Name:

Chemistry 153B – Winter 2024
Midterm 2 –

I understand that academic integrity is highly valued at UCLA. Further, I understand that academic dishonesty, such as cheating and plagiarism, are violations of University policy and will be pursued by the appropriate campus administrator. Finally, my signature below signifies that the work included is my own, and that I completed this assignment honestly.

NAME:

UID:

Signature:

Question 1 (20 pts):

Question 2 (25 pts):

Question 3 (25 pts):

Question 4 (30 pts):

Cheat sheet (2 pts extra credit):

WRITE YOUR ANSWERS IN THE BOXES. ANSWERS OUTSIDE OF THE BOXES WILL BE IGNORED

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For multiple choice, answer by writing a capital letter in the box. Throughout the exam, write short answer responses either inside the boxes or on images as instructed. Answers outside of boxes or images where directed will be ignored.

Question 1 – Multiple choice (20 pts).

1.1: Which of these statements about mismatch repair is **NOT** true? (4 pts):

A: Dam methylase activity is very low during replication
B: MutH cleaves only at hemi-methylated GATC sites
C: MutH only cleaves DNA upon activation by MutL
D: MutU/UvrD helps separate DNA strands after cleavage
E: Eukaryotes do not use MutS for mismatch repair

1.2: The role of DnaA in bacterial DNA replication is (4 pts):

A: Melting the DNA at an origin of replication by stretching it
B: Unwinding the DNA ahead of the replication fork
C: Loading the replicative helicase onto single-stranded DNA
D: Installing primers on the lagging strand during DNA synthesis
E: Loading sliding clamps behind DNA polymerases

1.3: Spontaneous deamination commonly generates DNA defects that include (4 pts):

A: Bulky adducts
B: Interstrand crosslinks
C: Pyrimidine dimers
D: 8-oxo-dG bases
E: Uracil bases

1.4: Which of these statements about Rho-dependent transcription termination is **NOT** true? (4 pts):

A: Rho halts the synthesis of transcripts with a Rho utilization (*rut*) sequence
B: Rho is a hexameric ring-shaped helicase
C: Rho translocation on RNA is slower than RNA synthesis by RNA polymerase
D: Rho uses the energy from ATP hydrolysis to translocate on RNA
E: Rho collides with RNA polymerase to halt transcription

1.5: The misincorporation of riboNTPs during eukaryotic DNA synthesis is mainly resolved by: (4 pts)

A: RNase H and enzymes that process Okazaki fragments
B: 3' to 5' proofreading activity of DNA polymerases δ (delta)
C: 5' to 3' proofreading activity of DNA polymerase I
D: RNA polymerase backtracking and hydrolysis
E: Enzymes that handle nucleotide excision repair

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Question 2 – DNA Replication (25 pts).

2.1: Do the following statements apply to DNA Replication in Bacteria, Eukaryotes, Both Bacteria and Eukaryotes, or Neither Bacteria nor Eukaryotes?
Write "Bacteria", "Eukaryotes", "Both", or "Neither" as appropriate in the boxes below (1.5 pts each).

	Sliding clamps unwind DNA at the replication fork.
	Replication fork polymerases are physically linked together by a flexible protein.
	Primase is fused to a polymerase that extends upon primers with short DNAs.
	Primers can be removed by a nuclease that acts on RNA:DNA hybrids.
	Telomerase is required to prevent the progressive shortening of the genome.
	DNA strands can be synthesized by DNA polymerases in the absence of a primer.
	DNA Polymerase I synthesizes both the leading and lagging strands.
	Nucleosomes are reassembled behind the replication fork.

2.2: The replisome of the bacteriophage T4 (a virus), pictured below adjacent to question 2.3, is strikingly similar to the replisomes of bacteria and eukaryotes that we have studied in class. Write "leading" and "lagging" next to DNA strands on the right-hand side of the image below (3 pts).

2.3: Based on your knowledge of DNA replication, give brief descriptions for the likely roles (5 words max. each) of components labeled A-E on the image of the bacteriophage T4 replisome (5 pts).

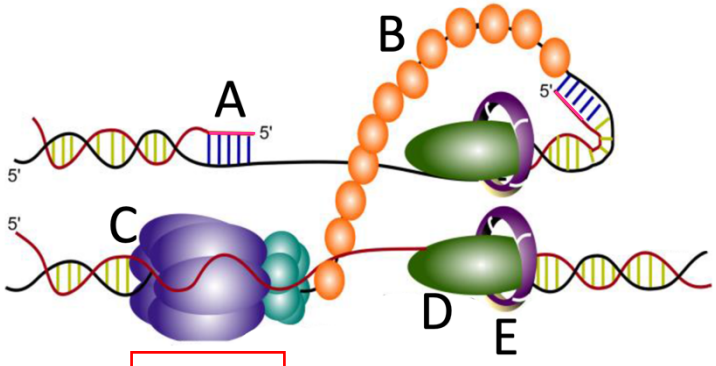
A:

B:

C:

D:

E:



2.4: In the red box, draw an arrow to denote the direction of movement of the replication fork (2 pts).

2.5: What are two essential components of replisomes that we have seen in class but are either not labeled with a letter in the image or are not shown in the image? (3 pts, 1.5 pts each).

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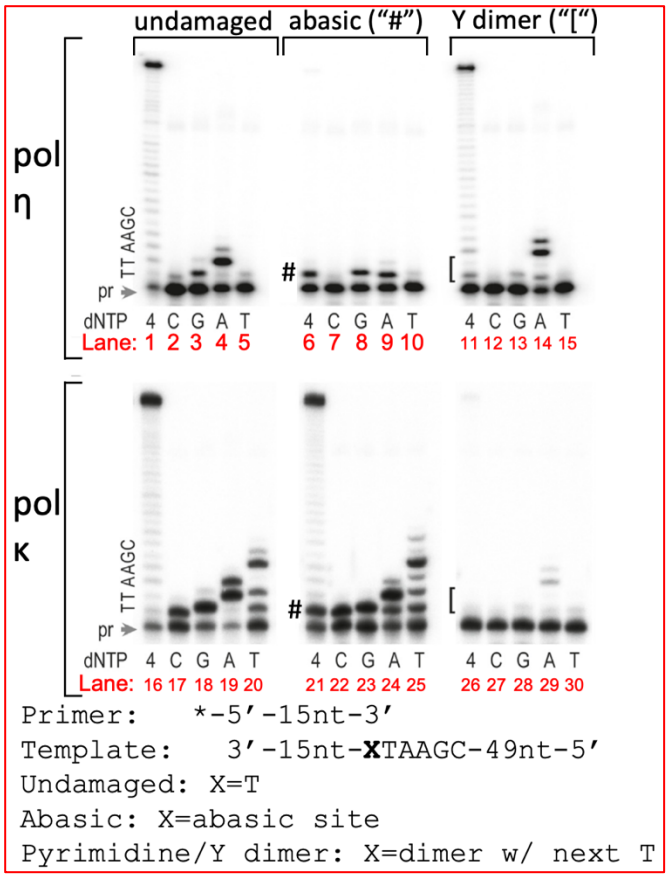
Question 3 – Mismatch Repair (25 pts). Researchers have purified pol η (eta) and pol κ (kappa) to understand their roles in translesion bypass. Polymerases were mixed with a template (undamaged or damaged, as noted above the gel), primer ("pr"), and either a mix of all 4 dNTPs ("4") or a single dNTP (noted below lanes) – see red box to the right. The sequences of the templates (varied in the gels from left to right) are shown below the gel. Lane numbers are noted in red below each gel.

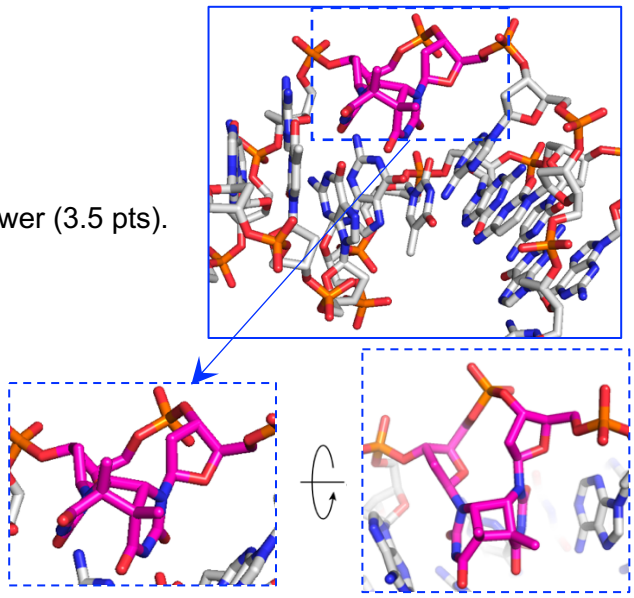


3.1. What polymerase is more processive on undamaged DNA? Explain why and cite two lanes that support your answer (3.5 pts).

3.2: What polymerase copies undamaged DNA with higher fidelity? Explain why and cite two or more lanes that support your answer (3.5 pts).

3.3: What polymerase is more efficient at abasic site bypass? Explain why and cite two or more lanes that support your answer (3.5 pts).

3.4: What polymerase is more efficient at pyrimidine dimer bypass? Explain why and cite two lanes that support your answer (3.5 pts).



3.5: Name the eukaryotic pathway discussed in class that repairs abasic sites (3 pts).

3.6: Name the eukaryotic pathway discussed in class that repairs pyrimidine dimers (3 pts).

3.7: What kind of DNA damage is seen in the blue boxes? The damage is shown in magenta. (2 pts)

3.8: Based on the data above, why are pol η or pol κ not ideal as primary leading/lagging strand polymerases, and instead are best suited for synthesizing only brief stretches of DNA near a site of damage?

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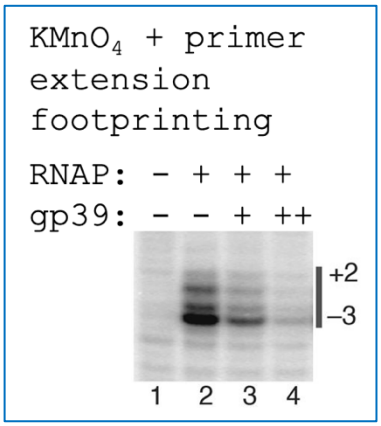
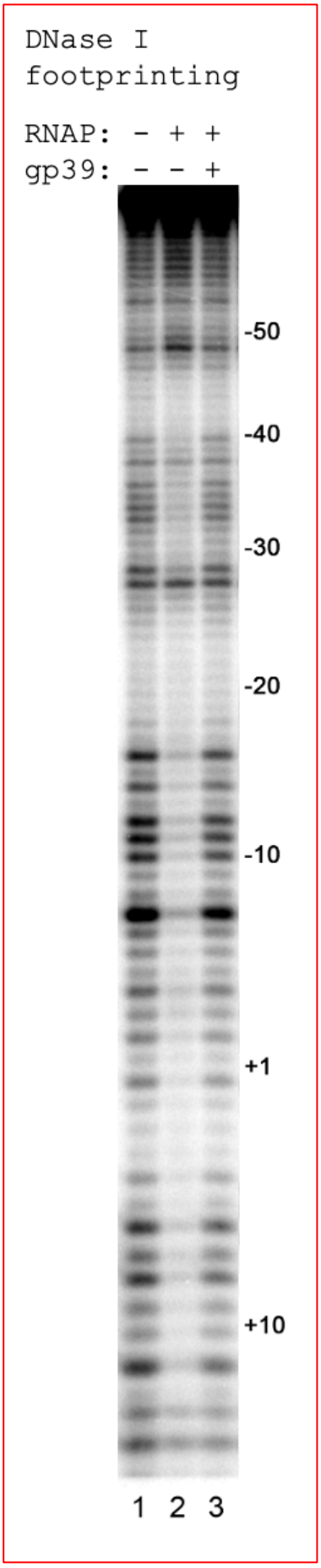
Question 4 – Transcription (30 pts). Researchers used biochemical and structural approaches to study the mechanism by which a bacteriophage (virus) protein, named gp39, affects transcription in the bacterium *T. thermophilus*.

Part 1: Researchers mixed purified bacterial RNA polymerase holoenzyme together with σ^{70} ("RNAP") and double-stranded DNA in the presence or absence of gp39, and conducted $KMnO_4$ (permanganate) footprinting and primer extension (see blue box). No NTPs were included. Numbers on the side of the gel are relative to the transcription start site.





4.1: What information do lanes 1 and 2 provide regarding the impact of RNAP on the status of DNA ($KMnO_4$ footprinting data, blue box)? (5 pts)

4.2: Based on the $KMnO_4$ footprinting data (+ is some gp39, ++ is more gp39), how does gp39 influence the interaction between RNA polymerase and DNA? (5 pts)

Part 2: A DNase I footprinting experiment (red box) was then conducted with RNAP and double-stranded DNA in the presence or absence of gp39. DNase I was included in all reactions, and components included or excluded is listed as +/- above lanes. No NTPs were included. Numbers listed on the side of the gel are relative to the transcription start site.

4.3: From the DNase I footprinting experiment, list all regions the DNA that are bound by RNAP in the absence of gp39 (5 pts).

4.4: Based on the DNase I footprinting experiment, how does gp39 affect the interaction of RNAP with DNA? (5 pts).

Question 4 continues on the next page.

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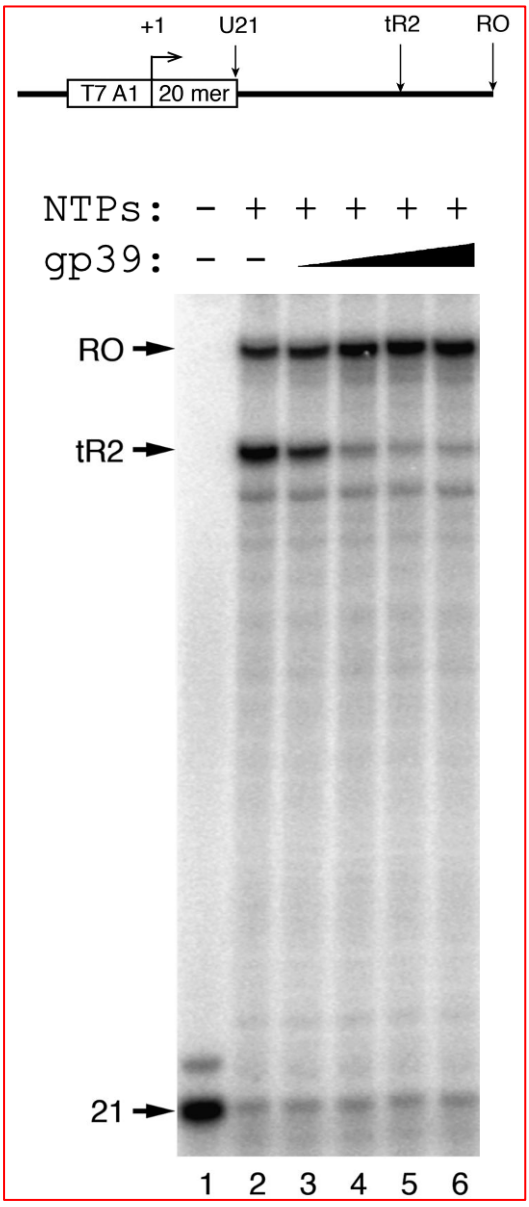
Part 3: In addition to the activities shown above, gp39 was found to influence other stages of transcription. To better characterize the impact of gp39 on transcription, RNAP holoenzyme complexes was combined with a double-stranded DNA template, which is diagrammed on the top of the red box the right. Reactions were paused after the synthesis of a 20-nt long RNA through the omission of UTP from the original synthesis reaction (the first template "A" is at position 21), which is noted as "-" NTPs. All four NTPs were then added back to the reaction ("+" NTPs) in the presence or absence of gp39, and RNA products were separated on a gel and visualized (bottom, red box). The concentration of gp39 increases in reactions 3-6 from left to right as denoted above the gel.



4.5: What is the significance of the "RO" band? (3 pts)

4.6: What is the significance of the "tR2" band? (3 pts)

4.7: Based on this gel, name a specific phase of the transcription cycle that is affected by gp39 and describe the impact (4 pts)

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