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

2. _____ An organism that gets its energy from chemical (inorganic) reactions and faxes carbon from CO₂ for growth is a ... (1 point)
   A. Photoautotroph
   B. Photoheterotroph
   C. Chemoautotroph
   D. Chemoheterotroph
   E. All of the above

3. _____ Species evolve by ... (1 point)
   A. advancement
   B. progression
   C. diversification
   D. regression
   E. force of will

4. _____ Which of the following is usually the best sequence (at least to start) for use in phylogenetic analyses? (1 point)
   A. Signal recognition particle RNA
   B. Glyceraldehyde phosphate dehydrogenase
   C. Large-subunit ribosomal RNA
   D. Small-subunit ribosomal RNA
   E. Ribonuclease P RNA

5. _____ The gene sequence you answered in question 4 cannot generally be used to analyze strains of a species or species of a genus because ... (1 point)
   A. these genes are too highly conserved
   B. these genes are too variable
   C. these genes are transferred by interbreeding
   D. these genes do not exhibit clock-like behavior
   E. these genes aren’t found in all species

6. _____ Which of the following algorithms can be used to generate a tree from a distance matrix? (1 point)
   A. Bootstrapping
   B. Neighbor-joining
   C. Jukes and Cantor
   D. Phylogenetic covariation
   E. Fitch-Margoliash

7. _____ A Field Guide is a kind of ______________ (1 point)
   A. Evolutionary model
   B. Binomial nomenclature
   C. Taxonomy
   D. Phylogenetic tree
   E. All of the above

8. _____ The universal tree was rooted by phylogenetic analysis of ... (1 point)
   A. small subunit ribosomal RNA
   B. precambrian fossils
   C. entire genomes
   D. morphological and phenotypic traits
   E. pairs of anciently-duplicated genes

9. _____ Which of the following organelles originated by endosymbiosis of an early Eukarya and a Gram-positive bacterium? (1 point)
   A. Nucleolus
   B. Chloroplasts
   C. cilia, flagella, centrioles and spindle apparatus
   D. Mitochondria
   E. None of the above
10. Answer the following questions from this ssu-rRNA-based tree (1 point each)

a. Which sequence is most similar to that of *M. funiformis*? ___________________________________________________________________

b. What is the outgroup in this tree? ____________________________

c. What is the approximate evolutionary distance between *P. sottacetonis* and *P. brassicae*? _________________

d. What is the approximate evolutionary distance between *S. ruminantium* and *S. silvacetica*? _________________

e. Circle the last common ancestor of the genus *Megamonas* and label it “E”.

f. Circle the least common ancestor of the genus *Pectinatus* and label it “F”.

g. Circle the least reliable branch on the tree and label it “G”.

h. Which is the most primitive sequence (not considering the outgroup)? _________________________________

i. Which is the least primitive sequence (not considering the outgroup)? _________________________________

11. On the tree in question above, what does the number “56” mean on the branch leading to *P. sottacetonis* and *P. brassicae*? How was this number determined? (Describe the process, don’t just name it.) (5 points)
12. Answer the following questions from this ssu-rRNA-based tree (1 point each)

![Tree Diagram]

- **a.** Which sequence is most closely related to *Methanoseta*? __________________________________________________________________________________________
- **b.** All of these are Archaea. What might have been used to place the root? __________________________________________________________________________________________
- **c.** Which is the most primitive of the Euryarchaeal sequences? __________________________________________________________________________________________
- **d.** Circle the last common ancestor of the methanogens (their names all start with “Methano-” and label it “D”).
- **e.** Circle the two major phylogenetic groups of Crenarchaea (1 point per circle).
- **f.** What is the approximate evolutionary distance between *Thermococcus* and *Pyrobaculum*? __________________________________________________________________________________________
- **g.** *Methanococcus* in this tree is represented by the species *Methanococcus voltae*. Draw on the tree what it might look like if a second species, *Methanococcus olentangii*, was also included.

**THOUGHT QUESTIONS**

13. The tree of below is based on valine tRNA synthetase sequences - the enzyme that charges the appropriate valine-specific tRNAs with valine. Sequences in black are from Bacteria, those in purple are from Archaea, and those in green are from eukaryotic nuclei. These sequences are highly conserved, and so the alignment of these sequences looks very good. Each of the proteins was tested for valine charging ability (i.e. they are all real valine-specific tRNA synthetases), and each of these organisms contains only a single valine tRNA synthetase. How does this tree differ from the ssu-rRNA “3 Kingdom” tree, and how would you explain this? (The root of the tree was identified using a tRNA synthetase specific for a different amino acid, isoleucine.) (5 points)

Sce = Saccharomyces cerevisiae  
Hsa = Homo sapiens  
Aae = Aquifex aeolicus  
Bsu = Bacillus subtilis  
Mge = Mycoplasma genitalium  
Mpn = Mycoplasma pneumoniae  
Hin = Haemophilus influenzae  
Eco = Escherichia coli  
Mtu = Mycobacterium tuberculosis  
Ssp = Synechococcus sp. PCC6803  
Ctr = Chlamydia trachomatis  
Hpy = Helicobacter pylori  
Bbu = Borrelia burgdorferi  
Tpa = Treponema pallidum  
Afu = Archaeoglobus fulgidus  
Mth = Methanobacterium thermoautotrophicum  
Mja = Methanococcus jannaschii  
Pho = Pyrococcus horikoshii  

V = valine-specific tRNA synthetase
14. The tree below is from Graur and Pupko, 2001 Molecular Evolution (18:1143), and includes a number of members of the family *Bacillus*. The point of the paper is to make an argument about one of these species: *Bacillus permians* (highlighted). This species was supposedly grown from a spore taken from the middle of a halite (salt) rock dating 250 million years old by Vreeland, et al. Vreeland argued that this spore had survived for all those years after being trapped in salt crystals when the salt lake it was in dried up, and went to amazing lengths to prevent the possibility of contamination. Graur argues that this cannot be the case, based on the tree below. What line of reasoning do you think Graur used? (5 points)
Use this page to complete any answers you need extra space for.
1. Align the following sequences: (5 points)

   Anabaena  G A C U G A A A C G G A U
   Nostoc     A C U G A A A U C G A C
   Microcystis A U U G A A A G A U
   Arthrospira A A U U G A A A U G A U

2. Align the following RNAs: (4 points)

   Thermococcus
   U C
   U * G
   U - A
   C - G A U

   Sulfolobus
   G A
   G - C
   A - U
   G • U A

   Pyrobaculum
   U
   C
   C - G
   A - U
   A - U A U

3. Draw the secondary structures of the A. stylex and A. temura RNAs in this alignment: (4 points)

   ( ( ( ( ( - - - - ) ) ) ) ) - - - -
   A. coli  G G A C C - U U C G G G U A C C U A U -
   A. temura G U C - - - U U U - - - G - G C U A U -

   A. coli
   U C
   U G
   C - G
   C - G
   A - U
   G - C
   G - C U A U
4. Create a similarity matrix from the following alignment, using a 2-parameter model in which transversions count as a full difference (-1.0), transitions count as half a difference (-0.5), and gaps are ignored. You can use either decimal numbers (e.g. 0.85) or fractions (e.g. $8.5/10$) in your answers. (5 points)

<table>
<thead>
<tr>
<th></th>
<th>Bacillus</th>
<th>Staphylococcus</th>
<th>Streptococcus</th>
<th>Clostridium</th>
<th>Eubacterium</th>
</tr>
</thead>
</table>

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!** (32 points)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>---</td>
<td>---</td>
<td>---</td>
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<td>---</td>
</tr>
<tr>
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<td>---</td>
<td>---</td>
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</tr>
<tr>
<td>C</td>
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<td>0.5</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>D</td>
<td>0.8</td>
<td>0.7</td>
<td>0.8</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>E</td>
<td>0.9</td>
<td>0.8</td>
<td>0.9</td>
<td>0.5</td>
<td>---</td>
</tr>
</tbody>
</table>