

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise and clear. I have given you more space than you should need.

You do not need a calculator for this exam, and no other study aids or materials are permitted.

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Honor Pledge: When you have finished the exam, please write out the following sentence and sign it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. DNA Replication (40 pts):

(a; 8 pts) What is the basis of the "protein footprinting" assay that Naktinis et al. (1996) used to assess interactions among the *E. coli* DNA polymerase III core, β , and γ subunits?

(b; 4 pts) What is the biochemical rationale for gapped DNA but not nicked DNA stabilizing the β core ?

(c; 12 pts) Sketch the “trombone model” for the dimeric DNA replication fork at the instant that the lagging strand polymerase completes the synthesis of an Okazaki fragment. Be sure to label your 5’s and 3’s. Include the Pol III cores, primase, helicase, τ complex/clamp loader, and sliding clamps.

(d; 6 pts) Briefly describe two fundamental differences between the bacterial and eukaryotic cell cycles.

The 3'→5' exonuclease activity of DNA polymerases is essential for improving fidelity over the level that is possible without this dissipative kinetic proofreading.

(e; 4 pts) The exo activity removes a mismatched 3' end only 10-fold faster than a correct 3' end. How is it then that the exo can contribute a factor of about 10^3 to fidelity?

(f; 6 pts) A proposed proofreading mechanism holds that phosphate could be used as the exonucleolytic nucleophile instead of the usual H_2O , giving a dNDP product. Arguing from thermodynamics, discuss why this is unlikely to be correct.

2. Protein-nucleic acid interaction, chromatin (35 pts):

(a; 7 pts) Increasing the salt concentration from 10 mM to 1 M generally reduces the affinity of proteins for nucleic acids. Is this effect primarily enthalpic or entropic? Briefly explain its origin.

(b; 12 pts) Give one-sentence descriptions of (1) direct readout, (2) indirect readout, and (3) recognition via deformability. For two of the three cases give a very brief description of a protein-DNA complex that clearly illustrates the mode of recognition.

(c; 12 pts) Why do zinc finger DNA binding proteins always contain at least two zinc finger modules? Why are zinc fingers particularly suitable for engineering a desired sequence specificity? Why are eukaryotic transcription factors often heterodimers?

(d; 4 pts) What is the cause of rotational positioning of nucleosomes?

3. Methods (25 pts):

(a; 10 pts) What is the fundamental equation for a simple protein-DNA binding curve? Describe an experiment you could use to measure the dissociation constant. Be specific in terms of how you would measure fractional saturation (i.e. give an equation for θ).

(b; 15 pts) Much recent work on the histone code hypothesis is based on chromatin immunoprecipitation, the ChIP assay. Given an antibody specific for acetylated Lysine 20 of histone H3 (an arbitrary example for concreteness), describe how you could use ChIP to find out whether this particular histone modification is associated with activation of YFG. If there were a Serine 21 next to K20, how might this experiment fail?

Page	Score
1	/12
2	/18
3	/17
4	/24
5	/14
6	/15
Total	/100

Score for the page _____