

## (I) MAXAM GILBERT METHOD:

- The chemical cleavage method devised by Maxam and Gilbert starts with a DNA that is labelled at one end of one strand with  $^{32}\text{P}$  (Radio labelled)
- Polynucleotide Kinase is usually used to add  $^{32}\text{P}$  at its 5' hydroxyl terminus.
- The labelled DNA is then broken preferentially at one of the four nucleotides.
- The conditions are chosen so that on average of one break is made per chain.
- Each broken chain yields a radioactive fragment extending from the  $^{32}\text{P}$  label to one of the positions of that base.

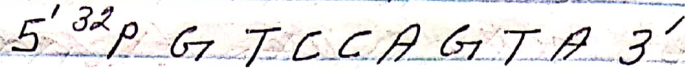


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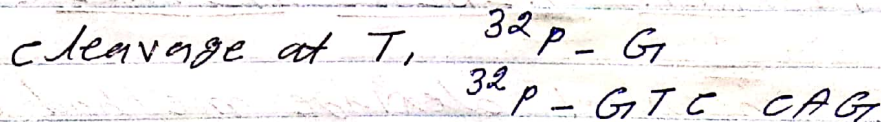
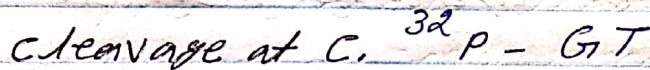
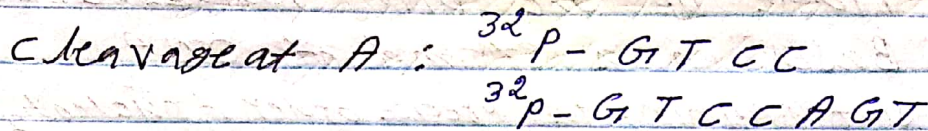
- Fragments are produced for every position of the base.

Example:-

If the sequence is



- The radioactive fragments produced by specific cleavage on the 5' side of each of the four bases would be



- The fragments in each mixture are then separated by polyacrylamide gel electrophoresis.
- which has the capacity to resolve DNA molecules differing in length by just one nucleotide.
- Next is to look at an autoradiogram of the gel for the above result the DNA is specifically cleaved by the following reagents.

