

## Independent Project Report Guidelines – Fall 2015:

Please use the **report form** provided on the microbiology webpage (see entry below this one) and **submit your report electronically** (send to microbes@mac.com.). All reports must be submitted as word files (.doc or .docx). Other formats are incompatible with our computers and appear as seriously garbled (word perfect) or unreadable (vista).

Independent project reports must be written in the **third person, past tense**. For example, a **correct** report form would read – "Isolation of the subject culture was accomplished by streaking nutrient agar plates with samples taken from well-isolated colonies. A pure culture was obtained after three transfers." An example of an **incorrect** form would read – "I take a sample from my air plate. I streak that on my new nutrient agar plate. After three tries I have my pure culture." **DO NOT USE** first person, personal pronouns including "I" or "my", "we" or "our".

**Introduction** – This section **explains why the project was initiated**, and should include some background information about the environment under investigation, conditions existing, or concerns about behaviors, potential for infection, etc. This section must also **include a hypothesis**, i.e., an educated guess addressing what the investigator expects to find. For example, if the project involves identification of organisms from water, conduct research to learn what types of bacteria are commonly found in water. Include some information about these organism types in your introduction, and then write a hypothesis, e.g., "Since bacteria in the genera *Escherichia*, *Pseudomonas*, *Acinetobacter* and *Aeromonas* are common inhabitants of water, a sample taken from a local pond is likely to include one or more of these organism types." The introduction is usually one or a few paragraphs in length, and must include references in the text (author, date) for all factual statements (see laboratory manual pages 19-21). **DO NOT include page numbers with references in the text.**

**Materials and Methods** – This section describes how the investigation was conducted, but is written by microbiologists, for microbiologists, so does not include all the details. For example, it is appropriate to include reference to a Gram-stain, but inappropriate to include the steps/reagents used for this preparation. Enzymatic tests used in the investigation are listed in paragraph form, but details about how these tests work (pH indicators, bubbles, black precipitate, etc.) are not included.

The materials and methods section should include **three paragraphs** as follows: The first paragraph addresses sample collection, culture isolation, and steps used to determine colony and cell morphology (including Gram stain, KOH test and indirect stain).

The second paragraph addresses DNA preparation and analysis. This includes extraction of DNA (cells boiled in 10mM Tris buffer, or boiled and beaten with glass beads), amplification of 16S ribosomal-RNA genes using the PCR (bacteria primers were Bacteria 8-Forward and Enteric 1530-Reverse, and fungus primers were ITS-4 and ITS-5), gel electrophoresis of PCR product DNA (to determine success of the PCR) and purification of DNA samples using QIAquick Gel Purification Kits (Qiagen). After the DNA was purified, it was transported to the UC Davis, DNA sequencing facility (Storer Hall). The sequencing primers used for bacteria included 8-forward, 533-forward, and 1530-reverse, while the sequencing primers used for fungi included either ITS-4 or ITS-5. Electropherograms were received via email, and were evaluated and edited using Mac OSX and 4Peaks (or other software as specified), and the comparison of sequence data to information available in public databases was accomplished through the use of the NCBI BLAST algorithm.

The third paragraph addresses any enzymatic tests used during the investigation. All cultures should be subjected to catalase and oxidase tests, metabolism type (fermentation VS respiration) should be determined using the MR-VP test for Gram-positive cultures, and the O/F test for Gram-negative cultures. Acid production from various carbohydrates requires the use of agar **slants** for *Staphylococcus*,

*Micrococcus*, *Bacillus* and multiple other genera, so **do not inoculate carbohydrate deeps** without checking with your instructor first. Remember that respiratory cultures do not ferment, so should not be inoculated into any carbohydrate deeps, and that our carbohydrate deeps cannot be used to determine single carbon source utilization because they all contain peptone in addition to carbohydrate.

**DO NOT include any results in your materials/methods section!**

**Results** – This section includes all findings (data and results), but should also be divided into three paragraphs. The first paragraph addresses colony morphology, KOH test, stain results and cell morphology. This information must be provided in paragraph form, but illustrations or photographs may also be included (if they are your own).

Paragraph two addresses DNA findings (BLAST results) and should be similar to the information recorded for PUNK2 (last page of the report form). Include genus and species names for the entry showing greatest similarity to your query, % similarity, ratio of bases matching pairwise, accession number, bit score and taxonomic lineage. Your BLAST query sequence should be at least 1400 bases in length (bacteria) or around 500- 600 bases in length (fungi).

Paragraph three addresses data and results obtained for all enzymatic tests conducted (minimum included here = catalase test, oxidase test, MR-VP tests, and/or O/F tests).

**Discussion** – This section relates findings to the introduction, and should include a complete description of the culture or cultures isolated (conclusions reached about these cultures following data analysis). This section may also include additional information gained through research (important aspects of a particular isolate). Excellent sources for additional information include NCBI PubMed and Highwire Press (Stanford University). Information gained from research articles must be referenced appropriately, e.g., the format indicated in the laboratory manual. The discussion may address a specific hypothesis, indicating if or not it appears valid, may describe changes in taxonomic status, provide an historical perspective or may suggest areas for additional investigation. The discussion is usually completed in one or a few paragraphs.

**Acknowledgements** – This section is appropriate, though not required, and would be included following the discussion section. Funding in support of student projects has been provided by the Sierra College Foundation and the North Valley and Mountain Biotechnology Center, at American River College. Personnel in the UC Davis DNA Sequencing Facility have also been extremely helpful.

**Literature Cited** – This section includes all sources of information, e.g., the Microbiology Laboratory Syllabus, the Bergey's Manual, and any other references used. Authors are listed in alphabetical order following the format described in the laboratory manual. When referencing the Bergey's Manual, use the name of the author listed for the chapter being cited, and please follow instructions.

The Independent Project is worth 70 points, maximum and will be graded on format as well as content. Reports will be accepted no later than 12:00 midnight on the last day of the semester. Reports may be written individually or with partners, but everyone is expected to participate. All microorganisms being investigated are to be submitted as living, pure cultures, on or before the day of the lecture final exam. A completed copy of the Semester Project Data Record (laboratory manual pages 23-24) is required as a part of this project (see grading rubric form below).

## Bio 4 Semester Project Grading Sheet

Name(s): \_\_\_\_\_

Lab Section: \_\_\_\_\_

**Project Template used (2 points)** Points \_\_\_\_\_

**Independent Project Data Record Submitted**  
with Pure Culture Submitted (if appropriate) **(10 points)** Points \_\_\_\_\_

### Write-up (38 points):

**Title** (2 points) Points \_\_\_\_\_

**Abstract** (5 points) Points \_\_\_\_\_

**Introduction** (5 points) Points \_\_\_\_\_

**Materials & Methods** (6 points) Points \_\_\_\_\_

**Results** (8 points) Points \_\_\_\_\_

**Discussion** (10 points) Points \_\_\_\_\_

**Literature Cited** (2 point) Points \_\_\_\_\_

**Project Completed and Submitted (20 points)** Points \_\_\_\_\_

### “Penalty Box” (Point Deductions)

- Grammar, spelling, and syntax problems (up to 7 pts.)
- Organization problems (ie: conclusions in your results section, etc.) (up to 7 pts.)
- Analysis problems (ie: your results do not corroborate your conclusions; conflicting results that are not acknowledged) (Up to 7 pts.)
- Inappropriate Citations or References (Up to 2 points)

Total Point Deductions \_\_\_\_\_

Total Points \_\_\_\_\_