Molecular Biology Techniques & its Applications

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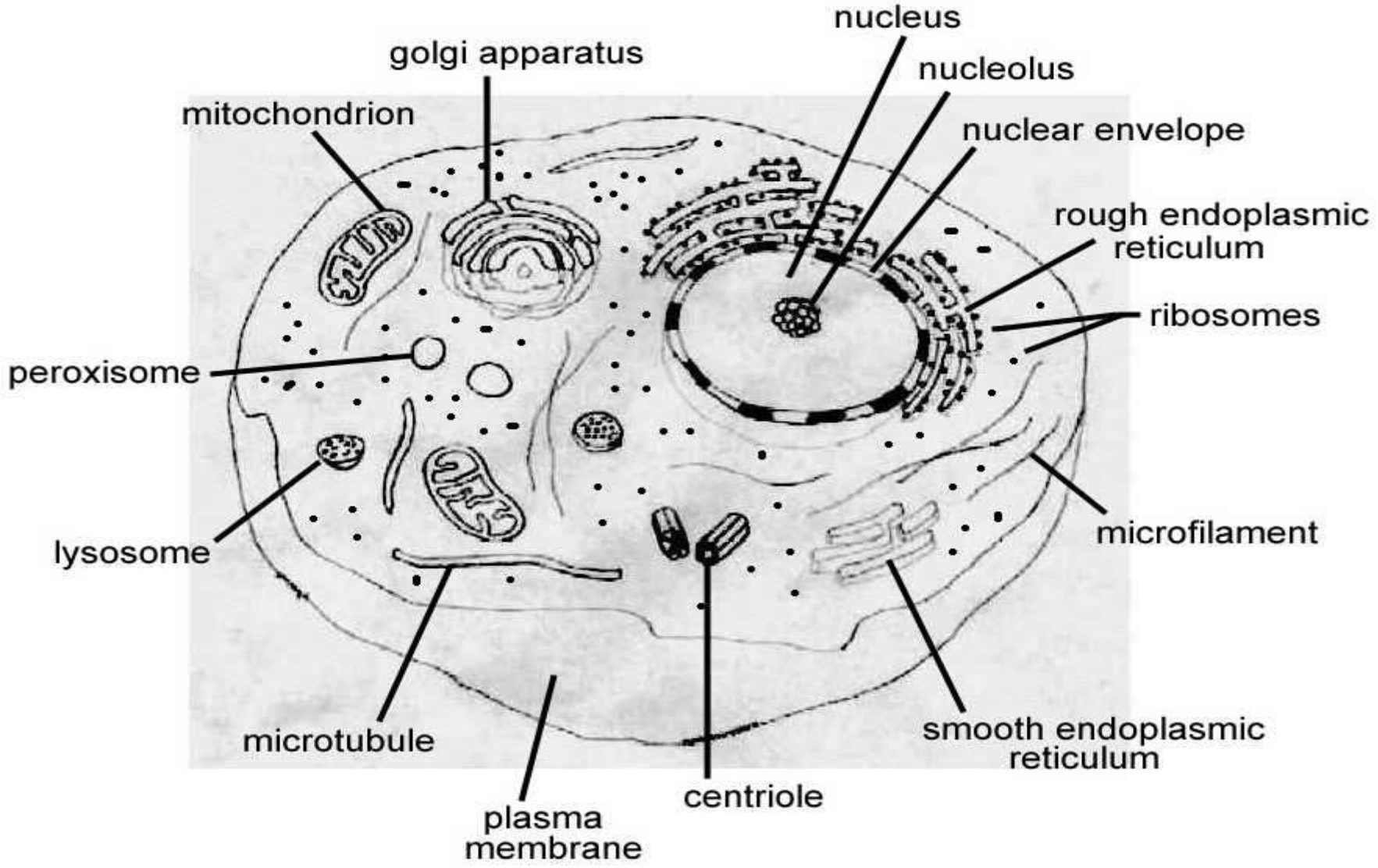
Eukaryotic cell

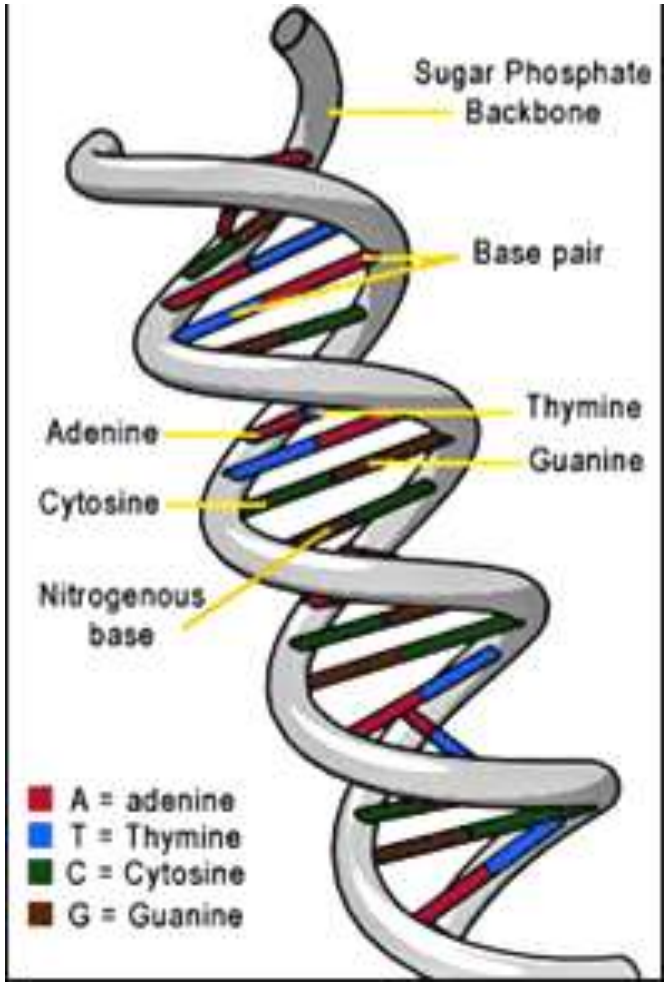


Terminology

- Nucleic acid: Biological molecules (RNA and DNA) that allow organisms to reproduce
- Gene: Basic physical and functional units of heredity located on the chromosomes consisting of specific sequences of DNA bases
- Genes encode instructions on how to make proteins
- Genotype: The genetic makeup of an organism
- Phenotype: the physical expressed traits of an organism

DNA

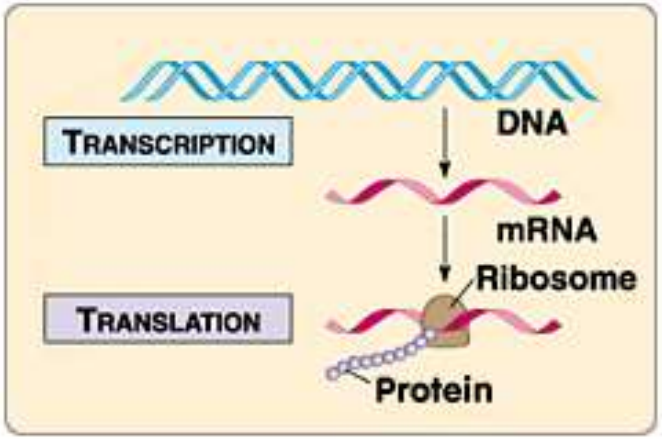
- Contained in the nucleus
- Arranged in 22 chromosomes, plus two sex chromosomes
- Two copies of each
- 99.9% identical to other humans.
- Around 2m DNA, enough to travel to sun and back 600 times!
- Therefore, very tightly packed



- 5' C-G-A-T-T-G-C-A-A-C-G-A-T-G-C 3'
- | | | | | | | | | | | | | |
- 3' G-C-T-A-A-C-G-T-T-G-C-T-A-C-G 5'

DNA function

- Carries the blueprint for life
- Duplication for new cells
- Make proteins for biological functions:

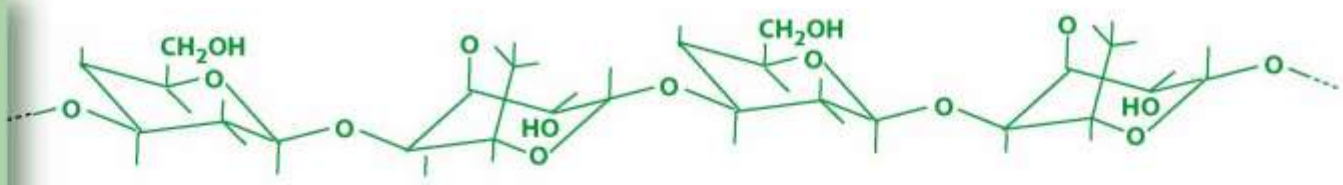


Gel Electrophoresis

- **Gel electrophoresis is a technique used to separate DNA fragments according to their size.**
- **DNA samples are loaded into wells (indentations) at one end of a gel, and an electric current is applied to pull them through the gel.**
- **DNA fragments are negatively charged, so they move towards the positive electrode.**

•Agarose Gel

Agarose is extracted from seaweed, and is a linear polymer of sugar molecules.

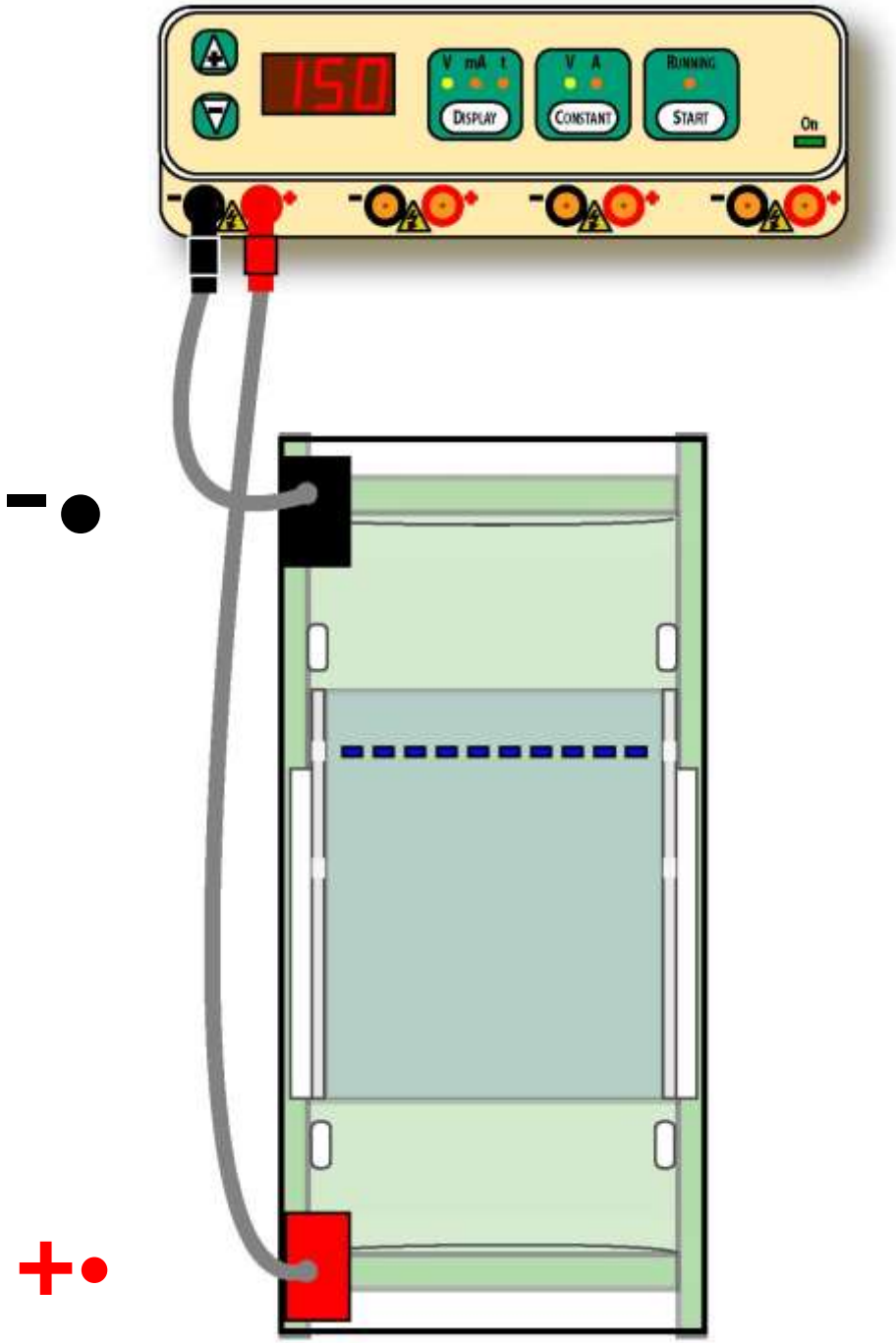


When heated in water, the agarose polymers are flexible and the mixture of agarose and water liquid.

Lower concentration of agarose

When the agarose and water cool, the agarose polymers form a matrix. When this happens, the mixture becomes more solid and gel-like. The concentration of agarose determines how dense the matrix is.

Higher concentration of agarose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- **DNA and Electrical Current**

- **Many DNA fragments of the same size migrate together on the gel as a “band”**

Techniques of Molecular Genetics Have Revolutionized Biology

- **Recombinant DNA Technology—Genetic Engineering—Biotechnology:**
  - Locating, isolating, altering, and studying DNA segments
- **Biotechnology:**
  - Using recombinant DNA technology to develop new biological products

Cutting and Joining DNA Fragments

- Restriction enzymes: recognizing and cutting DNA at specific nucleotide sequences
  - Palindromic sequences
  - Immune system of bacteria
- Type II restriction enzyme: most useful enzyme
- By adding methyl groups to the recognition sequence to protect itself from being digested by its own enzyme in bacteria

Cutting and Joining DNA Fragments

- Cohesive ends: fragments with short, single-stranded overhanging ends

- Blunt ends: even-length ends from both single strands

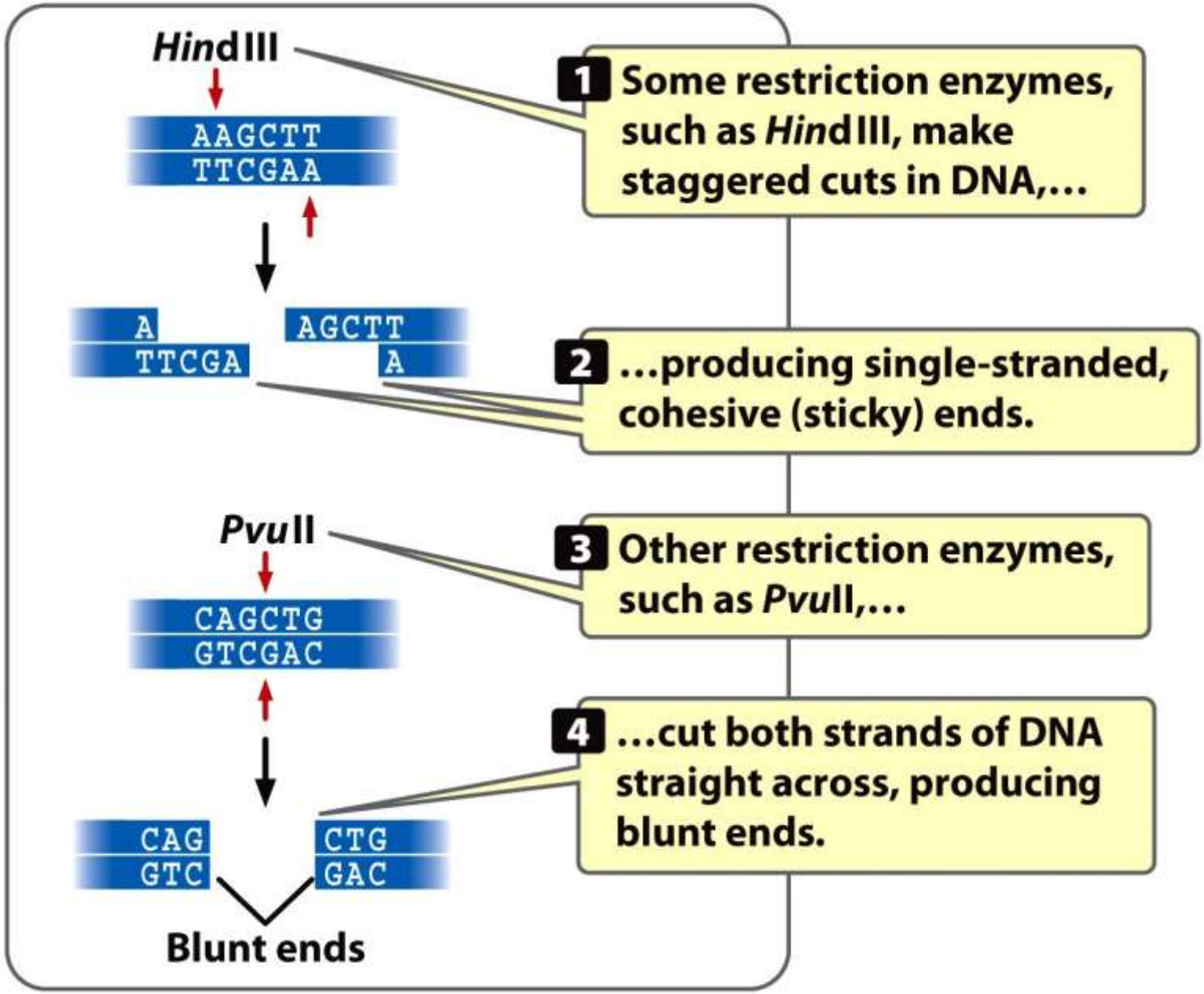

Figure 19-2a
Genetics: A Conceptual Approach, Third Edition

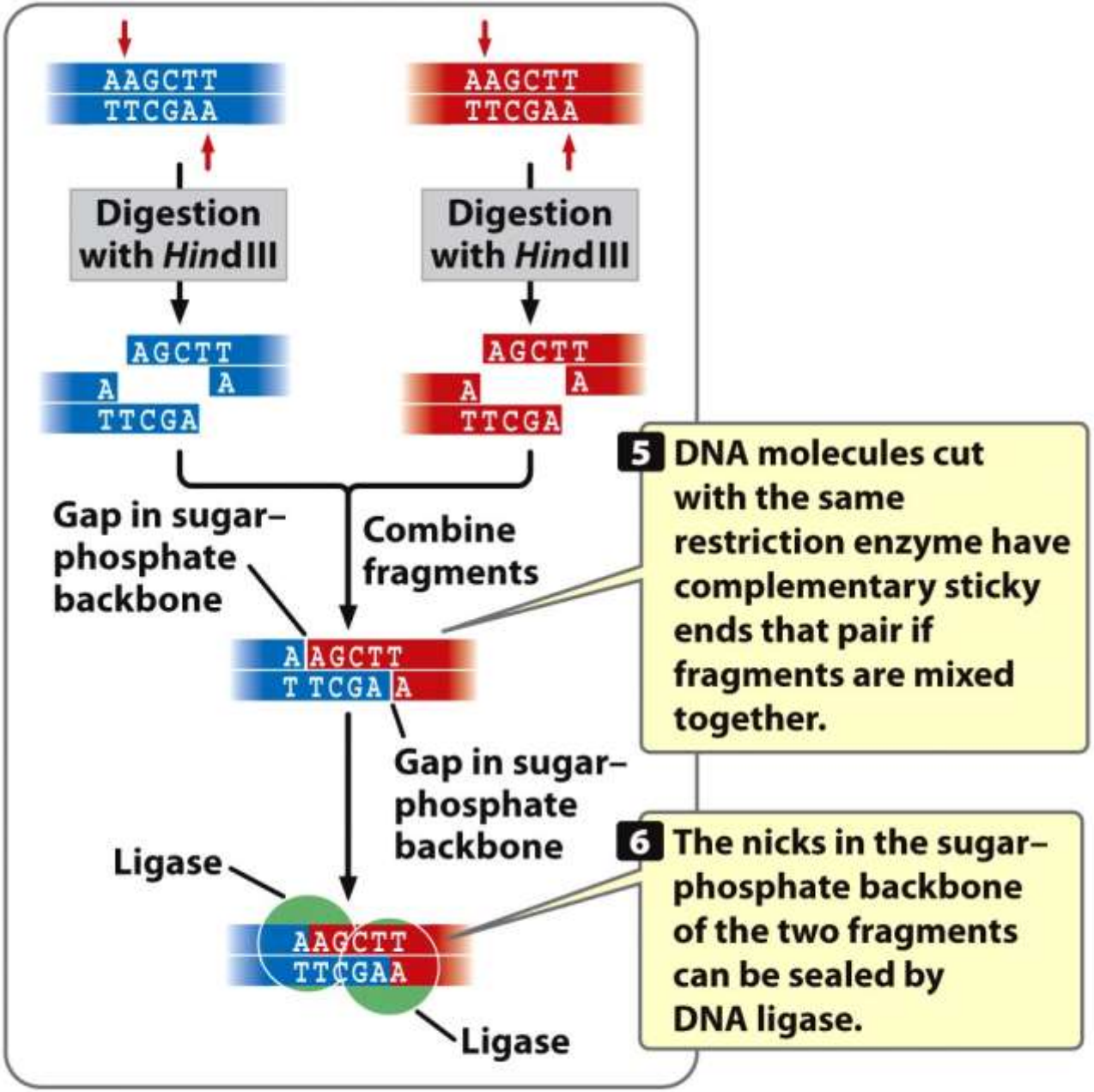

Figure 19-2b
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Viewing DNA Fragments

- **Gel electrophoresis-**separation of DNA fragments by size through a gel medium
  - **Smaller fragments migrate faster**

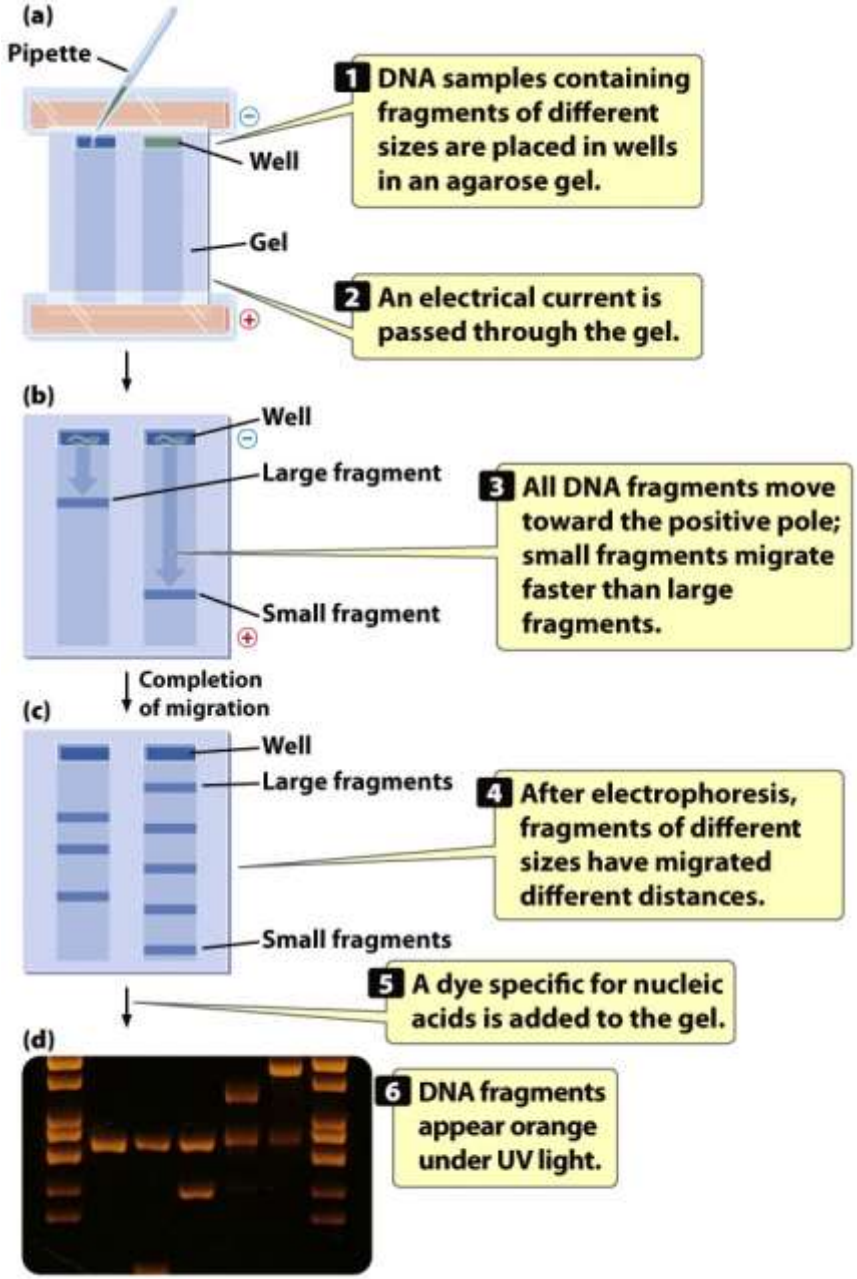

Figure 19-3
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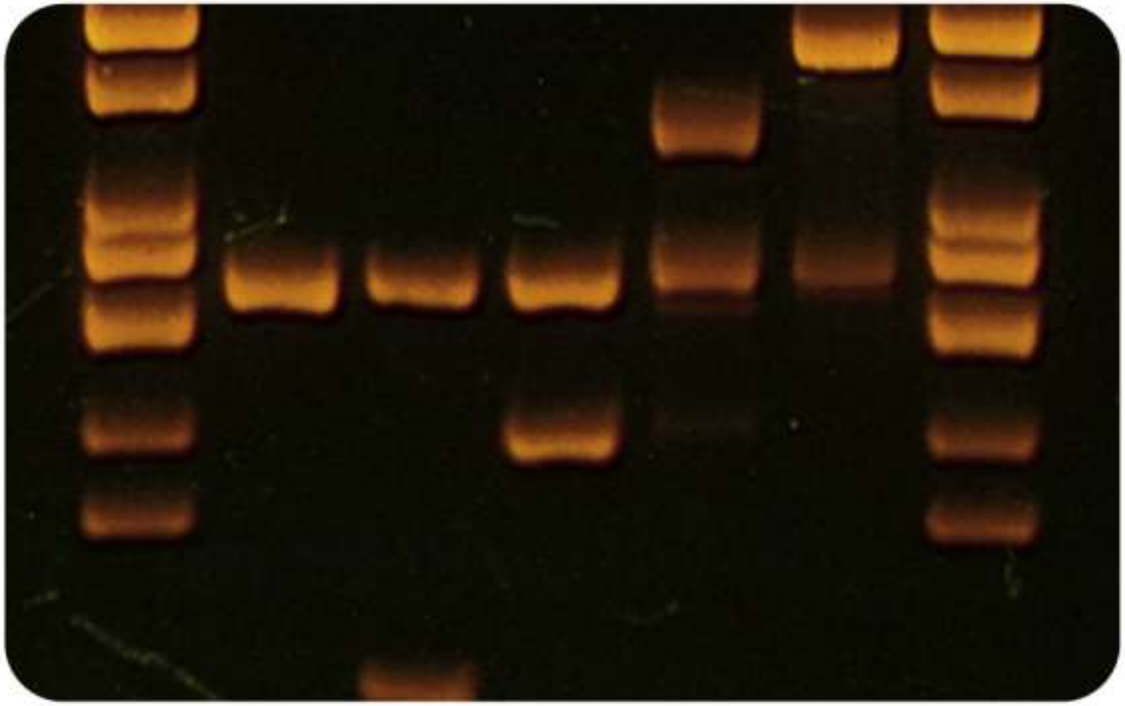

6 DNA fragments appear orange under UV light.

Figure 19-3d
Genetics: A Conceptual Approach, Third Edition

Viewing DNA Fragments

- **Locating DNA fragments with Southern blotting and probes**
  - **Probe:** DNA or RNA with a base sequence complementary to a sequence in the gene of interest
    - Is usually labeled for easy detection
      - Radioactive P^{32}
      - Fluorescent tag

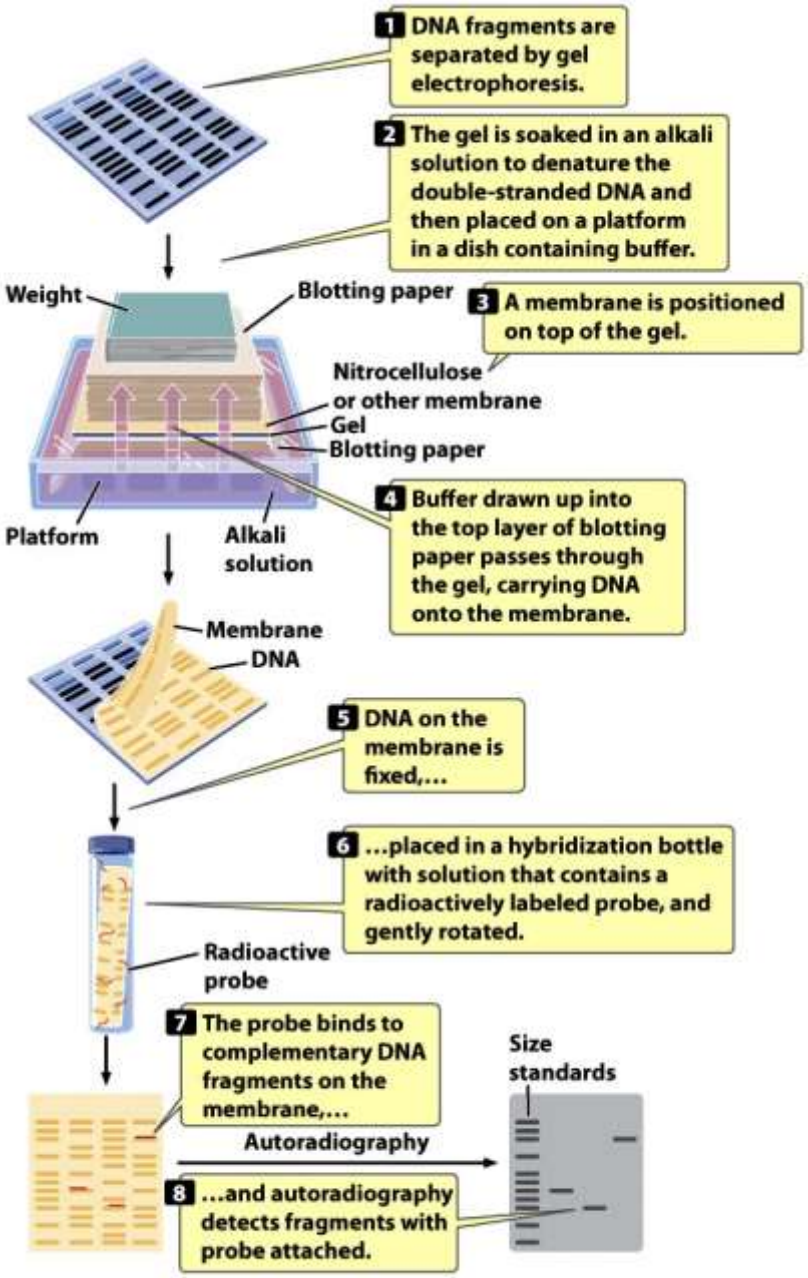

Figure 19-4
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Cloning Genes

- **Gene cloning:** amplifying a specific piece of DNA via a bacteria cell

- **Cloning vector:** a replicating DNA molecule attached with a foreign DNA fragment to be introduced into a cell
  - Has features that make it easier to insert DNA and select for presence of vector in cell.
    - Origin of replication
    - Antibiotic resistance gene
    - Cloning site

Cloning Genes

- Plasmid vectors
- Linkers: synthetic DNA fragments containing restriction sites
- Transformation of host cells with plasmids
- Selectable markers are used to confirm whether the cells have been transformed or not.

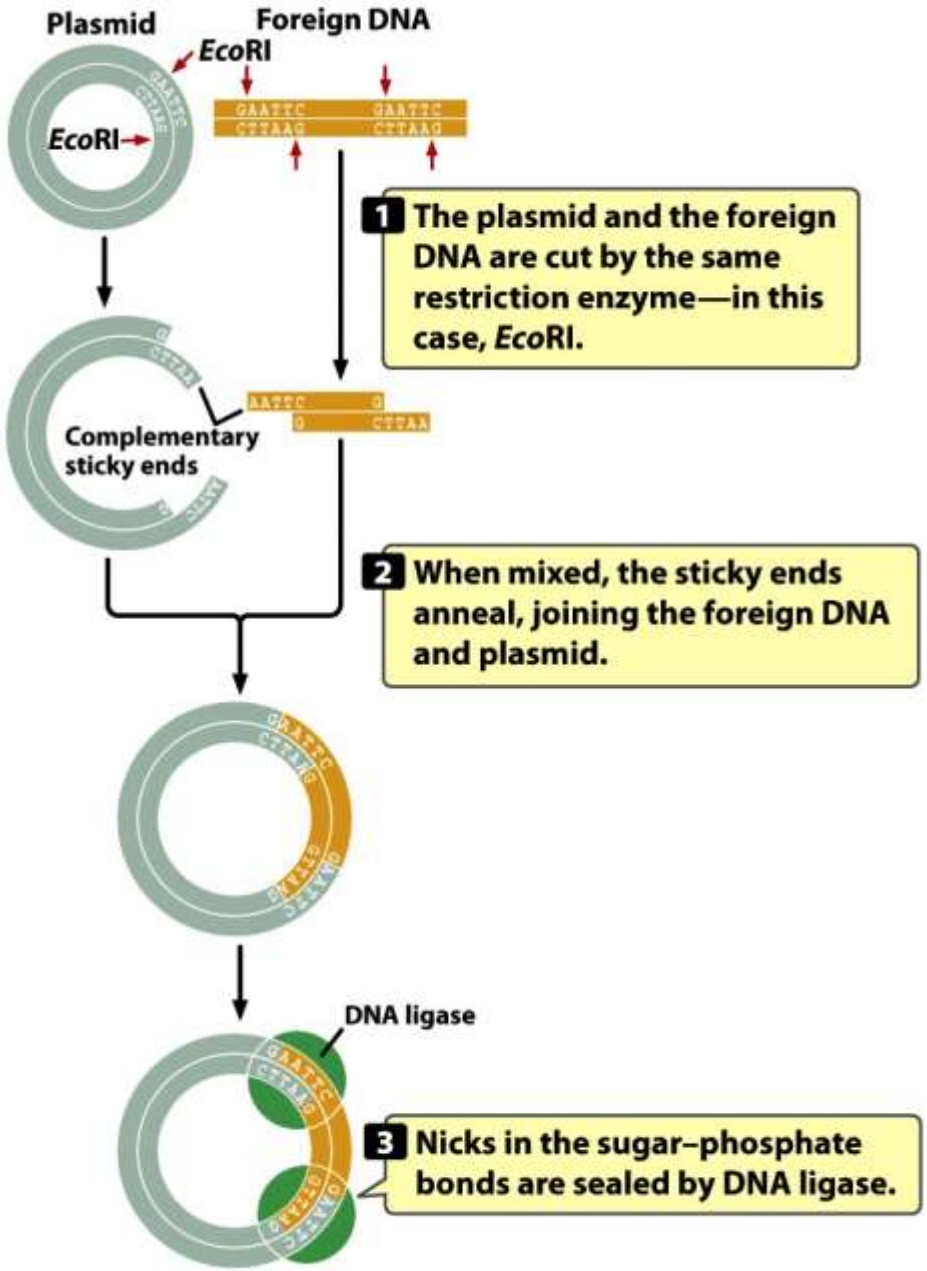

Figure 19-7
Genetics: A Conceptual Approach, Third Edition

Amplifying DNA fragments with the polymerase chain reaction (PCR)

- **Taq polymerase:** stable DNA polymerase at high temperature
- Researcher designs specific oligonucleotide primers that serve as the ends of the amplified fragment
- Very similar to replication

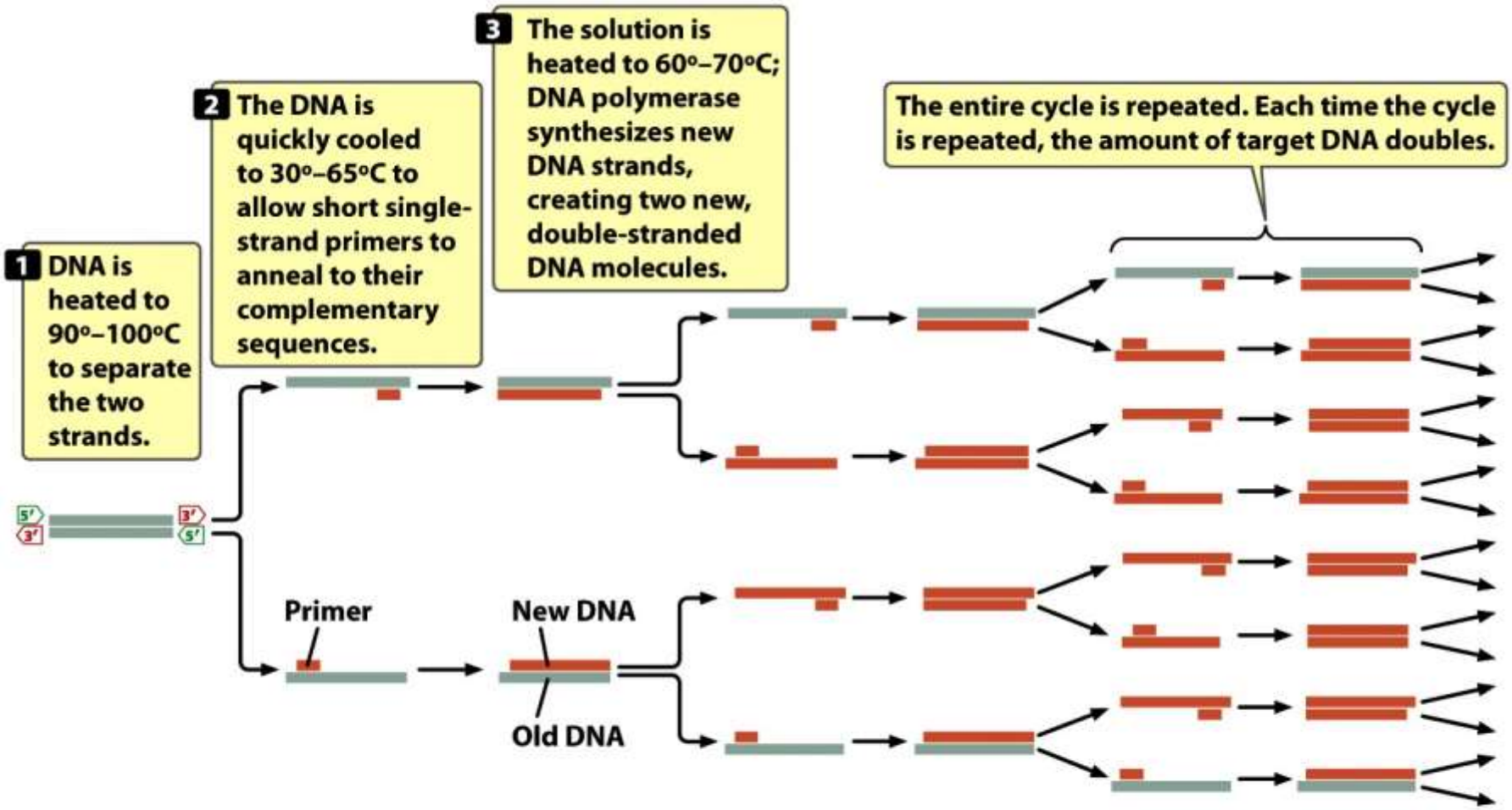

Figure 19-11
Genetics: A Conceptual Approach, Third Edition

Gene Libraries

- **Gene library:** a collection of clones containing all the DNA fragments from one source
  - Creating a genomic DNA library
  - **cDNA library:** consisting only of those DNA sequences that are transcribed into mRNA
    - Creating a cDNA library

Molecular Biology Applications

- Agriculture: pathogen detection, plant breeding programs, GMO detection, cultivar identification
- Animal husbandry: detection and treatment of infections, genetic selection, sex identification
- Human health: genetic diseases, infectious diseases, cancer diseases, etc.
- Environment & Ecology: Species identification, symbiotic interactions, assessment of biodiversity
- Forensic science: individual and familial identification, scene of crime


Thank you