**Independent Project Report Guidelines – Summer 2007:**

Please use the report form provided on the microbiology web page and submit your report electronically, i.e., send to hwilson@sierracollege.edu or microbes@mac.com. All reports must be submitted as word files (.doc). 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. Example of **correct** 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."
Example of **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."

**Introduction –** This section explains why the project was initiated, and may include background information about a condition, organism type, subject individual, etc. or propose a specific hypothesis to be tested. The introduction is usually one or a few paragraphs in length.

**Materials and Methods –** This section describes how the investigation was conducted, but is written by microbiologists, for microbiologists, so does not include all 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.

Methods used in the DNA analysis include the following, in the order listed:
1. Extraction of DNA with cells boiled in 10mM Tris buffer (pH 8.0), or boiled and beaten with glass beads.
2. Amplification of 16S ribosomal-DNA using the PCR and Taq Master Mix (Qiagen). Bacteria primers were Bacteria 8-Forward and Universal 1492-Reverse, and fungus primers were 0817-Forward and 1536-Reverse (Operon).
3. Gel electrophoresis of PCR product DNA (total volume) in agarose and TBE buffer.
4. Purification of DNA samples with QIAquick Gel Purification Kits (Qiagen).
5. Submission of purified DNA samples to the $^{UC}$DNA Sequencing Facility, Storer Hall, University of California, Davis. Sequencing primers were 8-For and 1492-Rev for all bacterial samples and 0817-F and 1536-R for all fungi samples.
6. Electropherogram evaluation and editing using Mac OSX and 4Peaks.
7. Comparison of sequence data to information available in public databases through the NCBI BLAST algorithm.

Inclusion of this information is required, but individual variation is expected. Additional information is available in the lab manual and in the "Biotechnology" article available on the Bio. Sci. home page (Blue button, bottom of page). The materials and methods section must be written in paragraph form. **Do not** "copy and paste" the above list into your report.

**Results** – This section includes all findings e.g., stain results, cell and colony morphology, enzymatic test results, percent similarity with sequences in the public databases, etc. This information is usually provided in paragraph form, but illustrations, tables or photographs may also be included. Be certain that results are not included in the materials and methods section.

DNA analysis is available for all student projects, and sequence data from two primers (Bacteria 8-Forward and Universal 1492-Reverse or 0817-Forward and 1536-Reverse) is currently posted on the microbiology website (see web based laboratory assignments, Summer 2007 ".ab1" files. Electropherogram analysis does not require Mac OSX or 4Peaks, but will require computer technology and software capable of reading ".ab1" files. For maximum accuracy, edit and combine the two sequences provided. Your BLAST query sequence should be at least 1400 bases in length (bacteria) or around 800-900 bases in length (fungi).

**Discussion** – This section relates findings to the introduction, but may also include information gained through research. 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 use in any ASM publication. The discussion may address a specific hypotheses, 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, in bold. 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 $^{UC}$DNA Sequencing Facility have 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 use in any ASM publication. When referencing the Bergey's Manual, use the name of the author listed for the chapter being cited.

The Independent Project is worth 50 points, maximum and will be graded on format as well as content. Reports will be accepted no later than 5:00 pm, Saturday, June 30$^{th}$. You may work 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 Thursday, June, 28$^{th}$.

If you are interested in receiving a copy of the Sierra College Journal of Microbiology – Summer Edition, please indicate this when submitting your entry. The Journal will be printed after grades have been submitted and the Editor has completed final formatting (sometime during July).