Honor pledge: "*I have neither given nor received unauthorized aid on this test.*"

Signed : _____ Date : _____

Name : _____

1. What are the 3 primary evolutionary branches of life? (5 points)

2. A *photoheterotroph* gets carbon from _____ and energy from _____. (1 point)

    A. light / organic compounds

    B. organic compounds / light

    C. CO_2 / light

    D. light / inorganic compounds

    E. none of the above

3. The fact that all living things share a highly conserved core of biochemistry implies that life .... (1 point)

    A. can't work any other way

    B. originated 3 times, once for each of the major branches

    C. originated elsewhere and traveled to Earth

    D. has a common ancestry

    E. none of the above

4. Species evolve by .... (1 point)

    A. diversification

    B. progression

    C. climbing the evolutionary ladder

    D. increased complexity

    E. all of the above

5. Which of the following is *not* part of a taxonomy? (1 point)

    A. Grouping

    B. Treeing

    C. Identifying

    D. Naming

    E. Trick question - *all* of these are part of any taxonomy

6. . Which of the following are *not* required for a molecular sequence to exhibit good clock-like behavior? (1 point)

    A. It must be made of many independently-evolving "bits"

    B. It must be functionally constant

    C. It must be long enough to be statistically meaningful

    D. It must have appropriate evolutionary variation

    E. Trick question - *all* of these are required

7. *Prokaryotic* means .... (1 point)

    A. bacterial

    B. microbial

    C. not eukaryotic

    D. Gram positive or negative

    E. none of the above

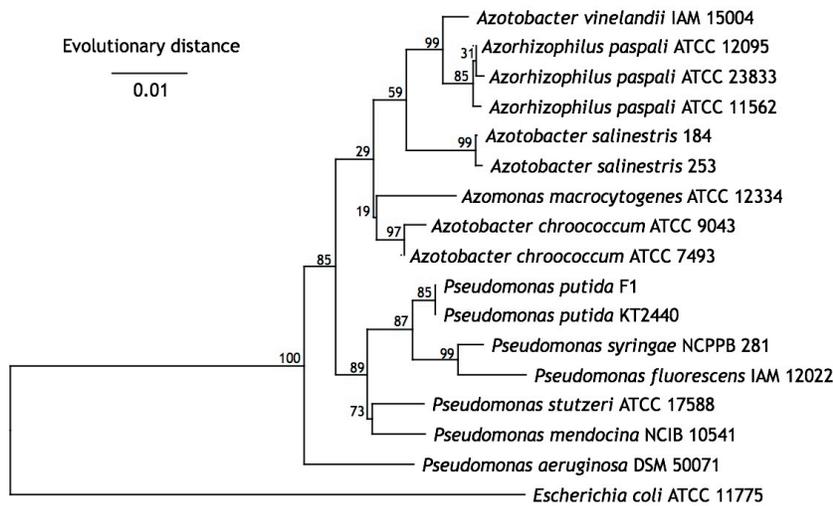8. An *outgroup* is a sequence (or sequences) that .... (1 point)

    A. is known not to be in the group being analyzed

    B. has been outcast from the "cool" group

    C. has moved by horizontal transfer

    D. does not exhibit clock-like behavior

    E. None of the above

9. ES-2 turned out to be a member of which phylogenetic group?. (1 point)

    A. *Clostridium/Eubacterium*

    B. Crenarchaea

    C. Enterobacteria

   D. Bacteroides/Flavobacteria/Cytophaga

    E. none of the above

10. Which of the following is a *substitution model?* (1 point)

   A. The Jukes and Cantor equation

   B. position weighting

   C. The 6-parameter model

   D. Kimura 2-parameter method

   E. All of the above

11. What are the two kinds of gaps? (1 point)

   A. indels & missing data

   B. redundant bases & variable positions

   C. hyphens & tildes

   D. unclear sequences calls & wobble positions

   E. none of the above

12. ssu-rRNA is generally not useful for phylogenetic analysis of .... (1 point)

   A. multicellular organisms

   B. organisms from very different habitats

   C. very distantly-related organisms

   D. very closely-related organisms

   E. ssu-rRNA is not generally useful for any of these

13. Alternatives to ssu-rRNA that are sometimes useful include all of these *except* .... (1 point)

   A. rRNA gene spacers

   B. RNase P RNA

   C. Protein sequences

   D. catenated alignments

   E. all of the above can sometimes be useful

14. The root of the "universal" tree divides organisms into .... (1 point)

   A. Bacteria and Eukarya vs Archaea

   B. Bacteria and Archaea vs Eukarya

   C. Bacteria vs Archaea and Eukarya

   D. Eukaryotes vs Prokaryotes

   E. none of the above

15. Which of the following is *not true* about horizontal transfer? (1 point)

   A. It occurs mostly with genes for selectable phenotypes

   B. It occurs most often between closely-related organisms

   C. It occurs between organisms of all kinds

   D. It was probably more common early in life's history

   E. All of these are true

16. What is an *bootstrapping*, and how is it used in a phylogenetic tree? (5 points)

17. Answer the following questions from this tree (1 point each)

Evolutionary distance
0.01

- *Azotobacter vinelandii* IAM 15004
- *Azorhizophilus paspali* ATCC 12095
- *Azorhizophilus paspali* ATCC 23833
- *Azorhizophilus paspali* ATCC 11562
- *Azotobacter salinestris* 184
- *Azotobacter salinestris* 253
- *Azomonas macrocytogenes* ATCC 12334
- *Azotobacter chroococcum* ATCC 9043
- *Azotobacter chroococcum* ATCC 7493
- *Pseudomonas putida* F1
- *Pseudomonas putida* KT2440
- *Pseudomonas syringae* NCPPB 281
- *Pseudomonas fluorescens* IAM 12022
- *Pseudomonas stutzeri* ATCC 17588
- *Pseudomonas mendocina* NCIB 10541
- *Pseudomonas aeruginosa* DSM 50071
- *Escherichia coli* ATCC 11775

a. Which sequence is most similar to that of *A. macrocytogenes* ATCC 12334? _____

b. What is the outgroup? _____

c. Which is the *most* primitive sequence (excluding the outgroup)? _____

d. Circle the least-reliable branch on the tree and label it "D".

e. What is the approximate evolutionary distance between *P. putida* F1 and *P. stutzeri* ATCC 17588 ? _____

f. Circle the last common ancestor of the genus *Azorhiziphilus* and label it "F".

18. Answer the following questions from this tree (1 point each)
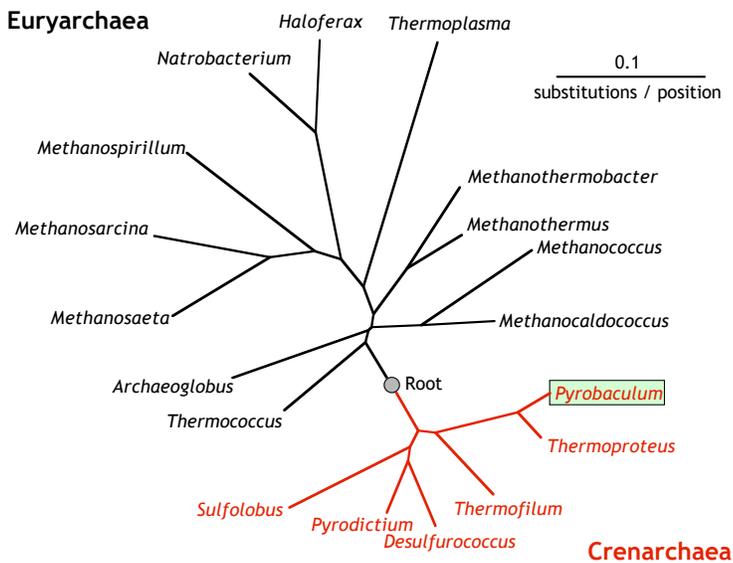
a. Which sequence is most closely related to *Methanothermus*? _____

b. These are all Archaea. What might have been used to place the root? _____

c. Which is the most primitive sequences? _____

d. What is the approximate evolutionary distance between *Pyrobaculum* and *Archaeoglobus*? _____

e. Circle the last common ancestor of the Euryarchaea _____

19. Describe *either* lipid profiling (FAME) *or* RFLP analysis. (5 points)
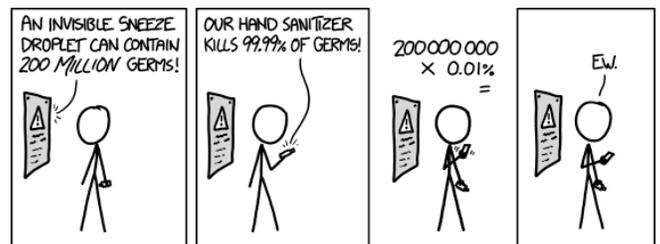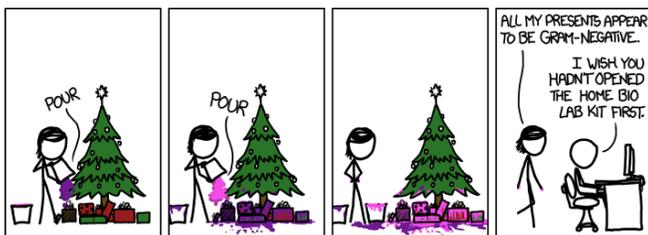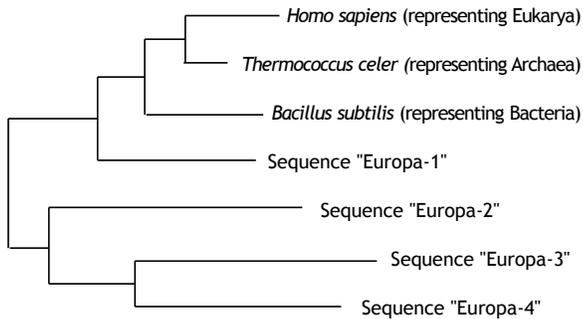

(OK, so this one only works if you see it in color)

xkcd.com

THOUGHT QUESTIONS

20. Looking back to the tree in Question 17, what problem do you see with the taxonomy of these organisms? (5 points)

21. The Europa Explorer, in the year 2035, returns to Earth with a sample of sediment drilled from beneath the icy crust of this moon of Jupiter. Attempts to cultivate anything from the sample fail, but you successfully use small-subunit ribosomal RNA-targeted primers and PCR to obtain 4 reasonable-looking ribosomal RNA sequences (called Europa-1, Europa-2, Europa-3, and Europa-4) from the sample. Below is a tree of these sequences relative to some representative Earth sequences. What can you tell me about how life on Europa and Earth are related, assuming you're able to show that the sequences are not contamination from Earth, are clean sequences (no sequencing errors) and are properly aligned? (5 points)

```
                        ┌──── Homo sapiens (representing Eukarya)
                    ┌───┤
                ┌───┤   └──── Thermococcus celer (representing Archaea)
                │   │
            ┌───┤   └──────── Bacillus subtilis (representing Bacteria)
            │   │
            │   └──────────── Sequence "Europa-1"
        ┌───┤
        │   └──────────────── Sequence "Europa-2"
────────┤
        │   ┌──────────────── Sequence "Europa-3"
        └───┤
            └──────────────── Sequence "Europa-4"
```

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The rules: You are free to use any notes, books, or online material while taking this take-home exam. You are also free to use a calculator and ruler or other drawing material. You are NOT allowed to get (or give) help of any kind from (or to) anybody. If you have questions about the exam, send an email to Dr. Brown at james_brown@ncsu.edu. You MUST turn this completed take-home portion of the exam in with the rest of the exam when you take it, unless you take the exam in the DELTA offices or via remote proctor.

Signed : _____   Date : _____

Name : _____

1. Align the following sequences: (4 points)

```
Hafnia     U A U G A A U A A U
Serratia   U U U G A A U G A U
Shigella   U C U G A A C A U A C
Vibrio     U U G A A U G A C
```

2. Align the following RNAs: (5 points)

```
     U              U C              C U
  U     U        U     G         U       G
   A - U          U • G           C - G
   C - G            U             C - G
   C - G          C - G           C - G
   G - C          G - C           G • U
   Bacillus       Krypidia        Listeria
```

3. Draw the secondary structures of the *Snowella* and *Lyngbya* RNAs in this alignment: (5 points)

```
              - ( ( ( - ( ( - - - - ) ) ) ) ) - - -
 Cyanobium    U C G A U G C U U U - G C U U G U U -
 Snowella     U C A A - A U G A A A G U U U G U - -
  Lyngbya     - U C C U C - U U C G - G G G G U U U
```

```
     U
  U     U
   C - G
   G - C
       U
   A - U
   G • U
  U C - G U U
   Cyanobium
```

4. Create a similarity matrix from the following alignment, using a 2-parameter model in which transversions count as a full difference (-1.0), and transitions count as half a difference (-0.5). Gaps should be excluded from comparison (i.e. counted as neither a match nor mismatch) (5 points)

```
  Bartonella  G A G A G A G A G A
  Martelella  G G G A G A G A G A
      Afipia  G U G A G A U A G G
    Brucella  G C G A G A U A G A
 Acetobacter  G - G A A A A A A U
```

5. Convert this phenogram into a dendrogram. **Please keep the tree to the same scale.** (5 points)
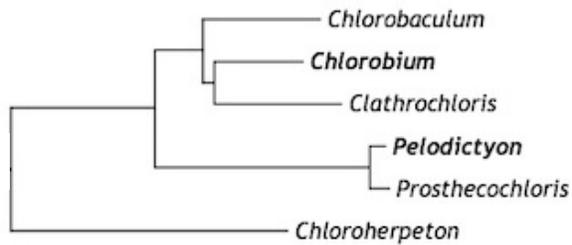
6. Use the following distance matrix to generate a tree (with branch lengths) using the neighbor-joining method. SHOW **ALL** OF YOUR WORK! (26 points)

	A	B	C	D	E	F
A	---	---	---	---	---	---
B	0.3	---	---	---	---	---
C	0.6	0.5	---	---	---	---
D	0.9	0.8	0.9	---	---	---
E	1.1	1.0	1.1	0.8	---	---
F	0.9	0.8	0.9	1.0	1.2	---

(CONTINUE YOUR WORK HERE)