INSTRUCTOR TIPS and SUGGESTIONS FOR TEACHING BACTERIAL GENETICS AT A HIGHER LEVEL

CHAPTER ONE  The Molecules that Make up a Cell

This chapter is designed to introduce students to what molecules are used to make up a cell, where different structures are located and how cells grow and divide.

A. The main concepts are:

1. Cells are made up of very few types of molecules.

2. The type of molecule used for a specific structure takes into account the properties of the molecule and the functions it will perform when it is part of the cell. For example, lipids are well suited to form cell membranes because they self-associate to form a stable barrier between inside the cell and outside the cell. The fluidity can be changed simply by changing the location and number of cis and trans bonds in the fatty acids.

3. The different locations in a cell indicate that spatial relationships must be taken into account in cell processes. Where a molecule resides is very important. Different compartments have different properties, functions and resident molecules.

4. Cell growth and division indicates that temporal constraints can apply to specific processes. Not only must certain molecules/structures be present, they must be present in the right place at the right time.

B. Activities

Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer to a specific question in writing and have them do this without their books or notes.

For example ask your students to:

1. Draw the different compartments of a prokaryotic cell.
2. Identify by name the cellular compartments and describe their function.
3. Draw a specific structure and identify the molecules making up that structure.

Teaching Bacterial Genetics at a Higher Level

A. Expandable Concepts:

1. Very little attention is given to how the molecules of the cell are built or imported into the cell. More information on these biosynthetic pathways could be taught, including how lipids are constructed and how carbohydrates are built or broken down.

2. More of the physiology of how bacterial cells grow and what components are rate limiting at different growth rates could be explained. The Copenhagen school of bacterial physiology and their experiments are great examples of these types of experiments. Even though these experiments are older, they are still the gold standard in physiology experiments and careful measurements of physiological parameters.

3. Students are introduced to the different structures that can be found on/in cells. Very little detail is given on what each structure is used for. Details on any of the structures can be
presented. For example, a description of flagella can be followed by a description of chemotaxis or a description of fimbriae can be followed by a discussion of how bacterial cells attach to eukaryotic cells. This latter example can be used to introduce pathogenesis.

4. More details on what happens in each compartment, what reactions take place, how molecules get to their final destination can be presented. This can include the physiology of the compartments, discussion of different types of proteins or a discussion of protein secretion.

5. A discussion of practical techniques such as preparation of pure cultures, diluting and plating bacteria or serial dilutions could be included.

6. A discussion of different types of bacterial growth media and how to identify nutritional requirements (auxanography) could be included.

7. A discussion of eukaryotes, prokaryotes and archaea cells and their major differences/similarities could be included.

B. Activities

Identify research articles addressing concepts in the chapter. Using the research efforts described in these articles, teach your students how to:

- Identify the hypothesis tested.
- Identify the means by which the hypothesis is tested (experimental approaches used).
- Determine which controls were used and why they were used.
- Analyze the results and the conclusions drawn from the results.
- Identify the new hypothesis to be tested based on the conclusions drawn.

Use Active learning activities to enhance retention of the information generated in the above tasks:

- Have groups of students take responsibility for one of the above tasks and then communicate what they have learned to the other groups of students. OR,
- Remove the abstract from the research article before it is handed out to the students. After completing the above tasks, ask the students to write the abstract for the article.

Examples of research articles:

CHAPTER TWO  The Bacterial DNA Molecule

This chapter is designed to teach the chemical structures of DNA and RNA and how DNA molecules are replicated.

A. The main concepts are:

1. The structure of a DNA molecule including the backbone and pairing of bases.

2. How DNA and RNA differ.

3. How a DNA molecule is replicated and what constraints the structure puts on the replication process (a continuation from Chapter One of how the properties of a molecule influence the functions it can perform in the cell).

4. A description of the proteins, including their function, that make up the replication machinery.

5. How all of the steps in replication are coordinated.

B. Activities

Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer in writing and have them do this without their books or notes.

For example ask your students to:

1. Draw a purine and pyrimidine.
2. Draw a nucleotide and nucleoside.
3. Draw a phosphodiester bond.
4. Draw adenine hydrogen bonded to thymine and cytosine hydrogen bonded to guanine.
5. Describe the role of a specific enzyme in the replication process and show where it is this enzyme exerts its effect.

The goal is to have the students be able to recall (not just recognize) the important chemical features of the DNA molecule so that later on in the course they will be able to visualize where different molecular processes impact the DNA molecule.

Teaching Bacterial Genetics at a Higher Level

A. Expandable Concepts

1. The biosynthesis of nucleotides could be considered in more detail. This could include the pathways that result in ribose, deoxyribose and the bases as well as the recycling of the component molecules.

2. A more in depth discussion of supercoiling, including the mathematics that describe supercoiled molecules could be taught. Additionally, the consequences of supercoiling on transcription caould be considered.
3. The organization of bacterial chromosomes and the genes they have in common could be taught. This could include practical problems using the sequenced genomes in Genbank or on the TIGR website.

4. Using the proteins of DNA replication as molecular tools could be taught in greater detail to make the course more molecular.

5. Methods used to isolate/study DNA molecules (DNA isolation, gel electrophoresis, centrifugation, pulse-field gel electrophoresis) could be described in detail.

6. Structures formed by specific DNA sequences and how certain proteins exploit these structures could be taught. This could include bent DNA, cruciform DNA or hairpin formation in DNA.

7. Nucleases that degrade DNA/RNA could be considered.

8. Regulation of bacterial chromosome replication could be considered. The differences/similarities between *E. coli* and *B. subtilis* provide good examples.

9. Techniques such as DNA denaturation and melting curves, heteroduplex formation and centrifugation techniques could be explored.

10. The termination of bacterial chromosome replication and regulation of termination could be examined.

11. The coordination of the cell cycle and DNA replication could be considered.

12. Antibiotics that affect DNA replication or DNA supercoiling could be taught.

**B. Activities**

Identify research articles addressing concepts in this chapter. Use these articles as described in Activities for Chapter One.

Examples of research articles:


**CHAPTER THREE  Mutations**

This chapter describes in detail the meat and potatoes of genetics, how do mutations arise and how many different kinds of mutation are there.
A. The main concepts are:

1. What are mutations and how do we describe them (gain of function, loss of function, macrosions, microlesions, etc.).

2. Genotype and phenotype, what are they and how are they written.

3. Descriptions of all of the classes of mutations.

4. How mutations are formed (the chemistry of mutation).

5. Reverting mutations.

B. Activities

Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer in writing and have them do this without their books or notes.

For example ask your students to:

1. Draw a pyrimidine dimer. Which type of pyrimidine dimer is most common and why?
2. Draw the tautomer of a nucleotide. How can this tautomer cause a mutation?
3. Describe how spontaneous mutations arise.
4. Show specifically the impact of a methylating agent.
5. Name the mutagens that primarily cause microlesions and describe how they cause the microlesion.
6. Give an example of a base analogue and show how it impacts a DNA molecule.

Teaching Bacterial Genetics at a Higher Level

A. Expandable Concepts

1. The uses of mutants could be investigated in more detail. This could include the DNA polymerase mutants that increase the speed of the enzyme but decrease the fidelity and the mutants that increase fidelity but decrease speed. It could also take the form of some of the different biological systems that have been examined using mutants.

2. Construction and importance of isogenic strains could be taught.

3. A more techniques orientated approach to isolating mutants could be included.

4. The importance of independently isolated mutants and the conclusions that can be drawn from them could be considered.

5. When and how to use mutagens could be investigated. What advantage/disadvantages do mutagens offer?

6. What uses can suppressor mutations be put to? What do suppressors tell you about the primary mutation?
B. Activities

Identify research articles addressing concepts in this chapter. Use these articles as described in Activities for Chapter One.

Examples of research articles:


CHAPTER FOUR DNA Repair

This chapter describes how DNA can be damaged and what the cell does to deal with this damage.

A. The main concepts are:

1. Many things will cause DNA damage in a predictable manner, based on what part of the DNA molecule is affected.
2. The cell must decide if it will reverse the damage, excise the damaged DNA or tolerate the damage and keep growing.
3. Mechanisms that reverse, excise and repair or tolerate the damage are described in detail.

B. Activities

Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer in writing and have them do this without their books or notes.

For example ask your students to:

1. Contrast and compare the processes of photoreactivation and UvrABC directed nucleotide excision repair.
2. Describe the proposed impact of a defective methyl directed mismatch repair system in humans.
3. Describe the mechanisms available to tolerate DNA damage.
4. Give an example of a glycosylase and describe its function.

Teaching Bacterial Genetics at a Higher Level

A. Expandable Concepts

1. The experiments that identified what genes are involved in repair of what lesions could be taught.
2. The importance of DNA repair genes and colon cancer could be taught as an example of how bacterial research impacts on other fields.

3. The chemistry of DNA repair (which base is impacted why it is impacted) could be examined in more detail.

2. Regulation of the genes coding for DNA repair enzymes could be taught.

B. Activities

Identify research articles addressing concepts in this chapter. Use these articles as described in Activities for Chapter One.

Examples of research articles:


**CHAPTER FIVE  Recombination**

This chapter describes the process of recombination, which is essential to understanding genetics.

A. The main concepts are:

1. What is homologous recombination and how does the process work?

2. What is site-specific recombination, how does the process work and how is it different from homologous recombination?

B. Activities

Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer in writing and have them do this without their books or notes.

For example ask your students to:

1. Describe the enzymatic properties of RecA
2. Describe the enzymatic properties of RecBCD
3. Draw the formation of heteroduplex DNA
4. Contrast and compare between RecBCD and Integrase
5. Design an experiment demonstrating the requirement for integrase in the integration of lambda bacteriophage genome in to the *E. coli* genome.
Teaching Bacterial Genetics at a Higher Level

A. Expandable Concepts

1. More time could be spent discussing recombination frequencies and what they imply.
2. More techniques, including deletion mapping could be included.
3. More time could be spent on complementation. This could include practical problems.
4. The recombination systems of phage (lambda red, RecE and rac, rec proteins of T4 and T7) and how they have helped identify host recombination genes could be taught.
5. Isolating Rec- mutants of *E. coli* could be included. This could include selections and screens, and predicted phenotypes of Rec- mutants.

B. Activities

Identify research articles addressing concepts in this chapter. Use these articles as described in Activities for Chapter One.

Examples of research articles:


CHAPTER SIX Transposition

This chapter describes transposons and how they move from one piece of DNA to another.

A. The main concepts are:

1. A description of the many different classes of bacterial transposons.
2. A description of the two reactions that result in transposon movement, replicative transposition and non-replicative transposition.
3. How have transposons been developed as molecular tools.

B. Activities
Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer in writing and have them do this without their books or notes.

For example ask your students to:

1. Describe an experiment testing the hypothesis that a DNA molecule suffered an insertion.
2. Draw a transposon and label the features essential to this element’s movement.
3. What is a transposase and how does it function?

Teaching Bacterial Genetics at a Higher Level

A. Expandable Concepts

1. How transposons have played a role in evolution and the nature of that role could be considered.

2. Techniques such as transposon mutagenesis or cloning transposon insertions could be included.

3. The genetic uses of transposons in nontraditional experimental systems and setting up genetic systems in novel organisms could be considered.

B. Activities

Identify research articles addressing concepts in this chapter. Use these articles as described in Activities for Chapter One.

Examples of research articles:


CHAPTER SEVEN  Bacteriophage

This chapter describes the lifecycles of several different bacteriophage including lambda, P1, M13 and T4. These phage were chosen because of either the amount and type of information known about its lifecycle or because the phage is important to bacterial genetics and molecular biology.

A. The main concepts are:

1. A general description of phage lifecycles including phage structure, how phage know what cell to infect and the lytic vs lysogenic decision.

2. A description of the lambda lifecycle including the lytic-lysogenic decision, which was the first developmental switch understood at the molecular level.
3. A description of the M13 lifecycle, including a comparison/contrast with lambda.

4. A description of the P1 lifecycle, including transducing particles.

5. A description of T4 as an example of the T phage and lytic phage in general. A bit of historical information about T4 is included to illustrate the importance of this phage.

B. Activities

Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer in writing and have them do this without their books or notes.

For example ask your students to:

1. Identify three different aspects between λ's and M13 lifecycle
2. What does M13 need to successfully infect an E. coli cell?
3. What controls the decision by λ to go lytic or lysogenic?
4. How do P1 and λ differ in their lysogenic state?

Teaching Bacterial Genetics at a Higher Level

A. Expandable concepts

1. Phage have a rich history in bacterial genetics. More of the work on T7 (Benzer work), lambda, the T-even phage and Mu could be considered. The use of phages to study DNA replication is a rich topic in phage biology.

2. Phage techniques such as the production of a phage lysate, single-burst experiments or the discovery of Chi sites for recombination could be considered.

3. Practical techniques such as the genetic test for lysogeny could be examined.

4. The lambda immunity region, the different lambdoid phages and other aspects of phage genetics have provided immeasurable amounts of information to biology and molecular biology.

5. T7-phage encoded RNA polymerase and its uses in molecular biology could be included as an example of current uses of phage knowledge.

6. Lysogenic phage and bacterial pathogenesis in Vibrio cholera are a great example of the medical relevance of phage.

B. Activities

Identify research articles addressing concepts in this chapter. Use these articles as described in Activities for Chapter One.

Examples of research articles:

CHAPTER EIGHT – Transduction

This chapter describes generalized and specialized transduction.

A. The main concepts are:

1. A description of generalized transduction and specialized transduction.
2. P1 as an example of a generalized transducing phage.
3. Selection vs screen.
4. The uses for transduction-determining gene linkage and order, constructing strains and localized mutagenesis.
5. Lambda as an example of a specialized transducing phage.
6. Genetic uses of specialized transducing phage-merodiploids and moving mutations from one DNA molecule to another.

B. Activities

Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer in writing and have them do this without their books or notes.

For example ask your students to:

1. What is it about P1’s lifecycle that contributes to the formation of transducing particles?
2. How does generalized and specialized transduction differ?
3. Can a specialized transducing particle be used to move any mutation from one DNA molecule to another? Why or why not?

Teaching Bacterial Genetics at a Higher Level

A. Expandable concepts

1. T4 transduction and the ability to move larger pieces of DNA could be taught.
2. How do you tell if a phage is a generalized transducing phage? Designing experiments to determine this would provide good practical experience to the students.
3. P22 and its infection of *S. typhimurium* provides a great example of many different aspects of phage. P22 is the most robust transducing phage known. P22 biology has similarity to both lambda and P1. Many very sophisticated techniques have been developed using P22.

B. Activities

Identify research articles addressing concepts in this chapter. Use these articles as described in Activities for Chapter One.

Examples of research articles:


**CHAPTER NINE Natural Plasmids**

This chapter is designed to introduce the features of naturally occurring plasmids. It is designed to lead into Chapters 13 and 14 which introduce plasmids as cloning vectors and using cloning vectors in molecular biology.

A. The main concepts are:

1. What features/genes are required for a plasmid?

2. What other features/genes can be found in plasmids?

3. Some concerns of plasmids, including copy number, incompatibility, amplification, host range and how plasmids move from cell to cell. Many of these features are useful in designing cloning vectors for molecular biology.

B. Activities

Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer in writing and have them do this without their books or notes.

For example ask your students to:

1. What is a plasmid copy number? Plasmid incompatibility?
2. What features would you expect to find on a naturally occurring plasmid? Why these features?
3. What other genes can be carried by plasmids?
Teaching Bacterial Genetics at a Higher Level

A. Expandable concepts

1. The ori regions of plasmids frequently have regulatory functions as well as serving as the place DNA replication begins. The functions of the ori region aside from DNA replication could be discussed.

b. How do you determine incompatibility groups?

c. How do you find the ori region of a plasmid? Design experiments for this problem.

d. How do you determine the requirements for plasmid replication (both protein and nucleic acid determinants)?

B. Activities

Identify research articles addressing concepts in this chapter. Use these articles as described in Activities for Chapter One.

Examples of research articles:


CHAPTER TEN – Conjugation

This chapter is designed to introduce the concept of moving plasmids from one cell type to another using the process of conjugation.

A. The main concepts are:

1. To describe F and R factors.

2. To describe how conjugation takes place and some of the molecules involved in the process.

3. To describe F, F’ and Hfr’s.

4. To describe the genetic uses of F, F’ and Hfr’s including gene mapping and merodiploid construction.

B. Activities

Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer in writing and have them do this without their books or notes.
For example ask your students to:

1. Describe the prominent features of an F plasmid
2. Hypothesize as to how an R plasmid might acquire antibiotic resistance
3. Describe how F' plasmids form and what they might be used for
4. Illustrate two ways by which Hfr strains might form

**Teaching Bacterial Genetics at a Higher Level**

A. Expandable concepts

1. How do you determine if a plasmid is self-transmissible?
2. How do you determine if a plasmid is mobilizable?
3. What are triparental matings and why are they so important in many experimental bacterial systems?
4. Other types of transmissible DNA (such as those in *Bacteriodes*) could be taught.

B. Activities

Identify research articles addressing concepts in this chapter. Use these articles as described in Activities for Chapter One.

Examples of research articles:


**CHAPTER ELEVEN  Transformation**

This chapter describes both natural and artificial transformation. It describes several examples of natural transformation and how the processes are regulated. It also discusses artificial transformation as an introduction to cloning.

A. The main concepts are:
1. The lifecycle of *B subtilis*, including its natural competency is described. The regulation of its natural competency process is described to illustrate several types of gene regulation.

2. Several different types of machinery for natural transformation are described.

3. Several different types of artificial competency are described as well as how they are useful in molecular biology.

B. Activities

Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer in writing and have them do this without their books or notes.

For example ask your students to:

1. What does it mean to establish competency?
2. What is needed to establish competency?
3. Can all bacteria be naturally transformed? Why or why not?
4. What regulates the establishment of competency?
5. Describe the enzymes needed for a natural transformation process.

Teaching Bacterial Genetics at a Higher Level

A. Expandable concepts

1. The discovery of transformation could be taught in greater detail.

2. The experiments used to construct and support the models of natural transformation could be discussed. This could include a discussion of how to isolate mutants defective in transformation.

B. Activities

Identify research articles addressing concepts in this chapter. Use these articles as described in Activities for Chapter One.

Examples of research articles:


CHAPTER TWELVE  Gene Regulation
This chapter describes several different types of bacterial gene regulation. It is placed late in the book so that many of the processes and techniques of genetics that are used to study regulation have been described. We have found that students can have difficulty understanding regulation. Placing it later in the course gives them time to adjust to the language and techniques used to describe and investigate regulation.

A. The main concepts are:

1. Transcriptional vs translational vs post-translational regulation.
2. Operons and regulons.
3. Regulation of \textit{lac} by LacI and cAMP/CRP.
4. Attenuation in \textit{trp}.
5. The multiple regulatory events of heat shock.
6. The multiple regulatory events of SOS.
7. Signal transduction

B. Activities

Use short and informal writing exercises at the beginning of each lecture period to enhance concept retention. Give students five minutes to complete their answer in writing and have them do this without their books or notes.

For example ask your students to:

1. Analyze the original data describing the Jacob and Monod model
2. Describe the role of cAMP/CRP in regulating the /lac operon
3. Describe how attenuation works
4. Contrast and compare the regulation of heat shock and SOS

Teaching Bacterial Genetics at a Higher Level

A. Expandable concepts

1. A more in depth discussion of translation and the resulting proteins could be taught.
2. There are a plethora of techniques for identifying regulatory proteins. These include both genetic and biochemical techniques. Any combination of these could be discussed in detail.
3. The antibiotics that block transcription and translation could be discussed.

B. Activities

Identify research articles addressing concepts in this chapter. Use these articles as described in Activities for Chapter One.

Examples of research articles:
CHAPTER THIRTEEN  Plasmids, Bacteriophage and Transposons as Tools

(Please note that activities for Chapters 13, 14 and 15 are grouped together and follow Chapter 15 below.)

This chapter synthesizes the information in Chapters 6, 7 and 9 to show how basic biological phenomenon can be used and exploited for molecular studies of unknown, complex systems.

A. The main concepts are:

1. A description of cloning and what makes a good cloning vector.

2. Examples of different types of cloning vectors and what types of experiments can be performed with them.

3. The adaptation of transposons for use as molecular tools.

Teaching Bacterial Genetics at a Higher Level

1. Many plasmid and phage vectors have been developed for molecular techniques. Any of these would provide a better understanding of what types of information are useful to know about a cloned gene.

2. Several different transposons (Tn5, Tn10, Mu and Himar) have been exploited to make vectors to study aspects of uncharacterized genes and proteins. These could be added to the content of this chapter.

CHAPTER FOURTEEN  DNA Cloning

(Please note that activities for Chapters 13, 14 and 15 are grouped together and follow Chapter 15 below.)
This chapter describes the reactions and enzymes of cloning. It relies on the information provided in the previous 13 chapters. It is meant as an introduction to cloning to give students an idea of what is involved in the process.

A. The main concepts are:

1. How is DNA isolated?

2. How is DNA cut into pieces (restriction enzymes)?

3. What happens at the ends of the DNA molecules during cloning?

4. Visualizing DNA cloning.

5. Making libraries.

6. PCR and its uses.

7. DNA sequencing and what we can learn from analyzing sequences.

Teaching Bacterial Genetics at a Higher Level

1. The techniques for identifying the clones you want (complementation, hybridization, immunological techniques, etc.) could be discussed in detail. These are only briefly mentioned in the text.

2. The uses and advantages of ordered gene libraries such as the Kohara phage could be discussed.

3. Restriction mapping could be expanded upon. This could include practical problems and having the students figure out a restriction map if they are given raw data.

4. Applications of genetic engineering (plants and GMOs, bacteria that degrade toxic compounds, production of drugs, synthetic vaccines) could be discussed.

5. The many and varied practical uses for PCR technology (i.e. forensics) could be taught.

CHAPTER FIFTEEN Bioinformatics and Proteomics

(Please note that activities for Chapters 13, 14 and 15 are grouped together and follow Chapter 15 below.)

This chapter provides an introduction into whole genome analysis. It briefly describes some of the newest techniques of molecular biology that are currently being developed.

A. The main concepts are:

1. What are the goals of bioinformatics?

2. What is the raw material of bioinformatics and how do you get this information?
3. What kind of information can be gained from studying whole genomes?

4. What is proteomics and what are the techniques used to generate information (2-D gels and microarrays)?

Teaching Bacterial Genetics at a Higher Level

1. Actual examples of experiments using bioinformatics could be presented.

2. Computer based problems that use sequenced genomes can be incorporated. These could include comparisons of different genomes, a determination of how many bacterial genomes contain certain types of gene (i.e. an essential gene vs. a nonessential gene).

3. Examples of the uses for microarrays could be discussed. These could include biological problems such as what genes are expressed under a specific stress condition or medical problems such as what genes are expressed in a productive infection by a pathogen.
In addition to traditional tests and informal writing assignments, we use projects, such as the one described below, as a means to engage students in the learning process. These types of activities require that the students synthesize and integrate related concepts and communicate to us by writing what they have learned.

Sample Project

You have been emailed a nucleotide sequence; it is attached as a Word document. Please perform the following procedures using the Web sites given below, and answer the questions. If you need assistance, please arrange to contact us during office hours.

1) Please translate your sequence in all 6 reading frames and **identify** the longest open reading frame. Please print out and attach all six reading frames. What features define a sequence as an open reading frame?


2) Using BLAST, find the 10 most homologous nucleotide sequences and print them out and attach. Print out the most homologous nucleotide sequence alignment. What is the proposed function of your gene? What would be the phenotype of an *E. coli* cell mutant for this gene function (please cite references to support your answers to both questions).

http://blast.genome.ad.jp/

3) Using ClustalW, please align your sequence and the most homologous sequence from a Gram+ organism. If a Gram- organism does not have sequence homologous to yours, please use a Gram- organism other than *E. coli*. Please do this alignment at the:
   A)nucleotide level
   B)amino acid level

From this alignment analysis, determine which features are similar.

http://www.ebi.ac.uk/clustalw/ Enter each sequence with a name in the following format:
   >name of seq1; on the next line, paste in your first sequence.
   New line: >name of seq2; on the next line, paste in your second sequence

4) Please identify any promoters, ribosomal binding sites, and transcriptional termination sequences in your sequence. If your sequence does not appear to have these regulatory sequences, then propose why they are lacking.

5) Please generate a restriction map for your sequence; print out and attach. What restriction sites within your gene would allow you to clone the largest portion of your gene into a cloning vector? Are these restriction enzymes commercially available? From which company? Which cloning vector would you use and why?

http://www.firstmarket.com/cutter/cut2.html

6) Design primers to amplify your sequence from chromosomal DNA. What is the T_m (melting temperature) for your primers? Will this work for PCR? Why?

7) Are there any motifs such as ATP-binding sites, DNA-binding sites, serine active sites, etc. in your sequence? Please highlight and label those sequences in your attachment and tell us what they mean. Does your sequence contain a single gene or is it an operon? How do you know if your sequence has a single gene or an operon?
Other helpful Web Sites
Pedro's Biomolecular Research Tools:
http://www.public.iastate.edu:80/~pedro/research_tools.html

ExPasy http://ca.expasy.org/

GenomeNet http://www.genome.ad.jp/