## 2.11 DNA sequencing

The term *DNA sequencing* encompasses biochemical methods for determining the order of the nucleotide bases, adenine, guanine, cytosine and thymine, in a DNA oligonucleotide.

The methodologies for DNA sequencing:

The *chain termination method* (by Sanger), in which the sequence of a ssDNA molecule is determined by enzymatic synthesis of complementary polynucleotide chains, these chains terminating at specific nucleotide positions.

The *chemical degradation method* (by Maxam and Gilbert), in which the sequence of a dsDNA molecule is determined by treatment with chemicals that cut the molecule at specific nucleotide positions.

The *pyrosequencing method*, in which the addition of a deoxynucleotide to the end of the growing strand is detectable because it is accompanied by the release of a flash of light.

**Chain termination method** (Sanger's method)

Chain termination method relies on the use of dideoxyribonucleoside triphosphates, derivatives of the normal deoxyribonucleoside triphosphates that lack the 3′ hydroxyl group.

Purified DNA is synthesized *in vitro* in a mixture that contains single-stranded molecules of the DNA to be sequenced, the enzyme DNA polymerase, a short primer DNA to enable the polymerase to start DNA synthesis, and the four deoxyribonucleoside triphosphates (dATP, dCTP, dGTP, dTTP: A, C, G, and T). If a dideoxyribonucleotide analog of one of these nucleotides is also present in the nucleotide mixture, it can become incorporated into a growing DNA chain. Because this chain now lacks a 3′ OH group, the addition of the next nucleotide is blocked, and the DNA chain terminates at that point.

To determine the complete sequence of a DNA fragment, the double-stranded DNA is first separated into its single strands and one of the strands is used as the template for sequencing. Four different chain-terminating dideoxyribonucleoside triphosphates (ddATP, ddCTP, ddGTP, ddTTP) are used in four separate DNA synthesis reactions on copies of the same single-stranded DNA template. Each reaction produces a set of DNA copies that terminate at different points in the sequence. The products of these four reactions are separated by electrophoresis in four parallel lanes of a polyacrylamide gel. The newly synthesized fragments are detected by a label (either radioactive or fluorescent) that has been incorporated either into the primer or into one of the deoxyribonucleoside triphosphates used to extend the DNA chain. In each lane, the bands represent fragments that have terminated at a given nucleotide but at different positions in the DNA. By reading off the bands in order, starting at the bottom of the gel and working across all lanes, the DNA sequence of the newly synthesized strand can be determined.

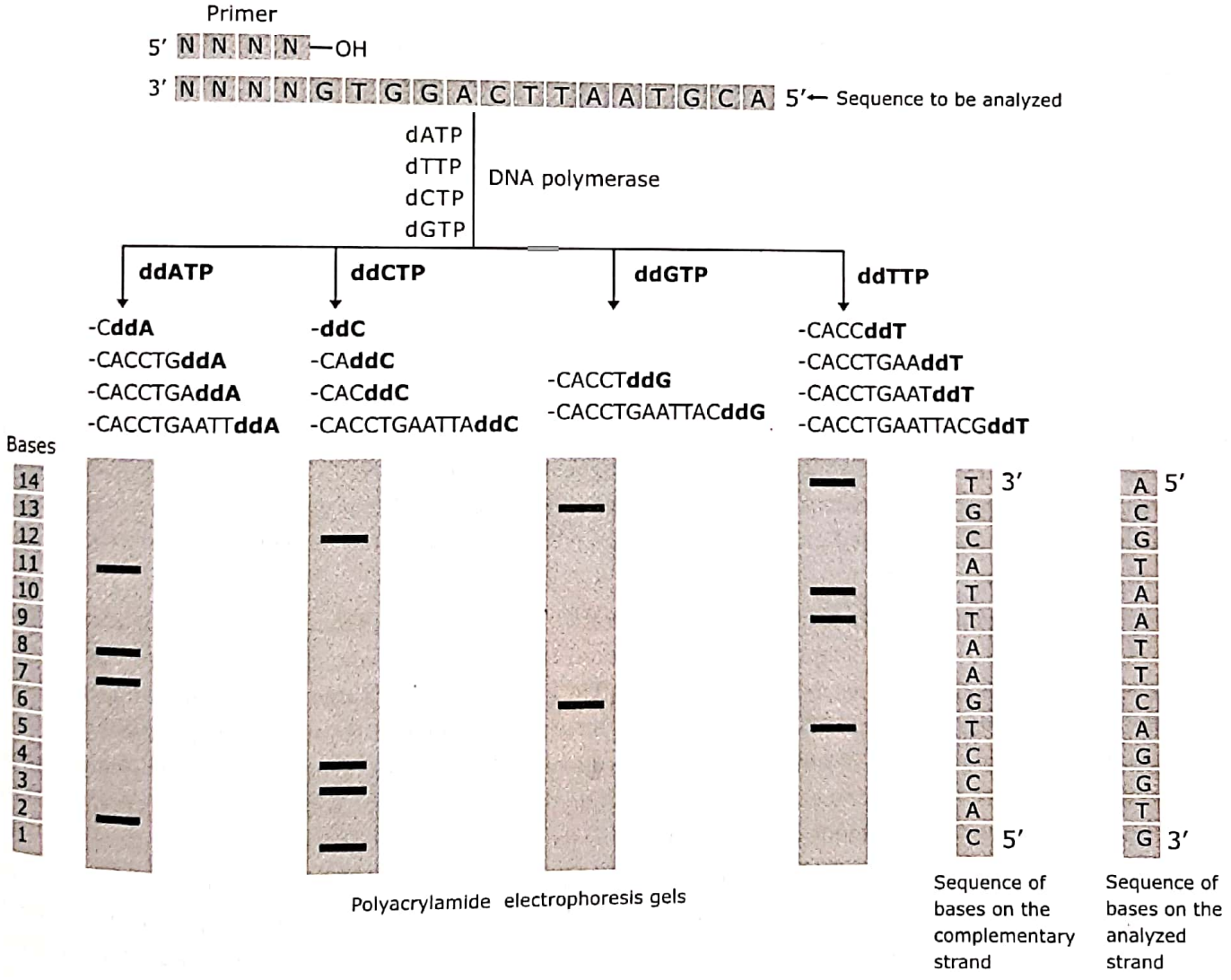


**Figure 2.25** DNA sequencing by chain termination method (Sanger method).