Name ________________
Period ________________
Date ________________
Science

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.

```
- - - - - G D V A K G K K T F V Q K C A Q C H T V E N G G K H K V G P N L W G L F G R K T
- G V P A G D V E K G K K I F V Q R C A Q C H T V E A G G K H K V G P N L H G L F G R K T
```

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.

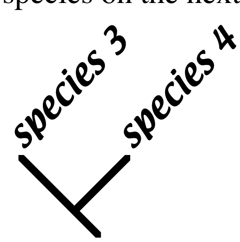
Tuna & Fly: 24

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*.

Name _____

AMINO ACID SEQUENCES IN CYTOCHROME-C PROTEINS FROM DIFFERENT SPECIES

Amino Acid Number ---->	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59
Human, Chimpanzee.	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	I	M	K	C	S	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	Y	S	Y	T	A	A
Rhesus monkey.....	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	I	M	K	C	S	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	Y	S	Y	T	A	A
Horse	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	F	T	Y	T	D	A
Donkey	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	F	S	Y	T	D	A
Common zebra	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	F	S	Y	T	D	A
Pig, Cow, Sheep...	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	F	S	Y	T	D	A
Dog.....	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	F	S	Y	T	D	A
Gray whale	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	V	G	F	S	Y	T	D	A
Rabbit.....	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	V	G	F	S	Y	T	D	A
Kangaroo.....	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	N	G	I	F	G	R	K	T	G	Q	A	P	G	F	T	Y	T	D	A
Chicken, Turkey...	-	-	-	-	-	-	-	-	-	G	D	I	E	K	G	K	K	I	F	V	Q	K	C	S	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	E	G	F	S	Y	T	D	A
Penguin.....	-	-	-	-	-	-	-	-	-	G	D	I	E	K	G	K	K	I	F	V	Q	K	C	S	O	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	I	F	G	R	K	T	G	Q	A	E	G	F	S	Y	T	D	A
Pekin duck.....	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	S	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	E	G	F	S	Y	T	D	A
Snapping turtle...	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	N	G	L	I	G	R	K	T	G	Q	A	E	G	F	S	Y	T	E	A
Rattlesnake.....	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	S	M	K	C	G	T	C	H	T	V	E	E	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	V	G	Y	S	Y	T	A	A
Bullfrog.....	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	C	E	K	G	G	K	H	K	V	G	P	N	L	Y	G	L	I	G	R	K	T	G	Q	A	A	G	F	S	Y	T	D	A
Tuna.....	-	-	-	-	-	-	-	-	-	G	D	V	A	K	G	K	K	T	F	V	Q	K	C	A	Q	C	H	T	V	E	N	G	G	K	H	K	V	G	P	N	L	W	G	L	F	G	R	K	T	G	Q	A	E	G	Y	S	Y	T	D	A
Screwworm fly.....	-	-	-	-	G	V	P	A	G	D	V	E	K	G	K	K	I	F	V	Q	R	C	A	Q	C	H	T	V	E	A	G	G	K	H	K	V	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	A	G	F	A	Y	T	N	A
Silkworm moth.....	-	-	-	-	G	V	P	A	G	N	A	E	N	G	K	K	I	F	V	Q	R	C	A	Q	C	H	T	V	E	A	G	G	K	H	K	V	G	P	N	L	H	G	F	Y	G	R	K	T	G	Q	A	P	G	F	S	Y	S	N	A
Tomato horn “worm”	-	-	-	-	G	V	P	A	G	N	A	D	N	G	K	K	I	F	V	Q	R	C	A	Q	C	H	T	V	E	A	G	G	K	H	K	V	G	P	N	L	H	G	F	F	G	R	K	T	G	Q	A	P	G	F	S	Y	S	N	A
Wheat	A	S	F	S	E	A	P	P	G	N	P	D	A	G	A	K	I	F	K	T	K	C	A	Q	C	H	T	V	D	A	G	A	G	H	K	Q	G	P	N	L	H	G	L	F	G	R	Q	S	G	T	T	A	G	Y	S	Y	S	A	A
Rice	A	S	F	S	E	A	P	P	G	N	P	K	A	G	E	K	I	F	K	T	K	C	A	Q	C	H	T	V	D	K	G	A	G	H	K	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)	-	-	-	T	E	F	K	A	G	S	A	K	K	G	A	T	L	F	K	T	R	C	E	L	C	H	T	V	E	K	G	G	P	H	K	V	G	P	N	L	H	G	I	F	G	R	H	S	G	Q	A	Q	G	Y	S	Y	T	D	A
Candida yeast(Fungus)	-	-	P	A	P	F	E	0	G	S	A	K	K	G	A	T	L	F	K	T	R	C	A	E	C	H	T	I	E	A	G	G	P	H	K	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)	-	-	-	-	G	F	S	A	G	D	S	K	K	G	A	N	L	F	K	T	R	C	A	E	C	H	G	E	G	G	N	L	T	Q	K	I	G	P	A	L	H	G	L	F	G	R	K	T	G	S	V	D	G	Y	A	Y	T	D	A

[CONTINUED FROM ABOVE]

Amino Acid Number->	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118
Human, Chimpanzee.	N	K	N	K	G	I	I	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	V	G	I	K	K	K	E	E	R	A	D	L	I	A	Y	L	K	K	A	T	N	E
Rhesus monkey.....	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	V	G	I	K	K	K	E	E	R	A	D	L	I	A	Y	L	K	K	A	T	N	E
Horse	N	K	N	K	G	I	T	W	K	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	T	E	R	E	D	L	I	A	Y	L	K	K	A	T	N	E
Donkey	N	K	N	K	G	I	T	W	K	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	T	E	R	E	D	L	I	A	Y	L	K	K	A	T	N	E
Common zebra	N	K	N	K	G	I	T	W	K	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	T	E	R	E	D	L	I	A	Y	L	K	K	A	T	N	E
Pig, Cow, Sheep...	N	K	N	K	G	I	T	W	G	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	G	E	R	E	D	L	I	A	Y	L	K	K	A	T	N	E
Dog.....	N	K	N	K	G	I	T	W	G	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	T	G	E	R	A	D	L	I	A	Y	L	K	K	A	T	K	E
Gray whale.....	N	K	N	K	G	I	T	W	G	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	G	E	R	A	D	L	I	A	Y	L	K	K	A	T	N	E
Rabbit.....	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	D	E	R	A	D	L	I	A	Y	L	K	K	A	T	N	E
Kangaroo.....	N	K	N	K	G	I	I	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	G	E	R	A	D	L	I	A	Y	L	K	K	A	T	N	E
Chicken, Turkey...	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	S	E	R	V	D	L	I	A	Y	L	K	D	A	T	S	K
Penguin.....	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	S	E	R	A	D	L	I	A	Y	L	K	D	A	T	S	K
Pekin duck.....	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	S	E	R	A	D	L	I	A	Y	L	K	D	A	T	A	K
Snapping turtle...	N	K	N	K	G	I	T	W	G	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	A	E	R	A	D	L	I	A	Y	L	K	D	A	T	S	K
Rattlesnake.....	N	K	N	K	G	I	I	W	G	D	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	V	F	T	G	L	K	K	K	K	E	R	T	D	L	I	A	Y	L	K	E	A	T	A	K
Bullfrog.....	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	G	E	R	Q	D	L	I	A	Y	L	K	S	A	C	S	K
Tuna.....	N	K	S	K	G	I	V	W	N	N	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	G	E	R	Q	D	L	V	A	Y	L	K	S	A	T	S	-
Screwworm fly.....	N	K	A	K	G	I	T	W	Q	D	D	T	L	F	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	L	K	K	P	N	E	R	G	D	L	I	A	Y	L	K	S	A	T	K	-
Silkworm moth.....	N	K	A	K	G	I	T	W	G	D	D	T	L	F	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	V	F	A	G	L	K	K	A	N	E	R	A	D	L	I	A	Y	L	K	E	S	T	K	-
Tomato horn “worm”	N	K	A	K	G	I	T	W	Q	D	D	T	L	F	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	V	F	A	G	L	K	K	A	N	E	R	A	D	L	I	A	Y	L	K	Q	A	T	K		
Wheat.....	N	K	N	K	A	V	E	W	E	E	N	T	L	Y	D	Y	L	L	N	P	K	K	Y	I	P	G	T	K	M	V	F	P	G	L	K	K	P	Q	D	R	A	D	L	I	A	Y	L	K	K	A	T	S	S
Rice	N	K	N	M	A	V	I	W	E	E	N	T	L	Y	D	Y	L	L	N	P	K	K	Y	I	P	G	T	K	M	V	F	P	G	L	K	K	P	Q	E	R	A	D	L	I	S	Y	L	K	E	A	T	S	-
Baker's yeast.....	N	I	K	K	N	V	L	W	D	E	N	N	M	S	E	Y	L	T	N	P	K	K	Y	I	P	G	T	K	M	A	F	G	G	L	K	K	E	K	D	R	N	D	L	I	T	Y	L	K	K	A	C	E	-
Candida yeast.....	N	K	R	A	G	V	E	W	A	E	P	T	M	S	D	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	A	F	G	G	L	K	K	A	K	D	R	N	D	L	V	T	Y	M	L	E	A	S	K	-
Neurospora (Fungus)	N	K	Q	K	G	I	T	W	D	E	N	T	L	F	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	A	F	G	G	L	K	K	D	K	D	R	N	D	I	I	T	F	M	K	E	A	T	A	-

AMINO ACID SYMBOLS
A = Alanine
C = Cysteine
D = Aspartic acid
E = Glutamic acid
F = Phenylalanine
G = Glycine
H = Histidine
I = Isoleucine
K = Lysine
L = Leucine
M = Methionine
N = Asparagine
P = Proline
Q = Glutamine
R = Arginine
S = Serine
T = Threonine
V = Valine
W = Tryptophan
Y = Tyrosine

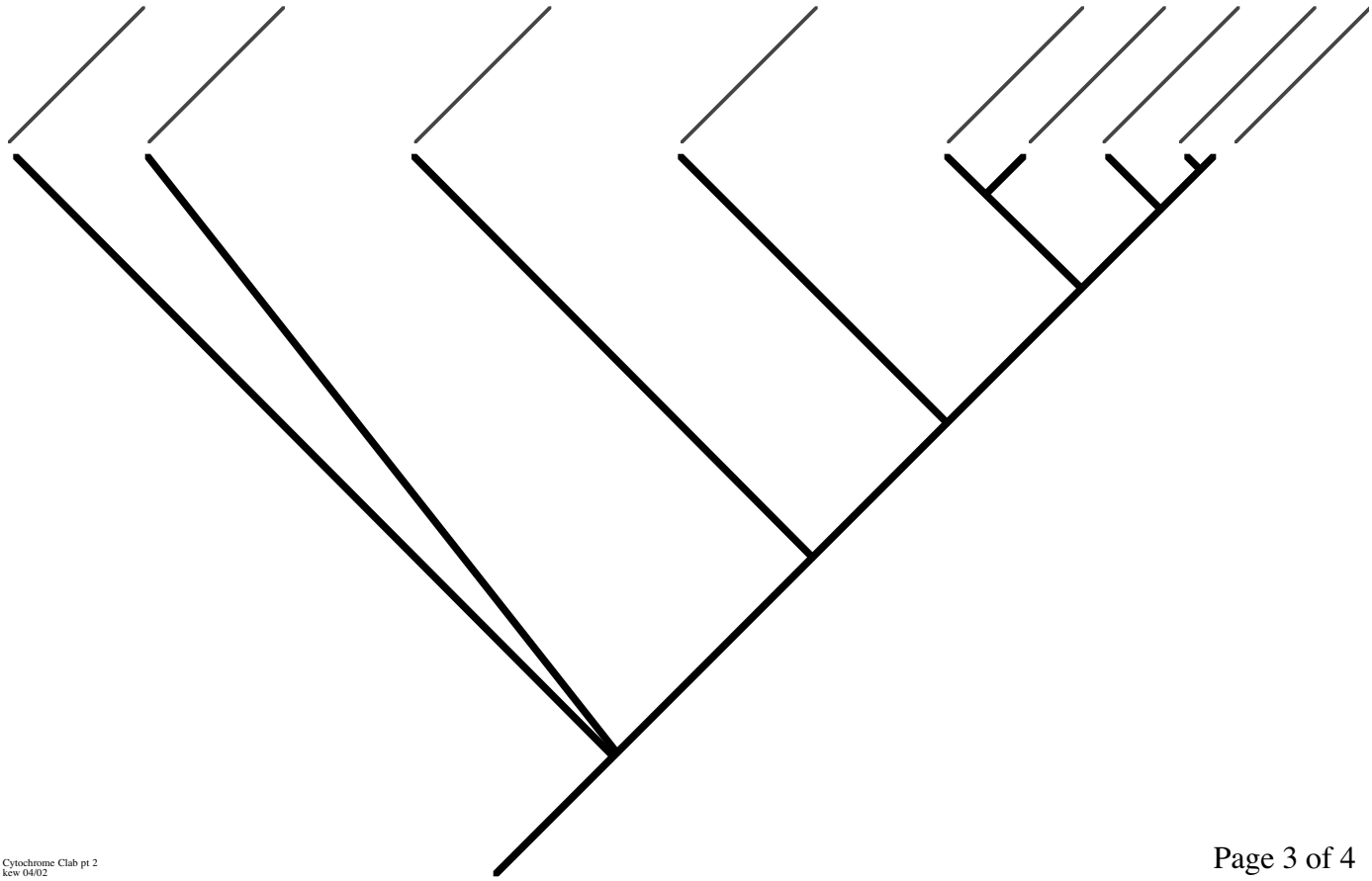
Symbols in light blue or gray represent amino acids which show NO differences in any organism on the list, so you can ignore them.
(Adapted from Strahler, Arthur, Science & Earth History, 1987, p. 348)

Name _____

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

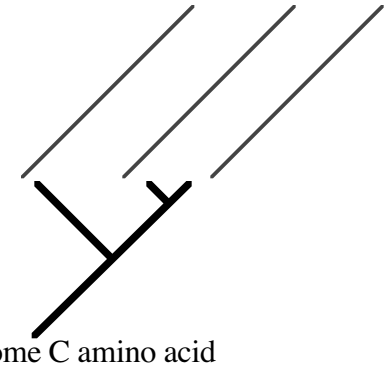


Name _____

QUESTIONS:

1. How many Cytochrome C amino acid sequence differences are there between chickens and turkeys? _____

2. Make a branching tree, or cladogram for chickens, penguins, and turkeys.



3. a. Predict the number of Cytochrome C amino acid sequence differences you would expect to see between
   i. horse and zebra _____
   ii. donkey and zebra _____

   b. What other information did you use to make this prediction?

   _____

   _____

4. Use this Cytochrome C sequence difference data to add a branch to the tree on page 3.

	horse	penguin	snake	tuna	silkworm moth	yeast	wheat
tomato hornworm moth	26	18	24	28	5	42	38

5. List three other things used to determine how organisms are related to each other.

   _____

   _____

   _____

6. Explain why more closely related organisms have more similar Cytochrome C.

   _____

   _____

   _____

   _____

   _____

7. Other data, including other genes, suggests that fungi are more closely related to animals than plants. What are some reasons that the Cytochrome C data suggests that fungi, plants, and animals are equally distantly related?

   _____

   _____

   _____

   _____