DNA Technology

Chapter 20

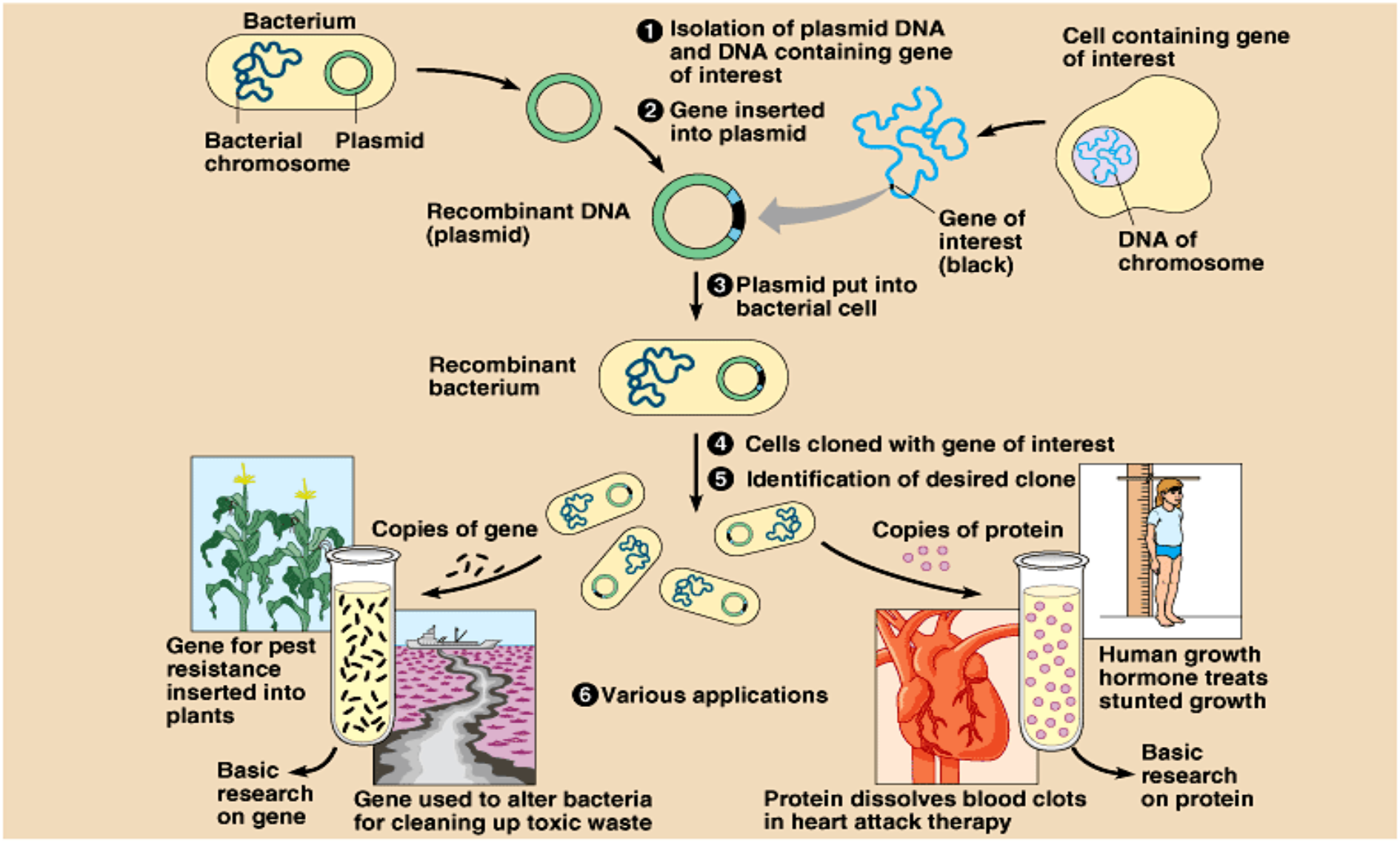
Questions

- Why is making a eukaryotic DNA strand from mRNA better than just using the original DNA?
- What is the benefit of using genomic libraries?
- How would you find 1 unique DNA strand out of a plate of several thousand?
- How could you determine if a gene is active or inactive at certain times in development?
- Would you fix genes if it meant getting rid of diseases?
  - What about the fact that evolution relies on genetic variation for survival to continue?

DNA Technology

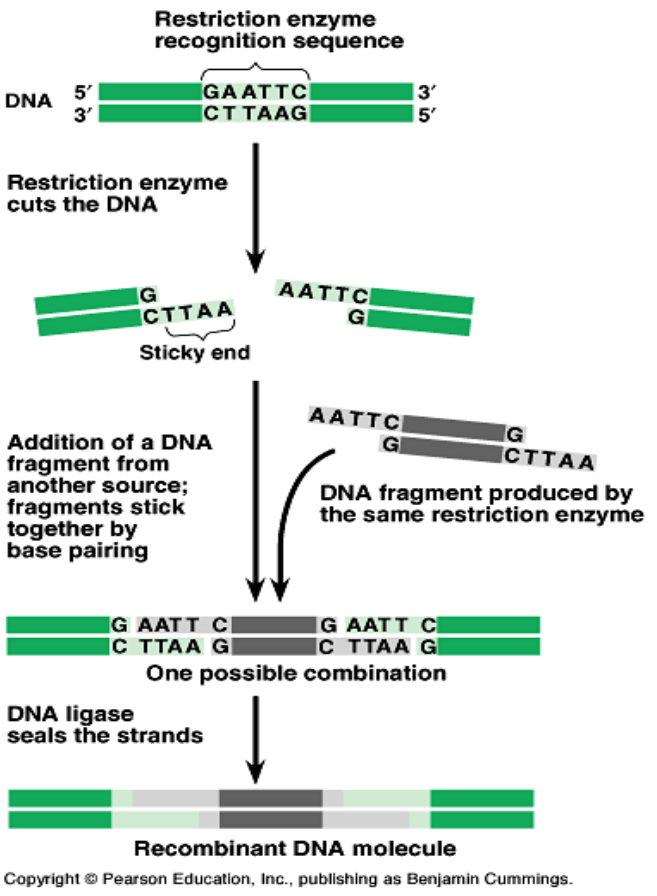
- **Genetic engineering: direct manipulation of genes for practical purposes**
- **Recombinant DNA: DNA in which genes from 2 different sources are linked**
- **Biotechnology: manipulation of organisms or their components to perform practical tasks or provide useful products**

Bacterial plasmids in gene cloning



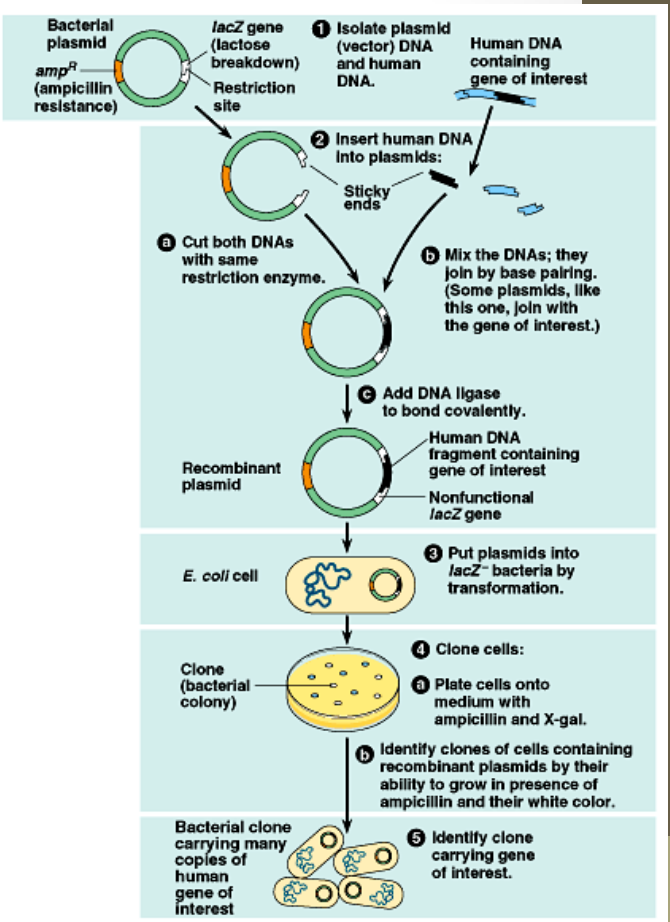
DNA Cloning

- Restriction enzymes (endonucleases): in nature, these enzymes protect bacteria from intruding DNA; they cut up the DNA (restriction); very specific
- Restriction site: recognition sequence for a particular restriction enzyme
- Restriction fragments: segments of DNA cut by restriction enzymes in a reproducible way
- Sticky end: short extensions of restriction fragments
- DNA ligase: enzyme that can join the sticky ends of DNA fragments
- Cloning vector: DNA molecule that can carry foreign DNA into a cell and replicate there (usually bacterial plasmids)



Steps for eukaryotic gene cloning

- **Isolation of cloning vector (bacterial plasmid) and gene-source DNA (gene of interest)**
- **Insertion of gene-source DNA into the cloning vector using the same restriction enzyme; bind the fragmented DNA with DNA ligase**
- **Introduction of cloning vector into cells (transformation by bacterial cells)**
- **Cloning of cells (and foreign genes)**
- **Identification of cell clones carrying the gene of interest**

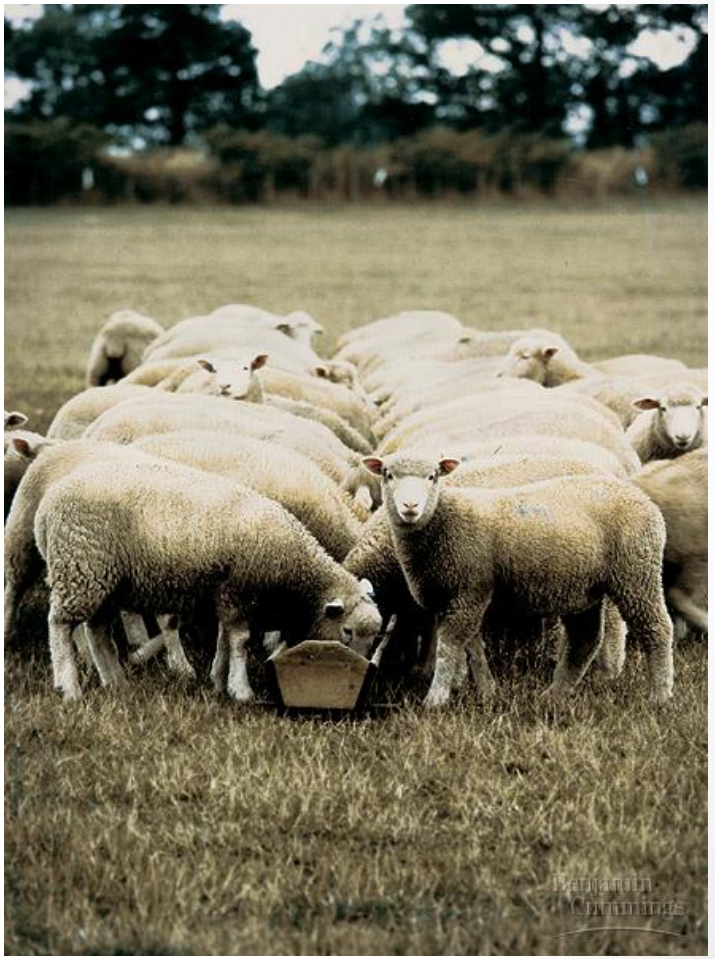


Storing Cloned Genes

- Genomic libraries: complete set of plasmids containing cell clones
  - Cloned genes can also be stored in phages or large plasmids
- cDNA: complimentary DNA made from mRNA that contains the consecutive coding sequence of a gene (no introns)
- Nucleic acid hybridization: using a complementary DNA strand to attach to a gene of interest in a genomic library
  - Radioactive probe attaches to the desired gene and then a photographic film is used to view where the gene is located in the library

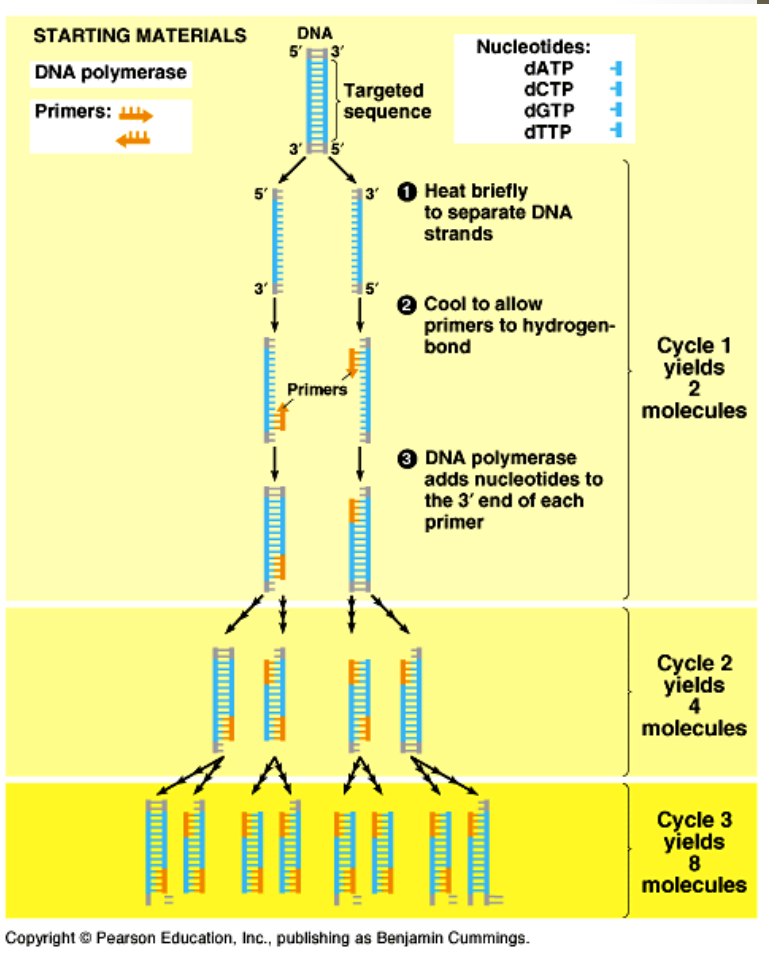
DNA Analysis & Genomics

- **DNA technology allows us to study the sequence, expression, and function of a gene**
- **Examples:**
  - **PCR (polymerase chain reaction)**
  - **Gel electrophoresis**
  - **Restriction fragment analysis (RFLPs)**
  - **Southern blotting**
  - **DNA sequencing**
  - **Human genome project**



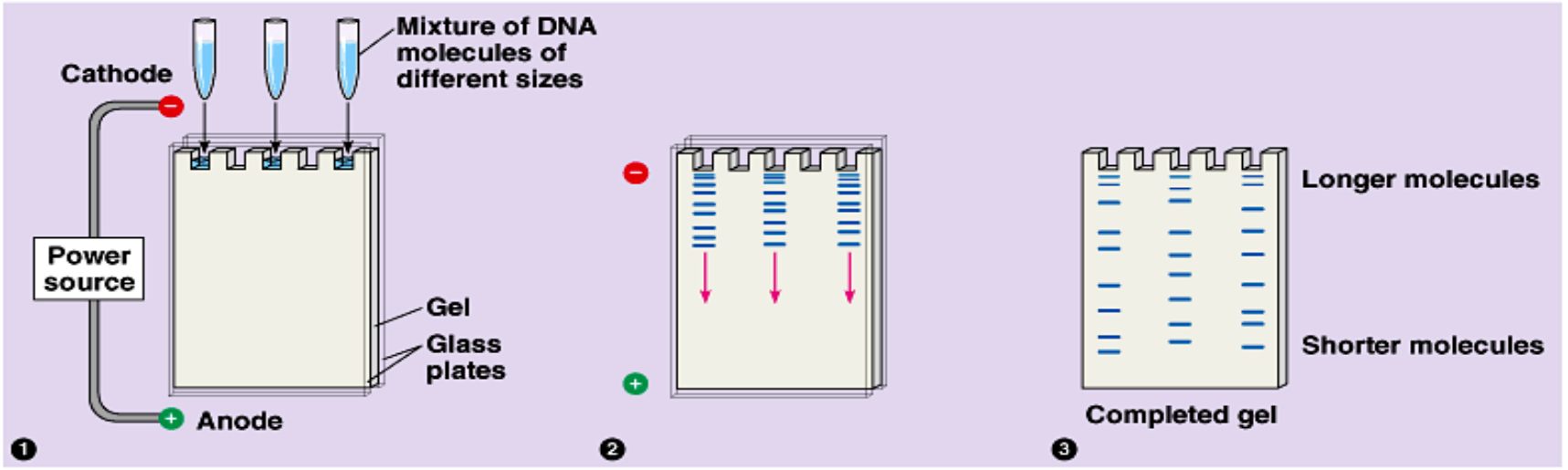
Polymerase chain reaction (PCR)

- **Amplification of any piece of DNA without cells (in vitro)**
- **Materials: heat, DNA polymerase, nucleotides, single-stranded DNA primers**
- **Applications: fossils, forensics, prenatal diagnosis, etc.**



DNA Analysis

- **<u>Gel electrophoresis</u>: separates nucleic acids or proteins on the basis of size or electrical charge creating DNA bands of the same length**



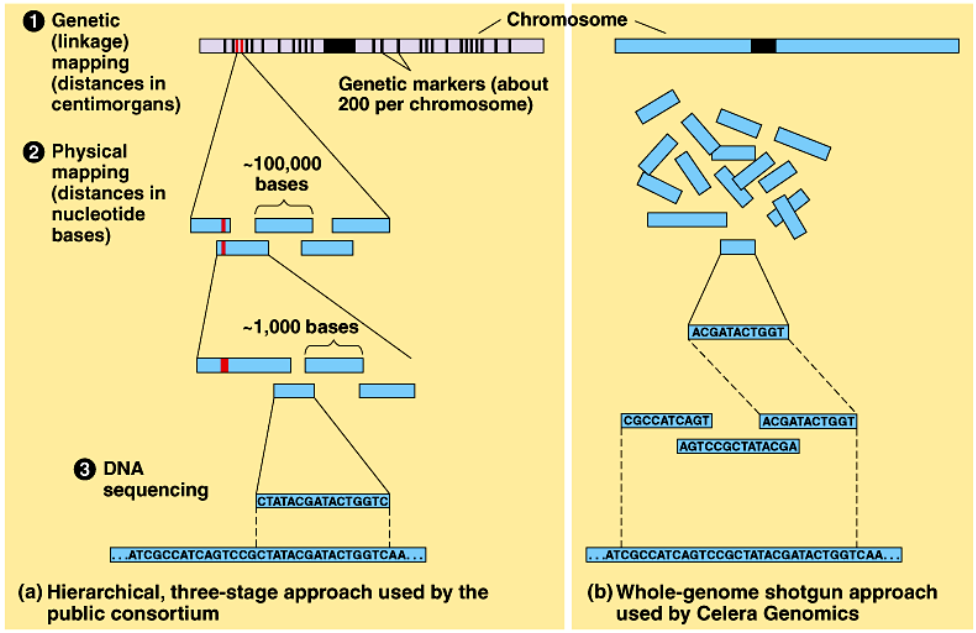
Southern Blotting

- Used when there are too many pieces of DNA fragments to be seen with normal gel electrophoresis
- Combination of gel electrophoresis and nucleic acid hybridization

- Uses: identifying carriers of genetic disorders

DNA Sequencing

- Determination of nucleotide sequences (Sanger method, sequencing machine)
- Genomics: the study of genomes based on DNA sequences
- Human Genome Project



DNA Sequencing and Gene Expression

- Dideoxy Chain Termination Method: using a single strand of DNA to determine the exact sequence of the DNA strand

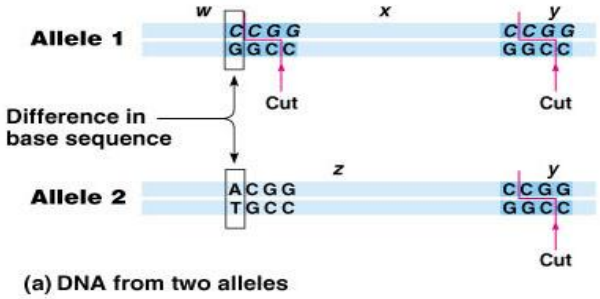
- RT-PCR: taking mRNA from developmental stages of organisms to determine which genes are active at different times
  - Pull out the mRNA, make DNA strand, amplify specific gene of interest, and use gel electrophoresis to see the strands

DNA Sequencing

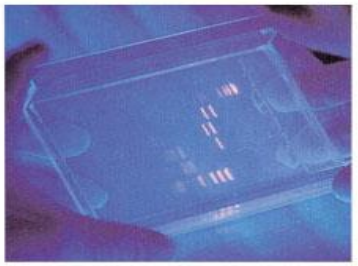
- Sequencing Video

Restriction fragment analysis

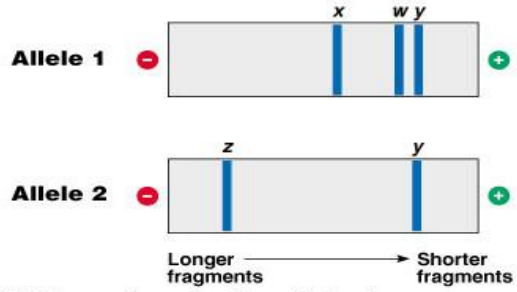
- **Restriction fragment length polymorphisms (RFLPs): specific sizes of restriction fragments that are used to distinguish between individuals**
- **DNA Fingerprinting**
- **Short tandem repeats (STRs): variation from person to person; more sensitive method than the RFLP analysis**



(a) DNA from two alleles



(c) Completed gel



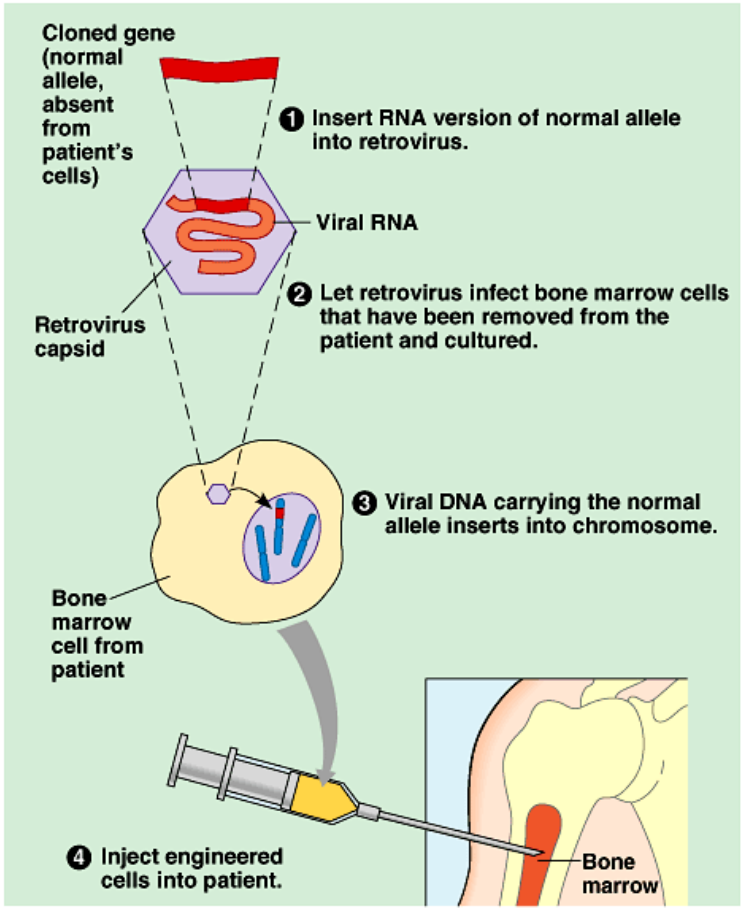
(b) Electrophoresis of restriction fragments

Animal Cloning

- Steps
  - Mammary cell donor DNA is added to an egg striped of its DNA
  - Cells are fused and grown in culture
  - Embryo is added to surrogate mother
  - Baby is genetically identical to mammary cell donor

Practical DNA Technology Uses

- Diagnosis of disease
- Human gene therapy
- Stem cells
- Pharmaceutical products (vaccines)
- Forensics
- Animal husbandry (transgenic organisms)
- Genetic engineering in plants
- Ethical concerns?



Gene Therapy

Ch21: Genomes and Their Evolution

Branches of Science

- Genomics = study of whole sets of genes and their interactions
- Bioinformatics = application of computational methods to the storage and analysis of biological data

Genome Sequencing

- Roughly locating where genes are on a chromosome

- Construct a linkage map
  - Order of markers (RFLPs or STRs) and relative distances between genes
- Construct a physical map
  - Ordering fragments by determining where they overlap with each other
- Sequence the DNA
  - Determine the order of nucleotides in a strand of DNA

Genome Sequencing

- Currently use the whole genome shot-gun approach
  - Skips the linkage and physical mapping stage and moves right to sequencing DNA using computer programs

Human genome project

- Started in 1990 and concluded in 2003

- Repetitive DNA that includes transposable elements – 44%
- Repetitive DNA that does not include transposable elements – 15%
- Introns – 24%
- Unique noncoding DNA – 15%
- Exons – 1.5%

Transposable Elements

- Transposons = segments of DNA that may be cut and moved from its original location on a chromosome to a new location
  - “Wandering DNA segments”

- Importance – can lead to a recombination of genes if the transposon is inserted into an exon
  - One of the mechanisms for evolution

Video

- Barbara McClintock – Great minds
- Discovery Video