**DNA FINGERPRINTING**

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27. **DNA fingerprinting**

* **It is obtaining the images of the DNA of man on an X-ray film**
* **It is similar to taking thumb impression**
* **Impression of fingerprint taken on a paper is used to identify persons**
* **Images of DNA bands captured on an x-ray film are used to identify individuals and hence the method is called DNA fingerprinting**
* **It is also called DNA profiling or molecular fingerprinting**
* **DNA fingerprinting was developed by Alec Jeffreys in 1984**
* **In fingerprinting, thumb impression is taken on a paper using ink or dye**
* **In DNA fingerprinting images of DNA bands are captured on an x-ray film after allowing the seperated DNAs on a nitrocellulose paper to hybridize with a known radio labelled probe**
* **As DNA print is unique and permanent like the thumb impression, DNA fingerprinting has been used in the identification of individuals**
* **The DNA prints of human beings are generally called DNA fingerprinting**
* **The DNA prints of animals are called Zoo blot**
* **The DNA prints of plants are called Garden blot**
* **The DNA fingerprinting technique uses Satellite DNA (Junk DNA) between the genes for DNA fingerprinting**
* **Stellite DNAs of different individuals are hybridized with a single type of radiolabelled probe DNA by using Southern Blotting method**
* **Then an autoradiogram is taken to get a DNA print pattern of the individuals**
* **Fro this DNA pattern, the in dividuals are distinguished on the basis of bands in their DNA prints**

1. **DNA fingerprinting is also called DNA profiling or molecular fingerprinting**
2. **Finger prints of animlas: The DNA prints of animals are called Zoo blot**
3. **Finger prints of Plants: The DNA prints of plants are called Garden blot**
4. **Satellite DNA:**

* **Satellite DNA is a non-coding region of eukaryotic cells having tandem repeats of a sequence**
* **It is also called mini-satellite**
* **It is rich is GC pairs and 9-40 bp in size**
* **Some minisatellites exist in a single copy but others represent 2 or more copies**
* **Each and every minisatellite differs in length from the corresponding minisatellite of an unrelated individual**
* **This is because of variation in the number of tandem repeats by deletion or addition of one or more sequences**
* **It causes variations in length of DNA fragments in the genomic DNA**
* **The number of tandem repeats vary from individual to individual so that they are known as variable number of tandem repeats (VNTR)**
* **The VNTR serves asa marker key fr identification in DNA Fingerprinting**

1. **Methodology of Fingerprinting:**

**The DNA fingerprinting involves 9steps**

1. **Isolation of cell DNA:**

* **DNA is isolated from the source material (girl) and the suspected individuals (boys)**
* **Any biological materials such as drop of blood, saliva, semen, teeth, bones, tissues, hairs with roots etc., found at the scene of crime is used to isolate DNA**
* **Blood or semen clotted on cloths, vaginal swabs taken from rape victims, hairs or fresh cells or blood are best for DNA fingerprinting**

1. **Restriction digestion**

* **The cell DNAs are separately cut with a restriction enzyme to generate short fragments**

1. **PCR amplification**

* **Since samples provide a small amount of DNA, PCR is performed in a PCR machine to get several copies of DNA for DNA fingerprinting**

1. **Gel Electrophoresis**

* **The DNA restriction digest of the suspected persons and source aterial are poured in to separate wells of an electrophoresis gel and electrphoresed**
* **The ministellites separate according to their lengths**

1. **Southern blotting and Baking**

* **The separated DNA fragments are transferred to a nylon membrane or a nitrocellulose filter paper by placing it over the gel**
* **This process is called Southern blotting**
* **The nitrocellulose filter having DNA fragments is dried in between dry filter papers at high temperature**
* **This process is called baking**

1. **Selection of DNA probe**

* **Dr. Lalji singh at the Centre for Cellular and Molecular Biology in Hyderabad has developed a DNA probe from minisatellite DNA of banded Krait (Bungarus fasciatus) , an Indian poisonous snake and named it BKm probe**
* **The BKm probe is being used for DNA fingerprinting in India**

1. **Filter hybridization**

* **The nitrocellulose filter is placed in an alkali solution to denature the duplex DNA fragments**
* **Then it is placed in the hybridization solution containing the probe DNA**
* **The probe DNA binds with appropriate minisatellite and forms duplex DNAs**

1. **Autoradiography**

* **After hybridization, the filter is washed with a wash solution to remove unboun probes**
* **Then an X-ray film uismplaced over the filter for about 23 hours**
* **The radioactivity of the probes makes dark spots on the X-ray film**
* **Therefore, an irreguilar ladder of dark spots develops on the X-ray film, for each and every DNA samle**
* **Each ladder represents a DNA print of an in dividual**
* **All DNA prints on the X-ray film together constitute a DNA fingerprint pattern**

1. **Analysis of DNA print pattern**

* **In case of rape and murder all bands of seminal DNA or hairs-DNA should match perfectly with DNA print of the accused**
* **If the match is 100%, the individual is found to be responsible for the victim**
* **All bands of source material never math with the bands of DNA print of any person who did not involve in the case**
* **If all bands of DNA print obtained from vaginal swab match with the bands of DNA print of a person A with 100% certainly.**
* **The persons B and C’s DNA prints never match with the DNA print of X**
* **So A is believed to be the accused**
* **To determine the parentage of a child, 50% of bands in the child’s DNA print should match with those of the father’s DNA print and the rest of the bands should match with those the mother’s DNA print**
* **This is because of the fact that half of the DNA of the child came from the father and the other half of the DNA came from the mother**
* **If some of the bands in the child’s DNA print do not correspond with either the alleged father or alleged mother, then it is certain that they are not its real parents**

Vaginal swab of a raped victim

Suspected persons

**DNA DNA DNA DNA**

**Add Restriction enzymes**

**Get Restriction Restriction Restriction Restriction**

**fragments fragments fragments fragments**

**( X ) Run PCR (A) Run PCR (B)Run PCR (C) Run PCR**

**X**

**A**

**B Electrophoresis gel**

**C**

**Electrophoresis**

**Southern blotting**

**Baking**

**Alkali treatment**

**Hybridization**

**Post –hybridization washing**

**Autoradiography**

**DNA fingerprint pattern**

1. **Applications of fingerprinting**
2. **To settle disputed parentage:**

**When courts cannot decide who is the real father or mother or both of a child, it is difficult to settle the case. But DNA fingerprinting is used to settle the case**

**Ex: A lade named Mala and her husband Babu missed their daughter. But they suspected the other couple have stolen their daughter**

**One of the 8 bands in the DNA print of the daughter , 4 matched with the DNA print of Mala and the other 4 matched with Babu DNA print**

**No one of the bands match with the bands in DNA print of those couple**

**On the basuis of DNA fingerprinting evidence, the court ordered to keep the daughter with Babu and Mala who are their real parents**

1. **To settle murder cases**

**A man who was innocent was in the jail. By using DNA fingerprinting, he was set free from jail**

1. **To trace the Date of murder**

* **DNA fingerprinting is done in eggs, maggots and adult flies captured from the murder place to detectthe date of murder (because within an hour of deth, flies come and infest the body)**
* **If the murder took place in the same day, the flies DNA fingerprinting will be negative and if on the previous day, the adult flies show th positive fingerprints.**
* **If before two days, several generations of flies are positive in the DNA fingerprinting analysis**

1. **To distinguish rapist**

**DNAs taken from the vaginal swab and semen or blood of doubtful personsmatches with the DNA print of any one of the man , it is confirmed that the particular man is accused for the rape**

1. **Pedigree analysis**

* **DNA fingerprinting is used in pedigree analysis in cat, dog, horses and man**

1. **Migration pattern**

**It is used to assess the pattern of migration of ancient populations**

1. **Genetic analysis**

**It is also employed in genetic analysis of various strains of agricultural and crops and animals**

1. **CDFD (The Centre for DNA Fingerprinting and Diagnostics**

* **It is the centre for DBNA fingerprinting in India.**
* **It is functioning in the Cellular and Molecular Biology Department (CCMB) , Hyderabad**
* **This centre has DNA fingerprinting facilities at International level**
* **If a sample is to be accepted by the CDFD, it should be forwarded by the Honourable court or police above the rank of iNspector**
* **The CDFD takes 4- 7 days to send the report of DNA fingerprint to the concerned party which report is accepted by all courts in India**

1. **Zoo plot and its uses:**

* **Zoo blot is obtaining the images of the DNA of animal on an x-ray film**
* **Zoo blot can be performed with Southern blotting and Northern blotting**
* **Zoo blot is used to demonstrate the presence of a particular gene in different species of vertebrates**
* **It helps to study the expression of genes associated with some genetic diseases**

1. **Garden plot and its uses:**

**Garden blot is obtaining the images of the DNA of plant on an x-ray film.**

**It is a slight variant of usual DNA fingerprinting**

**Garden bklot can be performed with Southern blotting or Northern blotting**

**Garden blot is used to demonstrate the presence of a particular gene in different plant species**

**It helps to identify similarities between different plant species in relation to specific genes**

**Garden blot is very useful to identify new DNA markers from plant genomes**

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