

You have 50 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 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: At the end of the exam time, please write out the following sentence and sign it, or talk to me about it:

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

(+1 pt)

**1. DNA Structure and Base Pairing (25 pts):**

(a; 15 pts) Draw a plausible G•A-U triple base pair with at least two H-bonds between the G and the Watson-Crick A-U pair.

(b; 10 pts) Enter “A” for A-form, “B” for B-form. or “Neither” or “Both” in the in the table below.

|   |          |
|---|----------|
| Original Watson-Crick Model   | <b>B</b> |
| Helical rise and base pair spacing are the same                     |          |
| Helix axis runs through “empty space”                               |          |
| Stabilized primarily by base stacking                               |          |
| Large base pair inclination   |          |
| C2'-endo sugar pucker   |          |
| Characteristic of dsRNA   |          |
| Each base pair has a pseudodyad axis that intersects the helix axis |          |
| The helix axis is nearly perpendicular to the base pair plane       |          |
| “Cavernous” major groove  |          |
| Looking into the minor groove, left strand runs 5'→3' bottom to top |          |

## 2. Secondary and tertiary structure (20 pts):

(a; 16 pts) Propose a secondary structure for the RNA below. Each line is a sequence from a homologous RNA, with changes boxed and in bold. The underlined regions are single-stranded by nuclease mapping, and X-link indicates a psoralen crosslink.

5' GCAUG AACUG AAAAG UUGGU AUUCG UAACC CAUGC 3' X-link

5' GCAUG AAC**C**G AA**A**GG UUG**G**C AUUCG U**G**ACC CAUGC 3'

5' G**G**AUG AACUG **C**AAAG UUG**G**C AUUCG U**G**ACC CAU**C**C 3'

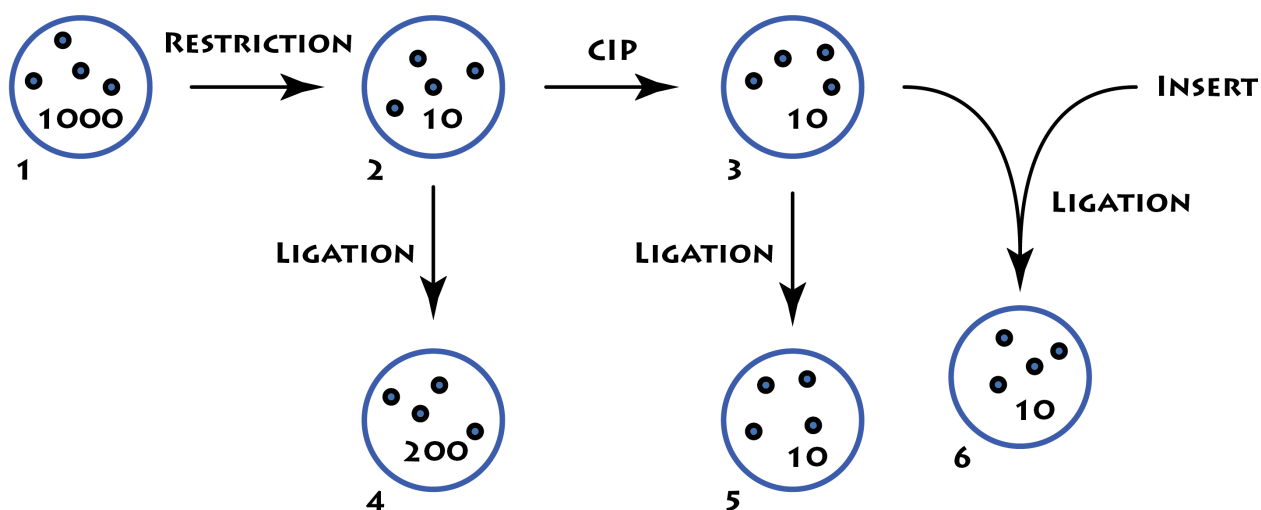
5' G**C**UG AACUG **U**AAAG UUG**A**C AUUCG UGA**U**C CA**G**GC 3'

5' G**C**UG AA**G**UG AAA**A**C UUG**A**C AUUCG UGA**U**C CA**G**GC 3'

(b; 4 pts) Why is a divalent cation like  $Mg^{++}$  typically required for the formation of RNA tertiary structure?

### 3. Basic Cloning (19 pts)

(a; 15 pts) The cloning experiment below did not work. Each circle indicates a plate of bacteria, with the number inside the plate being the number of colonies. All colonies are blue. Which plate gives an undesirable answer, and what should it have looked like? There are at least two possibilities for what went wrong. What are they, and how could you distinguish them or solve them in the next cloning attempt? [Hint: if your research director had been willing to spring for a phosphatase from an Antarctic shrimp instead of a cow, one of the possibilities could have been prevented.]



(b; 4 pts) Give a reason that 5' overhangs are generally more useful in cloning or manipulation of restriction fragments than 3' overhangs.

**3. DNA topology (24 pts):**

(a; 18 pts) Draw three plasmids with the following properties:

1.  $\Delta Lk = -4$ ,  $\Delta Tw = 0$ , plectonemic superhelix
2.  $\Delta Lk = +3$ ,  $\Delta Tw = 0$ , toroidal superhelix
3.  $\Delta Lk = -3$ , 3 turns of the helix unwound to make denaturation bubble.

(c; 6 pts) What are the linking number changes introduced by Type I and Type II topoisomerases? Topoisomerase poisons are useful anti-cancer agents. What makes a topoisomerase halted in the middle of its catalytic cycle particularly toxic to a cell (e.g. as opposed to a random metabolic enzyme that is inactivated by a drug)?

**4. DNA Flexibility (12 pts):**

(a; 12 pts) Define  $\rho$ ,  $P$ , and  $L$  in the equation  $\langle \rho \rangle = P(1 - e^{-L/P})$ . Which one do we control as experimentalists? Sketch an typical structure for a DNA with  $L = 3P$  that has the average value of  $\rho$ .

| Page  | Score |
|-------|-------|
| 1     |       |
| 2     |       |
| 3     |       |
| 4     |       |
| 5     |       |
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Score for the page\_\_\_\_\_