confers a selectable marker itself (e.g., the ability to grow on a substrate not normally utilized by the host). Also, antibodies to the target protein when tagged with a radiolabel or fluorescent label can be used to identify colonies expressing the target protein.

An important tool in working with both proteins and DNA fragments or plasmids is *electrophoresis*. In protein electrophoresis an electric field is applied to a solution containing proteins placed at the top of a gel (typically made of polyacrylamide). The proteins migrate through the porous structure of the gel in a direction and at a speed that reflect both the size and the net change of the molecule. The gel reduces the effects of convection, although thermally induced convection can be problematic in large gels. For proteins SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) is commonly used. In this technique the proteins are denatured by heating with SDS and mercaptoethanol. Mercaptoethanol is a reducing agent to break disulfide bands. Individual polypeptide chains form a complex with SDS, which is negatively charged. The negatively charged complex then migrates through the gel at a rate that reflects the molecular weight of the polypeptide. In a typical SDS-PAGE gel there is a lane with polymers of known molecular weight. Other lanes will have samples with unknown proteins. After a defined period of time (typically a few hours) the process is stopped and the gel examined. Each protein forms a band. With some types of electrophoresis the band is highlighted by use of stains. Smaller molecules travel a greater distance. The molecular weight of a protein band in the unknown sample can be estimated by comparing to the lane with the molecular weight standard. The relative amount of a protein can be determined by the intensity of the band.

Various forms of gel electrophoresis can be used to determine if a protein from a clone is being produced and at what relative level. A particularly useful procedure is an *immunoblot* or *Western* blot. Here a nondenaturing gel is run to separate proteins, they are blotted onto nitrocellulose paper, and the protein identified by binding to a specific antibody followed by a radioactive marker that binds antigen-antibody complexes. The band is visualized by exposure of the nitrocellulose paper to x-ray film. Because of the high degree of specificity of antibodies, a positive band is good confirmation that the protein band represents a target product rather than another protein of similar molecular weight.

Similarly DNA molecules can be separated by gel electrophoresis using agarose or polyacrylamide. DNA is negatively charged. DNA fragments (e.g., from digestion with restriction enzymes) can be separated by molecular weight as well as plasmids from larger DNA elements. The DNA separates based on molecular weight. The DNA can be recovered by simply cutting out the part of the gel corresponding to the band for the desired DNA and eluting. The DNA is invisible unless stained. One approach is to expose DNA to a dye that fluoresces when under ultraviolet light.

With shotgun cloning (which produces a large *gene library* or *gene bank*), a desired colony can be isolated by using radiolabeled RNA or DNA probes complementary to the cloned gene. Such a procedure involves transferring colonies to nitrocellulose filter paper, where they are lysed. After lysis, the DNA spills out to bind on the filter paper. The paper is flooded with the probe; the probe only binds to the DNA with a complementary sequence (hybridization) and excess probe is washed away. If the filter is covered with x-ray film, radioactivity will expose the film (visible as a black spot), identifying which colonies had the donor DNA. This procedure identifies colonies that have been transformed with the desired DNA; expression has to be established in separate experiments.