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Recent Advancements in Genetics: Pioneering New Horizons

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ABSTRACT
Human genetics is the study of hereditary and inheritance. Many diseases are genetic in origin and many are influenced by the genetic make-up of an individual. The modern arena is flourished with many advanced modalities which are not only diagnostic but also curative in nature. The recent advances in genetics and applied technology are miraculous and has a wide spectrum of application. Few of the important genetic advances like PCR, DNA finger-printing, In - situ hybridization, pharmacogenetics are reviewed in the present article.
Keywords: *Human genetics, advanced modalities, PCR, DNA fingerprinting, pharmacogenetics, In – situ hybridization*

Introduction and scope of genetics

Human beings first appeared in this planet some 50,000 years ago. Starting from the very early ancestors, the characters of one generation are transmitted to the next generations. The course of evolution with transmission and omission of characteristics from one generation to next, leads to the rise of *"Homo sapien sapiens"*, the present days human-beings, regarded as "The most intelligent animal in the earth". Man's interest and curiosity to know the proximity of offspring to their parents in relation to appearance and behavior leads to the evolution of "Genetics."

"Human Genetics" can be defined as, "The branch of medical science which deals with the study of hereditary material inherited from one generation to another."[1] It is a well established and proven fact that, "DNA is the basic hereditary material and is located in the genes." So Genetics, can also be defined as, "The study of genes and of the principles that govern the passage of genes from one generation to the next."[2] It also deals with the development of genetic defects, mutate-ons and its transmission to the next generation.

Many diseases and disorders are genetic in origin and are influenced by the genetic makeup of an individual. The genetic constituent of an individual can predispose a person to a specific disorder or condition. Mutations in the gene can leads to certain genetic disorders. It was only after Watson and Crick proposed the "Double helical model of DNA" in the year in 1953, genetics is explored to a greater depth and which gives rise to many advanced genetic modalities like PCR,

DNA fingerprinting, In- situ hybridization, pharmacogenetics, DNA probes etc. The application and advantages of this technique are reviewed in brief in this article.

POLYMERASE CHAIN REACTION OR PCR

It is a molecular biology technique to amplify a single or a few copies of a piece of DNA upto several orders of magnitude (10^{11}- 10^{12}) copies of a particular DNA sequence. It was developed by Kary mullis and Michael Smith in chemistry in 1993.[3] The amplification of DNA sequence by utilization of the host cellular mechanism, (*in vivo*) is called as "cell based cloning" or "recombinanat DNA technology". When genes are cloned in *in vitro* by non-cellular techniques in machines is called as polymerase chain reaction.[4]

Concept of PCR technique

DNA replication needs at least a single molecule of a double stranded DNA to begin with. The two strands are separated (denatured) with regulation of temperature. Enzymes, nucleotides and primers are added to make-up a mixture in the PCR machine (thermo-cyclers). Once added, the nucleotide gets arranged on each of the denatured DNA single strand. Thus the new complementary strand along with the old strand together forms the double helix. The nucleotides are attached one by one to the primer to the 3' end. The primers are actually very short DNA sequences called deoxyoligonucleotides. The DNA fragments, primers, nucleotides and DNA polymerase enzyme (the heat stable "Taq polymerase" derived from *Thermus aquaticus*) are all incubated in the machine and required temperature for amplification is maintained externally.[2, 4]

Each cycle of replication is repeated with fresh denaturation of the double helix and annealing of added nucleotides to the annealed primers. This results in the replication of a DNA segment in an exponential proportion. PCR thermo cyclers are automatic and not to be set again after each round of amplification.

Applications of PCR

- Amplification of small amounts of DNA for further analysis by DNA fingerprinting.
- The analysis of ancient DNA from fossils.
- Mapping the human (and other species) genome.
- The isolation of a particular gene of interest from a tissue sample.
- Generation of probes: large amount of probes can be synthesized by this technique.
- Production of DNA for sequencing: Target DNA in clone is amplified using appropriate primers and then its sequence determined. Helpful in conditions where amount of DNA is small.
- Analysis of mutations: Deletions and insertions in a gene can be detected by differences in size of amplified product.
- Diagnosis of monogenic diseases (single gene disorders): For pre-natal diagnosis, PCR is used to amplify DNA from fetal cells obtained from amniotic fluid. PCR has also proved very important in carrier testing.
- Detection of microorganisms: Especially of organisms and viruses that are difficult to culture or take long time to culture or dangerous to culture.
- The PCR has even made it possible to analyze DNA from microscope slides of tissue preserved years before.
- Detection of microbial genes responsible for some aspect of pathogenesis or antibiotic resistance.
- Crucial forensic evidence may often be present in very small quantities, e.g. one human hair, body fluid stain (blood, saliva, semen). PCR can generate sufficient DNA from a single cell.

Limitations of PCR: PCR is an extremely sensitive technique but is prone to contamination from extraneous DNA, leading to false positive results. Another potential problem is due to cross-contamination between samples. It is for this

reason that sample preparation, running PCR and post-amplification detection must be carried out in separate rooms. Concentration of Mg is very crucial as low Mg2+ leads to low yields (or no yield) and high Mg2+ leads to accumulation of nonspecific products.[5] Non-specific binding of primers and primer-primer dimmer formation are other possible reasons for unexpected results. Reagents and equipments are costly, hence can't be afforded by small laboratories.[6]

IN SITU HYBRIDIZATION

In situ hybridization (ISH) is a type of hybridization that uses a labeled complementary DNA or RNA strand (i.e. probe) to localize a specific DNA or RNA sequence in a portion or section of tissue (in situ), in the entire tissues, in cells and in circulating tumor cells.[7] It is a powerful technique for identifying specific mRNA species within individual cells in tissue sections, providing insight into physiological processes and disease pathogenesis.

This technique allows for precise location of a specific segment of nucleic acid within a histologic section. The underlying basis of ISH is that nucleic acids, if preserved adequately within a histologic specimen, can be detected through the application of a complementary strand of a nucleic acid.

In situ hybridization probes: Double stranded DNA (dsDNA) probes, Single stranded DNA (ssDNA) probes, RNA probes (Riboprobes), Synthetic oligonucleotides

Labeling techniques : Radioactive isotopes - ^{32}P, ^{36}S, ^{3}H and Non- radioactive labels – biotin, digoxigenin, Fluoroscent dyes (FISH).[8]

Applications

- Microbiology:- to identify morphology, population and structure of microorganisms.
- Pathology:- for pathogen profiling, to identify abnormal gene expression.
- Developmental biology:- gene expression profiling in embryonic tissues.
- Karyotyping:- unique fluorescent pattern can be seen on individual chromosomes, helps in the identification of chromosomal abnormalities.[9,10]

DNA FINGERPRINTING

In all humans the genome comprises of the coding as well as non-coding regions. In the non coding DNA regions the sequences are very repetitive and are called tandemly repeated DNA sequences. The collection of these repetitive sequences imparts unique identities to individuals. The pattern of occurrence, length and number of these repeats are unique and specific for each individual. The concept of DNA fingerprinting is based on the above principle.[11]

The technique begins with obtaining DNA from a source that may be a body fluid, cells or sequestrated dead tissue. Obtained DNA is cleaved into smaller fragments with the help of endonuclease enzyme. The action of the endonuclease enzyme differs in different individuals as the enzyme cuts individual genome at different places due to the presence of different tandem repeat sequences in different individuals. These ununiform cut in the genome give rise to DNA fragment of different lengths in individuals. The fragments of DNA are subsequently separated by agarose gel electrophoresis. Southern blotting is then applied to transfer the band on to nitrocellulose.

Applications:- DNA fingerprint is an important tool for identification of individuals, criminal investigations, settlement of disputed paternity etc.[12]

PHARMACOGENETICS

Pharmacogenetics is the term used to denote the science about how hereditary affects the response to drugs. It is basically the science which deals with the interaction of exogenously administered drugs with the cells, their interactions, adsorption and metabolism which are under genetic influence.[13]

It has been recognized for more than 50 years that genetic differences between people contribute to inter-individual differences in the response to many commonly used drugs. The intensive study of pharmacogenomics helps to understand the role of genetic factors for variation in drug response. The metabolism of drugs are also influenced by hereditary. The hypersensitivity or adverse drug reaction is also influenced by number of genes.[13,14]

NUCLEIC ACID PROBES

Nucleic acid probes are small stretches of DNA that can be derived from various sources. Radiolabeled probes helps to recognize complementary sequences in DNA or RNA molecule. This helps to identify and isolate the specific DNA sequences from an organism.[15]

Detection of DNA segments with Nucleic acid Hybridization

The following steps are followed sequentially to identify DNA segments of interest from a given genomic population.

- After the DNA molecules are extracted, they are digested with application of restriction enzyme so that they are cleaved into multiple segment of different sizes.
- The DNA sample is run in electrophoresis where the fragments are arranged according to their sizes along the gel.
- Bands appear on the gel at specific intervals depending on the molecular weights of the fragments.
- These bands are stained and visualized directly in the gel.[16]

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- Bands appear on the gel at specific intervals depending on the molecular weights of the fragments.
- These bands are stained and visualized directly in the gel.
- These bands can be isolated for analyzing their DNA sequence. A particular gene (DNA segment) can be identified within those bands with the help of radio tagged molecular probes that bind to definite denatured strand.

A particular segment in a band in the gel can b e identified by hybridization with molecular probes. This process requires transferring of the band from the gel to a nitrocellulose paper. This transferring technique is called as “Blotting”.

- Southern blotting:- blotting of the DNA bands on nitrocellulose paper.
- Northern blotting:- blotting of the mRNA bands on nitrocellulose paper.
- Western blotting:- blotting of protein on nitrocellulose paper[17]

Uses:- These probes in the diagnosis of infectious diseases and identification of specific casual organism.

Conclusion

Genetics is one of the youngest branch of medical science and it is blossoming day by day to encompass a great scope for futher researches contributing to the human health. The recent and advanced modalities are having a wide spectrum of applications. These techniques are more specific and sensitive when compared to the conventional techniques of screening and

detection. The limitations of these techniques are high cost, technique sensitivity and requirement of highly advanced set up. Researchers are continuously working to overcome these limitations. Many researches are being done with the use of cost effective set up and promising results are seen.

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