Question 1

You are studying the mechanism of DNA polymerase loading at eukaryotic DNA replication origins. Unlike the situation in bacteria, the three different eukaryotic DNA polymerases are not tightly associated with one another and you are interested in how they are all brought to the origin to assemble the replication fork.

Your ability to address this question is greatly facilitated by the recent development of a completely purified set of proteins that will initiate DNA replication from an *S. cerevisiae* (Baker's yeast) origin of replication.

Your first goal is to determine the order of assembly of the 3 DNA polymerases during initiation.

(A) Describe an assay you would use to monitor the assembly of the DNA polymerases at the origin. You have access to all the purified proteins required for replication initiation including the three purified DNA polymerases in labeled and unlabeled forms.

A gel filtration assay can be used to measure the assembly of DNA polymerases at the origin. This assay would require an origin containing plasmid DNA and 32P-labeled DNA polymerases, which are incubated with the DNA to allow loading. To measure assembled polymerases, one must separate bound (to DNA) polymerases from unbound (free) by using gel filtration (bound polymerases will elute first with DNA, whereas free will elute later in the fractions). In order to determine whether protein is DNA associated or free, the amount of radioactivity present in the fractions is determined by using a scintillation counter, which will indicate the presence of the labeled polymerase. By performing this analysis with all combinations of polymerases alone and with other polymerases, each time labeling the polymerase one is assaying with 32P, one can determine which polymerase(s) can load by themselves, which can load in the presence of other polymerases, as well as the relative order of loading.

Using your assay, you determine what effect leaving out one polymerase has on the assembly of the other two DNA polymerases at the origin. You obtain the following results:

DNA Polymerases Added	DNA Polymerases Assembled
Pol α/Primase, Pol ε	**Pol ε**
Pol α/Primase, Pol δ	**Pol δ**
Pol δ, Pol ε	**Pol δ, Pol ε**
Pol α/Primase, Pol δ, Pol ε	**Pol α/Primase, Pol δ, Pol ε**

(B) What can you conclude about the order of loading of the three DNA polymerases based on these data? Briefly explain the logic behind your conclusions.

In order for the Polα/Primase to load, both Pol ε and Pol δ must be already loaded. Pol ε and Pol δ can load independently of one another and Pol α/Primase. Pol α/Primase only loads in the last experiment when both Pol ε and Pol δ are present, and cannot load in the first two lanes when only one or the other is present.

The simplest hypothesis is that Pol ε and Pol δ both can load by themselves as seen in the first three lanes. The relative order of Pol ε and Pol δ loading cannot be determined from the data present. The data suggests that Pol ε and Pol δ can load by themselves, although it is possible that the loading of Pol ε and Pol δ requires either Pol α/Primase or the other Pol.

You find that if you add 300 mM NaCl to your binding assays you obtain a different set of results.

DNA Polymerases Added	DNA Polymerases Assembled	
	50 mM NaCl	300 mM NaCl
Pol α/Primase, Pol ε	Pol ε	none
Pol α/Primase, Pol δ	Pol δ	none
Pol δ, Pol ε	Pol δ, Pol ε	none
Pol α/Primase, Pol δ, Pol ε	Pol α/Prim, Pol δ, Pol ε	Pol α/Prim, Pol δ, Pol ε

(C) Propose a model that explains the effect of NaCl on your assay.

Only the most stable complex can load onto DNA in the presence of 300 mM salt. The combination of interactions that occur when Pol α/Primase, Pol δ and Pol ε are all present makes the complex more stable than the complexes formed with a subset of the polymerases present. By interacting with proteins and the DNA, NaCl can compete for charge interactions between the proteins and DNA and between the different proteins (that is why salt gradients are used in ion exchange columns, etc). Therefore, only a very stable complex stays associated with the DNA in high salt.

You want to determine whether DNA synthesis activity is required to assemble the DNA polymerases onto the DNA. To address this question, you take advantage of the fact that your assay monitors association with the DNA template and not DNA synthesis.

You perform your assays again using mutant DNA polymerases that have a single point mutation that prevents DNA synthesis by altering binding to a catalytic Mg^{+2} but does not alter the binding to substrate. At the same time you also produce a Pol α/Primase that has a similar point mutation in its Primase subunit.

You assay these mutant proteins in your template association assay and get the following results. Mutant subunits are indicated in *italics* and with an asterisk (*).

DNA Polymerases Added	**DNA Polymerases Assembled**	
	50 mM NaCl	**300 mM NaCl**
Pol α/Primase, *Pol* δ*, Pol ε	**Pol α/Prim, *Pol* δ*, Pol ε**	**Pol α/Prim, *Pol* δ*, Pol ε**
Pol α/Primase, Pol δ, *Pol* ε*	**Pol α/Prim, Pol δ, *Pol* ε***	**Pol α/Prim, Pol δ, *Pol* ε***
***Pol* α*/Primase, Pol δ, Pol ε**	***Pol* α*/Prim, Pol δ, Pol ε**	***Pol* α*/Prim, Pol δ, Pol ε**
Pol α/*Primase, Pol δ, Pol ε**	**Pol α/*Prim**, Pol δ, Pol ε**	**none**

(D) How can you explain the requirement of primase activity for DNA polymerase δ and ε association with the origin at high salt? Why do you see a different result with the Pol α mutant?

Formation of the salt-resistant, stable complex of Pol α/Primase, Pol δ, and Pol ε is dependent on a primer/template junction. A mutation in the Primase subunit eliminates its ability to make the RNA primer, therefore there is no primer/template junction and the complex is no longer stable in high salt.

(E) Based on your hypothesis and your knowledge of DNA polymerases, what other factors would you expect to be **specifically required** for the primase-dependent, salt-resistant binding of the DNA polymerases δ and ε but not for the low-salt binding? Explain your choice.

The eukaryotic sliding clamp, PCNA; the eukaryotic homolog of SSB, RPA (replication factor A); or RFC (replication factor C), the eukaryotic clamp loader for PCNA. Each of these factors would be expected to facilitate the association of DNA Pol ε and Pol δ with the primed template. Because the primed template is important for the stable complex, it is likely that other proteins required for the DNA Pol δ and ε association with primed templates would facilitate the formation of the stable complex.

Question 2

You have discovered a new single-cell, eukaryotic organism (which you decide to name *Redsoxus rulius*) and you want to figure out what proteins are involved in DNA replication. You decide to do a screen for temperature sensitive replication mutants (you're really looking forward to that month off in the Bahamas) so you mutagenize your *Redsoxus rulius*, grow at the non-permissive temperature with ^{3}H-thymidine, wash away the unincorporated ^{3}H-thymidine, put the cells in the freezer for a month, and then take them out and plate the cells at the permissive temperature.

(A) When looking for DNA replication mutants, why do you look for temperature sensitive mutants rather than gene deletions?

If you delete an essential gene, the organism is dead and while this is an interesting piece of information, all it tells you is that gene is essential. It doesn't tell you what the gene (or resultant protein) is involved in. It is useful to create a conditional mutant of an essential gene so that you can do experiments with that mutant, controlling when the protein is inactive and when it's not.

You pick a mutant that grows to analyze further (you name your mutant *Schilling1,* abbreviated *Sch1*). First, you want to test for DNA replication at the non-permissive temperature.

(B) You incubate your *SchI* mutant cells at the non-permissive temperature for two generations and then test for DNA synthesis. What type of assay would you use? Why?

A filter binding assay is a quick, quantitative assay for nucleotide incorporation. It doesn't give you any information about the length of synthesis, but in this case you don't need to know that information, you just want to know whether nucleotide incorporation is occurring or not.

(C) You find that no DNA synthesis occurs in your assay at the non-permissive temperature. You want to ask next whether your mutant is affecting the initiation or elongation steps of DNA replication. Your lab mate has developed a method to arrest this organism at two points in the cell cycle: in G1-phase and in the middle of S-phase.

How could you take advantage of the ability to arrest your *Sch1* cells at these two stages of the cell cycle to determine what stage of replication your mutant is required for? Describe the results you would get if your mutant were defective in the initiation of DNA replication.

To test whether your mutant is involved in initiation or replication, you would arrest the cells in either G1 or mid-S phase at the permissive temperature, then during the arrest, shift to the non-permissive temperature and release the cells at the non-permissive temperature. If your mutant is in the initiation phase of DNA replication, then shifting your cells to the non-permissive temperature in G1 and releasing the cells, should end in no DNA replication occurring, whereas arresting and releasing cells (at the non-permissive temp) in the middle of DNA replication should not affect the ability of the cells to replicate (and DNA replication should continue). If your mutant protein is involved in DNA replication, then no DNA replication should occur after you arrest and release in mid S-phase.

(D) Your results indicate that your mutation is only involved in the initiation of replication. Your next goal is to use the *Sch1* mutant cells to purify the protein whose activity is lost in the mutant extract. How would you do this? Be sure to describe the controls that you would need to include to ensure that your approach will work.

Take wildtype cells, fractionate and test your fractions with extracts made from your mutant cells (a form of complementation assay). Test for DNA synthesis. Must do experiments at 42degrees Celsius so that mutant protein in mutant extract is inactivated. Make sure that you do not get DNA synthesis in your mutant extract alone at 42 degrees.

(E) After many rounds of column chromatography you have a column fraction that you are convinced is fully purified. When you separate the proteins in the fraction on an SDS-polyacrylamide gel, you find that there are two proteins in 1:1 ratio. Explain how you could be convinced that the activity is pure despite more than one protein being present in the purified fractions?

If your <u>specific activity</u> stops increasing with subsequent purifications then you can consider your activity to be as pure as it's going to get (even if you see more than one protein in your fraction).

(F) After you finish your purification, you test if your purified protein has origin DNA binding, DNA helicase, or DNA unwinding activity. In each case you find no activity.

You discuss this result with your advisor and she reminds you that one of the chromatography steps that you performed during the end of the purification separated the two proteins in your final fraction. You did not pursue this fractionation method further, however, because neither of the separated proteins complemented the mutant extract. Nevertheless, your advisor suggests that you test each of the separated proteins for the same activities.

You are surprised to find that fractions containing the larger of the two proteins have a robust helicase activity. How can you explain this result?

The smaller protein is somehow inhibiting the helicase activity of the larger protein. When the smaller protein is not around, the larger protein can perform helicase activity.

G) You are curious to determine which of the two proteins in your purified fraction is altered in the original mutant strain. Because you had to do all your biochemical complementation assays at the non-permissive temperature, you anticipate that the purified protein is also temperature sensitive. Indeed, when you test the fractions you are purifying from the mutant cells you find that they can only complement the extracts derived from the mutant cells at 30°C but not at 42°C.

After you finish purifying the activity you find the same two proteins in the final fraction. On a hunch you test this fraction for helicase activity at 30°C and 42°C. You are excited to find that you only see helicase activity at 42°C.

How can you explain this finding? Based on your understanding of DNA replication, propose a function for the proteins in your purified fraction. Be sure to explain how these functions explain the results of your studies of 3H-thymidine incorporation by the mutant strain.

To reiterate first part of question (I thought it was a little confusing): So you go back to your mutant cells and do the same purification steps as you did in the wildtype cells. You test to make sure your purified activity displays temperature sensitivity by doing your complementation assay at 30 degrees and 42 degrees. This is a good control.

When you test your purified proteins for helicase activity you only see helicase activity at 42 degrees because the smaller protein is the one that is mutant (ts). At 42 degrees, the smaller protein is inactive, therefore the larger protein is not inhibitied, whereas at 30 degrees, the smaller protein is active and inhibits the helicase activity of the larger protein. This suggests that the larger of the two proteins is DNA helicase and the smaller protein is its helicase loader – when the loader and the helicase are together, the loader inhibits the helicase (the loader must dissociate from the helicase but the factor needed for loader dissociation is not in the fraction). The helicase has some latent ability to load onto DNA without its loader and so can perform its function in a DNA helicase assay when by itself. This result makes sense because your already knew that your mutant was involved in initiation, not replication, which is true of the helicase loader, not the helicase.

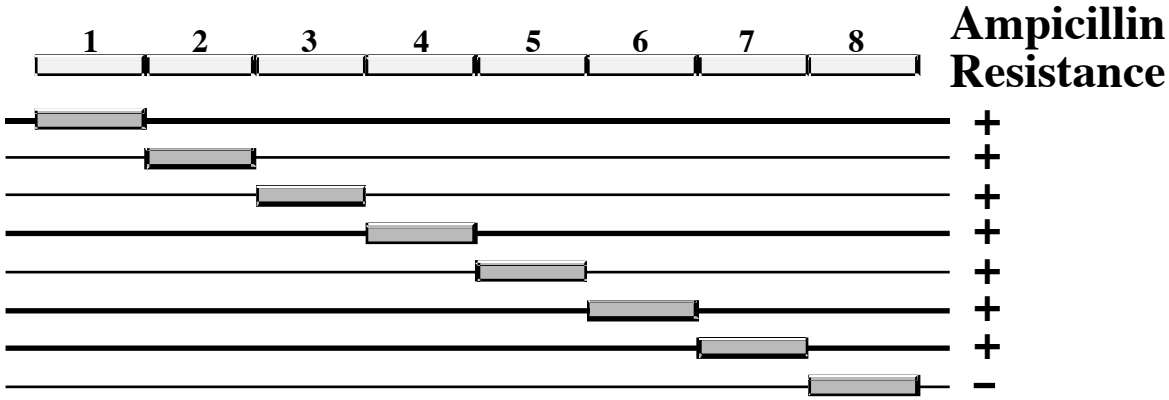
Question 3

You are studying the chromosomal replication of a new strain of archaebacteria isolated from deep sea sulpher vents called *Stinkus aquaticus*. As a first step in dissecting the replication process you want to identify the origin of replication for this organism.

Because it is a unicellular organism you use the plasmid transformation technique to look for the Stinkus replicator. You find one chromosomal DNA fragment that

consistently confers the ability to transform *Stinkus* to an ampicillin resistant state when ligated into a plasmid that otherwise cannot confer ampicillin resistance.

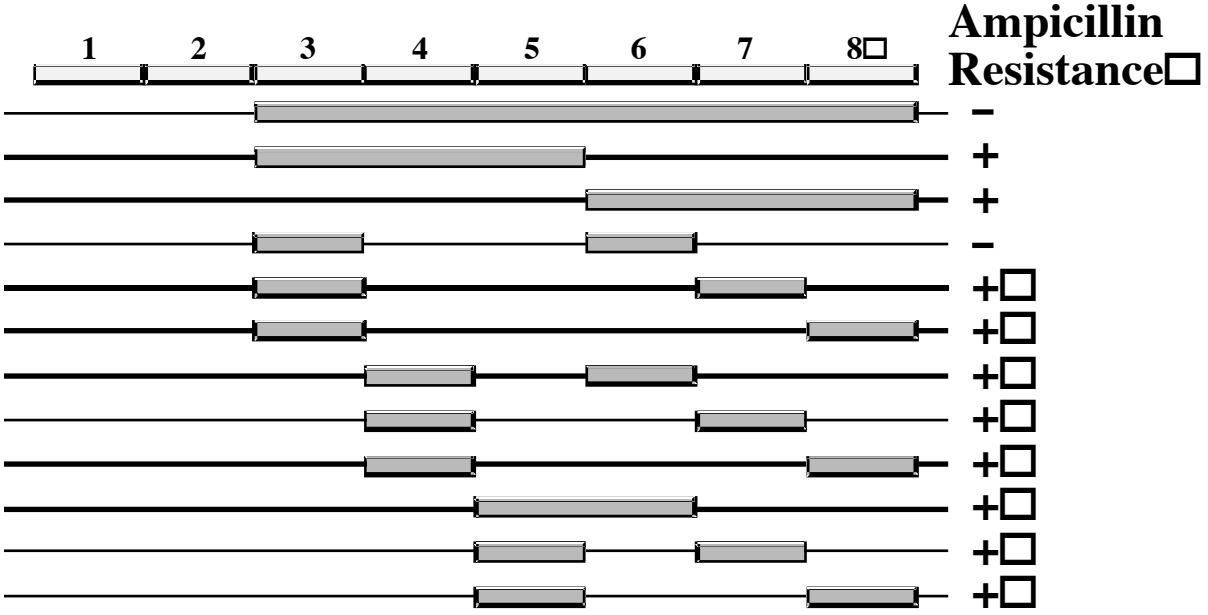
To map the sequences involved in origin function more carefully, you make a series of substitution mutants across the region and obtain the following results.



(A) What can you conclude about the DNA elements involved in the Stinkus chromosomal origin of replication? In particular, indicate what part of the DNA is necessary and what part is sufficient for replicator function.

This data suggests that region 2 is necessary for function (per the correction I made to the problem set on 3/1/05). The entire region (1-8) is sufficient for function (you found this out just by reading the introduction)

Surprised at the minimal region that the initial analysis revealed, you construct a number of other substitution mutations to investigate the region to the right of element 2. You obtain the following results.



(B) What additional elements of the replicator does this analysis uncover? Briefly explain why these elements were not observed in the first analysis.

This data suggests that replicator function depends on having a functional copy of either region 3 or region 6. These elements were not uncovered in the first analysis because only one element was mutated at a time. Since element 3 and 6 are redundant, mutating an individual element would not affect replicator function.

You have identified a *Stinkus* protein that is related to DnaA. You suspect that this protein might be the *Stinkus* initiator protein and want to test your hypothesis. As a first step you perform a gel shift experiment and find that the *Stinkus* DnaA protein binds to the fragment of DNA you identified in the transformation assay.

(C) Describe an experiment that would determine the region of the *Stinkus* replicator the DnaA-related protein recognizes. You have access to any DNA molecule that you need as well as a standard array of restriction enzymes and other nucleases. Describe all DNA and protein molecules used in your assay.

DNaseI footprinting analysis or a gel shift assay would allow you to determine the region bound by the Stinkus DnaA-related protein.

<u>DNase I footprinting:</u>

- **Labeled DNA molecule (on one end of one strand) corresponding to regions 1-8 (replicator)**
- **Add the Stinkus DnaA-like protein to half of your sample and allow it to bind; run a control sample without protein.**

- **Treat both samples with DnaseI**
- **Run samples on a denaturing polyacrylamide gel**
- **Dry gel and expose to film (autoradiogram)**
- **Results: The DNA to which the protein binds will be protected from DnaseI cleavage, and will appear as a blank region on the autoradiogram**

Gel Shift Assay:

- **Labeled probe corresponding to the replicator (1-8)**
- **Protein of interest (Stinkus DnaA-like protein)**
- **Competition of labeled probe with unlabeled DNA corresponding to regions 2, 3 and 6**
- **Run products on a nondenaturing gel and expose to film.**
- **Results: The mobility of the labeled probe should be shifted upon incubation of the probe with the Stinkus protein. Unlabeled DNA corresponding to the Stinkus protein binding site should compete for the protein; this will lead to an elimination of the mobility shift.**

You find that the *Stinkus* DnaA protein binds to element 2 that you identified in your mutagenesis experiment (part **A**). Having identified a function for element 2, you want to determine the function for the remainder of the origin.

(D) What function would you consider a likely possibility for the other regions of the origin?

Elements 3 and 6 are likely to be sites of DNA unwinding (analogous to the 13mers of E. coli oriC)

(E) Briefly describe an experiment to test your hypothesis?

DNA unwinding assay:

- **Label one end of one strand of dsDNA molecule containing regions 3 and 6**
- **Add the Stinkus DnaA like protein to allow unwinding to occur.**
- **Do a control with no added Stinkus protein (no unwinding)**
- **Add a nuclease that specifically cleaves ssDNA**
- **Run products on a denaturing gel, dry, and expose to film**
- **Results: unwinding at region 3 and 6 will produce a region of single stranded DNA that is susceptible to nuclease cleavage; a series of bands resulting from nuclease cleavage will appear on the autorad. The control lane should not unwind and should have only one band (corresponding to the full lenth DNA fragment).**

Question 4

Your introductory lab course has set up an Ames Test experiment for you to conduct. You are given two strains to study. Strain A has a single base substitution, while Strain B has two frameshift mutations. Both strains' mutations are in genes required for synthesizing histidine. Therefore, mutant strains do not grow on media lacking this amino acid. You plate cells from each strain onto minimal media plates lacking histidine and then expose them to the indicated mutagens.

	average colonies per plate	
Mutagen	**Strain A**	**Strain B**
Control (no mutagen)	35	~5
UV (1 sec)	45	30
UV (5 sec)	500	2000
UV (10 sec)	1000	2000+
MMS	350	12
9-aminoacridine	40	150

(A) In this experiment, you are exposing the cells to mutagens, yet the assay counts colonies that grow, and in order to grow the cells cannot be mutant. Please explain this apparent contradiction.

You are using the mutagens to make mutations that reverse the original mutations in Strains A and B. Counting colonies that grow is what tells you how many mutations were made, because the mutations are RESTORING the genes to their wild type sequences or other changes that restore function. This is why the cells can grow and be counted.

NOTE: Reversion does not have to restore the exact wild type sequence. A compensatory mutation could also restore the histidine pathway and give you the growth phenotype.

(B) Why do you see colonies appearing on the control plates? Propose an explanation for the difference in the control frequencies observed between Strains A and B.

These colonies arise from spontaneous reversions. Strain A had a single base substitution, while Strain B had two frameshift mutations. Logically, it should be easier to reverse the single base substitution than the two frameshifts. This explains the higher incidence of spontaneous reversions in Strain A.

(C) A chemical is classified as a mutagen if it produces twice the number of mutants that occur spontaneously. From the data above, suggest whether UV light, MMS, and 9-aminoacridine act as effective mutagens for each strain. What do these results indicate about they types of mutations induced by each of these treatments?

For Strain A, 5+ seconds of UV light and MMS both acts as effective mutagens. For Strain B, 1+ seconds of UV light and 9-aminoacridine both are good mutagens, while the MMS is marginal. This indicates that MMS probably induces base substiutions, while 9-aminoacridine induces frameshifts. UV light, is apparently capable of inducing both types of mutations.

(D) Your lab partner asks you why you didn't just start with wild type (His+) cells, mutagenize them, and look for His- cells. How do you justify using the mutant reversion studies instead of his suggested approach? Identify a couple of specific advantages to the reversion analysis approach. What are the limitations of determining mutagenic specificities (i.e. of deciding whether something is or is not a mutagen) by reversion studies?

The lab partner's suggestion is much harder and less quantitative. Imagine starting with a lawn of cells the are all wild type. When you apply mutagen, some will die from their mutations, but how do you find those cells? The reversions study allows you to identify a mutation based on the fact that that cell can restore the ability to grow under selective conditions.

Reversion studies are often quite sensitive because they provide a simple, genetic selection for mutants in a specific target gene (for example, in the Ames test the selection is for His+). They also allow you to test for specific types of mutations (the earlier parts of the question had you distinguish between mutagens that make frameshift mutations and those that make base susbtitutions, for example).

That any particular type of mutation will only be reverted by certain classes of mutagens can also be a limitation to reversion studies. Therefore, it is necessary to test a potential mutagen for reversion.

Question 5

To identify proteins involved in DNA repair in a newly identified bacterial strain, you decide to isolate mutant versions of this strain that have a mutator phenotype. In the context of this analysis, you define mutant strain as a mutator strain if it has at least a 10-fold higher frequency of mutations than the starting strain, as determined from an *in vivo* reversion assay, similar to that used in the Ames test. The starting test strain you use for the reversion assay carries a missense mutation in a gene required for arginine biosynthesis; as a result, this strain is an arginine auxotroph.

(A) Name **four** genes (or the protein encoded by these genes) (use *E. coli* nomenclature) that are likely to give the **most** elevated frequency of mutations (e.g. >100-fold increase) by this assay. Briefly justify your answers.
mutS, mutL, mutH, and proofreading exonuclease of PolIII.
An explanation of why the above mutations yield the MOST elevated frequency of mutations is necessary. mutS, mutL, and mutH are involved in mismatch repair. A mutation in any of these would cause a 1000x increase in frequency of mutations. A mutation in the proofreading exonuclease of PolIII would also cause a 1000x increase in frequency of mutations.
Genes necessary for BER and NER were not accepted because the frequency of mutation in these mutants is significantly lower than in the mutants above in the absence of a mutagen.
(Note: dam- was also accepted, though a mutation in this gene would cause a 500x increase in mutation frequency, which is not as high as the mutations mentioned above.)

As a result of this genetic screen, you isolate cells that constitutively express proteins required for trans-lesion DNA synthesis (TLS). These cells express the proteins in the absence of induction by a DNA damaging agent. The TLS proteins themselves are, however, perfectly normal in this strain.

(B) Why do these cells have a mutator phenotype? Explain your answer.

TLS is an error prone and will cause the incorporation of incorrect nucleotides during replication.
TLS genes are normally repressed. However, in these cells, TLS is constitutively active. Due to this, TLS proteins are free to act and therefore cause increased mutation.

In the process of characterizing the strain that constitutively expresses the TLS proteins (used in part b), you notice that one clone derived from this strain has an even HIGHER mutation rate that the parent strain. You observe this high mutation frequency in many different types of *in vivo* assays, regardless of the nature of the "tester" allele used in experiment. Further analysis of this hyper-mutable strain reveals that it carries a mutation in the *umuC* gene.

(C) What characteristic of the UmuC (polymerase V) protein could be altered in the mutant version to explain the phenotype? Briefly explain your answer.

UmuC is a low fidelity DNA polymerase and also has low processivity. However, a mutation that increases the processivity of UmuC will allow it to polymerize much longer than normal.
The longer UmuC polymerizes DNA, the more incorrect nucleotides it incorporates, increasing the mutation frequency.
Note: Other explanations were accepted, such as a mutation in the proofreading subunit of UmuC or a mutation making UmuC even more error prone. However, UmuC does not possess proofreading activity. Additionally, a mutation making it more error prone may be unlikely as it incorporates nucleotides almost randomly without consideration for proper base pairing.

(D) Design an experiment to test your hypothesis for the nature of the defect in the UmuC protein. Explain specifically what results you would expect in your experiment with the mutant protein if your hypothesis was correct. Compare these data with those you find in control experiments using the wild-type UmuC protein.
A processivity assay can test our hypothesis that UmuC obtained a mutation increasing its processivity.
A proper substrate would include a long single stranded template with a short complementary primer radioactively labeled at its 5' end. Incubate UmuC and labeled substrate in an equimolar ratio.
After incubation, add dNTPs and 1000x cold substrate (ssDNA template and unlabeled primer). Allow reaction to proceed and run the products out on a denaturing gel. Dry onto paper and expose to X ray film.
A mutant increasing processivity in UmuC will yield a long DNA product, indicating its ability to polymerize a long stretch of DNA in a single binding event. Wild type UmuC will yield a short DNA product, indicating low processivity in this enzyme.

Question 6

You are a UROP in a lab that studies mismatch repair. You are working with a grad student whose project is to identify the proteins required for mismatch repair in a recently identified prokaryote collected off the coast of Cape Cod. The grad student has already fractionated the cell extract and identified several fractions(A through D) that when combined can successfully repair a mismatched DNA substrate.

(A) Briefly describe 2 types of columns that can be used to fractionate cell extracts and explain how they work.

Ion exchange-separates by charge
Gel filtration-separates by size and shape
Affinity-separates out specific molecules that bind to an antibody, particular substrate, etc. that is attached to the column

While the grad student is away on vacation, she wants you to determine what kind of proteins are in each fraction. You decide to do an *in vitro* DNA repair assay using the following substrate.

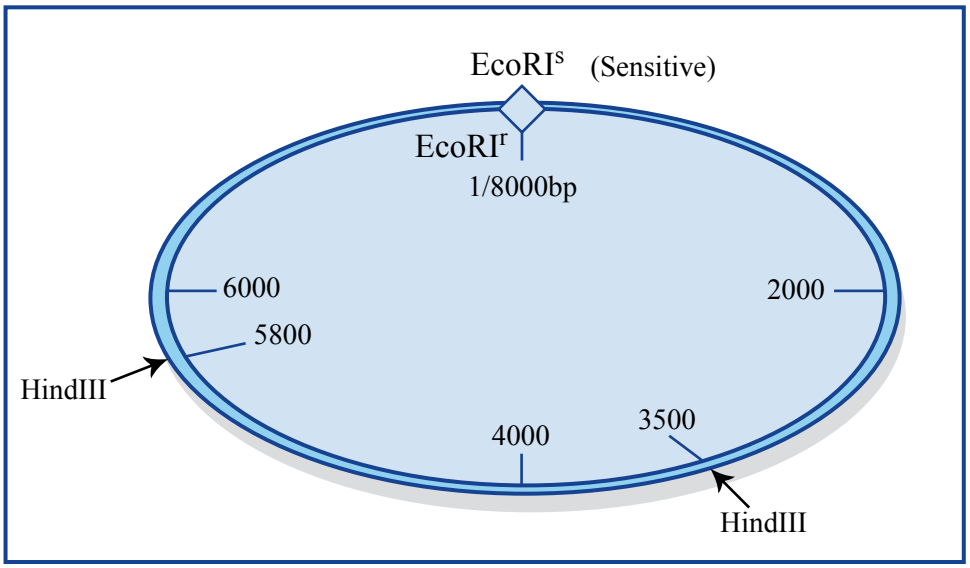
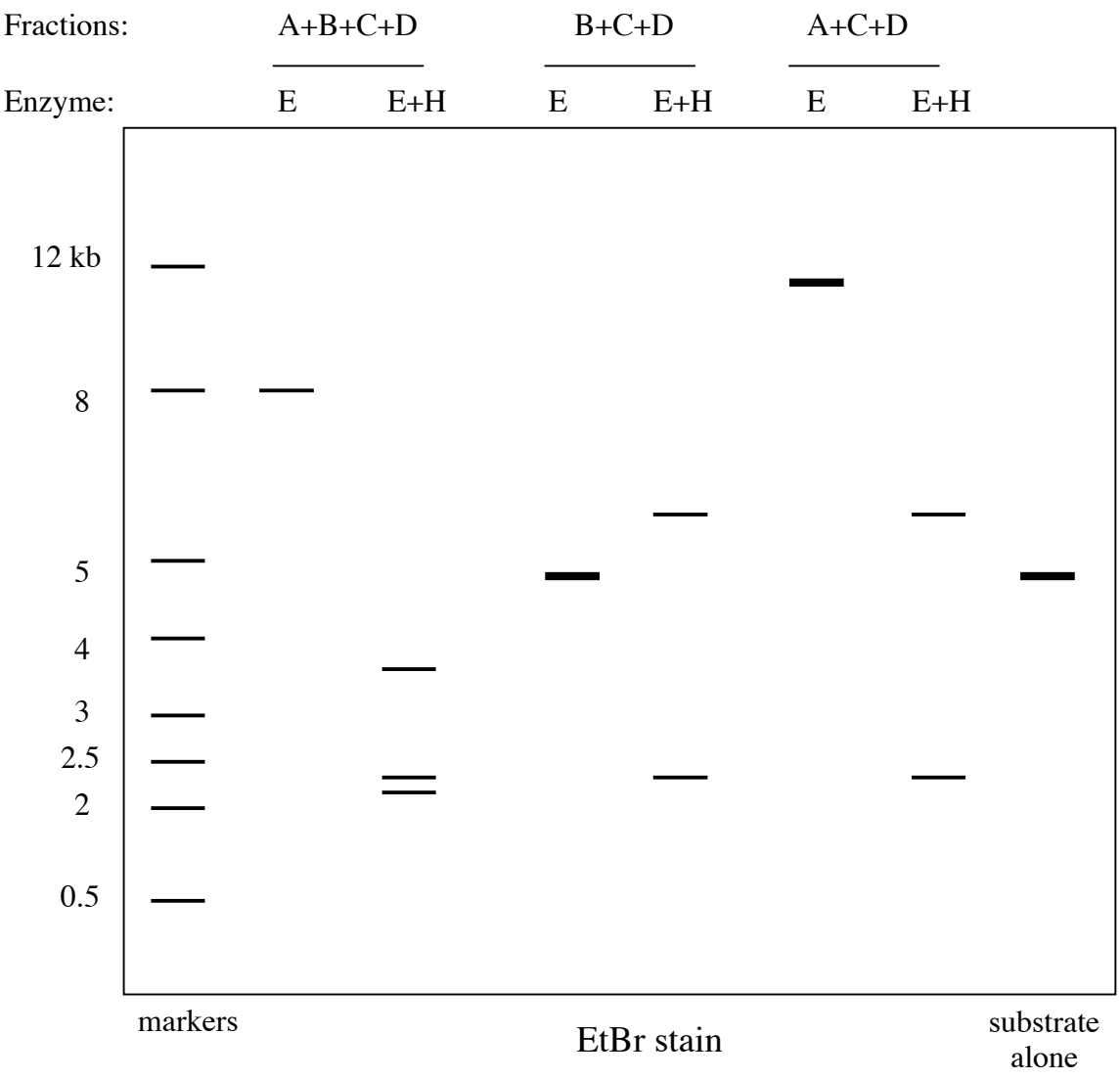


Figure by MIT OCW.

You incubate the repair substrate with each of the different fraction combinations in the presence of ATP and the four dNTPs. After the incubation, you recover the DNA and digest each sample with EcoRI (lanes marked E) or EcoRI and HindIII (lanes marked E+H) and run the resulting cleaved DNA molecules on an agarose gel. A picture of this agarose gel is shown below. The lanes are labeled at the bottom of the gel. The lane marked "Markers" contains DNA fragment size markers, and the lane marked "Substrate" contains the uncut substrate DNA (which migrates faster than 8 kb because it is supercoiled).



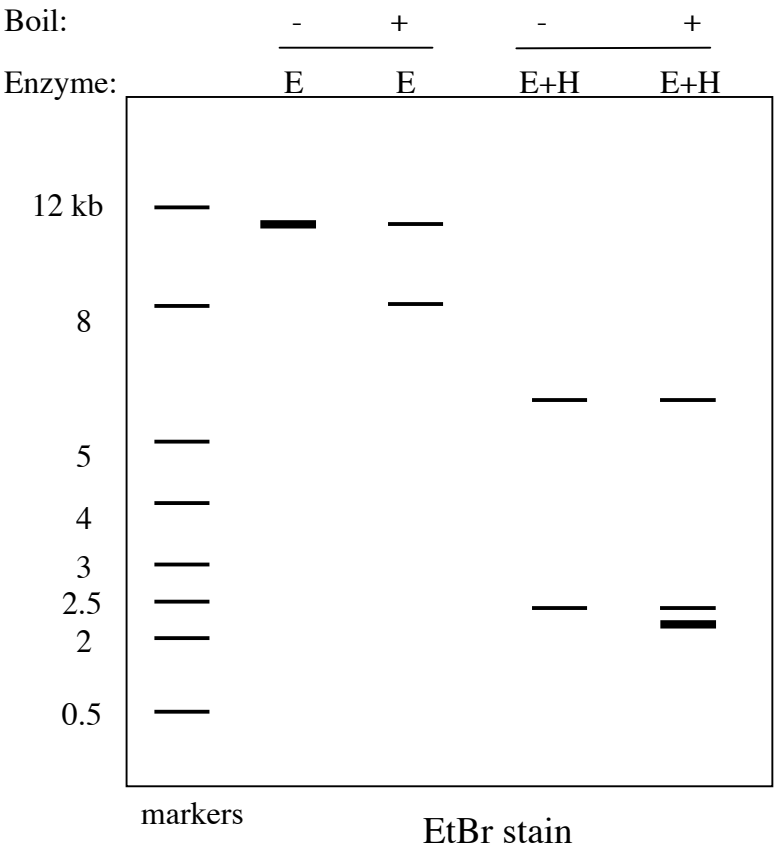
(B) Which fraction is likely to contain a MutS-like protein? Why?

Fraction A because in its absence repair does not occur.

(C) What other proteins could be contained in Fraction A?

MutH or MutL because they are also both required for repair.

You are confused by the results of the A+C+D fractions. To better understand what is going on you boil each sample after the digestion reaction. You see the following results for Fractions A+C+D.



(D) Based on this data, what protein might be contained in Fraction B? Explain.

Fraction B could contain any protein required for mismatch repair after MutH nicks the unmethylated strand: helicase II, an exonuclease, SSB, a DNA polymerase, and DNA ligase. Without one or more of these, a nick in the mismatched strand would persist. A nick in one strand of the supercoiled circular DNA would cause the plasmid to relax and run more slowly than both the supercoiled plasmid and the linearized plasmid. Boiling the samples separates the ds DNA.

(E) To distinguish between the possible proteins in Fraction B, you decide to do a simple DNA incorporation assay with Fraction B to narrow down what kind of protein it contains. What assay would you do and what results would you expect for the possible proteins in Fraction B?

You could do a filter binding assay to ask for DNA polymerase activity. Add fraction B to a PTJ substrate and the appropriate buffer. Start the reaction by adding labeled dNTPs. Separate dNTPs from DNA by spotting an aliquot of the reaction onto filter paper, wash, and measure the amount of label retained on the filter. If Fraction B contains a DNA polymerase, you will see incorporation by this assay. If one or more of the other proteins but no DNA polymerase are in Fraction B, you will not see incorporation by this assay.