cytochrome C Amino Acid Sequence



ESTIONS:					Γ	Name	
anow many v	vtochrome C	amino acid s	eanence				
differences are							
Make a branc	hing tree, or	cladogram fo	or chickens,	penguins, ar	nd turkeys.		
a. Predict th	ne number of	Cytochrome you would ex	C amino ac pect to see b	id between i.	horse and z	ebra	
4		J = 0.0 = 0.0	F		donkey and		
o. What oth	er information	on did you use	e to make thi		•		
		J		1			
Use this Cyto	chrome C se	quence differe	ence data to	add a brancl	h to the tree o	on page 3.	
	horse	penguin	snake	tuna	silkworm	yeast	wheat
tomato	HOISE	pengum	SHAKE	tuna	moth	yeası	wneat
hornworm	26	18	24	28	5	42	38
moth							
i ist three othe	er things use	d to determine	e now organi	isms are reia	ited to each o	otner.	
List tince our							
	nore closely	related organ	icme have m	nore similar	Cytochrome	C	
Explain why i	nore closely	related organ	isms have m	nore similar	Cytochrome	C.	
	nore closely	related organ	isms have m	nore similar	Cytochrome	C.	
	nore closely	related organ	isms have n	nore similar	Cytochrome	C.	
	nore closely	related organ	isms have m	nore similar	Cytochrome	C.	
	more closely	related organ	isms have m	nore similar	Cytochrome	C.	
	nore closely	related organ	isms have m	nore similar	Cytochrome	C.	
	cluding othe	r genes, sugge	ests that fung	gi are more o	closely relate	d to animals	
Explain why reconstruction of the data, in what are som	cluding othe	r genes, sugge	ests that fung	gi are more o	closely relate	d to animals	
Explain why reconstruction of the data, in what are som	cluding othe	r genes, sugge	ests that fung	gi are more o	closely relate	d to animals	