

49 Exams High: 98 Low: 29

A (100-90) - 2

B (89-80) - 9

C (79-70) - 13

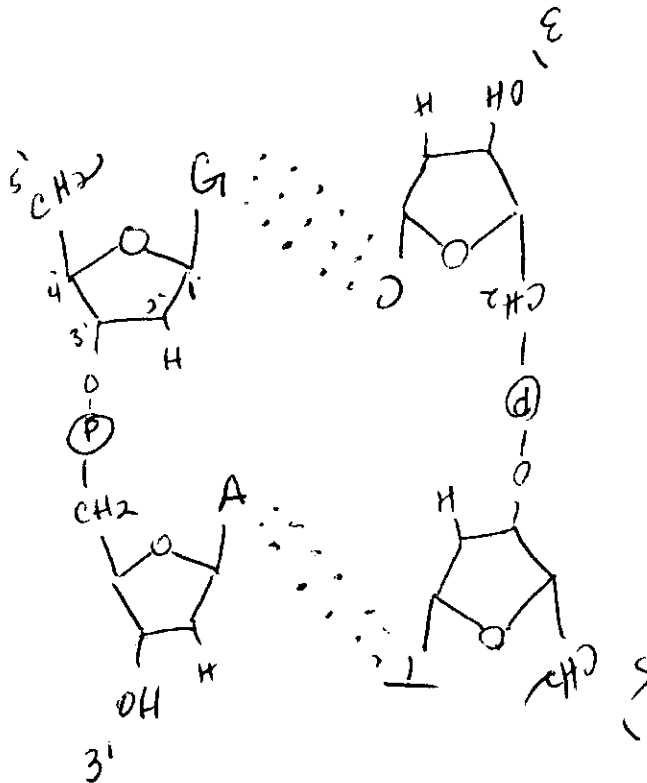
D (69-60) - 13

F (59-) - 12

NAME: Key

MICR/MBMB 460: First Hourly Exam - 2009

1. (10 pts) Draw a **double stranded** DNA molecule in which one strand corresponds to 3'-AG-5'. Indicate the position of the nitrogenous base with the appropriate letter abbreviation (there is no need to draw the base) and the phosphate group with a "P" (there is no need to draw the oxygens). Number the carbons on the sugar of one of the nucleotides and indicate the appropriate hydrogen bonds between base pairs. Make sure to label the 5' and 3' ends of the molecule.



- Had to have nucleotides actually linked 5' → 3'
- Needed to be 5'-G-A-3'

2. (2 pts) What would be the effect of a cell acquiring a nonsense mutation in the middle of the gene for ribonucleotide reductase?

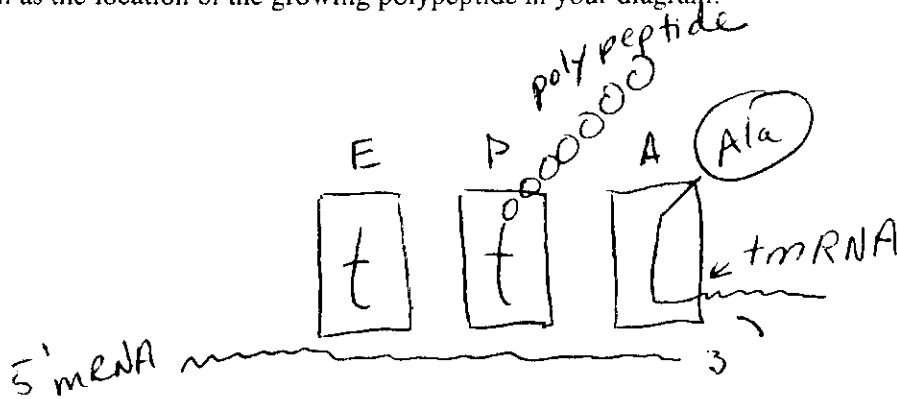
Ribonucleotide reductase would be nonfunctional, thus no dNTPs would be produced

3. (4 pts) Briefly describe how the mutation above could be suppressed?

Nonsense suppressor tRNA - mutation in anticodon of tRNA to recognize nonsense codon introduced into gene for ribonucleotide reductase.

4. (4 pts) During protein trafficking, inner membrane bound proteins must be assisted by the **SecB / Signal Recognition / Twin Arginine Transport** (circle one) pathway during active translation due to the polypeptide's **hydrophilic / hydrophobic / acidic** (circle one) nature.

5. (6 pts) Draw how the ribosome would look upon reaching the 3'-end of a mRNA that does not contain a stop codon. You do NOT have to differentiate between the 50S and 30S, but you MUST include the acceptor (A), exit (E), and peptidyl (P) sites, the molecules residing in each of these sites, as well as the location of the growing polypeptide in your diagram.



6. (8 pts) For the DNA below "P" indicates the promoter region and transcription is from **right to left**. With this information, answer questions (a) through (c).

5' -AAAAGCGATCTATCGCTTAGATTGGGCCGGTGTACATGTGCCTCCTGGAGGA [P] 3' template
 3' -TTTTCGCTAGATAGCGAATCTAACCCGGCCACATGTACACGGAGGACCTCCT 5' coding

mRNA - 5' UCC UCC AGGAGG CAC AUG U¹ A² A³ C⁴ G⁵ G⁶ C⁷ C⁸ A⁹ U¹⁰ C¹¹ U¹² A¹³ A¹⁴ G¹⁵ C¹⁶ G¹⁷ U¹⁸ ...

Start Stop

a) If the RBS is 5'-AGGAGG-3', how many amino acids would be in the polypeptide encoded by this gene?

6

b) While the -10 region is not indicated in this promoter region, what would be the consequence of the sequence being mutated to 5'-TACCGA-3'?

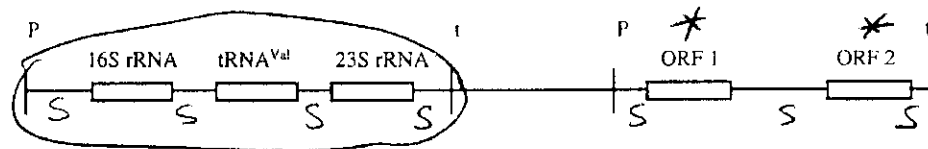
promoter region would not be easily denatured

c) What would be the consensus sequence of the 3'-end of the 16S rRNA molecule in this organism? Make sure to label the appropriate 3' and 5' ends.

3'-UCCUCC-5' or

5'-CCUCCU-3'

7. (8 pts) From the following graphic of a DNA locus:



- How many spacers will be transcribed? *7 or 5 + 1 leader + 1 tail*
- How many spacers will be translated? *0*
- How many citrons are encoded? ** 2*
- Circle the transcriptional unit that will be processed and modified post-transcription.

8. (6 pts) Genotype vs Phenotype:

a) Describe the phenotype (in words) of an *E. coli* strain that has the following genotype: *alaF*, *bla*, *galD*.

Ala⁻: alanine auxotroph - cannot synthesize alanine
Amp^R: resistant to ampicillin
Gal⁻: galactose auxotroph - cannot utilize galactose

b) Would this organism form colonies on the following plates? (Just yes or no for each)

Minimal medium (no amino acids) + galactose + alanine? *No*

Minimal medium (no amino acids) + glucose + ampicillin? *No*

Minimal medium (no amino acids) + galactose (no glucose) + ampicillin? *No*

9. (4 pts)

a) You have created a methylation minus *E. coli* mutant that is unable to methylate its own DNA. In one to two sentences describe what effect if any would this have on replication of the chromosome?

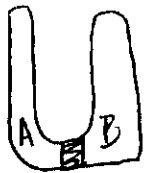
DnaA will not recognize oric, so DNA replication cannot begin

(b) In one to two sentences describe what effect if any this mutation would have on nucleotide excision repair.

No effect - Nucleotide excision does not use methylation state as an indicator.

10. (4 pts) During conjugation, RepA (relaxase) coupling protein (circle one) nicks the F DNA at the *oriT* and attaches itself to the 5'-end IS3 / 3'-end (circle one) of the nicked strand, facilitating transfer of the displaced single strand to the recipient cell.

11. (4 pts) You conduct a U-tube experiment with two bacterial strains and discover that genetic transfer still occurs even if you filter out whole cells. What genetic transfer event likely occurred and what type of genetic transfer event cannot be responsible?



→ No cell to cell contact. Transformation must have occurred and conjugation could not have occurred.

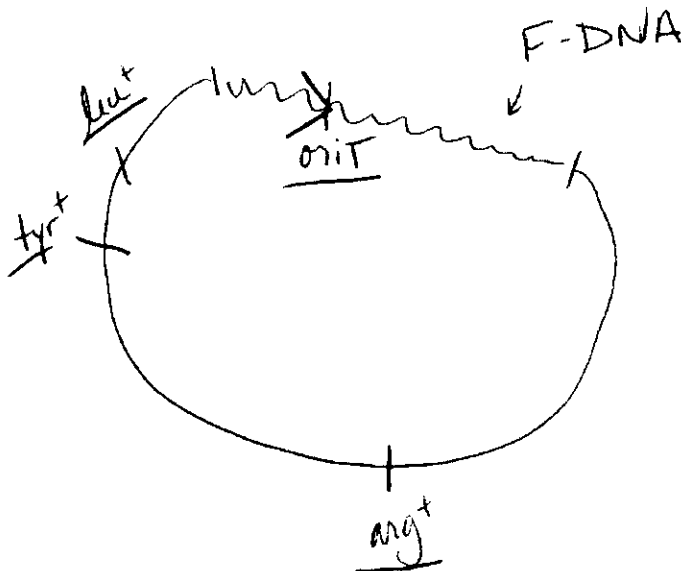
12. (6 pts) During Rho dependent transcriptional termination, Rho binds to the *ter* site (rut site) inverted repeat region (circle the correct answer) on the DNA / polypeptide / RNA (circle one) and dissociates the transcription bubble once it reaches RNA polymerase using its DNA:RNA helicase / endonuclease / RNase H (circle one) activity.

13. (8 pts) You conduct a conjugation experiment in the lab between an F^+ *E. coli* strain (the donor) and an F^- recipient. Unfortunately, you only know that the donor is F^+ (possesses F DNA); you do not know whether the donor is actually F^+ , Hfr or F' . The donor has the phenotype $Arg^+ Tyr^+ Leu^+$ while the recipient is $Arg^- Tyr^- Leu^-$. Following the conjugation event, 90% of the transconjugants were $Arg^- Tyr^- Leu^+$ and 10% of the transconjugants were $Arg^+ Tyr^+ Leu^+$.

a) The only explanation for these results is that your donor strain is F^+ / Hfr / F' . (Circle only one)

b) How many of the transconjugants do you expect to become F^+ ? 0 or $\leq 10\%$.

c) DRAW the location of the F DNA in the donor relative to the *arg*⁺, *tyr*⁺ and *leu*⁺ genes. Make sure to indicate the *oriT* region and provide an arrow indicating the direction of transfer.



cannot determine order of *leu*⁺ + *tyr*⁺, but *arg*⁺ is farther away from *oriT*.

14. (10 pts) Below are some factual and false statements regarding DNA replication in prokaryotes. Number the factual statements in **chronological order** as they occur in *E. coli*. Note that not all of the steps have been represented.

possibly interchangeable

- ~~DNA Pol I synthesizes most of the leading and lagging strands~~
- ~~DnaA binds to hemimethylated *oriC*.~~
- 5 Replication is terminated by Tus protein binding to DnaB of the fork
- 4 DNA Pol I uses 5' → 3' exonuclease activity to remove RNA primers
- ~~Replication is terminated by Tus protein binding to DNA Pol III of fork~~
- ~~DnaB synthesizes the primer~~
- 3 DNA Pol III synthesizes most of the leading and lagging strands
- 2 DnaG synthesizes the primer
- ~~PriA prevents the duplex from reannealing~~
- ~~DNA Pol I uses 3' → 5' exonuclease activity to remove RNA primers~~
- 1 DnaA binds to methylated *oriC*

15. (6 pts) You have isolated an *E. coli* strain that possesses **non-functional** LexA and RecA proteins. Briefly describe how these mutations would affect cells exposed to UV light as well as those cells NOT exposed to UV light. In your answer, you must include: SOS, DNA Pol IV and DNA Pol V. (Words only, please. No drawings.)

If LexA is nonfunctional, then NO repression of SOS genes. Would get transcription of genes for DNA Pol IV and V. Thus cells would acquire numerous mutations due to error prone replication regardless of UV light exposure. Since RecA non-functional, no functional DNA Pol V produced.

16. (10 pts) Briefly describe how the copy number of the plasmid ColEI is regulated in *E. coli*. In your answer, you must include RNAI, RNAII, P_{RNAI}, P_{RNAII}, and RNaseH.

ColEI copy number is regulated by the availability of RNAII. IF RNAII is free, RNase H processes it into a free 3'-OH molecule for replication priming. As the plasmid copy number increases, so does the level of RNAI. RNAI is antisense to RNAII and when the two molecules complementary bind to one another, RNaseH cannot process RNAII. This antisense binding prevents the primer for replication from being formed. As the copy number increases more RNAI is formed because P_{RNAI} is stronger than P_{RNAII}. Only untargeted mut.