



## MB 451 Microbial Diversity

# Final Exam

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

Name : KEY

Signed : \_\_\_\_\_ Date : \_\_\_\_\_

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

*Archaea, Bacteria, Eukarya*

### Multiple choice (2 points each)

B 2. The pink filamentous organism isolated from Octopus Spring was named ...

- A. *Thermus aquaticus*
- B. *Thermocrinus ruber*
- C. *Caulobacter crescentus*
- D. *Thauera phenolyticus*
- E. *Pelagibacter ubique*

A 3. The filamentous Bacteria that cause trouble (bulking and foaming) in wastewater treatment are ...

- A. *Chloroflexi*
- B. *Streptomyces*
- C. cyanobacteria
- D. *Arthromitis*
- E. epsilon proteobacteria

C 4. Proper teeth cleaning and oral hygiene results in ...

- A. an overall decrease in oral microbe populations
- B. an increase in oral pathogen populations
- C. a decrease in oral pathogen populations
- D. no change in oral pathogen populations
- E. an elimination of oral microbes

C 5. Proteorhodopsin is a ...

- A. sensory opsin
- B. light-driven chloride pump
- C. light-driven proton pump
- D. light-detecting calcium channel
- E. pseudogene

E 6. Which of the following is *not* an example of horizontal transfer?

- A. Genes in the nucleus encoding chloroplast proteins
- B. Antibiotic resistance plasmids in a pathogen
- C. Pathogenicity islands in a pathogen
- D. proteorhodopsin in SAR 86
- E. genes for photosynthesis in cyanobacteria

A 7. The term "metagenomics" refers to ...

- A. the genetic composition of an ecosystem
- B. the complete genome of a particular organism
- C. the similarities and differences between genomes
- D. hypothetical generic genome sequences
- E. none of the above

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- D 8. In *Caulobacter* swarmer cells, the chromosome is located with the origin here, the terminus there.
- A. in the middle of the cell, also in the middle
  - B. both are randomly placed anywhere in the nucleoid
  - C. both are located at the end opposite the flagellum
  - D. at the flagellated end, at the other end**
  - E. Trick question! Swarmer cells don't have chromosomes!

- A 9. The homologs of actin and tubulin in Bacteria are ...
- A. mreB and FtsZ**
  - B. found in the centrioles
  - C. not components of the cytoskeleton in Bacteria
  - D. components of the flagellum and motor
  - E. Bacteria lack homologs of these proteins

- E 10. Which of these dates is closest to the earliest evidence for life on Earth?
- A. 6000 years ago
  - B. 540 million years ago
  - C. 1 billion years ago
  - D. 2 billion years ago
  - E. 3 billion years ago**

- E 11. The most common genera of Bacteria in the human gut (or at least feces) are ...
- A. *Escherichia* and *Clostridium*
  - B. *Escherichia* and *Bacteroides*
  - C. *Clostridium* and *Lactobacillus*
  - D. *Lactobacillus* and *Bacteroides*
  - E. *Bacteroides* and *Clostridium***

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Short answer (5 points each)

12. rRNA PCR products from pure cultures, for example those we generated in lab, can be sequenced directly. But in most molecular phylogenetic analyses of environmental samples the rRNA PCR products are cloned before sequencing. Why is this necessary?

*Because the PCR product is a mixture of rRNA sequences, not a single sequence. Cloning is required to separate these into individual sequences so they can be sequenced.*

13. A **chimera** is a sequence partly from one source and partly from another. Explain how these are generated in the case of rRNA PCR reactions from microbial populations.

*Chimeras are generated when a DNA polymerase stalls partway through replication of the PCR product. The resulting partial-length DNA can serve as a primer rather than template in the next round of PCR, especially if it ends in a conserved region of the rRNA. If the partial-length DNA primes on the sequence from a different organism, then the result will be an rRNA molecule with the sequence from one organism at one end but another organism at the other. This isn't an issue when working from pure cultures because the only templates are all the same sequence - if this process occurs, it just results in the same, correct sequence instead of a chimera.*

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14. Describe, in one sentence, one way in which horizontally-transferred “foreign” genes can be identified in a microbial genome.

*Example answer:*

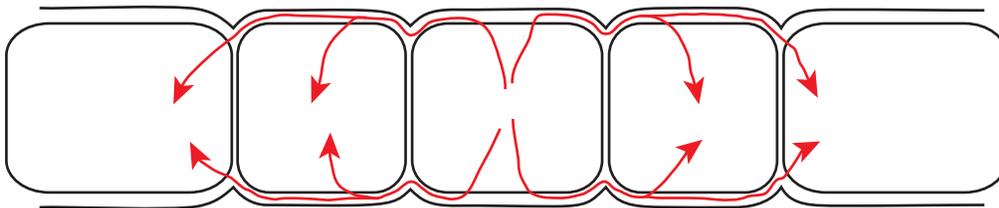
*One way is to look for genes (usually clusters of genes) in which the G+C content is significantly different than in the rest of the genome.*

15. All known members of the bacterial phylum *Chlamydiae* are obligate intracellular parasites, and yet rRNA sequences from *Chlamydiae* show up frequently in rRNA-based surveys of environmental samples, especially from aquatic environments and wastewater sludge. How can you explain this?

*Because these Chlamydia infect unicellular or at least very small eukaryotes, like amoeba.*

16. Vegetative and heterocyst cells in a filament of the cyanobacterium *Anabaena* exchange a variety of molecules, including fixed carbon (produced by photosynthesis in vegetative cells) and fixed nitrogen (produced by nitrogen fixation in heterocysts). How is it proposed this material is exchanged from cell to cell in the filament? Make sure to draw a picture.

*By secretion from the producing cells into the common periplasm of the filaments, which serves as a “conduit” for diffusion of these molecules from one cell to another.*



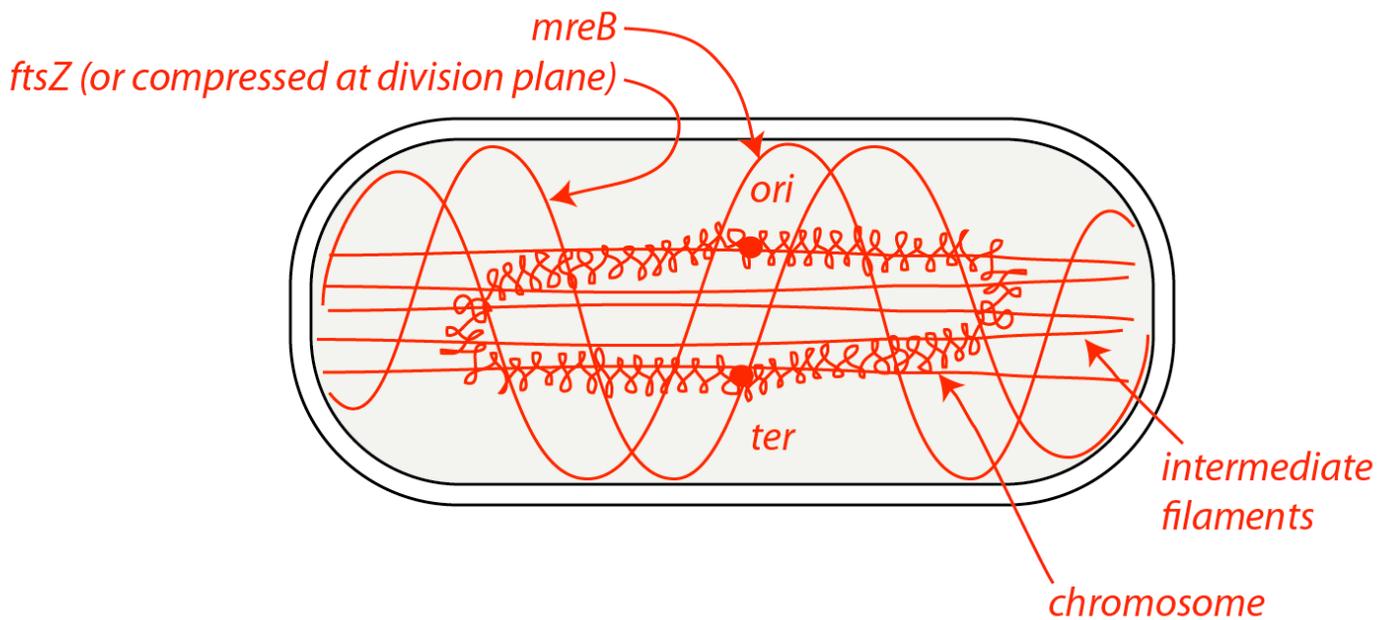
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Essay questions (10 points each)

17. Describe the last common ancestor from the perspective of Carl Woese in his paper "Interpreting the universal phylogenetic tree".

*According to the view described in this paper, the last common ancestor was a progenote, predating accurate translation and therefore the tight linkage between genotype and phenotype. Progenotes would evolve as communities rather than independent evolutionary lineages.*

18. Draw in the cytoskeleton in this diagram of *E. coli*. Be sure to label the parts. Now add in a drawing of the chromosome, assuming the cell is not growing. Be sure to label the *ori* and *ter* regions.



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19. Describe one of the papers discussed in class (since the second midterm) in detail. A list of these papers is on the last page of this exam. YOU ARE NOT ALLOWED TO USE THE TWO REVIEW ARTICLES ASSIGNED AS REQUIRED READINGS (Woese 2000 and Thanbichler & Shapiro 2008). Be sure to include the purpose (hypothesis, problem, question, ...), approach, results, and conclusion.

*Example answer:*

*A new form of phototrophy - SAR86 (DeLong lab)*

*Purpose: to determine the phenotype/capabilities/ecological niche of SAR 86, a hugely abundant member of the ocean surface community*

*Approach: Genomics - they cloned large fragments of genome DNA (>100kbp, in cosmids) from marine surface water and screened the clones for the presence of SAR 86 rRNA genes. When they found one, they sequenced the entire fragment in hopes that they would find metabolic genes that might tell them about what SAR 86 can do.*

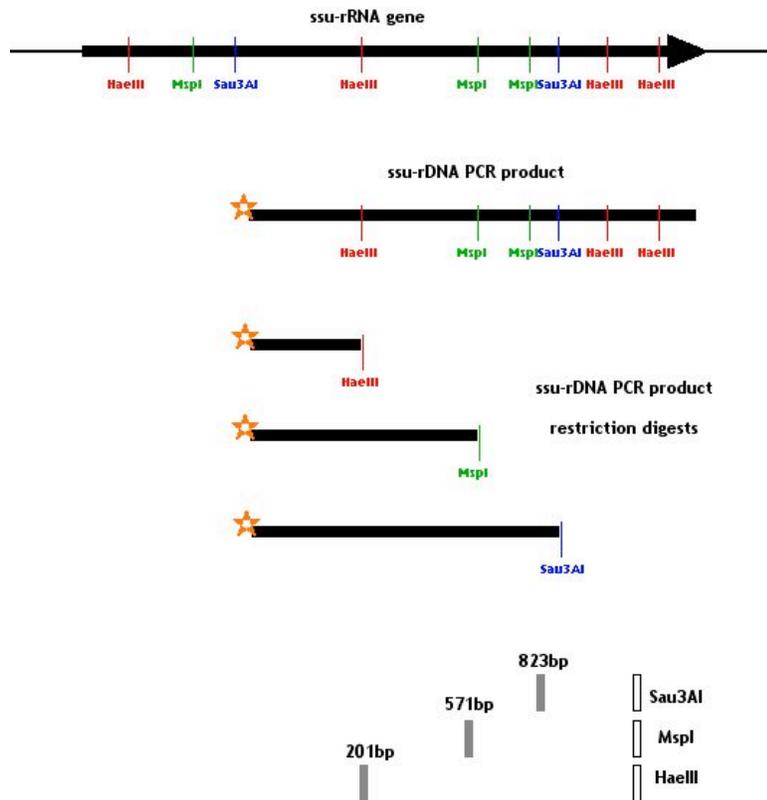
*Results: They found what looks like a functional rhodopsin gene. This "proteorhodopsin" was expressed in E.coli and shown to bind retinal and pump protons, and do so quickly like a real proton-pump rather than a sensory rhodopsin.*

*Conclusion: SAR 86 is a phototroph, using proteorhodopsin rather than traditional chlorophyll-based photosystems. Whether it is also autotrophic remained unknown.*

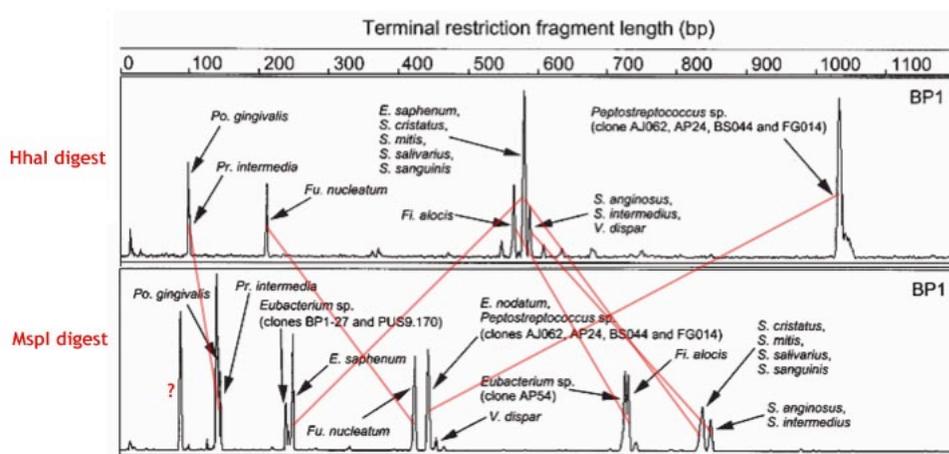
20. Choose one of the following methods to describe in detail: 454 sequencing, denaturing gradient gel electrophoresis, stable isotope probing, or terminal RFLP.

*Example answer: Terminal Restriction Fragment Length Polymorphism (t-RFLP) analysis*

*t-RFLP is a method similar to DGGE in that it generates fingerprints of a populations, but unlike DGGE, the bands can (ideally) be assigned to specific organisms directly, without the need for sequencing. You amplify the ssu-rRNA with some set of primers, e.g. 515F and 1492R (as in lab), and one of the primers (515F in this example) is fluorescently labeled. You digest this ssu-rDNA with several different restriction enzymes and separate the products out on a sequencing gel:*



*The sizes of the labeled fragments are compared to a database of potential fragments of ssu-rRNA sequences that would be generated from PCR products from those primers digested with those enzymes. A computer program sifts through the database and identify the organisms based on the observed (from the gel) sizes of the labeled fragments.*



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21. You've discovered a novel species of fish in a deep-sea methane (CH<sub>4</sub>) seep. This fish completely lacks a digestive tract, including both gullet and anus (the mouth opens to the gills but no further). However, it gets along just fine, swimming around in methane-infused water. You hypothesize that it's absorbing both methane and oxygen from the water by its gills, and living by methane oxidation (CH<sub>4</sub> + O<sub>2</sub> -> CO<sub>2</sub> + H<sub>2</sub>O). Recognizing that the fish also needs an organic nitrogen source (which would normally be acquired in the diet), you further hypothesize that the fish is fixing nitrogen from the ammonia also present in small amounts in the seep environment. You dissect one of these fish, and discover a grossly enlarged liver filling the space where the GI tract normally would be. Microscopic examination of the cells of this organ shows distinct spherical and rod-shaped bacterial endosymbionts, and a rod-shaped bacterial symbiont in the interstitial spaces. (1) How would you identify these apparent symbionts, and (2) how would you determine which (if any) of these are carrying out methane oxidation and/or fixing nitrogen? There is no need to describe how the general techniques involved work, but be sure to provide the specifics required to make these techniques provide the answers you're looking for.

*Example answer:*

*(1) Identifying the symbionts - DNA from the organ could be isolated & PCR amplified using ssu-rRNA primers, and the PCR products cloned and a bunch of them sequenced. Fluorescent probes against bacterial or archaeal rRNA sequences would be made & used to FISH-probe samples of the organ to identify which of these sequences represent each type of symbiont.*

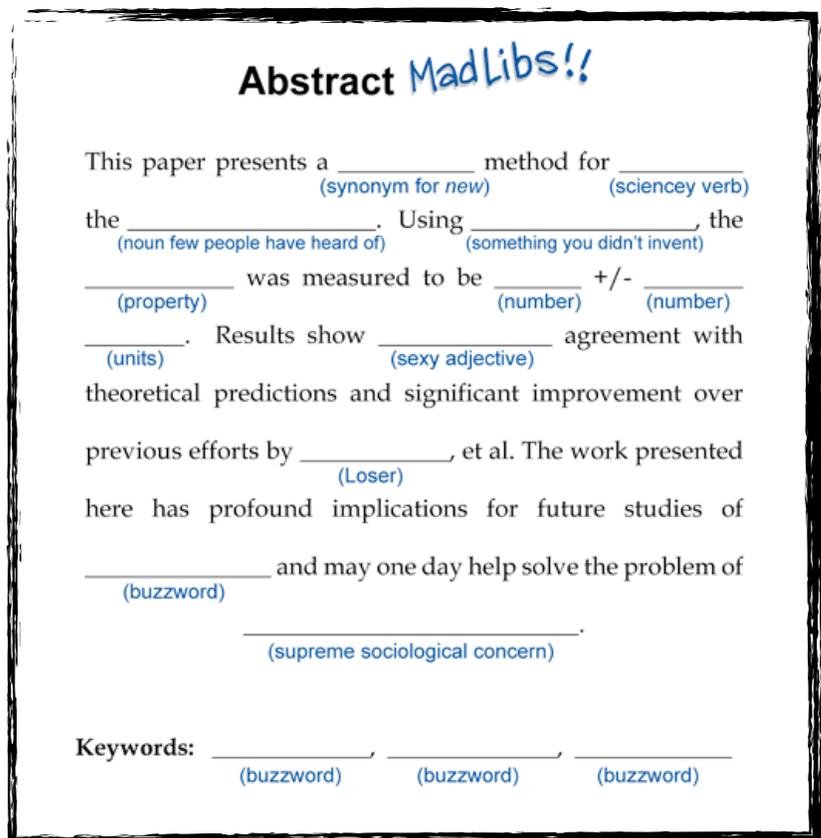
*(2) Determining which of these organisms carries out methane oxidation & nitrogen (ammonia) acquisition. Each of these sequences could be run on a DGGE gel to see where each of them migrates. Then a SIP experiment using <sup>13</sup>C-methane could be used to label the methane oxidizer - DGGE would be used to identify the PCR product from the heavy rRNA PCR product. Another SIP experiment using <sup>15</sup>N-ammonium could be used to label the rRNAs of the ammonium acquirer.*

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USE THIS PAGE TO FINISH ANY ANSWERS YOU'RE BEING LONG-WINDED ABOUT

## List of papers covered in class

- Reysenbach AL, Wickham GS & Pace NR 1994 Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park. *Appl. Env. Microbiol.* 60:2133-2199
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- Suau A, Bonnet R, Sutren M, Godon J-J, Gibson GR, Collins MD and Doré J. 1999 Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl. Env. Microbiol.* 65:4799-4807.
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- Huber JA, Mark Welch DB, Morrison HG, Huse SM, Neal PR, Butterfield DA & Sogin ML 2007 Microbial population structures in the deep marine biosphere. *Science* 318:97-100
- Yim LC, Hongmei J, Aitchison JC & Pointing SB 2006 Highly diverse community structure in a remote central Tibetan geothermal spring does not display monotonic variation to thermal stress. *FEMS Microbiol Ecol.* 57:80-91
- Sakamoto M, Huang Y, Ohnishi M, Umeda M, Ishikawa I & Benno Y. 2004 Changes in oral microbial profiles after periodontal treatment as determined by molecular analysis of 16S rRNA genes. *J. Med. Microbiol.* 53:563-571.
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- Thanbichler M & Shapiro L 2008 Getting organized - how bacterial cells move proteins and DNA. *Nat. Rev Microbiol.* 6:28-40
- Margulis L, Jorgensen JZ, Dolan S, Kolchinsky R, Rainey FA, & Lo S-C. 1998 The Arthromitis stage of *Bacillus cereus*: Intestinal symbionts of animals. *Proc. Natl. Acad. Sci. USA* 95:1236-1241.
- Jensen GB, Hansen BM, Eilenberg J and Mahillon J, 2003 *Environ. Microbiol.* The hidden lifestyles of *Bacillus cereus* and relatives.
- Huber H, Hohn MJ, Rachel R, Fuchs T Wimmer VC and Stetter KO. 2002 A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature* 417:63-67
- Waters E, et al. 2003 The genome of Nanoarchaeum equitans: insights into early evolution and derived parasitism. *Proc. Natl. Acad. Sci. USA* 100:12984-12988
- JE Berleman, J Scott, T Chumley & JR Kirby 2008 Predataxis behavior in *Myxococcus xanthus*. *PNAS* 105:17127-17132
- V Mariscal, A Herrero & E Flores 2007 Continuous periplasm in a filamentous heterocyst-forming cyanobacterium. *Mol Microbiol* 65:1139-1145
- Rasmussen, B. 2000 Filamentous microfossils in a 3,235-million-year-old volcanogenic massive sulphide deposit. *Nature* 405:676-679
- Woese CR 2000 Interpreting the universal phylogenetic tree. *PNAS* 97:8392



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