Hybridization based markers

- Hybridization based marker technology use cDNA, cloned DNA elements or synthetic oilgonucleotides as probes, which are labeled with radioisotopes or with conjugated enzymes that catalyze a coloured reaction to hybridize DNA.
- The DNA, either cleaved with restriction enzyme or amplified by PCR, are separated by gel electrophoresis and transferred to a solid support matrix by Southern hybridization and hybridized mainly with labeled probe of known origin and sequence.
- RFLP is representative of this type of technology. Restriction polymorphism occurs when mutation remove an existing restriction site or create a new restriction site. These alterations are detected by using a labeled probe

Restriction Fragment Length Polymorphism (RFLP)

- RFLP was the first technique which enables the detection of polymorphism at the DNA sequence level. In this method, DNA is digested with restriction enzymes that cut the DNA at specific sequences, electrophoreed, blotted on a membrane and probed with a labeled oligonucleotide.
- The DNA sequence variation detected by this method was termed restriction fragment length polymorphism.

Advantages

- RFLP are co dominant markers, enabling heterozygote to be distinguished from homozygote.
- The method is simple and no sequence specific information is required.
- They are reliable markers in linkage analysis and can be easily determined if linked
- Trait is present in a homozygous or heterozygous state in individual, an information highly desirable for recessive traits.
- This technique has been successfully employed for tagging gene of economic value.

Limitations

- Utility of RFLP has been hampered due to the large amount of DNA required for
- restriction digestion and southern blotting.
- The requirement of radioactive isotope makes the analysis relatively expensive and hazardous.

PCR based markers

- It is a PCR method, whereby a specific sequence of nucleotides within a double stranded DNA is amplified.
- The sequence is identified by the use of short synthetic oligonucleotides that are complementary to the terminal regions of the DNA sequence to be amplified; these oligonucleotides are extended by the thermostable Taq DNA polymerase on the DNA template.

PCR with Arbitrary Primers

- In this category of PCR based markers, the random or arbitrary primers are used for DNA fingerprinting. Random primers of different lengths and sequences are mainly used for this purpose.
- Primer sequence is mainly selected in such a way that it binds with most probability to their complementary sequence on the template DNA.
- The molecular markers of this category are divided on the basis of their primer lengths like RAPD (Random Amplified Polymorphic DNA), AP-PCR (Arbitrary Primed-PCR), DAF (DNA amplification Fingerprinting), and AFLP (Amplified Fragement Length Polymorphism).

Random Amplified Polymorphic DNA (RAPD

- A PCR based molecular marker technique in which a single type of random decamer primers are used to amplify a set of DNA segment distributed randomly throughout the genome.
- In this technique, a single species of decamer (10 bp) primers are used to exploit random sites from the genome and resulted in polymorphism between two distinct species.

Advantages

- Only a small amount of DNA is required for PCR amplification.
- No need of radioactivity as RFLP
- There is no need of prior genomic information for designing primer
- The primers are random and can be used across a large variety of species.

Disadvantages

- It is a dominant type of marker system and this causes a loss of information relative to marker which shows codominance.
- It is not a reproducible method because of short primer length, that binds to nonspecific sites in the genome and makes the results inconvenient for data interpretation.

DNA Amplification Fingerprinting (DAF)

- DNA Amplification Fingerprinting (DAF) is one of the recent amplification based nucleic acid scanning techniques. DAF utilizes primers of 5-12 nts of length of arbitrary sequence.
- Primers as short as 5 nts in length can produce complex banding patterns that are resolved by polyacrylamide gel electrophoresis and silver staining.
- DAF uses low stringency amplification condition, so that primers can anneal arbitrarily at multiple sites on template DNA strand and initiate DNA synthesis.
- DAF does not depend on cloning or DNA sequence information and can generate fingerprints from DNA of viral, bacterial, fungal, plant and animal origins.
- DAF can be used to assess genetic diversity, to identify genotype and also for segregation and linkage analysis.

Amplified Fragment Length Polymorphism (AFLP)

- It is based on the PCR amplification of genomic restriction fragments generated by specific restriction enzymes and oligonucleotide adaptors of a few nucleotide bases.

- AFLP is a combination of RAPD and RFLP methods, but detects a ten fold greater number of loci than those detected by RAPD analysis, thus the AFLP have the capacity to rapidly screen thousands of independent genetic loci.
- It is novel DNA fingerprinting technique in which no prior sequence knowledge is required for amplification.
- The number of DNA fragments in single reaction can be tuned by selection of specific primer sets.
- AFLP technique uses stringent reaction condition for primer annealing and combines the reliability of RFLP technique with the power of PCR technique.

Advantages

- AFLP is highly reproducible over RADP and RFLP.
- AFLPs are faster, less labour intensive and provide more information than RFLPs.
- Compared to RAPD, fewer primers are needed to screen all possible sites.
- AFLPs can be codominant marker like RFLPs. So it can be used for the discrimination of heterozygotes from homozygotes. AFLP analysis is especially useful in screening backcross individuals.
- AFLPs are extremely useful as a tool for DNA fingerprinting and also for cloning and mapping of variety specific genomic sequences.
- AFLP has wide spread application including assessment of genetic diversity studies, construction and saturation of linkage maps and tagging of genes.

Disadvantages

- AFLP generate huge quantity of information, which may need automated analysis therefore computer technology.
- AFLP markers display dominance.

PCR with Sequence Specific Primers

- An approach opposite to the PCR with arbitrary primers is to design primers to target specific genome. These primers are based on sequence specific knowledge of the genome and bind to a particular complementary sequence because of their long length and specificity.
- Sequence Tagged Sites (STS)
- Sequence tagged sites are primers that are based on some degree of sequence knowledge of genome under study. Sequence tagged site primers target and amplify a specific region of the genome. A sequence tagged site is general term given to a marker defined by its primer sequences amplified from the arbitrary primers.
- Example of STSs are
- Sequence Tagged Microsatellites (STMs)
- Sequence Characterized Amplified Region (SCAR)
- Cleaved Amplified Polymorphic Sequence (CAPS)

Advantages

- Only a limited quantity of DNA is required for this process.
- highly polymorphic in nature
- Good analytical resolution and high reproducibility because of the longer primer sequences.

- Codominant in nature that is useful in distinguishing between homozygotes from heterozygotes.
- Evenly distributed throughout the genome.

Disadvantage

- One obvious drawback of SSR marker is the development of polymorphic SSR marker because time, cost and skill is required for construction and screening of library and then sequencing of the hybridized clone to determine the flanking region of the repeats for SSR primer designing.

Sequence Characterized Amplified Regions (S CAR)

- A technique in which RAPD marker termini are sequenced and longer primers are designed (15-30bp long) for specific amplification of a particular locus.

Advantages

- PCR products of SCAR produce a clear banding pattern then RAPDs.
- Usually dominant but can be converted into codominant markers that can be used for genetic mapping.

Disadvantage

- There is no need to design and screen a library to pick up a sequence for primer synthesis, but sequencing efforts are required to find out the end sequences of RAPD products to synthesize specific primers for amplification.
- So skill, efforts and expenses are required to design specific primers for each locus.

Cleaved Amplified Polymorphic Sequence (CAPS)

- If specific primers designed for STMS, EST and STMS do not show polymorphism, than the amplified products are digested with a particular restriction enzyme and scored for polymorphism which is termed as Cleaved Amplified Polymorphic Sequences.

Advantages

- Codominant in ‘nature’ so the information can be effectively used for genetic mapping.
- CAPSs are reproducible because of specific and long primer sequences.

Disadvantages

- To design a primer some genomic sequence specific knowledge is required.
- Efforts and expenses are required to design specific primers for each locus.