# Chapters 12 & 10 Homework

*Ch.12: 2, 3, 6, 7, 13, 19, 24, 36; Ch. 10: 14, 15, 17, 18, 25, 32-37*

2. Based on the information in Figure 12-6, how does Gal4 regulate four different *GAL* genes at the same time? Contrast this mechanism with how the Lac repressor controls the expression of three genes.

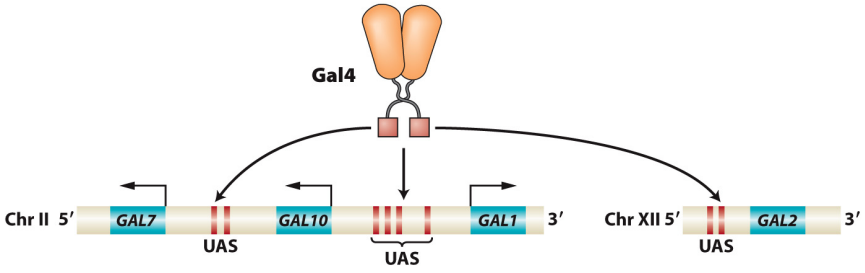


**Figure 12-6**
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3. In any experiment, controls are essential in order to determine the specific effect of changing some parameter. In Figure 12-7, which constructs are the "controls" that serve to establish the principle that activation domains are modular and interchangeable?

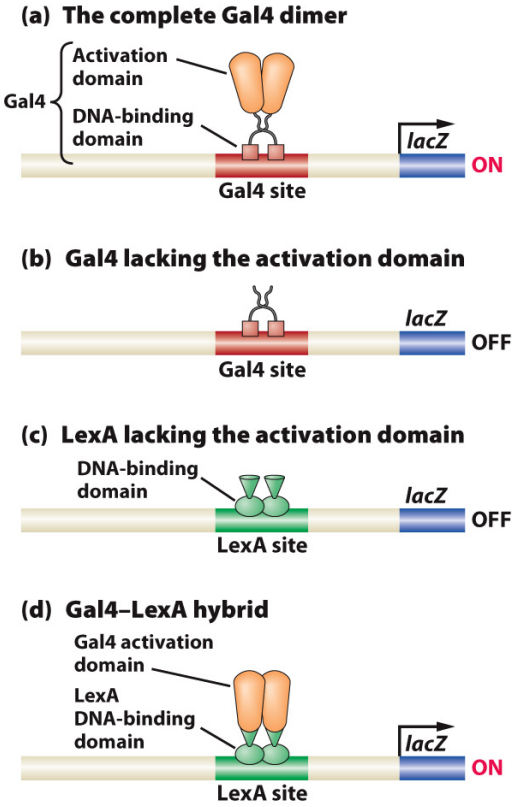


**Figure 12-7**
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6. What is the conceptual connection between Figures 12-12 and 12-19?

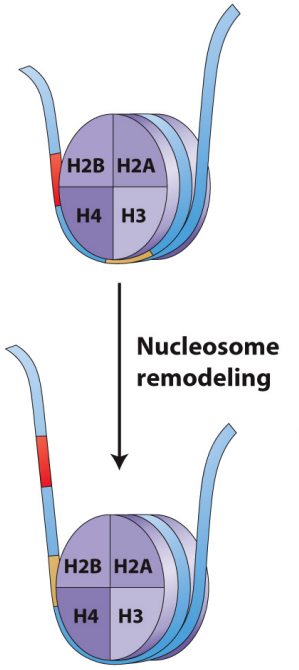


**Figure 12-12**
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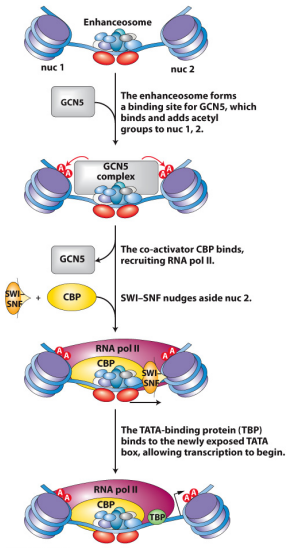


**Figure 12-19**
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7. In Figure 12-19, where is the TATA box located before the enhanceosome forms at the top of the figure?

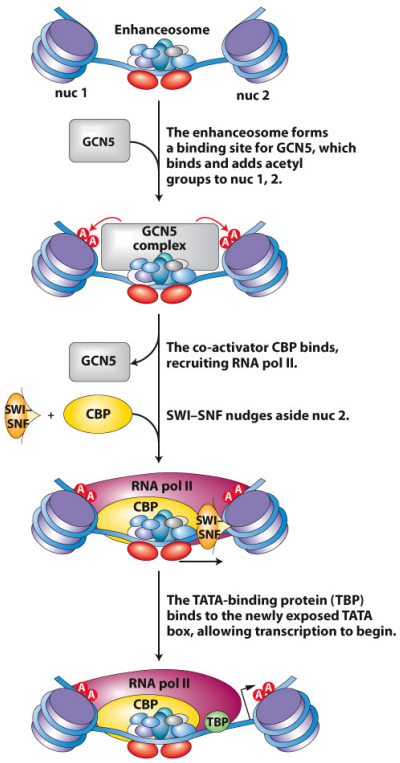


**Figure 12-19**
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13. Predict and explain the effect on GAL1 transcription, in the presence of galactose alone, of the following mutations:
    a. Deletion of one Gal4-binding site in the GAL1 UAS element
    b. Deletion of all four Gal4-binding sites in the GAL1 UAS element
    c. Deletion of the Mig1-binding site upstream of GAL1 d. Deletion of the Gal4 activation domain.
    d. Deletion of the GAL80 gene
    e. Deletion of the GAL1 promoter
    f. Deletion of the GAL3 gene

19. What is meant by the term *epigenetic inheritance*? What are two examples of such inheritance?

24. What is the fundamental difference in how bacterial and eukaryotic genes are regulated?

36. Null alleles (mutant genes) produce no protein product. This is a genetic change. However, epigenetically silenced genes also produce no protein product. How does one determine experimentally whether a gene has been silenced by mutation or has been silenced epigenetically?

14. Why is ligase needed to make recombinant DNA? What would be the immediate consequence in the cloning process if someone forgot to add it?

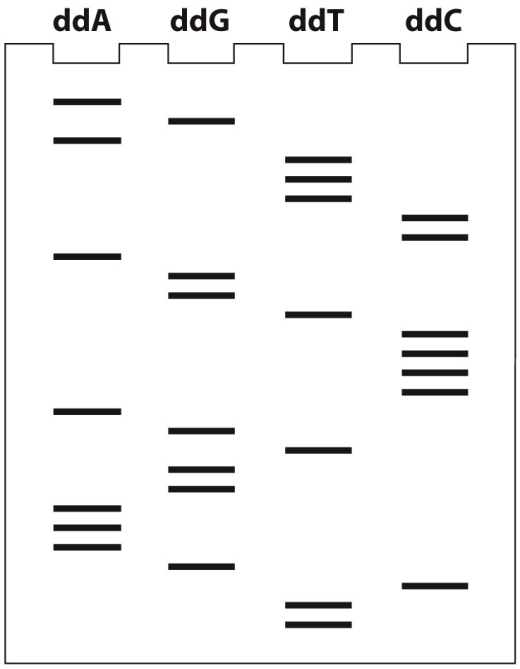
15. In the PCR process, if we assume that each cycle takes 5 minutes, how many-fold amplification would be accomplished in 1 hour?

17. You obtain the DNA sequence of a mutant of a 2-kb gene in which you are interested and it shows base differences at three positions, all in different codons. One is a silent change, but the other two are missense changes (they encode new amino acids). How would you demonstrate that these changes are real mutations and not sequencing errors? (Assume that sequencing is about 99.9 percent accurate.)

18. In a T-DNA transformation of a plant with a transgene from a fungus (not found in plants), the presumptive transgenic plant does not express the expected phenotype of the transgene. How would you demonstrate that the transgene is in fact present? How would you demonstrate that the transgene was expressed?

25. In yeast, you have sequenced a piece of wild-type DNA and it clearly contains a gene, but you do not know what gene it is. Therefore, to investigate further, you would like to find out its mutant phenotype. How would you use the cloned wild-type gene to do so? Show your experimental steps clearly.

32. A cloned fragment of DNA was sequenced by using the dideoxy chain-termination method. A part of the autoradiogram of the sequencing gel is represented here.




**Problem 10-32**
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a. Deduce the nucleotide sequence of the DNA nucleotide chain synthesized from the primer. Label the 5' and 3' ends.
b. Deduce the nucleotide sequence of the DNA nucleotide chain used as the template strand. Label the 5' and 3' ends.
c. Write out the nucleotide sequence of the DNA double helix (label the 5' and 3' ends).

33. The cDNA clone for the human gene encoding tyrosinase was radioactively labeled and used in a Southern analysis of *EcoRI*-digested genomic DNA of wild-type mice. Three mouse fragments were found to be radioactive (were bound by the probe). When albino mice were used in this Southern analysis, no genomic fragments bound to the probe. Explain these results in relation to the nature of the wild-type and mutant mouse alleles.

34. Transgenic tobacco plants were obtained in which the vector Ti plasmid was designed to insert the gene of interest plus an adjacent kanamycin-resistance gene. The inheritance of chromosomal insertion was followed by testing progeny for kanamycin resistance. Two plants typified the results obtained generally. When plant 1 was backcrossed with wild-type tobacco, 50 percent of the progeny were kanamycin resistant and 50 percent were sensitive. When plant 2 was backcrossed with the wild type, 75 percent of the progeny were kanamycin resistant and 25 percent were sensitive. What must have been the difference between the two transgenic plants? What would you predict about the situation regarding the gene of interest?

35. A cystic-fibrosis mutation in a certain pedigree is due to a single nucleotide-pair change. This change destroys an *EcoRI* restriction site normally found in this position. How would you use this information in counseling members of this family about their likelihood of being carriers? State the precise experiments needed. Assume that you find that a woman in this family is a carrier, and it transpires that she is married to an unrelated man who also is a heterozygote for cystic fibrosis, but, in his case, it is a different mutation in the same gene. How would you counsel this couple about the risks of a child's having cystic fibrosis?

36. Bacterial glucuronidase converts a colorless substance called X-Gluc into a bright blue indigo pigment. The gene for glucuronidase also works in plants if given a plant promoter region. How would you use this gene as a reporter gene to find the tissues in which a plant gene that you have just cloned is normally active? (Assume that X-Gluc is easily taken up by the plant tissues.)

37. The plant *Arabidopsis thaliana* was transformed by using the Ti plasmid into which a kanamycin-resistance gene had been inserted in the T-DNA region. Two kanamycin-resistant colonies (A and B) were selected, and plants were regenerated from them. The plants were allowed to self-pollinate, and the results were as follows:

Plant A selfed → 3/4 progeny resistant to kanamycin; 1/4 progeny sensitive to kanamycin
Plant B selfed → 5/16 progeny resistant to kanamycin; 1/16 progeny sensitive to kanamycin

a. Draw the relevant plant chromosomes in both plants.
b. Explain the two different ratios.