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. EcoRI from Escherichia coli

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

Steps associated with the electrophoresis technique.

Fig. 10.2 Revealing the patterns of DNA with electrophoresis

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

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)

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

**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
Recombinant DNA

- Recombinant
- Applications
- Cloning vectors
- Cloning host

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)

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

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

Recombinant DNA

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

An example of a plasmid vector.

Fig. 10.7 Methods and applications of genetic technology

Fig. 10.8 Partial map of the pBR322 plasmid of *E. coli*
Important protein products generated by recombinant DNA technology.

### Table 10.2 Current protein products from recombinant DNA technology

<table>
<thead>
<tr>
<th>Recombinant Organisms</th>
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<td>Transgenic plants</td>
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<td>Transgenic animals</td>
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</tr>
<tr>
<td>Pseudomonas syringae</td>
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<td>Pseudomonas fluorescens</td>
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<tr>
<td>- Prevents frost crystals from forming on plants</td>
<td>- Contains an insecticide gene</td>
<td></td>
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</tbody>
</table>

**Recombinant Organisms**

- Modified bacteria and viruses
- Transgenic plants
- Transgenic animals

**Modified bacteria**

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

**Transgenic plants**

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

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

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

**Fig. 10.11 Bioengineering of plants.**
Examples of other transgenic plants that include tobacco, garden pea, and rice.

Table 10.3 Examples of engineering plants

<table>
<thead>
<tr>
<th>Plant</th>
<th>Trait</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco (Nicotiana)</td>
<td>Herbicide resistance</td>
<td>Tobacco plants in the experiments have been identified with a gene that provides protection against herbicides</td>
</tr>
<tr>
<td>Pea (Pisum sativum)</td>
<td>Pest resistance</td>
<td>Pea plants were resistant to pests</td>
</tr>
<tr>
<td>Oryza sativa (rice)</td>
<td>Added nutritional value</td>
<td>Rice plants were enriched with nutrients</td>
</tr>
</tbody>
</table>

Table 31

Transgenic animals

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

Table 32

Therapy

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

Table 33

**Antisense RNA or DNA**
- Prevent the synthesis of an unwanted protein
- Targets mRNA

**Triplex DNA**
- Prevents transcription
- Targets double stranded DNA

Table 34

Examples of the mechanism for antisense DNA and triplex DNA

Fig. 10.14 Mechanisms of antisense DNA and triplex DNA
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

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

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

Genome Analysis

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

Comparing the fingerprints for different individuals.

Fig. 10.15 DNA fingerprints: the bar codes of life