

**You and your oral microflora BLAST assignment – example of student responses shown in italics below; Suggested point values for questions 4 and 5 are indicated**

*Follow the instructions in your Oral microflora handout to complete this assignment.*

Please provide the following information about your sequence:

1. The name of your sequence (e.g. SC): *\_NOTE: This information was requested as we checked student results against the results we obtained performing a BLAST with their sequences; \_SP\_\_\_\_\_*
2. The amount of DNA (volume) you used when you set up your sequencing reaction: *\_\_\_\_\_ 2 microliters \_\_\_\_\_* (i.e. which recipe did you follow?)
3. The length of the sequence you pasted into the BLAST algorithm: *\_\_\_\_\_ 270 letters \_\_\_\_\_*
4. Identify the top hit for your sequence using the BLAST algorithm (note, if your top hit is an “uncultured bacterium, indicate that here, and refer to the “Uncultured bacterium” instructions in step 5 below to answer these questions). (1 point for section a-e)
  - a. Accession number: *\_\_\_\_\_ GU561418.1 \_\_\_\_\_*
  - b. Description: *\_\_\_\_\_ Neisseria subflava strain GHT1 16S ribosomal RNA gene, partial sequence \_\_\_\_\_*
  - c. E-value: *\_\_\_\_\_ 2e-126 \_\_\_\_\_*
  - d. Query coverage: *\_\_\_\_\_ 95% \_\_\_\_\_*
  - e. Max identity: *\_\_\_\_\_ 99% \_\_\_\_\_*

The following information can be found by clicking on the hyperlink for your top hit’s “Max Score”, which will take you to the actual alignment of your “query” sequence to the “subject” sequence that it matched in the database. (1 point section f-h)

- f. Identities: *\_\_\_\_\_ 256-259 99% \_\_\_\_\_*
- g. Gaps: *\_\_\_\_\_ 2/259 1% \_\_\_\_\_*
- h. Were all of the nucleotides that you submitted used to find a match in the database? In other words, if your query sequence was 245 nucleotides, were all 245 nucleotides accounted for in the identities equation, or for example, were only 150 nucleotides used? *\_\_\_\_\_ no, only 259 of 270 \_\_\_\_\_*

i. If only a portion of your query sequence was used, what could that mean about the strength of your match? (1 point)\_Students should recognize that the fewer nucleotides from their query sequence used in the final match could indicate a weaker match. (Example of student response: *that the match is less strong since it did not incorporate all of the nucleotides*) \_\_\_\_\_

5. Now that you know the identity of one of the organisms in your mouth, you need to find a little bit more information about this organism. Use the websites listed below to help you find information about your newly identified oral microflora. (NOTE: if your top hits are “uncultured bacterium”, see number 6 below) (7 points total)

a. Is it gram positive or gram negative? (1 point)\_NOTE: This will vary depending on the organism identified above \_\_(*Gram negative*) \_\_\_\_\_

b. Is it typically found in the human mouth? (If not, please indicate where it is normally found, and why you think you might have found it in your sample). (1 point)\_If successfully isolated bacteria from the mouth, this should be YES! \_\_(*yes, in the upper respiratory tract and oral cavity*) \_

c. Can it be found elsewhere on the human body? If so, where else can it be found? (1 point)\_This will again vary depending on the organism; student response should be reasonable \_\_\_\_\_

d. Find one other interesting fact about this organism, and indicate where you found this information. (4 point, 2 points each part)\_Student response should be reasonable, and source of information must be a valid scientific source. \_\_  
Example response: *\_is a rare opportunistic pathogen and has been associated with endocarditis, bacteremia, meningitis, septic arthritis, endophthalmitis, and septicemia.* \_\_\_\_\_

<http://www.thelabrat.com/restriction/sources/Neisseriasubflava.shtml> \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

6. **“Uncultured bacterium” instructions.** If your top hit is an uncultured bacterium, repeat your BLAST search with the following changes: In the “Choose Search Set” section, be sure that you have checked the box marked “Uncultured/environmental sample sequences” next to “Exclude”. This will exclude unknown species from the database search and should provide you with the information to answer question 5 above.

### **Some useful websites for finding information about microorganisms.**

NCBI PubMed, a resource for scientific literature (experimental research and scientific reviews):

<http://www.ncbi.nlm.nih.gov/pubmed/>

NCBI Bookshelf, a resource of scientific textbooks: <http://www.ncbi.nlm.nih.gov/books>

Microbeworld, a website sponsored by the American Society for Microbiology ([www.asm.org](http://www.asm.org))

to enhance the understanding of microbiology, and to provide access to the latest in microbial research: <http://www.microbeworld.org/>

Centers for Disease Control and Prevention, a division of the federal Department of Health and Human Services: [www.cdc.gov](http://www.cdc.gov)