Figure 20.2 Overview of gene cloning with a bacterial plasmid.

Figure 20.3 Making recombinant DNA

Figure 20.4 Cloning a human gene

Figure 20.6 Genomic libraries
Figure 20.5 Screening a library with a nucleic acid probe

Figure 20.7 Polymerase chain reaction (PCR)

Figure 20.8 Gel electrophoresis

Figure 20.9 RFLP of sickle-cell allele & normal β-globin
**Figure 20.10 Southern blotting**

1. Preparation of restriction fragments
2. Gel electrophoresis
3. Blotting

Radioactively labeled probe for β-globin gene is added to solution in a plastic bag

- Probe hybridizes to fragments containing normal or mutant β-globin
- Fragment from sickle-cell β-globin allele
- Fragment from normal β-globin allele

4. Hybridization with radioactive probe
5. Autoradiography

**Figure 20.12 DNA sequencing using dideoxy nucleotides**

1. Isolate mRNA.

2. Make cDNA by reverse transcription, using fluorescently labeled nucleotides.

3. Apply the cDNA mixture to a microarray, a microscope slide on which copies of single-stranded DNA fragments from the organism’s genes are fixed, a different gene in each spot. The cDNA hybridizes with any complementary DNA on the microarray.

4. Rinse off excess cDNA; scan microarray for fluorescence. Each fluorescent spot (yellow) represents a gene expressed in the tissue sample.

**Figure 20.14 DNA microarray analysis of gene expression**

1. Tissue sample
2. mRNA molecules
3. Labeled cDNA molecules (single strands)
4. DNA microarray

Size of an actual DNA microarray with all the genes of yeast (6,400 spots)

13 loci amplified for submission to CODIS
Figure 20.16 Gene therapy using a retroviral vector

1. Insert RNA version of normal allele into retrovirus.
2. Let retrovirus infect bone marrow cells that have been removed from the patient and cultured.
3. Viral DNA carrying the normal allele inserts into chromosome.
4. Inject engineered cells into patient.

Bone marrow cell from patient

Figure 20.16 Gene therapy using a retroviral vector