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DNA Testing

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The process of identification of DNA from the given sample was known as genetic profiling. Genetic profiling was a process in which characteristic of DNA was determined from the given DNA sample. Genetic profile of particular allele size at several loci was analysed from the given sample of DNA. McEwen Identifying criminal, paternity and maternity, breast cancer or deafness whether it was in the family, in checking animal pedigrees and identifying people in high risk professions from the given DNA sample were a few applications in forensic science. According to this, from the source of DNA obtained in crime scene an individual was excluded or included. It was very essential to take care in keeping the sample out of contamination as it may sometime gives false results.

The two techniques that were used in DNA profiling were Restriction Fragment Length Polymorphism (RFLP) and Short Tandem Repeat (STR) profiling. The current experiment is subjected to STR based on PCR where number of copies of small portion of DNA was made out by using an enzyme. With the help of other enzyme the portion of DNA was cut into number of fragments and then separated by electrophoresis.

Proteins that help in arranging new DNA strands using pre-existing or template strands were known as DNA polymerase and the process was known as polymerization. To the growing strand new nucleotide was added by DNA polymerase with the help of template sequence strand.

The growing DNA strand, with appropriate base pair formed at each position was assembled in 5' to 3' direction. Whereas the other nucleotides were added to the free end of the 3' hydroxyl group.

DNA polymerases became an important enzyme in polymerase Chain Reaction because of its high specificity, fidelity and thermo stability. The performance of PCR process in genotyping was influenced by the choice of DNA polymerase. Certain enzymes were affected by the varying concentration of humic acid which was considered as an inhibitor of PCR reaction. Among them Taq polymerase was an enzyme that was affected differentially with varying concentration of humic acid. Polymerase Chain Reaction was inhibited by the binding of inhibitor to the active site of DNA polymerase. There were even certain routes where PCR process can be inhibited like DNA template binding and interaction with polymerase during primer extension.

The activity of DNA polymerase was inhibited by certain substances called DNA polymerase inhibitory substances. Substances that can act as an inhibitory substances were Fatty acids, phosphonoacetic acid, bilirubin, bile salts etc.

Inhibition of DNA polymerases take place at location of magnesium where some DNA polymerase requires as a cofactor. As a result, the availability of Mg^{2+} or interference of inhibitor with Mg^{2+} to the DNA polymerase can inhibit PCR process. Original samples such as saliva, blood, soil, fabrics etc. may contain inhibitors. Inhibitors like L-Homoserylaminoethanol (Hse-Gly-ol) and 6-Anilinouracils act as a selective inhibitor of DNA polymerase and DNA Polymerase III respectively.

Nucleic acid amplification was inhibited by various factors that include components of body fluids, reagents encountered in forensic and clinical sciences, food particles, and environmental compounds. The other inhibitors that contribute in inhibition were constituents of bacterial cells, non-target DNA, contaminants, and laboratory compounds such as laboratory plastic ware, cellulose, glove powder, and pollen. Mineral oil was identified to have an inhibitory effect on PCR process.

Most commonly known inhibitors in environmental samples like soil or marine sediments were found to be humic compounds that mainly include humic acid and fulvic acid. Humic compounds contaminate exposed material to the environment. The characteristic of humic acid was blackish brown in colour, an alkali-soluble but insoluble in acid and could be seen as a reddish brown fragment in soil. Inhibition by humic compounds could take place, because of the bonding of phenolic groups present in humic compounds to amides to form quinine, which denatures biological molecules, again covalently binds to DNA or proteins. Humic acids found in the soil enter into the amplification process where Taq polymerase is inactivated and cannot be removed completely.

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