

(i) Purines (A & G) are cleaved by dimethylsulphate, which methylates adenine at N-3 and guanine at N-7. The glycosidic bond of methylated purines are broken by heating at neutral pH. The sugar backbone is broken by heating in alkali.

(ii) Pyrimidines (C & T) are cleaved by hydrazine. Their backbone is cleaved by piperidine.

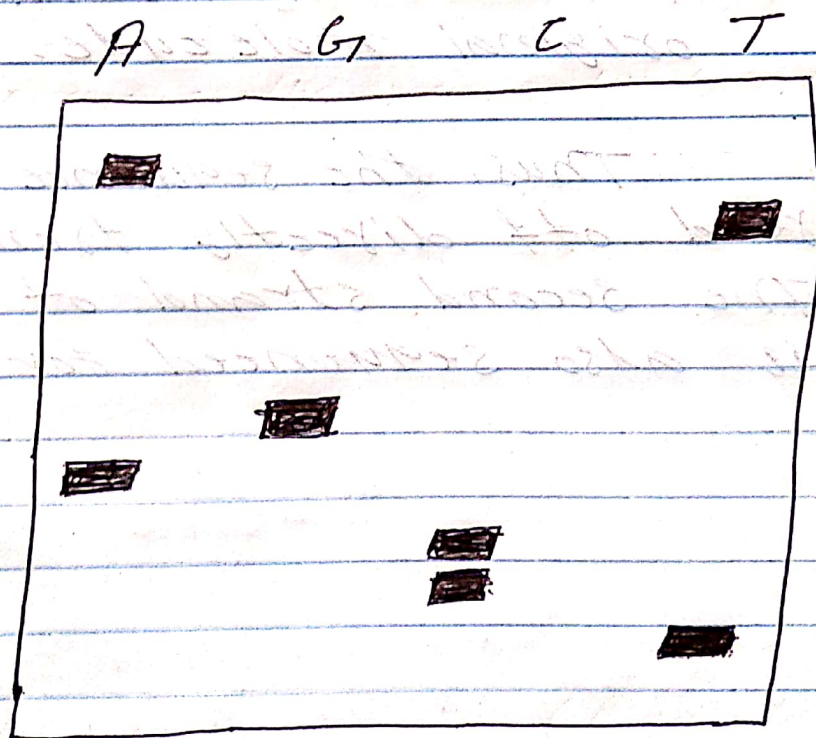


Diagram of a gel showing the radioactive fragments formed by specific cleavage of $5' \text{-}^{32}\text{P} \text{GTCCAGTA} 3'$

The position of A and G in the single strands are determined by the following rules:-

- (I) If a band containing a nucleotide is present in both the A+G lane and the G-only lane, then G exists at position $n+1$ in the original molecule.
- (II) If a band containing n nucleotides is present only in the A+G lane, then A exists at position $n+1$ in the original molecule.

Thus, the sequence can be read off directly from the gel.

The second strand of the molecule is also sequenced for confirmation.