8.5. How many different hybridization probes must you make to ensure that at least one corresponds to a set of four codons encoding the amino acid sequence val-leu-trp-lys?

8.6. You wish to develop a genetically engineered *E. coli* producing a peptide hormone. You know the amino acid sequence of the peptide. Describe the sequence of steps you would use to obtain a culture expressing the gene as a peptide hormone.

8.7. You wish to produce a small protein using *E. coli*. You know the amino acid sequence of the protein. The protein converts a colorless substrate into a blue product. You have access to a high-copy-number plasmid with a penicillin-resistant gene and normal reagents for genetic engineering. Describe how you would engineer *E. coli* to produce this protein. Consider: source of donor DNA; regulatory elements that need to be included; how the donor and vector DNA are combined; how the vector DNA is inserted; and how you would select for a genetically engineered cell to use in production.

8.8. You wish to express a particular peptide in *E. coli* using a high-copy-number plasmid. You have the amino acid sequence for the peptide.

- **a.** Explain the experimental process for generating and selecting the genetically engineered *E. coli* using restriction enzymes, ligase, *E. coli,* plasmid with neomycin resistance, and the known amino acid sequence.
- **b.** What control elements would you place on the plasmid to regulate expression and to prevent read-through?

8.9. **a.** There are three primary methods for obtaining donor DNA when doing genetic engineering. What are those methods (two- to six-word descriptions of each are acceptable)?

- **b.** You need to produce a protein from humans in *E. coli*. You do not know the primary amino acid sequence. You suspect that introns are present. Which method will you use to obtain the donor DNA?

8.10. What is the difference between "transduction" and "transformation" when discussing genes transfer to bacteria?

8.11. You wish to isolate a thymidine auxotrophic mutant of *E. coli*. Describe briefly what experiments you would do to accomplish this.

8.12. For the DNA sequence, TAGGATCATAAGCCA, and using a primer, "ATCC," sketch what the corresponding sequencing gel should look like.