Chapter 10 . Genetic Engineering

Tools and Techniques

- 1. Enzymes
- 2. Analysis of DNA
- 3. Nucleic acid hybridization
- 4. Synthesizing DNA
- 5. Polymerase Chain Reaction

1. Enzymes

- Restriction endonuclease
- Ligase
- Reverse transcriptase
  - cDNA

Restriction endonuclease

- Originates in bacterial cells
- Many different types exist
- Natural function is to protect the bacterium from foreign DNA (bacteriophage)
- Recognizes 4 to 10 base pairs (palindromic sequence)
- Cleaves DNA at the phosphate-sugar bond → generates "sticky ends"
- Used in the cloning method
- Ex. *Eco*RI from *Escherichia coli*

The function of a restriction endonuclease or enzyme.

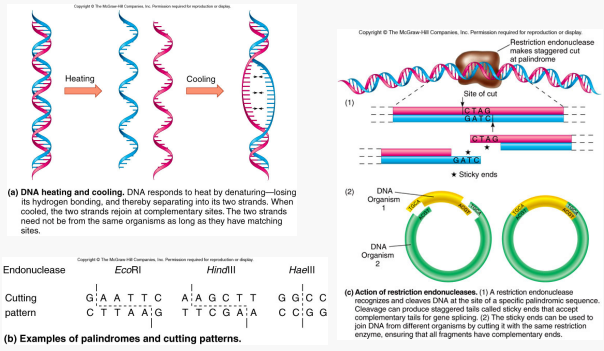

Fig. 10.1 Some useful properties of DNA

Ligase:

- Link DNA fragments
- Seals "sticky ends" by rejoin the phosphate -sugar bonds
- Used in the cloning method

Reverse transcriptase (retroviruses)

- Converts RNA to DNA
- Ex. Complementary DNA (cDNA)
  - Required for eucaryote gene expression
  - mRNA to cDNA; No introns are present

- Electrophoresis
- Hybridization and probes
- Sequencing
- Polymerase Chain Reaction

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Analysis of DNA

Electrophoresis:

- Separation of DNA based on size
- Negative charge DNA (phosphate group) migrates to positive electrode
- Usefulness
  - Characterizing DNA fragment (**RFLP**)
  - Fingerprinting

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Steps associated with the electrophoresis technique.

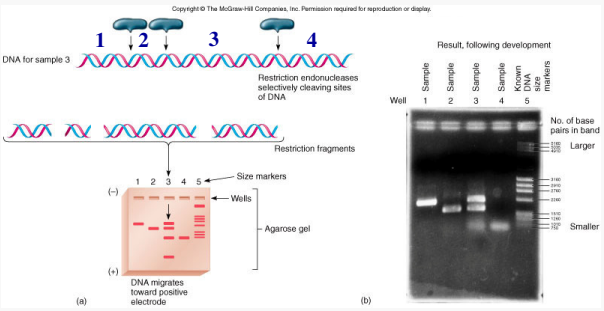

Fig. 10.2 Revealing the patterns of DNA with electrophoresis

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Analysis of DNA

Hybridization and probes:

- Complementary sites on two different nucleic acids bind or hybridize (ssDNA with ssDNA or RNA)

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Analysis of DNA

Probes:

- Small stretches of nucleic acid with a known sequence called an oligonucleotide
- Single stranded
- Detects specific nucleotide sequences in unknown nucleic acid samples
- Probes – reporter molecules (radioactivity, luminescent, etc)

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Analysis of DNA

Southern blot:

- Method for detecting an unknown sample of DNA
- Incorporates restriction endonuclease, electrophoresis, denaturing, transfer to filter, probing, and visual detection.

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A Southern blot separates DNA by electrophoresis, denatures and transfers the DNA to filter paper, and uses probes to visualize hybridization.

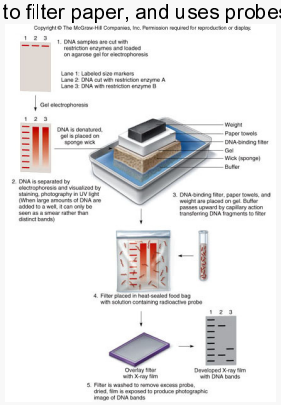

Fig. 10.3 Conducting a Southern blot hybridization test.

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Alternate hybridization methods can be used to detect unknown bacteria or virus.

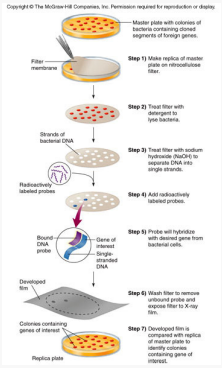

Fig. 10.4 A hybridization test relies on the action of microbe-specific probes

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Analysis of DNA

Sequencing:

- Provide the identity and order of nucleotides (bases) for all types of DNA
- Method
  - Sanger method
    - Synthesis of a complementary strand
    - Primers
    - Each dideoxynucleotide (dd) – no oxygen at C3 in the sugar → when added will stop reaction
    - Electrophoresis

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The Sanger method of sequencing DNA.

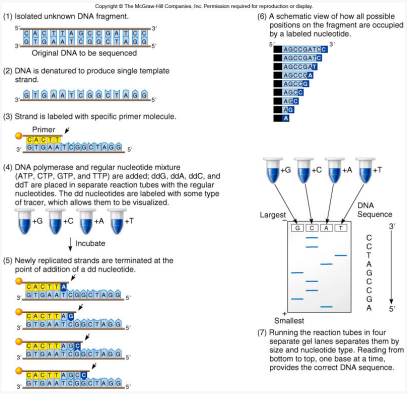

Fig. 10.5 Steps in a Sanger DNA sequence technique

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Polymerase Chain Reaction (PCR)

- Specific amplification of DNA
- Involves a denaturing (95 C), priming (annealing, 55-65 C), and extension (72 C) cycle
- 30 cycles are sufficient for detection of DNA
- Can be used to detect disease or infectious agents

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A schematic of the PCR reaction and its products

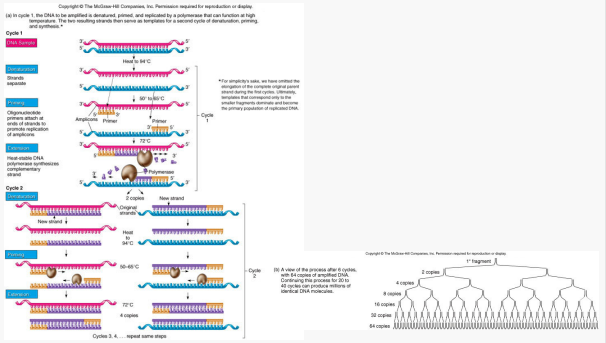

Fig. 10.6 Diagram of the polymerase chain reaction

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Recombinant DNA

- Recombinant
- Applications
- Cloning vectors
- Cloning host

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Recombinant DNA

Recombinant:

- When a cloning host receives a vector containing the gene of interest
- A single cloning host containing the gene of interest is called a clone

Applications:

- Protein production
- Alter organisms normal function
- Source of DNA (synthesis)

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Practical applications of recombinant technology include the development of pharmaceuticals, genetically modified organisms, and forensic techniques.

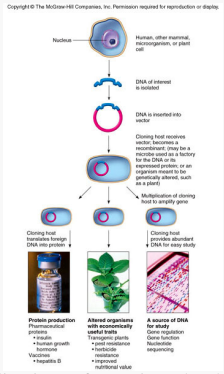

Fig. 10.7 Methods and applications of genetic technology

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Recombinant DNA

Cloning vectors:

- Carry a significant piece of the donor DNA (gene of interest)
- Readily accepted DNA by the cloning host
- Attributes:
  - 1. Contain an origin of replication (ORI)
  - 2. Must accept DNA of desired size (>10 kb)
  - 3. Contain a selective antibiotic resistant gene
- Ex. Plasmids, phages

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An example of a plasmid vector.

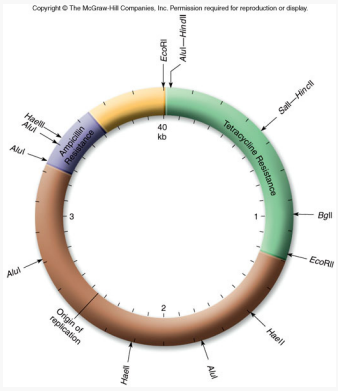

Fig. 10.8 Partial map of the pBR322 plasmid of *E. coli*

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Recombinant DNA

Cloning host

- Bacteria (procaryote)
  - *Escherichia coli*
  - Bacteria will not excess introns from eucaryotic DNA and no modification of proteins
- Yeast (eucaryote)
  - *Saccharomyces cerevisiae*
  - Will excess introns

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Important protein products generated by recombinant DNA technology.

TABLE 10.2 Current Protein Products from Recombinant DNA Technology

Immune Treatments
Interferons—peptides used to treat some types of cancer, multiple sclerosis, and viral infections such as hepatitis and genital warts
Interleukins—types of cytokines that regulate the immune function of white blood cells; used in cancer treatment
Orthoclone—an immune suppressant in transplant patients
Macrophage-colony-stimulating factor (GM-CSF)—used to stimulate bone marrow activity after bone marrow grafts
Tumor necrosis factor (TNF)—used to treat cancer
Granulocyte-colony-stimulating factor (Neupogen)—developed for treating cancer patients suffering from low neutrophil counts

Hormones
Erythropoietin (EPO)—a peptide that stimulates bone marrow used to treat some forms of anemia
Tissue plasminogen activating factor (tPA)—can dissolve potentially dangerous blood clots
Hemoglobin A—form of artificial blood to be used in place of real blood for transfusions
Factor VIII—needed as replacement blood-clotting factor in type A hemophilia
Relaxin—an aid to childbirth
Human growth hormone (HGH)—stimulates growth in children with dwarfism; prevents wasting syndrome

Enzymes
rH DNase (pulmozyme)—a treatment that can break down the thick lung secretions of cystic fibrosis
Antitrypsin—replacement therapy to benefit emphysema patients
PEG-SOD—a form of superoxide dismutase that minimizes damage to brain tissue after severe trauma

Vaccines
Vaccines for hepatitis B and *Haemophilus influenzae* Type b meningitis
Experimental malaria and AIDS vaccines based on recombinant surface antigens

Miscellaneous
Bovine growth hormone or bovine somatotropin (BST)—given to cows to increase milk production
Apolipoprotein—to deter the development of fatty deposits in the arteries and to prevent strokes and heart attacks
Spider silk—a light, tough fabric for parachutes and bulletproof vests

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Table 10.2 Current protein products from recombinant DNA technology

Recombinant Organisms

- Modified bacteria and viruses
- Transgenic plants
- Transgenic animals

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Modified bacteria

- *Pseudomonas syringae*
  - Prevents frost crystals from forming on plants
- *Pseudomonas fluorescens*
  - Contains an insecticide gene

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The construction of a recombinant in order to produce the human alpha-2a interferon.

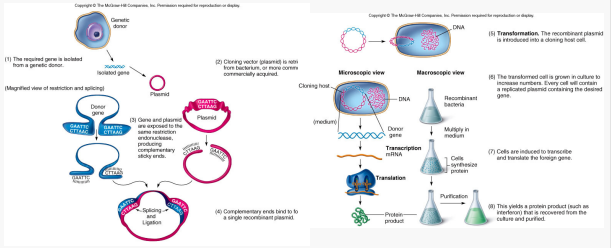

Fig. 10.9 Steps in recombinant DNA, gene cloning, and product retrieval.

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Transgenic plants

- *Agrobacterium tumefaciens*
  - Tumor inducing (Ti) plasmid contains gene of interest, and is integrated into plant chromosome
  - Ex. tobacco, garden pea, rice

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Schematic of *Agrobacterium tumefaciens* transferring and integrating the Ti plasmid into the plant chromosome.

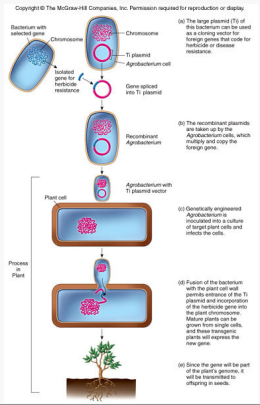

Fig. 10.11 Bioengineering of plants

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Examples of other transgenic plants that include tobacco, garden pea, and rice.

TABLE 10.3 Examples of Engineered (Transgenic) Plants

Plant	Trait	Results
Nicotiana tabacum (tobacco)	Herbicide resistance	Tobacco plants in the upper row have been transformed with a gene that provides protection against Buctril, a systemic herbicide. Plants in the lower row are normal and not transformed. Both groups were sprayed with Buctril and allowed to sit for 6 days. (The control plants at the beginning of each row were sprayed with a blank mixture lacking Buctril.)
Pisum sativum (garden pea)	Pest protection	Pea plants were engineered with a gene that prevents digestion of the seed starch (see seeds on the left in the photo). This gene keeps tiny insects called weevils from feeding on the seeds. Seeds on the right are from plants that were not engineered and are suffering from weevil damage (note holes).
Oryza sativa (rice)	Added nutritional value	The golden rice grains seen in the photo have been genetically engineered to produce beta-carotene, a precursor to vitamin A. Lack of vitamin A leads to over 1 million deaths and 300,000 cases of blindness a year.

Table 10.3 Examples of engineering plants

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Transgenic animals

- Knockout mouse
  - Tailor-made genetic defects
    - Cystic fibrosis
    - Gaucher's disease
    - Alzheimer's disease
    - Sickle-cell anemia
  - Pharmaceutical production

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Therapy

Gene therapy:

- Repair a genetic defect
  - *Ex vivo* strategy
  - *In vivo* strategy
- Severe immunodeficiency disease
- Cystic fibrosis
- Sickle anemia

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Representation of the *ex vivo* strategy.

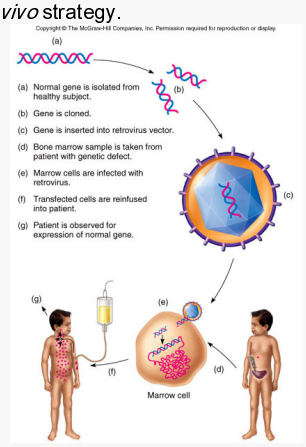

Fig. 10.13 Protocol for the *ex vivo* type of gene

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Therapy

- **Antisense RNA or DNA**
  - Prevent the synthesis of an unwanted protein
  - Targets mRNA
- **Triplex DNA**
  - Prevents transcription
  - Targets double stranded DNA

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Examples of the mechanism for antisense DNA and triplex DNA.

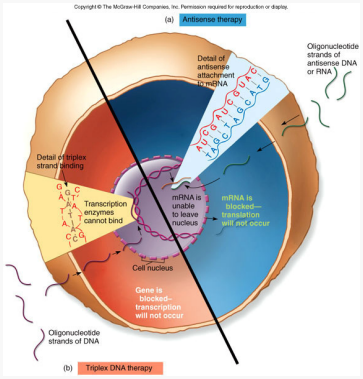

Fig. 10.14 Mechanisms of antisense DNA and triplex DNA

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Genome Analysis

Maps:

- Determine the location of particular genes (locus) on the chromosome
- Determine differences in chromosomal regions (alleles)
  - Types of maps
  - Genomics and bioinformatics

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Types of maps

- Linkage
  - Shows the relative proximity and location of genes
- Physical
  - Shows the proximity and size of genes
- Sequence
  - Shows the exact order of bases

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Genomic and bioinformatics

- New discipline of study as a result of the enormous data generated by maps
  - Analyze and classify genes
  - Determine protein sequences
  - Determine the function of the genes

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Genome Analysis

Fingerprinting:

- Emphasizes the differences in the entire genome
- Techniques
  - Endonucleases
  - PCR
  - Southern blot
- Uses
  - Forensic medicine
  - Identify hereditary disease

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Comparing the fingerprints for different individuals.

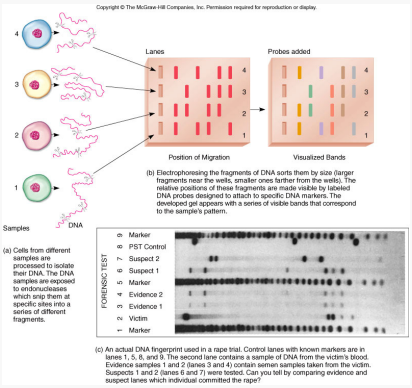

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Fig. 10.15 DNA fingerprints:the bar codes of life