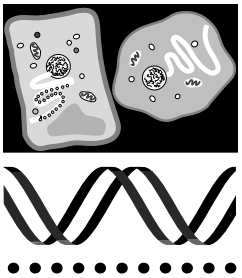
3 Recombinant DNA



Genetic Engineering

Among the most important techniques of modern biotechnology are those used to move individual genes from one organism to another. These techniques can move a specific gene among dissimilar organisms that cannot interbreed. For example, genes for cold tolerance can be moved from a fish to a plant. Collectively, the techniques for manipulating and moving genetic material are known as **genetic engineering**. In agriculture, genetic engineering is often applied as part of a traditional breeding program.

Genetic engineering begins with a gene and a host plant or animal. The end product is a new plant or animal with a gene from another organism, known as a **transgenic** organism. Eight major steps are required.

1. A source of genes which confer the desired trait is found (usually an organism).
2. DNA is removed from a donor organism cells and cut into fragments.
3. DNA fragments are grouped and sorted by size using electrophoresis, and a fragment containing the desired gene is isolated.
4. The fragment containing the gene is joined to other DNA that makes it possible to move the gene into a new organism.
5. The altered DNA is placed into cells.
6. The transformed cells are developed into a useful transgenic organism.
7. The transgenic organism is grown and tested.
8. The gene is transferred to the progeny.

Many techniques are used to accomplish these steps, including gene mapping, DNA sequencing, genetic engineering, immunoassay, tissue culture, field test design, and many others.

The process of finding and selecting genes is critical and difficult. A fragment that contains a gene is minute, and part of a very long DNA molecule. Matching a known trait with a specific sequence of DNA is about like finding a particular straw in a mountain of hay. Nevertheless, a DNA sequence and a trait (or a protein responsible for a trait) can be matched, and the DNA that contains a gene can be isolated. Techniques for accomplishing this are discussed in Lesson 4, *Selecting Genes*.

A recombinant DNA overview

To be inserted into a host cell, an isolated gene is usually combined with additional DNA from another organism. The combination allows the foreign gene to function, replicate, and be inheritable. Often, the additional DNA is a self-replicating closed loop, called a **plasmid**, that comes from a bacterium. DNA that is combined from several different organisms is called **recombinant DNA**.

The process of recombining DNA begins when millions of copies of the original source DNA are

The Genetic Engineering Process

1. Find donor organism and identify genes

2. Remove DNA from donor and cut into fragments

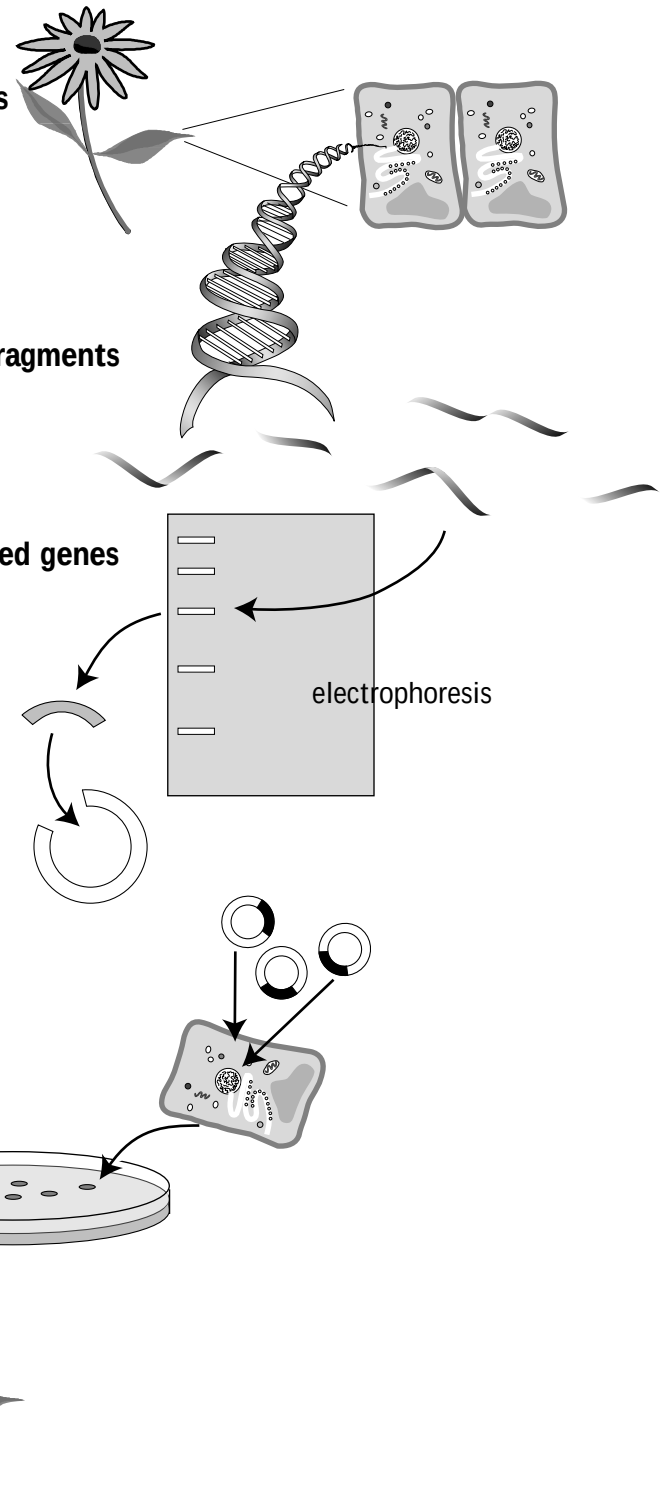
3. Sort fragments by size and locate desired genes

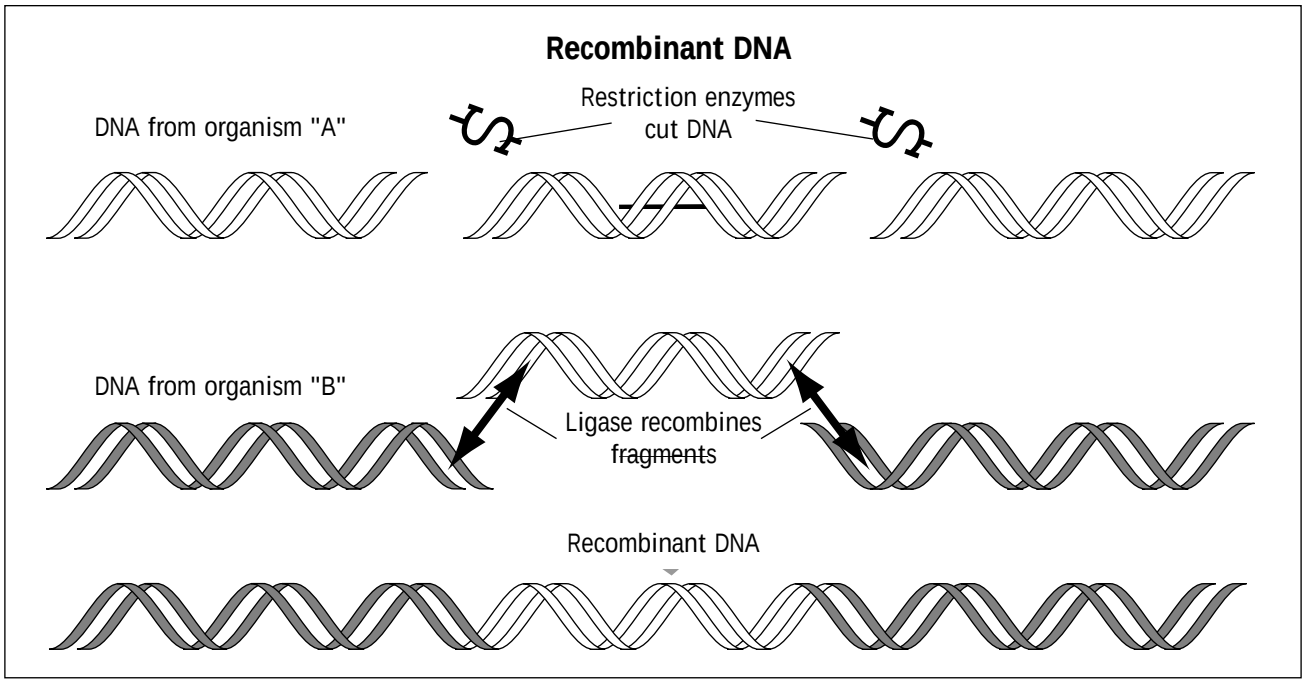
4. Create recombinate DNA

5. Transfer recombinant DNA into cells

6. Grow transformed cells

7. Grow transgenic organism



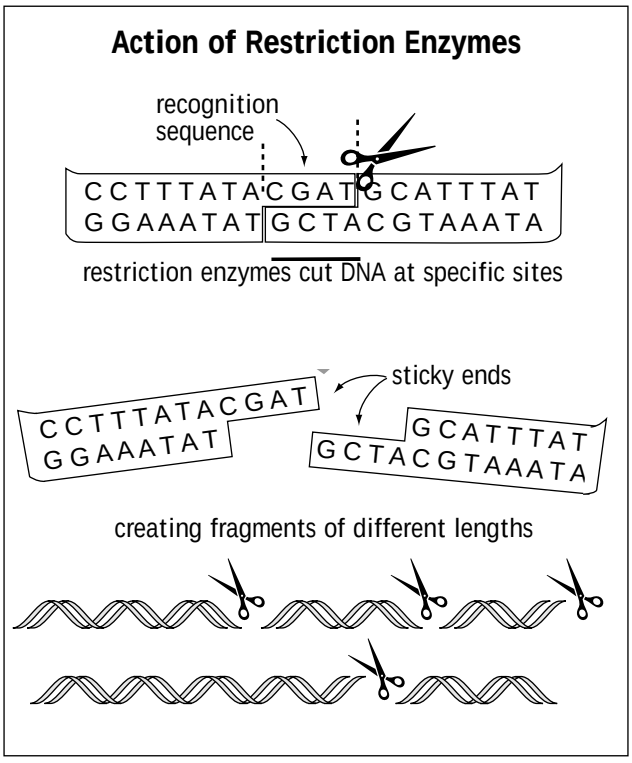


cut into fragments. The fragments are created using enzymes, called **restriction enzymes**, which cleave the DNA double strand. Restriction enzymes leave special ends on the fragments they produce. The ends, called **sticky ends**, can be spliced to other DNA. The source DNA is cut in many locations, and the resulting fragments are sorted and grouped by size using a process called **gel electrophoresis**. The identity of the gene-containing fragments has been worked out during the process of selecting a gene. Once the fragments are grouped and sorted, the fragments containing the gene can be found and isolated.

The sticky ends of the fragments containing the gene are then spliced to other DNA, often a plasmid that has been cut to produce matching sticky ends. Another enzyme, called a **ligase**, brings the correct sticky ends together and fuses them. The result is recombinant DNA composed of DNA from sources in several different organisms.

Restriction enzymes

Restriction enzymes are specialized proteins found in bacteria, which use them as a defense against viruses. Each restriction enzyme recognizes a specific code sequence on DNA double strands, such as the CGAT/GCTA sequence in the example. At the **recognition sequence**, the enzymes break both strands' phosphodiester bonds.



The hydrogen bonds release, and a fragment is generated. Many restriction enzymes are known which cut at different recognition sequences, and two different enzymes may be used to cut on both sides of a gene. Restriction enzymes offer two advantages in cutting DNA. First, the cut ends are usually "sticky," so that they can often be rejoined to complementary ends of other DNA fragments that were cut with the same restriction enzyme. Second, the enzymes usually cut at specific, known base sequences on the DNA strand.

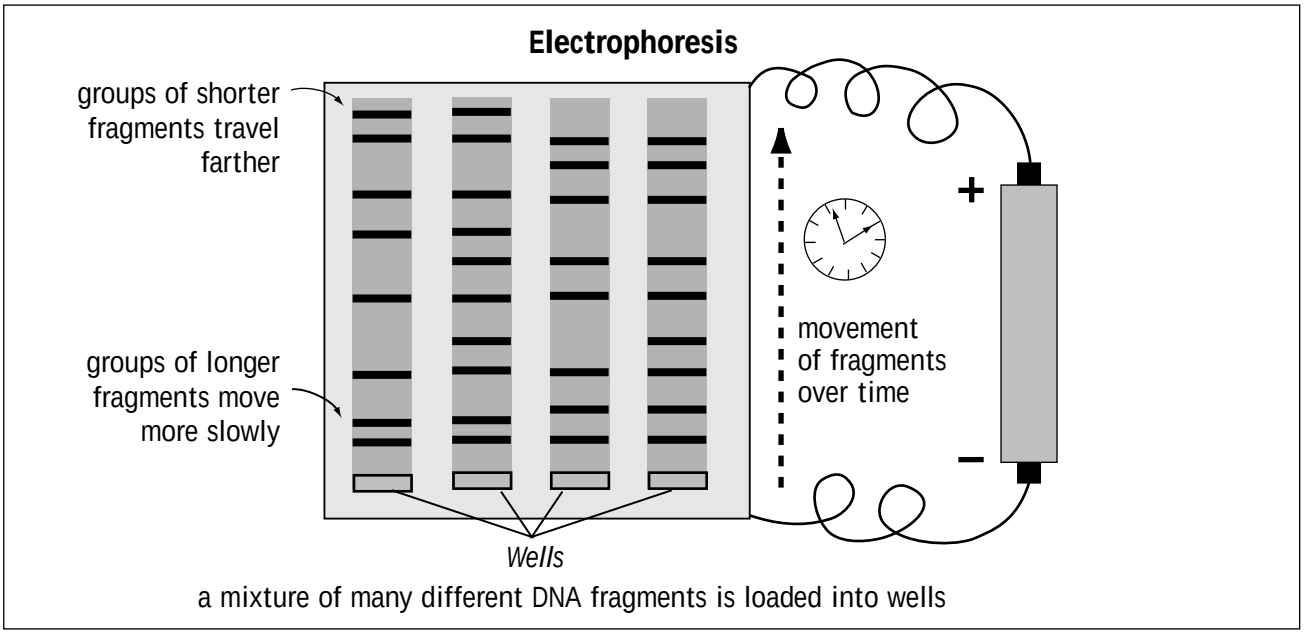
Electrophoresis

A restriction enzyme cuts the DNA strand at recognition sequences for that enzyme. Many recognition sequences may occur along the strand, so many fragments of different lengths may be generated. The desired gene occurs on only one of these fragments. To obtain fragments that contain the desired gene, the DNA must be grouped and sorted according to size. **Electrophoresis** is the process used to group and sort DNA fragments.

The DNA which has been cut into fragments with a restriction enzyme is called a **restriction digest**. The digest is made with millions of copies of DNA extracted from cells of the same organism, from a culture of bacteria, or from a strain of viruses. After the DNA is digested by the enzyme, the solution contains millions of copies of each size of fragment.

To group and sort these fragments, samples of the restriction digest are placed in wells at one end of an electrically charged gel. The wells are at the negatively charged end. The negative charge repels negatively charged DNA, and the positive charge at the far end attracts it. The charge moves the DNA fragments through the gel.

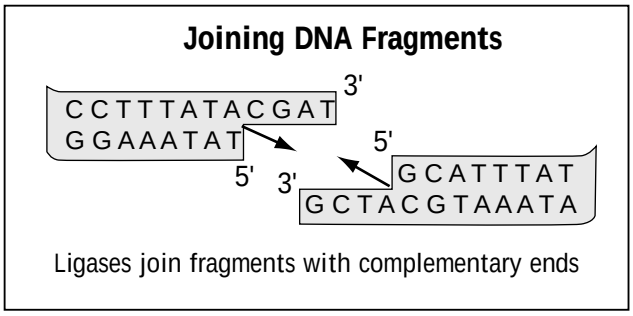
The gel resists the flow of strands through it, but it does not resist the movement of all strands equally. The larger the strand, the greater the resistance, and the more slowly it travels. The smaller the strand, the farther it travels in a given amount of time. After a time, the groups of short strands migrate to near the far end of the gel, and the groups of long strands remain nearer the wells. Strands of the same size move at the same rate, and so stay together. The end result is an array of fragments that are sorted according to size. This array provides important information about



the DNA that was cut, and grouping the fragments makes it possible to obtain many copies of the desired DNA.

Ligases

Once the gene is cut out, it must be combined with other DNA which will make it viable in the cell. The tool that allows DNA fragments to be recombined is another set of enzymes called **ligases**. Ligases are enzymes which repair DNA in the cell. Ligases establish a phosphodiester bond between 5′ and 3′ ends of complementary sequences, such the CGAT and GCTA sequences in the example below. Fragment ends which were cut by the same restriction enzyme have such complementary sequences.



When fragments with complementary ends are placed in a solution containing a ligase, recombination occurs at random. Many recombinations are possible, and a fraction of these contain the desired recombinant DNA. For example, if one of the fragments with complementary ends was a loop-shaped plasmid which restriction enzymes have opened into a linear piece, ligases can join a new gene to both ends and remake a loop with the new gene in it.

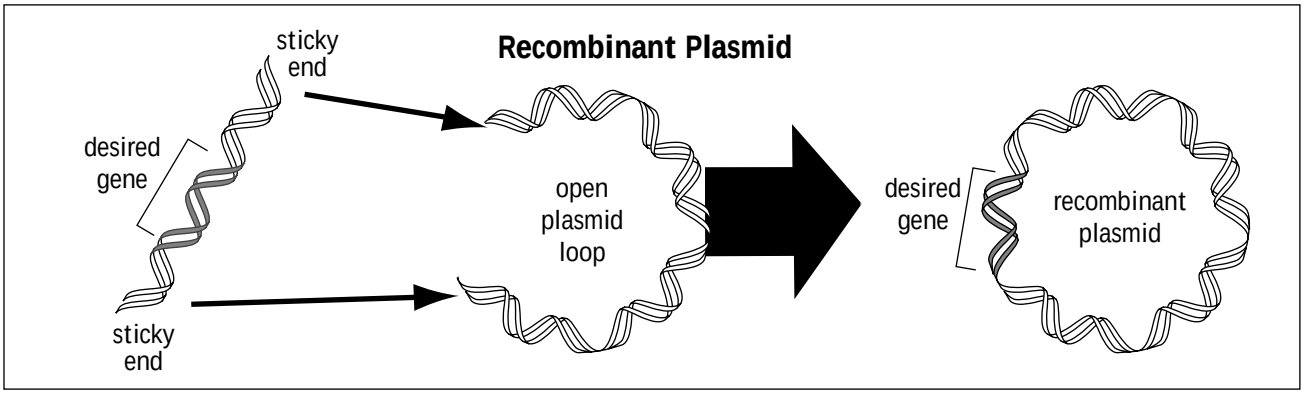
Recombinant plasmids

DNA is often cut and spliced into special DNA molecules that can replicate and function inside the cells. The most commonly used special DNA molecule is a loop called a **plasmid**.

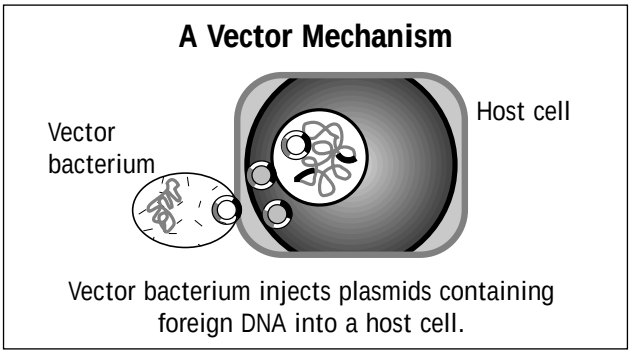
Plasmids are found naturally in bacteria and some yeasts. They contain a critical base sequence, called an **origin of replication**, where DNA replication can begin. The origin of replication and several genes allow plasmids to copy themselves inside the cell. When they copy themselves, they also copy genes that have been spliced into them.

Vectors

Recombinant plasmids can ferry a desirable gene into certain kinds of cells under special circumstances. For example, certain bacterial cells can accept and retain plasmids directly through the cell wall. Many kinds of cells, however, cannot accept foreign DNA directly, so other methods of moving DNA into cells are necessary. One method involves using organisms that inject DNA into cells. A biological system for carrying functional DNA into a cell is called a **vector**. Vectors can be plasmids alone, but they can also be viruses or



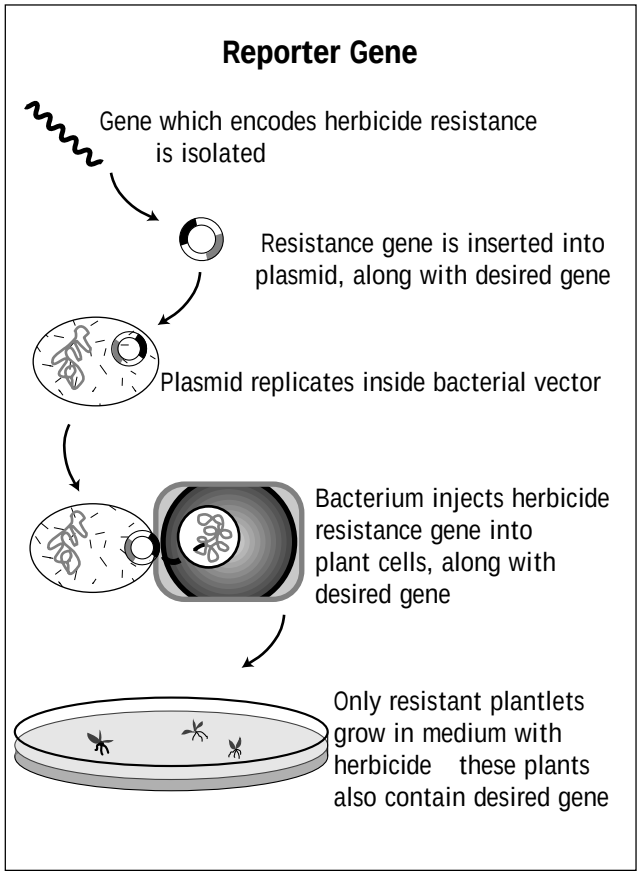
certain bacteria. Lesson 4, *Gene Transfer*, discusses vectors in greater detail.



Reporter genes

Before scientists try to grow a transgenic plant or animal from transformed cells, they need to be sure the cells do incorporate the new DNA. Unfortunately, transformation is rarely obvious in cells. For example, a fungal resistance trait is not apparent until a plant is propagated and successfully resists fungal attack. Scientists require early evidence at the cellular level to assure that the cells are transformed successfully.

To identify transformed cells, a **reporter gene** may be added to a recombinant plasmid before it is inserted into the cell. When the plasmid replicates inside the cell and the reporter gene begins to function, the cell produces proteins encoded by the reporter gene. Reporter genes code for expression of a protein that produces an observable trait at an early stage, such as the ability to grow in the presence of an antibiotic or herbicide. A reporter gene may also encode a detectable protein the plant does not normally make. The reporter and desired genes are linked, so cells carrying the reporter gene probably also carry the desired gene.



Terms

gel electrophoresis – an electrical process used to group and sort DNA fragments according to their size. The process uses a low voltage direct current to cause all DNA fragments of the same length to migrate at the same rate. Shorter fragments migrate through the gel more rapidly, and longer fragments migrate more slowly.

ligase – an enzyme that can fuse complementary ends of DNA to form a recombinant molecule.

origin of replication – a specialized base sequence on the DNA strand which enables DNA replication to begin.

plasmid – a loop–shaped DNA molecule, separate from the chromosome, that can replicate inside a cell.

recombinant DNA – DNA formed by combining DNA from several sources.

reporter gene – a gene added to recombinant DNA which gives clear and early indication that an organism has been genetically transformed. Reporter genes commonly code for expression of a protein that produces an easily observable result.

recognition sequence – the DNA code sequence which is recognized and cut by a restriction enzyme.

restriction digest – DNA which has been cut into fragments with restriction enzymes.

restriction enzyme – an enzyme which cleaves DNA at a specific code sequence.

sticky ends – ends of DNA that have been cut with restriction enzymes. Sticky ends will stick to another end with a complementary base sequence, such as an end cut with the same restriction enzyme.

transgenic – having a gene from another non–interbreeding organism.

vector – living microorganisms or specialized pieces of DNA which carry foreign DNA into a cell and allow it to be integrated as a functional unit into a cell.

Sources and Resources

Videotapes on Recombinant DNA

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Education at Cold Spring Harbor Laboratory – www.cshl.org/public/educat.html.

The Iowa Biotech Educator – a quarterly newsletter for educators incorporating biotechnology into their curricula, www.biotech.iastate.edu/ia_biotech_educator.

National Centre for Biotechnology Education (NCBE) – University of Reading, United Kingdom.www.ncbe.reading.ac.uk. Information and activities for teachers.

The Why Files **–** on-line reports from University of Wisconsin– whyfiles.news.wisc.edu/062ag_gene_eng/index.html.