CHAPTER 20
BIOTECHNOLOGY

Learning objectives

DNA Cloning

1. Describe the natural function of restriction enzymes and explain how they are used in recombinant DNA technology.
2. Explain how the creation of sticky ends by restriction enzymes is useful in producing a recombinant DNA molecule.
3. Outline the procedures for cloning a eukaryotic gene in a bacterial plasmid.
4. Explain the rationale for including a gene for antibiotic resistance and a gene that codes for a hydrolytic enzyme in the plasmid.
5. Define and distinguish between genomic libraries using plasmids, phages, and cDNA.
6. Describe the role of an expression vector.
7. Describe two advantages of using yeast cells instead of bacteria as hosts for cloning or expressing eukaryotic genes.
8. Describe the structure and function of a yeast artificial chromosome (YAC).
9. Describe two techniques to introduce recombinant DNA into eukaryotic cells.
10. Describe the polymerase chain reaction (PCR) and explain the advantages and limitations of this procedure.

Using DNA Technology to Explore Gene Function

11. Explain how gel electrophoresis is used to analyze nucleic acids and to distinguish between two alleles of a gene.
12. Describe the process of nucleic acid hybridization.
13. Describe the Southern blotting procedure and explain how it can be used to identify the heterozygous carriers of a mutant allele.
14. Explain how Northern blotting or the reverse transcriptase-polymerase chain reaction (RT-PCR) can be used to determine how expression of a gene changes at different stages of embryonic development.
15. State two questions that could be addressed through genome-wide expression studies.
16. Explain how *in vitro* mutagenesis and RNA interference help researchers to discover the functions of some genes.

Organismal Cloning

17. Distinguish between gene cloning, cell cloning, and organismal cloning.
18. Explain why it is so much easier to clone plants than animals.
19. Describe how nuclear transplantation was used to produce Dolly, the first cloned sheep.
20. Explain why cloned animals are so likely to have defects.
21. Distinguish between reproductive cloning and therapeutic cloning.
22. Distinguish between embryonic and adult stem cells.

Practical Applications of DNA Technology

23. Describe how DNA technology has medical applications in the diagnosis of genetic disease, the development of gene therapy, vaccine production, and the development of pharmaceutical products.
24. Explain how genetic markers can be used to detect abnormal alleles of a gene that has not yet been cloned.
25. Define a single nucleotide polymorphism. Explain how an SNP may produce a restriction fragment length polymorphism (RFLP).
26. Describe how gene therapy was used to treat severe combined immunodeficiency (SCID), with mixed success.
27. Describe an example of a transgenic animal used as a pharmaceutical factory.
28. Explain how DNA technology can be used to improve the nutritional value of crops and to develop plants that can produce pharmaceutical products.
29. Explain how DNA technology is used in the forensic sciences.
30. Describe how gene manipulation has practical applications in environmental cleanup and agriculture.
31. Describe how plant genes can be manipulated using the Ti plasmid carried by *Agrobacterium* as a vector.
32. Discuss the safety and ethical questions related to recombinant DNA studies and the biotechnology industry.
33. Describe the current controversy over genetically modified (GM) foods.