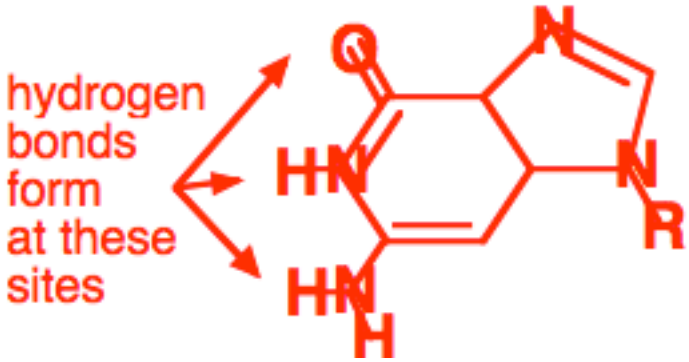
Name _____

Microbial Genetics
Bi 410/510
Winter 2025 Exam I

1.) Draw the structure of guanine. Indicate where the hydrogen bonds form with cytosine. (5pts)



2.) Thermosensitive mutations exist for several of the *E. coli* proteins associated with DNA replication. Describe the role that each of the following proteins play during semiconservative replication AND predict what would happen to replication if that protein were inactivated. EXPLAIN your rationale. (15pts)

DnaB helicase:
separates and unwinds the two DNA strands as replication progresses. Following DnaB inactivation, the replication fork would rapidly be arrested.

DnaG primase:
Repeatedly synthesizes a short RNA primer that allows the lagging strand polymerase to reinitiate on the lagging strand template. Following DnaG inactivation, the replication fork would either arrest or, if it continued, the lagging strand synthesis would not occur.

DNA Ligase:
Joins the Okazaki fragments on the nascent lagging strand following synthesis. Following inactivation of DNA Ligase, the synthesis on the nascent lagging strand would remain discontinuous (contain nicks).

Pol I:
Removes the RNA primer on each Okazaki fragment and resynthesizes it with DNA on the lagging strand. Following inactivation of Pol I, the synthesis on the nascent lagging strand would remain discontinuous (contain nicks).

DNA Gyrase topoisomerase:
Relieves the positive supercoiling induced by replication forks progressing along the DNA. Following inactivation of DNA Gyrase, replication would arrest as the supercoiling stress would poccurring.

3.) *Escherichia coli* replication machinery makes a mistake about once every 10_{10} times it incorporates a nucleotide. *E. coli* O157 is a pathogenic strain that was isolated from hamburgers at Jack in the Box restaurants in the late 1990's that caused severe fevers and even death in several customers. Its genome contains approximately $5 * 10^6$ basepairs. How many changes (mutations) would be expected to occur in its genome each time it replicates? Show your work. (5pts)

$(5 * 10^6$ bases / genome) * (1 error/ $1 * 10^{10}$ bases) =
$(5 * 10^6$ bases / genome) * (1 error/ $1 * 10^{10}$ bases) =
$5 * 10^{-4}$ errors / genome

4.) The actual error (mutation) rate per cell division in *E. coli* strain O157 was measured and it was found to make a mistake about once every 10^6 times it incorporates a nucleotide. How many changes (mutations) occur in its genome each time it replicates? Show your work. (5pts)

$(5 * 10^6$ bases / genome) * (1 error/ $1 * 10^6$ bases) =
$(5 * 10^6$ bases / genome) * (1 error/ $1 * 10^6$ bases) =
5 * errors / genome

5.) The *mutS* gene of *E. coli* O157 is inactivated by a mutation. MutS is part of the methyl directed mismatch repair system. Describe how DNA methylation allows replication to correct errors that were made during replication. (5pts)

Methylation occurs at GATC sites on the DNA. Since the methylation process takes some time to occur, the methyl-directed mismatch repair system is able to identify which strand is the newly replicated (unmethylated) strand of the DNA. Mismatched base pairs are then excised from the unmethylated, daughter strand and the region is then re-replicated to correct the error.

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.

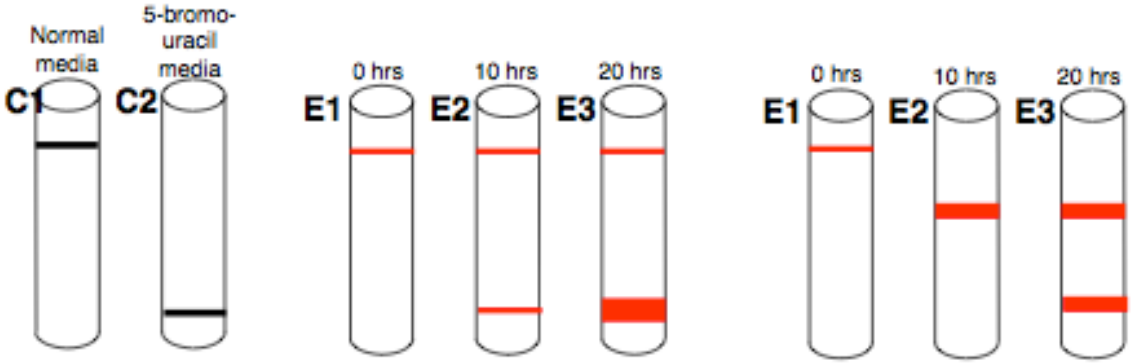
A soil bacteria that was isolated replicates in every 10 hours when grown in lab cultures.
To examine whether replication occurs conservatively or semiconservatively in this bacteria, you decide to utilize a variation of the approach that Meselson-Stahl originally used to examine this question in *E. coli*. For your controls, you grow the bacteria in two separate culture media for several generations.
C1.) One culture is grown in normal media
C2.) The other culture is grown in media that contains 5-bromouracil, an analog of thymine that has a much higher buoyant density.

For your experimental analysis, you inoculate a third culture of the bacteria in normal media and allow it to grow for 2 days. At this time, you then transfer the cells into media containing 5-bromouracil. To examine the mode of replication, you collect a portion of the cells

E1) immediately after changing the media,
E2) 10 hours after you changed the media, and
E3) 20 hours after you changed the media.
You then lyse the cells and load the cell lysates (and DNA) from each sample in neutral CsCl gradients before centrifuging them to equilibrium. The results of your controls, C1 and C2, are shown below.

7.) On the tubes below, clearly indicate where the DNA would be expected appear if the bacteria replicate conservatively? (5pts)

8.) On the tubes below, clearly indicate where the to DNA would be expected to appear if the bacteria replicate semi-conservatively? (5pts)



9.) What DNA sequences are important for factor independent transcriptional termination? How are these thought to promote transcription termination? (5pts) Termination by this mechanism relies upon an inverted repeat sequence that is followed by a stretch of UUUUs in the RNA transcript. Transcription of the inverted repeats produces a hairpin in the RNA that destabilizes the RNA polymerase enough to dissociate it when followed by a string of UA base pairs in the transcription bubble. The UA base pairs are less stable than GC base pairs due to the lower number of hydrogen bonds formed between these base pairs

10.) What DNA sequences are important for factor dependent transcriptional termination? How are these thought to promote transcription termination? (5pts) Factor dependant termination occurs when a protein factor, such a Rho, binds to a specific sequence on the RNA transcript. In the case of Rho, the *rut* sequence is bound. The Rho factor is an RNA helicase that, in effect, chases or follows the RNA polymerase and dislodges it at specific pause sites downstream on the transcript. Since Rho can only bind to *rut* when translation is not "hiding" the rut sequence, this also provides a mechanism to regulate the transcription of polycistronic messages.

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

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     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 caggggggcc cgggtccacc gggcccccga gccttataaa      1140
     gccctcaggg acctgaagga ggcgcggggg cttctcgcca aagacctgag cgttctggcc      1200
     ctgagggaag gccttggcct cccgcccggc gacgacccca acc
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gcggccaaga ccatcaactt cggggtcctc tacggcatgt cggcccaacc 3'

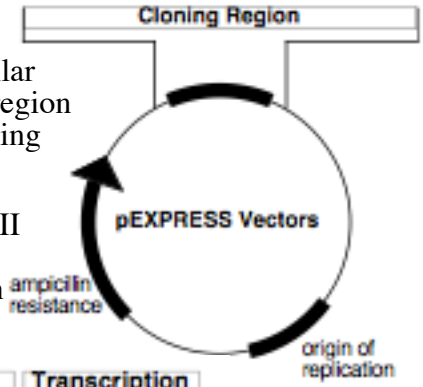
11.) Design two 15 base primers that you could use in a PCR reaction to amplify the open reading frame of this gene from the hyperthermophylic bacteria? Indicate the 5' and 3' ends of the primers. (6pts)

5'atgctcagatctacc3'
5'ggttggggtcgtcgc3'

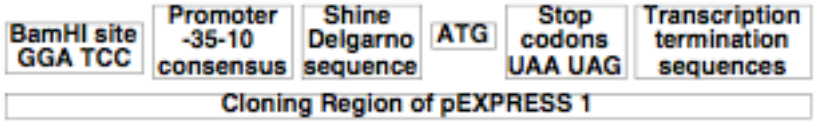
12.) You know that you will need to clone your PCR product into a BamHI restriction site, GGATCC, found on the expression vector. Modify your primers below such that you will be able to easily clone the PCR product into a BamHI site on an expression vector. (4pts)

5'(NNNNNN)GGATCC atgctcagatctacc3'
5'(NNNNNN)GGATCC ggttggggtcgtcgc3'

Your biotech company is using 4 different experimental cloning vectors called pEXPRESS1, pEXPRESS2, pEXPRESS3, and pEXPRESS4. All the vectors have similar constructions (shown to the right). However, the cloning region on each vector is slightly different. Each of the vector cloning regions is shown below.

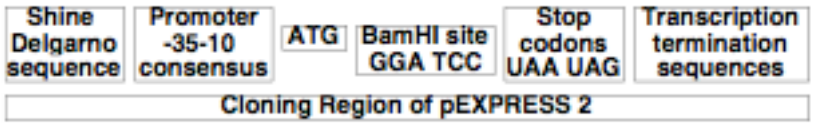


13.) For each vector, determine if the Coagulation factor III product is likely to be expressed when your PCR product is cloned into the Bam HI site. Explain Why or Why not in each case. (10 pts)



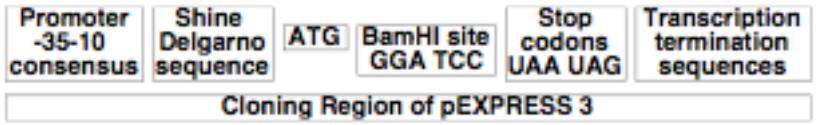
pEXPRESS1:

The polymerase will not be expressed when the PCR product is cloned into the Bam HI site because the gene will be upstream of the promoter and ribosome binding site that are needed for transcription and translation to occur.



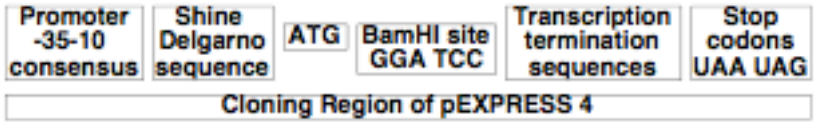
pEXPRESS2:

The polymerase will not be expressed when the PCR product is cloned into the Bam HI site because the ribosome binding site that is needed for transcription is upstream of the transcriptional promoter. Thus the mRNA containingtranslated because it does not contain the Shine Delgarno sequence.



pEXPRESS3:

The polymerase will be expressed when the PCR product is cloned into the Bam HI site. The open reading frame, when cloned into the BamHI site, has a transcriptional promoter, the mRNA have a ribosome binding site before the first start codon as well as additional stop codons prior to the end of the transcript.



pEXPRESS4:

The polymerase will probably be expressed when the PCR product is cloned into the Bam HI site. If the open reading frame includes its own stop codon, the gene should be expressed normally.

The Luria-Delbruck experiment demonstrated that bacteria can become resistant to bacteriophage T1 infection through random mutations, rather than through a directed change. You decide to try and see if the random mutation hypothesis also applies to how bacteria gain resistance to the antibiotic, rifampicin.

You do the following experiment. You inoculate 200 mls of media with a dilute bacterial culture. Then, you split the culture A) growing 100 mls in a single flask, and B) growing the other half in 100 separate, 1 ml test tubes. After allowing the cultures to grow for several hours, you spread A) 1ml aliquots from the single flask on 100 plates that contain rifampicin, and B) the 100 1ml cultures onto 100 plates that contain rifampicin. You allow each set to incubate overnight and count the number of rifampicin resistant colonies on each plate in the morning.

Five representative plates from A) the single 100 ml flask are shown below:

14.) If mutations arose primarily through a process of directed change, CLEARLY diagram how you would expect to five representative plates to look from B) the 100 individually grown 1ml cultures. Explain why. (4pts)

The experiment would predict that each cell has an equal probability of generating resistance to the antibiotic WHEN exposed to it. Since all cells were exposed to the antibiotic at the same time, each plate should have roughly equal numbers of mutations

15.) If mutations primarily arose randomly within growing populations, CLEARLY diagram how you would expect to five representative plates to look from B) the 100 individually grown 1ml cultures. Explain why. (4pts)

The experiment would predict that mutations occur randomly within the population prior to any exposure to the antibiotic...some of these mutations confer resistance to the antibiotic and depending on when they first occur in each culture, a given culture may have many (if the mutation occurred early) or few to no (if the mutation occurred late to never) resistant mutants. The number on each plate should vary significantly.

16.) Does this represent a positive or negative selection? (2pts)
positive. Gain of function

17.) If 20 out of your 100 plates of 1ml cultures had no colonies on them, and the cultures had 10^8 cells/ml, calculate the mutation rate (a) for generating resistence to rifampicin using the Poisson expression, where the probability of having i mutations per culture is represented by $P_{(i)} = (m^i e^{-m})/ i!$ and $a = m/n$. (5pts).
20 out of 100 cultures had 0 mutations.
So the probability of having zero mutations (P_i) is 20/100,
and i= 0 mutational events per culture in this situation.

P_i = $(m^i e^{-m})/ i!$
(20/100) = $(m^0 e^{-m})/ 0!$
0.2 = $(1 e^{-m})/ 1$
0.2 = e^{-m}
-ln(0.2) = m
.916 = m

1.6 mutational events per culture
and each culture has 1 x 10^8 cells

a = m/N
a = 1.6 mutational events/1 x 10^8 cells
a = 1.6 x 10^{-8} rif^R mutational events per cell division

18.) Looking back at your notebook, you see that when you selected for histidine auxotrophs that were no longer able to make their own histidine, mutants arose once in every 100,000 cell divisions.

How does this compare with the mutation rate for obtaining rifampicin resistance? Why are these mutation rates so different if you are using the same strain of bacteria? (5pts)

His- mutants arise more frequently. Histidine metabolism is likely to be controlled by many genes, and a mutation anywhere in this large target of genetic information could inactivate any one of the gene products that are required to make toluene.

Resistance to rifampicin probably requires a very specific mutation at a unique or rare site in one gene that confers the new ability to resist rifampicin. So the chance that this mutation occurs is smaller since the target number of bases that could mutate is so small.