X107A Test #2, Fall 2000, Answer Key

Very nice! 14 = A; 13.5 = A-; 13 = B+; 11 = B; 10 = B-; 9 = C+; 7 = C; 5 = C-.
Mean = 13.4, median = 14.5 (only 0.5 below that for Test 1). Top scores = 18, 2 at 17.

Estimate your current course grade by averaging the letter grades on the two tests so far. If they differ, round towards #2. The final will be weighted equally with these two, i.e., as 1/3 of total grade.

Brief answer key. If there are questions, we can discuss them in class or privately.

1. The moving Pol opens up the DNA (unwinds it) ahead of the Pol. If there is any resistance of the DNA, it must compensate by overwinding at that region, i.e, ahead of the Pol. (Weaver p 169; Ch 6 handout Sect K.)

> Behind the moving Pol, the DNA re-winds, and compensating negative supercoiling should occur.
>
> This applies to elongation phase, as the Pol is moving forward. It is not correct during formation of the open complex. At that point, the DNA is opened, and overwinding would be induced both in front of and behind the Pol.

2. a. The control lane should show a series of bands at all possible lengths. The experimental lane should be similar, but with 29 bands missing, over the indicated region. (No, I didn't count how many you showed! But you should label the result.) The result should be something like Weaver's Fig 5.32, bottom frame. The region involved here is just in front of *argF*; given the spacing shown on the map, it would just about fit in the 43 nt space between ORF8 and *argF*.

b. The region covered by the regulatory protein closely corresponds to where RNA Pol binds. It is hard to imagine how a protein covering that whole region could help Pol bind (activator protein, positive control). It is easier to imagine that it interferes with Pol binding (repressor protein; negative control).

> The question asked "most likely"; I did not accept "can't tell". I think the clue was fairly strong, and most of you agreed.

c. It looks like a ρ-independent transcriptional terminator, to mark the end of a transcript. The key features are the GC-rich hairpin, followed immediately by a run of U. It is from the transcript for ORF4; it is the end of a transcript "near the beginning of ORF5".

d. It is not likely to be involved in making arginine. Logically, one expects Arg to repress synthesis of enzymes to make Arg, but the data says that Arg induces ORF4. This would be expected if the ORF4 gene product helps to utilize exogenous Arg, or helps to degrade excess Arg.

> The function of this product is not known. From its sequence, they suggest that it is a membrane protein, and speculate that it is involved in Arg transport.

Based on: R Sanchez et al, Organization and expression of a Thermus thermophilus arginine cluster: presence of unidentified open reading frames and absence of a Shine-Dalgarno sequence. J Bacteriol 182:5911-5915, 10/00. Both Figures on the test are from their Fig 1.

3. Deletion expts change the spacing between those DNA regions that remain; linker scanning expts do not. Thus a good interpretation is that the regions that the linker scanning points to as important are important -- and so is their spacing. Thus the results would point to two binding sites, with a critical spacing between them.

The -10 and -35 promoter elements would be a model here. However, note that there is no info about what the sites are for. The results give no clue as to whether the sites are for Pol or activator proteins, or whatever.

It is not fair to say that linker scanning is "more accurate". If you omitted the deletion expts, you would not learn about the critical spacing. Both expts contribute useful info.

One might argue that any particular linker scanning experiment could give a "wrong answer", if the linker sequence was satisfactory. This is plausible, but quickly becomes unreasonable with any significant amount of data. Even if a single linker is tested, it is unlikely that that linker is ok at any position.

4. a. You should show the gene (*proP*), the start site (+1), the promoter (presumed to be at -10 and -35), the Fis binding site (at -41), and the CRP binding site (at -121).

There is no basis for showing an operator (or did I miss something?), and I took off if you did show one.

It is good to label the positions with nucleotide numbers, but I did not grade on this. (I intended that you should show the numbers, but the example I gave you did not have them.)

Showing the proteins in your sketch here was unnecessary, and sometimes was actually confusing.

b. The key idea is that cis dominance refers to a mutation having an effect only on genes on its own chromosome. [Careful with terminology. "Same chromosome" is correct; "same strand" or "same gene" is not. And "mutation" cannot be replaced with "mutant" (Ch 1 handout).] This typically occurs for mutations in DNA "sites" that bind proteins (rather than for genes that code for proteins). In this case, you can use the binding site for Fis or for CRP (or even for σ). It is shown by using two marker genes, so you can tell which chromosome is functioning.

The system as provided only has one gene, but you can add your own reporter genes. In fact, the given gene is not easy to measure (a transport protein), so I will choose to use two marker genes, the common z and a genes from the *lac* operon. In the following diploid, all regulatory elements are from the *proP* gene, but the proteins are from the *lac* operon.

There are many possible examples; here is one. + means that the gene or site is wild type (functional); - means that it is not.

C^+	F^+	P^+	z^+	a^-
C^+	F^-	P^+	z^-	a^+

In this case, we have a defective Fis-binding site (F) on the lower chromosome. As a result, the a enzyme cannot be made even when the Fis protein is present; the only a^+ allele is behind a defective Fis-binding site. However, at the same time, the z enzyme can be made, since the chromosome containing the z^+ allele has a Fis-binding site.

> You should end up with something logically equivalent to Ch 7 hw #1cd. But the point was to translate that concept to one of the current regulatory sites.
>
> If you tried to present this without a drawing of the chromosomes, that makes it difficult. I did not take off for lack of a drawing per se, but a drawing greatly increases clarity. I suspect some were confused by your own lack of a drawing.
>
> I realized later that Weaver is not very clear about the experimental approach, say in Fig 7.4. But we were clear in the handout, in class, and in homework. It is odd that he left out the experimental aspects here, given his usual emphasis on such. I am also surprised that no one said anything. In any case, I did expect a level of presentation consistent with handout, but I gave good partial credit, if it was clear you understood the conceptual point.

c. The complex should include RNA Pol with σ bound to the promoter, Fis bound to its site and to a Pol α subunit, and CRP bound to its site and to the other Pol α subunit. The DNA should be bent, to allow CRP to bind to its site and to Pol.

> I didn't require that you show the DNA bent, but it is hard to imagine the complex without bending the DNA. The distance form p to the CRP site is a long distance for a protein to cover. Most of you did show bent DNA.
>
> Based on: S M McLeod et al, Coactivation of the RpoS-dependent proP P2 promoter by Fis and cyclic AMP receptor protein. J Bacteriol 182:4180-4187, 8/00.

5. a. The upper line must be the template, so it is paired with the bases shown. That strand must be 3′ to 5′ (left to right), to be antiparallel with the given RNA strand.

b. Show a nucleoside triphosphate, with its α-phosphate (the one nearest the sugar) attacking the 3′-OH of the growing strand that is given. Pyrophosphate (PP_i) is released.