1. Protein-DNA Interaction, Bioinformatics (30 pts):

(a; 12 pts) Here is the binding equilibrium for a protein that dimerizes when it binds the DNA, which is the case for leucine zipper proteins. For simplicity, we assume there is no single binding (i.e. no stable PD) and that total [P] = $[P]_{free}$.

$$P + P + D \underset{}{\overset{K_d}{\rightleftharpoons}} P_2D$$

$$K_d = \frac{[P]^2[D]}{[P_2D]}$$

Recalling that we know the total DNA concentration D_T and that all of the DNA must be either free or bound, substitute for $[D]$ to obtain an equation including only K_d, $[P]$, $[P_2D]$, and D_T. Solve for the fraction of DNA bound by protein. What concentration $[P]$ would give 50 % binding?

Score for the page_____

(b; 8 pts) DNA looping is important in transcriptional regulation. How can a bending protein exert "action at a distance" by binding within a loop? Looping can be detected by DNAse I footprinting. One can sometimes distinguish looping from independent binding to two sites by observing periodic changes in the footprinting of the DNA between the two sites. What do you think might cause these changes?

(c; 10 pts) The results below are from a BLAST search. What is the meaning of the "Expect" value? What do the "+" signs mean in the output? What about the dashes in the second query line?

```
Score =  201 bits (511),  Expect = 2e-50

Query  1    MRIILLGAPGAGKGTQAQFIMEKYGIPQISTGDMLRAAVKAGSELGLKAKEIMDAGKLVT  60
            MR++LLG P AGKGTQA  I+ KY IP ISTGDMLR+ +K G+ LG KAKE MD G LV
Sbjct  1    MRLLLLGPPSAGKGTQASGIVNKYHIPHISTGDMLRSNIKQGTALGNKAKEYMDQGLLVP  60

Query  61   DELVIALLKERITQEDCRDGFLLDGFPRTIPQA----DAMKEAGIKVDYVLEFDVPDELI  116
            DELV+A++++R+ Q+DC++GFLLDGFPRT+ QA    D + + G+ +D V+  +VP   +
Sbjct  61   DELVVAIVEDRLQQDDCQEGFLLDGFPRTVVQAKALDDVLDKMGVTLDKVVSIEVPKGTL  120
```

Score for the page_____

2. DNA Replication (39 pts):

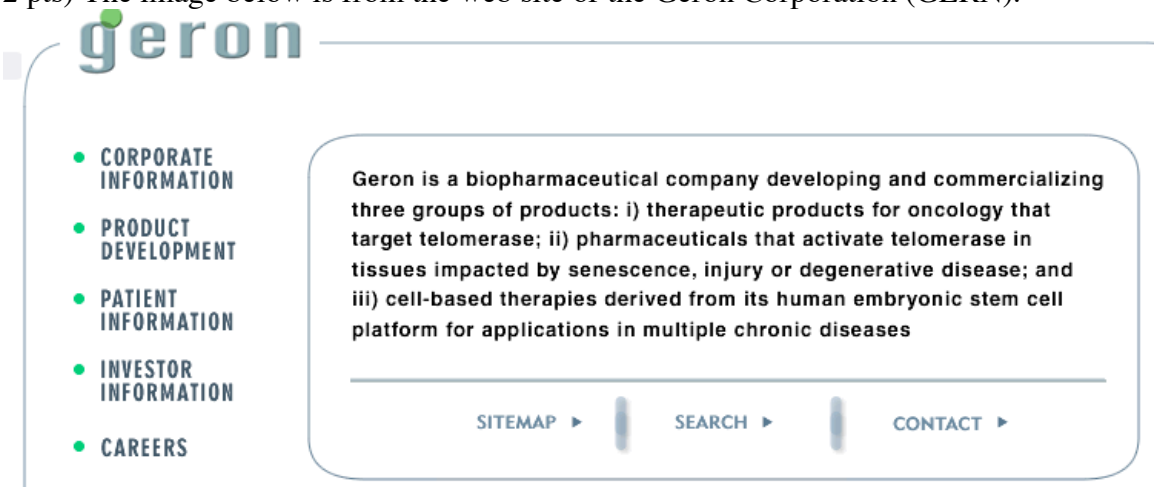
(a; 4 pts) What aspect of the replication fork is captured by the phrase "trombone model?"

(b; 11 pts) Why does kinetic proofreading require an irreversible step? What is the irreversible step in DNA polymerase proofreading? How can DNA polymerase proofreading be described in terms of a molecular clock?

Score for the page_____

(c; 6 pts) Why did primers for lagging strand replication evolve to be made of RNA?

(d; 12 pts) The image below is from the web site of the Geron Corporation (GERN).



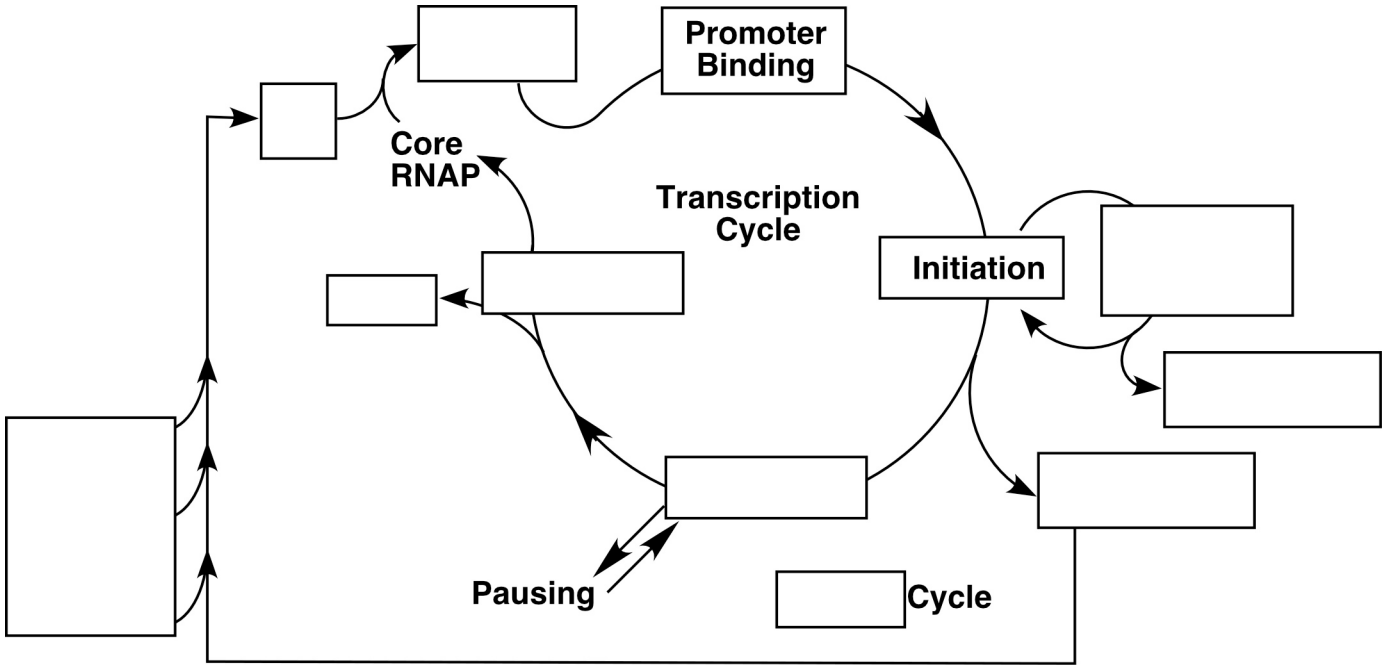
Why would one want to treat cancer by inhibiting telomerase? What might be some of the risks, especially for young people? Why might one want to activate telomerase? What are the risks?

Score for the page_____

(e; 6 pts) Explain why a eukaryotic cell that has lost checkpoint control does not die, but may become hypersensitive to DNA damage agents.

3. Transcription and Regulation (31 pts):

(a; 12 pts) Fill in the boxes in the schematic below. The same answer may appear more than once.



Score for the page_____

(b; 6 pts) We speculated that abortive initiation may be mechanistically inevitable. What is abortive initiation? Why is promoter escape difficult?

(c; 13 pts) What is an AAA+ protein? One example we have looked at is the transcriptional activator NtrC, which is part of a two-component signalling system. What is the name for the downstream partner (i.e. NtrC) in these systems? Describe some evidence that the mechanistic basis of NtrC activation of the $E\sigma^{54}$ holoenzyme is that it alters the conformation of σ^{54}, as opposed to NtrC simply acting as a helicase to open up the DNA. Finally, how is the NtrC activation signal turned off?

Page	Score
1	
2	
3	
4	
5	
6	
Total	

Score for the page_____