CHAPTER 20 LECTURE SLIDES

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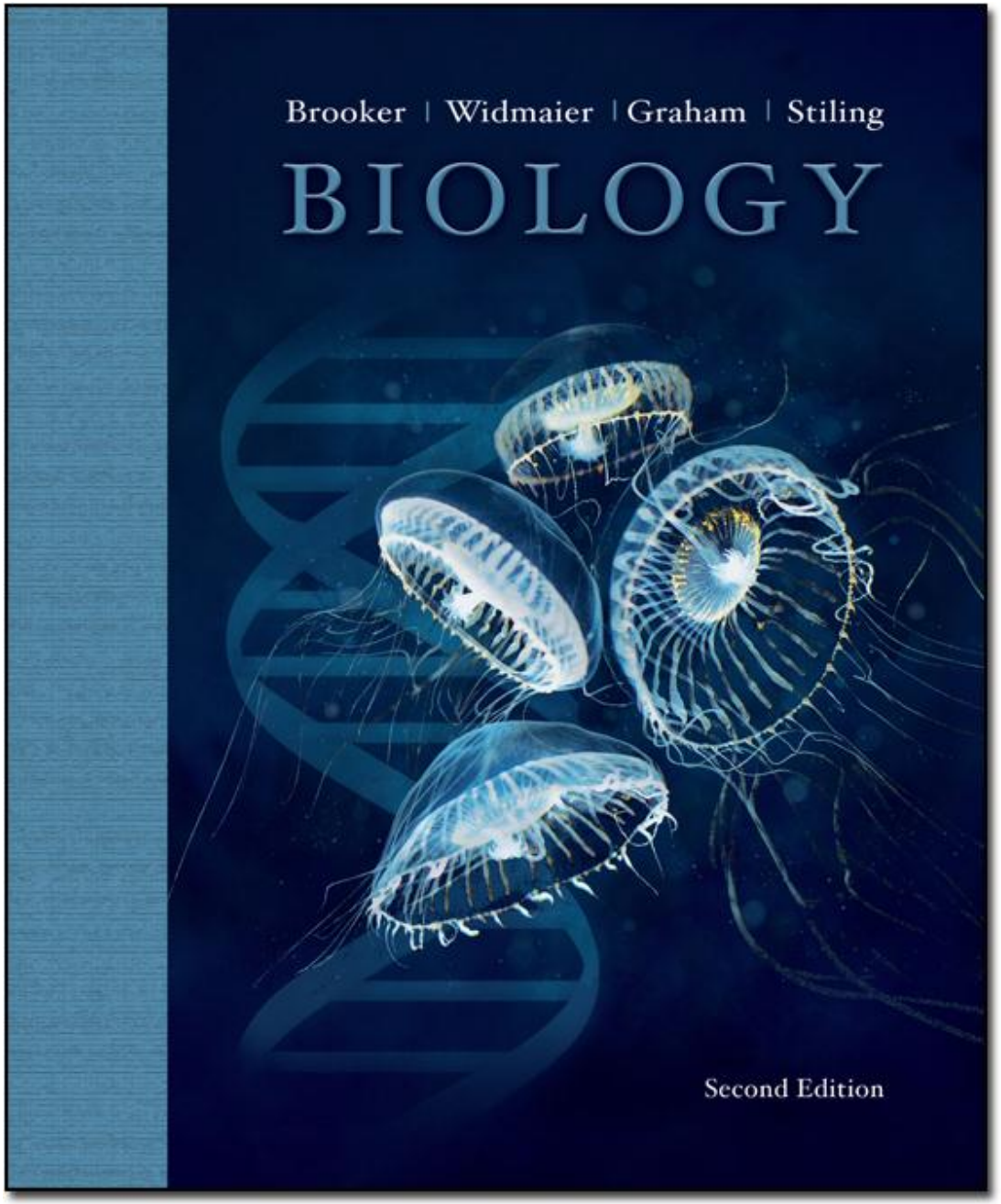
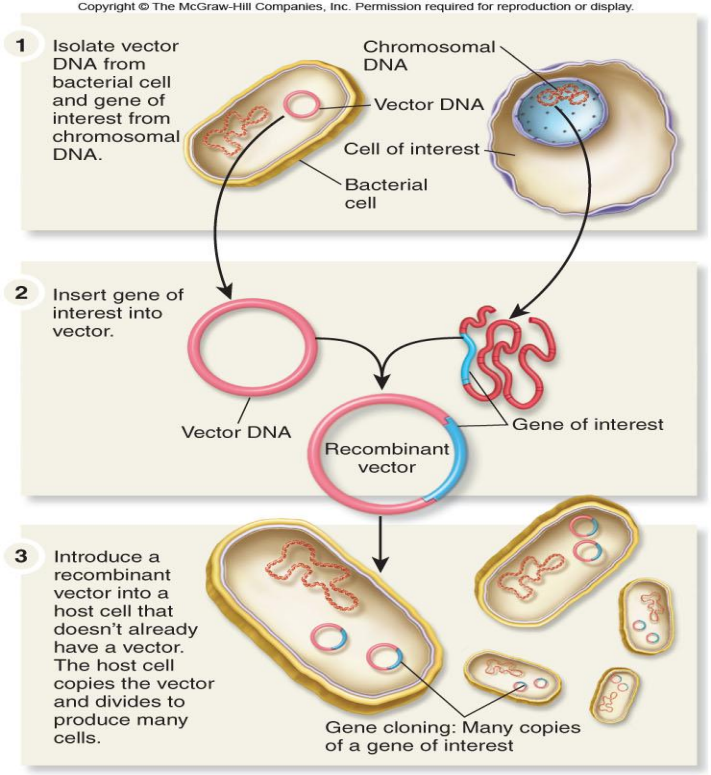
To run the animations you must be in **Slideshow View**. Use the buttons on the animation to play, pause, and turn audio/text on or off. Please note: once you have used any of the animation functions (such as Play or Pause), you must first click in the white background before you advance the next slide.



- Recombinant DNA technology
  - Use of laboratory techniques to isolate and manipulate fragments of DNA
- Recombinant DNA contains DNA from 2 or more sources
- Once inside a host cell, recombinant molecules are replicated to produce identical copies or clones

Gene cloning

- Procedures that lead to the formation of many copies of a particular gene
- Why?
  - Want copies of a gene for study or use
  - Obtain lots of gene product- mRNA or protein



4 Gene cloning is done to achieve one of two main goals:

Producing large amounts of DNA of a specific gene	Expressing the cloned gene to produce the encoded protein
Examples • Cloned genes provide enough DNA for DNA sequencing. The sequence of a gene can help us understand how a gene works and identify mutations that cause diseases. • Cloned DNA can be used as a probe to identify the same gene or similar genes in other organisms.	*Examples* • Large amounts of the protein can be purified to study its structure and function. • Cloned genes can be introduced into bacteria or livestock to make pharmaceutical products such as insulin. • Cloned genes can be introduced into plants and animals to alter their traits. • Cloned genes can be used to treat diseases—a clinical approach called gene therapy.

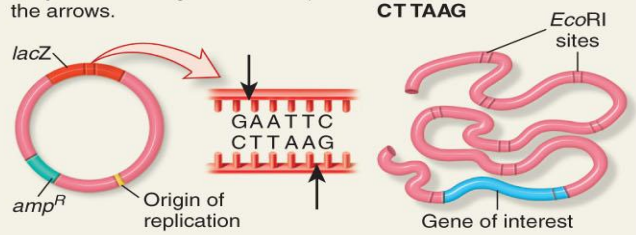
Step 1 in gene cloning

- Vector DNA acts as a carrier for the DNA segment to be cloned
- When a vector is introduced into a living cell, it can replicate making many copies
- Common vectors are plasmid or viral
- Also need the gene of interest from chromosomal DNA

Step 2

- Insert chromosomal DNA into vector
- Cut DNA using restriction enzymes or restriction endonucleases
  - Made naturally by bacteria as protection against bacteriophages
  - Cuts at specific known restriction sites
  - Most restriction sites palindromic
  - May produce sticky ends
  - DNA ligase must be used to permanently link DNA
- Result may be
  - Recircularized vector with no gene of interest inserted
  - Recombinant vector with gene of interest inserted

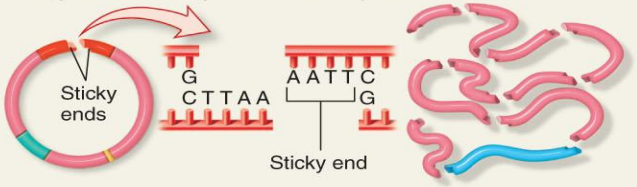
1 Cut vector and chromosomal DNA with *Eco*RI, a restriction enzyme that recognizes the sequence **GAATTC** and cuts at the arrows.



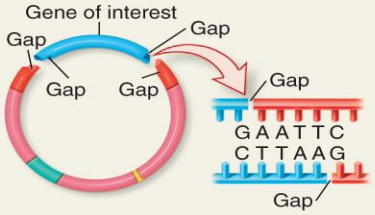
Vector DNA has one *Eco*RI site.

Chromosomal DNA has many *Eco*RI sites.

The restriction enzyme opens up the vector and cuts the chromosomal DNA into many fragments with short single-stranded regions called sticky ends.

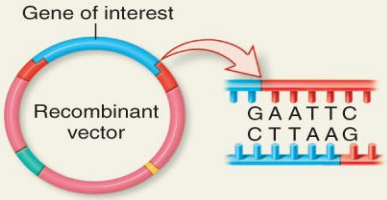


2 Allow sticky ends to hydrogen-bond with each other due to complementary sequences.



In this example, a fragment of DNA carrying the gene of interest has hydrogen-bonded to the vector. Four gaps are found where covalent bonds in the DNA backbone are missing.

3 Add DNA ligase to close the gaps by catalyzing the formation of covalent bonds in the DNA backbone.

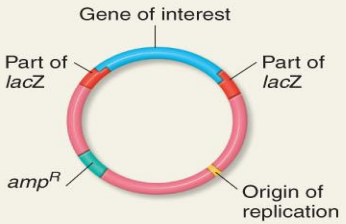


Step 3 – actual cloning

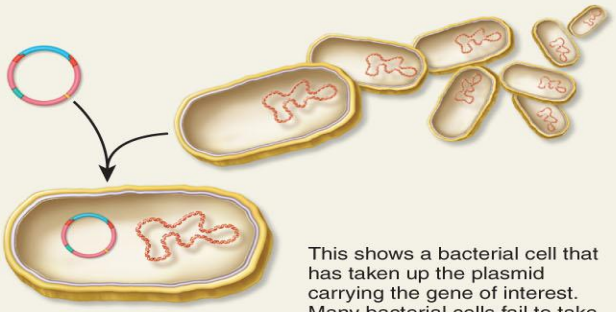
- Goal for recombinant vector to be taken up by bacteria
  - Some will take up a single plasmid
  - Most cells fail to take up a plasmid
- Vector carries a selectable marker
  - Presence of antibiotics selects for cells expressing amp^R gene – contains plasmid
  - amp^R gene codes for b-lactamase that degrades ampicillin, which normally kills bacteria

- After treatment, only cells with the plasmid will grow on plates treated with ampicillin
  - To eliminate recircularized vectors from further examination, lacZ gene part of vector
  - Insertion of chromosomal DNA disrupts lacZ gene
  - lacZ codes for b-galactosidase which cleaves colorless X-Gal into a blue dye
    - Recircularized plasmids will form blue colonies
    - Recombinant vectors will form white colonies

1 Mix plasmid DNA with many *E.coli* cells that have been treated with agents that make them permeable to DNA.

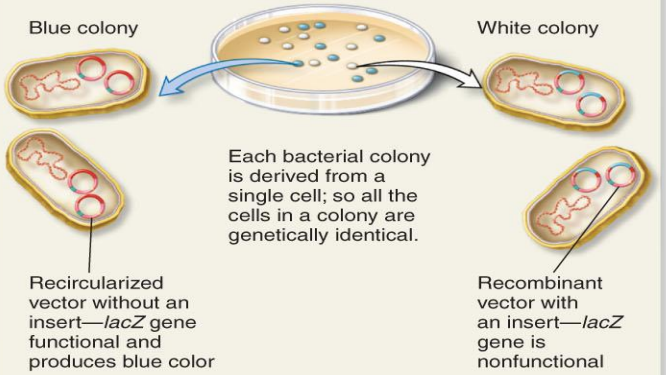


In this example, the gene of interest was inserted into a plasmid. This separates the *lacZ* gene and renders it nonfunctional. It is also possible for any other chromosomal DNA fragment to be inserted into the plasmid, or the plasmid may recircularize without an insert.



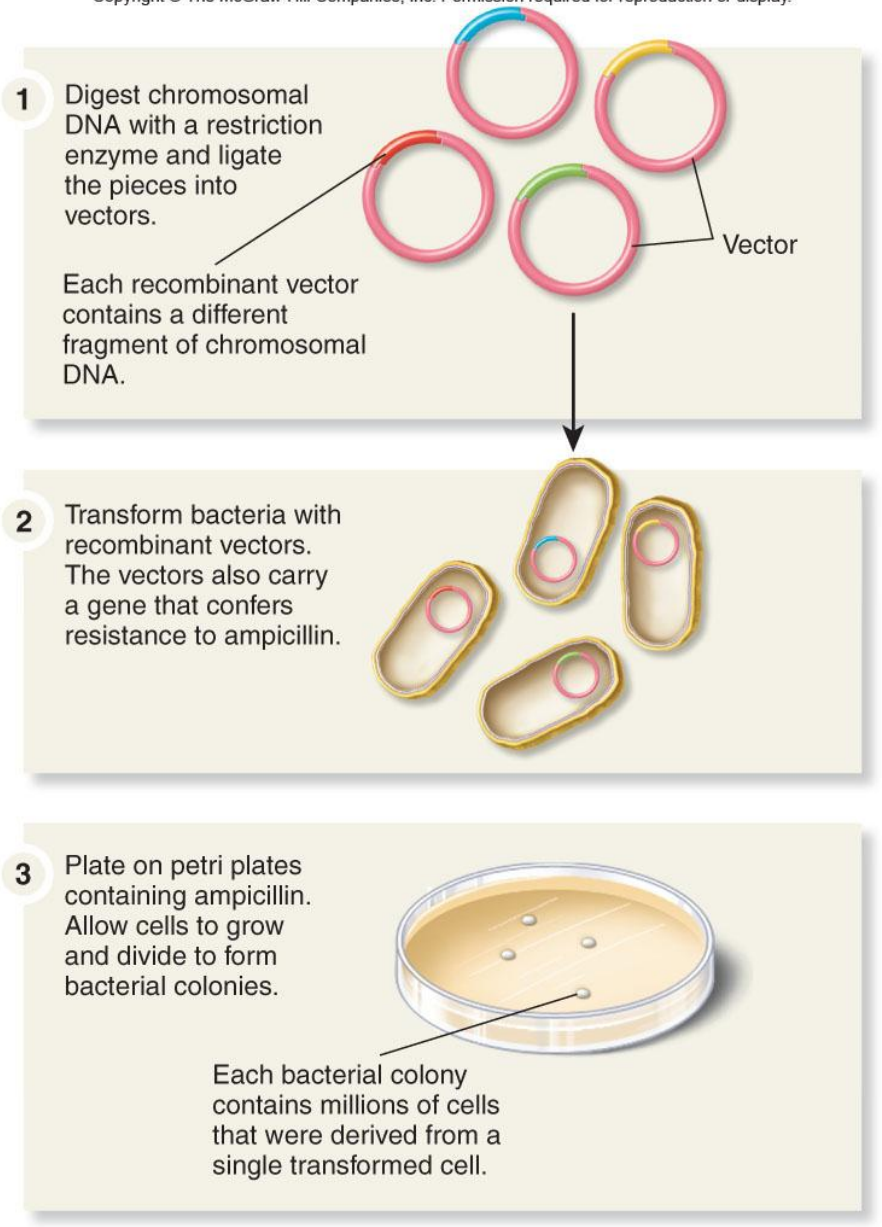
This shows a bacterial cell that has taken up the plasmid carrying the gene of interest. Many bacterial cells fail to take up a plasmid.

2 Plate cells on media containing X-Gal and ampicillin. Incubate overnight. Note: The *ampR* gene allows bacteria to grow in the presence of ampicillin. The *lacZ* gene encodes β-galactosidase that degrades X-gal to produce a blue color.



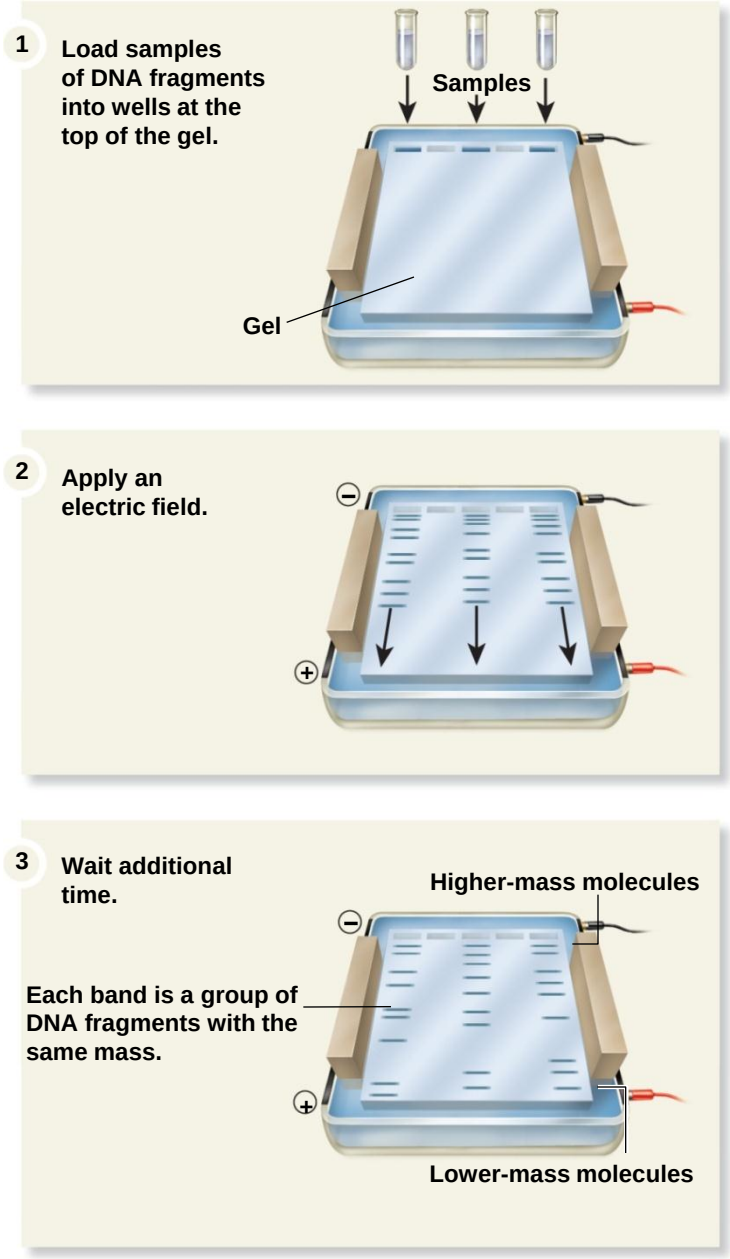
DNA library

- Treatment of chromosomal DNA with restriction enzymes yields tens of thousands of different fragments
- DNA library- collection of many recombinant vectors each with a fragment of chromosomal DNA
- 2 types of common DNA libraries
  - Genomic – inserts derived from chromosomal DNA
  - cDNA – use reverse transcriptase to make DNA from mRNA of interest (complementary DNA) - lacks introns so simpler to use



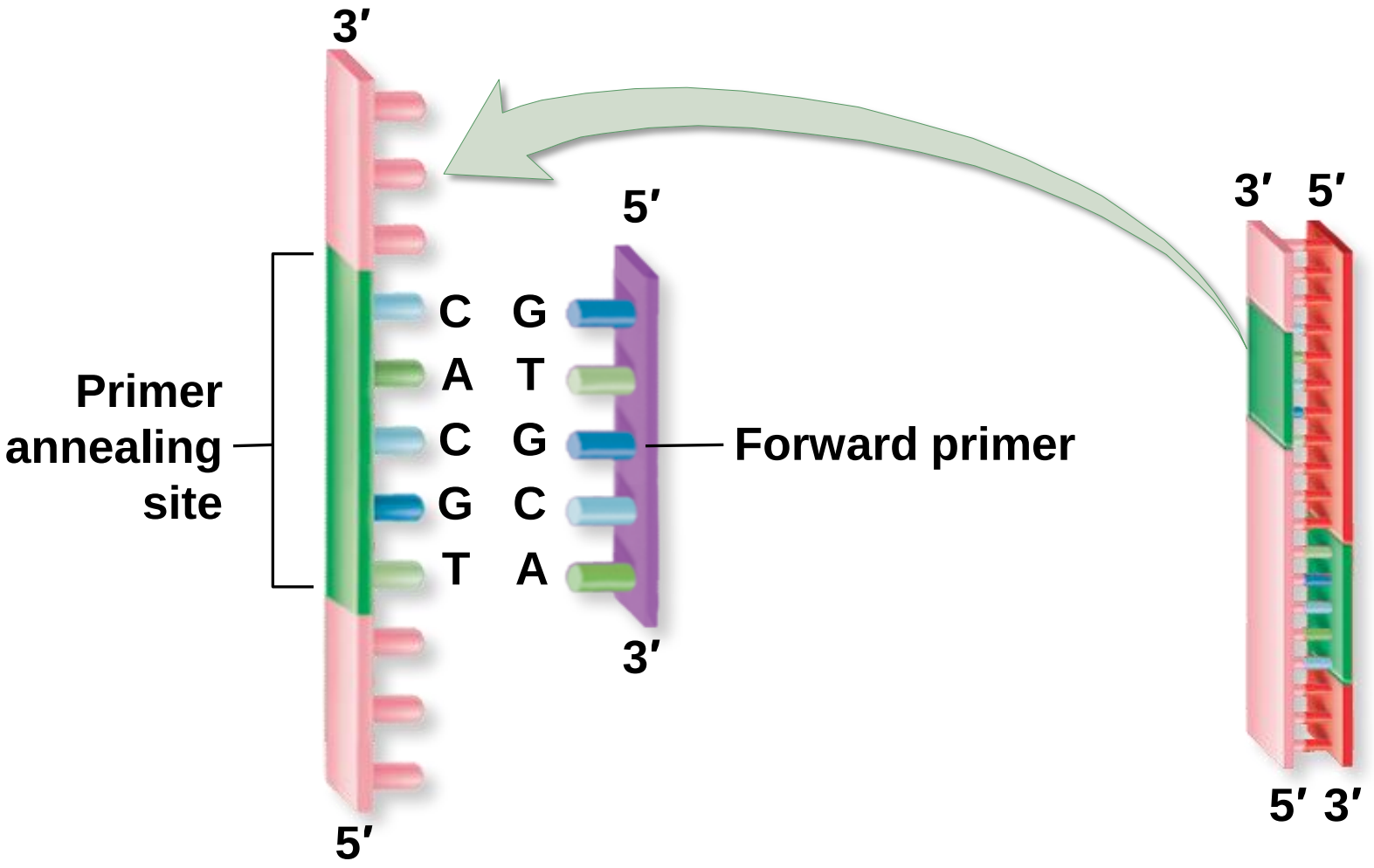
Electrophoresis

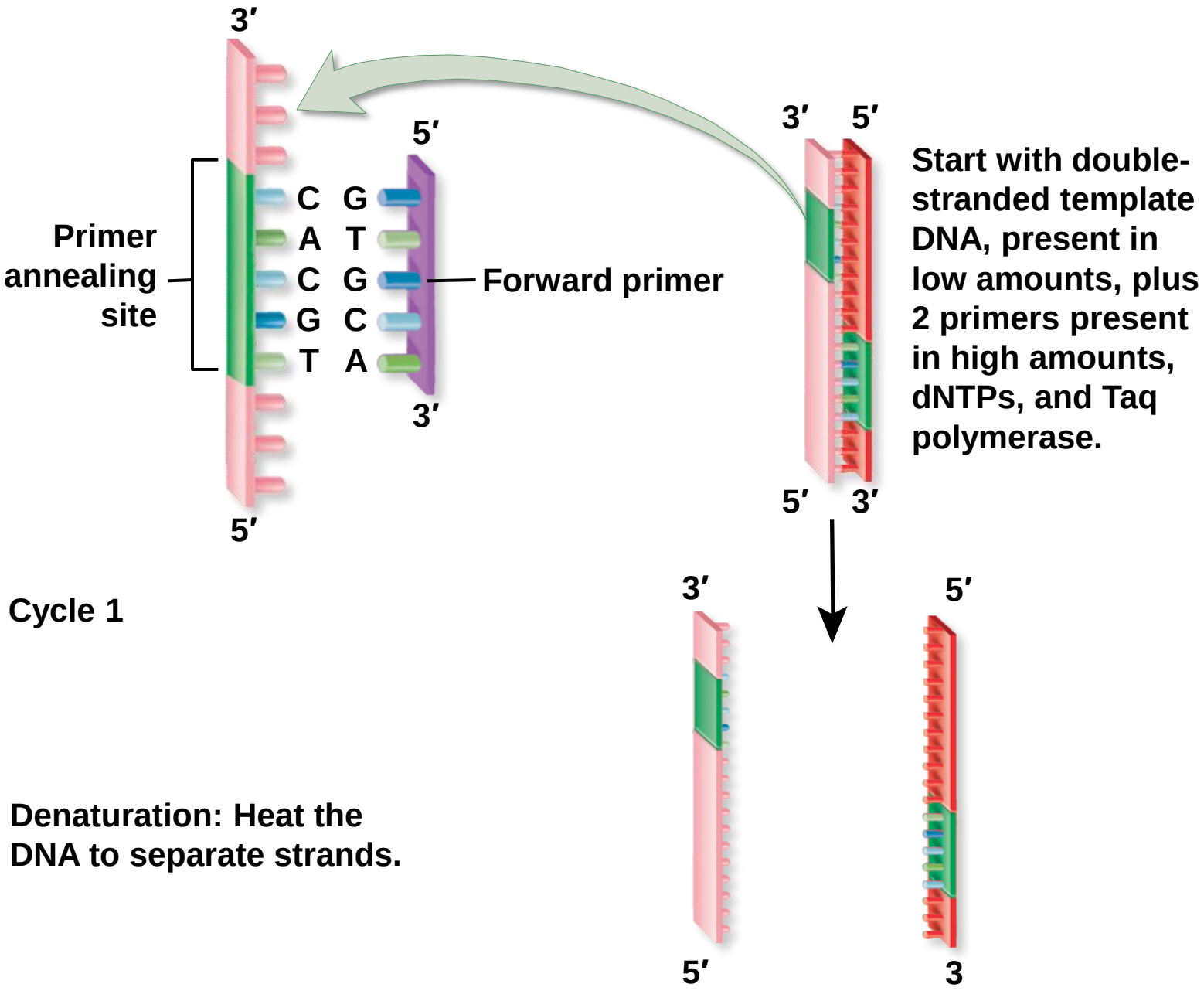
- Technique that is used to separate macromolecules, such as DNA and proteins, on a gel
- Can be used to separate molecules based on their charge, size/length, and mass

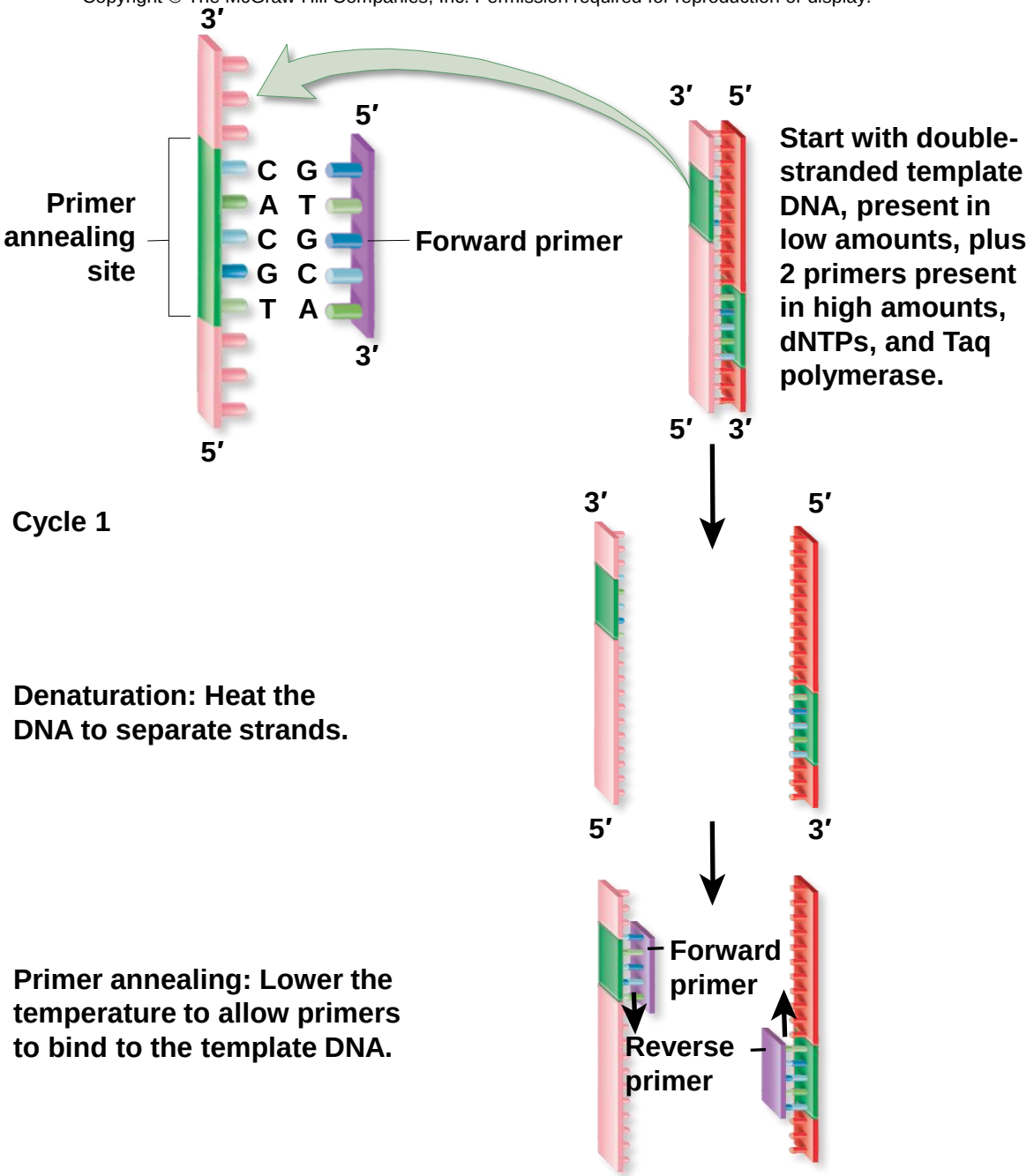


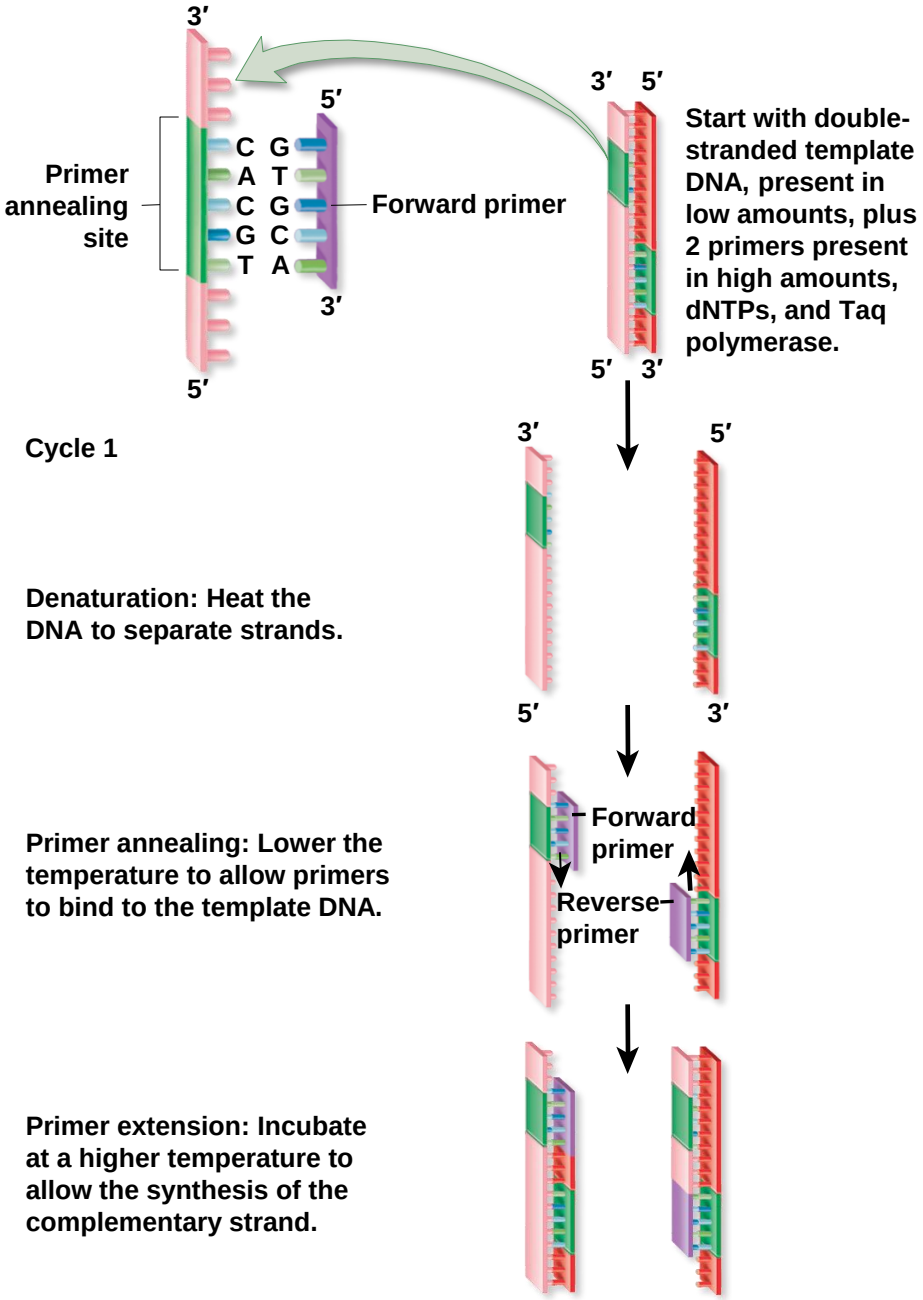
Polymerase chain reaction (PCR)

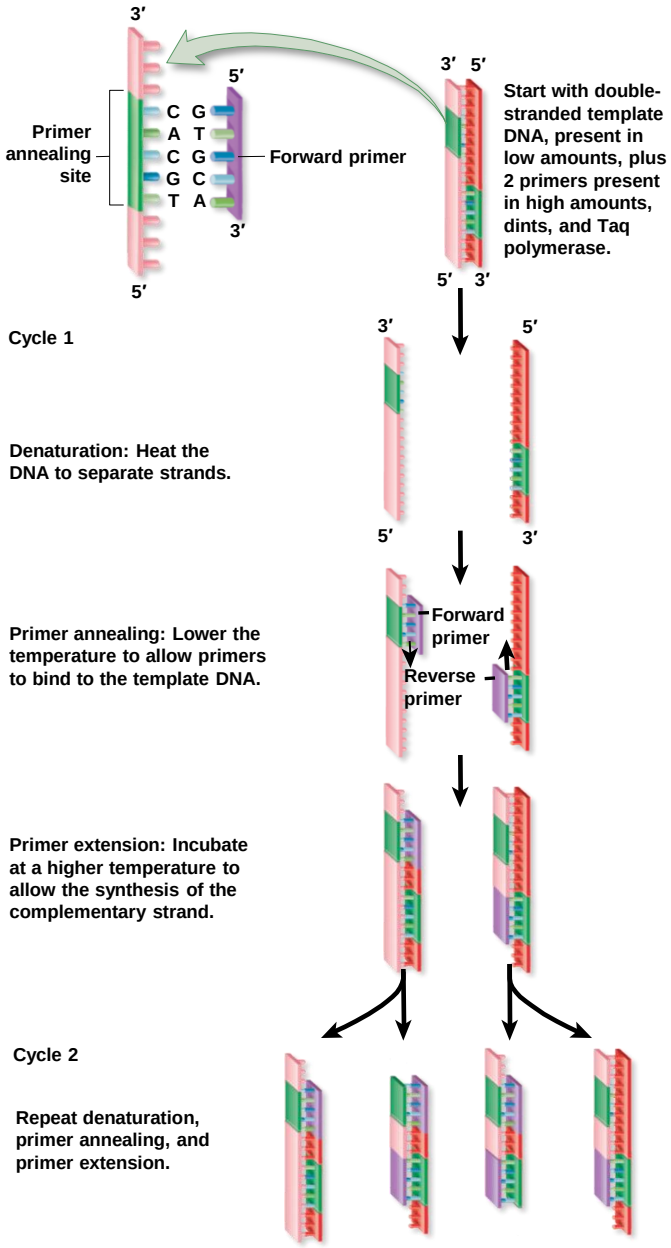
- Copy DNA without vectors and host cells
- Goal to make many copies of DNA in a defined region
- Uses high concentration of two primers that are complementary to sequences at the ends of the DNA region to be amplified, deoxynucleoside triphosphates (dNTPs), and a heat-stable form of DNA polymerase called *Taq* polymerase
- Sample of DNA taken through repeated cycles of denaturation, annealing and synthesis
  - Thermocycler automates this process
- After 30 cycles of amplification, a DNA sample will increase 2^{30}-fold

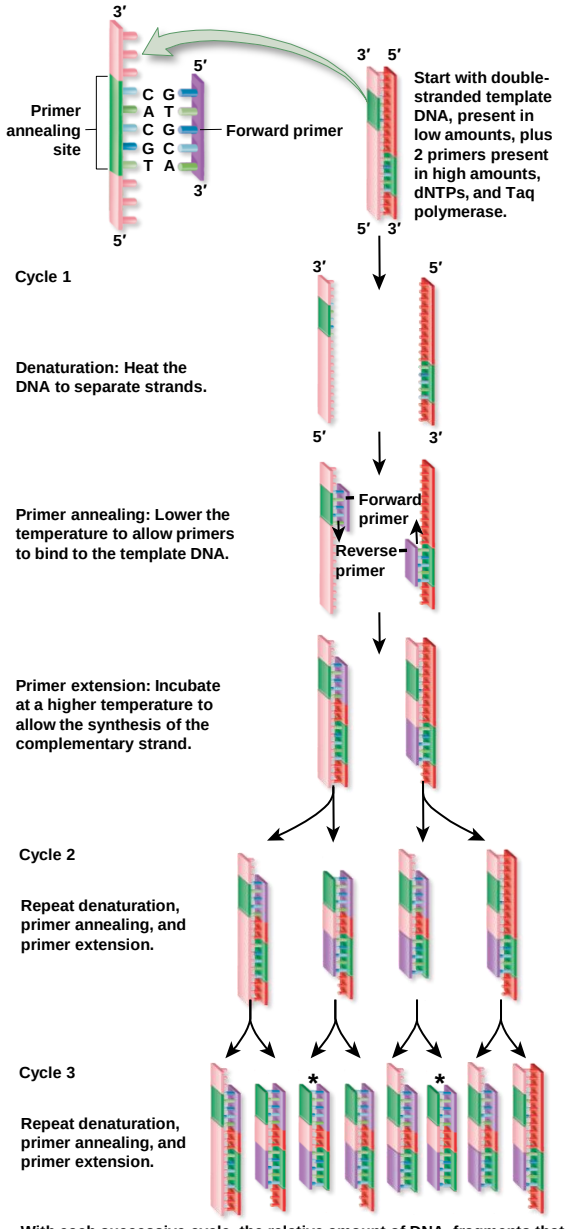




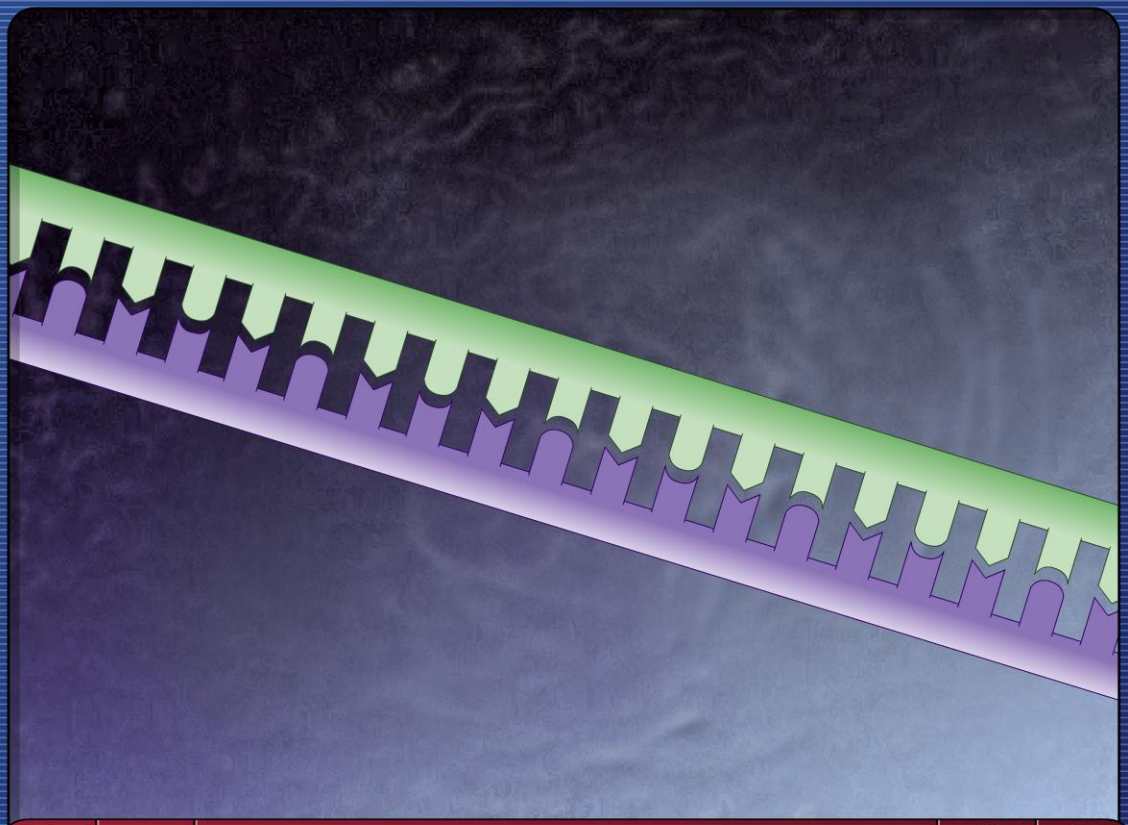








Polymerase Chain Reaction



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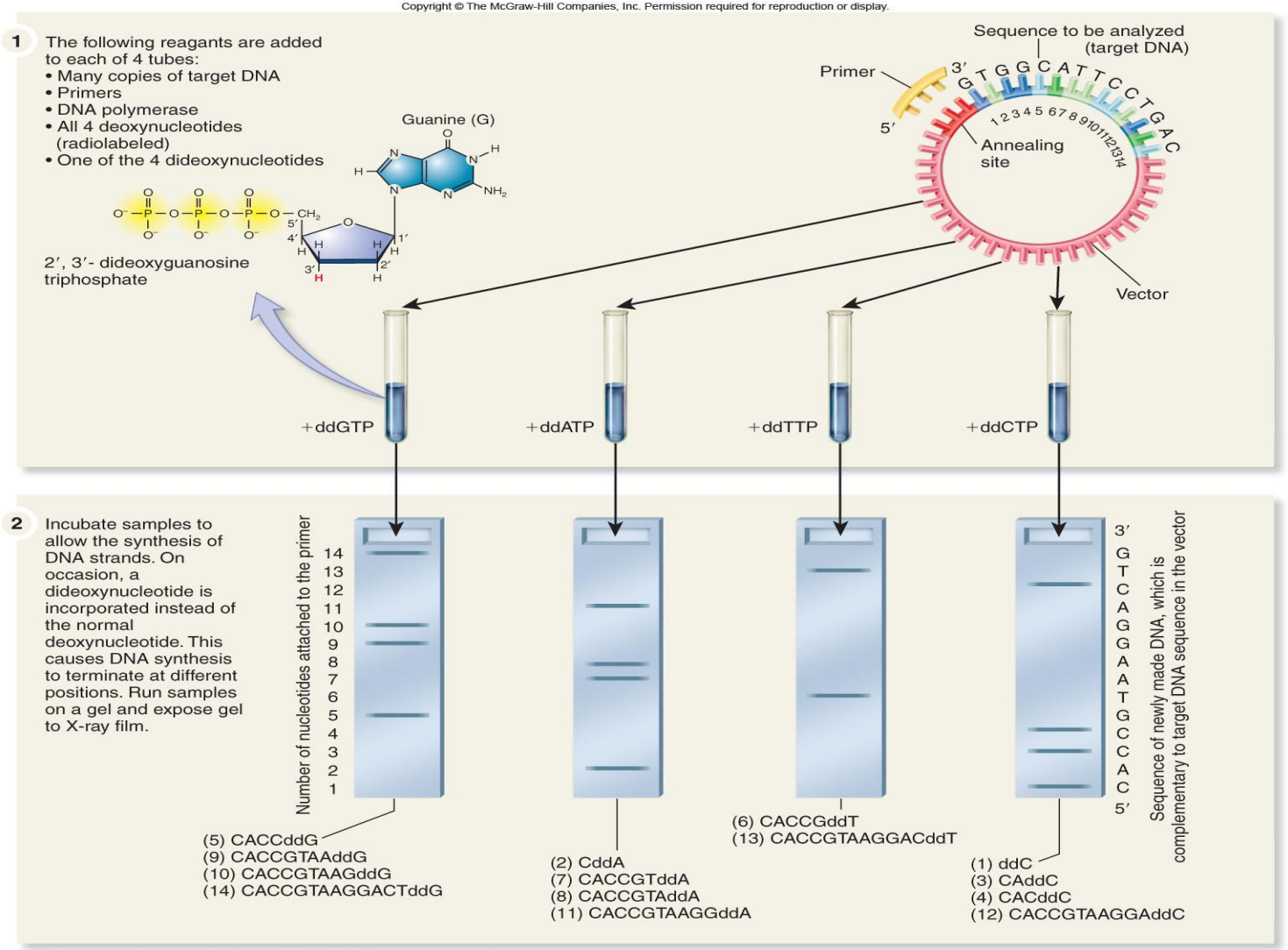
The polymerase chain reaction is a method for making many copies of a specific segment of DNA, starting with a very small amount.

Genomics

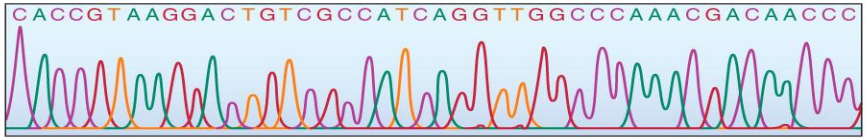
- Refers to the molecular analysis of the entire genome of a species
- 2 phases
  - Mapping of genome
  - Functional genomics
    - Studying expression – which genes turned on or off in particular cells

DNA sequencing

- Determines base sequence of DNA
- Dideoxy chain-termination method or dideoxy sequencing
  - Dideoxynucleoside triphosphates (ddNTPs) are missing the 3' –OH group and will terminate the chain
  - 4 tubes with many copies of single stranded DNA of interest
    - Each tube has a different radiolabelled dNTP
  - DNA polymerase will make complementary strand until dNTP inserted and chain terminates
  - After electrophoresis, DNA sequence can be read by reading which base is at the end of the DNA strand
- Procedure has been automated using fluorescent dyes in one tube

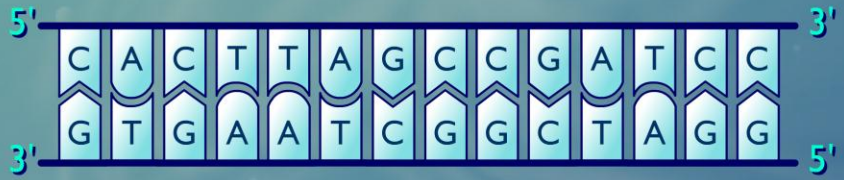


(a) The procedure used in traditional dideoxy sequencing



(b) Output from automated dideoxy sequencing

Sanger Sequencing



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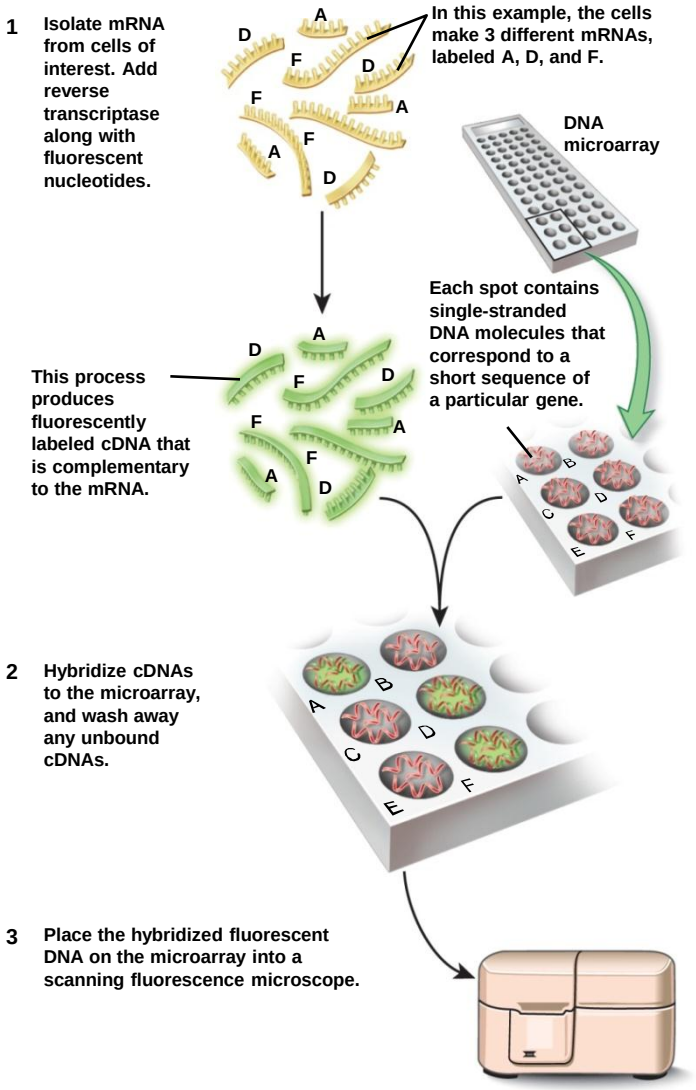
To sequence DNA by the chain termination method, the DNA must first be obtained in single-stranded form.

A Microarray Can Identify Which Genes Are Transcribed by a Cell

- DNA microarray or gene chip
- Used to monitor the expression of thousands of genes simultaneously
- Short sequences of known genes attached to spots on slide
- Goal to find out which genes are transcribed into mRNA in particular sample of cells
- mRNA isolated from those cells and used to make fluorescently labeled cDNA
- cDNAs that are complementary to the DNAs in the microarray will hybridize
- If the fluorescence intensity in a spot is high, a large amount of cDNA was in the sample that hybridized to the DNA at this location

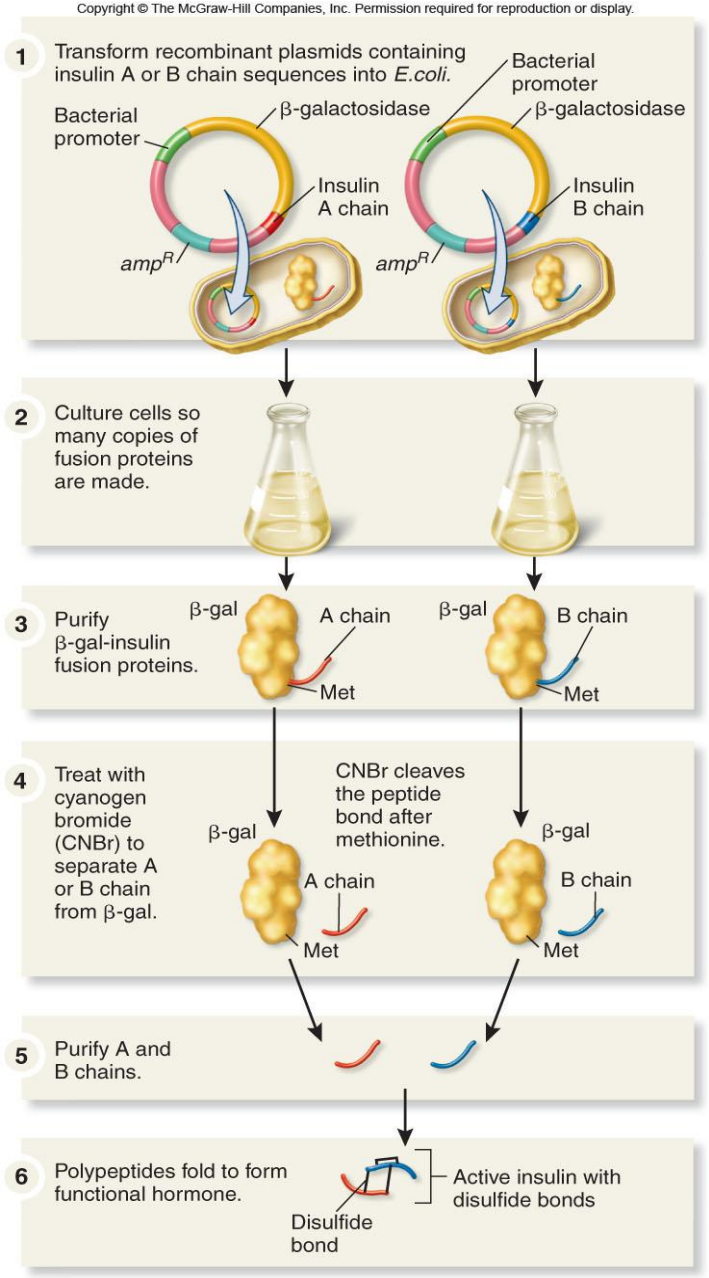


Biotechnology

- Technologies that involve the use of living organisms to benefit humans
- Use began about 12,000 years ago with livestock domestication
- More recently associated with molecular genetics

Insulin

- In 1982, US FDA approved sale of human insulin made by recombinant bacteria
- Prior to 1982, insulin isolated from cattle
  - Some people developed allergies and had to use cadaver insulin
- Insulin composed of 2 polypeptides – A and B
  - A and B coding sequence inserted into *E.coli*
  - Fusion proteins extracted and β-galactosidase removed
  - Purified A and B chain mixed to form functional protein



Bioremediation

- Use of microorganisms or plants to detoxify pollutants in the environment
- Enzymes produced by microorganism can alter or transform toxic pollutant structure
- May degrade toxic form into less complex, nontoxic metabolites
- 1980, first patented recombinant microorganism
  - Not a commercial success

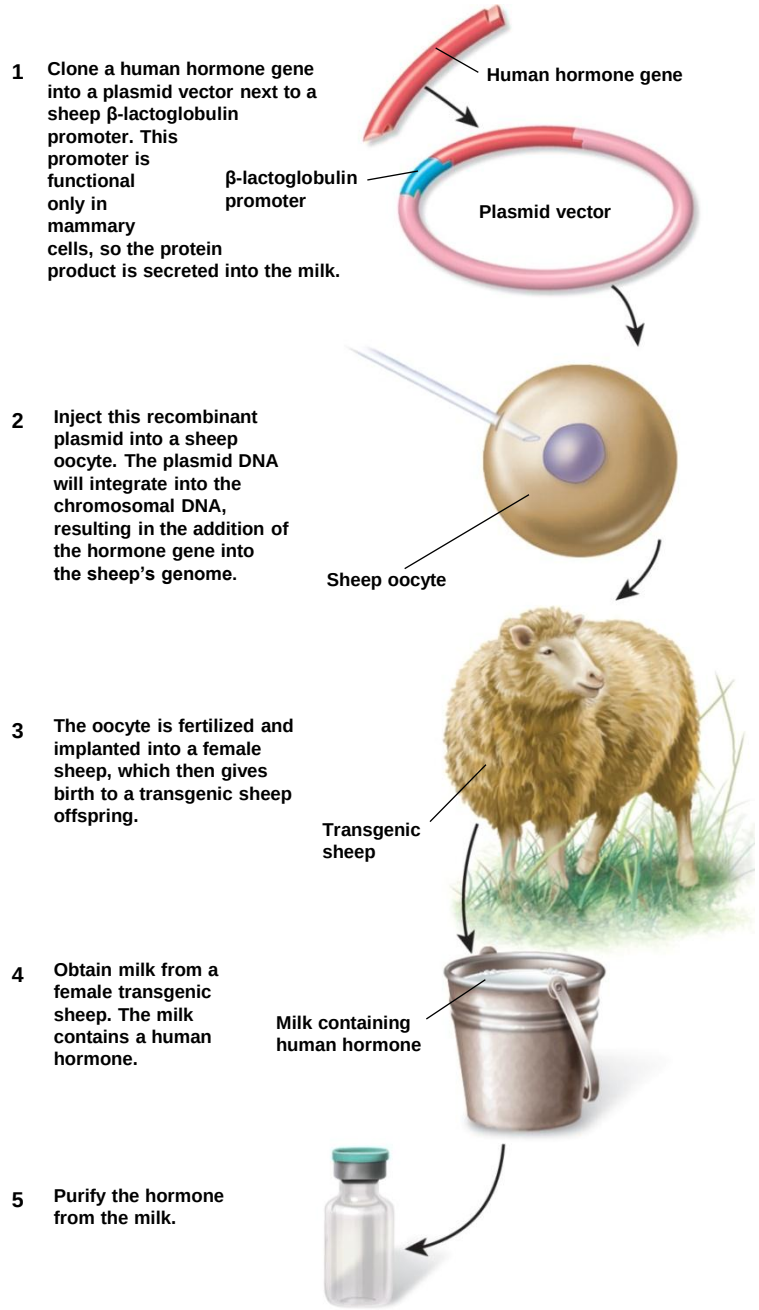
Transgenics

- An organism that carries genes introduced using molecular techniques such as gene cloning
  - Genetically modified organisms (GMOs)
- Gene replacement – cloned gene recombines with normal gene on a chromosome
  - Only 1 of 2 copies replaced creating hetrozygote
  - Heterozygotes can be crossed to yield homozygotes
- Gene knockout – if cloned gene is a mutation that inactivates function, homozygote will not have gene function
- Application in studying human disease
  - Used as models to study disease
  - Used to test effect of therapies

Molecular pharming

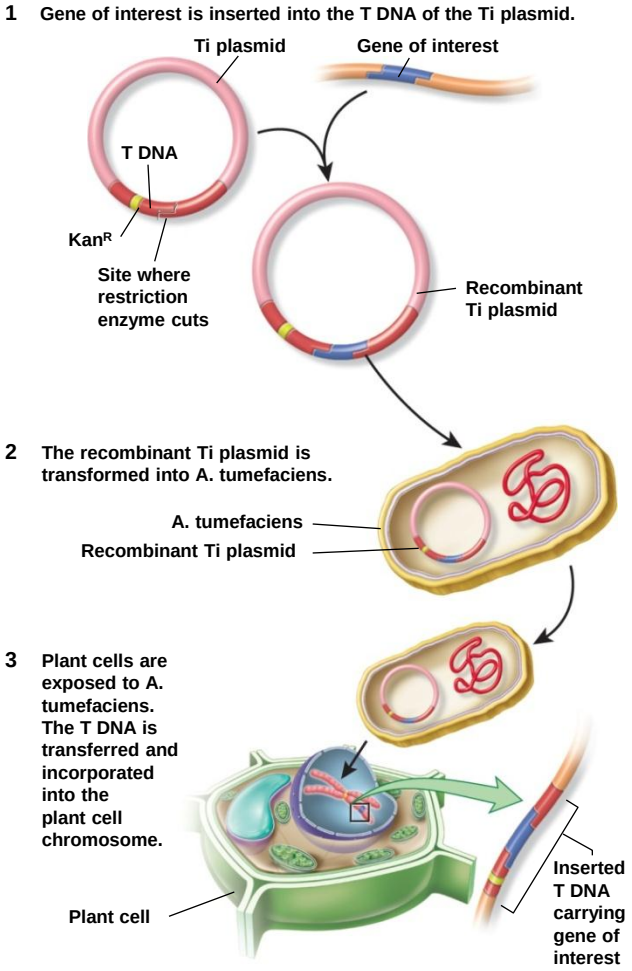
- Production of medically important proteins in livestock mammary glands
- Certain proteins more likely to function when expressed in mammals
  - Post-translational modification
  - Degraded or improperly folded in bacteria
  - High yield in cows

- Strategy to clone gene next to the promoter of a gene specifically expressed in mammary cells
- Vector injected into oocyte, fertilized and implanted
- Milk containing hormone purified for protein



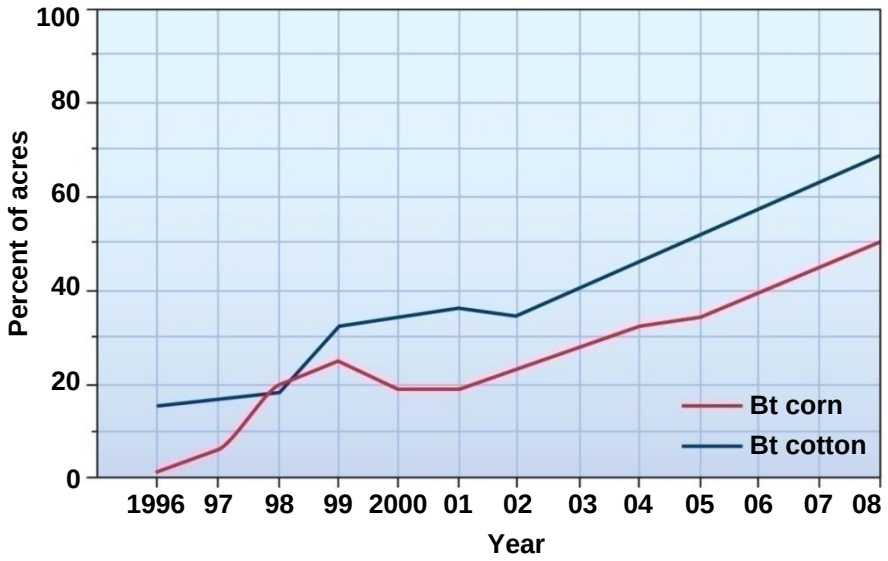
Transgenic plants

- Somewhat easier because plant cells are totipotent
- Cloned genes can be introduced into somatic tissue and entire plant regenerated with hormonal treatments
- *Agrobacterium tumefaciens* naturally infects plant cells and causes tumors
  - Contains Ti plasmid that integrates into host chromosome
  - Codes for plant growth hormones that form crown gall tumor
- Ti plasmid modified to introduce cloned genes
  - Kan^R used as a selectable marker for kanamycin resistance
  - Contain convenient restriction sites
- Transformed cells plated on media with kanamycin (kills nontransformed cells) and carbenicillin (kills *Agrobacterium*)



- Successful example of the use of transgenic plants has involved the introduction of genes from *Bacillus thuringiensis (Bt)*
- Bacterium produces toxins that kill certain types of caterpillars and beetles and has been widely used as an insecticide for several decades
- Such Bt varieties of plants produce the toxins themselves and therefore are resistant to many types of caterpillars and beetles

(a) A field of Bt corn

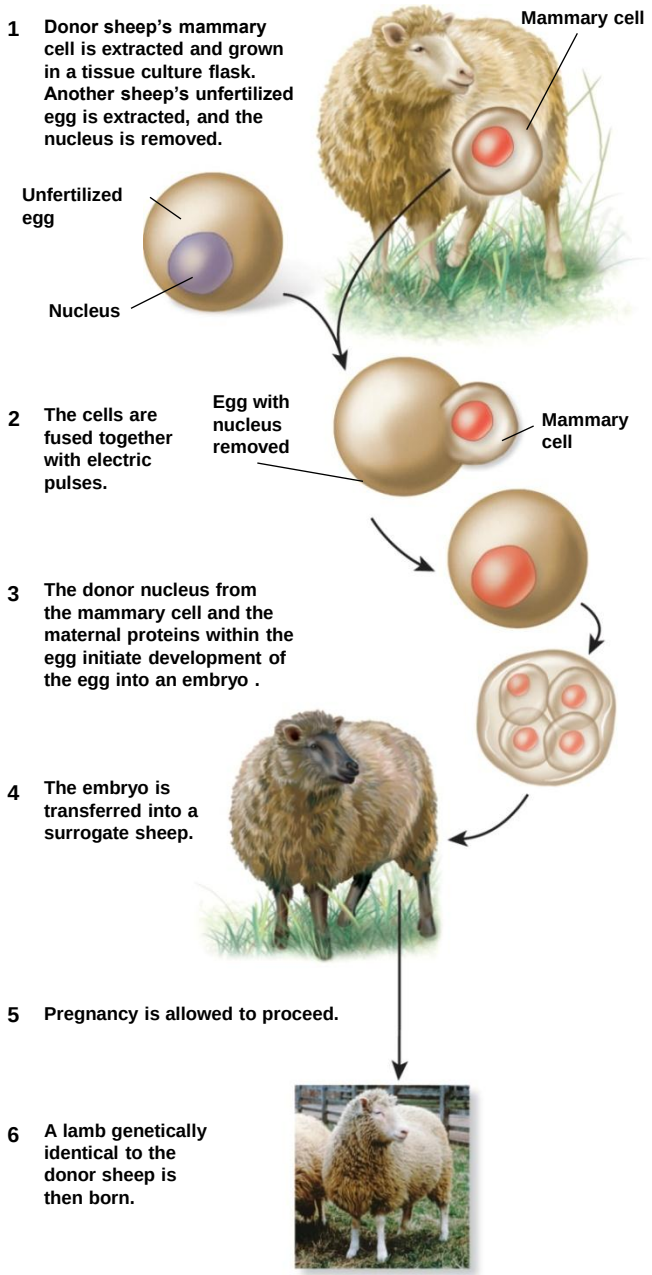


(b) Bt corn and Bt cotton usage since 1996

a: © Bill Barksdale/Agestockusa/Age fotostock

Cloning mammals

- Identical twins are genetic clones from a single fertilized egg that split early in development
- Plants can be cloned from somatic cells
- Believed for decades that mammalian somatic cells could not be used for cloning
- 1996, Dolly is the first cloned lamb

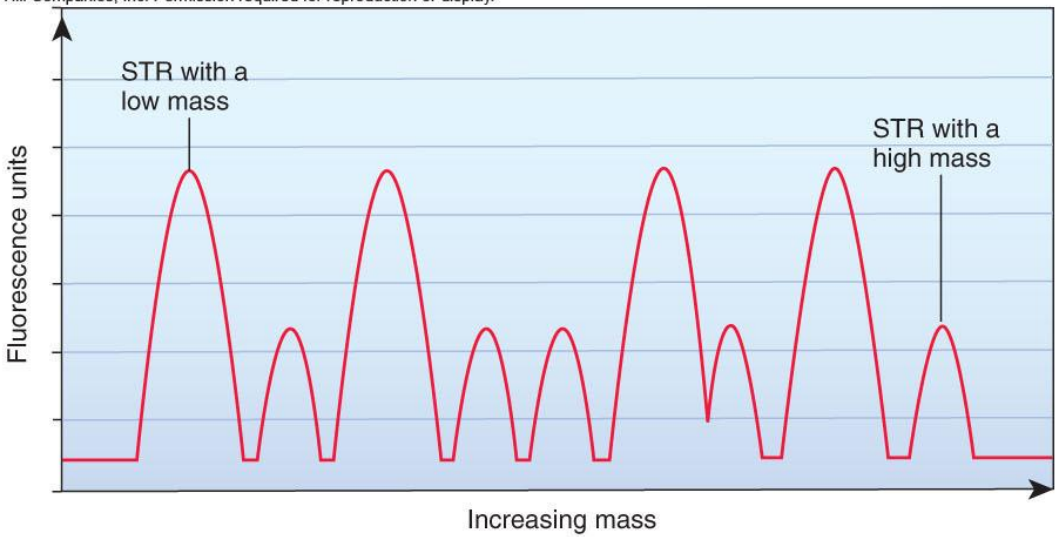


- Mammary cells from adult sheep removed
- Fused diploid mammary cell with enucleated sheep oocyte
- Zygote implanted
- Dolly and donor were almost genetically identical
  - Same set of genes
  - Minor differences due to differences in mitochondrial DNA and maternal effect genes
- Achieved in several mammal species

DNA fingerprinting

- Identifies and distinguishes among individuals based on variations in their DNA
- Chromosomal DNA produces series of bands on a gel
- Unique pattern of bands used
- Automated using PCR to amplify short tandem repeat sequences (STRs)
  - Such tandem repeat sequences are found at specific locations in the genomes of all species, and the number of repeats at each spot tends to vary from one individual to the next

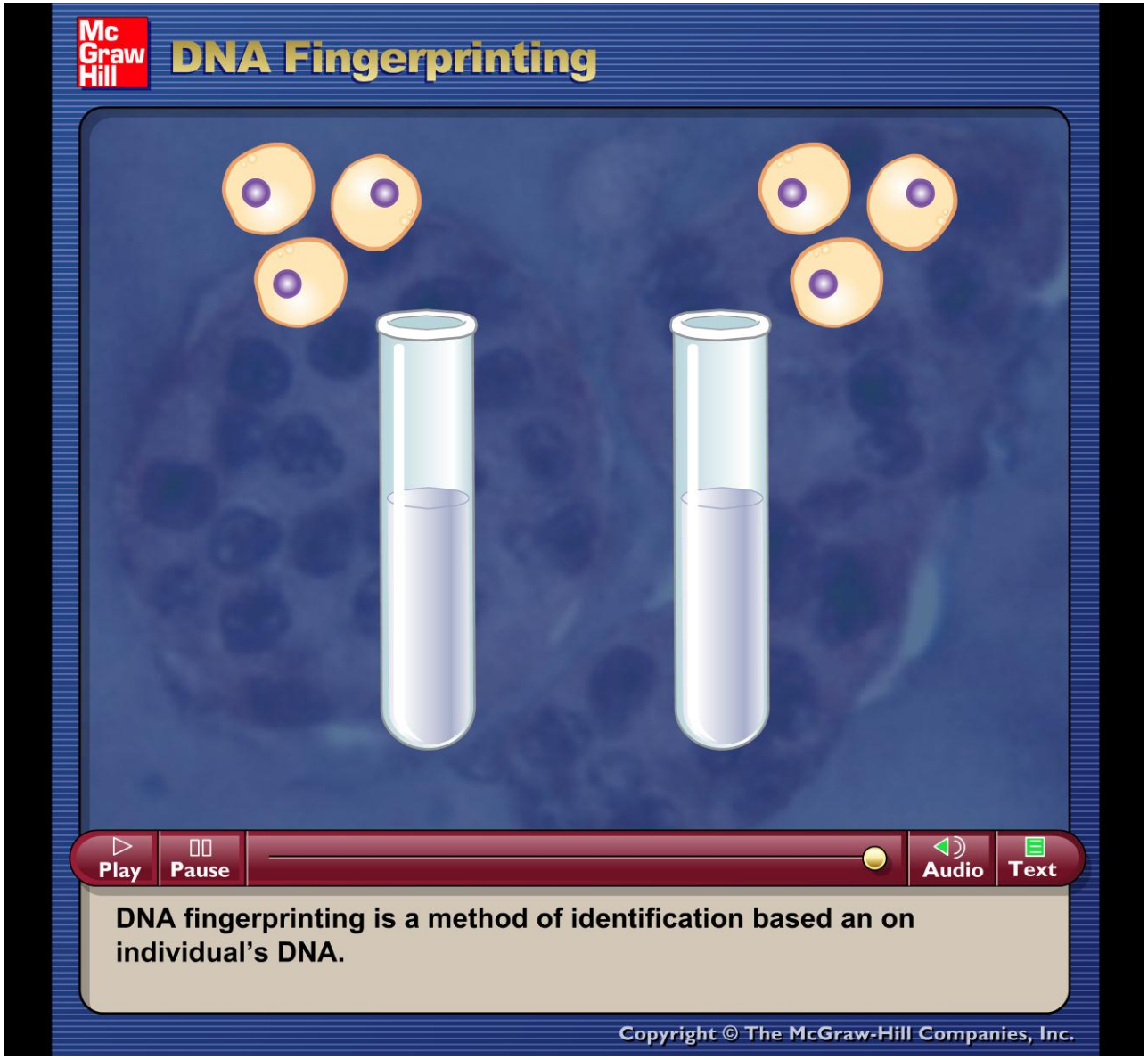
(a) Traditional DNA fingerprinting



(b) Automated DNA fingerprinting

a: Leonard Lessin/Peter Arnold, Inc.

- Uses
  - Identify different species of bacteria and fungi
  - Forensics – 1986 first use in US court system
  - Paternity testing and other family relationships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DNA Fingerprinting
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DNA fingerprinting is a method of identification based an on individual's DNA.
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Blaese and Colleagues Performed the First Gene Therapy to Treat ADA Deficiency

- Gene therapy introduces cloned genes into living cells to cure disease
- More than 4,000 diseases involve a single gene abnormality
- Adenosine deaminase deficiency prevents proper metabolism of nucleosides
- Deoxyadenosine accumulates and results in destruction in B and T cells
- Leads to severe combined immunodeficiency syndrome (SCID)
  - Fatal at early age (1 or 2 years old)

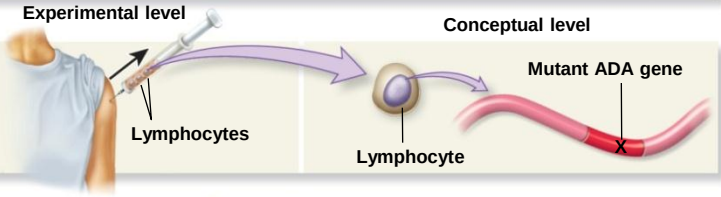
- 3 treatment approaches
  - Bone marrow transplant
  - Purified ADA enzyme
  - Gene therapy
- Gene therapy
  - September 14, 1990
  - Remove lymphocytes from girl
  - Treat with retroviral vector containing ADA gene
  - Return cells to her bloodstream
- Results suggest that this first gene therapy trial may offer benefit but patients also treated with ADA

FEATURE INVESTIGATION

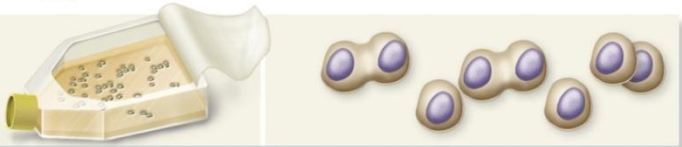
HYPOTHESIS Infecting lymphocytes with a retrovirus containing the normal ADA gene will correct the inherited deficiency of the mutant ADA gene in patients with ADA deficiency.

KEY MATERIALS A retrovirus with the normal ADA gene.

1 Remove ADA-deficient lymphocytes from the patient with severe combined immunodeficiency disease (SCID).

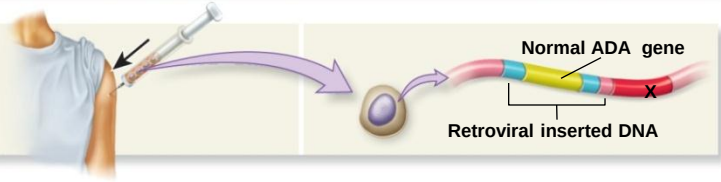


2 Culture the cells in a laboratory.

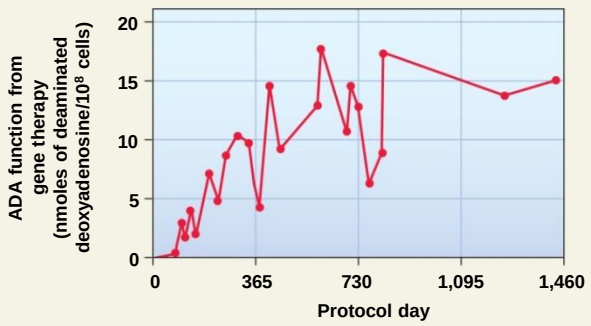


3 Infect the cells with a retrovirus that contains the normal ADA gene. As described in Chapter 18, retroviruses insert their DNA into the host cell chromosome as part of their reproductive cycle.

4 Infuse the ADA-gene-corrected lymphocytes back into the SCID patient.



5 THE DATA



6 CONCLUSION The introduction of a cloned ADA gene into lymphocytes via gene therapy resulted in higher ADA function, even after 4 years.

7 SOURCE Blaese, Robert M. et al. 1995. T lymphocyte-directed gene therapy for ADA-SCID: Initial trial results after 4 years. Science 270:475–480.