PROBLEM SET 5 – DNA Repair, Cloning & Molecular techniques
(covering lectures 10 & 11 and QS 6 from week 6)

1. Wild type *Drosophila* fruit flies have red eyes. You have conducted an experiment in which a wild type male *Drosophila* fruit fly has been irradiated with X-rays and mated to a red-eyed female bearing the clB chromosome (which carries a recessive lethal mutation, the Dominant eye marker Bar, and chromosomal rearrangements preventing recombination). One red-eyed daughter with Bar eyes is selected and mated to a wild type male. This cross produces the following offspring in the following ratios:
1/3 clB females
1/3 wild type females
1/3 white-eyed males
Explain this ratio in terms of the genetic effect of X-rays

2. A man employed by a toxic chemical waste disposal service becomes the father of a male child with hemophilia (a disorder caused by recessive mutations on the X-chromosome). There is no history of hemophilia in the man's or his wife's ancestors. Another man, also employed by the same toxic chemical waste disposal service has an achondroplastic dwarf child (caused by an autosomal dominant mutation) - a condition nowhere recoded in his, or in that of his wife's ancestry. Both men sue their employer for damages claiming that the mutations resulting in the conditions of their children were induced by the toxic waste. You are asked to testify in court. What do you say in each case?

3. One of the jobs of the Hiroshima-Nagasaki Atomic Bomb Casualty Commission was to assess the genetic consequences of the bomb blasts. One of the first things they studied was the sex ratio in the offspring of the survivors. Why do you think they did so?

4. Imagine that the first deoxyadenosine methyltransferase mutant (*dam*$^-$) of *E. coli* has been discovered through a screen in which colonies from mutagen-treated cells were plated on petri dishes, and many individual colonies were assayed biochemically for deoxyadenosine methyltransferase activity using DNA as a substrate. Nothing else is known about the mutant. You wish to determine if the *dam*$^-$ mutation results in an increased mutation rate (i.e., results in an increase in the spontaneous frequency of mutation in other genes). Describe how you could test for such a "mutator" phenotype. Assume, if it would be helpful, that you can easily introduce mutations in other genes into the *dam*$^-$ strain.

5. *E. coli* strains bearing a mutation of the deoxyadenosine methylase gene (*dam*$^-$) show elevated mutation rates relative to wild type cells, owing to a defect in mismatch repair (for reasons discussed in class). However, the production of very high levels of this enzyme in *E. coli* cells also results in increased mutation rates relative to wild type *E. coli* strains. From your knowledge of the normal role that this enzyme plays in mismatch repair processes, propose an explanation for this finding.

6. The bacterial species *E. coli*, *B. thuringiensis* and *P. furiosus* can be infected by several different lytic bacterial viruses, including λ virus. When any of these bacterial species are infected with λ virus, the virus makes many new copies of itself inside the bacterial cell and ultimately lyses the bacterial cell. You have also identified a strain of λ virus that bears a single missense mutation relative to wild type λ virus which renders the mutant unable to lyse *B. thuringiensis* or *P. furiosus*. However, this mutant can infect *E. coli* cells. You now wish to explore the mechanism of mismatch repair in *B. thuringiensis* and *P. furiosus.* To perform this analysis you prepare λ virus DNA from both your wild type and mutant strains by growing them on wild type *E. coli* cells and on a deoxyadenosine methylase mutant (*dam*$^-$) strain of *E. coli* (which is unable to methylate DNA). After preparing the viral

DNA, you heat the DNA at high temperature to denature it, and then make several hybrid DNA molecules (DNA heteroduplexes) by mixing various combinations of the mutant and WT viral DNA prepared from the WT and *dam-* mutant strains of *E. coli*. These hybrid viral DNA molecules are then mixed with viral proteins to make infectious viral strains and used to infect *B. thuringiensis* and *P. furiosus* with the results shown below:

DNA heteroduplex	Ability to lyse *P. furiosus*	Ability to lyse *B. thuringiensis*
――――――― ▬ ▬ ▬	-	+
▬▬▬▬▬▬ - - - - - - - -	++	+

methylated WT λ DNA: ▬▬▬▬▬▬
unmethylated WT λ DNA: ―――――――
methylated mutant λ DNA: ▬ ▬ ▬
unmethylated mutant λ DNA: - - - - - - - -

++ = complete lysis (all infected cells lysed)
\+ = partial lysis (~1/2 of infected cells lysed)
\- = no lysis

Explain what these results indicate in terms of the mechanism of strand discrimination during mismatch repair in *B. thuringiensis* and *P. furiosus*.

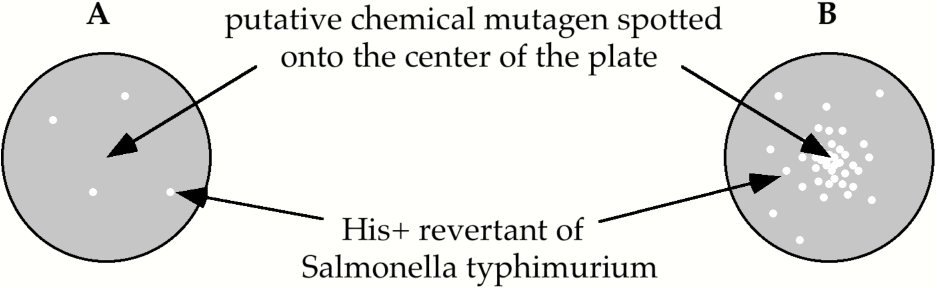
7. Hereditary non-polyposis colon cancer (HNPCC) is a hereditary colon cancer in humans. Tumors in individuals with HNPCC have a high level of DNA instability (where DNA synthesis has an unusually high error rate). At the time the first HNPCC gene was discovered, very little was known about which genes were responsible for DNA damage repair in humans, but many of the analogous E. coli genes were known. One class of E. coli genes involved in DNA repair was the mut family of genes. A group of researchers hypothesized that human homologs of the E. coli mut genes may be responsible for HNPCC.

7a. What is the normal function of the mut genes in E. coli? Given the tumor phenotype of HNPCC, why was the mut gene family a good place to start looking?

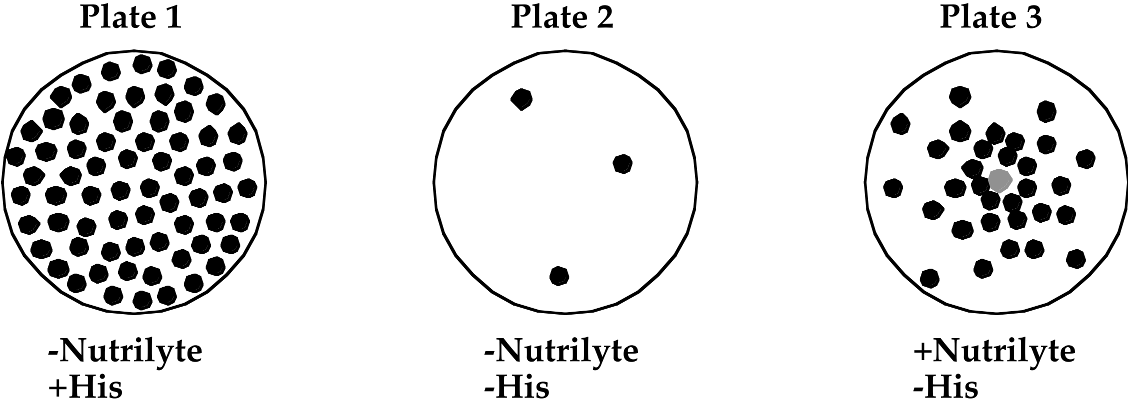
7b. Using some of the molecular techniques you have learned about, how could you identify the human homologs of the yeast mut genes? Keep in mind that this work was done prior to the complete sequencing of the human genome, so doing a simple DNA sequence search online was not an option.

8. The Ames test is a simple low cost method for detecting chemical mutagens. In its original form this test was carried out by uniformly plating a his$^-$ strain of the bacterium Salmonella typhimurium (bearing a mutation in the gene encoding an enzyme involved in synthesis of the amino acid histidine) onto agar plates lacking histidine, and then spotting a drop of the chemical to be tested onto the center of the plate. If the chemical is not a mutagen, typically only a few colonies will arise on the plate, and the locations of these colonies will be random with respect to the location of the chemical spotted onto the plate (figure A below). These colonies correspond to rare His$^+$ reversion mutations which restore function to the histidine biosynthetic gene. If, on the other hand, the chemical being tested is a mutagen, a great many more colonies will arise (due to the increased chance of reverting the mutation reponsible for the his$^-$ phenotype), and these colonies will be clustered in the vicinity of the chemical under investigation (because the concentration of the chemical will be highest where it is spotted onto the plate; figure B below). Although for many chemicals there was good correspondence

between the results of the Ames test and the known ability of the chemical to induce mutations in higher eukaryotes (i.e., mice, humans, etc.), there were also many chemicals that did not act as mutagens in the Ames test, but were well known to act as mutagens in higher eukaryotes. Can you propose an explanation for this observation?



9. You work for a company that specializes in healthy frozen lunches called Lean Yummy Tummy. The legal department has been negotiating with an herbalist who claims that he has discovered an "all natural" compound Nutrilyte which helps digestion. To investigate the safety of this compound your supervisor asks you to assess Nutrilyte's mutagenicity using the Ames Test using a *Salmonella* strain bearing a missense mutation reducing its ability to produce histidine. Results of this analysis are shown below.



9a. What can you conclude about the mutagenicity of Nutrilyte?

9b. Name at least one other test you could carry out to verify your findings.

9c. You decide to pick several colonies from plate 3 and grow them in liquid media lacking histidine. None of the colonies is able to grow under these conditions. However, when you add Nutrilyte to the growth media, all of the colonies are capable of growth. Does this result affect your conclusions regarding the mutagenicity of Nutrilyte? Briefly explain why.

10. A single-stranded DNA has the following sequence:

5'ATAGATTGCTATTCG3'

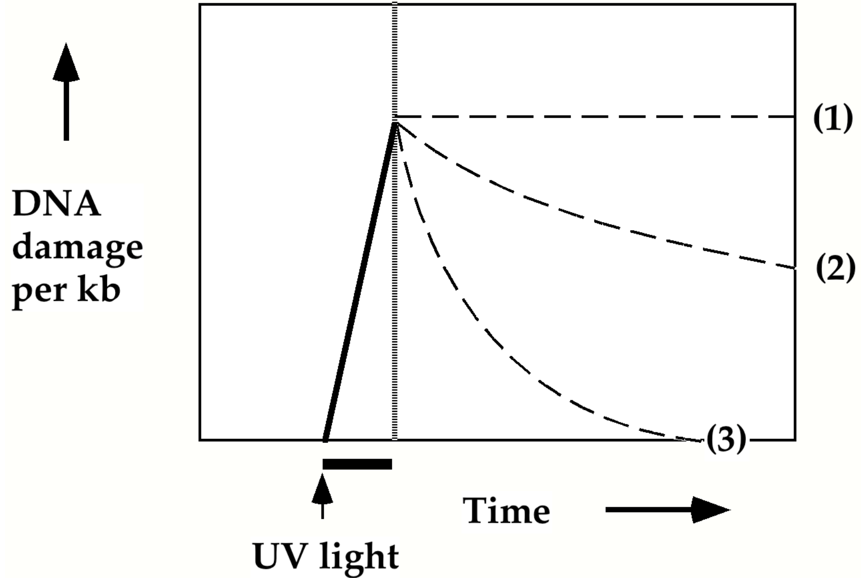
10a. How many different mutagenic lesions of this DNA molecule are possible after exposure to UV light?

10b. How would your answer change if this sequence was exposed to X-rays instead of UV light (increase or decrease)?

10c. Circle all of the genes/enzymes in the list below that function in the repair of pyrimidine dimers:

uvrA	DNA polymerase	uvrC	photolyase	mutL
uvrB	DNA ligase	mutS	mutH	EcoRI
uvrD	deoxyadenosine methylase			

11. The following graph illustrates the time course of DNA repair in *E. coli* following UV-light induced DNA damage. Each curve on the graph represents the result of a particular experiment carried out using either wild type or UV-sensitive *E. coli* mutants.



Given your knowledge of UV-induced DNA damage repair in *E. coli*, choose the curve (1, 2, or 3) from the above graph that most closely resembles the outcome you would expect from the *E. coli* cell types and experimental conditions described in the table below (you can use the same curve multiple times):

E. coli Cell Type	Experimental Conditions	Corresponding Curve (1, 2, or 3)
wild type	in blue (300-500nm) light	
wild type	in dark	
uvrA mutant	in dark	
phr mutant	in blue (300-500nm) light	

12. An ampicillin-resistant, tetracycline-resistant plasmid is cleaved with Eco RI, which cuts within the ampicillin gene. The cut plasmid is ligated with Eco RI digested *Drosophila* DNA to prepare a genomic library. The mixture is used to transform *E. coli*.

12a. Which antibiotic should be added to the medium to select cells that have incorporated a plasmid?

12b. What antibiotic-resistance pattern should be selected to obtain plasmids containing *Drosophila* inserts?

12c. How can you explain the presence of colonies that are resistant to both antibiotics?

13. You are working on tryptophan metabolism in E. coli and wish to conduct an experiment with the TrpE gene (which is required for growth on plates lacking tryptophan). Although this gene was cloned by complementation years ago, and could be obtained by asking any one of a number of laboratories to send it to you, you happen to have a trpE mutant strain (which fails to grow on plates lacking tryptophan) in your lab and decide to simply clone the gene using the same complementation cloning approach. To do this, you prepare a library from Bam HI digested *E. coli* genomic DNA. Although the original cloning of TrpE made use of a library composed of Bam HI partial digest products, you don't see any reason for this and proceed to make your library from complete Bam HI digest products. This library is then used to transform trpE mutant cells, and the desired colonies are selected by plating on media lacking tryptophan. Surprisingly, you find that none of the transformants are able to grow on plates lacking tryptophan. How can you account for these conflicting results?

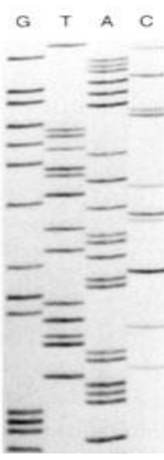
14. Wild type E. coli cells can grow in the absence of valine, or in the presence of low levels of valine, but NOT in the presence of high levels of this amino acid (presumably because high intracellular levels of valine are toxic). Genetic studies of valine metabolism in E. coli have revealed two sorts of conditional mutant. One type of mutant (mutant A) can grow in the presence of low levels of valine, but not in its absence. The other type of mutant (mutant B) can grow in the presence of both low and high levels of valine. Type B mutants can also grow in the absence of this amino acid. You wish to clone the gene corresponding to these two mutant categories and hypothesize that class A mutants are defective in valine synthesis (the mutations are recessive to wild type), and that the B class mutants are defective in a pump responsible for importing valine from the media. However, after thinking about this, you realize that the mutations responsible for the B class could be of two sorts, which you call B1 and B2. The B1 sort might simply be defective in pumping valine (recessive to wild type), whereas the B2 sort might reverse the flow of the valine pump (pumping it out of the cell; this sort of mutation might well be dominant to wild type). With this information in mind, please answer the following questions:

14a. How do you suppose the genetic screens were carried out to identify the mutants falling into the A and B categories?

14b. How would clone the gene corresponding to mutant category A?

14c. How would you clone the gene corresponding to mutant category B (be sure to address both possible explanations for this mutant type; assume that the B2 mutants ARE dominant to wild type)?

15. You used the Sanger method to sequence your favorite gene. You do not have enough money to buy an automated sequencer, so you run your sequencing products out on a gel (pictured below). What is the sequence of your gene? Denote the 5' and 3' ends.



PROBLEM SET 5 – DNA Repair, Cloning & Molecular techniques
(covering lectures 10 & 11 and QS 6 from week 6)

Answers posted by Craig Bierle. Attend office hours for explanations/questions.

1. Wild type *Drosophila* fruit flies have red eyes. You have conducted an experiment in which a wild type male *Drosophila* fruit fly has been irradiated with X-rays and mated to a red-eyed female bearing the clB chromosome (which carries a recessive lethal mutation, the Dominant eye marker Bar, and chromosomal rearrangements preventing recombination). One red-eyed daughter with Bar eyes is selected and mated to a wild type male. This cross produces the following offspring in the following ratios:
1/3 clB females
1/3 wild type females
1/3 white-eyed males
Explain this ratio in terms of the genetic effect of X-rays

The X-rays must have produced an X-linked recessive mutation resulting in white eyes in one of the sperm cells of the irradiated male.

2. A man employed by a toxic chemical waste disposal service becomes the father of a male child with hemophilia (a disorder caused by recessive mutations on the X-chromosome). There is no history of hemophilia in the man's or his wife's ancestors. Another man, also employed by the same toxic chemical waste disposal service has an achondroplastic dwarf child (caused by an autosomal dominant mutation) - a condition nowhere recoded in his, or in that of his wife's ancestry. Both men sue their employer for damages claiming that the mutations resulting in the conditions of their children were induced by the toxic waste. You are asked to testify in court. What do you say in each case?

Hemophilia is X linked—germ line mutations the first worker's X chromosome would never be observed in a male child. In the second case, since neither parent is affected, an autosomal dominant mutation may have arisen in the germ line of either parent. The case would be supported if the waste was a known carcinogen.

3. One of the jobs of the Hiroshima-Nagasaki Atomic Bomb Casualty Commission was to assess the genetic consequences of the bomb blasts. One of the first things they studied was the sex ratio in the offspring of the survivors. Why do you think they did so?

Exposure to a mutagen would increase the rate of recessive lethal mutations on all chromosomes. Because males are hemizygous X, these mutations would result in fewer males than females. The fewer males, the more severe the exposure.

4. Imagine that the first deoxyadenosine methyltransferase mutant (*dam*$^-$) of *E. coli* has been discovered through a screen in which colonies from mutagen-treated cells were plated on petri dishes, and many individual colonies were assayed biochemically for deoxyadenosine methyltransferase activity using DNA as a substrate. Nothing else is known about the mutant. You wish to determine if the *dam*$^-$ mutation results in an increased mutation rate (i.e., results in an increase in the spontaneous frequency of mutation in other genes). Describe how you could test for such a "mutator" phenotype. Assume, if it would be helpful, that you can easily introduce mutations in other genes into the *dam*$^-$ strain.

One possibility is to observe the frequency of mutation in a second gene that can be selected for. For example, introduce a point mutation into both *dam*⁻ and wt E. coli in a gene required for leucine biosynthesis (*leu*-). Mutagenize both, plate equal cell numbers on media lacking leucine, and count the number of colonies. Then calculate # revertants/cells mutagenized.

5. *E. coli* strains bearing a mutation of the deoxyadenosine methylase gene (*dam*⁻) show elevated mutation rates relative to wild type cells, owing to a defect in mismatch repair (for reasons discussed in class). However, the production of very high levels of this enzyme in *E. coli* cells also results in increased mutation rates relative to wild type *E. coli* strains. From your knowledge of the normal role that this enzyme plays in mismatch repair processes, propose an explanation for this finding.

Increased methylating activity could decrease the time during which the newly polymerized DNA is hemimethylated. The mismatch repair enzymes would then have less of an opportunity to find a hemimethylated strand of DNA and fewer mismatches may be repaired.

6. The bacterial species *E. coli*, *B. thuringiensis* and *P. furiosus* can be infected by several different lytic bacterial viruses, including λ virus. When any of these bacterial species are infected with λ virus, the virus makes many new copies of itself inside the bacterial cell and ultimately lyses the bacterial cell. You have also identified a strain of λ virus that bears a single missense mutation relative to wild type λ virus which renders the mutant unable to lyse *B. thuringiensis* or *P. furiosus*. However, this mutant can infect *E. coli* cells. You now wish to explore the mechanism of mismatch repair in *B. thuringiensis* and *P. furiosus.* To perform this analysis you prepare λ virus DNA from both your wild type and mutant strains by growing them on wild type *E. coli* cells and on a deoxyadenosine methylase mutant (*dam*⁻) strain of *E. coli* (which is unable to methylate DNA). After preparing the viral DNA, you heat the DNA at high temperature to denature it, and then make several hybrid DNA molecules (DNA heteroduplexes) by mixing various combinations of the mutant and WT viral DNA prepared from the WT and *dam*- mutant strains of *E. coli*. These hybrid viral DNA molecules are then mixed with viral proteins to make infectious viral strains and used to infect *B. thuringiensis* and *P. furiosus* with the results shown below:

DNA heteroduplex	Ability to lyse *P. furiosus*	Ability to lyse *B. thuringiensis*
———— / ▬ ▬ ▬	-	+
▬▬▬▬ / - - - - - - - -	++	+

methylated WT λ DNA: ▬▬▬▬

unmethylated WT λ DNA: ————

methylated mutant λ DNA: ▬ ▬ ▬

unmethylated mutant λ DNA: - - - - - - - -

++ = complete lysis (all infected cells lysed)

+ = partial lysis (~1/2 of infected cells lysed)

- = no lysis

Explain what these results indicate in terms of the mechanism of strand discrimination during mismatch repair in *B. thuringiensis* and *P. furiosus*.

P. fusiosus-DNA repair system is similar to that of E. coli, repairing based off of the methylated strand. *B. thuringiensis*-mismatch repair does not appear to be using methylation as a signal, so the bacteria must not use the same mechanism for strand determination. This causes the strand used to template repair to be random.

7. Hereditary non-polyposis colon cancer (HNPCC) is a hereditary colon cancer in humans. Tumors in individuals with HNPCC have a high level of DNA instability (where DNA synthesis has an unusually high error rate). At the time the first HNPCC gene was discovered, very little was known about which genes were responsible for DNA damage repair in humans, but many of the analogous E. coli genes were known. One class of E. coli genes involved in DNA repair was the mut family of genes. A group of researchers hypothesized that human homologs of the E. coli mut genes may be responsible for HNPCC.

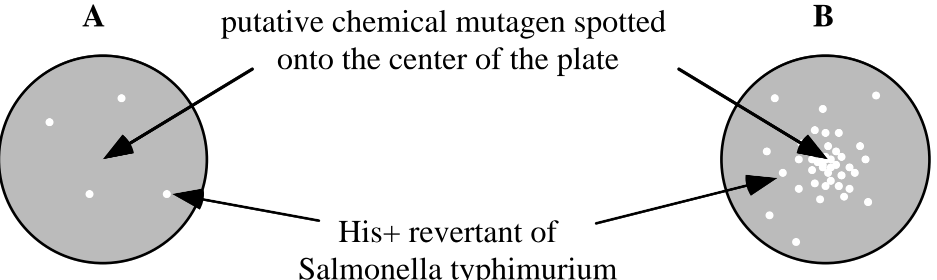
7a. What is the normal function of the mut genes in E. coli? Given the tumor phenotype of HNPCC, why was the mut gene family a good place to start looking?

E. coli with a mut phenotype have an increased mutation rate—the genes are often related in DNA damage repair. Tumors are the result of somatic mutations—hereditary defects in damage repair are a strong candidate for causing the accumulation of tumor causing mutations.

7b. Using some of the molecular techniques you have learned about, how could you identify the human homologs of the yeast mut genes? Keep in mind that this work was done prior to the complete sequencing of the human genome, so doing a simple DNA sequence search online was not an option.

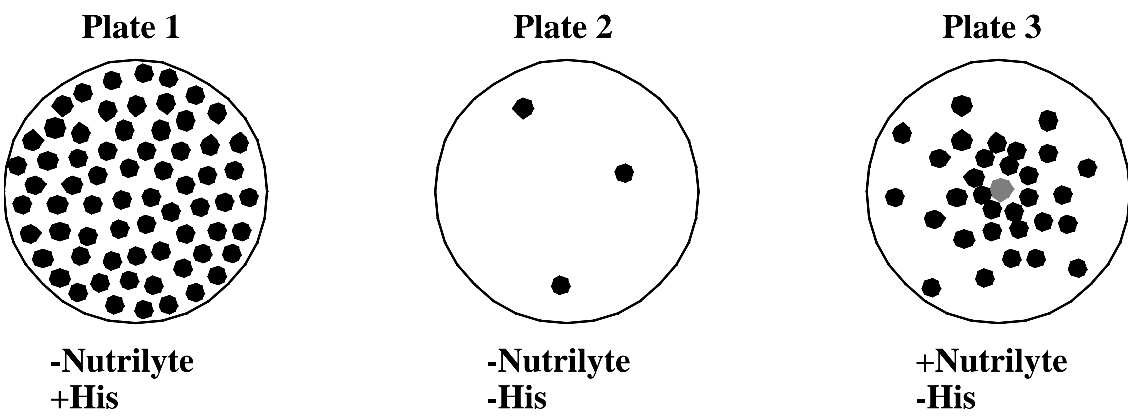
One example-generate a library of large fragments of the human genome. Introduce these fragments into mut bacteria and see which fragments cause a decrease in mutator phenotype of the cells.

8. The Ames test is a simple low cost method for detecting chemical mutagens. In its original form this test was carried out by uniformly plating a his$^-$ strain of the bacterium Salmonella typhimurium (bearing a mutation in the gene encoding an enzyme involved in synthesis of the amino acid histidine) onto agar plates lacking histidine, and then spotting a drop of the chemical to be tested onto the center of the plate. If the chemical is not a mutagen, typically only a few colonies will arise on the plate, and the locations of these colonies will be random with respect to the location of the chemical spotted onto the plate (figure A below). These colonies correspond to rare His$^+$ reversion mutations which restore function to the histidine biosynthetic gene. If, on the other hand, the chemical being tested is a mutagen, a great many more colonies will arise (due to the increased chance of reverting the mutation reponsible for the his$^-$ phenotype), and these colonies will be clustered in the vicinity of the chemical under investigation (because the concentration of the chemical will be highest where it is spotted onto the plate; figure B below). Although for many chemicals there was good correspondence between the results of the Ames test and the known ability of the chemical to induce mutations in higher eukaryotes (i.e., mice, humans, etc.), there were also many chemicals that did not act as mutagens in the Ames test, but were well known to act as mutagens in higher eukaryotes. Can you propose an explanation for this observation?



Many chemicals that are not directly mutagenic are metabolized to strongly mutagenic derivatives in eukaryotic cells. By incubating the chemical with Eukaryotic cell extract before plating, the mutagenic compound that is a byproduct of Eukaryotic metabolism will be present and its effect on the bacteria can be quantified.

9. You work for a company that specializes in healthy frozen lunches called Lean Yummy Tummy. The legal department has been negotiating with an herbalist who claims that he has discovered an "all natural" compound Nutrilyte which helps digestion. To investigate the safety of this compound your supervisor asks you to assess Nutrilyte's mutagenicity using the Ames Test using a *Salmonella* strain bearing a missense mutation reducing its ability to produce histidine. Results of this analysis are shown below.



9a. What can you conclude about the mutagenicity of Nutrilyte?

Based on the data above, one concludes that Nutrilyte is mutagenic.

9b. Name at least one other test you could carry out to verify your findings.

A number of possibilities exist—test the capacity of Nutrilyte to mutagenize mammalian cells by feeding them and screening for chromosomal damage or conversion into cells able to produce tumors when introduced into an animal. You could also feed to flies and screen for the prevalence of recessive lethal mutations on the X chromosome. You could also repeat the Ames test with other bacteria containing mutations in other metabolic or antibiotic resistance genes.

9c. You decide to pick several colonies from plate 3 and grow them in liquid media lacking histidine. None of the colonies is able to grow under these conditions. However, when you add Nutrilyte to the

growth media, all of the colonies are capable of growth. Does this result affect your conclusions regarding the mutagenicity of Nutrilyte? Briefly explain why.

The colonies inability to grow without Nutrilyte indicates that the bacteria have not mutated to obtain a revertant phenotype but rather receive something (possibly his) from the compound that allows their growth.

10. A single-stranded DNA has the following sequence:

5'ATAGATTGCTATTCG3'

10a. How many different mutagenic lesions of this DNA molecule are possible after exposure to UV light?

UV forms dimers between adjacent pyrimidines. There are four potential dimers in this strand.

10b. How would your answer change if this sequence was exposed to X-rays instead of UV light (increase or decrease)?

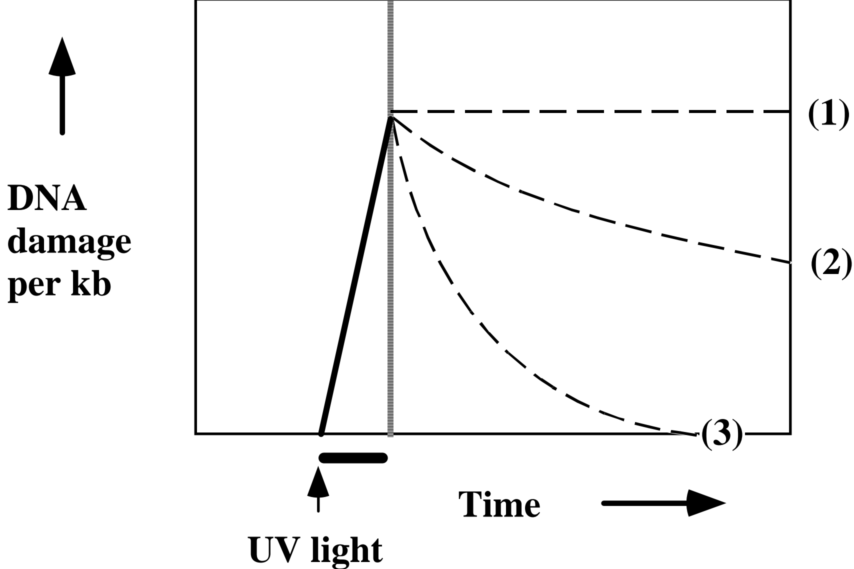
X rays cause breakage in the phosphodiester backbone of the DNA molecule and could result in many more possible changes.

10c. Circle all of the genes/enzymes in the list below that function in the repair of pyrimidine dimers:

uvrA	DNA polymerase	uvrC	photolyase	mutL
uvrB	DNA ligase	mutS	mutH	EcoRI
uvrD	deoxyadenosine methylase			

Both light dependant and light independent mechanisms may be used: uvrA; uvrB; uvrC; uvrD; photolyase; DNA polymerase; DNA ligase

11. The following graph illustrates the time course of DNA repair in *E. coli* following UV-light induced DNA damage. Each curve on the graph represents the result of a particular experiment carried out using either wild type or UV-sensitive *E. coli* mutants.



Given your knowledge of UV-induced DNA damage repair in *E. coli*, choose the curve (1, 2, or 3) from the above graph that most closely resembles the outcome you would expect from the *E. coli* cell types and experimental conditions described in the table below (you can use the same curve multiple times):

E. coli Cell Type	Experimental Conditions	Corresponding Curve (1, 2, or 3)
wild type	in blue (300-500nm) light	
wild type	in dark	
uvrA mutant	in dark	
phr mutant	in blue (300-500nm) light	

a. 3, b. 2, c. 1, d. 2

12. An ampicillin-resistant, tetracycline-resistant plasmid is cleaved with Eco RI, which cuts within the ampicillin gene. The cut plasmid is ligated with Eco RI digested *Drosophila* DNA to prepare a genomic library. The mixture is used to transform *E. coli*.

12a. Which antibiotic should be added to the medium to select cells that have incorporated a plasmid?

tetracycline

12b. What antibiotic-resistance pattern should be selected to obtain plasmids containing *Drosophila* inserts?

tetracycline resistant, ampicillin-sensitive

12c. How can you explain the presence of colonies that are resistant to both antibiotics?

The original plasmid failed to incorporate a fragment of the drosophila genome and ligated back shut.

13. You are working on tryptophan metabolism in E. coli and wish to conduct an experiment with the TrpE gene (which is required for growth on plates lacking tryptophan). Although this gene was cloned by complementation years ago, and could be obtained by asking any one of a number of laboratories to send it to you, you happen to have a trpE mutant strain (which fails to grow on plates lacking tryptophan) in your lab and decide to simply clone the gene using the same complementation cloning approach. To do this, you prepare a library from Bam HI digested *E. coli* genomic DNA. Although the original cloning of TrpE made use of a library composed of Bam HI partial digest products, you don't see any reason for this and proceed to make your library from complete Bam HI digest products. This library is then used to transform trpE mutant cells, and the desired colonies are selected by plating on media lacking tryptophan. Surprisingly, you find that none of the transformants are able to grow on plates lacking tryptophan. How can you account for these conflicting results?

The original digest was partial—your original clone happens to have a Bam HI site inside of the TrpE coding sequence that you disrupt when you are attempting to clone into a new plasmid by doing a complete digest of the plasmid.

14. Wild type E. coli cells can grow in the absence of valine, or in the presence of low levels of valine, but NOT in the presence of high levels of this amino acid (presumably because high intracellular levels of valine are toxic). Genetic studies of valine metabolism in E. coli have revealed two sorts of conditional mutant. One type of mutant (mutant A) can grow in the presence of low levels of valine, but not in its absence. The other type of mutant (mutant B) can grow in the presence of both low and high levels of valine. Type B mutants can also grow in the absence of this amino acid. You wish to clone the gene corresponding to these two mutant categories and hypothesize that class A mutants are defective in valine synthesis (the mutations are recessive to wild type), and that the B class mutants are defective in a pump responsible for importing valine from the media. However, after thinking about this, you realize that the mutations responsible for the B class could be of two sorts, which you call B1 and B2. The B1 sort might simply be defective in pumping valine (recessive to wild type), whereas the B2 sort might reverse the flow of the valine pump (pumping it out of the cell; this sort of mutation might well be dominant to wild type). With this information in mind, please answer the following questions:

14a. How do you suppose the genetic screens were carried out to identify the mutants falling into the A and B categories?

To find type B mutants-mutagenize and plate with high levels of valine. To find type A mutants, grow in the presence of low levels of valine, replica plate, and screen for colonies that cannot grow in the absence of valine.

14b. How would clone the gene corresponding to mutant category A?

Begin with a type A mutant and transform it with a library constructed from the *E. coli* genome. Screen for colonies that are capable of growth in the absence of valine.

14c. How would you clone the gene corresponding to mutant category B (be sure to address both possible explanations for this mutant type; assume that the B2 mutants ARE dominant to wild type)?

B1 class mutants are recessive to wild type and you could do a similar complementation cloning screen as in b. Transform in a wild-type E. coli library, selecting for transformants using a resistance gene that is also found on the plasmid. Then grow on plates lacking or with low levels of lysine, replicate plating onto plates with high levels of valine. Look for colonies that fail to grow when in high concentrations—these contain your mutants of interest.

Since B2 is dominant to wild type, a similar yet slightly different approach could be used. Generate a library from B2 mutants and introduce into wild type cells. Grow these on plates with high valine concentrations—only those with the B2 fragment present will be able to grow.

15. You used the Sanger method to sequence your favorite gene. You do not have enough money to buy an automated sequencer, so you run your sequencing products out on a gel (pictured below). What is the sequence of your gene? Denote the 5' and 3' ends.

5' GAGGGAAATCAATTCTGTGAACGATAATCCAGTCATTGATGTTGCCAGAGACAAAGCT 3'