**DNA Sequencing**

Dideoxynucleotide Chain Terminators

The reaction mixture containing newly synthesized, labelled DNA is then divided into four aliquots, representing the four dNTPs adenine, cytosine, quanine and thymine (A, C, ***G*** and T).

In addition to all of the dNTPs being present in the tube containing **A** (A tube), an analogue of dATP (2’,3’-dideoxyadenosine triphosphate, ddATP) is added.

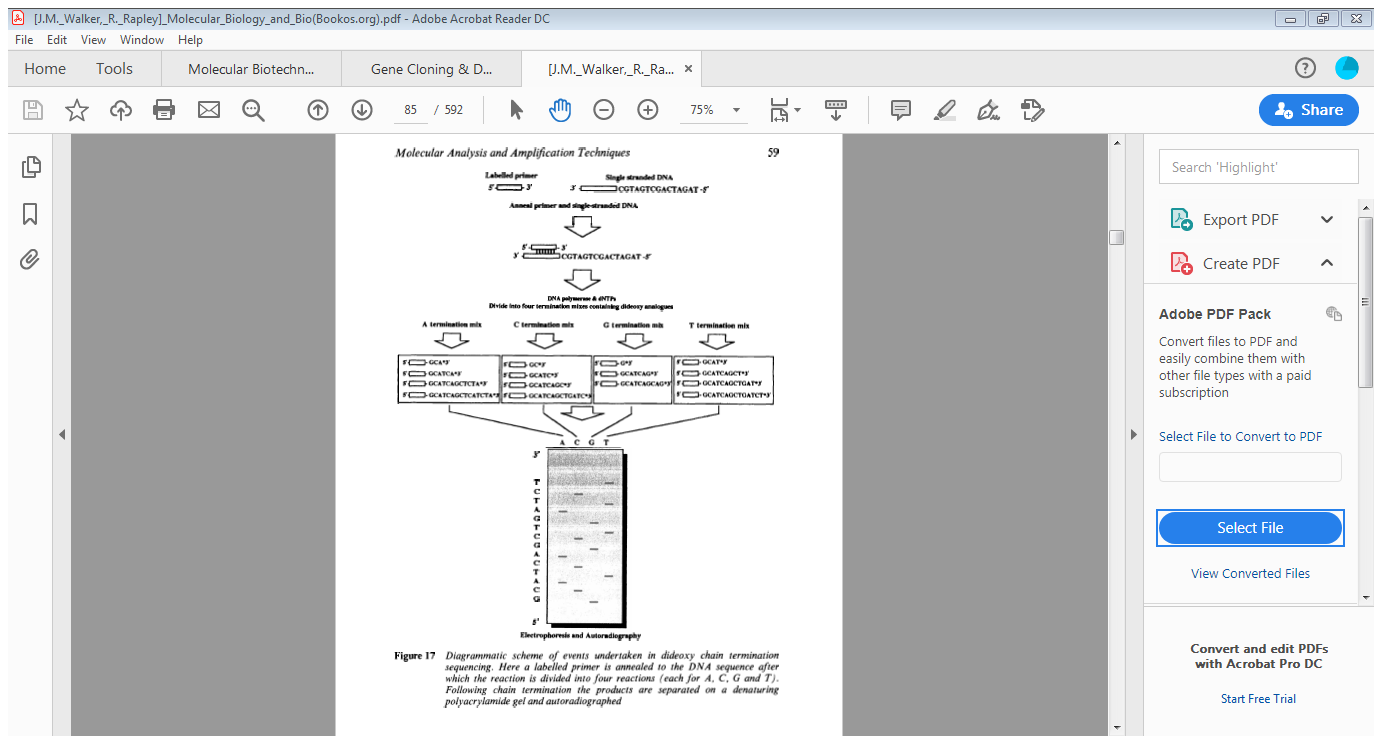
This is similar to A but has no 3’ hydroxyl group and so will terminate the growing chain since a 5‘ to 3‘ phosphodiester linkage cannot be formed without a 3’ hydroxyl group.

The situation for tube C is identical except that ddCTP is added; similarly, the G and T tubes contain ddGTP and ddTTP, respectively.

In case of Sangers method, primer is radioactively labelled. As the reaction proceeds four sets of DNA sequence are generated, each terminating at a different type of base, but all having a common *5‘* end (the primer). The four labelled and chain terminated samples are then denatured by heating and loaded next to each other on a polyacrylamide gel for electrophoresis. Electrophoresis is performed at approximately 70°C in the presence of urea, to prevent renaturation of the DNA.

Very thin, long gels are used for maximum resolution over a wide range of fragment lengths. After electrophoresis, the positions of radioactive DNA bands on the gel are

determined by autoradiography. Since every band in the track from the dideoxyadenosine triphosphate sample must contain molecules which terminate at adenine, and that those in the ddCTP terminate at cytosine, etc., it is possible to read the sequence of the newly synthesized strand from the autoradiogram, provided that the gel can resolve differences in length equal to a single nucleotide. Under ideal conditions, sequences up to about 300 bases in length can be read from one gel.



**Maxam and Gilbert Sequencing**

The chemical cleavage method **of** DNA sequencing developed by Maxam and Gilbert is often used for sequencing small fragments of DNA such as oligonucleotides. **A radioactive label is added to either the 3' or the 5' end of a double-stranded DNA preparation.** The strands are then separated by electrophoresis under denaturing conditions and analysed separately. DNA labelled at one end is divided into four aliquots and each is treated with chemicals which act on specific bases by methylation or removal of the base. The conditions are selected ***so*** that, on average, each molecule is modified at only one position along its length, and every base in the DNA strand therefore has an equal chance of being modified. Following the modification reactions, the separate samples are cleaved by **piperidine**, which breaks phosphodiester bonds exclusively at the 5' side of nucleotides whose base has been modified.

The result is similar to that produced by the Sanger method. Each sample contains radiolabelled molecules of differing lengths, however all the labelled ends are common. The other end is cut at the same type of base. Analysis of the reaction products by electrophoresis is as described for the Sanger method.

**Reference**

1. **A.** M. Maxam and W. Gilbert, ***Proc. Natl. Acad. Sci. USA,* 1977,74560.**
2. Walker and Rapley, Molecualr Biology and and Biotechnology, 4th Edition, RSC, London