PROBLEM SET 5

1. The electrophoretic mobility of a naturally occurring negatively supertwisted covalently-closed circular DNA 5040 base pairs long is measured in the presence of varying concentrations of an intercalating agent chloroquine. At a concentration of chloroquine in which 360 molecules bind per circular DNA molecule, the DNA was found to migrate at a speed identical with that of a fully relaxed or nicked circular DNA 5,040 base pairs long. Assuming the each molecule of chloroquine that binds to the DNA unwinds the helical twist by 26° upon binding, how many supertwists (writhes) were present in the original DNA sample. What is the average number of writhes are present after binding 360 molecules of chloroquine?
2. Which of the following reactions can be mediated by a prokaryotic type I or a prokaryotic type II DNA topoisomerase? For each mechanism, state which class of topoisomerase, or neither or both, can catalyze the reaction shown.

A. Renaturation of complementary single stranded circles.

\[
\begin{array}{c}
\text{\textbullet} \quad + \quad \bigcirc \quad \rightarrow \quad \bigcirc
\end{array}
\]

B. Catenation of duplex circles.

\[
\begin{array}{c}
\bigcirc \quad + \quad \bigcirc \quad \rightarrow \quad \bigcirc \bigcirc
\end{array}
\]

C. Knotting of single-stranded circles.

\[
\begin{array}{c}
\bigcirc \quad \rightarrow \quad \bigcirc \bigcirc
\end{array}
\]

D. Catenation of a single-stranded circle and an intact duplex circle.

\[
\begin{array}{c}
\bigcirc \quad + \quad \bigcirc \bigcirc \quad \rightarrow \quad \bigcirc \bigcirc
\end{array}
\]

E. Relaxation of a covalently-closed-circular DNA with a single negative writhe.

\[
\begin{array}{c}
\bigcirc \quad \rightarrow \quad \bigcirc
\end{array}
\]

F. Decatenation of interlocked circular duplexes.

\[
\begin{array}{c}
\bigcirc \bigcirc \quad \rightarrow \quad \bigcirc \quad + \quad \bigcirc
\end{array}
\]
G. Resolution of two duplex circles joined in a Holliday structure.
3. List, in order, the enzymatic steps that *Escherichia coli* DNA polymerase I would catalyze using the following DNA template/primer under physiological conditions of nucleotide precursors (0.1 mM each), salt (0.15 M), temperature (37°C), pH (7.5) and divalent ions (5 mM Mg^{++}):
4. Measurements of helical twist in both crystal structures of B-form DNAs and in solution have indicated that the twist angle varies greatly from one base pair to the next as a function of the actual DNA sequence. The helical twist angle between different base pairs can vary from as little as 25° to as much at 47° compared to the average of 34.6° for B-DNA in solution (10.4 base pairs per turn). Describe the primary force causing the deviation of the helical twist angle from the average and describe how this force changes the helical twist. Describe two other changes in the structure of DNA observed in crystals from those in the classical Watson-Crick B-DNA caused by these forces.
5. Unlike prokaryotes, where the superhelix density of DNA is the result of the combined action of two topoisomerases, DNA gyrase and topoisomerase I, the superhelical density of DNA isolated from eukaryotes is the result of the topological folding of eukaryotic DNA in chromatin. Many aspects of chromatin folding contribute to the average superhelical density measured in eukaryotic DNA. Assuming an average of 200 base pairs of DNA per nucleosome (140 base pairs of core DNA and 60 base pairs of linker DNA) estimate the number of writhes each of the following aspects of chromatin folding would contribute to the total writhe of DNA per nucleosome.

<table>
<thead>
<tr>
<th>DNA folding in chromatin</th>
<th>Number of writhes per nucleosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wrapping of nucleosomal DNA in left handed solenoidal path around core histones twice per nucleosome</td>
<td></td>
</tr>
<tr>
<td>Reduction in the pitch of DNA from 10.4 bases per turn to 10.0 base pairs per turn in 140 base pair of core DNA.</td>
<td></td>
</tr>
<tr>
<td>Wrapping of the linker DNA in a left handed solenoidal path in the 300 Å diameter chromatin fiber (assume a pitch of 6 nucleosomes per turn of the solenoid).</td>
<td></td>
</tr>
<tr>
<td>Total writhe expected per nucleosome (sum of above)</td>
<td></td>
</tr>
</tbody>
</table>
6. List three similarities and three differences between the mechanism of initiation of DNA replication at oriC in bacteria and initiation of SV40 DNA replication by the T-antigen.

Similarity 1

Similarity 2

Similarity 3

Difference 1

Difference 2

Difference 3
7. Circle whether each of the following properties is true or false for the base-pairs found in B-DNA crystals:

The base pairs contain complementary rather than identical bases
TRUE or FALSE

The base pairs are flat-coplanar structures
TRUE or FALSE

Base pairs are connected by non-covalent hydrogen bonds
TRUE or FALSE

Purines are in syn conformation about glycosylic bonds
TRUE or FALSE

There is a constant twist angle between each base pair in the DNA
TRUE or FALSE

An obtuse angle between the two glycosylic bonds on the paired bases gives rise to a major and minor groove
TRUE or FALSE

The bases have a propeller twist with respect to each other giving rise to a purine-purine clash
TRUE or FALSE
8. Explain the inverse relationship between the Twist and the Writhe in a covalently closed circular DNA molecule. Define both terms and give a formula relating their relative value assuming that the covalent structure of the DNA is not interrupted.

9. Diagram a hypothetical eukaryotic gene with 2 distributed enhancers, 3 exons and 2 introns. Diagram how it is differentially spliced to give two different protein forms. Label the following in your diagram:

1. enhancers
2. exons
3. introns
4. core promoter
5. cleavage/polyadenylation site
6. upstream promoter region
7. translational start
8. translational stop
9. 3' untranslated region
10. 5' untranslated region
11. transcriptional initiation site
12. transcriptional termination site(s)
10.  
A. Where in a typical mammalian cell is most mRNA processing believed to take place? Does it take place throughout the organelle or at specific locations?

B. Very recently (Nature 385, 357 (1997)) it was found that the C-terminal domain of the largest subunit of RNA polymerase II is required for efficient mRNA splicing and cleavage/polyadenylation. Why was this a surprising finding?

C. It was also found that this domain interacts with several splicing and polyadenylation factors. What functional consequences might the association of these factors with RNA polymerase II have for gene expression in the cell?

11. Differential gene expression is usually investigated by measuring changes in the levels of mRNA’s. Briefly discuss the advantages and disadvantages of choosing to measure this particular parameter among the many that can reflect differential gene expression.
12. Among the genes controlling yeast mating type, there are two silent copies of the alpha- and a-type mating hormone genes called HML-ALPHA and HMR-a and an active copy of one of the genes at the MAT locus in between the silent copies. In addition, there is a gene called HO that encodes an endonuclease that initiates the switching of the mating type gene.

A. Describe the role of histone and non-histone proteins that are responsible for repressing the silent mating type loci. Name the non-histone proteins and the site on the DNA where they act and describe their interaction with the nucleosomal histones on the silent genes.

C. Describe the gene products involved in the switching of mating types and describe their role in the expression of the HO locus.
13. Obtaining Gene & Genome Function from DNA Sequence (Davis)

Two different approaches have recently been developed to determine the phenotype of a disruption in virtually every region of the yeast genome. The first approach uses a transposon to generate, in a parallel fashion, a large number of random insertions in the genome, followed by serial analysis of each 500 base region by gel electrophoresis. The second approach uses a molecularly tagged deletion to generate, in a serial fashion, a precise deletion of any region by homologous recombination, followed by parallel analysis of the deleted regions by hybridization of the tag sequence to a DNA chip.

Suppose a new organism was being analyzed. Given the listed constraints, which of the methods - transposition or deletion - would be the preferred form of whole genome analysis? Circle the correct answer.

A. A very large number of growth conditions are to be analyzed
B. Only the essential genes are to be determined
C. Non-homologous recombination occurs at a much higher level than homologous recombination
D. A large number of small (10 bp) DNA binding sites are to be analyzed
E. There are a large number of tandem copies of genes to be analyzed

14. Provide a rationale for the complexity of pre-mRNA splicing, especially in contrast to the relative simplicity of self-splicing introns.
15. Semi-conservative DNA replication in both prokaryotes and eukaryotes is a complex process requiring many discrete enzymes, each catalyzing a specific kind of reaction. Discuss succinctly the following features of DNA replication. Please indicate the important similarities and differences that have been found between prokaryotes and eukaryotes.

A. Initiation of replication.
B. Formation of primers.
C. Semidiscontinuous DNA synthesis, i.e. leading and lagging strand synthesis.
D. Joining of discontinuously synthesized "Okazaki fragments."

16. State which of the following statements about type I and type II DNA topoisomerases are true or false:

<table>
<thead>
<tr>
<th>Statement</th>
<th>True or False</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I topoisomerases always require ATP in their reactions</td>
<td></td>
</tr>
<tr>
<td>Type I topoisomerases always require a divalent metal ion</td>
<td></td>
</tr>
<tr>
<td>Type I topoisomerases always cleave only one DNA strand during their reaction</td>
<td></td>
</tr>
<tr>
<td>Inhibitors of type I topoisomerases are useful anti-cancer drugs</td>
<td></td>
</tr>
<tr>
<td>Type II topoisomerases always change the linking number of DNA in steps of two</td>
<td></td>
</tr>
<tr>
<td>Type II topoisomerases always relax supercoiled DNA</td>
<td></td>
</tr>
<tr>
<td>Type II topoisomerases always cleave both strands of DNA during their reaction</td>
<td></td>
</tr>
<tr>
<td>Inhibitors of type II topoisomerases are useful anti-cancer drugs</td>
<td></td>
</tr>
</tbody>
</table>
17. State which of the following statements about the replication of the SV40 minichromosome in vitro is true or false:

(a) Replication is initiated at a specific nucleotide sequence and is bidirectional.  
True or False 

(b) The primase that catalyzes the synthesis of the initiating oligoribonucleotide resembles the E. coli primase in that it is associated with a DNA helicase as part of a primosome.  
True or False 

(c) A heterotrimeric single strand DNA binding protein is required.  
True or False 

(a) It requires two distinct DNA polymerases, Pol _ and Pol __  
True or False 

(e) An RNA copy of the SV40 chromosome serves transiently as a template.  
True or False 

(f) It proceeds by a semidiscontinuous mechanism.  
True or False 

(g) A type II topoisomerase is required to resolve the two circular products of the reaction.  
True or False 

(h) Proliferating Cell Nuclear Antigen (PCNA) serves to enhance the processivity of deoxynucleotidopolymerization by Pol __  
True or False 

(i) A DNA ligase is required.  
True or False
18. There are certain sites within the E. coli chromosome known as "hot spots" that have unusually high rates of point mutations. Many of these sites contain 5-methylcytosine. How do you explain the existence of such hot spots?

19. E. coli with a mutation in the rec A gene is unable to promote homologous (general) recombination (<10^-3 of wild type). Such mutants are also defective in the repair of DNA damage resulting from U.V. irradiation. How do you explain this finding?
20. The *E. coli* Replisome

Compare DNA polymerases (DNAP) with RNA polymerases (RNAP).

A. Cite 3 fundamental similarities

B. Complete table below with "yes" or "no" to identify differences

<table>
<thead>
<tr>
<th></th>
<th>DNAP</th>
<th>RNAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can start chains</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Uses ribonucleotides</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Uses deoxyribonucleotides</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Copies very long (multigenic) stretches of DNA</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Edits the copies made</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Processivity, inherent in the polymerase</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>(i.e. not dependent on auxiliary proteins)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uses T to match A</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Uses U to match A</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>
21. Surveying Physiological Differences in Gene Expression

DNA microarrays can be used to monitor thousands of genes in parallel by hybridization of complex nucleic acid mixtures to an ordered array of individual gene sequences.

a) Describe two properties of genes that can be measured using microarrays.

b) Describe how these properties are measured using these arrays.
Chuang, et al., *Science* (1997), **275**, 1468-1471. Ded1p is a newly discovered DEAD box protein in *S. cerevisiae*. Through several lines of evidence the authors have demonstrated that it functions during translation. Shown below are four polysome profiles from a yeast strain bearing a temperature-sensitive allele of the ded1 gene at different times after a shift to the non-permissive temperature. What do these profiles indicate is the step of translation at which Ded1p operates? Briefly explain your reasoning.