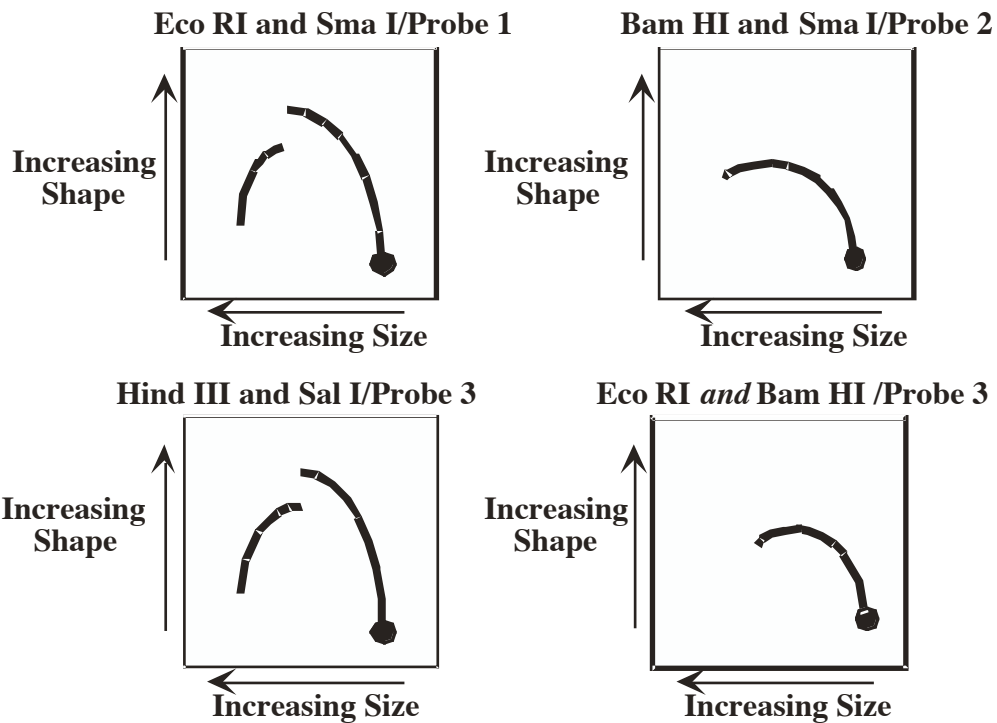
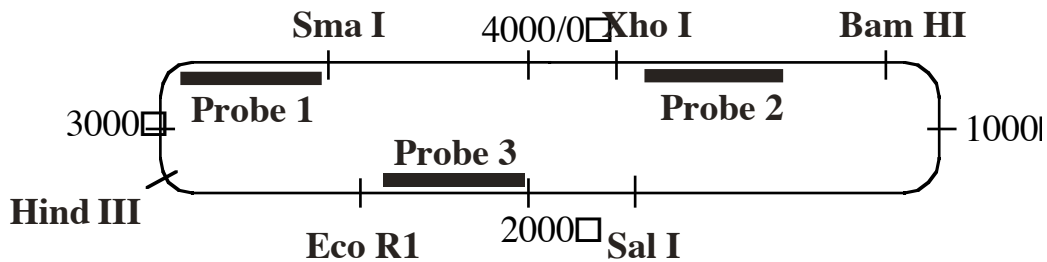


Name _____

Question 1 (28 Points). Your lab is studying a novel thermophilic eukaryote called *S. mokin* that has an optimum growth temperature of 75°C. Surprisingly, it has a circular genome and your student has decided to identify its origin(s) of replication.

Another student has found that some *S. mokin* cells contain a 4 kb plasmid that confers resistance to Chromium. Based on this finding, your student wants to use a plasmid rescue assay to identify the genomic origin in *S. mokin*. As a first step, they use 2D gel analysis to locate the origin of replication in the plasmid and obtain the following results.



1A (6 Points). Based on their data, what two enzymes do you suggest that your student use to cut the plasmid and genomic DNA for the plasmid rescue assay? Explain your reasoning.

Name _____

The bubble to y arc gels are the only gels that give you useful information in this experiment. They show that the origin is between EcoR1 and SmaI, and HindIII and SalI. As long as there is only one origin in this plasmid (as was stated in the question), the origin must be in the sequences that overlap these two regions, namely between EcoRI and HindIII. Some people wanted to take out the whole region between SmaI and SalI (they received half credit), but in this sort of experiment, the less cut out of the plasmid the better and the data clearly shows where the origin is and is not.

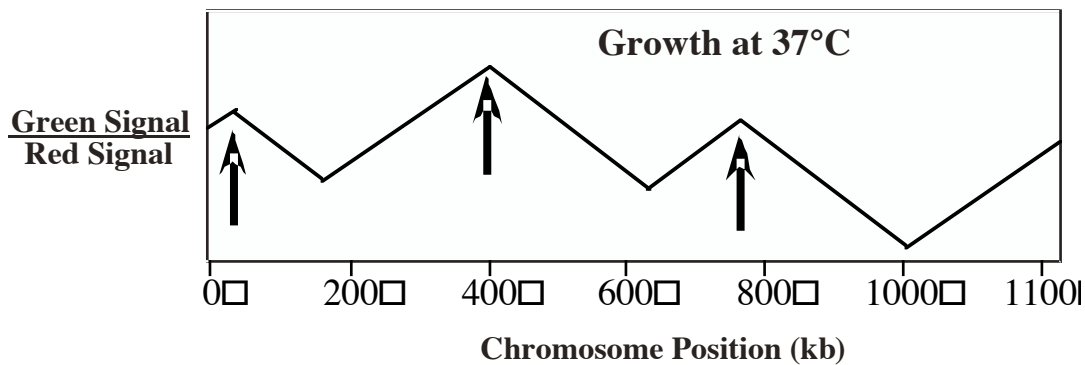
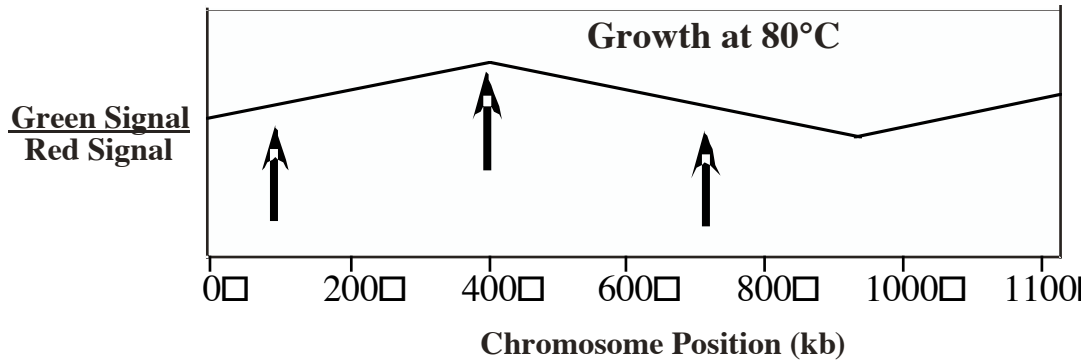
At the conclusion of your plasmid rescue assay you identify three non-overlapping DNA fragments that act as replicators. You are surprised since most archaeobacterial genomes have only a single origin of replication.

1B (6 points). You want to know if there are multiple origins in the *S. mokin* genome. You take advantage of a DNA microarray that was developed by another lab to study gene expression. However, before you will buy the DNA microarrays, you insist that your students identify a conditional initiation mutant. Why do you need this mutant to perform the microarray analysis of DNA replication?

Microarray analysis of DNA replication requires that you are able to arrest cells prior to the initiation of DNA replication and then release them from this arrest. This allows the investigator to: 1) have a population of unreplicated cells that can be used as an unreplicated control DNA sample and 2) obtain cells that are going through DNA replication synchronously. Partial credit was given for either of these two answers and full credit was given if both answers were included.

Because of the thermophilic nature of the *S. mokin* your students identify a urea (a mild protein denaturant) sensitive initiation mutant. With the mutant in hand, you buy the DNA microarrays and perform an assay to map origins at two different growth temperatures. The replicating DNA is labeled green and the unreplicated control DNA is labeled red. Your student obtains the following results (the arrows indicate the position of the replicator sequences that your student has already mapped).

Name _____



1C (4 Points). You are surprised at the two different results but pleased that at 37°C all three replicators are used. One of the first things you notice is the different slopes of the lines in the 37°C and 80°C data. If the peaks represent origins of replication, what aspect of DNA replication is represented by the slope of the lines? Hint: remember that the Y axis can also be thought of time of replication with the top being earliest and the bottom being latest).

The slope of the lines represents the speed of replication fork movement. A shallower slope indicates faster replication and a steeper slope indicates slower replication.

1D (6 Points). Based on your knowledge of the mechanisms that prevent origins from replicating the genome more than once per cell cycle, propose a reason for the different number of origins observed in the two microarray data sets.

At 80°C, replication is occurring more quickly and the two outside origins are being passively replicated. At 37°C, replication occurs slower, allowing all three origins to fire. High CDK activity means origins, either passively replicated or actively fired, can not reform pre-RC's during S phase.

1E (6 points). Your student points out that the plasmid rescue assay was done at 75°C rather than 37°C yet all three replicators were identified. How can you

Name _____

explain the finding that the temperature effects on origin usage were not observed in the plasmid rescue assay?

In plasmid rescue, each replicator stands alone in the plasmid and therefore can not be passively replicated by replication forks derived from an adjacent early firing origin.

Question 2 (24 points total)

You are studying mechanisms used by bacterial cells to avoid accumulating mutations. Using a reversion assay similar to the Ames test, you identify a mutant strain that has a 20 to 50-fold higher spontaneous mutation rate. You find that this strain carries a mutation that destroys the activity of the enzyme dUTPase; this enzyme recognizes dUTP and converts it into dUMP. You name the mutation *dut1*.

2A (6 points). You hypothesize that the *dut1* mutants have a higher than normal mutation rate because dUTP accumulates at high concentrations in these cells and is used for DNA synthesis. Design an assay to determine if the replicative polymerase from *E. coli* can use dUTP as a substrate. An ideal assay would allow you to quantify the frequency at which dUTP is incorporated compared to one of the normal precursors.

A good incorporation assay for this experiment would be a filter-binding assay because you just want to know quantitatively how much dUTP is being incorporated. Using a gel would work, but would be more work intensive. A filter-binding assay involves a DNA polymerase (in this case Pol III since we're working with *E. coli*), a PTJ, buffer, Mg²⁺, dNTPs, and dUTP. In one experiment, label dUTP (if doing ³²P must label on α P) and in another experiment label another dNTP to compare levels of incorporation. You would then spot an aliquot of the reaction onto a piece of positively charged filter paper, then wash with a salt buffer (washes away unused dNTP, but not DNA) and then do scintillation counting (if doing radioactivity). Half credit was given for the correct assay but no control (comparing to levels of incorporation of normal precursor).

2B (6 points). If your misincorporation hypothesis is correct, what class of mutations would you expect to occur at high frequency in the *dut1* mutant cells? Be as specific as possible.

In the *dut1* mutant, it is hypothesized that there are high levels of dUTP in the cell. This could lead to misincorporation of dUTP in place of any of the other dNTPs leading to base substitution mutations.

To further understand the mechanisms used by cells to avoid mutations, you decide to combine the *dut1* mutation with mutations in various genes known to be involved in DNA repair. Most of the cells carrying these pairs of mutations do not have a phenotype that is significantly more severe than the parent strains carrying the individual mutations. However, you find two genes (called *dre1* and *dre2*) in which the combination of the *dut1* mutation with a mutation in this

Name _____

gene causes a dramatic change: These doubly-mutant cells grow very slowly, and therefore form only tiny colonies. They also exhibit increased mutation frequencies, as determined by the His⁻ to His⁺ reversion assay. These data are shown in the table below.

Strain:	His ⁺ colonies spontaneous	His ⁺ colonies after treatment with low-dose UV
Wildtype	5	10
<i>dut1</i>	200	400
<i>dre1</i>	55	110
<i>dre2</i>	120	600
<i>dut1, dre1</i>	>1000	Not determined
<i>dut1, dre2</i>	>1000	Not determined
<i>dre1, dre2</i>	120	600

Additional experiments reveal that the *dre1* and *dre2* mutants are not defective in post-replication mismatch repair.

2C (6 points). What repair protein is most likely to be defective in the *dre1* mutant? Briefly explain your answer.

The most likely possibility is that the *dre1* mutant is uracil glycosylase. This is suggested by the *dre1dut1* double mutant results and the fact that *dre1* does not have a UV defect. In the double mutant, the uracil misincorporated into the genome would not be removed, leading to a high mutation rate. But in the *dre1* single mutant, uracil is not found in the genome too often so the incidence of spontaneous mutation in these mutants is not terribly high. Also, a mutant in uracil glycosylase would not have a UV repair defect.

2D (6 points). What repair protein could be defective in the *dre2* mutant? Briefly explain your choice and make sure your answer is consistent with that you gave in 2C.

The most likely possibility is that the *dre2* mutant is in the AP endonuclease. This is suggested by the *dre1dre2* double mutant results showing that mutations occur at the same frequency as *dre2*. AP endonuclease acts downstream of uracil glycosylase, thus the *dre1dre2* double mutant would have the same mutation rate as *dre2* alone. Furthermore, *dre2* mutants have a defect in UV damage repair, which makes sense because AP endonuclease is required for some UV damage repair. Lastly, if the *dre2* mutant is AP endonuclease, *dut1dre2* double mutants will have high spontaneous mutation rates because uracil will not be able to be removed from the genome.

Name _____

Question 3 (24 points total). You are studying telomere replication in a single cell eukaryote, *T. shorty*. This organism has the unusually short telomere repeat of 5' GCAC 3'. You want to identify mutants in the telomerase of this organism.

3A (4 Points). A colleague in the lab suggests you use the ³H-thymidine incorporation mutant screen that she had learned about being used to identify DNA replication mutants in other organisms. You reject this idea, however. Why can't this approach be used to identify the mutants you are looking for?

Mutants in telomerase will not be recovered by this screen. The screen works for *E. coli* replication mutants because these mutants do not allow any ³H-thymidine incorporation into their genomes and do not suffer the consequent massive DNA damage that WT cells do. This damage selectively kills WT cells but spares your replication mutant cells to be recovered. A telomerase mutant will not incorporate ³H-thymidine into its telomeres, but it will still incorporate it into its genome, thus both WT and telomerase mutants will die. You have no way of recovering telomerase mutants in this screen.

Many students pointed out that this telomere repeat does not contain a T, therefore ³H-thymidine would never be incorporated into the telomere. This is true, but it does not explain why the screen wouldn't work.

Using a clever fluorescent assay for telomere length you have identified three mutants (*TEL1*, 2, and 3) that show defects in maintaining their telomere length. You clone the wild-type version of each mutant gene and find that *TEL1* and *TEL3* encode proteins. *In contrast*, *TEL2* does not include any protein-coding regions greater than 6 amino acids.

3B (4 points). You suspect that *TEL2* encodes the telomerase RNA. Based on your knowledge of telomerase function, what **RNA** sequence would you predict would be encoded by this gene? Please indicate the polarity of the sequence.

The RNA sequence of the telomerase contains 1.5x the complement of the telomere repeat: 3' CGUGCG 5' (or in the more conventional 5'-> 3' directions, 5' GCGUGC 3').

Name _____

To demonstrate that the gene you have identified is the telomerase RNA you want to change the sequence of the *T. shorty* telomere. Your strategy is to replace the wild type gene with a mutant gene that changes the sequence of the telomere repeat. Your advisor suggests that you change the repeat to the sequence 5'GTAC3' because you can easily assay for the change with a restriction enzyme that cuts that sequence.

3C (4 Points). Before you make the mutant gene your advisor asks to see the change in the telomerase RNA that would be made by your mutant. Indicate below the altered RNA sequence you would propose and its polarity. Briefly explain the reasoning behind your changes.

Similar to part B, but the new RNA sequence to complement the new repeat is 3' CAUGCA 5'.

3D (6 Points). To look more carefully at telomerase function in this organism you purify Telomerase from *T. shorty* cells. Because of the small repeat, you want to investigate the processivity of your telomerase. Describe the assay you would use to determine the processivity of your telomerase. You have access to any DNA oligomers and radiolabeled nucleotide precursors that you need.

(2 points) The substrates for this reaction are 5'-labeled and unlabeled ssDNA oligomers. DNA polymerase requires a PTJ substrate, NOT telomerase!

(2 points) The sequence of the substrate molecules should be a number of copies of the telomere repeat sequence(5' GCAC 3') so that the telomerase can bind.

(2 points) the workings of the assay

- 1. Add telomerase to labeled substrate + buffer + Mg²⁺**
- 2. Add dNTPs + 1000x excess of unlabelled substrate.**
- 3. Allow reaction to proceed to completion.**
- 4. Separate the products on a denaturing gel. The length of the product, indicated by mobility in the gel, will indicate degree of processivity.**

Your lab mate has found that this organism has the unusual ability to extend telomeres even when cells are not in S phase. Surprisingly some of the newly added telomeric repeats are in the form of double stranded DNA. Since telomerase can only synthesize single stranded DNA you are sure that there must be another DNA polymerase involved.

You devise an assay that only detects the synthesis of the 2nd strand of DNA(not the strand synthesized by telomerase). Using this assay and wild type telomerase with

Name _____

and without the three primary eukaryotic DNA polymerases you obtain the following results:

Enzymes added	2 nd Strand Synthesis Activity
Telomerase	-
DNA Pol α /primase	-
DNA Pol δ	-
DNA Pol ϵ	-
Telomerase + Pol α /primase	+
Telomerase + Pol δ	-
Telomerase + Pol ϵ	-
Telomerase + Pol α /primase + Pol δ	++++
Telomerase + Pol α /primase + Pol ϵ	+

3E (6 points). Propose a model that explains your results. Be brief.

Many models were acceptable, but generally, these points needed to be covered:

- **Second strand synthesis requires that the first strand is synthesized by telomerase. Telomerase may recruit Pol α / primase to the site.**
- **Pol α / primase is required to synthesize primers and generate a PTJ for any further second strand synthesis by a polymerase.**
- **Pol δ but not Pol ϵ is able to synthesize more of the second strand. This may be because Pol α / primase exclusively recruits Pol δ to the site or forms a stable complex with it that allows Pol δ to be active.**

Question 4 (24 points). In prokaryotes and eukaryotes mismatch repair normally occurs during within the first few minutes after the DNA replication fork passes. In bacteria closely related to *E. coli*, the process is triggered by the hemi-methylated DNA that is generated during replication. In many other bacteria, and in eukaryotes, other mechanisms are used to couple DNA replication and mismatch repair.

4A (6 points). In *E. coli*, which type of mutation in the gene encoding the Dam methylase would you expect to cause a bigger defect in mismatch repair? (That is the highest spontaneous mutation frequency due to replication errors).

- (1) a hyperactive mutation in Dam causing it to methylate DNA as soon as it was replicated? or
- (2) a null mutation that inactivates the Dam enzyme?

Briefly justify your answer.

Mutation(1) will cause a bigger defect-no repair will occur because both strands are methylated. MutH will only nick unmethylated DNA. With a null mutant, neither strand will be methylated so the mismatch repair machinery will randomly choose one strand to repair off of which will result in repair occurring correctly half the time.

4B (6 points) You discover a new bacterial species that, like *E. coli*, uses adenine methylation to mark DNA for mismatch repair. However, in this species the Dam enzyme uses the sequence: 5' CGTACG. List **two ways** that the mismatch repair process in this bacteria needs to differ from the process in *E. coli* as a result of this sequence difference. Be as specific as possible.

1) Mut H needs to recognize the new sequence.

2) Because the sequence will occur less frequently in the genome(1/4096 vs. 1/256), the mismatch repair machinery needs to adapt to dealing with longer distances between the hemimethylated site and the mismatch site—for example, UvrD, endonuclease, and polymerase need to be more processive.

4C (6 points). You decide to survey numerous bacterial species to identify the in each case the sequence recognized by the endogenous Dam methylase. Using synthetic DNA fragments, gel mobility shift assays, and direct assays for the methylation of the DNA, you identify four new sequences recognized by the Dam enzymes from different organisms. These sequences are:

1. 5'TCGATCGA
2. 5'CATG
3. 5'GTACGT
4. 5'GxxATxxC (where x can be any nucleotide)

You show these sequences to your baymate, and she immediately predicts that in one of these bacteria, the Dam methylase is not used to mark newly-synthesized DNA for mismatch repair. Which sequence causes her to come to this conclusion and why?

Sequence 3 is not a palindrome-the methylation sites need to be the same on both strands so that the sequence can be recognized and methylation state assessed on both strands by MutH.

Partial credit was given if you wrote that sequence 1 wouldn't work because it would occur too infrequently in the genome.

Name _____

4D (6 points). Name a cellular process other than DNA replication that normally generates DNA carrying mismatches. Why are these mismatches less likely to be repaired by the MMR proteins than those arising during replication?

Homologous recombination-this cellular process occurs after replication so both strands will already be methylated. In addition, the replication machinery generally is required to recruit the MMR proteins.