Name _____

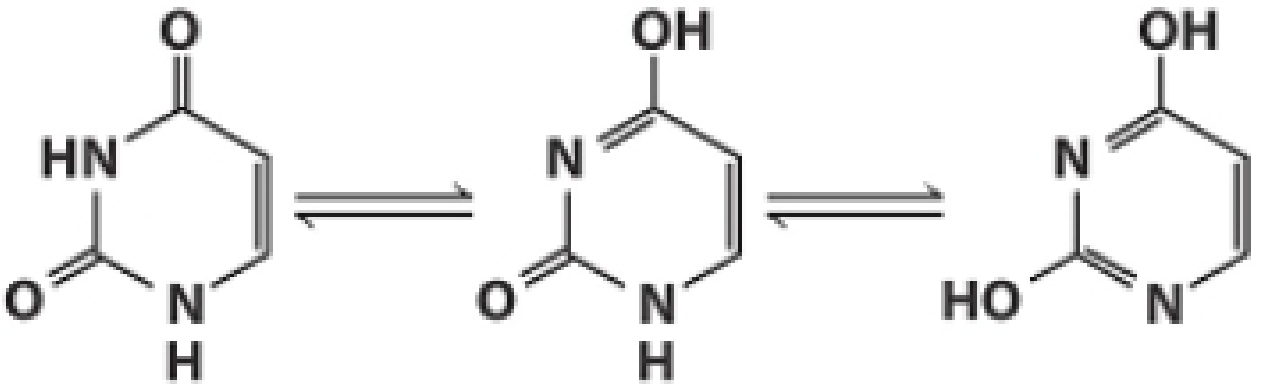
Spring 2005
Biochemistry 302
Exam 1 (90 points total)

Directions: This exam has twenty-two questions/problems totaling 90 points. Check to make sure you have all six pages. Some questions have multiple parts so read each one carefully. Succinct answers are preferred but should you feel compelled to write me a short story, use the back of each page. Feel free to include drawings or cartoons in your answers as you see fit. Partial credit will be given and some questions may have more than one plausible answer. If you get stuck move on to the next question and come back to tackle more challenging questions later. You have up to two hours to complete the exam but it shouldn't take you longer than an hour.

1a. What is the name of the base shown below (1 pt). *uracil*

1b. In what type of polynucleotide would this particular base normally be found (1 pt)? *RNA*

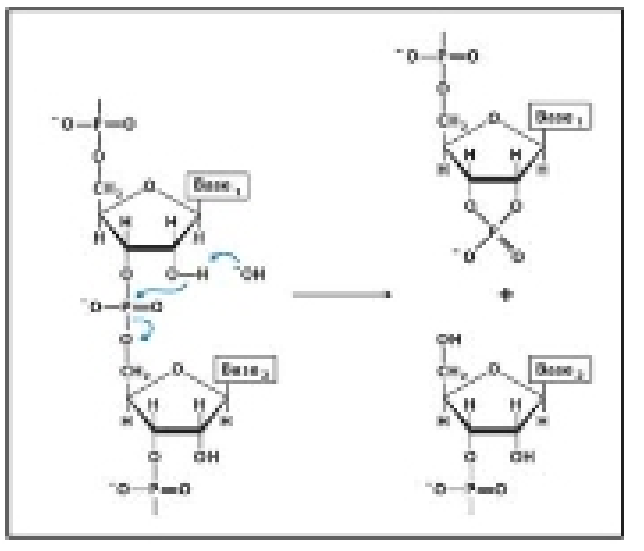
1c. Circle the tautomer that would be predicted to predominate at neutral pH (1 pt). *keto form*



keto

2. RNA has a characteristic structural feature that makes it prone to degradation under alkaline (i.e. basic) conditions. Using either words or by showing a reaction mechanism, describe the chemistry underlying this phenomenon. (4 pts)

Base activates the hydroxyl group linked to the 2′ carbon of ribose to facilitate nucleophilic attack on the phosphorus atom linked to the 3′OH. Electronic rearrangement breaks the phosphodiester backbone in RNA and generates a 2′,3′ cyclic monophosphate derivative in the process.



3. Polynucleotides adopt certain types of secondary structure depending upon their chemical composition. Carefully examine the polynucleotide sequences shown below and 1) predict the helical configuration that each would adopt assuming conditions of physiologic pH, ionic strength, and water content 2) provide a biochemical rationale for your prediction. (4 pts)

a. 5′-CGCGCGCGCG-3′
3′-GCGCGCGCGC-5′

Z-form
dsDNA with CG repeats

b. 5′-GUACCGUAGGCGACCCUACGGUAC-3′

A-form
self complementary RNA hairpin

4. DNA has the propensity to denature under conditions of high temperature or pH. Provide a chemical and thermodynamic explanation for why this is the case. (4 pts)

High temperature or pH (↑ [hydroxide ion]) disrupts Watson-Crick hydrogen bonding between base pairs resulting in strand separation. Conversion from dsDNA to ssDNA is an entropically favorable process augmented by increased temperature ($\Delta G = \Delta H - T\Delta S$).

5. Predict how and explain why the migration of a relaxed plasmid would differ from its supercoiled counterpart during non-denaturing electrophoresis? (3 pts)

   Because it has a more open and extended conformation, a relaxed plasmid would migrate more slowly than its more compact supercoiled counterpart.

6. In both prokaryotes and eukaryotes, zones of initiation of DNA replication exhibit a structural feature that makes them susceptible to strand separation. What is it? (2 pts)

   Abundance of A:T base pairs

7. For each of the functions described below, list the protein and/or enzyme of the *E. coli* replisome responsible on the line provided. Please use general names, which describe the activity rather than *E. coli*-specific gene names like DnaG or DnaB. (8 pts)

   a. Initiation of Okazaki fragment — *Primase*

   b. Relief of superhelical stress — *Topoisomerase or DNA gyrase*

   c. Unwinding of dsDNA template — *Helicase*

   d. Sealing of nicks in lagging strand — *DNA ligase*

8. What are the three different enzymatic activities present in *E. coli* DNA polymerase I? (3 pts)

   5′→3′ polymerization, 5′→3′ exonuclease (RNA primer removal), 3′→5′ exonuclease

9. *E coli* DNA polymerase III holoenzyme is much more processive than single chain DNA polymerase I. A) Define what is meant by processivity in kinetic terms and in the context of DNA synthesis (2 pts). B) Describe, in structural terms, the mechanism by which the holoenzyme acquires and maintains this distinguishing characteristic (3 pts).

   A) Number of nucleotides incorporated in primer/daughter strand per encounter of enzyme with DNA template strand.
   B) Pol III holenzyme is a multi-subunit complex. Certain subassemblies enhance its processivity. Subunits comprising the γ complex (or clamp loader) facilitate the formation of a circular clamp (dimer of β subunit) around the DNA. Protein-protein interaction between core polymerase and the β clamp, which presumably can slide along the DNA, tethers the holoenzyme to the DNA (or prevents its dissociation from the DNA).