BIOL313
Spring 2008
Pre-test/post-test

1. Marjorie Molecule, a Centenary student, is investigating a novel enzyme. She has cloned the gene that encodes the enzyme, placing it in a plasmid that can be replicated in E. coli. She decides to perform site-directed mutagenesis to better understand the role of various amino acids in the enzyme's function. To do her experiment, she mixes the following:
   The cloned gene/plasmid construct
   Two mutagenic primers
   Buffer
   Heat-resistant DNA polymerase

   a. What is the purpose of site-directed mutagenesis? A. To determine which amino acids in a protein are most susceptible to mutation. B. To determine which nucleotides in a gene are most susceptible to mutation. C. To introduce a desired change in nucleotide sequence in a gene. D. To introduce a desired change in nucleotide sequence in a particular tissue of an organism. E. None of the above.
   b. What did Marjorie omit from her reaction mix that is necessary for site-directed mutagenesis? A. Taq polymerase B. dNTPs C. ddNTPs D. template DNA E. Non-mutagenic primers

2. Marjorie later mixes her mutant gene/plasmid construct with competent *E. coli*.
   a. This process is referred to as A. transduction B. replication C. transvection D. transformation E. expression.
   b. The purpose of this step is to allow the bacteria A. to take up the DNA and replicate it as they divide. B. to take up the DNA and perform mismatch repair. C. to take up the DNA and degrade the nonmutant strand. D. to excrete enzymes that degrade the mutant strand. E. to excrete enzymes that replicate the DNA extracellularly.

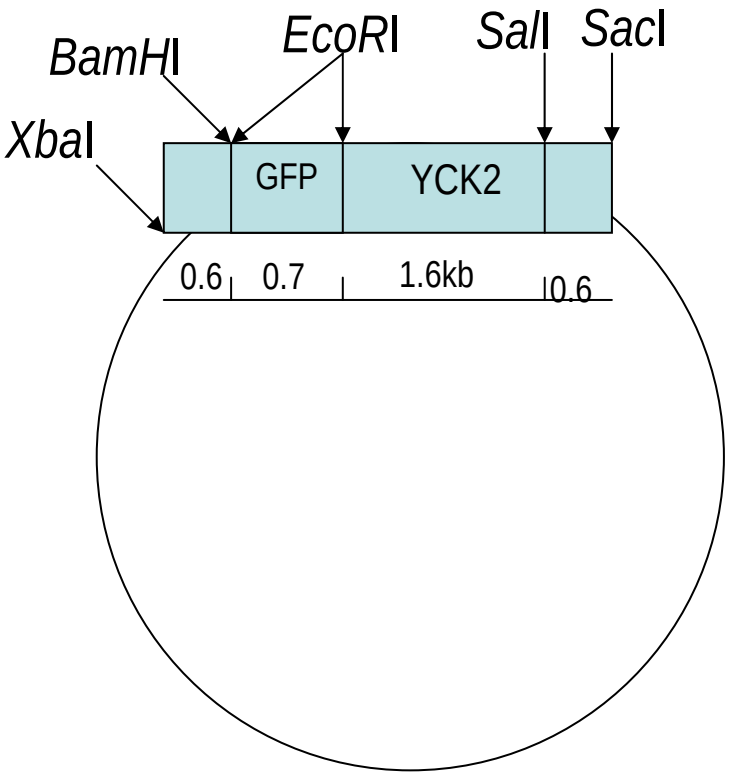
3. To recover her DNA, Marjorie performs a miniprep. Match the following miniprep components to their function in the miniprep procedure.
   a. Potassium acetate
   b. Solution containing SDS and NaOH
   c. Silica spin column
   d. RNase
   e. Water

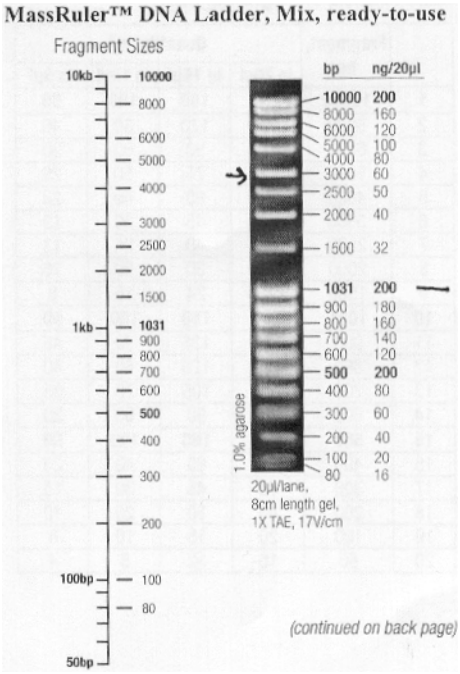
   Match with:
   _____ Cell lysis; DNA and protein denaturation
   _____ Plasmid elution
   _____ Plasmid renaturation
   _____ RNA degradation
   _____ Allows DNA binding in the presence of salt

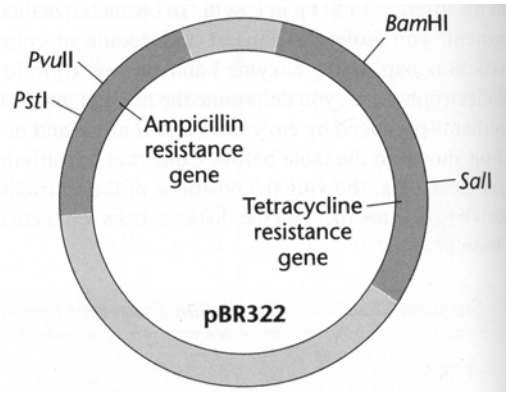
4. After performing the miniprep, Marjorie performs a restriction digest. A restriction digest A. degrades DNA nucleotide by nucleotide. B. degrades proteins amino acid by amino acid. C. cleaves DNA at particular nucleotide sequences. D. degrades proteins at particular amino acid sequences.

5. Later in her experiment, Marjorie examines some DNA fragments by gel electrophoresis as shown at left. Using the map of the plasmid and the mass ladder shown below, match the top DNA band from the sample on the gel to the appropriate DNA fragment from the plasmid. The body of the plasmid is 2.7 kb.

6. In question 5, what restriction enzymes were used to generate this fragment? A. *Xba*I and *BamH*I B. *BamH*I and *EcoR*I C. *Xba*I and *Sal*I D. *Xba*I and *Sac*I E. *BamH*I and *Sal*I F. *BamH*I and *Sac*I







7. An ampicillin-resistant, tetracycline-resistant plasmid, pBR322 (shown at left), is cleaved with *Pst*I, which cleaves within the ampicillin resistance gene. The cut plasmid is ligated with *Pst*I-digested *Drosophila* DNA to prepare a genomic library, and the mixture is used to transform *E. coli*.

A) Which antibiotic should be added to the medium to select cells that have incorporated a plasmid?

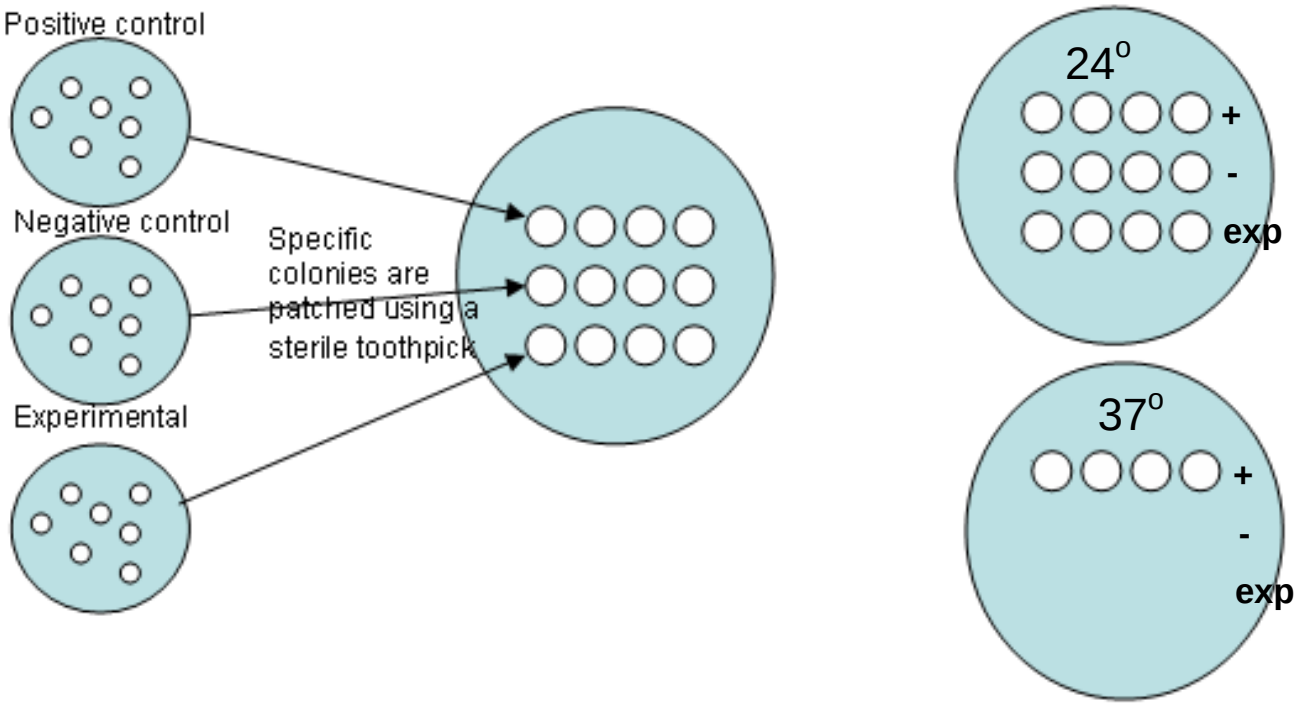
B) What cells should be selected to obtain plasmids containing *Drosophila* inserts?

8. In the Sanger method of DNA sequencing, 2',3'-dideoxyadenosine (ddATP) A. is used to radioactively label the DNA. B. blocks further DNA synthesis when it is incorporated into the newly synthesized DNA strand in place of dATP. C. blocks DNA synthesis at random positions along a DNA strand. D. has no effect on DNA synthesis when it is incorporated. E. none of the above.

9. YCK2 and YCK1 form a pair of genes that together are essential for life in yeast. If either YCK is functional, cells live, but if both YCK1 and YCK2 are deleted, cells do not survive. A yck1- yck2- yeast strain can survive if that strain also carries an episomal (plasmid) copy of either YCK1 or YCK2 (pYCK1 or pYCK2). This is termed complementation.

In the experiment shown below, a yck^{ts} strain of yeast was used to examine the function of a mutated form of YCK2. Yck^{ts} yeast have no YCK1 gene and have a temperature-sensitive YCK2 allele.

Yck^{ts} yeast were transformed with a plasmid copy of YCK2 (the positive control), an empty plasmid (the negative control), or a plasmid containing the mutant YCK2 gene (experimental sample). The yeast were grown at the permissive temperature of 24°C (middle plate). After replica plating, the yeast were grown either at 24°C or the restrictive temperature (37°C) (plates at right). The data shown below suggest that the YCK2 mutation induced in the experimental sample A. does not interfere with YCK2 function. B. partially interferes with YCK2 function C. inhibits YCK2 function D. It's not possible to say from the results shown. E. none of the above.



10. The compound 5-fluoroorotic acid, or 5-FOA, is toxic to cells that have the ability to convert orotic acid to orotodine-5'-monophosphate (a step in uradine synthesis). The reason for toxicity is that the 5-FOA enters the uridine biosynthetic pathway and is converted to the monophosphate derivative and then by the enzyme orotidine-5'phosphate decarboxylase (in yeast, the product of the URA3 gene) to 5-fluorouracil. 5-fluorouacil is a precursor of a very potent inhibitor of thymidylate synthase, so is toxic to cells. So, to summarize, 5-FOA kills cells that have a functioning uridine biosynthetic pathway, and specifically in yeast, a functional URA3 gene product.

We will be working with the YCK2 gene. YCK2 is one of a pair of genes that together are essential for life in yeast. If either YCK is functional, cells live, but if both YCK1 and YCK2 are deleted, cells do not survive. A $yck1^{-}$ $yck2^{-}$ yeast strain CAN survive if that strain also carries an episomal (plasmid) copy of either YCK1 or YCK2 (pYCK1 or pYCK2). This is termed complementation.

Given all of this information, predict whether each of the following yeast strains should grow on media containing 5-FOA.

$YCK1^{+}$ $YCK2^{+}$ $pURA3^{+}$
$yck1^{-}$ $YCK2^{+}$ $pURA3^{+}$
$yck1^{-}$ yck2- $pYCK1^{+}$, $URA3^{+}$

11. Find sequences for histone H3 from humans (*Homo sapiens*), mice (*Mus musculus*), and fruitflies (*Drosophila melanogaster*). Use ClustalW (align.genome.jp) to align the sequences. Which of the following sequences from histone H3 are conserved? A. MARTKQ B. EIRRY C. LVGLF D. RGERA E. All of the above.

12. In *Saccharomyces cerevisiae*, histone H3 is encoded by a gene called HHT2. Find the coding sequence of this gene. Which are the first six nucleotides? A. ATGGCC B. GCCAGA C. ACCACC D. AAAAAA E. MARTKQ

13. To what amino acids do these nucleotides correspond? A. Met-Lys B. Met-Ala C. Ala-Arg D. Lys-Lys E. Thr-Thr

14. Using the Structure database at NCBI, find the *Drosophila* core nucleosome. Find and highlight the sequence APVYLAAVMEYLAAEVLELAGNAARDN. What type of structure does it form? A. Random structure B. Beta sheet C. Alpha helix D. Hairpin loop E. Double helix

Rubric for assessment of lab report 1

Introduction	12
Materials and Methods	11
Results—Experimental Rationale and Description of Data	15
Figures, Tables and Graphs	15
Discussion	12
Writing	10

Introduction (12 points)

12 The introduction provided enough information to understand and appreciate the problem at hand, including background information to explain the interest of the question. The author has provided a brief overview of the question to be investigated and the experimental approach.
9 The introduction allows a partial understanding and appreciation of the problem at hand, but background information is not sufficient. The author has provided a brief overview of the question to be investigated and the experimental approach.
6 The introduction allows a partial understanding and appreciation of the problem at hand, but background information is not sufficient. The author has not provided an overview of the question to be investigated and/or the experimental approach.
3 The introduction allows only a very limited understanding and appreciation of the problem at hand. The author has not provided an overview of the question to be investigated and/or the experimental approach.

Materials and Methods (11 points)

11 The materials and methods included a thorough and comprehensive description of each experiment discussed in the report. The materials and methods included all of the information needed to repeat the experiments.
9 The materials and methods lacked minor information about one or more experiments discussed in the report.
5 The materials and methods lacked major information about one or more experiments discussed in the report.
3 More than one experiment was completely left out of the methods section. Very little of the information about the experiments were given.
0 Several experiments were not discussed. Few or no specific experimental details were given.

Results: The Experimental Rationale (5 points)

5 The reader is given a thorough description of the experimental rationale. Each section contains a broadly understood summary of the experiment. The summary includes the required attention to the question being addressed and the experimental approach used. It is clear “why” and “how” the experiment was done.
4 Each section contains a summary of the experiment. The summary includes a brief mention of question being addressed and the experimental approach used. In some cases the information is not easily understood. The reader might need to flip back to the methods or figures to understand the experiment.
3 Not all sections contain a brief summary of question being addressed and the experimental approach used. In some cases the author jumps immediately to summarizing the data. The reader is not given the information to understand the significance of the experiment.
1 The result section is mostly a collection of data with little information provided to the reader to explain the significance of the experiment.
0 Little attention was given beyond a quick statement of the result.

Results: A Complete Description of the Data (10 points)-

10 All of the data presented in the report is described accurately in the text. The results were presented in an extremely logical and effective manner. The description of the data is clear and easily understood by a reader not familiar with the system.
8 The data presented in the report is described accurately in the text. The description of the data is complete, but not easily understood by a reader not completely familiar with the system.
6 The data was presented for the most part in an effective manner, however some portions were unclear or missing.
3 The data was presented in a confusing or incomplete fashion. The author either misunderstood the results, failed to communicate them in an effective manner, or left significant portions of the data out of the report.
0 The description of the data was significantly lacking in quality. The author did not understand any of the data presented.

Figures, Tables and Graphs: Data Presentation (15 points)-

15 The figures, tables, and graphs are clear and well labeled. The titles and legends greatly clarified the figure or table. The title was descriptive and accurate. The legends explained all of the unidentified components in the picture, chart, or table.

12 The figures were relatively clear, but some labels are missing. The figures do, however, communicate all of the data. The titles and legends were adequate and mostly corresponded to the figure or table. The legends explained most of the unidentified components in the figure.
9 The figures provide an accurate representation of the data, but are not always clearly labeled. The titles and legends were incomplete or inappropriate in places. The legends often failed to explain many of the unidentified components in the picture, chart, or table.
5 The figures were difficult to read and unclearly (or un-) labeled. The titles were missing or inappropriate. The legends were missing or uninformative.
0 Most figures were illegible or missing. The data was not extractable in any form from the pictures, charts or tables. The majority of the figure legends and titles were illegible or missing for all of the figures or tables.

Discussion: Data analysis and Interpretation (12 points):

12 The author effectively interpreted the findings and expectations. The author provided a valid and insightful critique of the experiments and results.
9 The author provided a solid analysis of most of the data but did not interpret all experiments effectively. The author provided a sound critique of the experiments and results.
5 The author provided a limited analysis of the data; however, the author mostly reiterated the results without further expansion. The author failed to provide a thorough critique of the experiments and results.
2 The author did not provide an adequate analysis of the data. The author failed to provide an adequate critique of the experiments and results.
0 No attempt at data analysis or data interpretation was made.

Writing (10 points) - This report:

10 is a pleasure to read. It is crisp, clear and concise. Needs minor editing only.
8 is easy to read, but would need some editing to be an excellent example of a research report.
5 is hard to read in places. Some sentences had to be reread to get at the meaning. The topic under discussion was not always clearly presented to the reader.
2 is poorly written. Significant portions are sloppy or unclear. There are many misspellings and ambiguities. Even upon re-reading, sentences did not make sense.
0 Is very difficult to read. Most sections are unclear, ungrammatical and convoluted.

Rubric for assessment of lab report 2

Introduction	20
Materials and Methods	10
Results—Experimental Rationale and Description of Data	15
Figures, Tables and Graphs	15
Discussion	30
Writing	10

Introduction (20 points)

20 The introduction provided enough information to understand and appreciate the problem at hand, including background information to explain the interest of the question. The author has provided a brief overview of the question to be investigated and the experimental approach.
15 The introduction allows a partial understanding and appreciation of the problem at hand, but background information is not sufficient. The author has provided a brief overview of the question to be investigated and the experimental approach.
12 The introduction allows a partial understanding and appreciation of the problem at hand, but background information is not sufficient. The author has not provided an overview of the question to be investigated and/or the experimental approach.
6 The introduction allows only a very limited understanding and appreciation of the problem at hand. The author has not provided an overview of the question to be investigated and/or the experimental approach.

Materials and Methods (10 points)

10 The materials and methods included a thorough and comprehensive description of each experiment discussed in the report. The materials and methods included all of the information needed to repeat the experiments.
8 The materials and methods lacked minor information about one or more experiments discussed in the report.
5 The materials and methods lacked major information about one or more experiments discussed in the report.
2 More than one experiment was completely left out of the methods section. Very little of the information about the experiments were given.
0 Several experiments were not discussed. Few or no specific experimental details were given.

Results: The Experimental Rationale (5 points)

5 The reader is given a thorough description of the experimental rationale. Each section contains a broadly understood summary of the experiment. The summary includes the required attention to the question being addressed and the experimental approach used. It is clear “why” and “how” the experiment was done.
4 Each section contains a summary of the experiment. The summary includes a brief mention of question being addressed and the experimental approach used. In some cases the information is not easily understood. The reader might need to flip back to the methods or figures to understand the experiment.
3 Not all sections contain a brief summary of question being addressed and the experimental approach used. In some cases the author jumps immediately to summarizing the data. The reader is not given the information to understand the significance of the experiment.
1 The result section is mostly a collection of data with little information provided to the reader to explain the significance of the experiment.
0 Little attention was given beyond a quick statement of the result.

Results: A Complete Description of the Data (10 points)-

10 All of the data presented in the report is described accurately in the text. The results were presented in an extremely logical and effective manner. The description of the data is clear and easily understood by a reader not familiar with the system.
8 The data presented in the report is described accurately in the text. The description of the data is complete, but not easily understood by a reader not completely familiar with the system.
6 The data was presented for the most part in an effective manner, however some portions were unclear or missing.
3 The data was presented in a confusing or incomplete fashion. The author either misunderstood the results, failed to communicate them in an effective manner, or left significant portions of the data out of the report.
0 The description of the data was significantly lacking in quality. The author did not understand any of the data presented.

Figures, Tables and Graphs: Data Presentation (15 points)-

15 The figures, tables, and graphs are clear and well labeled. The titles and legends greatly clarified the figure or table. The title was descriptive and accurate. The legends explained all of the unidentified components in the picture, chart, or table.

12 The figures were relatively clear, but some labels are missing. The figures do, however, communicate all of the data. The titles and legends were adequate and mostly corresponded to the figure or table. The legends explained most of the unidentified components in the figure.

9 The figures provide an accurate representation of the data, but are not always clearly labeled. The titles and legends were incomplete or inappropriate in places. The legends often failed to explain many of the unidentified components in the picture, chart, or table.

5 The figures were difficult to read and unclearly (or un-) labeled. The titles were missing or inappropriate. The legends were missing or uninformative.

0 Most figures were illegible or missing. The data was not extractable in any form from the pictures, charts or tables. The majority of the figure legends and titles were illegible or missing for all of the figures or tables.

Discussion: Data analysis and Interpretation (30 points):

30 The author effectively interpreted the findings and expectations. The author interpreted the individual results and interpreted the overall implications of the findings, linking them back to the original hypothesis. The author provided a valid and insightful critique of the experiments and results.

25 The author effectively interpreted the findings and expectations. The author interpreted the individual results but did not interpret the overall implications of the findings. The author did not link the results to the original hypothesis. The author provided a valid and insightful critique of the experiments and results.

20 The author provided a solid analysis of most of the data but did not interpret all experiments effectively. The author provided a sound critique of the experiments and results.

15 The author provided a limited analysis of the data; however, the author mostly reiterated the results without further expansion. The author failed to provide a thorough critique of the experiments and results.

10 The author did not provide an adequate analysis of the data. The author failed to provide an adequate critique of the experiments and results.

0 No attempt at data analysis or data interpretation was made.

Writing (10 points) - This report:

10 is a pleasure to read. It is crisp, clear and concise. Needs minor editing only.

8 is easy to read, but would need some editing to be an excellent example of a research report.

5 is hard to read in places. Some sentences had to be reread to get at the meaning. The topic under discussion was not always clearly presented to the reader.

2 is poorly written. Significant portions are sloppy or unclear. There are many misspellings and ambiguities. Even upon re-reading, sentences did not make sense.

0 Is very difficult to read. Most sections are unclear, ungrammatical and convoluted.