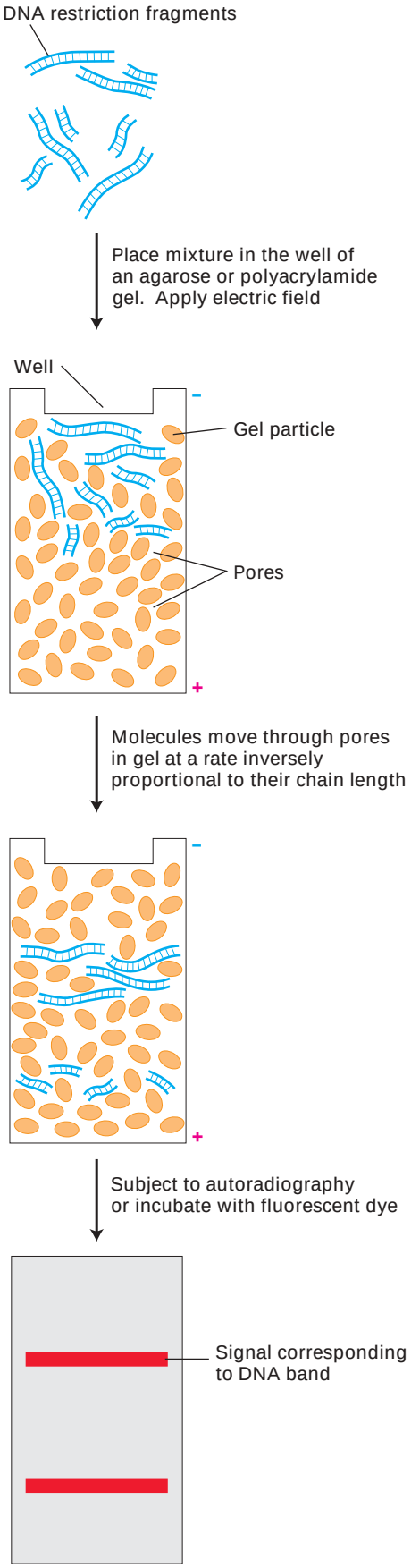
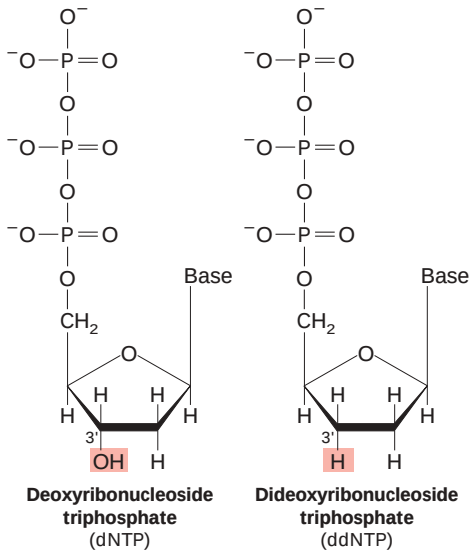


◀ EXPERIMENTAL FIGURE 9-21 **Gel electrophoresis separates DNA molecules of different lengths.** A gel is prepared by pouring a liquid containing either melted agarose or unpolymerized acrylamide between two glass plates a few millimeters apart. As the agarose solidifies or the acrylamide polymerizes into polyacrylamide, a gel matrix (orange ovals) forms consisting of long, tangled chains of polymers. The dimensions of the interconnecting channels, or pores, depend on the concentration of the agarose or acrylamide used to form the gel. The separated bands can be visualized by autoradiography (if the fragments are radiolabeled) or by addition of a fluorescent dye (e.g., ethidium bromide) that binds to DNA.

Cloned DNA Molecules Are Sequenced Rapidly by the Dideoxy Chain-Termination Method

The complete characterization of any cloned DNA fragment requires determination of its nucleotide sequence. F. Sanger and his colleagues developed the method now most commonly used to determine the exact nucleotide sequence of DNA fragments up to ≈500 nucleotides long. The basic idea behind this method is to synthesize from the DNA fragment to be sequenced a set of daughter strands that are labeled at one end and differ in length by one nucleotide. Separation of the truncated daughter strands by gel electrophoresis can then establish the nucleotide sequence of the original DNA fragment.

Synthesis of truncated daughter stands is accomplished by use of 2′,3′-dideoxyribonucleoside triphosphates (ddNTPs). These molecules, in contrast to normal deoxyribonucleotides (dNTPs), lack a 3′ hydroxyl group (Figure 9-22). Although ddNTPs can be incorporated into a growing DNA chain by



▲ FIGURE 9-22 **Structures of deoxyribonucleoside triphosphate (dNTP) and dideoxyribonucleoside triphosphate (ddNTP).** Incorporation of a ddNTP residue into a growing DNA strand terminates elongation at that point.