code is degenerate, the deduction of the actual nucleotide sequence is ambiguous. This ambiguity requires that a variety of probes be generated. Hybridization reactions require the donor DNA to be both fragmented and converted into single strands that can react with the single-stranded probes.

An alternative to hybridization is total *chemical synthesis* of a gene that corresponds to the desired protein. This alternative requires knowledge of the amino acid sequence of the desired protein. An artificial gene may code for exactly the same protein as in nature, even if the sequence of nucleotides on the artificial gene is not identical to the natural gene. Chemical synthesis also allows us to produce specifically modified natural proteins or potentially totally human-designed proteins.

Another method to obtain the desired gene is particularly useful for genes with *introns*. Since bacteria lack the cellular machinery to cutout introns and to do *m*-RNA splicing, eucaryotic genes with introns cannot be directly placed in bacteria to make a desired protein. Often we wish to make these proteins in bacteria, since the bacteria grow much more rapidly and are much easier and cheaper to culture. Often the processed *m*-RNA for the desired gene can be isolated directly from the donor organism's cytoplasm (using hybridization probes). Once the *m*-RNA is isolated, the enzyme *reverse transcriptase* (see Chapter 4) can be used to synthesize a DNA molecule with the corresponding nucleotide sequence; this molecule is called *complementary DNA* or *c DNA*.

Once the desired gene is isolated or made, it can be inserted into a small piece of carrier DNA called a *vector*. Typically, vectors are plasmids, although temperate viruses can be used. The process for preparing the donor DNA and vector for recombination and the actual joining of the DNA segments requires special enzymes (see Fig. 8.8); we have discussed these enzymes in our previous consideration of DNA replication and genetic recombination. A wide variety of *restriction enzymes* exist that will cut DNA at a different prespecified site. Most vectors have maps showing the various restriction sites; important examples are EcoR1 from *E. coli* and Bam H1 from *Bacillus amylofacieus*. Many restriction enzymes leave "sticky ends," a few nucleotides of single-stranded DNA projecting from the cut site. Pieces of DNA with complementary "sticky ends" naturally associate, and in a mixture of cut vector and donor DNA, some pieces of donor DNA will associate with vector DNA. *DNA ligase* can permanently join these ends.

The mixture bearing the desired vector–donor combinations is then moved into the recipient or host cell. In most cases this is done by transformation, although other techniques can be used if transformation of the host is difficult. Note that the construction of the desired vector–donor DNA usually results in a mixture (e.g., some vector molecules may be opened and rejoined without donor DNA being inserted, or multiple copies of donor DNA may be inserted, or DNA contaminants of the donor DNA mixture may become inserted into the vector). Consequently, an efficient method to screen transformants for those with the desired vector–donor DNA combination is important.

Most vectors contain selectable markers such as antibiotic resistance or the genes to make essential growth factors that have been removed by mutation from the host cell. In the latter case, growth in minimal medium is possible only in the presence of the plasmid. These selectable markers allow the isolation of genetic *clones* that have been successfully transformed. A further screening step is then necessary to ensure that the donor DNA is present and being *expressed* (i.e., a functional protein is being made from the donor DNA).