Frequently Asked Questions About the Unknown Projects
(EU, OU, PCR-ID)

The following are questions that were asked in previous semesters and the answers that were given. Reading them will help you prepare better reports although not all may apply to you or there may be other questions that are important and are not asked here. If you have a question, please email it to me (ebassiri@sas.upenn.edu) and I will try to answer it and add it to the list of FAQ for coming semesters. I have categorized the questions based on the project.

General Questions:

#1: Is there a specific way that you want us to follow when citing material in our reports?

Yes, we follow the rules shown in the article “Anatomy of a Citation” on the following Website: http://www2.liu.edu/cwis/cwp/library/workshop/citama.htm. Below are some examples taken from this Website for the most common reference citations:

Example of a scientific journal citation:


Example of a book article or chapter citation:


Example of a book citation:


Example of an encyclopedia article citation:


Examples of using a Website citation:


Example of using a journal article on the Internet

**#2:** Do we need to cite the Lab Manual throughout our report (for example, after descriptions of procedures)? Or is it sufficient to just cite it at the end as part of the bibliography?

If you cite all your references alphabetically in your bibliography and give them numbers starting from 1, then you can just put that number in parentheses after every time you need to refer to the content of the Lab Manual.

**#3:** Is it not necessary to go to the Biomedical library now that we have the documents on blackboard?

Not really. Some people prefer to see the hard copy of books and could use the library copies. But if you want to use the BlackBoard material, it is perfectly all right.

**#4:** Are there videos on some of the lab procedures that we can watch?

Yes, there are some videos available; e.g. general staining, Gram staining, nigrosin staining and KOH test. Check them out at the Biology Department's website (http://www.bio.upenn.edu/computing/media).

**#5:** What other sources of information can we find except the Bergey’s Manual?

One other good source of information is books.google.com. You can go to this site and put your species name in the search box. This brings up many books and gives you the first page number of the book that has a mention of the species. You may need to do some scrolling to find more information.

**Questions Regarding the EU Project**

**#1:** What exactly do I need for describing my EU and where can I find more information about it?

When writing your report, you should discuss your species as to its usual habitat, characteristics, economical values, pathogenicity, etc. Such information can usually be found in the “Bergey’s Manual of Systematic Bacteriology”. We also have uploaded some pdf files on the BlackBoard that describe and provide pertinent information about your EU genera and species. These files are searchable, so it would be very easy to research a specific species. Please first take a look at “How to Use Bergey’s Manual” and “Generalities of Enterobacteriaceae Family” and then research the genus of your EU unknown.

Remember that you cannot take sentences or paragraphs directly from these files (that would be plagiarism!) or from any other references that you may use in your research. You need to write them in your own words and give credit to the original writer by properly citing the reference (refer to General Questions #1 in this section). This procedure should be followed for ANY REPORT that you write in this lab.
#2: Can you give us more information about the format and length of the EU report?

Based on previous years, using Times or Times New Roman 12 pt font and double spacing, the approximate number of pages should be as follows:

- Cover sheet- 1 page
- Introduction- about 1 page
- Methods- about 3 or more pages depending on the number of drawings/pictures that you may want to include
- Results & Discussion:
  - Explaining how you got to the end result- about 1-2 pages
  - Summary data sheet- 1 page
  - Describing the species- about 2 pages
- Summary: 1-2 lines
- References- 4-5 refs.

The above are just suggestions. If you feel you want to write more and what you are writing is pertinent, by all means do so.
The weight of EU report is twice as much as each of your usual weekly reports.

**Questions Regarding the OU Project**

#1: How do I fill out my ID key?

To fill out your ID key, first look at the Gram stain and shape data you have already collected on your OU. As an example, suppose you have found your OU to be a Gram positive coccus. Then going back to the table of OU species in your Lab Manual, check to see which organisms in the list are positive cocci. You will note that 10 of them belong to this category and your OU is one of them. This means that you will limit your investigation to just these 10 species if you have a Gram positive coccus. The number would be only 2 if you have a Gram negative coccus, 6 if you have a Gram positive rod and 9 if you have a Gram negative rod. Your next job is to fill the ends of branches of the key that pertains to your OU. For example, if your OU is a Gram negative rod, then you need to place the 9 species mentioned before at the end of branches in the dichotomous key page that has Negative Rods as its heading. Note that it is possible that not all branch ends get filled with species’ names.

[If you have a negative coccus, you need to make a new key for yourself from scratch. Since there are only 2 such species in the table of species given, it is very easy to make a dichotomous key for them by finding a single biochemical test or observation that differentiates the two.]

Now that you know which page to fill, you should re-visit Constructing an Identification Key and use the Bergey’s Manual to find the differences between families, genera and species of the organisms that should go into your appropriate dichotomous key. Copies of Bergey’s Manual are on reserve in Biomedical Library but we have also posted the
appropriate genera of bacteria used in species table on the course BlackBoard.

#2: Do we need to make an ID key for all 4 types of bacteria (negative cocci, negative rods, positive rods, positive cocci) or just the type we obtained as our OU?

You only need to work on and include that in your report only an ID key for only the type of your OU.

#3: How can we be absolutely sure that we are working on the correct ID key?

Please note that the most important first two steps in identifying a bacterial species are (1) determining the correct Gram reaction and (2) determining the correct shape of the cell. If one or both of the above are wrong, the rest of the work on identifying the organism is useless. Many times, if the decolorization step is not done correctly (which happens quite frequently), the results will turn out to be wrong. Too much decolorization causes a Gram positive species to show up as Gram negative. Too little decolorization causes a Gram negative species to show up as Gram positive. At other times mixed Gram reaction is observed. One can redo the Gram and nigrosin stains a few times with different amounts of smear. Another easy way is to run your species along with the one from Gram Staining Lab that was similar in Gram reaction and shape to your unknown. We usually keep a plate of #1, #2, #3 and #4 species in the refrigerator.

#4: My bacterium is a negative coccus. Will the information in the Bergey's manual be enough to make this ID key?

Yes, the only negative cocci species given in the table of species belong to the genus *Neisseria* and that genus is also posted on the BlackBoard.

#5: I have a question about the key. I have read the instructions multiple times and I am really confused as to what we are supposed to be doing. On the chart provided for the positive cocci, there are more than 10 places so how do we know what to write at each place? Is it that you look up each of the 10 species and then just try to put them in the empty spots somewhere? How do you know what test to stop at?

I am going to take the example you have given above and go through it for you to make this clear: For these 10 species, you need to check their characteristics individually and keep placing them in the key. How is this done? Based on the dichotomous key given for positive cocci in the Lab Manual, the first characteristic that you need to research in Bergey's files is to see which ones are catalase positive and which ones are catalase negative. This test enables you to divide the original 10 species into two groups. Suppose you find 6 are catalase negative & 4 are catalase positive. You then need to check the first 6 for their oxygen requirements. Again you find such information in Bergey's files. If any one is an aerobe, you place its name under "A", if any one is a microaerophile, you place its name under "M" and the rest should be facultative anaerobes (FA) but you need to check the Bergey's to make sure. Now, for the FA group, your next test is hemolysis. Again you will find hemolytic reaction characteristics of each of these FA species in Bergey's. Next you check the optochin (an antibiotic) and
glycerol (a carbohydrate) tests. Then you go back to the 4 species that were catalase positive and check their oxygen requirements, etc, etc.

If you keep continuing this way, you will be able to place one species at the end of each branch. [Note that there may be more spots than species, so it is possible to have an empty branch end]. When your key is ready as such, you just need to perform tests (catalase, oxygen requirement, etc) on your OU and based on the results of each test, determine on which branch you land. If you land on a branch that still branches, you need to perform its particular test. If you land at the end of a branch, your work is done and you just look up what species was placed there, and that would be the species of your OU.

#6: How do we get the material necessary to do individual diagnostic tests on our OU?

It is best to do a diagnostic test on your OU early in the first lab session of the week based on your prepared ID key. At each session, you are going to fill a “Supply Request Form” (or SRF). Know exactly what supplies you need so you can perform your test. For example, if you are going to do a catalase test on your OU, you should ask for a slide and an H₂O₂ bottle, or if you want to determine the hemolytic reaction of your species, you need to ask for an SBA (Sheep Blood Agar) plate. Your TA will distribute the SRFs at the start of each lab and collect them after 15 minutes to be able to get you your requested supply as soon as he/she can.

#7: What is OU plating mentioned in the lab syllabus?

After working with several species and learning how to streak plates properly, each student is going to subculture his/her OU species onto a fresh BHI plate. Make sure you use the quadrant method. After incubation (at the temperature that you think is best for your species), your TA is going to grade your plate. If you were able to produce isolated single colonies on the 4th quadrant of this plate, you will get a full grade of 5. This grade is a part of your total OU grade. All your upcoming diagnostic test can be done off of this new plate. You may discard your older BHI plates at this point as (1) they are occupying valuable space in the lab refrigerator and (2) tests performed on old plates may not be reliable.

Questions Regarding the PCR-ID Project

#1: I cannot open my sequencing file. What should I do?

Note that all these files can be opened from within a word processing application such as Word after being downloaded to your computer although their extension is .seq. If you have difficulty opening the files, open Word first and then go to the File menu and open the file from there. If you still need help in downloading files from the course BlackBoard and using BLAST, please contact your TA.

#2: I am having trouble opening my PCR sequence. I am able to download the sequence, but when I try to open it using a text editor, it only appears as "NNNNN."
“NNNNNN” means your sample sent to the Sequencing Facilities did not produce any proper sequences. You and others who had this problem are given sequences from Replacement samples that you can use to write up your report.

#3: I cannot find my PCR-ID sequencing file on the BlackBoard. What should I do?

If you don’t find your file, contact your instructor or TA. Probably your sequencing results were not usable. You still need to write your report based on a Replacement file that will be given you.

#4: What is the grade weight of PCR-ID report?

The weight of the PCR-ID report is twice as much as each of your usual weekly reports.

Questions Regarding the Combined EU or OU and PCR-ID Projects

#1: Our instructor wants us to combine the EU and the PCR-ID reports since we used the same species in both cases. Will you give us a new guideline sheet for the requirements for combined report? I know we now have the two separate guidelines for the PCR and for the EU, but some of that stuff will overlap.

The format of this report is very much like the EU or OU report. I would like you to read the separate guidelines for the EU and the PCR-ID reports so as not to miss anything that should be included. Again, using Times or Times New Roman 12 pt font and double space, the approximate number of pages should be as follows:
- Cover sheet- 1 page
- Introduction- about 2 pages; include why is it important to diagnose the species of Enterobacteriaceae family? What methods are used for this purpose—i.e. conventional vs. molecular biology methods? Compare the methods (broad advantages and disadvantages of each).
- Materials and Methods- about 4-5 pages; include daily logs and overall flow diagram for the PCR part. This flow chart should be just 1 page, so a reader could easily grasp all that was done in one glance.
- Results and Discussion- include results of tests, a summary data sheet, and discuss the results of tests done for the Enterobacteriaceae family recognition, Enterotube (attach Coding sheet), gel electrophoresis (attach gel picture and describe what point it clarifies) and Blast (attach appropriate blast pages). Answer all the questions asked for this part in the PCR-ID report, and finally the characteristics of the species, its habitat, its pathogenicity, etc. Also discuss whether the two ID methods pointed to the same species. If not, suggest reasons for this discrepancy. Length of this part depends on the species and how much info you want to include.
- Summary- Just a final paragraph about the species and its name.
- References- 8-10 refs.
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