Applications of Gene cloning

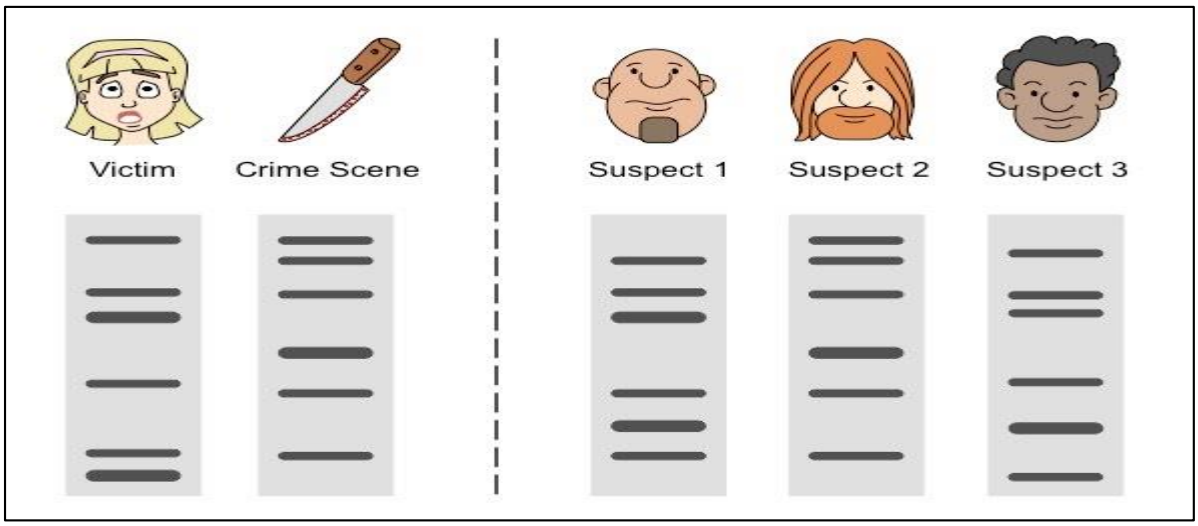
Gene cloning experiments can be useful in Forensics, Agriculture, Breeds, Foods, Medicals and Pharmaceuticals sciences.

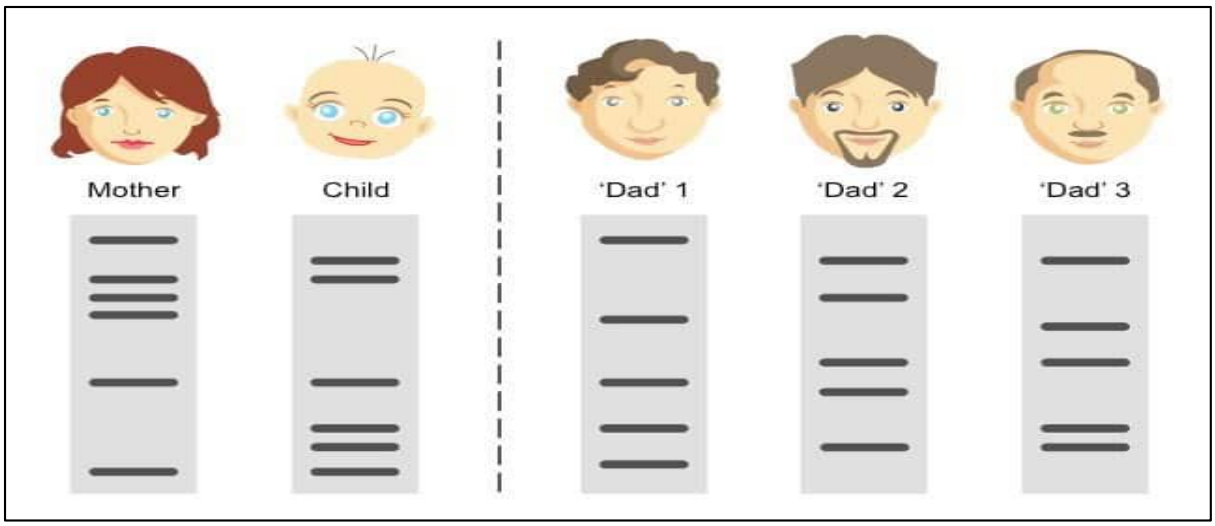
Forensics:

Any type of organism can be identified by examination of DNA sequences unique to that species. When DNA sequencing technologies progress farther, direct comparison of very large DNA segments, and possibly even whole genomes, will become feasible and practical and will allow precise individual identification. To identify individuals, forensic scientists scan 13 DNA regions (genetic loci with sequence repeats) that vary from person to person and use the data to create a DNA profile of that individual (called a DNA fingerprint/profiling).

Some examples of DNA uses for forensic identification:

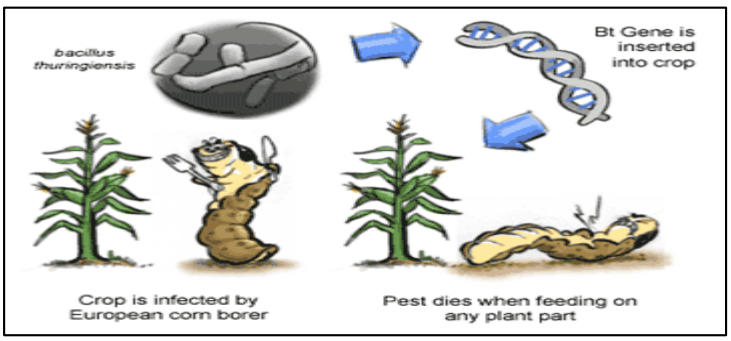
1) Identify potential suspects whose DNA may match evidence left at crime scenes
2) Exonerate persons wrongly accused of crimes
3) Identify crime and catastrophe victims
4) Establish paternity and other family relationships
5) Identify endangered and protected species as an aid to wildlife officials (could be used for prosecuting poachers)
6) Detect bacteria and other organisms that may pollute air, water, soil, and food
7) Match organ donors with recipients in transplant programs
8) Determine pedigree for seed or livestock breeds
9) Authenticate consumables such as caviar and wine.





Biotechnology:

Researchers can use laboratory techniques to clone the resistance genes and introduce them into elite varieties of domestic crops to protect them against pathogens and pests such as rusts, powdery mildew and Hessian fly. By making crops more disease resistant, it will help to improve yields and reduce the use of pesticides. The technique called "AgRenSeq" lets researchers made a library of resistance genes discovered in wild relatives of modern crops so they can rapidly identify sequences associated with disease fighting capability and so perform speed cloning. (video, https://t.co/ytMGIEm1Vk?amp=1)



After years of detailed study and analysis, the Food and Drug Administration (FDA) has concluded that meat and milk from clones of cattle, and goats, and the offspring of clones from any species traditionally consumed as food, are as safe to eat as food from conventionally bred animals. This conclusion stems from an extensive study of animal cloning and related food safety, culminating in the release of three FDA documents in January 2008: a risk assessment, a risk management plan, and guidance for industry. Dolly the sheep may have been the world's most famous clone, but she was not the first. Cloning creates a genetically identical copy of an animal or plant. Many animals - including frogs,

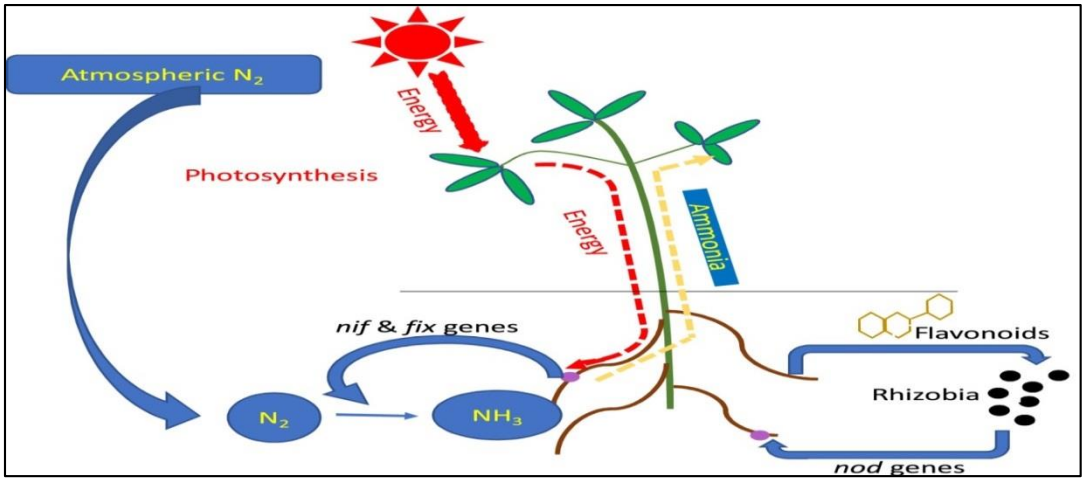
mice, sheep, and cows - had been cloned before Dolly. Now, there is some contradictory evidence about the safety of the animal cloning (will be discussed in the course B484, 2nd semester).

Agricultural Applications

The fundamental importance of dinitrogen fixation for world agriculture, in relation to projected energy supplies, population pressure and food requirements over the next decades, obliges scientists to reconsider ways of exploiting this biological process. Genetic manipulation offers several options in principle.

1) Cyano-bacteria like *Nostoc* are nitrogen fixing bacteria i.e. they fix the nitrogen in the air thus making them useful for the plants. Nitrogen fixing cyanobacteria along with the desired genes are used to increase the crop productivity and health. It thus decreases the use of fertilizers and hence agro chemical free crops are produced which are better in nutritional value.

2) Cloning in Bacteria facilitates nitrogen fixation in plants. Existing symbiotic systems such as the legumes and seemingly inefficient systems such as the grass associations could be improved; new symbioses could be developed by "nif gene" transfer to rhizosphere commensals or by somatic hybridization of appropriate plants to render plants independent of microbes by manipulation of expressable nif into the plant genome.



Medicals and Pharmaceuticals

Medical Applications:

Cloned bacteria play important role in the synthesis of vitamins, antibiotics, insulin, clotting factors, human growth hormone, cytokines (cell growth stimulants), and several anticancer drugs. Different genes for the treatment of diseases like cystic fibrosis and many other diseases like this can be synthesized and produced from cloned genes.

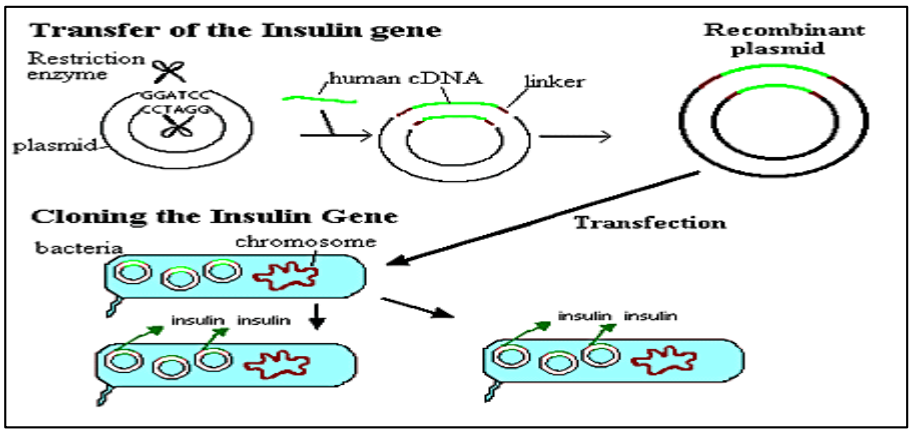
Genomic Library:

Molecular techniques are already routine in the diagnosis of genetic disease and the potential of the human genome project in allowing us to understand the functioning of the human body. From a gene library, it is possible to identify a clone containing the specific gene. This gene can be multiplied by growing the relevant clone in a culture. The base sequence in this gene can be found, as the methods for this purpose are known. From the base sequence, the sequence of amino acids in a polypeptide can be worked out on the basis of the triplet code.

Gene therapy:

The process in which defective gene is removed inserting new and healthy gene into its place is known as gene therapy.

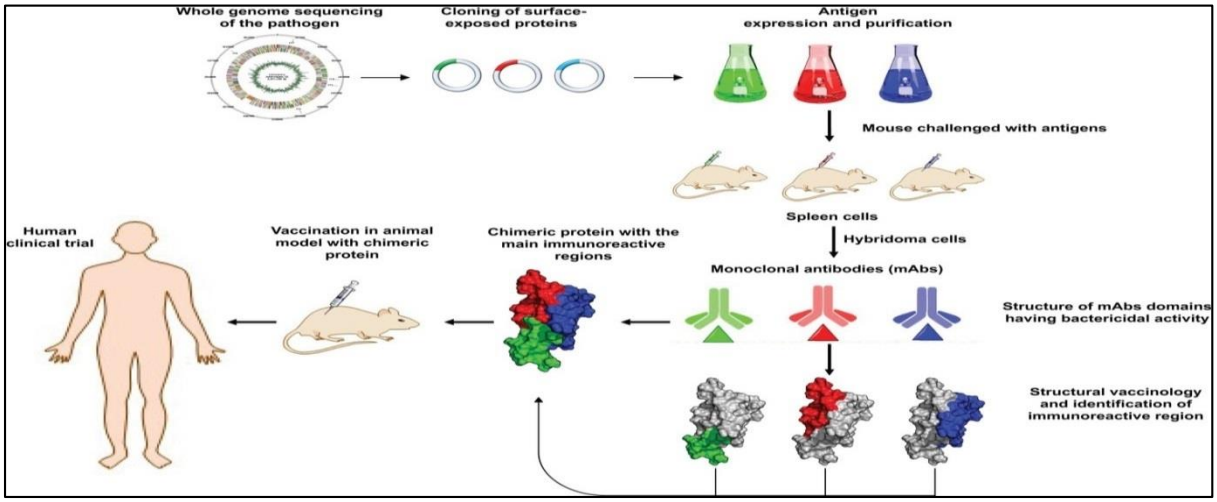
- Very deadly ailments like leukaemia can be treated using gene therapy in which defected gene is replaced. Sickle cell anaemia can also be treated using this process.
- As Plasmids are useful in cloning short segments of DNA, it can also be used to replicate proteins, such as the protein that codes for insulin, in large amounts. Additionally, plasmids are being investigated as a way to transfer genes into human cells as part of gene therapy. Cells may lack a specific protein if the patient has a hereditary disorder involving a gene mutation. Inserting a plasmid into DNA would allow cells to express a protein that they are lacking. Using transgenic animals to produce therapeutic products is the idea of "humanizing" animals as a donor of organs for use in xenotransplantation.



- Gene cloning has proved to be especially valuable in the production of safe and effective vaccines. Using this technology it is possible to clone specific proteins from a pathogen, for instance viral coat proteins from hepatitis B, produce these proteins in cultured cells and use them as a vaccine to elicit a protective immune response against the whole organism. These so-called subunit vaccines avoid many of the problems encountered with

killed or attenuated vaccine preparations; the absence of whole organisms avoids any risk of causing an infection and also reduces side effects.

Note: The use of cultured cells, whether bacteria or eukaryotic cells, offers a number of advantages. In many cases the only other possibility is to isolate the proteins from humans or animals. Proteins from humans carry the risk of transmitting disease, as in the case of HIV passed to hemophiliacs in factor VIII blood clotting factor. Proteins from other animals may not behave in exactly the same way as the human protein as in the case of insulin, one of the first pharmaceuticals to be produced by genetic engineering techniques.



Pharmaceutical applications:

Many important pharmaceutical products are proteins and gene cloning is already used extensively in their production. Bacteria, yeast and tissue culture cells can be used in the production of proteins and pharmaceuticals. Introducing the idea of biopharming; of using transgenic plants or animals to produce pharmaceutical proteins: this technology is in its infancy, but it holds the promise of fields of plants or flocks of sheep cheaply and efficiently producing valuable pharmaceutical products. In most cases it is envisaged that the pharmaceutical product would be harvested and purified, although in the case of edible vaccines it is hoped that it will be possible to deliver the vaccine orally by eating the plant. The advantages of this approach are not only in the acceptability of this method of delivery to people who have an aversion to hypodermic needles, but also in being able to grow, harvest and store vaccines using low-tech farming techniques rather than expensive hightech pharmaceutical technology.

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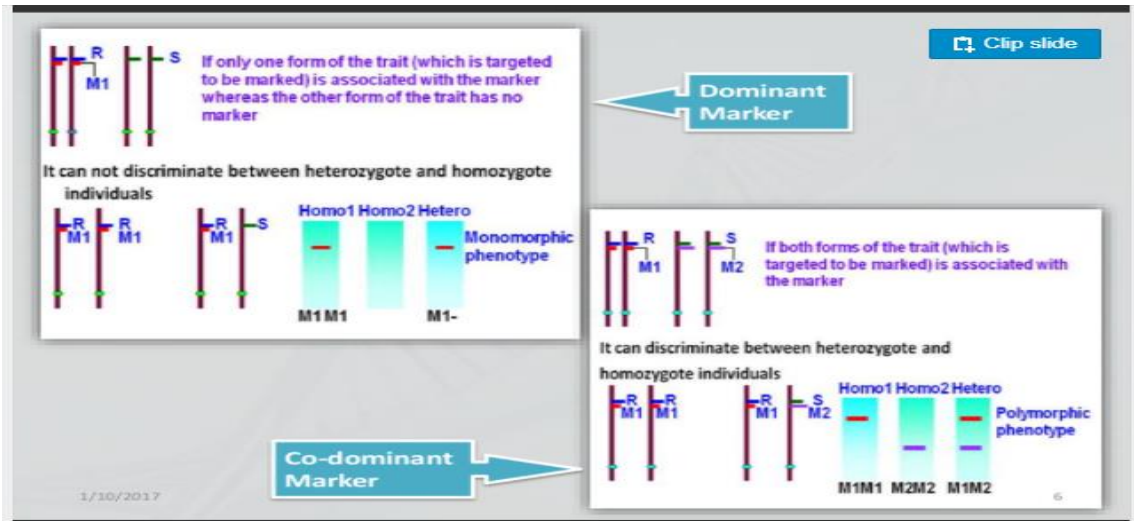
Genetic Markers

A genetic marker is a gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species. It can be described as a variation (which may arise due to mutation or alteration in the genomic loci) that can be observed. Generally, they do not represent the target genes themselves but act as 'signs' or 'flags'. Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred to as gene 'tags'. Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or 'linked' to genes controlling the trait. All genetic markers occupy specific genomic positions within chromosomes (like genes) called 'loci' (singular 'locus').

Types of genetic markers:

Markers can exhibit two modes of inheritance, i.e. dominant/recessive or co-dominant. If the genetic pattern of homo-zygotes can be distinguished from that of hetero-zygotes, then a marker is said to be co-dominant. Generally co-dominant markers are more informative than the dominant markers as they identify more than one allele. These markers allow for the amplification of particular sequence within the genome for comparison and analysis.



There are many types of genetic markers, each with particular limitations and strengths. Genetic markers may be morphological or molecular. Molecular genetic markers can be divided into 3 classes a) biochemical markers which detect variation at the gene product level such as changes in enzymes/isozymes, proteins and amino acids and b) Non-PCR (hybridization) and PCR-based molecular markers which detect variation at the DNA level such as nucleotide changes: deletion, duplication, inversion and/or insertion.

Molecular Marker

They are specific fragments of DNA that can be identified within the whole genome to detect the differences in DNA sequences occur among members of the same species. They are found at specific locations of the genome to reveal polymorphisms at the DNA level. They are used to 'flag' the position of a particular gene or the inheritance of a particular character. Molecular markers are phenotypically neutral. They are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA.

They are effective because they identify 1) an abundance of genetic linkage between identifiable locations within a chromosome and are able to be repeated for verification, 2) small changes within the mapping population enabling distinction between a mapping species, allowing for segregation of traits and identity, 3) particular locations on a chromosome, allowing for physical maps to be created and 4) how many alleles an organism has for a particular trait (bi allelic or poly allelic).

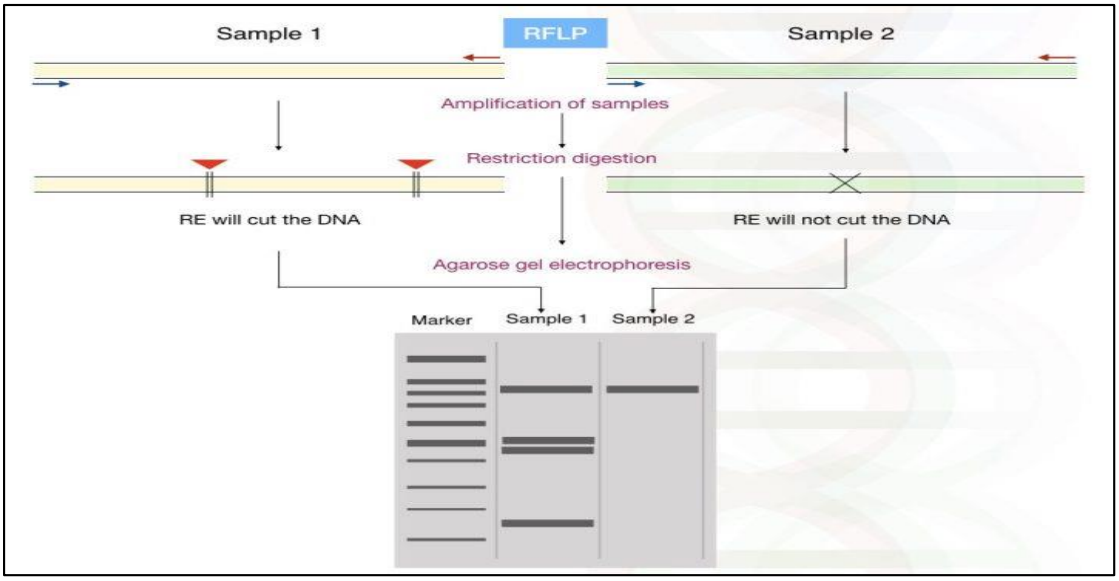
non-PCR and PCR-based molecular markers:

Restriction fragment length polymorphism (RFLP) is the first hybridization-based molecular marker system that was intensively used at the beginning of the molecular biology era in life science. In contrast, many PCR-based molecular marker detection methods have been developed such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), single nucleotide polymorphisms (SNP) and inter-simple sequence repeat (ISSR).

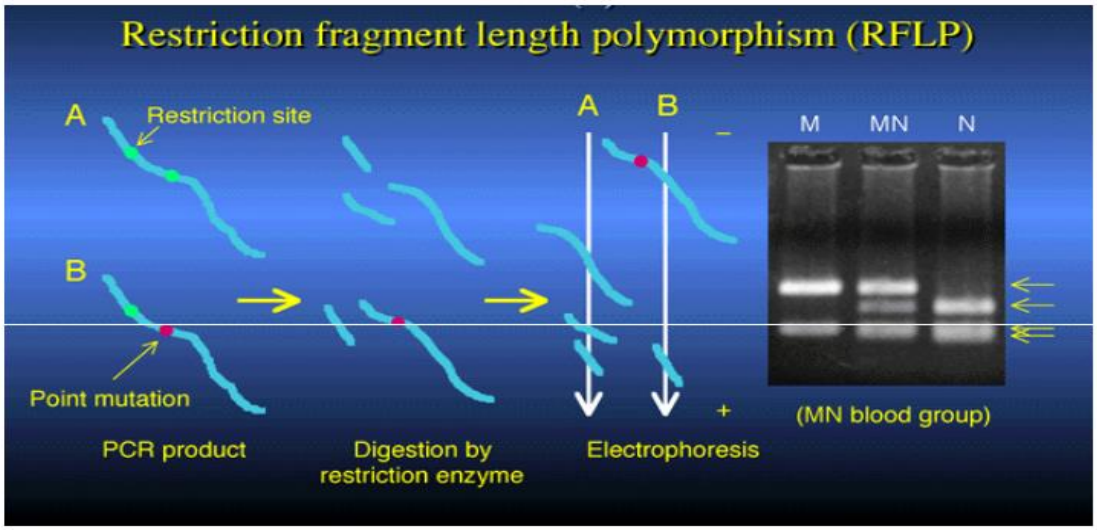
RFLP:

Restriction fragment length polymorphism (RFLP) is a technique that exploits variations in homologous DNA sequences, known as polymorphisms, in order to distinguish individuals, populations, or species or to pinpoint the locations of genes within a sequence.

In RFLP analysis, alterations in the same/ homologous DNA sequences can be detected by analyzing fragments of different length, digested with REase. REase recognize and cut DNA at specific sites. Different sized fragments are produced depending on whether the restriction site exists or not.



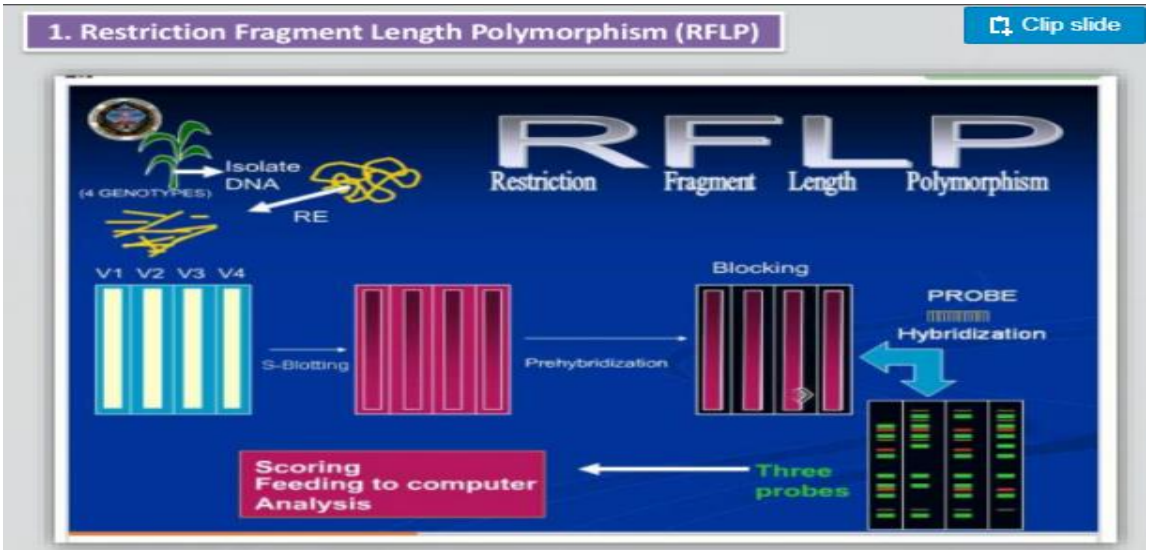
A single restriction endonuclease gives more specific results by cutting at one specific locus and produces fragments of different length.



DNA sample is digested into fragments by one or more restriction enzymes, and the resulting restriction fragments are then separated by gel electrophoresis according to their size. The length of different fragments is identified using blotting-based probe hybridization method**, which is now replaced by sequencing. The RFLP markers are highly locus-specific (due to REase) and co-dominant (as both alleles in the heterozygous mutant (as well as normal) can be detected).

RFLP analysis was an important early tool in genome mapping, localization of genes for genetic disorders, determination of risk for disease, and paternity testing.

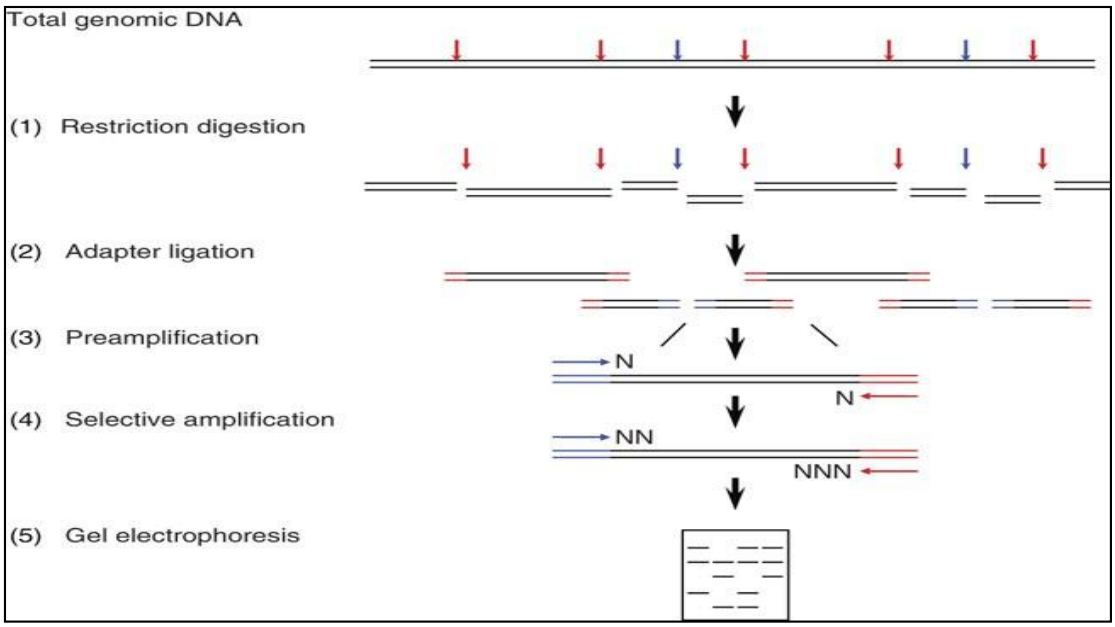
** The labelled DNA probes called an RFLP probe, used to hybridized with the digested fragments by the Southern blotting analysis.

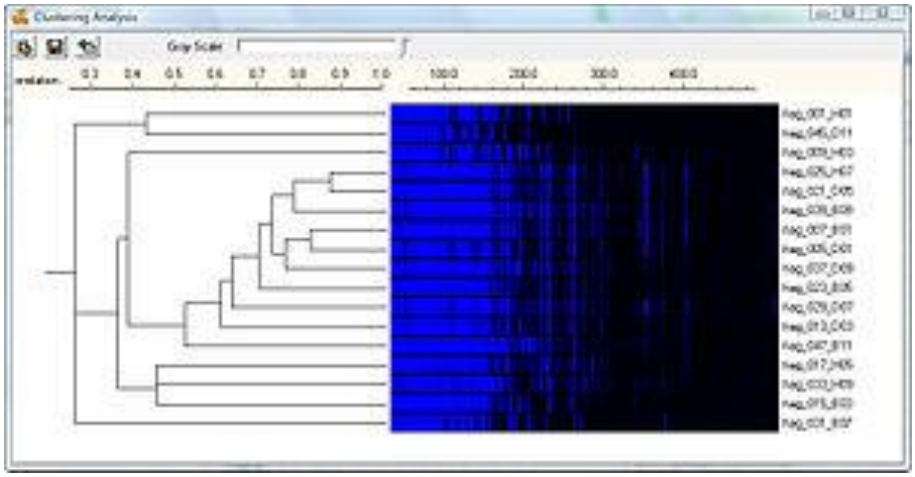


AFLP:

AFLP (PCR-based technique) is a dominant marker which uses restriction enzymes to digest genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments. A subset of the restriction fragments is then selected to be amplified. This selection is achieved by using primers complementary to the adaptor sequence, the restriction site sequence and a few nucleotides inside the restriction site fragments. The amplified fragments are separated and visualized on denaturing on agarose gel electrophoresis , either through autoradiography or fluorescence methodologies, or via automated capillary sequencing instruments.

The AFLP technology has the capability to detect various polymorphisms in different genomic regions simultaneously. It is also highly sensitive, reproducible and do not require any DNA sequence info from the studied organism. As a result, it is a used in genetics research for the identification of slight genetic variation in strains or closely related species of plants, fungi, animals, and bacteria, DNA fingerprinting, criminal and paternity tests, and in the practice of genetic engineering.



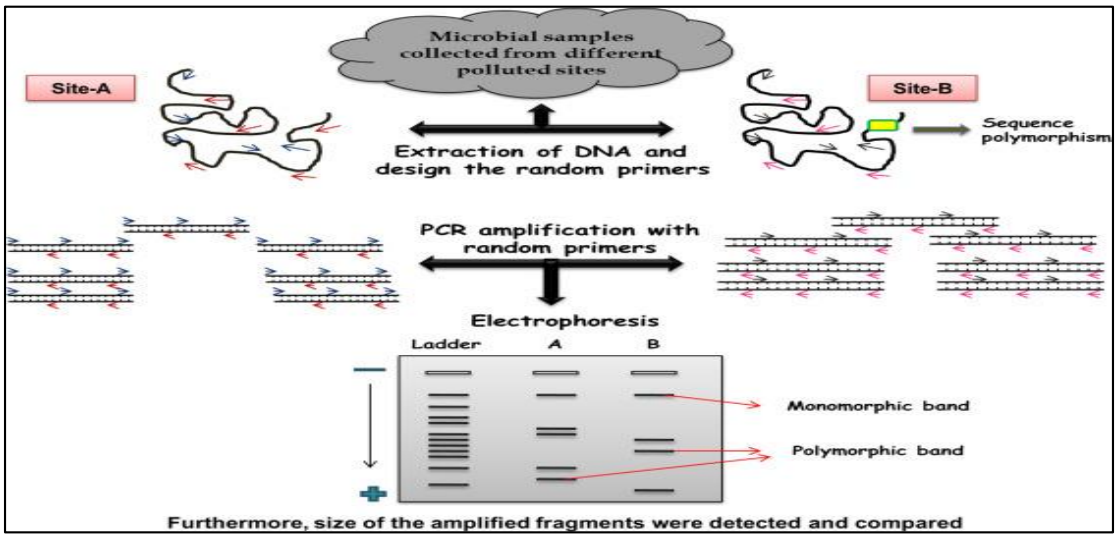


AFLP phylogeny analysis

RAPD:

Random Amplification of Polymorphic DNA is a PCR-based tool, in which the segments of DNA that are amplified are random. The scientist performing RAPD (dominant marker) creates several arbitrary, short primers (8–12 nucleotides), then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify. By resolving the resulting patterns, a semi-unique profile can be gleaned from an RAPD reaction. This technique is inexpensive, quick, easy, need low quantity of DNA and do not require any specific knowledge of the target (as the primers will bind somewhere in the sequence, but it is not certain exactly where). It is not suitable for forming a cDNA databank as it is not reproducible. In the previous decade, RAPD has been used to characterize, and trace, the phylogeny* of diverse plant and animal species.

* A phylogenetic tree or evolutionary tree is a branching diagram or "tree" showing the evolutionary relationships among various biological species. Their phylogenies are based upon similarities and differences in their physical or genetic characteristics.



SNP: Single-nucleotide polymorphism (SNP) represents a substitution of a single nucleotide at a specific position in the genome. It is formed due to INDEL (insertion and deletion) of a single nucleotide. It presents a sufficiently large fraction of the population (1% or more). For example, a C/T substitution in the DNA of plants (No 7) compared to the same region of DNA in other plants of the same species.

Plant	Sequence
1	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC
2	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC
3	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC
4	GAATTCCGCAATGCAGGTTAAGAGCTTTGTGAAAGAGGAAAACGAAAAAC
5	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC
6	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC
7	GAATTCCGCAATGCAGGTTAAGAGCTTTGTGAAAGAGGAAAACGAAAAAC
8	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC

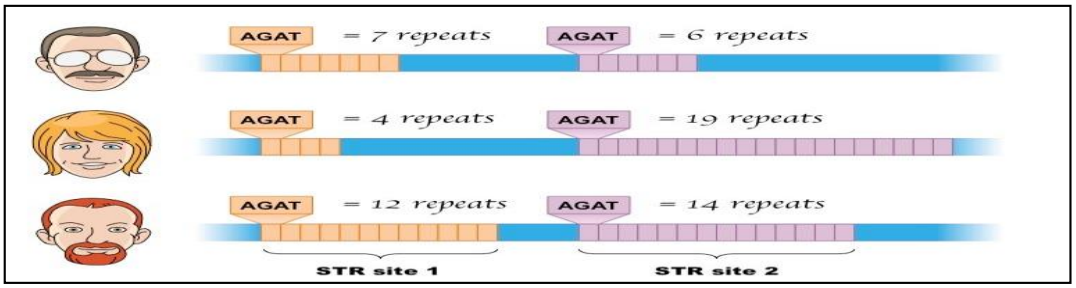
For example, at a specific base position in the human genome, the C nucleotide may appear in most individuals, but in a minority of individuals, the position is occupied by an A. This means that there is a SNP at this specific position, and the two possible nucleotide variations – C or A – are said to be the alleles for this specific position.

SNPs pinpoint differences in our susceptibility to a wide range of diseases (as sickle-cell anemia). The severity of illness and the way the body responds to treatments are also manifestations of genetic variations. For example, a single-base mutation in the APOE (apolipoprotein E) gene is associated with a lower risk for Alzheimer's disease. SNP can be

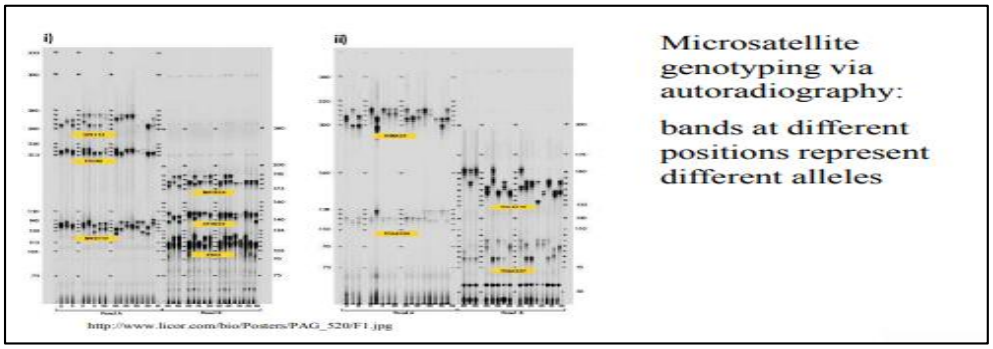
used to determine whether a genetic variant is associated with a disease or trait. Variations in the DNA sequences of humans can affect how humans develop diseases and respond to pathogens, chemicals, drugs, vaccines, and other agents. SNPs are also critical for personalized medicine.[21] Examples include biomedical research, forensics, pharmacogenetics, and disease causation.

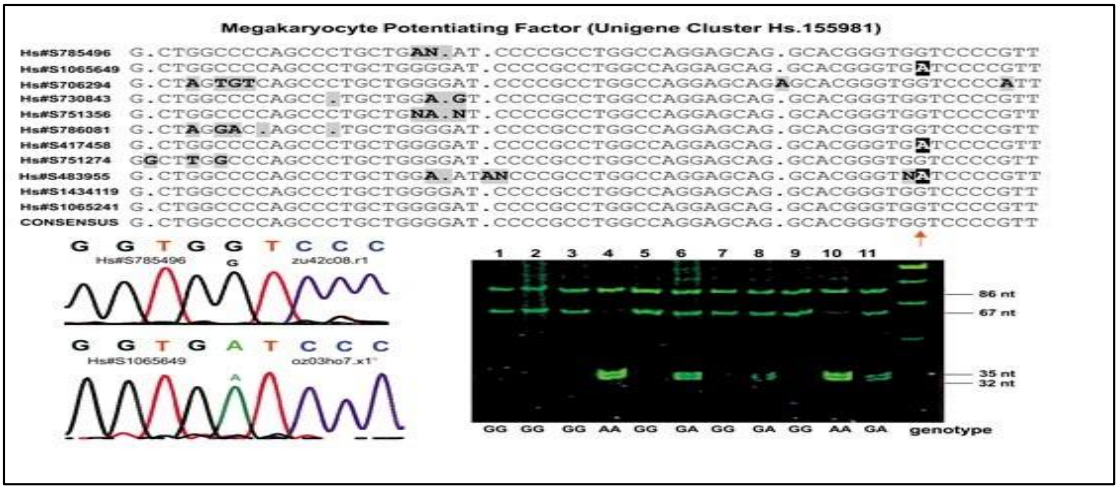
STR:

The short tandem repeats/ simple sequence repeats are also called a microsatellite. The microsatellite is the shorter repeats sequences present into genome thousands or millions of times. The repeats in STR are 2 to 6 base pairs per repeats. The STR is highly polymorphic and the number of repeats differs between individuals even though the loci are homologous. Because of this reason, the STR marker is the first choice in forensic analysis (PCR-based technique). Due to the difference in the repeat number each individual have different STR profile.



SSR: Simple sequence repeat is a microsatellite repetitive co-dominant DNA marker, typically ranging from 5 to 50 times in a genome. The marker is highly variable and highly reproducible. It is more similar to the short tandem repeats. The SSR is a PCR-based technique which consist of 1) amplify region by PCR (primers labeled via radioactivity or fluorescence), 2) separate PCR products according to size (polyacrylamide gel, capillary based systems), 3) determine size of amplified product (autoradiography, fluorescent traces) and score alleles. This technique is used for population studies.

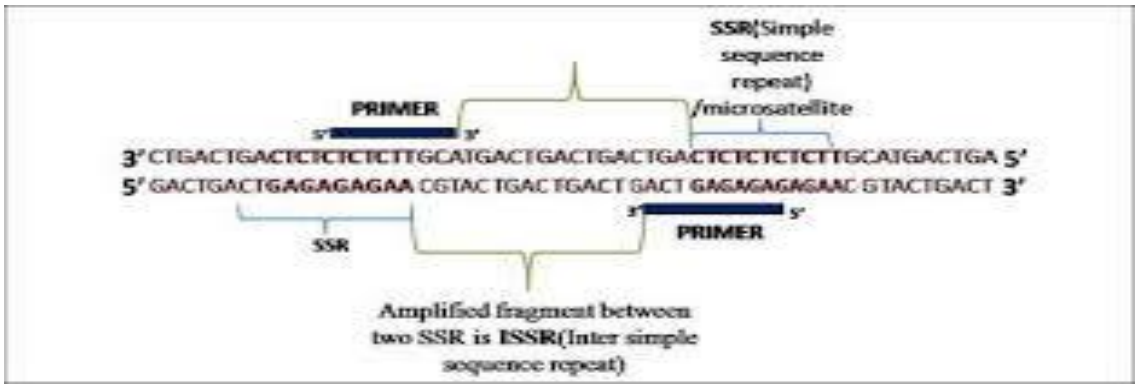




ISSR: The ISSR marker is the inter simple sequence repeat, the method is based on the PCR agarose gel electrophoresis, detected using the single primer amplification reaction. Between the oppositely oriented microsatellites, the sequence of 100 to 3000bp fragment of a sequence is called the ISSR.

Here the advantage of using the ISSR is that no prior sequence information is required for constructing the primers. Multiple loci are amplified because of the random distribution of ISSR within the genome. Also, the low quantity of template is sufficient to do PCR. However, it has lower reproducibility and non-homology of similar-sized fragments.

Because of the simple set up of the ISSR, it is employed in the gene mapping studies, clone identification, different strain identification and parental verification. It is a highly polymorphic marker and used in the genetic diversity studies, gene tagging, evolutionary study and phylogenetic studies. In this technique, the flanking non-repeated regions of the inversely oriented microsatellites are amplified using a single primer or pair of primers.



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