Total Score: ___/60

Name _____
Section Number _____

Homework 7
Due May 29th at the beginning of lecture

Instructions:

You are welcome to discuss concepts with your classmates but must compose your own answers. If you are unsure of the honor code for this course, please ask or look at the course website. http://www.its.caltech.edu/~bi1/Bi1__Micro-_to_Macro-Biology/Policies.html

The goal of this assignment is to help you understand a dense research paper and the molecular basis of cellular information processing. Many of the questions do not have a single correct answer. You will be given full credit as long as your answer is reasonable.

The answers must be legible and should not extend past the allotted space. Keep in mind that a few well-written sentences can give a higher score than a whole page of text.

This assignment is a little bit different than previous assignments. Firstly, it builds off of the 16S rRNA sequences we are analyzing as part of the mini-lab and requires you to construct a phylogenetic tree as well as thoughtfully consider the phylogenetic tree in the assigned paper. Secondly, we are providing you with a practice exam question to submit for grading typed as well as some ungraded (and not-submitted) comprehension and interpretation questions related to the journal article. Remember to write your full name and section number on each page you submit.

Part A: General phylogeny and 16S rRNA tree questions

1. Phylogenetic trees can often look different but show the same information. Which tree is different? (1 point)

A   B   C   D

A B C D   A B D C   B A C D   A D C B
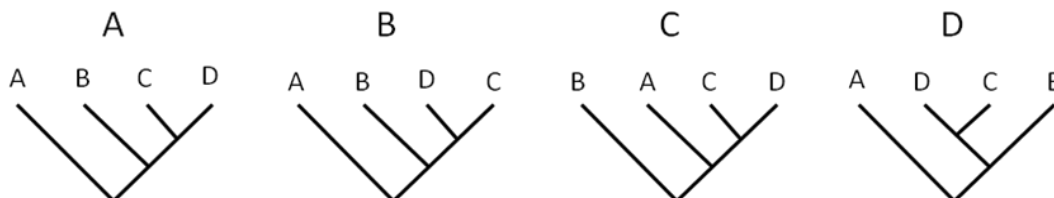
2. Below is an alignment of DNA sequences, like the ones we made in recitation. From the alignment draw a rooted and an unrooted phylogenetic tree, using the templates. In 2 or 3 sentences explain how you predict your phylogenies. In particular which positions were informative, which were not, and why. Draw an arrow where the root would be in your unrooted tree to make it the same as the rooted tree you drew. (4 points)

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        1 2 3 4 5 6
Seq 1  T G T A C C
Seq 2  G G A C T A
Seq 3  T G A A C G
Seq 4  G G A G C T
```
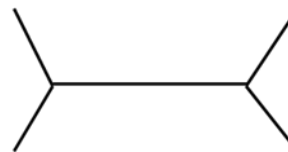
3. Please turn in a copy of the rRNA tree based off of your unknown sequence (this is the tree that you started in recitation). Make sure to include the appropriate modifications listed in the

tree making guide (found on the website). In your rRNA tree, what was the closest relative to your unknown sequence? Look up your unknown sequence's closest relative. What environment(s) is it found in? Is it a human pathogen? Why do you think we found a relative of this organism in a pond at Caltech? Did you find any other interesting information about this organism? (10 sentences) (10 points)

4. On your rRNA tree, there is an rRNA sequence isolated from a chloroplast. Where does it fall on the tree (ie. What domain and who is its closest relative? What is this evidence for? (5 sentences) (5 points)

Part B: Fantastic Fixers

Reading:

o Deep-Sea Archaea Fix and Share Nitrogen in Methane-Consuming Microbial Consortia (Dekas et. al.)
o Fantastic Fixers (Fulweiler)

*Questions **THAT WILL NOT BE GRADED**. You **do not need to turn these in**. However, we would encourage you to make sure you understand them – they explore important concepts of critically reading a paper. If you have trouble answering any of them, please go see a TA for help.*

1. What is a symbiosis? What are the symbiotic partners discussed in the paper? What was discovered about these partners that prompted this research?

2. Because the ANME-2 / DSS consortia cannot be cultured, the Orphan group grows them in enrichments. Why did the research combine FISH and nanoSIMS? (i.e. Why did they need both techniques to study the relationship between ANME-2 and DSS?)

3. Consider figure 1 in Dekas *et. al*. As pointed out in the paper, a different scale bar is used in each NanoSIMS figure. Do you think this is misleading when looking just at the images? Why or why not?

4. Study figure 3 in Dekas *et. al*. What does this figure show? In two sentences or less, explain why this is important. (Hint: Focus on the differences between archaea and bacteria.)

5. What is the major result of this study? Why is this result important?

6. How does the result of this study relate to the major biological principles we are covering in lectures?

Name _____

Section Number _____

7. Modern agriculture is releasing substantial amounts of fixed nitrogen into fresh water. This runoff eventually makes it into the oceans. What may be the long term impact of this nitrogen source? (Hint: Think of the different niches in the ocean.)

*Essay Question (**GRADED**). This question is meant to be practice for your exams. (Turn this in!) Please **TYPE** this and keep it less than 500 words.* (25 points)

Dekas *et al.* indicates that these consortia sometimes grow in an environment with reduced nitrogen present. We are told that fixing nitrogen is energetically more costly than many other possible processes. Come up with and discuss a hypothesis that explains why the archaea still fix nitrogen, despite the consequent retardation in growth (note this is a current area of research! There is no single right answer; you must explain your hypothesis in a rational way). Make sure this takes into account how the consortia make the energy balance work in the context of their microenvironment. What experiment could you do to test your hypothesis? Be as specific as possible. You will be graded on: 1.) clarity, 2.) logic, 3.) depth of thought, and 4.) completeness (both that of any individual argument and all of the different arguments).

   Hint: the things we are looking for while grading are:
- *A well-explained hypothesis including:*
  - *Why archaea fix nitrogen.*
  - *Talk about how your hypothesis fits with the ideas of niches and the local deep-see environment.*
  - *Talk about how the energy balance works.*
  - *What is the role of the bacteria in your hypothesis?*
- *Why the hypothesis explains the result (i.e. how your hypothesis explains that the archaea still fix nitrogen).*
- *Experiment*
  - *Explain what exactly your experiment tests and can make conclusions about. How does it make those conclusions? Is it conclusive?.*
  - *Which techniques will you use? (be specific.)*
  - *Controls.*
- *We will also be assigning points for the clarity of your writing.*

   Part C: Phylogeny Questions from Dekas et al.

1. As discussed in week 1, 16S rRNA phylogenies represent the history of life through vertical descent, the passage of genetic material from parent to daughter cell. Proteins can have evolutionary histories that are different than the 16S rRNA tree. Compare the 16S rRNA tree you made to the phylogeny of *nifD* in Figure 4 of Dekas et al. Does *nifD* have a different evolutionary history than your 16S rRNA tree? If so how do they differ? (Hint figure out which leaves on the *nifD* tree are from Bacteria or Archaea) (5 sentences) (5 points)

2. What are the possible mechanisms that would cause *nifD* to have a different evolutionary history than the 16S rRNA tree? (list 3)(Hint: look at David and Alm, 2011) (3 points)

3. The group Rhizobiales appears twice on the *nifD* tree. This means that members of the Rhizobiales have two copies of *nifD*. Based on their placement in the *nifD* phylogeny what

Total Score: ___/60

mechanism from question 2 would best explain this observation? Explain. (2-3 sentences) (3 points)

4. In *nifD* clade III, some copies of the genes are from Bacteria and others are from Archaea. What mechanism from question 2 would best explain this observation? Explain. (2-3 sentences) (2 points)

5. Based only on the phylogeny of *nifD* the authors were unable to unambiguously determine which member of the symbiosis was fixing nitrogen. What genomic evidence did they use to support their claim that the Archaeal partner was fixing nitrogen? (2-3 sentences) (2 points)