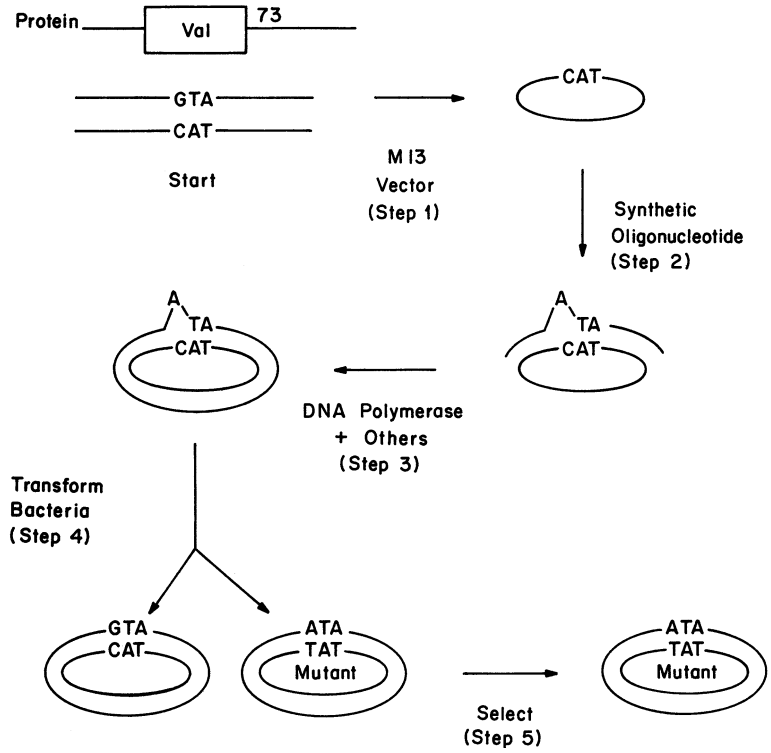

Figure 14.9. The general approach to site-directed mutagenesis is depicted. For this example the amino acid, valine, at position 73 of a particular protein, is to be replaced with isoleucine. The first step is to clone the gene into the single-stranded DNA vector, M13, and collect a working amount of gene. In the second step, an oligonucleotide essentially complementary to that region of the gene to be mutated is synthesized chemically. A typical length for such an oligonucleotide is 18 residues. However, this synthetic oligonucleotide contains one mismatched nucleotide so as to cause the desired mutation. In the third step, enzymes are added that complete the *in vitro* synthesis of a double-stranded vector. In the fourth step, the amount of double-stranded vector is amplified. In the last step, cells with the mutant gene are detected by hybridization with the synthetic oligonucleotide.

catalysis to unusual environments, such as in organic media rather than in aqueous solutions.

14.10. SUMMARY

The application of recombinant DNA technology at the commercial level requires a judicious choice of the proper host–vector system. *E. coli* greatly facilitates sophisticated genetic manipulations, but process or product considerations may suggest alternative hosts. *S. cerevisiae* is easy to culture and is already on the GRAS list, simplifying regulatory approval, although productivities are low with some proteins and hyperglycosylation