(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}P (Radiolabled)
- Polynucleotide Kinase is usually used to add ^{32}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 an average of one break is made per chain.
- Each broken chain yields a radioactive fragment extending from the ^{32}P label to one of the positions of that base.

~~base, and so~~

- Fragments are produced for every position of the base.

Example:-

If the sequence is

$5'\ ^{32}P\ G\ T\ C\ C\ A\ G\ T\ A\ 3'$

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

Cleavage at A : $^{32}P-G\ T\ C\ C$

$^{32}P-G\ T\ C\ C\ A\ G\ T$

Cleavage at G : $^{32}P-G\ T\ C\ C\ A$

Cleavage at C. $^{32}P-G\ T$

Cleavage at T, $^{32}P-G$

$^{32}P-G\ T\ C\ C\ A\ G$

- 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.

$5'\ G\ C\ G\ C\ T\ C\ A\ C\ T\ G\ 3'$

$1\ 2\ 3\ 4\ 5\ 6\ 7\ 8\ 9\ 10$