NAME_____ **#**_____ **DATE**_____

Lab – Biochemical Evidence of Evolution

Objectives: To examine amino acid sequences from different species and, using this information, determine the evolutionary relationships that may exist between them.

Background: The biochemical comparison of proteins is a technique used to determine evolutionary relationships among groups of organisms. Proteins are composed of specific sequences of amino acids, and this sequence determines the shape and nature of the protein. This sequence is determined by the sequence of nucleotide bases in the DNA molecule. Any mutation or change in the DNA sequence may cause a change in the amino acid sequence and therefore a change in the protein. The more amino acids two organisms have in common, the greater the chance that they are closely related by evolution and may have a more recent common ancestor. Conversely, the greater the time organisms have been diverging from a common ancestor, the greater the differences in their amino acid sequences which can change their proteins.

Two proteins are commonly studied in attempting to deduce evolutionary relationships from differences in amino acid sequences. One is **cytochrome c**, which is a protein used in cellular respiration, and the other is **hemoglobin**, which is the oxygen-carrying molecule found in red blood cells.

Procedure:

Part I. Cytochrome c

The cytochrome c molecule consists of a chain of 104 amino acids. Table 1 shows the same amino acid sequences of the cytochrome c molecules of nine vertebrates. The numbers along the side of the chart refer to the position of these amino acids in the chain. Each letter is an abbreviation for a specific amino acid in the chain.

1. Compare the amino acid sequence of human cytochrome c with that of the other 8 vertebrates. Circle each amino acid that differs from the human amino acid. Record the number of differences in the amino acid sequences in the space at the bottom of each column.
2. In Table 2, list the vertebrates ***in order*** from the ***fewest*** differences to the ***most*** differences.
    a. Frog and turtle cytochrome c molecules have the same number of differences from human cytochrome c. Which vertebrate (frog or turtle) will you put higher (more closely related to humans) on the list? (Hint – think about how each organism gets its oxygen from its environment).
3. ***AFTER*** you have completed Table 2, answer the 3 questions below.

1. According to the evidence, which organism is most closely related to humans? _____
2. Which organism is least closely related to humans? _____
3. Fully explain why you put Frog or Turtle as more closely related to humans.

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Table 1: Cytochrome c Amino Acid Sequences

AA#	Horse	Chicken	Tuna	Frog	**Human**	Shark	Turtle	Monkey	Rabbit
42	Q	Q	Q	Q	**Q**	Q	Q	Q	Q
43	A	A	A	A	**A**	A	A	A	A
44	P	E	E	A	**P**	Q	E	P	V
46	F	F	Y	F	**Y**	F	F	Y	F
47	T	S	S	S	**S**	S	S	S	S
49	T	T	T	T	**T**	T	T	T	T
50	D	D	D	D	**S**	D	E	A	D
53	K	K	K	K	**K**	K	K	K	K
54	N	N	S	N	**N**	S	N	N	N
55	K	K	K	K	**K**	K	K	K	K
56	G	G	G	G	**G**	G	G	G	G
57	I	I	I	I	**I**	I	I	I	I
58	T	T	V	T	**I**	T	T	T	T
60	K	G	N	G	**G**	Q	G	G	G
61	E	E	N	E	**E**	Q	E	E	E
62	E	D	D	D	**D**	E	E	D	D
63	T	T	T	T	**T**	T	T	T	T
64	L	L	L	L	**L**	L	L	L	L
65	M	M	M	M	**M**	R	M	M	M
66	E	E	E	E	**E**	I	E	E	E
100	K	D	S	S	**K**	K	D	K	K
101	A	A	A	A	**A**	T	A	A	A
102	T	T	T	C	**T**	A	T	T	T
103	N	S	S	S	**N**	A	S	N	N
104	E	K	---	K	**E**	S	K	E	E
# of differences					**XXXX XXXX**				

Table 2: Cytochrome c Amino Acid Sequence Differences Between Humans and Other Vertebrate Species

Species	**Number of differences from human cytochrome c**

Part II. Hemoglobin

Look at the amino acid sequences shown in Table 3 below. These sequences are portions of the hemoglobin molecules of five organisms. The portion of the chain shown is from amino acid number 87 to amino acid number 116 in a sequence of 146 amino acids.

1. Compare the amino acid sequence of human hemoglobin molecules with that of the other four vertebrates using the same procedure from cytochrome c.
2. Complete Table 4, making sure to list the animal species in *in order* from the ***fewest*** differences to the ***most*** differences from human hemoglobin.

Table 3: Hemoglobin Amino Acid Sequences

Amino Acid #	Human	Chimpanzee	Gorilla	Monkey	Horse
87	THR	THR	THR	GLN	ALA
88	LEU	LEU	LEU	LEU	LEU
89	SER	SER	SER	SER	SER
90	GLU	GLU	GLU	GLU	GLU
91	LEU	LEU	LEU	LEU	LEU
92	HIS	HIS	HIS	HIS	HIS
93	CYS	CYS	CYS	CYS	CYS
94	ASP	ASP	ASP	ASP	ASP
95	LYS	LYS	LYS	LYS	LYS
96	LEU	LEU	LEU	LEU	LEU
97	HIS	HIS	HIS	HIS	HIS
98	VAL	VAL	VAL	VAL	VAL
99	ASP	ASP	ASP	ASP	ASP
100	PRO	PRO	PRO	PRO	PRO
101	GLU	GLU	GLU	GLU	GLU
102	ASN	ASN	ASN	ASN	ASN
103	PHE	PHE	PHE	PHE	PHE
104	ARG	ARG	LYS	LYS	ARG
105	LEU	LEU	LEU	LEU	LEU
106	LEU	LEU	LEU	LEU	LEU
107	GLY	GLY	GLY	GLY	GLY
108	ASN	ASN	ASN	ASN	ASN
109	VAL	VAL	VAL	VAL	VAL
110	LEU	LEU	LEU	LEU	LEU
111	VAL	VAL	VAL	VAL	ALA
112	CYS	CYS	CYS	CYS	LEU
113	VAL	VAL	VAL	VAL	VAL
114	LEU	LEU	LEU	LEU	VAL
115	ALA	ALA	ALA	ALA	ALA
116	HIS	HIS	HIS	HIS	ARG
# of differences	XXXXXXXX				

Table 4: Differences between Human Hemoglobin and that of other Vertebrate Species

Species	Number of differences From human hemoglobin

4. Looking at hemoglobin, which vertebrate is most closely related to humans? _____

5. Which is least closely related to humans? _____

Conclusion/Analysis Questions:

1. Since evolutionary biologists look for patterns to determine the closeness of different species and how long ago those species split from a common ancestor, how can proteins act like "molecular clocks" to aid in this process?

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2. When the portions of the gorilla and human hemoglobin molecules were compared, there was only one difference in the amino acid sequence. Explain how this change could have happened?

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3. If the amino acid sequences are similar in gorillas and humans, will the nucleotide sequence of their DNA also be similar? Explain why or why not?

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4. Examine the Table 2 you completed in Part I. The values listed for the chicken and the horse differ by only one. Can you deduce from only this information that the chicken and the horse are closely related? Explain why or why not?

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5. How is biochemical comparison different from other methods of determining evolutionary relationships, such as anatomical or embryological? _____

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