

A Lot of Cutting and Pasting Going on Here... Recombinant DNA and Biotechnology

How Are Large DNA Molecules Analyzed?

- Naturally occurring enzymes that cleave and repair DNA are used in the laboratory to manipulate and recombine DNA.
- Restriction enzymes (restriction endonucleases) cut double-stranded DNA into smaller pieces.
- Bacteria use these as defense against DNA from bacteriophage.
- DNA is cut between the 3' hydroxyl group of one nucleotide and the 5' phosphate group of the next—restriction digestion.

- There are many restriction enzymes that cut DNA at specific base sequences—the recognition sequence, or restriction site.
- Restriction enzymes do not cut bacteria's own DNA because the recognition sequences are modified.
- Methylases add methyl groups after replication; makes sequence unrecognizable by restriction enzyme.

- Bacterial restriction enzymes can be isolated from cells.
- DNA from any organism will be cut wherever the recognition site occurs.
 - EcoRI (from *E. coli*) cuts DNA at a particular sequence.

- The sequence is palindromic—it reads the same in both directions from the 5' end.
- EcoRI occurs about once every four genes in prokaryotes. DNA can be chopped into small pieces containing a few genes.
- The EcoRI sequence does not occur anywhere in the genome of the phage T7. Thus it can survive in its host, *E. coli*.

- After DNA is cut, fragments of different sizes can be separated by gel electrophoresis.
- Mixture of fragments is placed on a well in a porous gel. An electric field is applied across the gel. Negatively charged DNA fragments move towards positive end.
- Smaller fragments move faster than larger ones.

- Electrophoresis provides information on:
 - Size of fragments. Fragments of known size provide comparison.
 - Presence of specific sequences. These can be determined using probes.
- DNA can then be denatured while in the gel, then transferred to a nylon filter to make a “blot.”

- DNA fingerprinting uses restriction analysis and electrophoresis to identify individuals.
- Works best with genes that are polymorphic—have multiple alleles.

- Two types of polymorphisms:
 - Single nucleotide polymorphisms (SNPs): inherited variation involving a single base
 - Short tandem repeats (STRs): moderately repetitive sequences side by side

- STRs are recognizable if they lie between two restriction sites.
- Several different STRs can be used to determine the unique pattern for an individual.
- DNA fingerprinting requires at least 1 µg of DNA (amount in about 100,000 human cells).
- This is not always available, so amplification by polymerase chain reaction (PCR) is used.

Polymerase Chain Reaction (PCR)

- To make multiple copies of a piece of DNA enzymatically
- Used to
 - Clone DNA for recombination
 - Amplify DNA to detectable levels
 - Sequence DNA
 - Diagnose genetic disease
 - Detect pathogens

What Is Recombinant DNA?

- DNA fragments can be rejoined by DNA ligase.
- Any two DNA sequences can be spliced.
- First done in 1973 with two *E. coli* plasmids; Recombinant DNA was born
- Some restriction enzymes cut both DNA strands exactly opposite each other.
- Others (such as EcoRI) make a staggered cut. Results in single-stranded “tails” at the ends of fragments.
- Tails are called sticky ends—can bind by base pairing to other sticky ends.
- Sticky ends of fragments that were cut by the same restriction enzyme are all the same—thus fragments from different species can be joined.
- When temperature is lowered, the fragments anneal—join by hydrogen bonding. Must be permanently spliced by DNA ligase.

How Are New Genes Inserted into Cells?

- Recombinant DNA technology can be used to clone, or make exact copies of genes.
- The gene can be used to make a protein—but it must first be inserted, or transfected, into host cells.
- The altered host cell is called transgenic.
- To determine which of the host cells contain the new sequence, the recombinant DNA is often tagged with reporter genes.
- Reporter genes have easily observed phenotypes or genetic markers.
- The first host cells used were bacteria, especially *E. coli*.
- Yeasts (*Saccharomyces*) are commonly used as eukaryotic hosts.
- Plant cells are also used—they have totipotency, the ability of any differentiated cell to develop into a new plant.

- The new DNA must also replicate as the host cell divides. It must become a segment with an origin of replication—a replicon or replication unit.
- New DNA can become part of a replicon in two ways:
 - Inserted near an origin of replication in host chromosome.
 - It can be part of a carrier sequence or vector that already has an origin of replication.
- A vector should have four characteristics:
 - Ability to replicate independently of the host cell
 - A recognition sequence for a restriction enzyme
 - A reporter gene
 - Small size in comparison with host's chromosomes
- Plasmids have all these characteristics.
- Plasmids are small, many have only one restriction site.
- Genes for antibiotic resistance can be used as reporter genes.
- And they have an origin of replication and can replicate independently.
- Plasmids can be used for genes of 10,000 bp or less. Most eukaryote genes are larger than this.
- Viruses can be used as vectors—e.g., bacteriophage. The genes that cause host cell to lyse can be cut out and replaced with other DNA.
- Bacterial plasmids don't work for yeasts because the origins of replication use different sequences.
- A yeast artificial chromosome (YAC) has been created: contains yeast origin of replication, plus yeast centromere and telomere sequences.
- Also contains artificial restriction sites and reporter genes
- A plasmid from the soil bacterium *Agrobacterium tumefaciens* is used as a vector for plant cells.
- Plasmid Ti (tumor inducing) causes crown gall.
- Plasmid has a region called T DNA, which inserts copies of itself into chromosomes of infected plants.
- T DNA has several restriction sites, where new DNA can be inserted.
- With altered T DNA, plasmid no longer causes tumors, but can still insert itself into host chromosomes.
- Usually only a small proportion of host cells take up the vector, and they may not have the appropriate sequence.
- Host cells with the desired sequence must be identifiable.
- One method:
 - *E. coli* is host; pBR322 plasmid is the vector.
 - Plasmid has genes for resistance to ampicillin and tetracycline.
 - Plasmid has only one restriction site for enzyme BamHI, within the gene for

tetracycline resistance.

- If new DNA is inserted at that restriction site, it inactivates the gene for tetracycline resistance.
- Plasmid then has gene for ampicillin resistance, but not for tetracycline. This can be used to select for host cells with new DNA.
- Other reporter genes:
 - Artificial vectors with restriction sites within the *lac* operon. If new DNA is inserted there, vector no longer carries its original function into the host cell.
 - Green fluorescent protein, which normally occurs in the jellyfish *Aequorea victoria*.

What Is Biotechnology?

- Biotechnology is the use of living cells to produce materials useful to people.
 - Examples: use of yeasts to brew beer and wine, use of bacteria to produce cheese, yogurt, etc.
 - Use of microbes to produce antibiotics such as penicillin, alcohol, and other products.
- Gene cloning is now used to produce proteins in large amounts.
- Almost any gene can be inserted into bacteria or yeasts, and the resulting cells induced to make large quantities of the product.
- Requires specialized vectors.
- Expression vectors are synthesized that include sequences needed for expression of the transgene in the host cell.
- Expression vectors can be modified by:
 - Inducible promoters; enhancers can also be added so that protein synthesis takes place at high rates.
 - Tissue-specific promoters
 - Signal sequences—e.g., a signal to secrete the product to the extracellular medium.
- Example of a medical application:
 - After wounds heal, blood clots are dissolved by plasmin. Plasmin is stored as an inactive form called plasminogen.
 - Conversion of plasminogen is activated by tissue plasminogen activator (TPA).
 - TPA can be used to treat strokes and heart attacks, but large quantities are needed—can be made using recombinant DNA technology.
- Pharming: production of medically useful proteins in milk.
- Transgenes for a protein are inserted into the egg of a domestic animal, next to the promoter for lactoglobulin—a protein in milk. The transgenic animal then produces large quantities of the protein in its milk.
- Through cultivation and selective breeding, humans have been altering the traits of plants

- and animals for thousands of years.
- Recombinant DNA technology has several advantages:
 - Specific genes can be targeted.
 - Any gene can be introduced into any other organism.
 - New organisms are generated quickly.
- Crop plants have been modified to produce their own insecticides:
 - The bacterium *Bacillus thuringiensis* produces a protein that kills insect larvae.
 - Dried preparation of *B. thuringiensis* are sold as a safe alternative to synthetic insecticides. The toxin is easily biodegradable.
- Genes for the toxin have been isolated, cloned, and modified, and inserted into plant cells using the Ti plasmid vector.
- Transgenic corn, cotton, soybeans, tomatoes, and other crops are being grown. Pesticide use is reduced.
- Some transgenic crops are resistant to herbicides.
- Glyphosate (Roundup) is widely used to kill weeds.
- Expression vectors have been used to make plants that synthesize so much of the target enzyme of glyphosate that they are unaffected by the herbicide.
- The gene has been inserted into corn, soybeans, and cotton.
- About half of U.S. crops of these plants contain this gene.
- Crops with improved nutritional characteristics:
 - Rice does not have β -carotene, but does have a precursor molecule.
 - Genes for enzymes that synthesize β -carotene from the precursor are taken from daffodils and inserted into rice by the Ti plasmid.
- The transgenic rice is yellow, and can supply β -carotene to improve the diets of many people.
- β -carotene is converted to vitamin A in the body.
- Recombinant DNA is also used to adapt a crop plant to an environment.
 - Example: plants that are salt-tolerant
- Genes from a protein that moves sodium ions into the central vacuole were isolated from *Arabidopsis* and inserted into tomato plants.
- Concerns over biotechnology:
 - Genetic manipulation is an unnatural interference in nature.
 - Genetically altered foods are unsafe to eat.
 - Genetically altered crop plants are dangerous to the environment.
- Advocates of biotechnology point out that all crop plants have been manipulated by humans.
- Advocates say that since only single genes for plant function are inserted into crop plants, they are still safe for human consumption.

- Genes that affect human nutrition may raise more concerns.
- Concern over environmental effects centers on escape of transgenes into wild populations:
 - For example, if the gene for herbicide resistance made its way into the weed plants.
- Beneficial insects can also be killed from eating plants with *B. thuringiensis* genes.