Automated DNA sequencers offer a number of advantages that are not particularly obvious. First, manual sequencing can generate excellent data, but even in the best sequencing laboratories poor autoradiographs are frequently produced that make sequence reading difficult or impossible. Usually the problem is related to the need to run different termination reactions in different tracks of the gel. Skilled DNA sequencers ignore bad sequencing tracks, but many laboratories do not. This leads to poor-quality sequence data. The use of a single-gel track for all four dideoxy reactions means that this problem is less acute in automated sequencing. Nevertheless, it is desirable to sequence a piece of DNA several times and on both strands, to eliminate errors caused by technical problems. It should be noted that long runs of the same nucleotide or a high G+C content can cause compression of the bands on a gel, necessitating manual reading of the data, even with an automated system. Note also that multiple, tandem short repeats, which are common in the DNA of higher eukaryotes, can reduce the fidelity of DNA copying, particularly with Taq DNA polymerase. The second advantage of automated DNA sequencers is that the output from them is in machine-readable form. This eliminates the errors that arise when DNA sequences are read and transcribed manually. A third advantage derives from the new generation of sequencers that have been introduced recently. In these sequencers, the slab gel is replaced with 48 or 96 capillaries filled with the gel matrix. The key feature of this system is that the equipment has been designed for use with robotics, thereby minimizing hands-on time and increasing throughput. With a 96-capillary sequencer, it is possible to sequence up to 750 000 nucleotides per day.

is redundant. However, not only do the two determinations serve as a check on one another, but, as will be seen shortly. It is necessary to use the other strand also in order to obtain the complete sequence).

The sequencing procedure is following. The sample containing the purified single strand is divided into two portions. To one portion (I) is added dimethylsulfate, which methylates purines, however, G is methylated five times more effectively than A. An essential future of the protocol is that the reaction is not carried to completion but only to the extent that above one purine by single strand is methylated. Since methylation occurs at random position, the particular A/G that is methylated differs in each strand. The methylated sample is mixed divided into two portions (Ia and Ib). Sample Ia is treated, a treatment that removes all methylated bases, leaving the deoxyribose. The sample is then treated with alkali, which cleaves the sugar phosphate chain at the site of the base that has been removed. This heating cleavage protocol generates a set of fragments of varying size, and the differing number of nucleotides in each fragment is determined by the different positions of the methylated G/A. Because G is methylated more often than A, sample Ia is said to contain the G only fragments. Sample Ib is not heated but instead with treated with dilute acid, which removes mainly methylated A (and some G). Then it is treated with alkali to cleave the sugar phosphate chain at the site where an A was removed. Thus, in sample Ib fragments are produced whose size is determined mainly by the position of the methylated A, but some G- these are called the A+G fragments. Note that every G-only fragment size is also present in the A+G collection.

The two samples Ia (G only) and Ib (A+G) are now electrophresed in a 20% polyacrylamide gel containing 8M urea, a denaturant that prevents hydrogen bonding and hence keep the fragments single stranded. After electrophoresis, the bands are located by placing autoradiographic film on the gel and exposing the films for several days. The single terminal ^{32}P atom, which was enzymatically added before the methylation reaction, is the sole source of radioactivity. Note that when a 5' ^{32}P labeled molecule is cleaved, only one of the two fragments produced contain ^{32}P and only that one is detected.

The position of A and G in the single strand is determined by the following rules: 1. If a band containing n nucleotides is present in both the (A+G) lane and the G only lane, then a G exists at position n+1 in the original molecule. 2. If a band containing n nucleotides is present in A+G lane, then an A exists at position n+1 in the original molecule.

So far, the analysis has used only sample I. Sample II is used to identify the position of cytosine (C) and thymine (T). This sample is also divided into two portions, II a and IIb, which are reacted with hydrazine in either dilute buffer (sample IIa) or in 2 M NaCl (sample IIb). Hydrazine react with C and T (but neither A nor G), but in 2 M NaCl the reactions is with C only. Cleavage at the site of hydrazine reaction is accomplished by treatment with peperidine, which breaks the sugar phosphate backbone at the 5' side of each base that has reacted with hydrazine. The sizes of the fragments are then determined by the positions of both C and T by C only. Thus,