

1 **Chapter 9**

Biotechnology and
Recombinant DNA

2 **Biotechnology and Recombinant DNA**

- Biotechnology: The use of microorganisms, cells, or cell components to make a product.
 - Foods, antibiotics, vitamins, enzymes
- Recombinant DNA (rDNA) technology: Insertion or modification of genes to produce desired proteins

3 **Biotechnology and Recombinant DNA**

- Vector: Self-replicating DNA used to carry the desired gene to a new cell
- Clone: Population of cells arising from one cell, each carries the new gene

4 **A Typical Genetic Modification Procedure**5 **A Typical Genetic Modification Procedure**6

- ✓ Differentiate biotechnology and recombinant DNA technology. 9-1
- ✓ In one sentence, describe how a vector and clone are used. 9-2
- ✓
- ✓

7 **Selection and Mutation**

- Selection: Culture a naturally occurring microbe that produces desired product
- Mutation: Mutagens cause mutations that might result in a microbe with a desirable trait
- Site-directed mutagenesis: Change a specific DNA code to change a protein
- Select and culture microbe with the desired mutation

8 **Restriction Enzymes**

- Cut specific sequences of DNA
- Destroy bacteriophage DNA in bacterial cells
- Cannot digest (host) DNA with methylated cytosines

9 10 **Restriction Enzyme & Recombinant DNA**11 **Vectors**

- Carry new DNA to desired cell
- Shuttle vectors can exist in several different species
- Plasmids and viruses can be used as vectors

12 **A Plasmid Vector Used for Cloning**13 **Polymerase Chain Reaction (PCR)**

- To make multiple copies of a piece of DNA enzymatically
- Used to
 - Clone DNA for recombination
 - Amplify DNA to detectable levels
 - Sequence DNA
 - Diagnose genetic disease
 - Detect pathogens

14 **PCR**

15 **PCR**

16 **PCR**

17

- ✓ How are selection and mutation used in biotechnology? 9-3
- ✓ What is the value of restriction enzymes in recombinant DNA technology? 9-4
- ✓ What criteria must a vector meet? 9-5
- ✓ Why is a vector used in recombinant DNA technology? 9-6
- ✓ For what is each of the following used in PCR: primer, DNA polymerase, 94°C? 9-7

18 **Inserting Foreign DNA into Cells**

- DNA can be inserted into a cell by
 - Electroporation -a mechanical method used to introduce polar molecules into a host cell through the cell membrane
 - Transformation- the genetic alteration of a cell
 - Protoplast fusion

19 **Process of Protoplast Fusion**

20 **Inserting Foreign DNA into Cells**

- DNA can be inserted into a cell by
 - Gene gun
 - Microinjection

21 **Obtaining DNA**

- Complementary DNA (cDNA) is made from mRNA by reverse transcriptase

22 **Obtaining DNA**

- Synthetic DNA is made by a DNA synthesis machine

23

- ✓ Contrast the five ways of putting DNA into a cell. 9-8
- ✓ What is the purpose of a genomic library? 9-9
- ✓ Why isn't cDNA synthetic? 9-10
- ✓ How are recombinant clones identified? 9-11
- ✓ What types of cells are used for cloning rDNA? 9-12
- ✓

24 **Therapeutic Applications**

- Human enzymes and other proteins
- Subunit vaccines
- Nonpathogenic viruses carrying genes for pathogen's antigens as DNA vaccines
- Gene therapy to replace defective or missing genes

25 **The Human Genome Project**

- Nucleotides have been sequenced
- Human Proteome Project may provide diagnostics and treatments
 - Reverse genetics: Block a gene to determine its function

26

- ✓ Explain how rDNA technology can be used to treat disease and to prevent disease. 9-13

- ✓ What is gene silencing? 9-14
- ✓ How are shotgun sequencing, bioinformatics, and proteomics related to the Human Genome Project? 9-15, 9-16

✓
✓

27 **Scientific Applications**

- Understanding DNA
- Sequencing organisms' genomes
- DNA fingerprinting for identification

28 **Southern Blotting**

29 **Southern Blotting**

30 **Southern Blotting**

31 **Forensic Microbiology**

- PCR
- Primer for a specific organism will cause application if that organism is present
- Real-time PCR: Newly made DNA tagged with a fluorescent dye; the levels of fluorescence can be measured after every PCR cycle
- Reverse-transcription (RT-PCR): Reverse transcriptase makes DNA from viral RNA or mRNA

32 **Safety Issues and Ethics of Using rDNA**

- Avoid accidental release
- Genetically modified crops must be safe for consumption and for the environment
- Who will have access to an individual's genetic information?