7. The restriction nucleases BamHI and PstI cut their recognition sequences as shown in Figure 7.

A. Indicate the 5' and 3' ends of the cut DNA molecules.
B. How would the ends be modified if you incubated the cut molecules with DNA polymerase in the presence of all four dNTPs?
C. After the reaction in part B, could you still join the BamHI ends together by incubation with T4 DNA ligase? Could you still join the PstI ends together? (T4 DNA ligase will join blunt ends together as well as cohesive ends.)
D. Will joining of the ends in part C regenerate the BamHI site? Will it regenerate the PstI site?

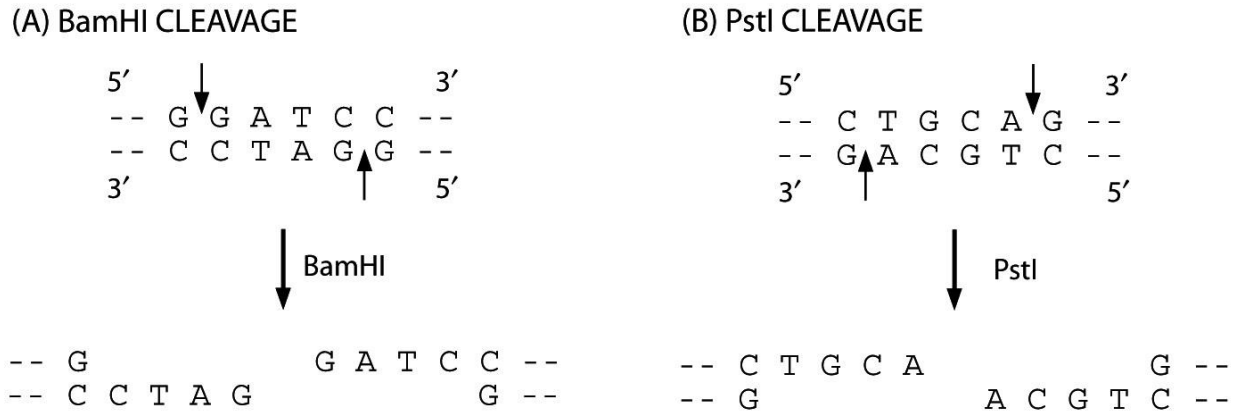

Figure 7 Restriction nuclease cleavage of DNA. (A) BamHI cleavage. (B) PstI cleavage. Only the nucleotides that form the recognition sites are shown.

8. The restriction nuclease EcoRI recognizes the sequence 5'-GAATTC and cleaves between the G and A to leave 5' protruding single strands (like BarnHI, see Figure 7). PstI, on the other hand, recognizes the sequence 5'-CTGCAG and cleaves between the A and G to leave 3' protruding single strands (see Figure 7). These two recognition sites are displayed on the helical representations of DNA in Figure 8.

A. For each restriction site indicate the position of cleavage on each strand of the DNA.

B. From the positions of the cleavage sites decide for each restriction nuclease whether you expect it to approach the recognition site from the major-groove side or from the minor-groove side.

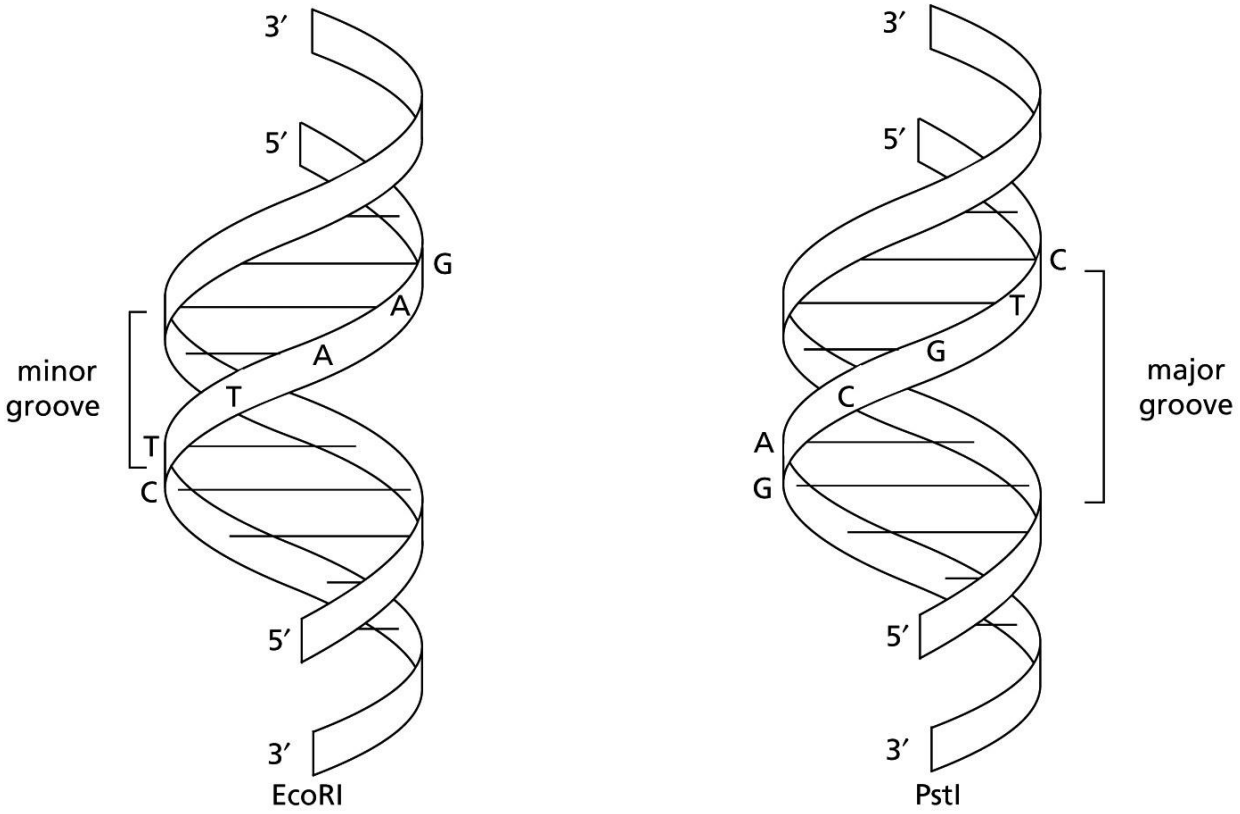

Figure 8 Restriction sites on heilcal DNA.

9. You wish to know whether the cDNA you have isolated and sequenced is the product of a unique gene or is made by a gene that is a member of a family of related genes. To address this question, you digest cell DNA with a restriction nuclease that cleaves the genomic DNA but not the cDNA, separate the fragments by gel electrophoresis, and visualize bands using radioactive cDNA as a probe. The Southern blot shows two bands, one of which hybridizes more strongly to the probe than the other.

You interpret the stronger hybridizing band as the gene that encodes your cDNA and the weaker band as a related gene. When you explain your result to your advisor, she cautions that you have not proven that there are two genes. She suggests that you repeat the Southern blot in duplicate, probing one with a radioactive segment from the 5' end of the cDNA and the other with a radioactive segment from the 3' end of the cDNA.

A. How might you get two hybridizing bands if the cDNA was the product of a unique gene?

B. What results would you expect from the experiment your advisor proposed if there were a single unique gene? If there were two related genes?

10. How would a DNA sequencing reaction be affected if the ratio of dideoxynucleoside triphosphates (ddNTPs) to deoxynucleoside triphosphates (dNTPs) were increased? What would the consequences be if the ratio were decreased?

11. Discuss the following statement: “From the nucleotide sequence of a cDNA clone, the complete amino acid sequence of a protein can be deduced by applying the genetic code. Thus, protein biochemistry has become superfluous because there is nothing more that can be learned by studying the protein.”