Chapters 20: DNA Technology

AP Biology 2013

1

Understanding DNA

- One of the greatest achievements: Human Genome Project (completed 2003)
- Sequencing of the genomes of more than 7,000 species was under way in 2010
  - Recombinant DNA technology makes this possible (DNA from two species are combined *in vitro*)
- Genetic Engineering - direct manipulation of genes for practical purposes
- Biotechnology - manipulation of genetic components to make useful products
  - Microarray - measures gene expression of many genes at once

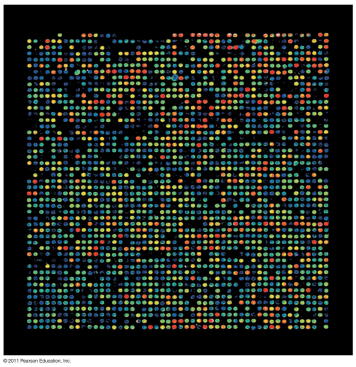


Fig. 20.1

2

DNA Cloning

- To work with specific genes, scientists must identical copies of genes
- Uses plasmids (small circular DNA molecules that replicate separately from the bacterial chromosome)
  - Called a cloning vector
- **Gene cloning** involves using bacteria to make multiple copies of a gene.

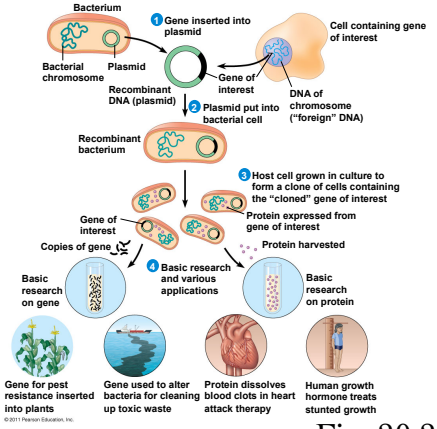


Fig. 20.2

- Foreign DNA is inserted into a plasmid and the recombinant plasmid is inserted into a bacterial chromosome

3

Restriction Enzymes

- **Restriction enzymes** cut DNA molecules at specific sequences called **restriction sites** to make **restriction fragments**
- Most useful restriction enzymes cut in a staggered way producing "sticky ends" that can bond with complementary fragments
- DNA ligase seals the bonds between restriction fragments

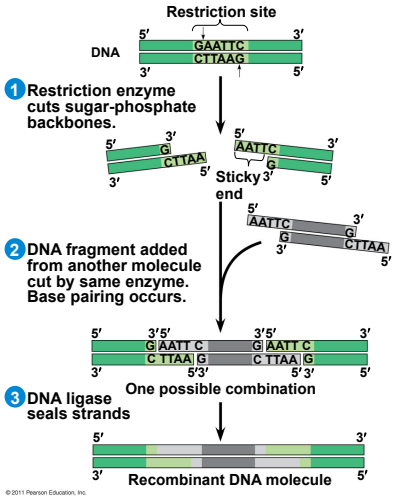
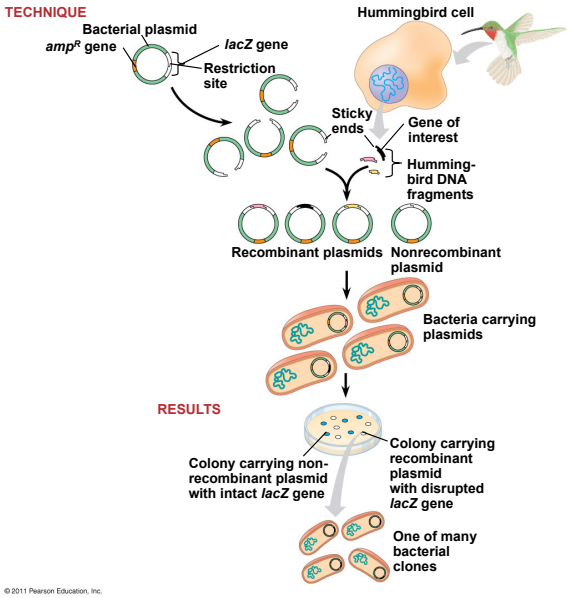


Fig. 20.3

Producing Clones

- In gene cloning, the original plasmid is called a **cloning vector** which is a DNA molecule that can carry foreign DNA into a host cell and replicate there.
- Ex. hummingbird β-globin gene
  - Hummingbird genomic DNA and bacterial plasmid are isolated and cut with the same restriction enzyme
  - Fragments are mixed and DNA ligase is added to bond the sticky ends
  - Some recombinant plasmids now contain hummingbird DNA
  - DNA mixture is added to bacteria
  - Bacteria are plated on a type of agar that selects for bacteria with recombinant plasmids
  - Results in cloning of many hummingbird DNA fragments (including the β-globin gene)



Producing Clones

Fig. 20.4

Genomic Libraries

- A genomic library is a collection of recombinant vector clones produced by cloning DNA fragments from an entire genome
- A complementary DNA (cDNA) library is made by cloning DNA made *in vitro* by reverse transcription of all the mRNA produced by a cell (does not include all DNA from the cell)

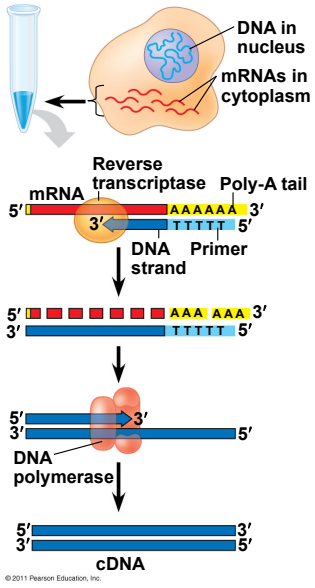


Fig. 20.6

Screening a Genomic Library

- Clone carrying the gene can be identified with a nucleic acid probe if its sequence is complimentary to the gene (**nucleic acid hybridization**)
  - Ex. if the desired gene is 5′ ··· CTCATCACCGGC··· 3′
    - Then the probe would be 3′ GAGTAGTGGCCG 5′
- DNA probe can be used to screen a large number of clones simultaneously and once identified, the clone carrying the gene can be cultured

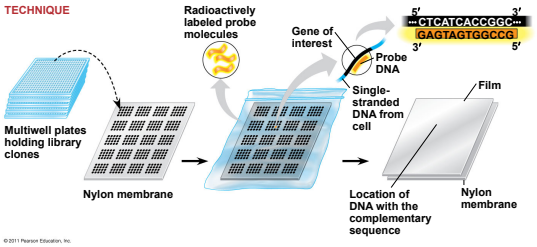


Fig. 20.7

DNA Amplification

- Polymerase Chain Reaction (PCR)
  - Produces many copies of a target segment of DNA
  - Uses primers that bracket the desired sequence

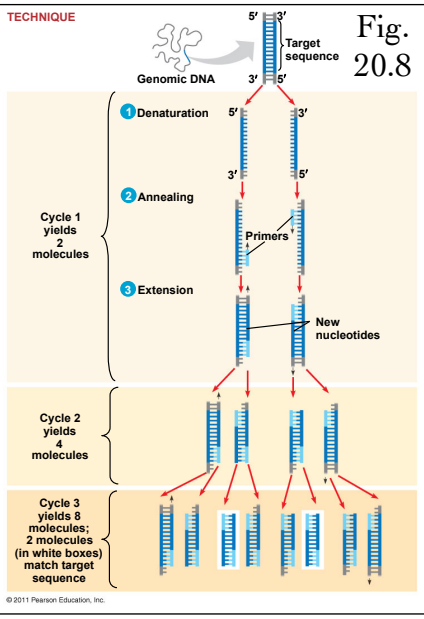


Fig. 20.8

Restriction Fragment Analysis

- Provides comparative information about DNA sequences
  - Useful for comparing two different DNA molecules such as two alleles for a gene.
- Gel electrophoresis - separates nucleic acids or proteins of different lengths or charges

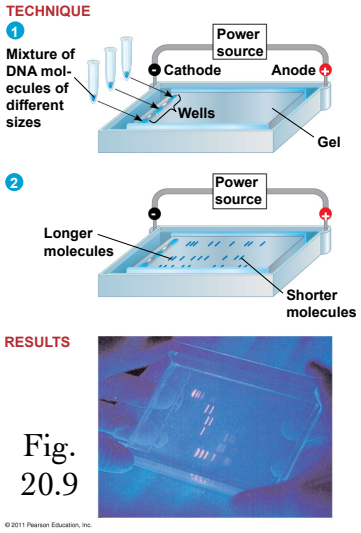
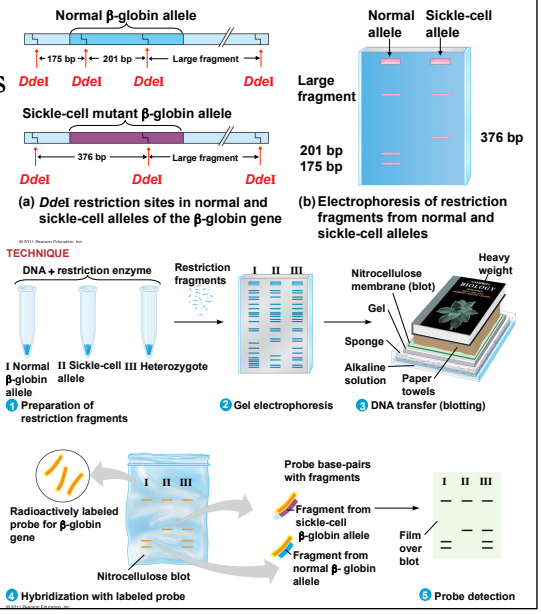


Fig. 20.9

10

Gel Electrophoresis

- Restriction fragment analysis can be used to compare two different DNA molecules (such as two alleles for a gene)
- Southern blotting combines gel electrophoresis with nucleic acid hybridization

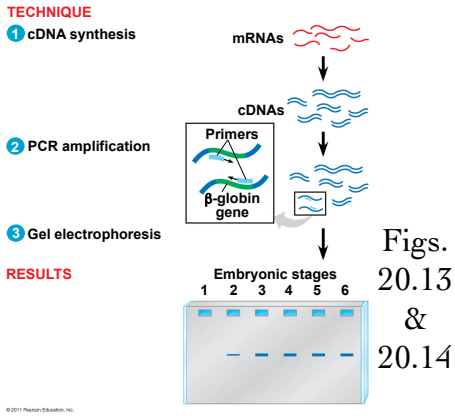


Figs. 20.10 & 20.11

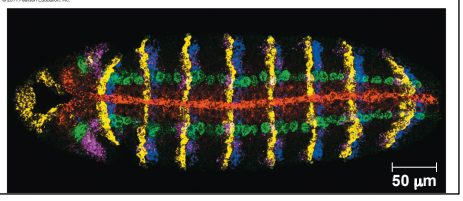
11

Studying Gene Expression

- Northern Blotting - combines gel electrophoresis of mRNA by hybridization
- Reverse transcriptase-polymerase chain reaction (RT-PCR) requires less mRNA than Northern blotting
- In situ hydridization uses fluorescent dyes attached to probes to identify the location of specific mRNAs in the intact organism



Figs. 20.13 & 20.14



12

DNA Microarray

- Allows scientists to automate the measure of expressed genes
- Allows for comparison of gene expression in different tissues, at different times, or under different conditions

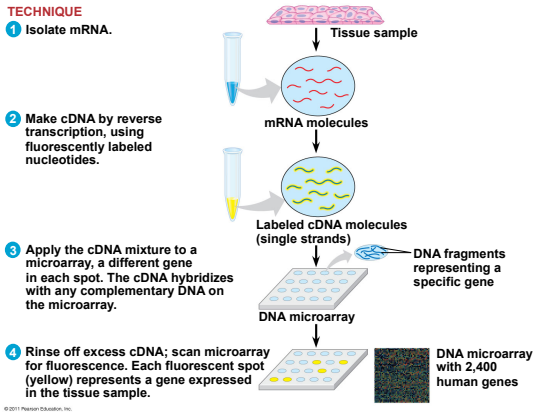


Fig. 20.15

13

Determining Gene Function

- In vitro mutagenesis introduces mutations into a cloned gene to disable the function and observe the consequences
- When the mutated gene is returned to the cell the phenotypes can be examined
- Hopefully these techniques can be used to produce stem cells
  - Totipotent cell is one that can generate a complete new organism

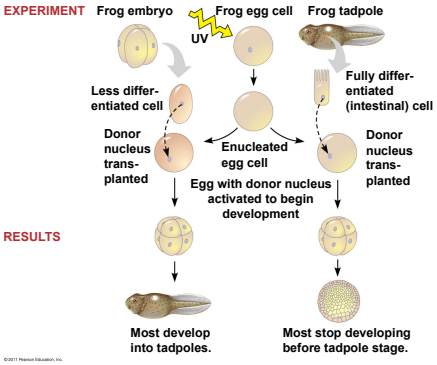


Fig. 20.18

14

Cloning Organisms

- Organismal cloning produces one or more organisms genetically identical to the "parent" that donated the single cell
- Nuclear transplantation - nucleus of an unfertilized egg or zygote is replaced with the nucleus of a differentiated cell
- 1997 - Dolly the sheep was born in Scotland (her death in 2003 and health problems may show that cloned cells are not as healthy)

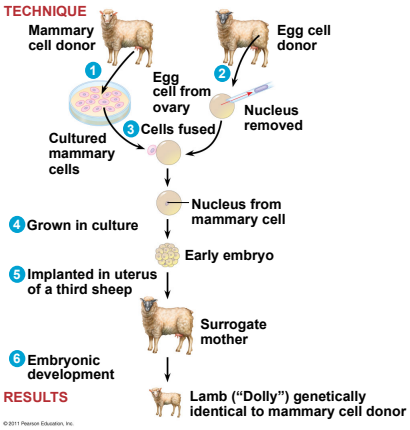


Fig. 20.19

15

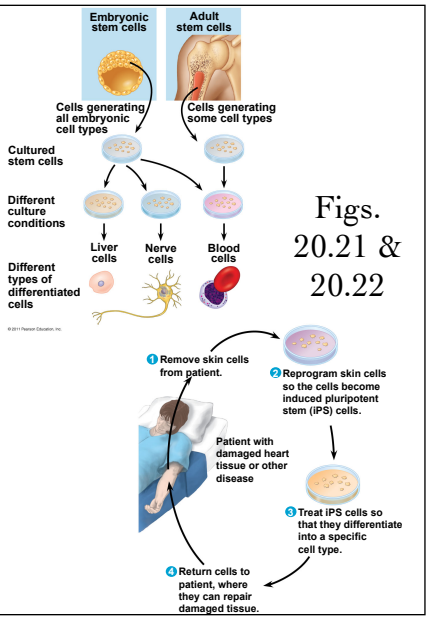
Problems with Animal Cloning

- Only a small percentage of cloned embryos have developed normally to birth (many exhibit defects)
- Epigenetic changes (acetylation of histones or methylation of DNA) must be reversed in donor nucleus in order for genes to be expressed appropriately

16

Stem Cells

- **Stem cell** is a unspecialized cell that can reproduce itself indefinitely and differentiate into specialized cells of one or more types
- Stem cells isolated from embryos at the blastocyst stage are called embryonic stem (ES) cells and can differentiate into all cell types
- Adult stem cells can replace non-reproducing specialized cells
- Researchers have used skin cells to produce ES cells by using viruses to introduce stem cell master regulatory genes. These cells are called iPS cells (induced pluripotent stem cells).



Figs. 20.21 & 20.22

17

Practical Applications

- Identification of genes whose mutation causes genetic diseases
- Diagnosis of genetic disorders
- Gene therapy - alteration of afflicted individual's genome (uses vectors to deliver genes to cells)
- Productions of pharmaceutical products (ex. hormones, vaccines) using **transgenic animals**
- DNA fingerprinting (CSI)
- Environmental cleanup
- Agriculture

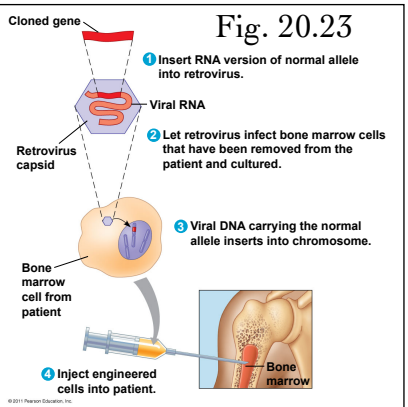


Fig. 20.23

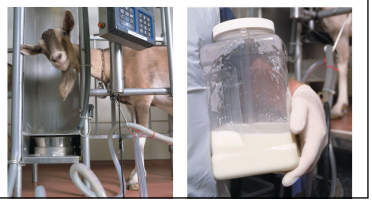


Fig. 20.24

18