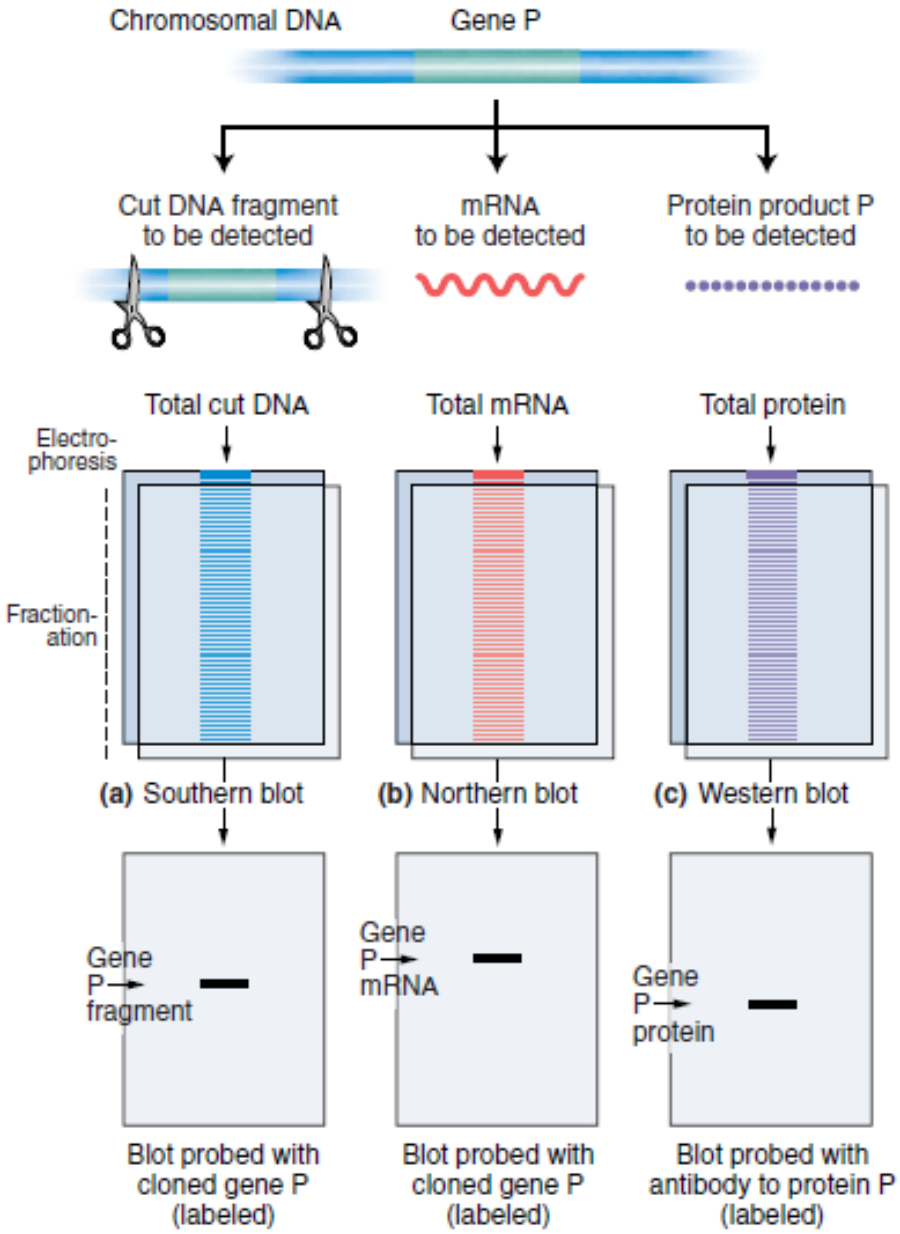


Figure 1-14 Probing DNA, RNA, and protein mixtures.

A **restriction map is a** linear map showing the order and distances of restriction endonuclease cut sites in a segment of DNA. The restriction sites represent small landmarks within the clone.
An example of one method used to create a restriction map of a clone is shown.

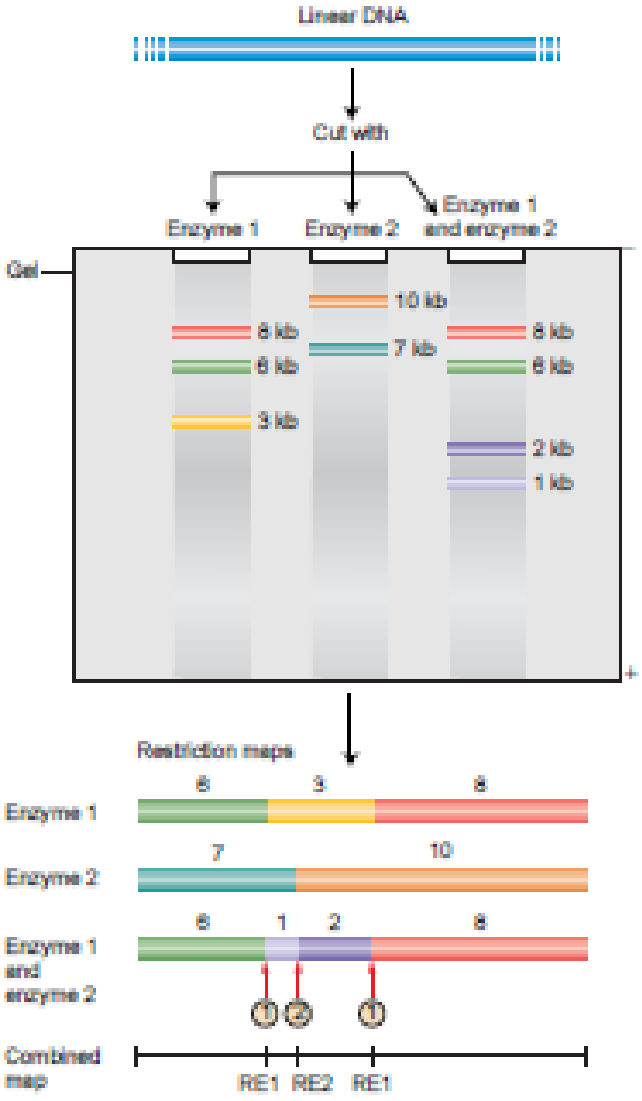


Figure 11-16 Restriction mapping by comparing electrophoretic separations of single and multiple digests. In this simplified example, digestion with enzyme 1 shows that there are two restriction sites for this enzyme but does not reveal whether the 3-kb segment generated by this enzyme is in the middle or on one of the ends of the digested sequence, which is 17 kb long. Combined digestion by both enzyme 1 (RE1) and enzyme 2 (RE2) leaves the 6- and 8-kb segments generated by enzyme 1 intact but cleaves the 3-kb fragment, showing that enzyme 2 cuts at a site within the 3-kb fragment, showing that the 3-kb fragment is in the middle. If the 3-kb segment were at one of the ends of the 17-kb sequence, digestion of the 17-kb sequence by enzyme 2 alone would yield a 1- or 2-kb fragment by cutting at the same site at which this enzyme cut to cleave the 3-kb fragment in the combined digestion by enzymes 1 and 2. Because this result is not the case, of the three restriction fragments produced by enzyme 1, the 3-kb fragment must lie in the middle. That the RE2 site lies closer to the 6-kb section than to the 8-kb section can be inferred from the 7- and 10-kb lengths of the enzyme 2 digestion.

Determining the base sequence of a DNA segment

- Obtaining the complete nucleotide sequence of a segment of DNA is often an important part of understanding the organization of a gene and its regulation, its relation to other genes, or the function of its encoded RNA or protein.

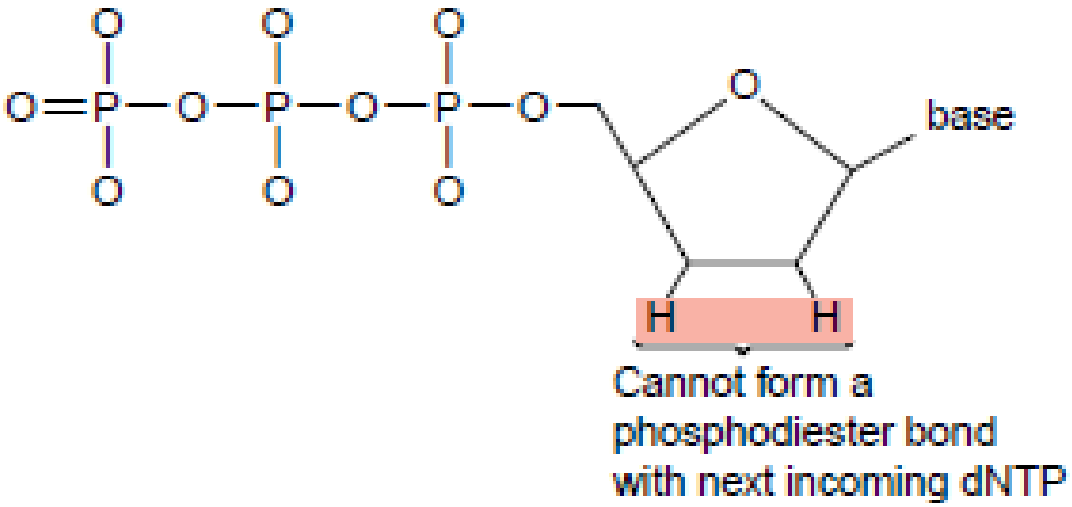
- Several techniques have been developed, but one of them is by far most used. It is called **dideoxy sequencing or, sometimes, Sanger sequencing** after its inventor. The term *dideoxy comes from a special* modified nucleotide, called a dideoxynucleotide triphosphate (generically, a ddNTP).

- This modified nucleotide is key to the Sanger technique because of its ability to block continued DNA synthesis.

- A dideoxynucleotide lacks the 3'-hydroxyl group as well as the 2'-hydroxyl group, which is also absent in a deoxynucleotide.

•For DNA synthesis to take place, the DNA polymerase must catalyze a condensation reaction between the 3'-hydroxyl group of the last nucleotide added to the growing chain and the 5'-phosphate group of the next nucleotide to be added, releasing water and forming a phosphodiester linkage with the 3'-carbon atom of the adjacent sugar.

•Because a dideoxynucleotide lacks the 3'-hydroxyl group, this reaction cannot take place, and therefore DNA synthesis is blocked at the point of addition



The logic of dideoxy sequencing:.

Suppose we want to read the sequence of a cloned DNA segment of, say, 5000 base pairs.

First, we denature the two strands of this segment.

Next, we create a primer for DNA synthesis that will hybridize to exactly one location on the cloned DNA segment and then add a special “cocktail” of DNA polymerase, normal nucleotide triphosphates (dATP, dCTP, dGTP, and dTTP), and a small amount of a special dideoxynucleotide for one of the four bases (for example, dideoxyadenosine triphosphate, abbreviated ddATP).

The polymerase will begin to synthesize the complementary DNA strand, starting from the primer, but will stop at any point at which the dideoxynucleotide triphosphate is incorporated into the growing DNA chain in place of the normal nucleotide triphosphate.

Suppose the DNA sequence of the DNA segment that we’re trying to sequence is:

Using the special DNA synthesis cocktail “spiked” with ddATP, for example, we will create a nested set of DNA fragments that have the same starting point but different end points because the fragments stop at whatever point the insertion of ddATP instead of dATP halted DNA replication.

5′	ATGGGATAGCTAATTGTTTACCGCCGGAGCCA	3′	Template DNA clone
3′	CGGCCTCGGT	5′	Primer for synthesis
	←		Direction of DNA synthesis
3′	*ATGGCGGCCTCGGT	5′	Dideoxy fragment 1
3′	*AATGGCGGCCTCGGT	5′	Dideoxy fragment 2
3′	*AAATGGCGGCCTCGGT	5′	Dideoxy fragment 3
3′	*ACAAATGGCGGCCTCGGT	5′	Dideoxy fragment 4
3′	*AACAAATGGCGGCCTCGGT	5′	Dideoxy fragment 5
3′	*ATTAACAAATGGCGGCCTCGGT	5′	Dideoxy fragment 6
3′	*ATCGATTAACAAATGGCGGCCTCGGT	5′	Dideoxy fragment 7
3′	*ACCCTATCGATTAACAAATGGCGGCCTCGGT	5′	Dideoxy fragment 8

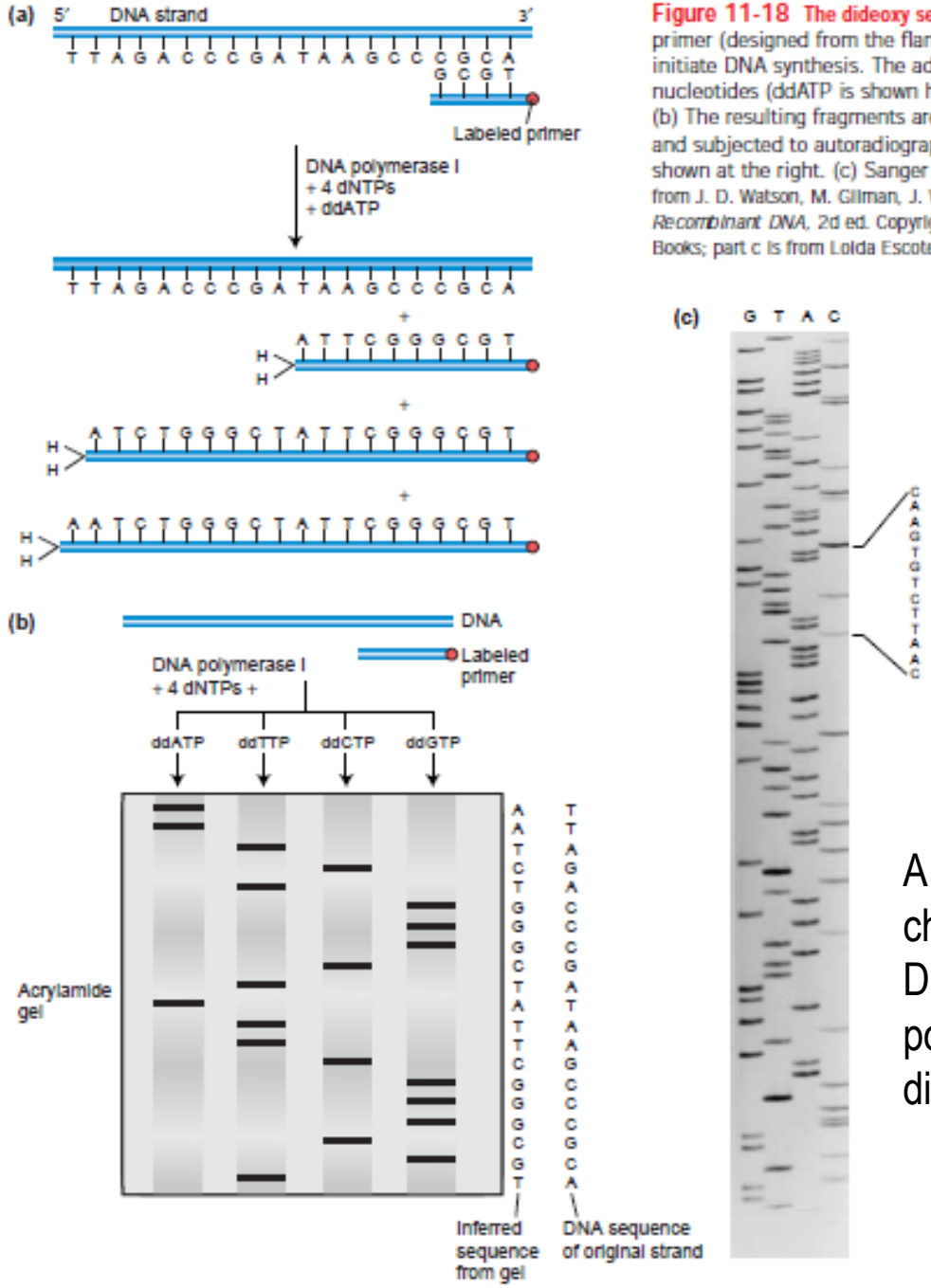
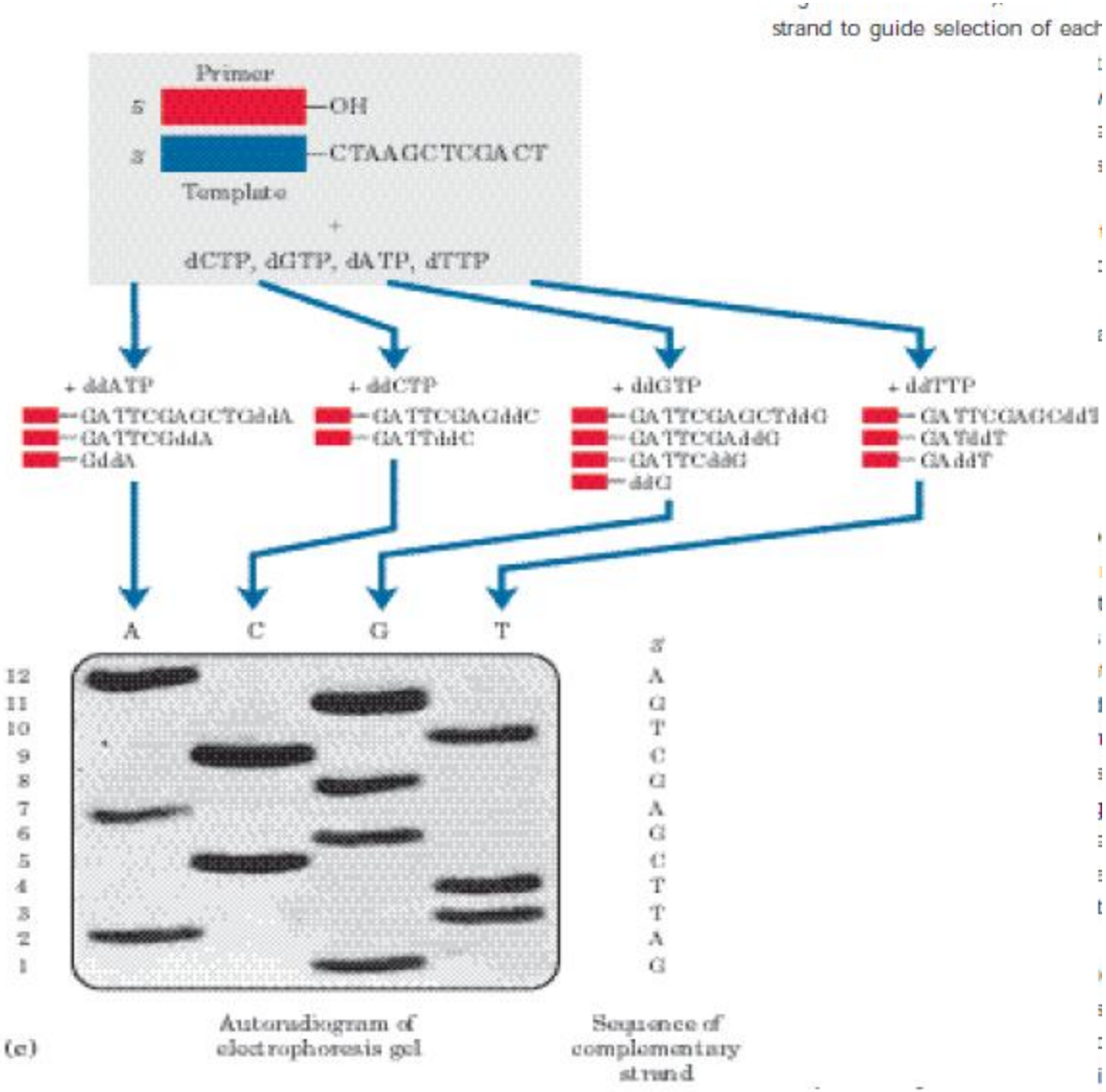


Figure 11-18 **The dideoxy sequencing method.** (a) A labeled primer (designed from the flanking vector sequence) is used to initiate DNA synthesis. The addition of four different dideoxy nucleotides (ddATP is shown here) randomly arrests synthesis. (b) The resulting fragments are separated electrophoretically and subjected to autoradiography. The inferred sequence is shown at the right. (c) Sanger sequencing gel. [Parts a and b from J. D. Watson, M. Gilman, J. Witkowski, and M. Zoller, *Recombinant DNA*, 2d ed. Copyright 1992 by Scientific American Books; part c is from Lolda Escote-Carlson.]

A cloned DNA segment can be sequenced by characterizing a serial set of truncated synthetic DNA fragments, each terminated at different positions corresponding to the incorporation of a dideoxynucleotide.



Result is a ladder of labeled DNA chains increasing in length by one, and so all we need do is read up the gel to read the DNA sequence of the synthesized strand in the 5’-to-3’ direction.

If the tag is a fluorescent dye and a different fluorescent color emitter is used for each of the four ddNTP reactions, then the four reactions can take place in the same test tube and the four sets of nested DNA chains can undergo electrophoresis together.

Thus, four times as many sequences can be produced in the same time as can be produced by running the reactions separately.

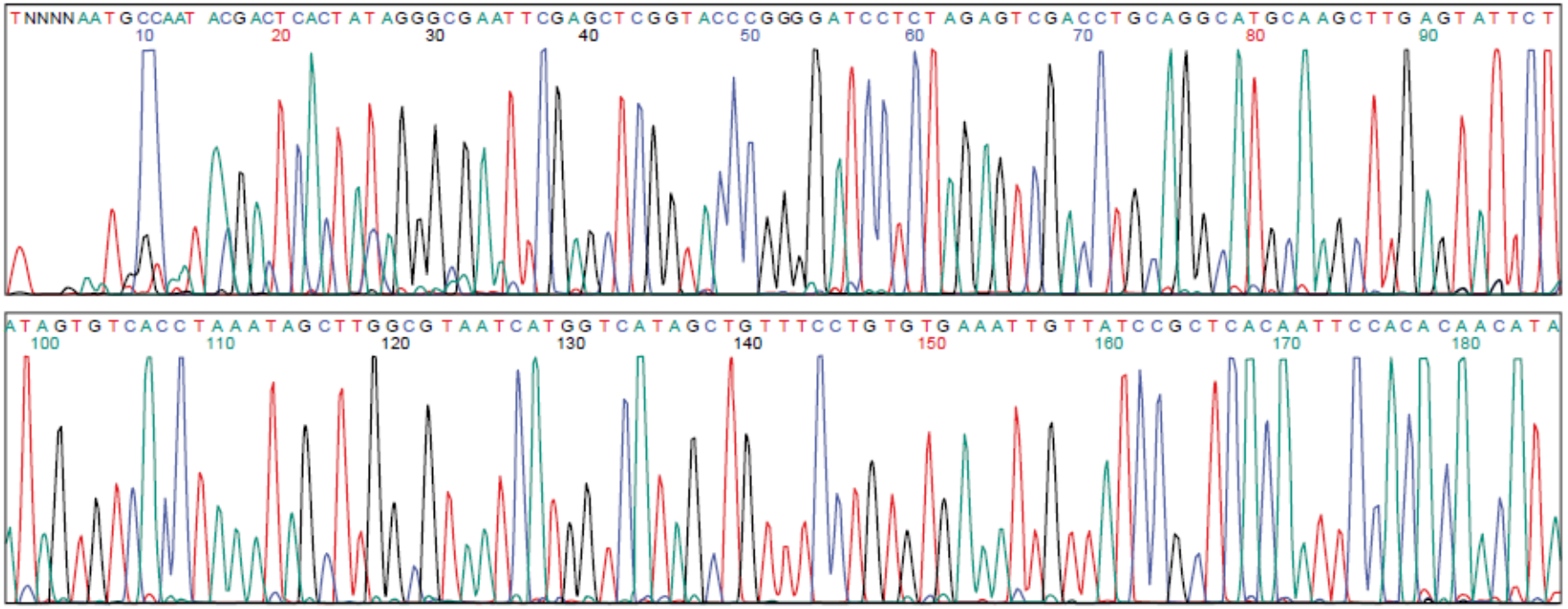


Figure 11-19 **Printout from an automatic sequencer that uses fluorescent dyes.** Each of the four colors represents a different base. N represents a base that cannot be assigned, because peaks are too low. Note that, if this were a gel as in Figure 11-18c, each of these peaks would correspond to one of the dark bands on the gel; in other words, these colored peaks represent a different readout of the same sort of data as are produced on a sequencing gel.