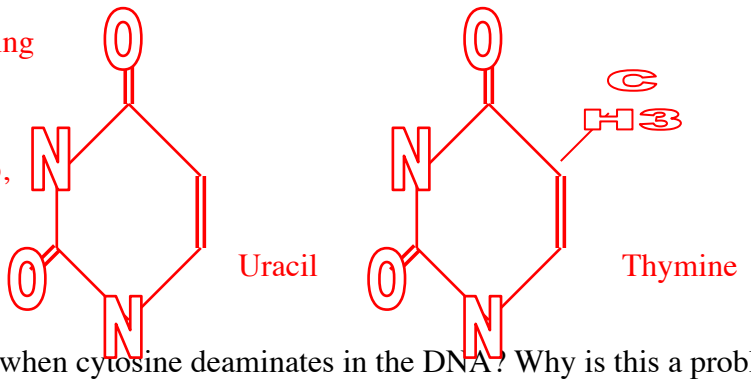
Name_____

Microbial genetics
Exam I
Winter 2023

1.) Draw the structures thymine, and uracil. (5pts)

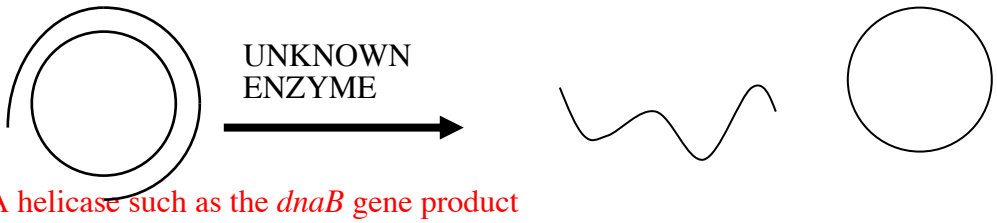
If you drew a six ring structure and remembered that thymine has an extra methyl group, you got almost full credit



2.) What happens when cytosine deaminates in the DNA? Why is this a problem for the cell? (3pts)

Cytosine deaminates to uracil. Uracil pairs like thymine and so it is potentially mutagenic to the cell if left unrepaired.

3.) An unknown replication enzyme is added to the following substrate and generates the following product. What is the enzyme called? (3pts)



Any DNA helicase such as the *dnaB* gene product

4.) By itself, PolIII, encoded by *dnaE*, incorporates the wrong base approximately once in every 10^5 bases. The measured rate that a misincorporated base results in a mutation is actually closer to once in every 10^{10} bases. Describe two general mechanisms or activities that operate to increase the fidelity of replication. (5pts)

dnaQ, proof-reading exonuclease subunit of the polIII holoenzyme cleaves mismatched bases.

methyl-directed mismatch repair system can excise mismatched base pairs from the daughter strand and then re-replicate the region shortly after replication occurs.

5.) *tus* and *ter* are involved in what cellular process? How do they function? (4pts)

replication termination.

Tus protein binds to *ter* sequences in the terminus region and act to block replication forks approaching from one direction while allowing replication forks approaching from the other direction to pass.

6.) The *E. coli* origin or replication contains multiple DnaA boxes and AT-rich 13-mers. Describe what role each of these plays in the initiation of replication? (5pts)

Multiple DnaA proteins bind in a sequence specific manner to the DnaA boxes and associated with each other. The DnaA binding induces stress on the surrounding helical DNA regions and promote strand separation at the AT-rich regions. These single stranded regions then serve as the binding sites for the delivery and loading of the DnaB helicase by DnaC to initiate the subsequent steps of establishing the replication forks.

You add an antibiotic that inhibits translation to a bacterial culture.
7.) How would you expect this to affect factor independent transcriptional termination? Why? (5pts)
It should not affect the transcription of these genes early on. Termination by this mechanism relies upon a hairpin and stretch of UUUUs in the RNA transcript that interacts with the RNA polymerase on its own to terminate transcription.

8.) How would you expect this to affect factor dependent transcriptional termination? Why? (5pts)
(Either increase or decrease was acceptable with justification)
It should increase the frequency that transcription terminates since, in the absence of translation, the termination factor, such as rho, would always have access to its binding site on the RNA transcript (in the case of rho, it binds to the rut sequence). When translation is actively occurring, the ribosomes may cover or hide the binding site preventing the termination factor from functioning. (Or you could say it will decrease frequency that transcription terminates if you thought that translation was needed to make the termination factor (such as Rho protein)

9.) A strain of *Samonella enterica* has approximately $6*10^6$ base pairs in its genome, and it can replicate and divide every 30 minutes. Assuming that it has one bidirectional origin of replication (just like *E. coli*), what's the minimum rate that the replication fork must be moving in bases per minute? (Show your work) (5pts)
(6*106 bp/genome) / 1 (genome/30minutes) / 2 replication forks =
200,000 bp/minute / 2 replication forks =
100,000 bp/min/replication fork

10.) You have isolated two mutants that do not contain any ß-galactosidase (the mutants do not grow on plates that have only lactose as a carbon source). They are interesting because when you sequence the gene for ß-galactosidase in these mutants, neither mutant contains a mutation in the coding region. You decide to sequence upstream of the start codon and find that mutant #1 has a deletion within the TATA box. Mutant #2 contains a point mutation in the Shine Delgarno sequence.

How can mutations in the TATA box or Shine Delgarno sequence result in a failure to express any ß-galactosidase enzyme? (5pts)

An intact TATA box is essential for transcription initiation to occur. Without it no ß-galactosidase transcript can be made.

The Shine Delgarno sequence is important for the initiation of translation. Without it no ß-galactosidase protein can be translated from the mRNA.

Ten years down the road….

The first round-trip space mission to Mars has just returned with large samples of soil and ice from the polar regions of Mars. The big news in all of the journals is that scientists found an organism that resembles bacteria on this planet and have cultured it out from one of the soil samples! The bacteria-like organisms are able to grow under culturing conditions very similar to the *E. coli* but seem to divide almost twice as fast.

At this point, scientists have examined the chemical make up the organism and found that its DNA is identical in structure and composition to that which is found on earth. However, nothing yet is known about how they replicate or divide.

NASA scientists have recently sent you a sample of the bacteria so that you can determine if the DNA is replicated by a similar mechanism to that which occurs on earth.

You remember the Meselson-Stahl experiment and decide that repeating their experiment on these new "bacteria" may be the best way to begin an analysis of replication mechanisms.

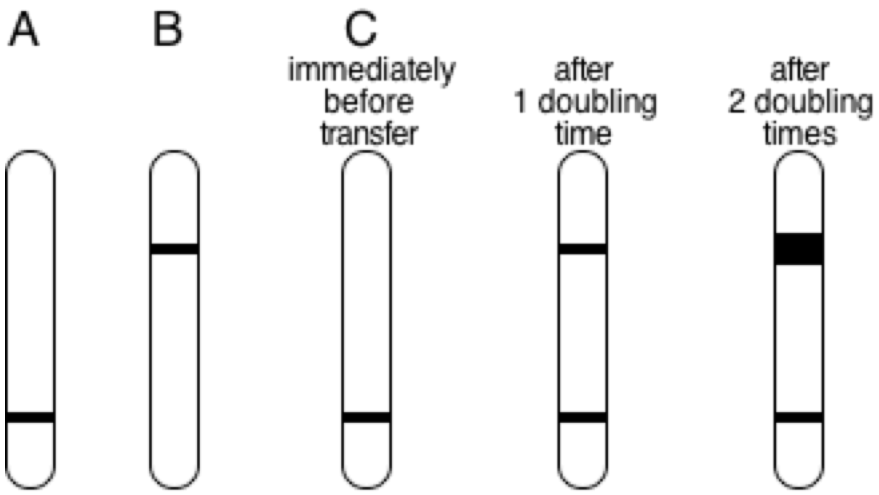
You grow three separate cultures of the bacteria.

A.) For the first culture, you grow the bacteria in media containing heavy isotopes of nitrogen (^{14}N) and carbon (^{13}C) to density label the DNA during growth.

B.) For the second culture, you grow the bacteria in normal media.

C.) For the third culture, you begin by growing the culture in the media containing heavy isotopes. Then, after several generations of growth in this media, you transfer the bacteria into normal media and takes samples at various times (I. immediately before transfer, II. after one doubling time in the normal media, and III. after two doubling times in the normal media).

You lyse the cells, isolate the DNA and centrifuge each sample to equilibrium in neutral CsCl gradients. Following centrifugation, you examine where the DNA ran in each gradient an observe the following results shown below:



11.) NASA needs to know. Do these bacteria replicate conservatively, semiconservatively, or distributively? (5 pts)

Conservatively

12.) What would you expect the results look like if the bacteria replicated by one of the alternative mechanisms? **Circle one** of the following replication mechanisms and then draw in where you would expect the bands to run. Explain your answer below. (5 pts)

Conservative. Semiconservative. Distributive.

Explain: After on generation in normal media, all DNA would contain one heavy and one light strand. After a second generation, replication of the light strand of the hybrid creates a completely light molecule, while replication of the heavy strand would remain a hybrid density.

Various size pieces of DNA that replicated either conservatively or semiconservatively would create a distribution of DNA that ranges in composition but gets gradually becomes less dense on average as it replicates in normal media.

In the course of your studies, you notice that the bacteria are able to grow on plates that contain toluene as the sole carbon source. Realizing the potential commercial value of this ability, you decide to try and learn more about the genes involved in this pathway by screening for spontaneous mutants that cannot grow on toluene.

13.) Briefly describe how you would conduct your screen. (6pts)
(May wish to mutagenize the bacteria first to enrich for mutants)
1st Plate the bacteria on normal (nonselective or glucose) media: Everybody grows.
2nd Replica plate the bacterial colonies onto minimal media plates that contain only toluene as a carbon source: *tol* mutants will not grow.
3rd determine which colonies didn't grow on the toluene plates and isolate these from your original nonselective plates: These are your mutants.

14.) Does this screen involve a positive selection or a negative selection? (4pts)
Negative selection

15.) Estimate the approximate frequency you expect to obtain mutants that cannot grow on toluene and describe the rationale for how you reached this number. (5pts)
(other numbers are possible within reason and with rationale provided)

If toluene metabolism involves 3 genes products encoded by genes of approximately 1000 bp/gene, and if ~ 1/3 of the basepirs in these gene products inactivate the gene products when mutated, this would give a target size of ~1000 potential bases in the genome that could be mutated and produce a tol- phenotype.

If the basal mutation rate is $1*10^{-9}$ mutation/bases replicated, and 1000 bases/cell, this would mean that you would need to screen ~
$1*10^{-9}$ mutation/bases replicated * 1000 bases/cell = $1*10^{-6}$ mutations/cell or 1,000,000 cells to see a tol- mutant/

16.) Describe three general methods you could use to increase the frequency with which you obtain mutants that cannot grow on toluene. (10pts)
mutagenize,
maybe use a mutS strain,
simply pass the strain for several generations before screening,
or perhaps you could use an enrichment strategy first.

Health concerns abound about the possibility that a foreign and potentially contagious bacteria could accidentally be released on earth. Data involving its susceptibility to various antibiotics and its mutation rates are important for the safety of the general public.

You find that the Mars bacteria is killed by treatment with rifampicin, a promising result. Not surprisingly however, you notice that if you plate enough bacteria, rifampicin resistant bacteria can be isolated. You decide to perform a fluctuation test to determine the mutation rate to rifampicin resistance.

You grow 30 individual, 1 ml cultures of bacteria and spread each on a plate containing rifampicin. By counting the bacteria from a separate culture, you determine that there were $1 * 10^8$ bacteria/ml at the time you plated them on rifampicin.

After spreading each 1ml culture on separate plate, you let them grow overnight. The next day, you count the following number of rifampicin resistant colonies that grew on each plate:

PLATE	# of rif^R colonies	PLATE	# of rif^R colonies	PLATE	# of rif^R colonies
#1	**1**	#11	**22**	#21	**2**
#2	**4**	#12	**16**	#22	**4**
#3	**0**	#13	**0**	#23	**0**
#4	**0**	#14	**0**	#24	**0**
#5	**0**	#15	**0**	#25	**0**
#6	**0**	#16	**0**	#26	**0**
#7	**6**	#17	**1**	#27	**9**
#8	**6**	#18	**2**	#28	**1**
#9	**1**	#19	**1**	#29	**1**
#10	**4**	#20	**1**	#30	**4**

Assuming that the mutations occur at random and that the mutation rate, $a = m/N$.

17.) Estimate the mutation rate for rif^R mutations occurring in the Martian bacteria by using the Poisson expression where the probability of having i mutations per culture is represented by $P_i = (m^i e^{-m})/ i!$. Show your work. (10pts)

12 out of 30 cultures had 0 mutations.
So the probability of having zero mutations (P_i) is 12/30,
and i= 0 mutational events per culture in this situation.

$P_i = (m^i e^{-m})/ i!$
$(12/30) = (m^0 e^{-m})/ 0!$
$0.4 = (1 e^{-m})/ 1$
$0.4 = e^{-m}$
$-\ln(0.4) = m$
$.916 = m$

.916 mutational events per culture
and each culture has 1×10^8 cells

$a = m/N$

$a = .916$ mutational events/1×10^8 cells
$a = 9.16 \times 10^{-9}$ rif^R mutational events per cell division

The gene below encodes a new DNA polymerase from a recently discovered hyperthermophylic bacteria that appears to have high processivity and high fidelity. Your working for a biotech company that is interested in selling the polymerase by over-expressing it and purifying the protein in E.coli Your task is to clone this gene into an expression vector that can be used to generate large quantities of the enzyme. It's genomic sequence, and the sequence immediately upstream and downstream of the gene are provided.

```
5'acctcctggg gctggcgcgc ctcgaggtcc cgggctacga ggcggacgac

     atgctcagat ctacctgcct gagggcgtcc ggttccagct ggcccttccc gaggggagaa        60
     gggaggcgtt tctaaaagcc cttcaggacg ctacccgggg gcgggtggtg gaagggtaac       120
     atgaggggga tgctgccccc ctttgagccc aagggccggg tcctcctggt ggacggccac       180
     cacctggcct accgcacctt ccacgccctg aagggcctca ccaccagccg gggggagccg       240
     gtgcaggcgg tctacggctt cgccaagagc ctcctcaagg ccctcaagga ggacggggac       300
     gcggtgatcg tggtctttga cgccaaggcc ccctccttcc gccacgaggc ctacgggggg       360
     tacaaggcgg gccgggcccc cacgccggag gactttcccc ggcaactcgc cctcatcaag       420
     gagctggtgg acctcctggg gctggcgcgc ctcgaggtcc cgggctacga ggcggacgac       480
     gtcctggcca gcctggccaa gaaggcggaa aaggagggct acgaggtccg catcctcacc       540
     gccgacaaag acctttacca gctcctttcc gaccgcatcc acgtcctcca ccccgagggg       600
     tacctcatca ccccggcctg gctttgggaa aagtacggcc tgaggcccga ccagtgggcc       660
     gactaccggg ccctgaccgg ggacgagtcc gacaaccttc ccggggtcaa gggcatcggg       720
     gagaagacgg cgaggaagct tctggaggag tgggggagcc tggaagccct cctcaagaac       780
     ctggaccggc tgaagcccgc catccgggag aagatcctgg cccacatgga cgatctgaag       840
     ctctcctggg acctggccaa ggtgcgcacc gacctgcccc tggaggtgga cttcgccaaa       900
     aggcgggagc ccgaccggga gaggcttagg gcctttctgg agaggcttga gtttggcagc       960
     ctcctccacg agttcggcct tctggaaagc cccaaggccc tggaggaggc cccctggccc      1020
     ccgccggaag gggccttcgt gggctttgtg ctttcccgca aggagcccat gtgggccgat      1080
     cttctggccc tggccgccgc cagggggggc cgggtccacc gggcccccga gccttataaa      1140
     gccctcaggg acctgaagga ggcgcggggg cttctcgcca aagacctgag cgttctggcc      1200
     ctgagggaag gccttggcct cccgcccggc gacgacccca acc

gcggccaaga ccatcaactt cggggtcctc tacggcatgt cggcccaacc 3'
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18.) Design two 15 base primers that you could use in a PCR reaction to amplify the open reading frame of this gene from the hyperthermophylic bacteria? Indicate the 5' and 3' ends of the primers. (6pts)

5'atgctcagat ctacc3'
5'ggt tggggtcgtc gc3'

19.) You know that you will need to clone your PCR product into a BamHI restriction site, GGATCC, found on the expression vector. Modify your primers below such that you will be able to easily clone the PCR product into a BamHI site on an expression vector. (4pts)

5'(NNNNNN)GGATCC atgctcagat ctacc3'
5'(NNNNNN)GGATCC ggt tggggtcgtc gc3'