

# Mader/Biology, 11/e – Chapter Outline

## Chapter 14

### 14.1 DNA Cloning

1. **Cloning** is the production of identical copies of DNA through some asexual means.
  - a. An underground stem or root sends up new shoots that are clones of the parent plant.
  - b. Members of a bacterial colony on a petri dish are clones because they all came from division of the same cell.
  - c. Human identical twins are clones; the original single embryo separate to become two individuals.
2. **Gene cloning** is production of many identical copies of the same gene.
  - a. If the inserted gene is replicated and expressed, we can recover the cloned gene or protein product.
  - b. Cloned genes have many research purposes: determining the base sequence between normal and mutated genes, altering the phenotype, obtaining the protein coded by a specific gene, etc.
  - c. Humans can be treated with **gene therapy**: alteration of the phenotype in a beneficial way.
  - d. Otherwise **transgenic organisms** are used to produce products desired by humans.
  - e. To clone DNA, scientists use recombinant DNA (rDNA) and polymerase chain reaction (PCR).
- A. Recombinant DNA Technology
  1. **Recombinant DNA (rDNA)** contains DNA from two or more different sources.
  2. To make rDNA, a technician selects a vector.
  3. A **vector** is a plasmid or a virus used to transfer foreign genetic material into a cell.
  4. A **plasmid** is a small accessory ring of DNA in the cytoplasm of some bacteria.
  5. Plasmids were discovered in research on reproduction of intestinal bacteria *Escherichia coli*.
  6. Introduction of foreign DNA into vector DNA to produce rDNA requires two enzymes.
    - a. **Restriction enzyme** is a bacterial enzyme that stops viral reproduction by cleaving viral DNA.
      - 1) The restriction enzyme is used to cut DNA at specific points during production of rDNA.
      - 2) It is called a restriction enzyme because it restricts growth of viruses but it acts as a molecular scissors to cleave any piece of DNA at a specific site.
      - 3) Restriction enzymes cleave vector (plasmid) and foreign (human) DNA.
      - 4) Cleaving DNA makes DNA fragments ending in short single-stranded segments with “sticky ends.”
      - 5) The “sticky ends” allow insertion of foreign DNA into vector DNA.
    - b. **DNA ligase** seals the foreign gene into the vector DNA.
      - 1) Treated cells take up plasmids, and then bacteria and plasmids reproduce.
      - 2) Eventually, there are many copies of the plasmid and many copies of the foreign gene.
      - 3) When DNA splicing is complete, an rDNA (recombinant DNA) molecule is formed.
  7. If the human gene is to express itself in a bacterium, the gene must be accompanied by the regulatory regions unique to bacteria and meet other requirements.
    - a. The gene cannot contain introns because bacteria do not have introns.
    - b. An enzyme called reverse transcriptase can be used to make a DNA copy of mRNA.
    - c. This DNA molecule is called **complementary DNA (cDNA)** and does not contain introns.
    - d. A laboratory DNA synthesizer can produce small pieces of DNA without introns.
- B. The Polymerase Chain Reaction (PCR)
  1. **Polymerase chain reaction (PCR)** can create millions of copies of a single gene or a specific piece of DNA in a test tube.
  2. PCR is very specific—the targeted DNA sequence can be less than one part in a million of the total

- DNA sample; therefore a single gene can be amplified using PCR.
3. PCR uses the enzyme DNA polymerase to carry out multiple replications (a chain reaction) of target DNA.
  4. PCR automation is possible because heat-resistant DNA polymerase from *Thermus aquaticus*, which grows in hot springs, is an enzyme that withstands the temperature necessary to separate double-stranded DNA.
  5. Three DNA analysis techniques are gel electrophoresis, short tandem repeat profiling, and “finger-printing.”
    - a. **Gel electrophoresis** separates DNA fragments according to their size and results in distinctive bands that can help identify individuals.
    - b. **Short tandem repeat (STR) profiling** is a technique used to analyze DNA.
      - 1) Advantages include not being limited to using restriction enzymes.
      - 2) Since the chromosomal locations of STRs are known, these are the only locations that are subjected to PCR and gel electrophoresis.
      - 3) The band patterns are different in each person because each individual has their own number of STR repeats at different locations.
    - c. **DNA fingerprinting** is a technique of fluorescently labeling DNA fragments.
      - 1) The STR are excited by a laser, emissions from DNA fragments are recorded in peaks and valleys.
  6. PCR amplification and DNA analysis is used to:
    - a. Detect viral infections, genetic disorders, and cancer.
    - b. Determine the nucleotide sequence of human genes, and, because it is inherited.
    - c. Associate samples with DNA of parents.
    - d. Investigate evolutionary connections.