Another approach to using a gene library to find genes that express proteins with certain functions is the use of display technologies. Most common are *phage displays* and *bacterial displays*. For example, a bacteriophage or bacterium may display on its surfaces proteins encoded from genes derived from the library. Each cell may display at most only a few of these gene products. An example of the use of such a system is the isolation of a cell producing a gene product that will bind to a particular molecule (*ligand*). The ligand can be bound to a surface, and only those cells expressing a protein on their surface that binds to that ligand can "stick" to the surface. These cells that stick can be recovered and propagated to make more copies of the gene and protein.

Although our focus is on amplifying the number of genes as a basis for producing target proteins or altering pathways, gene cloning is often done to obtain many copies of a particular gene. The amplification of the gene number facilitates gene sequencing and analysis. This amplification is particularly important for mapping genomes (e.g., the human genome project), for diagnosis of disease-causing organisms (both microbial and viral), for biologists studying evolution, and for forensic scientists.

An alternative technique to traditional gene cloning is a technique called the *polymerase chain reaction,* or *PCR*. PCR is the preferred method to amplify DNA. In this technique a target sequence of interest is a gene on double-stranded DNA.[‡] The technique requires that two short primer sequences (< 20 nucleotides) on either side of the target be known. If heat is applied, the complementary strands of double-stranded DNA separate. While separated, two pieces of chemically synthesized DNA (the primers) are added. Each primer binds to complementary sequences. A heat-stable DNA polymerase from a bacterium that grows in hot springs (the Taq polymerase) is added and quickly synthesizes from the primers the complementary DNA strands using the four nucleotides (A, G, T, and C) added to the reaction mixture. At the end of the cycle, there are two copies of the original gene. If the cycle is repeated, those two copies become four. Thirty cycles can be done in less than a day. Thus, from a single gene copy, 2^{30} copies of the gene can be generated, more than a billionfold increase. Thermal cyclers and PCR kits are commercially available.

This simple summary does not cover all the intricacies investigators often face in obtaining industrially useful clones. However, the procedures discussed here are applicable in most cases where plasmids are used as vectors to transform bacteria such as *E. coli.*

8.4.2. Genetic Engineering of Higher Organisms

The direct genetic engineering of higher organisms can be a great deal more difficult because of a lack of good effective techniques to introduce foreign DNA and of an understanding of host cell genetics. The reader should be aware of the techniques being developed to work with some of these host systems. The introduction of foreign DNA into higher organisms is usually termed *transfection*.

Some plants are subject to infection with the bacterium *Agrobacterium tumefaciens*. *A. tumefaciens* contains a plasmid that contains a section known as T-DNA. This T-DNA can integrate into the plant chromosome. If genes are inserted into the T-DNA region, they

[‡]For forensic studies or phylogenic studies on relationships of organisms a non-protein coding sequence may be the target.