Two 21bp dsDNA molecules are shown below. Which molecule will have the higher Tm if they were each placed in separate solutions with the same salt concentration. *Briefly explain your answer*. (10 points)

DNA molecule A: 5' ATAGCGTAGCTGTCGTATCGC 3'
3' TATCGCATCGACAGCATAGCG 5'

DNA molecule B: 5' GCGTAGGGCCGCTGCCTATAC 3'
3' CGCATCCCGGCGACGGATATG 5'

Molecule B would have the higher Tm because it has the greater G+C content as compared to Molecule A

- Considering the DNA sequences only as they are written above, what could you do to make Molecule A's Tm equal to Molecule B's Tm

Without changing the DNA sequence itself, you could place Molecule A in a solution with a higher salt concentration. This would increase the stability of the helix and increase the Tm

- In general terms, what two chemical interactions contribute to the stability of the DNA helical structure?

1. **Hydrogen bonds between the bases**
2. **Stacking interactions between the rings of adjacent base pairs**

Use your knowledge about basic molecular biology techniques to determine the major products produced when the DNA substrate shown below is subjected to the following treatments. After each treatment, write out the products produced: (10 points)

DNA Substrate: 5' AAAAACTG 3'
3' TTTTTGACGTATAGCG 5'

Treatments are done in the following order:
Step 1: DNA polymerase with all the dNTPS. The dCTP is alpha ^{32}P labeled deoxyCTP. This means that the alpha phosphate is the ^{32}P isotope

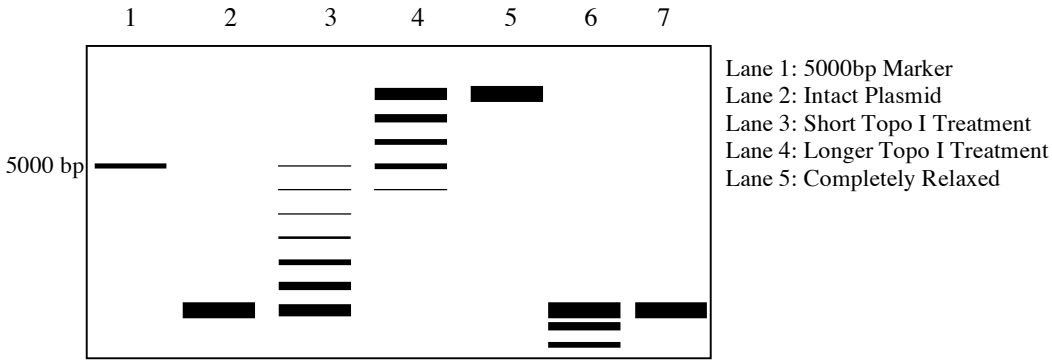
5' AAAAACTGCATATCGC3'
3' TTTTTGACGTATAGCG 5'

Step 2: Heat denaturation of the DNA products from step 1:

5' AAAAACTGCATATCGC3' **3' TTTTTGACGTATAGCG 5'**

The ssDNA piece on the left will have ^{32}P incorporated. The alpha phosphate remains in the newly formed DNA backbone, the beta and gamma phosphates are lost as pyrophosphate and ultimately cleaved into 2 Phosphates. The other strand was the template and the labeled dCTP was not incorporated into the old strand

You have a plasmid that is 5000bp in length. You carefully isolate this plasmid from *E.Coli* and find that it runs significantly faster (lane 2) than a linear 5000bp piece of marker DNA (lane 1).



You carefully mix your plasmid with *E. coli* topoisomerase I for varying amounts of time and run the results in lanes 3 and 4. The completely relaxed plasmid is shown in lane 5.

a. What is Lk° for this plasmid? **5000/10.5=~476**

b. Explain why the intact plasmid runs faster than the linear 5000bp piece of DNA?

It is supercoiled

c. Using the information found in lanes 3-5 on the gel determine:

- Is the plasmid negatively or positively supercoiled? **Negatively, *E.Coli* topo I can only relax negatively supercoiled DNA.**
- How many supercoils were present in the original intact plasmid you isolated? **9, each step up the topoisomer ladder is equal to a change in 1 Lk**
- What was Lk for the original intact plasmid that you isolated? **467=476+(-9)**

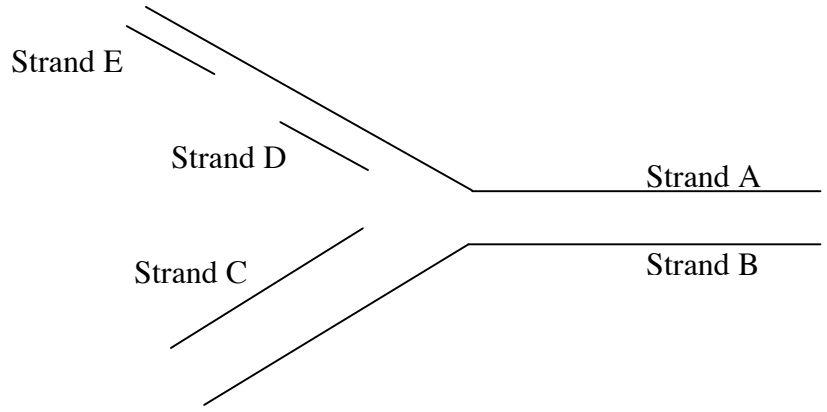
d. You treat the intact plasmid you originally isolated with *E. coli* gyrase and ATP. Show in lane 6 where the plasmid would migrate on the gel.

Gyrase adds negative supercoils, so the original plasmid would become more supercoiled and run even faster in the gel.

e. You treat the intact plasmid you originally isolated with *E. coli* gyrase and no ATP. Show in lane 7 where the plasmid would migrate on the gel.

Gyrase requires ATP to add negative supercoils. There would be no changes made to the plasmid.

The diagram below shows one replication fork. Label the following on the diagram:



A. Label the 5' ends of all the DNA strands pictured: **See Above**

B. Which strand serves as the template for leading strand synthesis?

Strand B is the template for leading strand synthesis

C. Which was synthesized first, strand D or E?

Strand E was the first Okazaki Fragment Made and then the Polymerase "jumped" back to make D

D. How is the gap between strands D and E closed? Be specific about the steps.

a. **RNAse H digests the RNA primer**
b. **DNA polymerase I adds new nucleotides to fill the Gap, using the template as a guid**
c. **DNA ligase seals the gap in the sugar-phosphate backbone between the new DNA and the Okazaki Fragment**

E. If a cell is treated with a chemical that inhibits the synthesis of UTP, why does that inhibit DNA replication if there is no U present in the DNA double helix?

It inhibits the formation of the RNA primer that is produced by Primase. Without a primer, DNA polymerase cannot start polymerizing new DNA