NCERT Class 12 Biology: Chapter – 6 Molecular Basis of Inheritance Part 12 (For CBSE, ICSE, IAS, NET, NRA 2022)

Question 7:

Give an account of the methods used in sequencing the human genome.

Answer:

1. DNA sequencing is the method used to detect the definite order of bases (Adenosine, Guanine, Thymine and Cytosine) with in the DNA.

2. Methods mainly used are:

A. Maxim Gilbert Method

B. Sanger Method

Maxim Gilbert Method:

In 1977 Maxim Gilbert devised for DNA sequencing.

Procedure of Maxim Gilbert Method:

1. At first the double helix structure of the DNA is denatured into a single stranded DNA by increasing the temperature.

2. The 5' end is labelled radioactively by Kinase reaction using gamma P ^{32}.

3. Cleave the DNA strand at specific positions using chemical reactions.

4. For cleavage at specific positions two chemicals are used followed by the addition of piperidene.

a. Dimethyl Sulphate: This chemical only attacks purines (Adenosine and Guanine) .

b. Hydrazine: This chemical only attacks pyrimidine (Cytosine and Thymine)

5. In Maxim and Gilbert experiment the chemicals cleaved G, A + G, C and C + T.

6. In four test tubes, four different size of DNA's are obtained.

7. Now, these DNA's are separated based on size by gel electrophoresis technique.

Advantage:

1. Purified DNA can be read easily.

2. DNA-Protein interactions can be analysed.

3. DNA stretches can also be sequenced.

4. Nucleic acid structure can also be analysed.

Disadvantage

1. This method is not used widely.

2. Toxic and radioactive chemicals are used.

3. The process is complex.

4. Not more than 500 base pair can be analysed.

SANGER METHOD:

1. In Sanger's method DNA that has to be sequenced is amplified.

2. Now, heat is used to denature the DNA to produce complementary and template strand for DNA sequencing.

3. A primer is than annealed to the 5' end of the template strand.

4. The primed DNA is then distributed equally among four test tubes.

5. In each of the test tube all four standard deoxynucleotides triphosphate (DNTP's) which are (dATP, dGTP, dCTP and dTTP) and DNA polymerase are added.

6. To each of the test tube only one of the four dideoxynucleotide (ddATP , ddGTP, ddCTP, ddTTP) are added.

7. The amount of dideoxynucleotide should be 100 times less than the deoxynucleotides so that the fragments can still transcribe.

8. The DNA fragments now formed, are denatured by heating and separated by the process of gel electrophoresis based on size difference.

9. The bands now formed are seen under UV light or X-ray and the sequence can be detected.

Advantages:

1. This the commonly used method.

2. Scientists were able to study the sequencing properly.

3. Various genetic disorders were detected.

Disadvantages:

1. Illegal use of DNA sequence information can be done.

Question 8:

List the various markers that are used in DNA finger printing.

Answer:

DNA markers are small regions of DNA sequence which are unique to an individual.

The various markers used in DNA fingerprinting are:

1. Restriction Fragment Length Polymorphism (RFLP's) :

It is a non-PCR based approach to identify the DNA sequencing. In this process DNA is digested at specific sites with restriction enzymes. The pattern band of fragments thus formed are separated by Gel electrophoresis and identified under X-ray or UV.

2. Random Amplified Polymorphic DNA (RAPD) :

It is a PCR based approach to find out specific DNA sequence. In this process many short primers are formed. From which random DNA segments are amplified using PCR (which means specific DNA sequence information is not required) . The band pattern formed from these DNA fragments are separated by Gel electrophoresis and identified under X-ray or UV.

3. Amplified Fragment Length Polymorphism:

In this process DNA is digested with the restriction endonuclease enzyme. Selective amplification of the fragments from a mixture of DNA fragments is done.

4. Southern Bloating:

In this process the fragments of the DNA are transferred to nylon sheath. These fragments are the mixed with radioactive isotope and the band of pattern formed are then visualised under UV or X-ray.

These are the various markers used to identify DNA sequencing.