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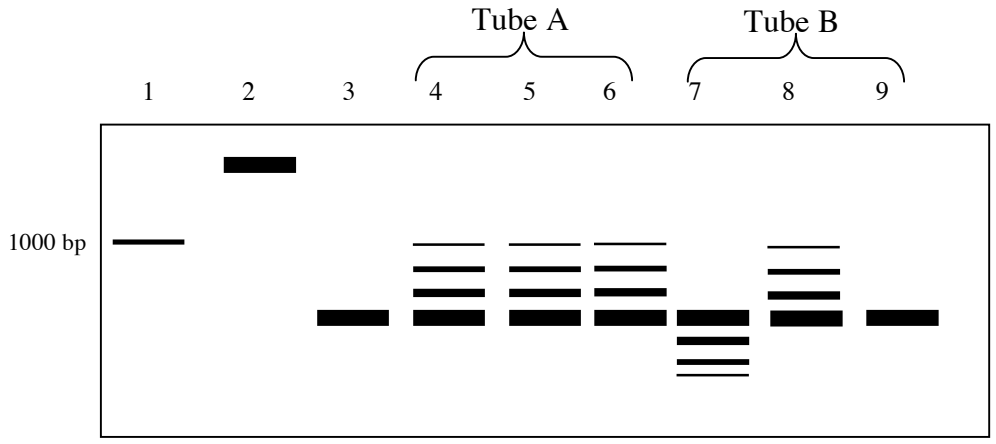
Biol312 Molecular Biology Problem Set 1

1. You study prokaryotic and eukaryotic topoisomerases in your lab. As you are getting ready to do some experiments, you discover that the labels have fallen off of two tubes of enzyme in your freezer and you no longer know what type of topoisomerase is contained in each tube. To determine which type of topoisomerases are in each tube, you pull out a **1000 bp negatively supercoiled closed circular piece of DNA** and a **1000 bp positively supercoiled closed circular piece of DNA** to run several different experiments. You label one tube Enzyme A and the other tube Enzyme B and incubate them with the two types of supercoiled DNA under various conditions. The DNA is isolated after each experiment and then run on an agarose gel. The results of your experiments are outlinde below.

Control Experiments:
- Lane 1: 1000 bp of linear DNA
- Lane 2: 1000 bp nicked, relaxed circular DNA
- Lane 3: 1000 bp supercoiled circular DNA (either positively or negatively supercoiled)

Experiments with Enzyme A:
- Lane 4: 1000 bp **negatively supercoiled DNA** after incubation with Enzyme A and ATP
- Lane 5: 1000 bp **positively supercoiled DNA** after incubation with Enzyme A and ATP
- Lane 6: 1000 bp **negatively supercoiled DNA** after incubation with Enzyme A and no ATP

Experiments with Enzyme B:
- Lane 7: 1000 bp **negatively supercoiled DNA** after incubation with Enzyme B and ATP
- Lane 8: 1000 bp **positively supercoiled DNA** after incubation with Enzyme B and ATP
- Lane 9: 1000 bp **negatively supercoiled DNA** after incubation with Enzyme B and no ATP



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Using the information on the previous page. Answer the following questions.

- What type of topoisomerase is Enzyme A (make sure to indicate whether it is prokaryotic or eukaryotic)? Be sure to explain your choice using the evidence shown in lanes 4-6 in the gel.

Tube A can relax both negative and positive supercoils (Lane 4 and 5). This indicates that it is likely a Eukaryotic topoisomerase. It also can relax the supercoils in the absence of ATP suggesting that it is a Topoisomerase I (Lane 6). So, Tube A likely contains Eukaryotic Topo I

- What type of topoisomerase is Enzyme B (make sure to indicate whether it is prokaryotic or eukaryotic)? Be sure to explain your choice using the evidence shown in lanes 7-9 in the gel.

Tube B increases the supercoiling of negatively supercoiled DNA (Lane 7) and relaxes positively supercoiled DNA (Lane 8). It requires ATP to do this (Lane 9). This suggests that it is the Prokaryotic Topo II protein Gyrase.

2. You have identified a new protein complex that you have called the "posisome" after its ability to bind and wrap DNA around itself forming one positive supercoil. In the lab, you are able to experimentally add five "posisome" proteins to a 15000 bp relaxed circular plasmid.

    - What is Lk° for this plasmid? **15000/10.5 = ~ 1428 (since it must be an integer, it is ok to round to 1428 or 1429)**

    After adding the five "posisome" proteins to the relaxed plasmid, you treat the protein/DNA complex with Eukaryotic Topoisomerase I. After treating the plasmid with Topo I to fully relax any supercoils, you remove the "posisome" proteins and examine the plasmid's final topological state

    - After removing the "posisome" proteins, are there supercoils present in the plasmid? **Yes**
    - If so, are they positive or negative? **Positive**
    - What is the final Lk?

    1428 = 1428 + (+5 supercoils from posisomes) + (-5 supercoils in DNA in response to posisomes)

    After treatment with topoisomerase I the 5 "–" supercoils will be removed, while the 5 positive supercoils remain bound to the posisimes. <u>Because the DNA backbone was broken and resealed by Topo I the LK has changed.</u>

    1433 = 1428 + (+5 supercoils from posisomes)

After removing the posisomes, the 5 positive supercoils remain in the DNA

1433 = 1428+ 5

Final Lk is 1433

- If you had treated the plasmid with Eukaryotic Topoisomerase II (w/ATP), rather than Topo I, to fully relax any supercoils, what would the final Lk be?

Topo II can only relax in steps of two so it could not remove all 5 of the supercoils in the DNA. It would either remove just 4 of the positive supercolis or add one more positive supercoil. Thus you would likely have a mixture of two different Lks in your DNA population.

1428 = 1428 + (+5 supercoils from posisomes) + (-5 supercoils in DNA in response to posisomes)

Remove only 4 of the "-" supercoils in 2 steps of 2 Lk changes:
1432 = 1428 + (+5 supercoils from posisomes) + (-1 supercoils); Final Lk= 1432

Add one more + supercoil in 3 steps of 2 Lk changes:
1434 = 1428 + (+5 supercoils from posisomes) + (+1 supercoils); Final Lk= 1434

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3. You are interested in the studying a DNA binding protein. In your lab you have a piece of dsDNA that contains the binding site for your protein, a purified supply of your protein, an antibody that binds to your DNA binding protein, and the lab supplies to end label your dsDNA fragment. Using these supplies you are going to design a gel shift assay to show that your DNA binding protein binds to DNA.

   - How would you end label your dsDNA probe?

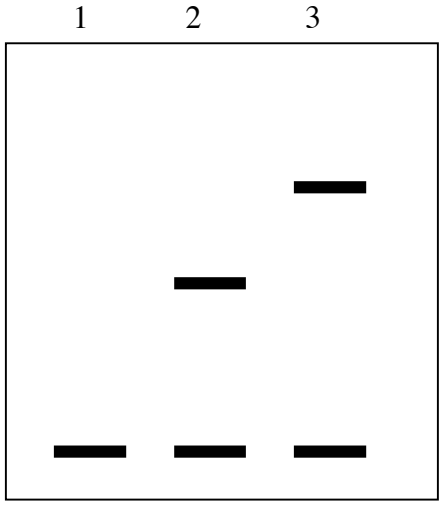
   You would use polynucleotide kinase to add ^{32}P to the OH group on the end of the DNA molecule. You must use a gamma labeled NTP in order for the DNA to become radiolabeled. The polynucleotide kinase adds the last of the three phosphates of the NTP to the DNA.

   - Briefly describe two experiments and one control that you would do to show that your DNA binding protein binds to the dsDNA fragment you have. Draw the predicted results of your experiments as they would appear on a polyacrylamide gel in the box below.

   Control: This is the DNA probe alone. It will show you how the DNA runs in the gel when it is not bound by the protein. (Lane 1)

   Experiment 1: If the protein binds to the DNA, then the protein/DNA complex will be larger than the DNA alone and thus run through the gel more slowly. This will shift the band up the gel. (Lane 2)

   Experiment 2: If you add the antibody to the protein-DNA complex, it will produce an even larger complex that will further slow the migration of the band in the gel. This "super shift" should be above the band seen in Lane 2. (Lane 3)



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