**review questions**

1. Describe a strategy using restriction endonucleases to clone a bacterial gene into a vector for propagation in *E. coli*. Assume that the target sequence is known. Describe the selection for *E. coli* cells that carry the cloned gene. Consider methods to minimize unwanted products.

2. Describe the features that make pUC19 a useful cloning vector.

3. Outline a strategy to clone a eukaryotic gene into a vector for expression in *E. coli*. Briefly describe the activity of the enzymes used in the process.

4. Describe how a library of open reading frames that represents a proteome is constructed by recombinational cloning.

5. A genomic DNA library of the bacterium *Pseudomonas putida* was constructed by partially digesting the genomic DNA with Sau3AI and inserting the fragments into pUC19 digested with BamHI. Why were two different restriction enzymes used in this experiment? How is the partial digestion performed, and what is the result? Why was a partial digestion used to construct the library?

6. How is the CRISPR-Cas system used to delete nucleotides at a specific site in a eukaryotic genome?

7. How is the CRISPR-Cas system used to insert a sequence at a specific site in a eukaryotic genome?

8. Outline the steps in a PCR cycle. What component of a PCR determines the specificity of the amplified product?

9. Describe how PCR is used to clone a specific gene.

10. Why is real-time PCR quantitative?

11. If your DNA synthesizer has an average coupling efficiency of 98.5%, what overall synthesis yield would you expect after the synthesis of a 50-mer oligonucleotide?

12. Suggest two different strategies for synthesizing a 0.5-kb gene.

13. What is a dideoxynucleotide? How is it used to determine the sequence of a DNA molecule?

14. Outline the basic features of pyrosequencing.

15. How are incorporated nucleotides recognized after each cycle of sequencing using reversible chain terminators? How does this differ from pyrosequencing?

16. What are some advantages of sequencing DNA by real-time single molecule synthesis?

17. Why are adaptors often ligated to DNA fragments prior to sequencing?

18. Describe how a cluster of thousands of copies of a DNA sequencing template can be generated on a solid support.

19. Define the following terms as they relate to genome sequencing: shotgun cloning, multiplexing, massive parallelization, barcode, Phred quality score, contig, scaffold, paired end reads, coverage, *de novo* sequencing, resequencing, reference genome, annotation, metagenome.

20. Outline a DNA microarray experiment. List some applications for this technology.

21. What are some of the advantages of using RNA sequencing rather than DNA microarrays to profile gene expression?

22. How are gene expression levels quantified using high-throughput RNA sequencing?

23. Explain why random hexamers used for RNA sequencing result in overrepresentation of sequences from the 5′ end of a gene.

24. How can 2D PAGE be used to identify proteins that are differentially expressed in two samples?

25. Describe some applications for protein microarrays.

26. What biological information may be provided by a protein microarray assay that is not provided by using a DNA microarray?

27. Describe two methods to determine protein-protein interactions.

28. What is an affinity tag?

29. What biological information may be provided by the tandem affinity purification tag system that is not provided by a two-hybrid assay?

30. Explain how metabolomics may be used to identify biomarkers of disease.