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# PCR in Linkage Analysis of Genetic Diseases

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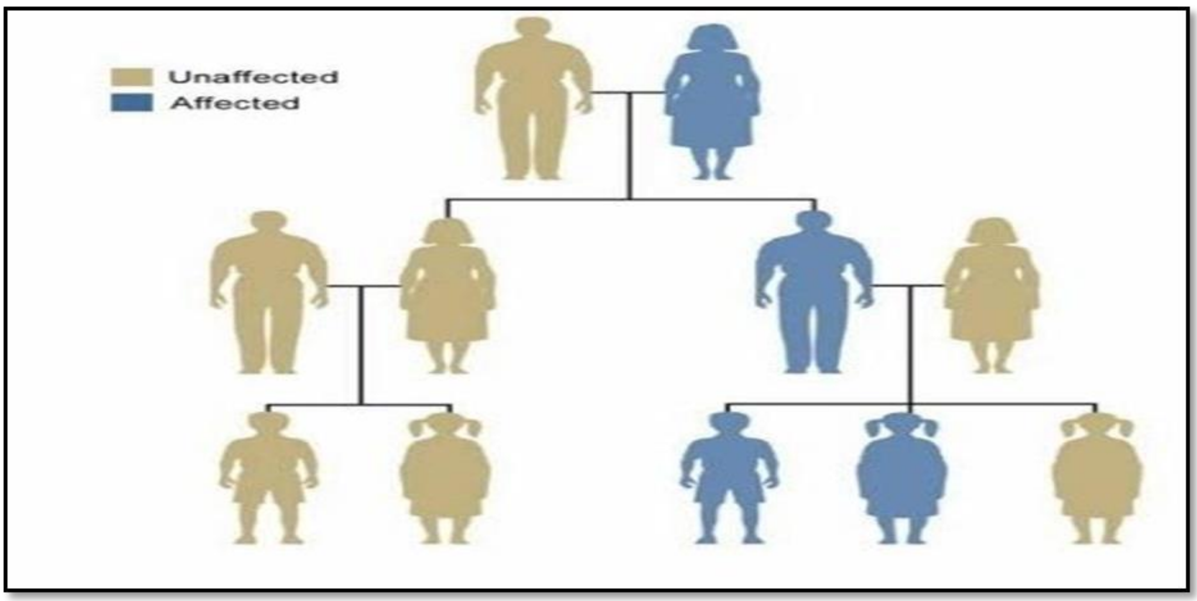
**ABSTRACT**

PCR also called as polymerase chain reaction plays an important role in amplifying a specific DNA segment from a complex template in an automated reaction and has made a molecular analysis more accessible both to basic research and diagnostic laboratories. Modifications of the basic PCR method using generic primers now allow DNA amplification even in the absence of specific nucleotide sequence information. It has proved extremely valuable for detecting the presence or absence of a given sequence. It has also been a useful parameter in the detection of infectious disease. It also plays an important role in the new approaches of Genetic mapping.

## INTRODUCTION

Genetic linkage analysis is a powerful tool to detect the chromosomal location of disease genes. It is based on the observation that genes that resides physically close on a chromosome remain linked during meiosis. For some of the neurological disorders for which the underlying biochemical defect was not known. The identification of the chromosomal location of the disease gene was the first step in its eventual isolation. Genes that are isolated in this way include examples such as alzheimers, Parkinson's, to the diseases of the ion channels leading to periodic paralysis to tumor syndrome such as neurofibromatosis.



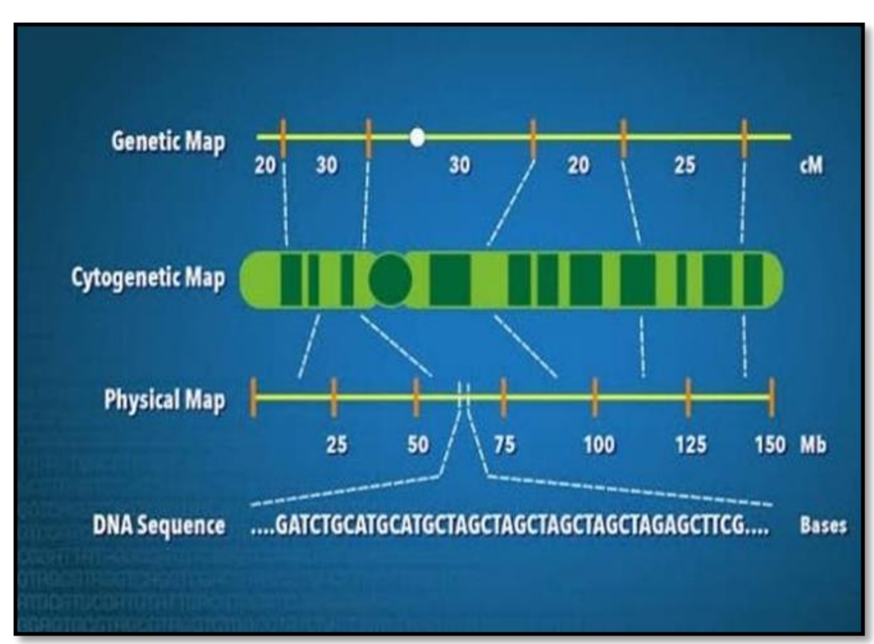

## LINKAGE ANALYSIS

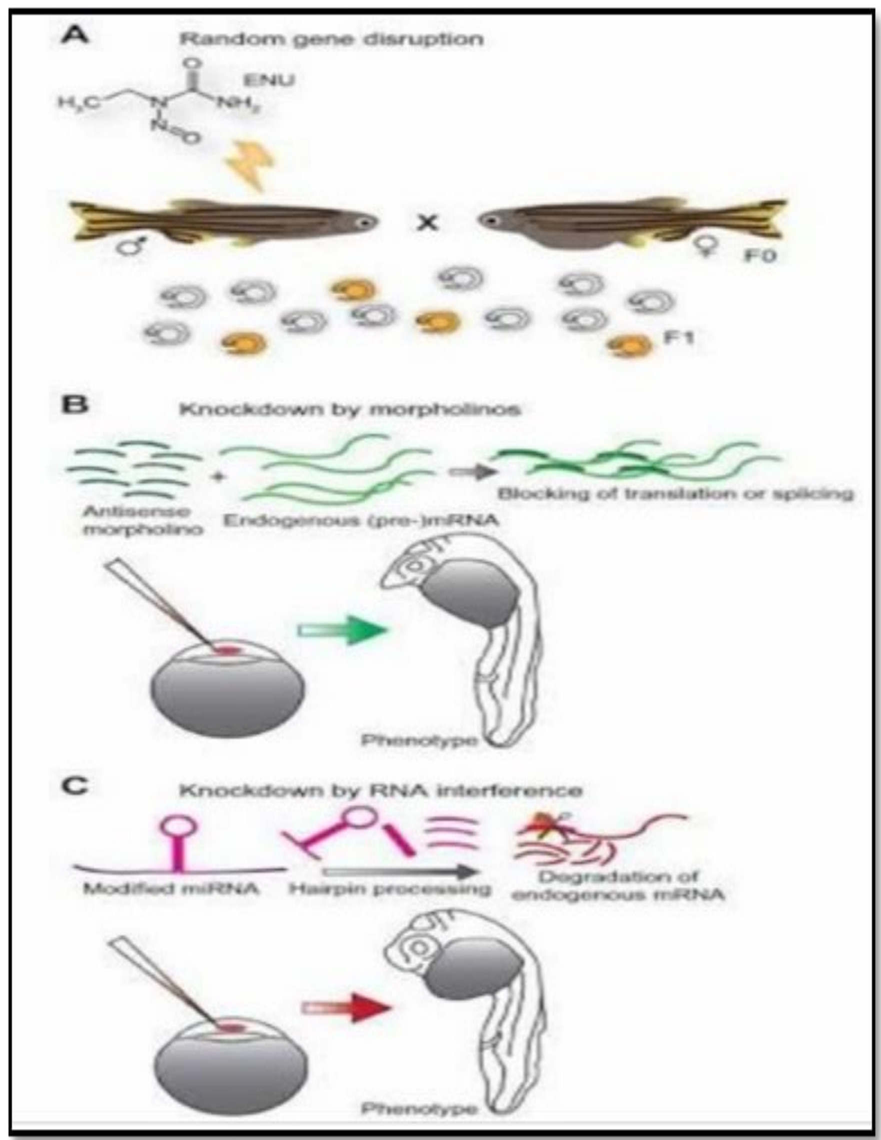
Linkage is the tendency for genes and genetic markers to be inherited together because of their location near one another on the same chromosome. It is based on the statistical method that infers linkage between two loci from segregation analysis in pedigrees. In most cases one loci is given to unknown disease gene locus and other to a locus defined by a cloned DNA probe, which has been previously mapped in the genome and helps in identifying a DNA polymorphism. This is also called as Marker locus. When Mendel observed an independent assortment of traits, he was fortunate to have chosen traits that were not localised close to one another on the same chromosome. This was proved by Mendel in his second law. The law was called as the law of independent assortments. Even in the case of drosophila genetics the Morgan showed that the degree of linkage increased with physical proximity of the genes and that linkage very correctly identified the 4 chromosomes in drosophila. The first trait in

human linked to a chromosome was actually sex gene itself. This was followed by the Dufy locus to chromosome after the observations it was very surprising that the Dufy locus is also a protein polymorphism linked to a neurological disease locus on the chromosome. With the ability to detect DNA polymorphism the study of genetic linkage blossomed because polymorphisms were not limited to the relatively rare protein polymorphisms. Linkage analysis plays an important role in finding genes that contribute to individual differences in the phenotypes. The phenotypes may be diseases and disorders or part of normal variation which is also called a gene hunting.

**GENETIC MAPS**

The discovery of str's made it feasible to generate a larger number of markers and then map them by genetic linkage analysis. In contrast to the physical maps that measures distances in base pairs of karyotypic maps that assigns the markers to chromosomal bands the genetic gives the relation between the distances in recombinational units. Male and female recombination rates are not identical and there are significant changes in the rate of recombination along specific chromosomal segments. Chromosomal regions with increased recombination are referred to as recombination hot spots. Genetic maps of the human genome have provided the backbone for the construction of physical maps of human chromosomes. They are now used to map disease trait in model organism such as egans, zebra fish and latest versions of human genetic and physical amps can be found electronically. Hence genetic aps plays an important role in linkage analysis of genetic diseases using PCR technique.






## GENETIC MARKERS

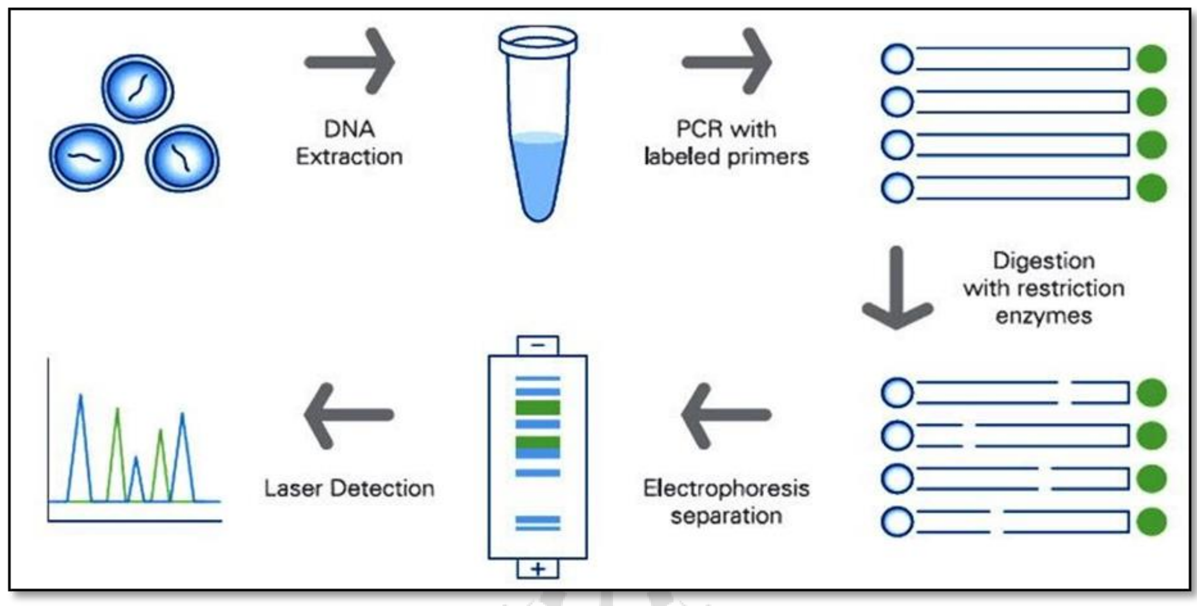
It is a gene or a DNA sequence with a known location on a chromosome and associated with a particular gene or trait. It can also be described as a variation which may arise due to mutation or alteration in the genomic loci that can be observed.

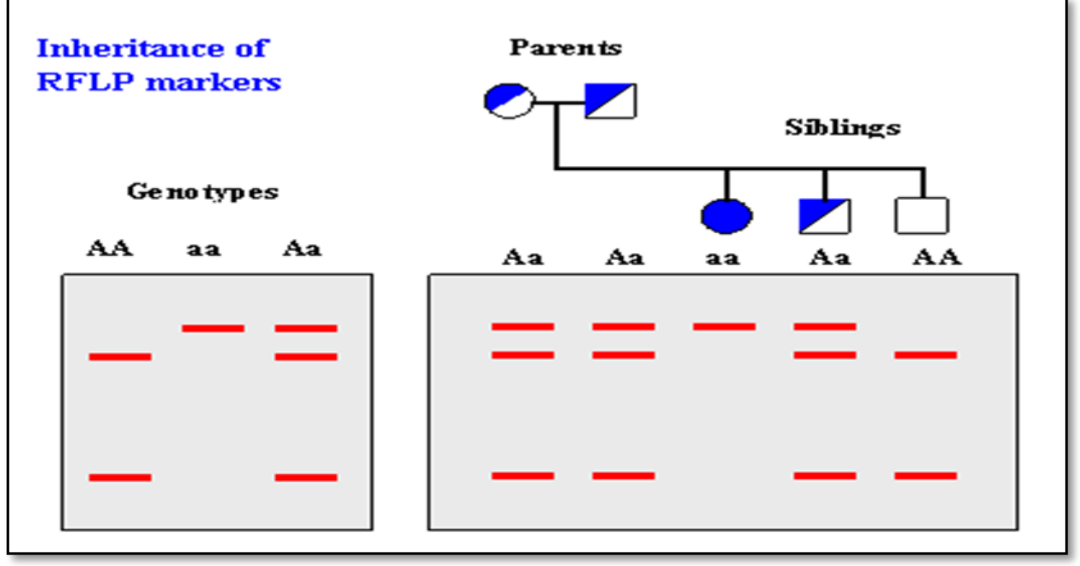
Genetic markers are broadly classified into mainly 4 major Types-

## RESTRICTION FRAGMENT LENGTH POLYMORPHISM.

With the help of restriction digestion by REase, alterations in the same/ homologous DNA sequences can be detected by analysing fragments of different length, digested with a restriction enzyme. A single restriction endonuclease gives more specific results by cutting at one specific locus and producing fragments of different length. See the figure how different

DNA fragments are created by restriction digestion. The length of different fragments is identified using blotting, which is now replaced by sequencing. RFLP is applicable in disease identification, genetic mapping, heterozygous detection and carrier identification. The RFLP markers are highly locus-specific and co-dominant. Why RFLP markers are locus specific? Because of the nature of the REase used in the RFLP.

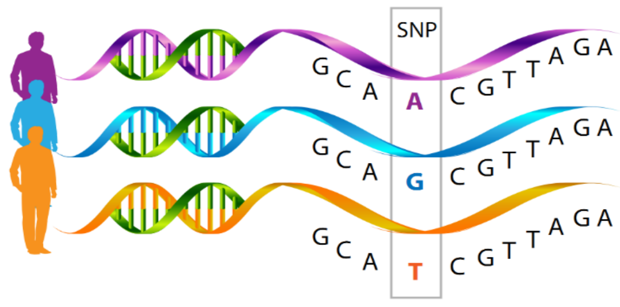


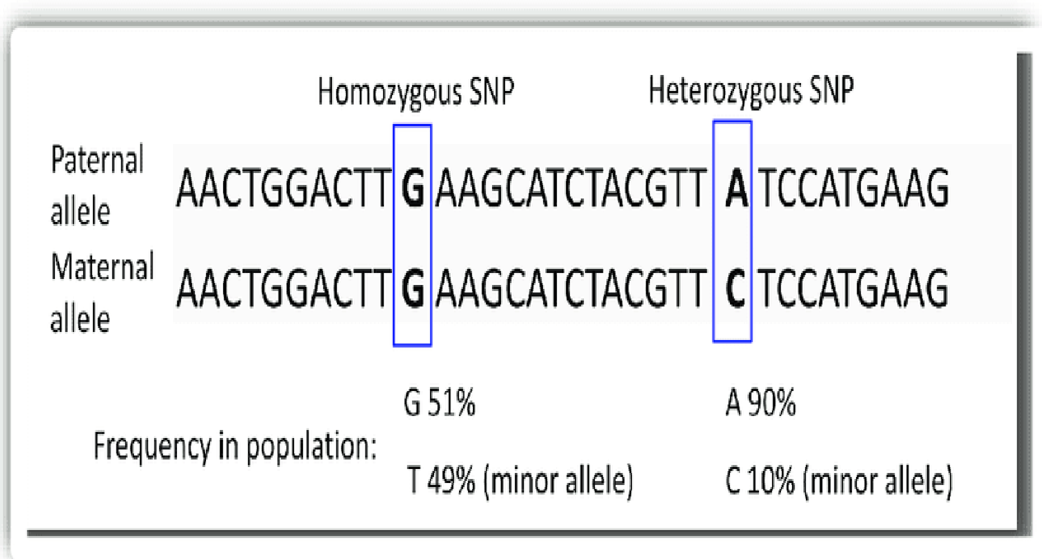




## SINGLE NUCLEOTIDE POLYMORPHISM

SNP is a single nucleotide polymorphism, type of unique marker in comparison with other markers enlisted here. Due to the addition or deletion of a single nucleotide in a genome, the SNP originated. Millions of SNPs are present in the genome, however, it may or may not be pathogenic. The SNP marker is used in the Genome-wide association studies. By comparing

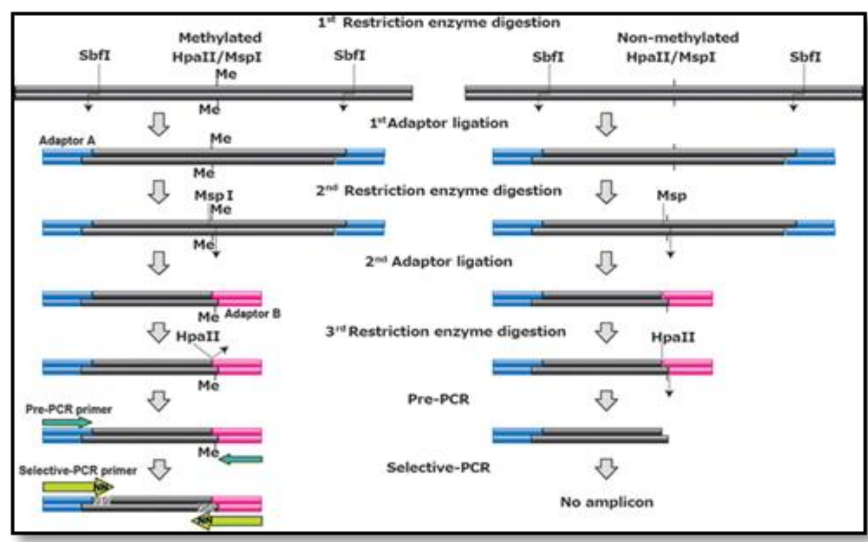
the case and control groups, the association of particular SNP associated with a particular disease can be determined.



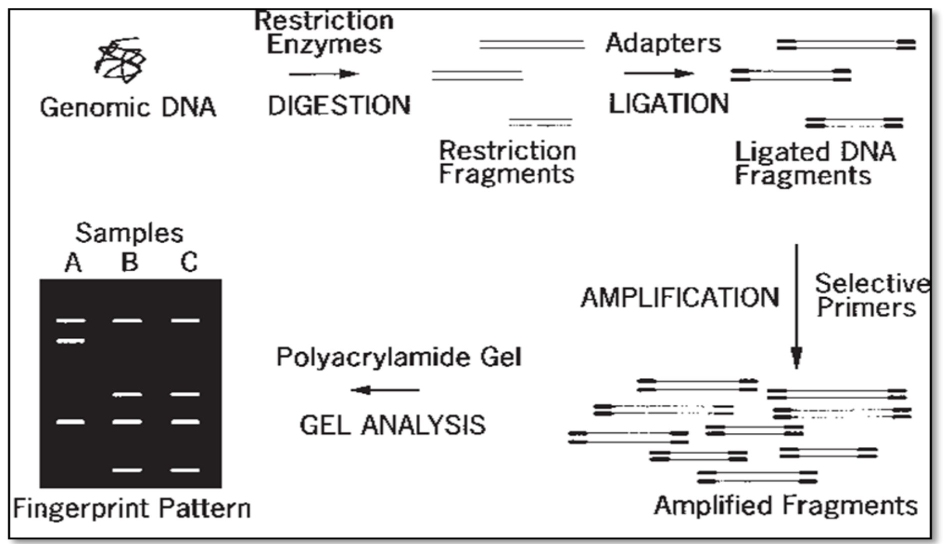




**AMPLIFIED FRAGMENT LENGTH POLYMORPHISM**

The AFLP technique was developed by Zabeau and Vos in the year 1993. The method is based on the amplification of the selected fragments followed by the digestion of the total genomic DNA of the selected organism. The process of the AFLP is restriction digestion of the genomic DNA, adapter ligation of the digested DNA fragments and amplification of DNA fragments in PCR using the adapter specific primers. The method was originally developed by the keygen in 1990. The genomic DNA is extracted using one of the DNA extraction methods. The DNA is digested with the help of the restriction endonuclease. The digested DNA is ligated with the short adapter sequences. The sequence of the adapters is known. The fragments are amplified using a set of primer. The results are analysed on agarose gel electrophoresis followed by autoradiography or we can use PAGE technique to differentiate multiple fragments of DNA.


1st Restriction enzyme digestion
Methylated HpaII/MspI
Non-methylated HpaII/MspI
SbfI
Me
1st Adaptor ligation
Adaptor A
2nd Restriction enzyme digestion
Msp I
Msp
2nd Adaptor ligation
Adaptor B
3rd Restriction enzyme digestion
HpaII
Pre-PCR
Pre-PCR primer
Selective-PCR
Selective-PCR primer
No amplicon

Size standard
Allele
100 bp
"Rest" DNA
1000 bp
Well


Genomic DNA
Restriction Enzymes
DIGESTION
Restriction Fragments
Adapters
LIGATION
Ligated DNA Fragments
Samples
A B C
Fingerprint Pattern
Polyacrylamide Gel
GEL ANALYSIS
AMPLIFICATION
Selective Primers
Amplified Fragments

**RANDOM AMPLIFICATION OF POLYMORPHIC DNA**

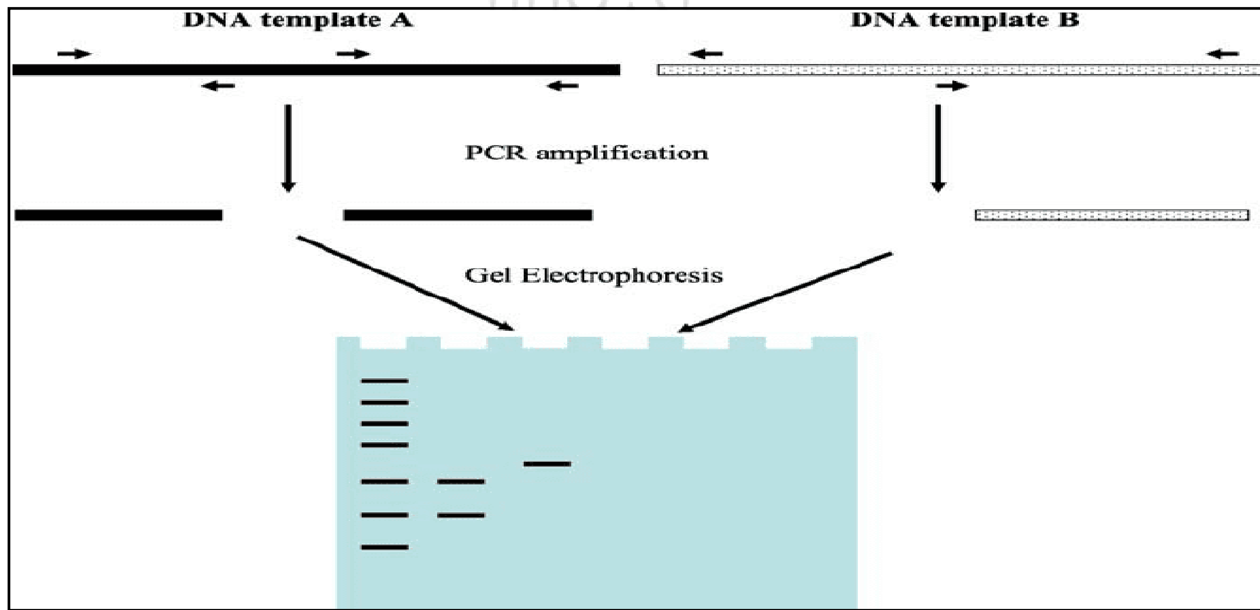
By using the short, arbitrary DNA primers, the part of the genomic DNAs is randomly amplified. Pronounced as a "rapid", it does not require any previous sequence information, the primer of 8 to 12mer randomly amplifies the region of the genomic DNA. If any mutation or alteration occurred in the region of the primer binding site, the primer can not bind to that location or the amplified fragment length decreases or increases. The RAPD fragments are between 0.2 to 5.0kb. It can be observed using the ethidium bromide-stained agarose gel or it can also be analyzed on polyacrylamide gel electrophoresis.

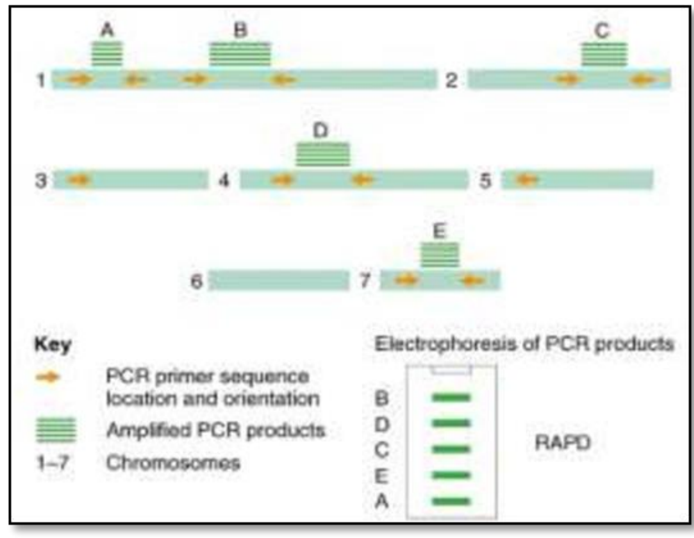
**ADVANTAGES**

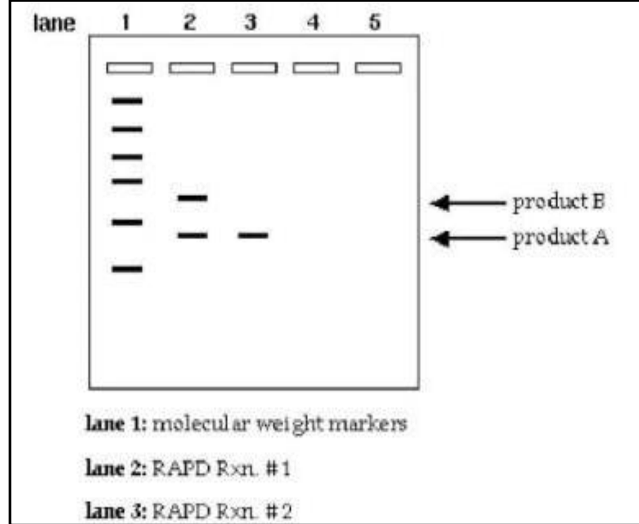
The RAPD markers are abundantly present into the genome and are distributed throughout the genome. No previous sequence information is needed. It can amplify low quantity of DNA.

**DISADVANTAGES**

The marker is not locus-specific. The sensitivity of the RAPD is also lower.






## REVERSE GENETIC APPROACH –

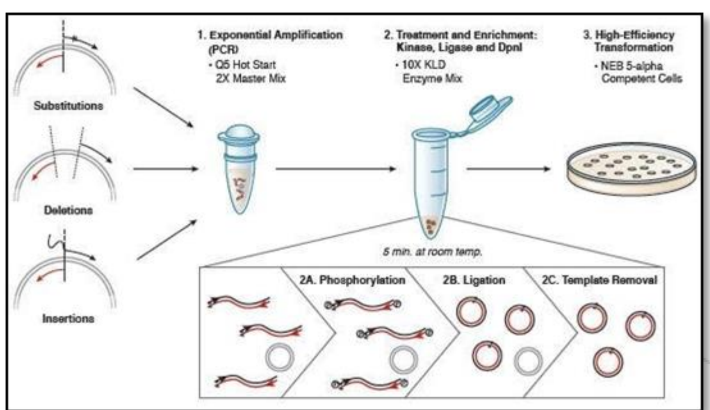
It also plays an important in linkage analysis of genetic Diseases using PCR. It is a type of molecular genetics which helps in Understanding their functions of gene by analyzing the phenotypic effects caused by genetically engineering Specific nucleic acid sequences within the gene. They generate large volumes of genomic sequences data relatively rapidly. They connect to a specific genetic Sequence with specific effects on the organisms. The reverse genetic approach is carried out by various Techniques which helps to learn a sequence on phenotype or to discover its biological function.

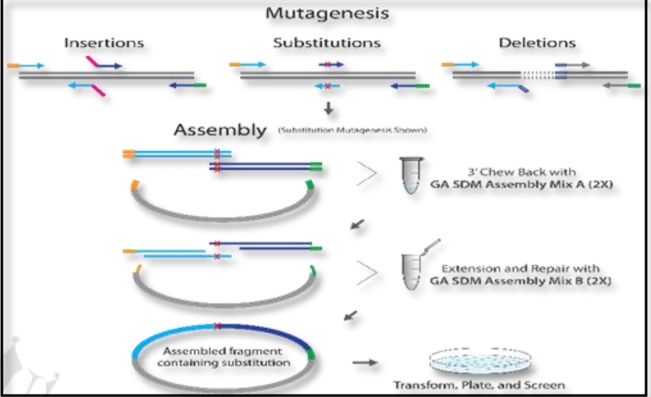
The various methods are as follows-

## DIRECTED DELETIONS AND POINT MUTATIONS

Site-directed mutagenesis is a sophisticated technique that can either change regulatory regions in the promoter of a gene or make subtle codon changes in the open reading frame to identify important amino residues for protein function. Alternatively, the technique can be used to create null alleles so that the gene is not functional. For example, deletion of a gene by gene targeting (gene knockout) can be done in some organisms, such as yeast, mice and moss. Unique among plants, in *Physcomitrella patens*, gene knockout via homologous recombination to create knockout moss is nearly as efficient as in yeast. In the case of the yeast model system directed deletions have created in every non-essential gene in the yeast genome. In the case of the plant model system huge mutant libraries have been created based on gene disruption constructs. In gene knock-in, the endogenous exon is replaced by an altered sequence of interest.

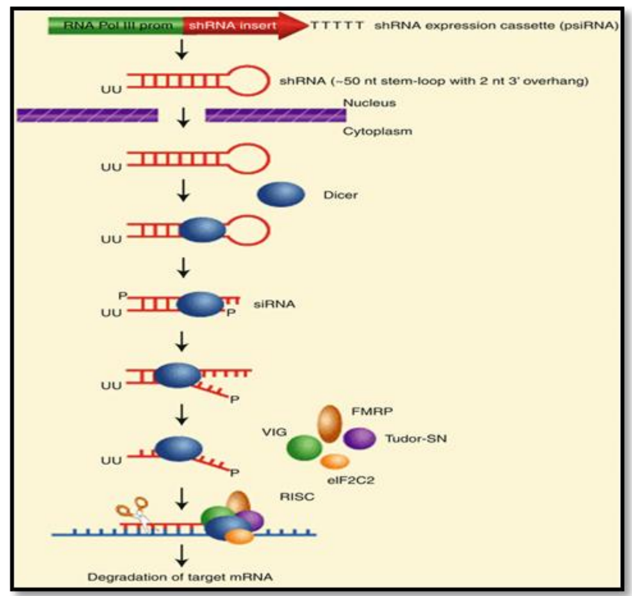
In some cases conditional alleles can be used so that the gene has normal function until the conditional allele is activated. This might entail 'knocking in' recombinase sites (such as lox or frt sites) that will cause a deletion at the gene of interest when a specific recombinase (such as CRE, FLP) is induced. Cre or Flp recombinases can be induced with chemical treatments, heart shock treatments or be restricted to a specific subset of tissues. Another technique that can be used is TILLING. This is a method that combines a standard and efficient technique of mutagenesis with a chemical mutagen such as Ethyl methane sulfonate (EMS) with a sensitive DNA screening technique that identifies single base mutations (also called point mutations) in a target gene.

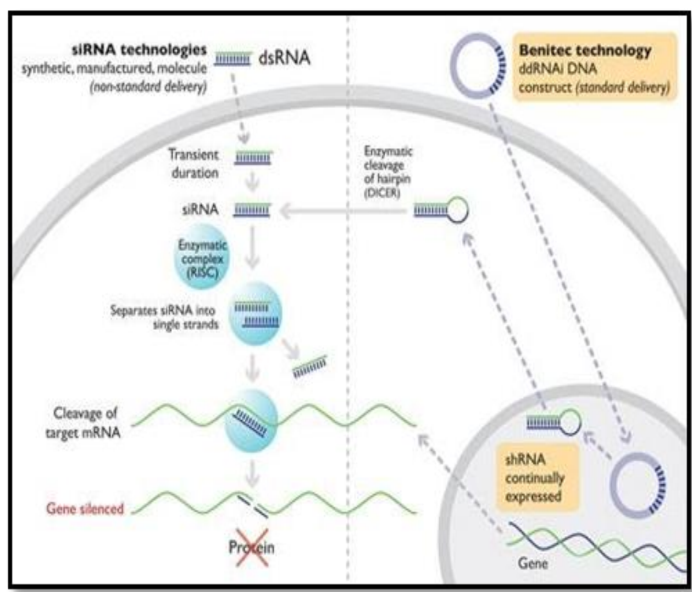



## GENE SILENCING

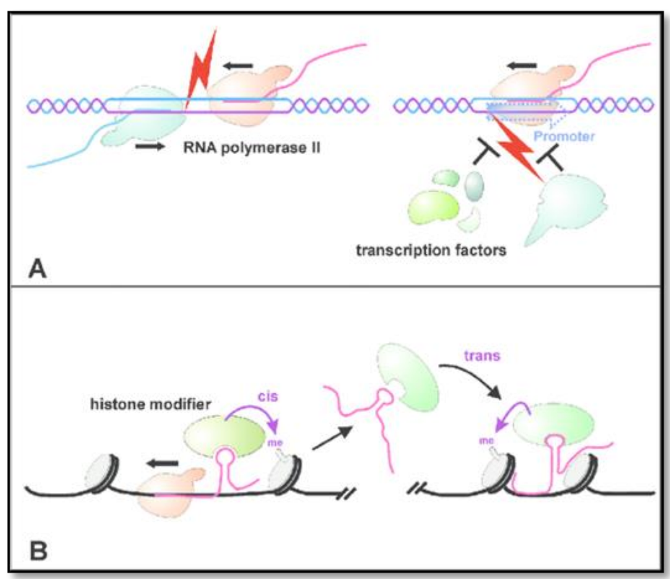
The discovery of gene silencing using double-stranded RNA, also known as RNA interference (RNAi), and the development of gene knockdown using Morpholino oligos, has made disrupting gene expression an accessible technique for many more investigators. This method is often referred to as a gene knockdown since the effects of these reagents are generally temporary, in contrast to gene knockouts which are permanent. RNAi creates a specific knockout effect without actually mutating the DNA of interest. In *C. elegans,* RNAi has been used to systematically interfere with the expression of most genes in the genome. RNAi acts by directing cellular systems to degrade target messenger RNA (mRNA). While RNA interference relies on cellular components for efficacy (e.g. the Dicer proteins, the RISC complex) a simple alternative for gene knockdown is Morpholino antisense oligos. Morpholinos bind and block access to the target mRNA without requiring the activity of cellular proteins and without necessarily accelerating mRNA degradation. Morpholinos are effective in systems ranging in complexity from cell-free translation in a test tube to *in vivo* studies in large animal models.

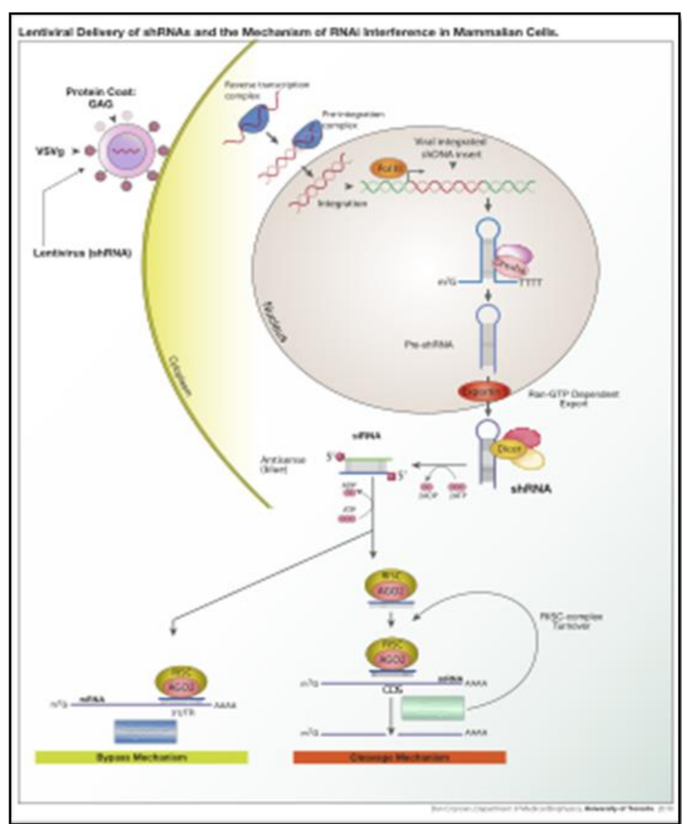



## INTERFERENCE USING TRANSGENES

A molecular genetic approach is the creation of transgenic organisms that overexpress a normal gene of interest. The resulting phenotype may reflect the normal function of the gene. Alternatively, it is possible to overexpress mutant forms of a gene that interfere with the normal (wildtype) genes function. For example, over expression of a mutant gene may result in high levels of a non-functional protein resulting in a dominant negative interaction with the wildtype protein. In this case the mutant version will out compete for the wildtype proteins partners resulting in a mutant phenotype. Other mutant forms can result in a protein that is abnormally regulated and constitutively active ('on' all the time). This might be due to removing a regulatory domain or mutating a specific amino residue that is reversibly modified (by phosphorylation methylation or ubiquitination). Either change is critical for modulating protein function and often result in informative phenotypes.




## LINKAGE DISEQUILIBRIUM

It is the occurrence of specific alleles at 2 loci with a Frequency greater than expected by chance they play an Important role or they serve as a powerful tool for Genetic mapping. They are also called as gametic disequilibrium or allelic association. It also results from natural selection or by chance. When a disease mutation arises on a founder chromosome and not much time has elapsed since the Mutation event the disease mutation will be in linkage

Equilibrium with alleles from loci to the gene. They are influenced by various factors such as selection, the rate of genetic recombination, mutation rate, Genetic rate, the system of mating population structure and genetic linkage. They are also in consideration while examining other Gene complexes encoding immunologically relevant Molecules.

## CHROMOSOMAL DISORDERS

Typically, somatic cells proliferate via division called mitosis while germ cells are produced through meiosis division. Meiosis involves a reductional division followed by an equational division, Meiosis I and II, respectively. Oogenesis begins in the female foetus at 12 weeks, but it is stopped in a stage of meiosis I (when the homologous chromosomes have replicated and paired as bivalents or tetrads) at about 20 weeks. At puberty usually, only one oocyte is released per month; a primary oocyte completes meiosis I and produces one secondary oocyte and one polar body. Chromosomal aberrations including numerical (due to errors at chromosome pairing and crossing-over) and structural damages lead to chromosomal disorders (Tables 2, 3 and 4; Fig. 2A and B). Aneuploidy is usually due to failure of segregation of chromosomes in meiosis I or meiosis II (non-disjunction, premature disjunction or anaphase lag); examples of numerical aberrations include Down syndrome (trisomy 21), Edwards's syndrome (trisomy 18), Patau syndrome (trisomy 13), Klinefelter syndrome (XXY syndrome), Turner syndrome (monosomy X) and trisomy X. Chromosomal errors in oocytes are increased dramatically with maternal age. Non-disjunction or chromosome lag during mitosis can lead to mosaicism.

## CONCLUSION

Any changes in DNA sequence could be pathogenic if it has an abnormal effect on biological pathways characterisation of basic analysis of diseases is required for accurate diagnosis. PCR is a powerful and a sensitive technique that can amplify very small amounts of DNA. This technique has showed many applications in several of biology and it has also been used in the diagnosis of inherited diseases on the DNA level. The use of genetic maps, genetic markers, reverse genetic approaches as brought a very vast change in the PCR linkage analysis of genetic diseases. Molecular diagnosis of Genetic disorders is noticed as the detection of pathogenic mutations in DNA or RNA samples are ranging widely. It has also helped the clinical researchers to find the fault and diagnose the genetic diseases so that they could offer an appropriate genetic test for diagnosis of diseases. Hence PCR in linkage analysis plays a vital role in genetic analysis of genetic diseases.

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