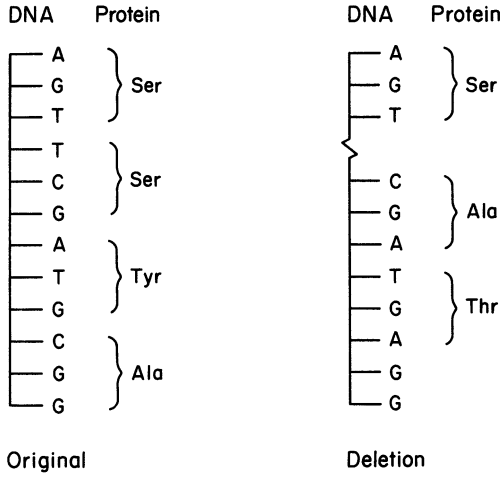

Figure 8.2. Effect of deletion of a base on the reading frame and the protein encoded.

The selection of a mutant with desirable properties is no easy task. Mutations are classified as *selectable* and *unselectable*. A selectable mutation confers upon the mutant an advantage for growth or survival under a specific set of environmental conditions; thus, the mutant can grow and the wild type will die. An unselectable mutant requires a cell-by-cell examination to find a mutant with the desired characteristics (e.g., green pigment). Even with mutagens, the frequency of mutation is sufficiently low to make prohibitive a brute-force screening effort for most unselectable mutants.

Selection can be direct or indirect. An example of direct selection would be to find a mutant resistant to an antibiotic or toxic compound. A culture fluid containing 10^8 to 10^{10} cells/ml is subjected to a mutagenic agent. A few drops of culture fluid are spread evenly on a plate, with the antibiotic incorporated into the gelled medium. Only antibiotic-resistant cells can grow, so any colonies that form must arise from antibiotic-resistant mutants. If one in a million cells has this particular mutation, we would expect to find about 10 to 100 colonies per plate if 0.1 ml of culture fluid was tested.

Indirect selection is used for isolating mutants that are deficient in their capacity to produce a necessary growth factor (e.g., an amino acid or a vitamin). Wild-type *E. coli* grow on glucose and mineral salts. *Auxotrophic* mutants would not grow on such a simple medium unless it were supplemented with the growth factor that the cell could no longer make (e.g., a lysine auxotroph has lost the capacity to make lysine, so lysine must be added to the glucose and salts to enable the cell to grow). The wild-type cell that needs no supplements to a minimal medium is called a *prototroph*. Consider the selection of a rare mutant cell that is auxotrophic for lysine from a population of wild-type cells. This cannot be done directly, since both cell types would grow in the minimal medium supplemented with lysine. A method that facilitates selection greatly is called *replicate plating* (see Fig. 8.3). A master plate using lysine-supplemented medium will grow both the auxotroph and wild-type cells. Once colonies are well formed on the master plate, an imprint is made on sterile velveteen. The bristles on the velveteen capture some of the cells from each colony. The orientation of the master plate is carefully noted. Then a test plate with minimal medium is pressed against the velveteen; some cells, at the point of each previous colony, then serve to inoculate