Slide 1

Biotechnology

Lecture 13

Slide 2

Learning Outcomes

- Recombinant DNA
- Restriction Enzymes
- Vectors
- Clones
- Subunit Vaccines
- Polymerase Chain Reaction

Slide 3

Biotechnology and Recombinant DNA

- Biotechnology:
  - The use of microorganisms, cells, or cell components to make a product
  - Foods, antibiotics, vitamins, enzymes
- Recombinant DNA Technology:
  - Insertion of genes to produce desired proteins
  - Often inserted into bacteria like E.coli

Slide 4

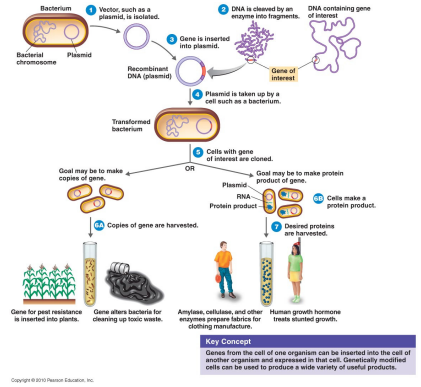


Figure 9.1.2

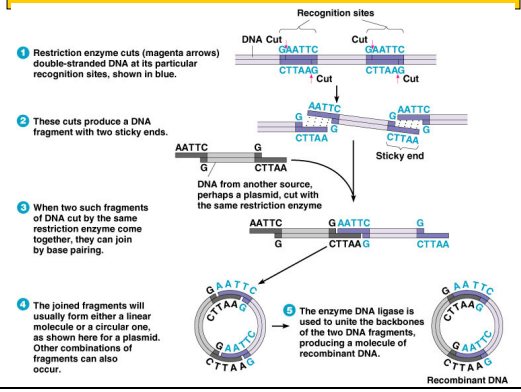
Slide 5

Restriction Enzymes

- Cut specific sequences of DNA
- Destroy bacteriophage DNA in bacterial cells
- Hundreds of restriction enzymes now known
  - Staggered cut
  - Sticky ends – un-paired bases

Slide 6

Sticky Ends



Slide 7

Plasmid Vectors

- Carry desired gene from one cell to cell for insertion
- Plasmids are used to insert genes into bacteria
- Genetically engineered viruses are used to insert genes into animal cells

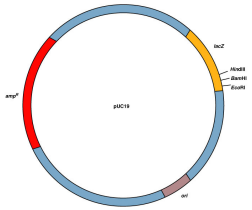


Figure 9.3

Slide 8

Polymerase Chain Reaction (PCR)

- To make multiple copies of a piece of DNA enzymatically
- Heat stable form of DNA Polymerase from *Thermus aquaticus*
- RNA primers synthesized to match known base sequences in DNA
- Deoxynucleotide Triphosphates
- Thermocycler

Slide 9

Chain Reaction

Incubate target DNA at 94°C 1 min

- DNA strands separate
- Incubate at 60°C 1 min
- Primers attach to single stranded DNA
- Incubate at 72°C 1 min
- DNA polymerase copies target DNA
- Repeat

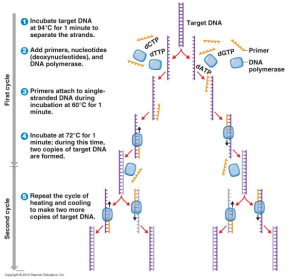


Figure 9.4.2

Slide 10

Applications of PCR

- Clone DNA for recombination
- Amplify DNA to detectable levels (CSI)
- Diagnose genetic disease
- Detect pathogens
- RNA primer synthesized specific sequence
  - unique to pathogen
  - Known mutation
  - Will only attach if primer anneals

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DNA can be inserted into a cell by:

- Transformation
  - cells take DNA from environment
  - $CaCl_2$ + Heat Shock
- Electroporation
  - Electrical currents form pores in plasma membrane
- Protoplast fusion
  - Enzymatically digest cell wall
  - Plant cells and algae
  - Cells fuse genes recombine
- Vectors
  - Modified viruses

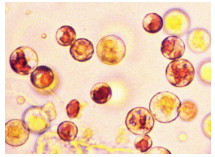


Figure 9.5b

Slide 12

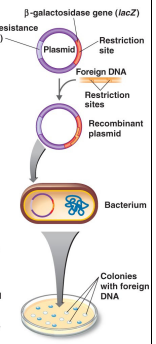
DNA insertion

- Gene gun
- Tungsten Bullets
  - Plant cells
- Microinjection
  - Animal cells

Figure 9.6 & 7

Slide 13

Genetic Engineering



1. Plasmid DNA and foreign DNA are both cut with the same restriction enzyme. The plasmid has the genes for lactose hydrolysis (the *lacZ* gene encodes the enzyme β-galactosidase) and ampicillin resistance.
2. Foreign DNA will insert into the *lacZ* gene. The bacterium receiving the plasmid vector will not produce the enzyme β-galactosidase if foreign DNA has been inserted into the plasmid.
3. The recombinant plasmid is introduced into a bacterium, which becomes ampicillin resistant.
4. All treated bacteria are spread on a nutrient agar plate containing ampicillin and a β-galactosidase substrate and incubated. The β-galactosidase substrate is called X-gal.
5. Only bacteria that picked up the plasmid will grow in the presence of ampicillin. Bacteria that hydrolyze X-gal produce galactose and an indigo compound. The indigo turns the colonies blue. Bacteria that cannot hydrolyze X-gal produce white colonies.

Figure 9.11.1

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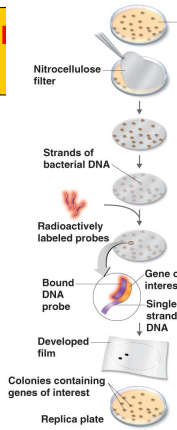
Making a Ge Product



1. Make replica of master plate on nitrocellulose filter.
2. Treat filter with detergent (SDS) to lyse bacteria.
3. Treat filter with sodium hydroxide (NaOH) to separate DNA into single strands.
4. Add radioactively labeled probes.
5. Probe will hybridize with desired gene from bacterial cells.
6. Wash filter to remove unbound probe and expose filter to X-ray film.
7. Compare developed film with replica of master plate to identify colonies containing gene of interest.

Figure 9.12.1

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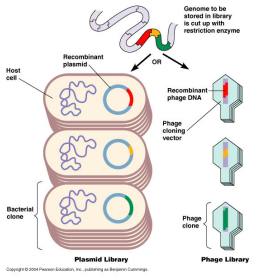
Gene Libraries

- Gene libraries
  - fragments of an entire genome stored in plasmids or phages
- cDNA is made from mRNA by reverse transcriptase



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Therapeutic Applications

- Subunit vaccines
- Nonpathogenic viruses carrying genes for pathogen's antigens as vaccines
- Gene therapy to replace defective or missing genes
- Human Genome Project
  - Nucleotides have been sequenced
  - Human Proteome Project may provide diagnostics and treatments

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Agricultural Applications

TABLE 9.2 **Some Agriculturally Important Products of Genetic Engineering**

Product	Comments
Agricultural Products	
Bt cotton and Bt corn	Plants have toxin-producing gene from *B. thuringiensis;* toxin kills insects that eat plants.
MacGregor tomatoes	Gene for pectin degradation is removed so fruits have longer shelf life.
Pseudomonas syringae, ice-minus bacterium	Lacks normal protein product that initiates undesirable ice formation on plants.
Pseudomonas fluorescens bacterium	Has toxin-producing gene from insect pathogen *Bacillus thuringiensis;* toxin kills root-eating insects that ingest bacteria.
Rhizobium meliloti bacterium	Modified for enhanced nitrogen fixation.
Round-Up® (glyphosate)-resistant crops	Plants have bacterial gene; allows use of herbicide on weeds without damaging crops.

Table 9.2

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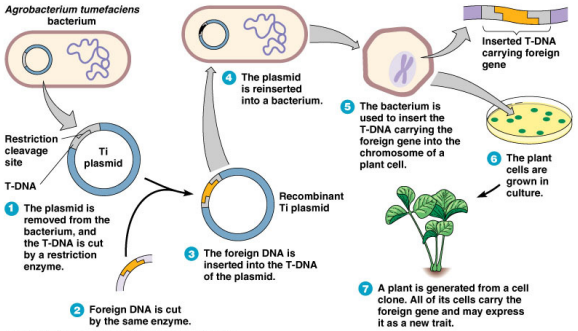
Genetic Engineering Using *Agrobacterium*



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Safety Issues and Ethics

- Avoid accidental release
- Genetically modified crops must be safe for consumption and for the environment
  - "Superweeds"
- Who will have access to an individual's genetic information?

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TABLE 9.1 **Some Pharmaceutical Products of Genetic Engineering**

Product	Comments
Alpha-interferon	Therapy for leukemia, melanoma, and hepatitis; produced by *E. coli* and *Saccharomyces cerevisiae* (yeast).
Antitrypsin	Assists emphysema patients; produced by genetically modified sheep.
Beta-interferon	Treatment for multiple sclerosis; produced by mammalian cell culture.
Bone morphogenic proteins	Induces new bone formation; useful in healing fractures and reconstructive surgery; produced by mammalian cell culture.
Colony-stimulating factor (CSF)	Counteracts effects of chemotherapy; improves resistance to infectious disease such as AIDS; treatment of leukemia; produced by *E. coli* and *S. cerevisiae*.
Epidermal growth factor (EGF)	Heals wounds, burns, ulcers; produced by *E. coli*.
Erythropoietin (EPO)	Treatment of anemia; produced by mammalian cell culture.
Factor VIII	Treatment of hemophilia; improves clotting; produced by mammalian cell culture.
Gamma-interferon	Treatment of chronic granulomatous disease; produced by *E. coli*.
Hepatitis B vaccine	Produced by *S. cerevisiae* that carries hepatitis-virus gene on a plasmid.
Human growth hormone (hGH)	Corrects growth deficiencies in children; produced by *E. coli*.
Human insulin	Therapy for diabetes; better tolerated than insulin extracted from animals; produced by *Escherichia coli*.

Table 9.1.1

Gardisil a Subunit Vaccines

- Gardasil offers women protection v HPV 6,11,16 and 18
  - Genital warts
  - Cervical Cancer
- Virus gene for HPV protein L1 moved to Yeast
- Yeast cells synthesize HPV protein
- Injected as a vaccine