

Potential of Microsatellites DNA Markes in Monitoring The Genetic Diversity of Fishes

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1. Introduction

Microsatellite DNA markers or simple sequence repeats (SSRs) (Tautz 1989), or short tandem repeat (STR) (Tautz, 1989; Edward et al., 1991) are known as the versatile, very popular and most desired genetic markers that found multiple applications in conservation genetics, genetic composition of biological populations, molecular ecology, developmental and evolutionary biology. The phenotypic characteristics of any organism are determined by the genes. Genetic variation results in the production of individuals that are distinct from one another at the organismic as well as molecular level. Stock is a panmictic population within a species that has related individuals and is genetically different from other such populations. Fishery biologists began dealing with economically significant marine organisms by utilizing these intra-specific groups, whom they were referred to as “stocks” (Shaklee et al., 1990). The crucial component in any management regime is the detection of distinct stocks (Ihseen et al., 1981; & Fetterolf et al., 1981). Genetic variety in the form of various alleles of many genes exists in the majority of natural populations. Population that is sexually reproducing, there aren't any two organisms that can be believed to have identical genotypes for every gene (Hartle and Clark 1989). A genetic marker is one whose genotype can be derived from the phenotype through genetic screening and it is a variant that is inherited genetically. DNA (molecular) and protein are the two major types of genetic markers. Nuclear DNA markers are now the most familiar markers to identify the genetic variation within the population of organisms such as in fishes. They are molecular genetic markers with short segments of about 1- base pairs in length, (Beckmann and Weber 1992). DNA is composed of di, tri, and tetra nucleotide repeats sequence. They are highly short, abundant, co-dominant, and hyper-variable with frequencies of 10, 20, and 100 bases and are widely spread in the genome of eukaryotes and they have multiple alleles in them (Mojekwu et al., 2013). Microsatellites have been classified into various categories concerning the type of repeated sequence present. Microsatellites in the population of fish provide critical information for example demographic bottleneck detection, genome, pedigree and parental analysis detection, genetic variation identification within and between stocks, genetic conservation, and QTL (quantitative trait loci) detection (Fjalestad et al., 2003; Olubunmi, 2019 & Chistiakov et al., 2005). Microsatellites have developed as the ideal marker due to their ease of assaying, accuracy, and high variability, and that's the reason they are used in research for populations

with high resolution (Tripathy, 2018). The primers designed in a species for the amplification of microsatellites also in adjacent species offer cross-amplification for homologous loci, which proves helpful in assessing populations that are on the edge of extinction or are small (Estoup 1998). Comprising the changes and interactions of countless genes has also been made possible by genomic research, and including certain biological phenomena has been made possible by genome analysis (Venkatesh et al., 2007 & Hudson et al., 1980). The procedure that is used to identify the genotype of the DNA marker of the fish is known as microsatellite genotyping. There are three basic steps in this procedure firstly microsatellite amplification, secondly specific design of the primer, and lastly the testing of Polymorphism. As primer is designed then amplification of such marker takes place. Currently, the amplification of the tiny DNA fragments is made possible via advanced thermo-cyclers by Polymerase chain reaction (PCR). In fisheries, microsatellite DNA markers contribute lots of necessary information such as genomic markers as well as diagnostics markers for diseases in fish (Duran et al. 2009). Although microsatellite markers provide multiple benefits, but they also have various disadvantages, and limitations and present several challenges that can severely suppress their application such as by complicating data analysis. Because of microsatellite flexibility to tackle a wide range of ecological queries overcome their disadvantages in many cases, even though all types of markers have their pitfalls. Luckily, through the procedure of isolation, many problems that are related to microsatellite markers can be condemned by selecting loci carefully. In this review, we will discuss the potential of microsatellite DNA markers in monitoring the genetic diversity in fish through their functioning, working, role in genetic diversity, genetic structure, microsatellite genotyping, advantages, and role in fish conservation, as well as its limitations.

2. Microsatellite DNA markers

A short DNA sequence that appears numerous times at various locations across an organism's DNA is referred to as a molecular DNA marker. These repeats are highly variable making them useful as markers or tags for specific locations (polymorphic locus or loci) (Okumus & Ciftci, 2004). The molecular markers based on DNA sequence polymorphisms play a significant role in the measurement of genetic variation and genetic analysis in fish. (Olubunmi et al., 2019). Based on the current scientific understanding as stated in Deepak et al. (2017), molecular markers can be grouped into three categories. The first category involves nucleic acid hybridization which relies on complementary base pairing and includes techniques such as restriction fragment length polymorphisms (RFLPs). The second category comprises techniques such as microsatellites, or SSRs, AFLP (amplified fragment length polymorphisms) RAPD (random amplification of polymorphic DNA), that use PCR-based DNA amplification methods. The third category is single nucleotide polymorphisms (SNPs) as described by Duran et al. (2009). The non-coding and neutral markers are microsatellites and AFLPs (Brown et al., 2003).

The modern DNA structural model was developed in 1950 by F. Crick, J. Watson, and M. Wilkins, who also introduced the idea of molecular genetics (Hallerman et al., 2003). Since then, advancements in the understanding of DNA and gene structure and function have led to their use in determining genetic diversity. Different types of DNA markers were developed during the 1980s. However, the first investigation into population genetics based on mitochondrial DNA was carried out in the early 1980s (Avisé et al., 1979). Later, the development of PCR technology facilitated the emergence of various methodologies for studying population genetics. These approaches encompassed not only DNA sequencing of the target regions but also techniques that examined length polymorphisms, including microsatellite DNA markers (Hansen, 2003).

Microsatellite DNA markers are molecular genetic markers with short sequences of around 1-6 base pairs in length (Beckmann et al., 1992). They are referred to as SSRs (Tautz et al. 1989) and STR (short tandem repeat) (Jeffreys et al., 1988; Edwards et al., 1991, & Weber et al., 1990) and broadly distributed over the genome of eukaryotes. They are highly abundant, small, and co-dominant with frequencies of 10 to 10 copies, 20 and 100 bases, and are widely distributed in the genome of eukaryotes and they have multiple alleles in them (Mojekwu et al., 2013). They are

hyper-variable, nuclear-encoded genetic markers that can be amplified by polymerase chain reaction or DNA sequencing markers (Phumichai et al., 2015 & Deepak et al., 2017). Microsatellite DNA markers have two main techniques to analyze (Okumus & Ciftci, 2004). Tautz (Tautz et al., 1989) and Weber and May first explained the polymorphism loci at microsatellite markers (Weber et al., 1989).

PCR plays a crucial role in amplifying alleles at microsatellite loci (Saiki et al., 1988). This technique enables the amplification of small samples of genomic DNA, allowing for the precise separation and sizing of alleles on a polyacrylamide gel or agarose gel. The resulting bands, typically appearing as one or two distinct bands, can then be utilized to quantify genetic variations within and between populations of a species. (Connel et al., 1997). Microsatellites allow for accurate differentiation based on electrophoresis (Muneer et al., 2009). Every locus is distinguished by a recognized DNA sequence that comprises both unique DNA segments and repetitive DNA motifs (Tautz et al., 1984). Simple sequence repeats, often made up of two to four nucleotides like (AC)_n or (GATA)_n, where n is between five and fifty, make up the repeating elements (Dewoody et al., 2000). Di-nucleotide repeats -GT and CA- are considered to be the most common microsatellites in vertebrates (Zardoya et al., 1996). To analyze single locus microsatellites specific primers flanking the repeat units are required and these sequences are obtained from genomic DNA libraries or are readily available in gene banks. These techniques are often used for developing microsatellite markers (Zane et al., 2002). Genetic markers serve as valuable tools for studying fish populations, offering valuable insights such as identifying genetic variations between and within stocks, monitoring genetic changes in stocks through gene tagging, detecting quantitative trait loci, and identifying demographic bottlenecks. (Chistiakov et al., 2005).

3. Functions of Microsatellite DNA Markers

For inscribing questions in evolution and population genetics microsatellite is one of the most adaptable and conventional markers (Flajoulot et al., 2005). Microsatellite marker is highly used in the correlated field of life sciences due to their fast detection protocol, high volume of polymorphism, and comparatively small size (Coe et al., 2009). Microsatellite has a wide variety of functions which include;

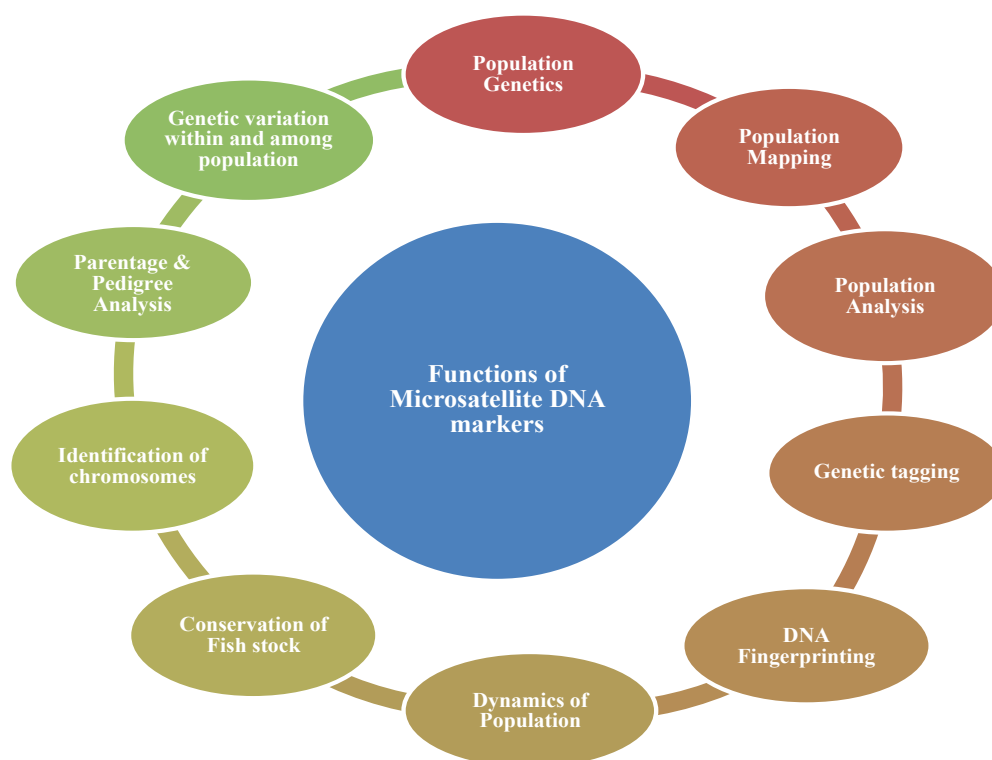


Fig.1. Showing functions of Microsatellite DNA markers

3.1. Parentage and Pedigree Analysis:

Microsatellite has mostly come out as a type of marker that is selected for pedigree analysis. In the task of a parent, microsatellites act as an effective instrument such as there are always hereditary alleles from both parents by each progeny. Broad genetic constitution details on the trait of examined discrete can be acquired with the use of multiple panel microsatellite markers (Duran et al., 2009; Olubunmi et al., 2019 & Sundaray et al., 2016). Microsatellite loci are used to rebuild pedigree in fish populations conceived from joint families (Norris et al., 1999; Morelli et al., 2007; Teng et al., 2020 & Olivatti et al., 2011). Microsatellite has modified applications such as assignment testing of population (Glover et al., 2010). In selective breeding, microsatellites provide us with various details in aquaculture for pedigree and parentage analysis such as genome mapping, regulating the genetic mutation in stock, observation of quantitative trait loci (QTL), an association of genetic variability among and within stock (Fjalestad et al., 2003; Subasinghe et al., 2003; Volckaert et al., 2008 & Olubunmi et al., 2019).

By using just two microsatellite loci and demonstrating the offspring accomplishment of mature males (cuckolders), Colbourne et al. (1996) conducted a parental analysis on eggs laid in a natural nest of bluegill sunfish (*Lepomis macrochirus*, Centrarchidae). Paternity in offspring from seven species of lekking cichlids is analyzed by (Kellogg et al., 1995) in a similar study. In the majority of the cases, these analyses circulate multiple paternity and in one exceptional condition, it divulged the offspring from one young was extracted from not less than six males. This level of study was not possible in the past due to the sharing of high levels of alleles recognized with less volatile markers. This kind of research will enable the proficiency of various living-history models in fish, which show a significant divergence in the phylogenetic perspectives among vertebrates to be calculated. In confined care schemes, the pedigree information is highly beneficial for farm management (O'Connell et al., 1997). In broad-scale pedigree analysis, the production of any path will increase with the amount of available molecular information. For example, for a higher number of alleles per locus we will use an increased number of loci that will increase the possibility of successful parentage assignments (Slate et al., 2000) and it will also increase both the correctness and accuracy of relatedness evaluation (Ritland, 1996). Microsatellites have been used to distinguish joint family groups and also to evaluate family/parentage locating in many species (Tripathy et al., 2018; Duran et al., 2009; & Al-Atiyat et al., 2012). Fractional allocation is another method for parentage analysis. Rather than regulating the single similar parent from a set of applicants, the fractional allocation method tries to fractionally allocate the offspring among non-excluded parents based on their likelihood parentage (Neff et al., 2001).

3.2. Genetics Variation of Closely Related Species:

Tautz (Powell et al., 1995) and Weber and May (Ma et al., 2010) first displayed the polymorphism at microsatellite loci. The polymerase chain reaction (Saiki et al., 1988) can increase the symbol of alleles at microsatellite loci from small genomic DNA specimens. These alleles segregate and correctly sort as one or two bands on a polyacrylamide gel, and these bands are used for evaluating genetic variation within and among individuals or species (Abdul-Muneer et al., 2014). Microsatellite is a dominant genetic indicator for evaluating the hereditary variation between and within individuals or species according to (Muneer, et al., 2011). For genetics population and stock isolation microsatellites is a precious marker (O'Connell et al., 1997, & Abdul Muneer et al., 2012). As stated by (Barbara et al., 2007) the triumph of assignable microsatellite loci was straightly associated with evolutionary relationships among groups are associated and transferable for the fish breed can be almost 70 percent in relative and 60% lowering between groups of similar families (Abdul Muneer et al., 2012 & Gopalakrishnan et al., 2009). Microsatellites use evaluation directly from DNA and are modified to survey the genetic diversity within and among the population (Ekerette et al., 2017).

3.3. Population Genetics:

According to Beckmann and Soller (Beckmann et al., 1990) the microsatellite markers are used in the population genetics of fish. Microsatellites as genetic molecular markers are dominant instruments to perceive the hereditary rareness of individuals, groups of populations, or breeds as stated by (Duncan et al., 2001; Amos et al., 2001; Avise, 1986; & Avise, 1994) and Linda and Paul (Askari et al., 2013). These are necessary for the analysis of the genetic diversity of various population groups (Collevatti et al., 1999). These molecular markers could give a powerful basis for the genetic diversity of fish assessment and locating of populations or species from various model and non-model organisms (Tripathy et al., 2018). Microsatellites having one locus in which both of the alleles express co-dominance declaration in heterozygotes have been extensively utilized for population studies (Abdul Muneer et al., 2009). With microsatellites only a few alleles are rigorously adapted for studies of population genetics, while for genome mapping the more changeable loci are ideal and to determine taxonomic vagueness in different taxa the stable or fewer polymorphic microsatellite loci and pedigree analysis are used (Carvalho et al., 1994). The variation in the microsatellite and sequence of freshwater and saltwater fishes for demographic genetics is described by (Chauhan, et al., 2007; Mandal, et al., 2018; & Xu, et al., 2020) and (Sinama et al., 2011; Abdul Muneer et al., 2012; & Muneer et al., 2011). Microsatellite shows the markers to select for genetic monitoring of farmed stock (Davis et al., 1998, & Hulata, 2001).

3.4. Genome Mapping:

By using various types of polymorphic markers the genetic linkage map has been established for several water-based species (Moen et al., 2008; Jiang et al., 2013; Liu et al., 2013; Wu et al., 2023; Zhu et al., 2019; Keong et al., 2014; & Hollenbeck et al., 2015). For genome mapping, divergence of population, pedigree and parentage analysis, and recognition of stock microsatellites as genetic markers have been used (Wright et al., 1995; Hansen et al., 2001; Liu et al., 2004; Webster et al., 2005; & Sanetra et al., 2009). Microsatellite markers have been utilized to develop the genetic maps by analyzing the reinterpretation and alleles separation via genotyping in common carp, cichlids, trout, zebrafish, and many other species. (O'Reilly et al., 1995) who recommend that microsatellites would be more useful for genomic identification. For establishing a genetic structure microsatellites have a strong use of various mapping tactics (comparative genetic, physical mapping, linkage mapping, and genomic tools) to include additional genes and markers. To locate the map the controlling of important features economically and finally cloned to classify the candidate gene.

3.5. Population Analysis:

Microsatellites have become the markers of option for population analysis of high constancy due to their high variance, facileness, and correctness of assaying according to (Estoup, et al., 1993). For isolation of stock and population genetics microsatellite markers are an effective instrument (Wright et al., 1995, & Abdul Muneer et al., 2012). Microsatellite loci are expensive instruments to examine genetic variability with application to management and population genetics (Oliveira et al., 2006).

3.6. Genetic Tagging:

Microsatellite markers are developing for a selected species providing several benefits, the genetic tags are the physical markers for parentage projects in assigning offspring to their birth parents. This process allows many families to be bred in a common aquarium from propagation to reaping size, an exercise that can crucially lower the amount and grind involved while removing any problem with common environmental results (Gjedrem, 2005). All of the progeny of bloodstock are essentially "tagged" by the genotyping of their DNA. A non-lethal tissue sample can be used to genetically recognize this youngster (for example, tag recovery), and parentage assessment can be used to determine an offspring's parents. This determines the age and hatchery stock of the tested offspring.

3.7. DNA Fingerprinting:

However, in-stock uses of DNA fingerprinting recognition and observing possible interchange in brood fish (Fjalestad et al., 2003; Sekino et al., 2001; & Alam et al., 2005). Microsatellite and mtDNA sequencing have been employed to evaluate the wild population and breeding of Japanese flounder (*Paralichthys olivaceus*) in contrast to French and Czech breeding populations of common carp (*Cyprinus carpio*) implementing allozymes and microsatellite analysis of the data of genetic diversity perceive a huge distinction among spot of two countries by Microsatellite marker.

3.8. Dynamics of Population:

By using microsatellite markers we can manage the genetic variability and vast diversity of innovational studies. Microsatellites are more fragile than allozymes for the advancement of population dynamics which include statistics bottleneck (Olubunmi et al., 2019). A method to reduce the population of brown trout in Denmark and to give gap microsatellite has been used (Zeng et al., 2017). Microsatellite loci are a benefit due to their high mutation rate that is used in genetic studies of population dynamics.

3.9. Conservation of Fish Stock:

With the help of microsatellite markers, polymorphism is achieved which supplies strong information that is used in the conservation of fish stock (Aida, 1921). For feasible yield and in the conservation of genetic variability molecular markers have informative applications which provide information on the molecular structure of fish species that will help locate distinctive stock and improvement of stock (Tripathi et al., 2011 & Ude et al., 2020). In examining inbreeding desolation microsatellites have essential approaches by detecting particular chromosomal regions that are answerable for inbreeding stress (Zhan et al., 2008). However, microsatellites would become the most suitable with cultivated species within a closed system where parents and their offspring can be controlled and traced. Microsatellite plays a remarkable role in the maintenance of fisheries and the conservation of species in their native populations.

3.10. Identification of Chromosomes:

Microsatellites have been verified successfully in the identification of various chromosome regions consisting of recognized quantitative traits loci gene which impact the capacity of rainbow trout to bear the infection-induced pancreatic Necrosis (IPN), determination of sex regions in channel catfish and also in the identification of relationship with stress-related plasma cortisol level and Basal plasma glucose volume in common carp (Waldbieser et al., 2001; Ozaki et al., 2001; & Tanck et al., 2001).

4. Genetic Diversity in Fishes & DNA Markers Potential

The genetic diversity among species is crucial in their ability to persist and adapt to their changing environments (Soule & Wilcox, 1980). Fish from freshwater and marine environments have more genetic variety than fish from freshwater environments (McCusker & Bentzen, 2010). Mitochondrial DNA (mtDNA) is a key DNA marker in phylogeography and population genetics. The recombination gap and rise in mutation particularly help to signal speciation, highlight the geographical separation of populations by a barrier that is often physical, and indicate whether there is a decrease or increase in population diversity (Avice, 1994). Since there are so many different species of fish, especially marine ones, it is possible to identify genetic hitchhiking in them. Most fish species are monitored using DNA markers for conservation, preservation, and organization (Avice, 1998; Moritz, 1994; Grant & Waples, 2000; Shaklee & Bentzen, 1998; Hauser et al., 2002). Fish have the greatest species diversity among vertebrates. The fish family has more than 32,000 species, and their number is enormous and incalculable. They may be found in various aquatic environments, including deep ocean bottoms at 6000 to 7000 meters and ecosystems in

the mountains at 2000 meters (Fishbase, 2014).

The IUCN Red List of 2014 revealed that 2172 varieties of fish are on the edge of disappearing due to overfishing, the degradation of natural ecosystems, which includes pollution, dam building, and the removal of water bodies for the benefit of humans (IUCN, 2014). Mutations are mostly responsible for the genetic diversity. The pace of evolution will eventually be very low if the mutation rate is low. Genetic diversity across organisms cannot exist if there are no mutations. Point mutations at certain DNA locations identify the genetic diversity. The use of DNA markers has been used to demonstrate these alterations. SNPs are recognized by sequencing genetic material, while mutations are most easily detected by electrophoresis on an agarose gel (Liu & Cordes, 2004). Using molecular markers makes it possible to keep track of and benefit from genetic variation throughout the whole genome. Because of this, the use of molecular markers in fish has made it possible to quickly track advancements in the exploration of inherited traits and closely related species, antecedent establishment, recognition of variety, construction of relationships in genes of fishes that are important for aquaculture, and identification of multi-factorial Loci associated with particular characteristics for marker aided selection.

Genetic indicators of Type 1 were formerly regarded as unsuitable to use for fish genetic research. Still, it is now proven that these indicators or markers have critical significance when dealing with natural populations as well as fish farming. These markers are now essential for comprehending linkage phenomena and for QTL mapping. They are useful for comparative research in genomes and also for the recognition of potentially suitable genes for quantitative characteristics in a variety of fish types used for the aquaculture industry. Numerous studies have looked at the molecular bases of many salmonid species' traits that are important commercially. For instance, when it encounters Atlantic salmon, QTL analysis was done for loci related to resistance to viral infections (Houston et al., 2008), flesh color and growth aspects (Baranski et al., 2010), salt tolerance (Norman et al., 2012), late sexual maturity (Gutierrez et al., 2014), etc. The first set of molecules includes those found in repetitive genomic sequences, PASA (PCR Amplification of Specific Alleles), PCR-RFLP, and SNP (Liu & Cordes, 2004). Only morphological descriptions of Romania's natural Salmonid varieties are known (Emil et al., 2011), and molecular-based research on these species is still in its early stages. Using the 16SrRNA and 12SrRNA sequences of genomes, Romanian salmonid varieties were separated phylogenetically. (Dudu et al., 2011) was one of the rare studies that looked at the genetic differentiation of salmonid fishes using the PCR-RFLP method. Even though the phylogeny and evolution of the salmonid fish family have been extensively studied, many questions remain. As a consequence, despite several investigations employing both morphological (Stearley & Smith, 1993) and genomic data (Kitano et al., 1997; Oohara et al., 1997; Crespi & Fulton, 2004), considering relationships on the genus stage, there persistently exist conflicting views (Dudu et al., 2011). The Coregoninae and Thymallinae subgroups came out of a common origin earlier than Salmoninae (including various genera such as Hucho, Salmo, Oncorhynchus, Salvelinus & Brachymystax), depending on the evolutionary study of early salmonid varieties. According to morphology and genetic proof, Coregoninae and Thymallinae had been believed to be the Salmonidae family's first extensions (Crespi & Fulton, 2004).

5. Microsatellite DNA marker Working

Microsatellites are ranging from 1 to 6 base pairs in size and are unitedly organized (Tautz et al., 1984 & Tautz et al. 1989). Regions that are gene-coded, non-gene sequences, and introns all have shown them (Liu et al., 2001). Microsatellites are the fastest evolving markers, as compared to point mutations they are 10 times faster, per generation mutation rate is 10^{-3} to 10^{-4} (Goldstein et al., 1995), and recent investigation found that in all fish species, they exist usually every 10 kb (Wright, 1993). PIC (Polymorphic Information Content) value of microsatellite markers is high i.e., PIC regards the marker value for determining population polymorphism. Microsatellite gives this benefit besides their excess, high Q polymorphism, small locus size, and even genomic distribution. SSRs are constructed reproducible and highly polymorphic by the processes of uneven chromosomal

recombination and/or slippage of DNA polymerase. Null allele's presence is the main limitation, in spite of the benefits of microsatellite markers. When the SSRs locus acquires mutations at the primer binding regions null alleles arise, but the microsatellite DNA itself not gets mutated. The locus presenting null alleles is usually eliminated.

Shutter or shadow bands are a significant drawback of microsatellite markers, which can result from PCR errors generated by slipped strands (Tautz et al. 1989) or amplification products' incomplete denaturation (Reilly et al., 1999). By radioactive marking of either of the two primers, SSRs are usually studied this way, both primers have a sequence that is complementary on both sides of the repeat unit array. In PCR reaction the repeat unit array is amplified by using these primers. The amplification products are streamed on a sequencing gel to resolve the length difference that results from samples that have a variable number of repeats among themselves. Then the gel is dried and developed overnight usually after X-ray film exposure. In order to determine allele size reference to standards and allelic ladder is used generally such as the M13 sequence. In the genome, these loci are distributed all over and seem to be highly abundant, contrary to some reports considering mini-satellites that in chromosomes telomeric regions tend to be clustered (Jeffreys et al., 1987& 1991; Royle et al., 1988). In DNA extraction of an elementary configuration, PCR analysis largely utilizes micro-tissue quantities, and the chloroform/ phenol steps that are laborious are usually eradicated, therefore in contrast to mini-satellites, SSRs is proportionally facile to isolate (e.g. McGregor et al., 1996). Furthermore, by using imaging systems (e.g. the Hitachi FMBIO system or Molecular Dynamic's STORMTM) and automated fluorescent sequencers per day a significant number of samples can be genotyped (maintaining accuracy and quality), which gives microsatellites the potential to increase the number of samples (O'Reilly & Wright 1995 and O'Reilly et al., 1996). The PCR technology was first developed in 1985, and during the past 100 years, the progress of amplification of DNA by employing the PCR technique has made it possible to study the genetic changes in fish populations during the past century (Ferguson & Denman 1988).

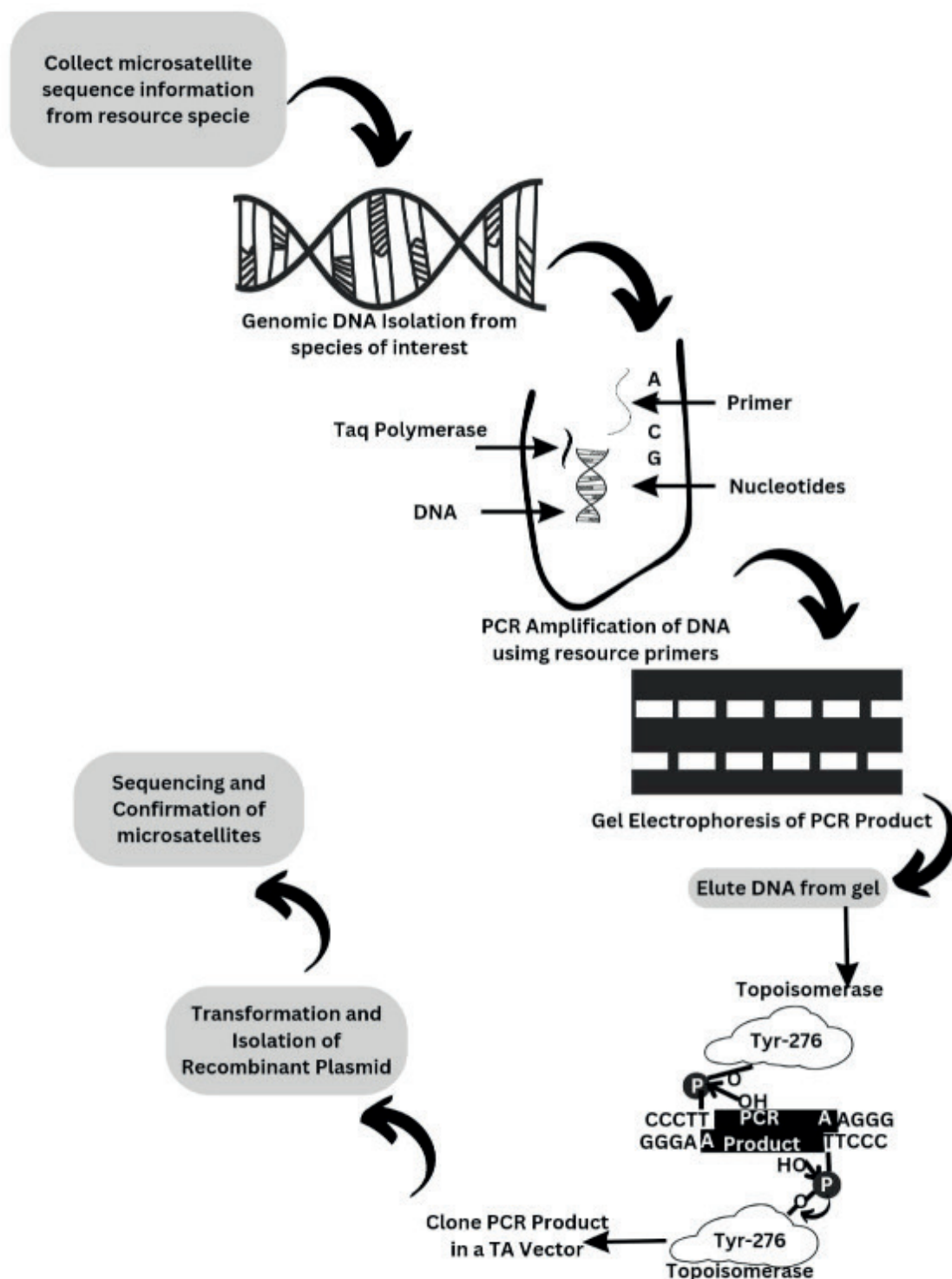


Fig.2. Showing Development of Microsatellite DNA Marker

5.1. Isolation:

Practice explanation is commonly used in the identification of repeat di-nucleotide loci and isolation (O'Reilly & Wright 1995). Shortly after, the DNA is broken down by enzymes, sorted by size on an agarose gel, and by cutting from the gel a particular size range (often it ranges between 300 to 600 bp) is eliminated. A vector such as pUC18 is used to link retrieved DNA. This procedure can be used to produce a partial genomic library and for cell transformation. Then a probe that contains repeating units, such as GT (n), is used to screen the library. The clones that efficiently hybridize with the GT (n) probe are then sequenced, and primers are developed to match unique sequences flanking the array. In spite of its simplicity, some significant moves are there to take,

that can considerably enhance the microsatellite numbers separated from a library.

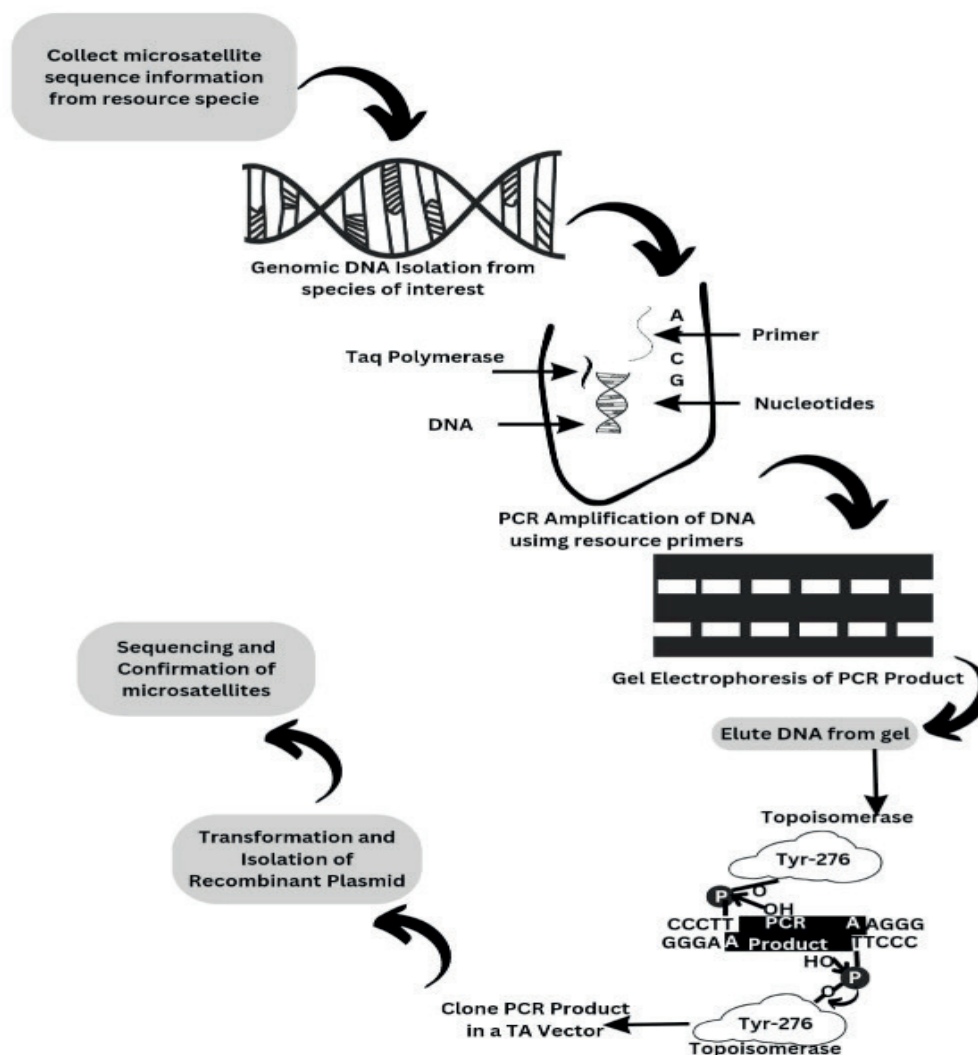


Fig.3. Traditional Method for Development Microsatellite DNA Marker

5.2. Technical Analysis & Potential Problems:

Beneath are technical aspects to consideration and troubles that may arise during microsatellite library creation.

1. It is suggested that during the library construction process DNA of high quality that has undergone purification via an organic extraction protocol.
2. If workable, focus on making a library with a colony including 20,000 or more. Mark down those libraries that can be constructed with less than 50 micrograms of DNA. Nevertheless, the time taken for microsatellite loci primers development can be lowered appreciably by increasing this quantity twofold (specifically if only one enzyme such as Mbot has been digested with the genomic DNA).
3. Prior to progressing with screening and cloning, it is important to examine the genomic digest to be utilized for multiplex copy elements such as Alut elements, accompanied by the single enzymes.
4. It is recommended to retain the size of the PCR product to less than 220 bp to avoid indefinite and vague alleles. If dealing with deteriorated or aged tissue such as scales or otolithor this is specifically significant, as amplification of products larger than 120 bp generally evinces unsuccessful.

5. Except for working on mapping, it is suggested to emphasize the isolation of tri- and tetra-nucleotide primers in various implementations.

6. More critically primer synthesis standard is the base on which the loci amplification capability is dependent than signature (stuttering) patterns. Consequently, it is serious to recognize an authentic supplier and keep away from making changes unless utterly mandatory.

7. To identify the consistent amplification of loci, it is suggested to allot enough resources and time through the development stage. The urge to utilize the first identified polymorphic markers must be ignored.

Progressively workers are shifting to loci that feature longer repeat units to make PCR products that can be examined effortlessly by exercising an automated scoring system and to lower the scoring error. In contrast to dinucleotide-based loci, these loci exhibit notably less stuttering, enabling more precise allele typing. Microsatellite alleles that are dinucleotide are exceedingly seen as a series of bands rather than individual discrete bands.

The phenomenon that is considered to emerge from slipped-strand miss-pairing is called stuttering (O'Reilly and Wright, 1995). Various protocols for enrichment have been set up that give instructions on the isolation of loci based on tri- and tetra nucleotides (Armour et al., 1995; Edwards et al., 1995; Karagyozev et al., 1993; Ostrander et al., 1992; Kijas et al., 1994 & Waldbieser, 1995). Contrasting to the ordinary "shotgun cloning" a technique that is used for the isolation of dinucleotide repeat loci, additional time is significantly needed for employing tri and tetra nucleotide loci, however, utilizing tri and tetra nucleotide loci facilitates remarkably by overcoming any starting developmental problems.

6. Microsatellite genotyping

In Fish Genome, the procedure for determining the individual genetic makeup for each microsatellite locus or Simple sequence repeat is known as genotyping (Guichoux et al., 2011). First, the fish species are collected and the caudal fin from each fish is taken and preserved in -20°C and 70% ethanol solution for further DNA extraction process (Wattanadilokchatkun et al., 2022). As the total genomic DNA of fish is isolated, 1% agarose gel electrophoresis and spectrophotometry vice versa used to identify the quality and quantity of the DNA (Zhang et al., 2016). Due to low DNA quantity, the PCR of stomach specimens failed and Microsatellite genotyping is only successful for dorsal fin, caudal fin, or muscle tissue samples of fish (Choi et al., 2021). For microsatellite genotyping, you need to design specific primers, amplify the microsatellite regions, and test for Polymorphism (Vieira et al., 2016).

6.1. Specific primer design:

Microsatellite markers are used for testing genotyping of fish. In order to fulfill this purpose first SSRs primers are designed for a closely related species. In SSRs primer it is mandatory to have nucleotides sequence first (Vieira et al., 2016). By the application of the Microsatellite identification (MISA) tool, SSRs motifs can be determined. SSRsL locator v1.0 is used to design the locus-specific primers. The primers can be forward primer or Reverse primer. They can be formed manually or automated (Vieira et al., 2016). A fluorescent dye marks the 5-end of the forward primer in each primer pair (Choi et al., 2021).

6.2. Amplification of microsatellite:

To confirm the effectiveness of the SSRs primers, it is necessary to perform PCR amplification. The SSRs-PCR reaction mixture, which has a volume of 20 µL, contains DNA polymerase, a reaction buffer with a concentration of 1x, a forward primer with a concentration of 0.5 µmol L⁻¹, dNTP mix with a concentration of 40 µmol L⁻¹, MgCl₂ with a concentration of 2 mmol L⁻¹, a reverse primer with a concentration of 0.5 µmol L⁻¹, and 50 ng of genomic DNA. The PCR instrument runs

the thermal cycling with these steps: 5 min of initial denaturation at about 94°C, 36 cycles of 50s denaturation at 94°C, 1 min annealing at optimal temperature, and 1 min extension at 72°C, and a final extension of 7 min at 72°C (Chowdhry et al., 2021). On a 1% agarose gel, the products of PCR were separated by electrophoresis. To ensure accuracy, the PCR amplification process is repeated at least three times for each sample, which helps to minimize the impact of any erroneous alleles. Once the PCR amplification products are fluorescently labeled, they are genotyped using an automated sequencer such as the ABI 3730XL from Applied Biosystems (Applied Biosystems, Foster City, CA, USA). This is typically done at the DNA sequencing service of a specialized laboratory such as Macrogen, Inc. The data obtained from the sequencer is then analyzed to determine the particular sequence of alleles present in the sample being tested. The determination of allelic size was carried out through the utilization of Peak Scanner version 1.0 software from Applied Biosystems, based in Foster City, California, USA. The genotypic data resulting from this process has been archived in the Dryad Digital Repository (Wattandilokchatkun et al., 2022).

6.3. Polymorphism testing:

By using PAGE (polyacrylamide gel electrophoresis) or agarose gel electrophoresis, the PCR results are visualized. PAGE genotyping provides good resolution however it is labor intensive. For conducting finely detailed analyses, specific SSRs primers that have been labeled with a fluorescence dye can be produced. These primers facilitate genotyping via capillary electrophoresis, which can be carried out using specialized instruments such as the ABI 3730 DNA Analyzer (Saha et al., 2017). In this scenario, the amplified DNA fragments obtained through PCR are introduced into capillaries filled with a polyacrylamide matrix. Subsequently, electrophoresis takes place within these capillaries, during which the emitted fluorescence is detected. This fluorescence data is utilized to determine the molecular weight of each PCR product. A visual representation called an electropherogram is created, displaying distinct peaks of luminescence that correspond to the amplified alleles (Saha et al., 2017). Microsatellite Genotyping helps to figure out the polymorphism of fishes by identifying the genetic diversity in fishes such as Siamese fighting fish as well as freshwater fishes and marine fishes (Wattanadilokchatkun et al., 2022).

7. Genome mapping

Genome mapping is used to record the synchrony, sequence, and spacing of genes or genetic markers present on chromosomes. The genome maps provide homologous regions in one species to be observed with mapped genes in another, in addition to this they also make it simpler to map genes and markers which are undetermined in their placement. The position of markers or genes is offered by genome mapping with reliable proof. Thus, it is possible to identify quantitative genes that have value for commerce using genetic markers, and this is exactly what has been done. These genes can be recognized being single genes that are transmitted according to the Mendelian manner. Other than that, they might refer to sections within a genome that have been identified as most responsible for the quantitative variability in a gene. Certain physical attributes and resistance to diseases may be regulated by only one gene, but numerous traits with commercial significance are influenced by several genes with modest combined effects.

AFLPs are an additional popular genetic marker for gene mapping. Although it is challenging to construct microsatellite markers, AFLP has in fact been chosen for certain crustaceans (Moore et al., 1999; Davis and Hetzel, 2000). One of the reasons AFLPs are so attractive is because a genetic map can be developed in a couple of months, instead of years. According to Young et al. (1998), a rainbow trout linkage map of roughly 500 markers has been made available, with most of the markers belonging to the AFLP category (Young et al., 1998). For lineage analysis and QTL mapping, AFLP markers might have some disadvantages because many of them seem to be expressed dominantly. The quantification technique of PCR known as densitometric PCR will be required to differentiate between homozygous and heterozygous genotypes due to the absence of co-dominant allelic expression for these areas (Ferguson and Danzmann, 1998). Microsatellite

loci are considered to be better than AFLPs and RAPDs also, for genome mapping as well as when looking for QTL (Sakamoto et al., 1999), despite being utilized more time and technically challenging to develop. This is because the first kind is co-dominantly inherited, while the second type inherits itself as recessives and dominants also (Okumuş et al., 2003).

7.1. QTL Mapping:

QTLs (Quantitative Trait Loci) are identified by evaluating phenotypes via linked marker maps. The process to detect markers associated with QTLs can yield notable advantages for traits that can be costly or challenging to quantify (such as feed conversion), are quantified only for one sex (like fecundity), can solely be calculated after the time of choice (reproductive traits), can solely be calculated after homicide the specimen (flesh quality), or might just be calculated on fish that are raised in an environment apart from the primary breeding stock (disease resistance) (Davis and Hetzel, 2000). Monitoring of genetic diversity and proximity among individuals, strains, and families is the goal of QTL mapping, and the results are used to inform marker-assisted operations aimed at improving production-related traits (Fjalestad et al., 2003). Genetic map construction enables the recognition of QTLs as well as the identification of markers for QTLs. Numerous technologies are accessible (Park and Moran, 1995) and might be implemented (Poompuang and Hallerman, 1997). By extracting a significant number (often more than 100 loci) of extremely diverse markers (typically microsatellite loci, AFLPs, or RAPDs), this method looks for the association among numerous production traits in each allele separately and in combination. Hypervariable microsatellite regions are perhaps the most potential way to generate molecular markers. Depending on known mapping studies, they seem to be extensively distributed in fish genomes and various in the majority of fish species.

8. DNA Markers Potential in Conservation of Fish Diversity

The research of microsatellite markers will allow the administration of fisheries and the preservation of organisms in their native populations, which offers helpful knowledge on fish species' genetic diversity and stock organization. Differentiating a species into genetically distinct populations is an important phase in evolution. This process is affected by biological, physical, and geographic hurdles as well as migration, selection, and genetic drift. Small and declining populations of endangered species make inbreeding and genetic diversity loss inevitable. Because genetic diversity is being lost and inbreeding is increasing, populations' capacity to adapt to environmental change is being lowered; Frankham (Kounnamasa, 2021) hypothesized that these genetic traits would increase the danger of extinction, particularly in vulnerable species with tiny populations. The low diversity of genes (hybrid deficit and divergence from Hardy-Weinberg equilibrium) and hereditary (positive value of FIS) in population genetic analysis demonstrates the effects of a genetic bottleneck brought on by overexploitation and habitat destruction. (Ranjan, 1997) "Supportive breeding" may increase an endangered species' natural numbers. A portion of the wild parents are used in this program to breed in captivity, and the offspring are then returned to the wild.

- In the holding facility, fish species progeny from various rivers must be recognized and stored in separate pools.

- Additionally, there shouldn't be a cap on the size of an effective breeding population or the sex ratio. A wide variety of courses in different lengths or decades provided at various times are preferable to a single class in a single size and year.

- To enhance the quantity of high-yielding communities and aid in the improvement of fish populations that are at risk of becoming extinct, cryo-preserved milt which was previously collected from multiple males and pooled could be applied. Gene banking using sperm cryopreservation is significantly more affordable, simple to operate, and less susceptible to the risk imposed by system failure or death related to illness than the captive breeding program. As a result, it ought to be a helpful addition to the captive breeding effort.

- The same rivers from which the genetic stocks are gathered should be used for independent breeding and ranching of the various genetic stocks.
- River segments with a resident population or those potentially acting as a sanctuary may be chosen for fish population ranching.
- For evaluating the effects of ranching, experimental fishing should be utilized from tracking parameters like catch per unit effort/area.

It is important to monitor the abnormalities in the genetic code or rate of alleles, particularly the incidence of uncommon allelomorphs throughout time (Willi et al., 2022, & O'Connell et al., 197). The basic genetic profiles of representative fish stock samples from the holding facility and the ranching zones should be preserved. To more fully evaluate the influence of genetic diversity, the baseline information obtained in this research by utilizing microsatellite markers may be beneficial. Because of their special qualities, including their high polymorphism, reproducibility, co-dominance, strong discriminatory power, specificity in PCR-based assays, wide distribution in the genome, and extraordinarily high allelic variation, microsatellite markers are extremely helpful. They are quickly becoming indispensable in fish biotechnology (Chao et al., 2022, & Olubunmi, 2019). Fish biotechnology uses it for various purposes, including genetic fingerprinting (Schlötterer, 2000). Using microsatellite markers will improve the management of wild fish populations (Agbebi et al., 2013). Managing wild fish populations, including trout and salmon, would benefit tremendously from approaches based on DNA markers at scale emerging from genetics of population and conservation. SSRs are the preferred markers in aquaculture for genetic management of cultured livestock in light of breeding projects, as they allow for the design of good crosses, the selection of genetically improved stocks, the reduction of inbreeding, and an increase in selection response (León-Bernabeu et al., 2021).

9. Expediency in Monitoring Fish Diversity

Microsatellite markers offer lots of advantages over other molecular markers like allozymes and mitochondrial DNA (mtDNA) and also provide more effective information than mtDNA or allozymes-type markers. These Mendelian inherited microsatellite molecular markers have a codominant nature (can distinguish heterozygotes from homozygotes), have high variability, are easy to isolate, and are highly polymorphic. All these properties make them very preferable, highly valued, and ideal for studying pedigree analysis, population composition, and stock structure determination in fisheries (Grover et al., 2016). These markers have the capability of observing the genetic variations and differences among very closely related species and can distinguish between genotypes (Gowrimanohari Rakkannan 2022). Thus, they provide an effective substitute for other markers in determining the sources of hybrid populations of migratory fish specimens. For QTL (Quantitative Trait Loci) identification and genome mapping, where lots of loci are needed (Okumus et al., 2003).

In the past decade, the use of DNA satellites to diagnose genetic mutations and deletions has increased. These markers are effective for genetic stock characterization, gene mapping, broodstock selection, mapping metric traits, and mapping the genetic loci associated with these traits in the fields of aquaculture and fisheries. (Xiao-Gu et al., 2006). These microsatellite markers provide very useful and effective information for developing efficient strategies for population analysis, fisheries stock management, and biodiversity conservation (Gowrimanohari & Rakkannan, 2022). The unique features of microsatellites have resulted in improvements in the fields of digital information retention and the development of automated detection and diagnostic mechanisms like fluorescence imaging equipment (O'Connell et al., 1997). Simple sequence repeats loci containing a huge number of alleles are very effective for the identification of population genetics, phylogeny, and mixed population parent-offspring (Gowrimanohari Rakkannan, 2022).

In short, microsatellite DNA markers can be used for identifying and monitoring genetic

variations within and between stocks, for pedigree analysis, parentage determination, and examining potential genetic changes in fish stocks (Mojekwu et al., 2020) and provide effective information for developing strategies for fisheries stock conservation and management.

10. Microsatellite Limitations

Despite the certain capability of microsatellites in fish biotechnology, they have several limitations in their application. Chance of error is one of the principal considerations in all data sets, involving genotyping errors that in population genetics possibly can prejudice the ultimate conclusions (Bonin et al., 2004). Several variables are reviewed that might produce microsatellite genotyping errors, such as contamination or Taq polymerase error, and reagent quality. Misinterpretation of banding patterns of alleles is the major outcome of those errors (Pompano et al., 2005).

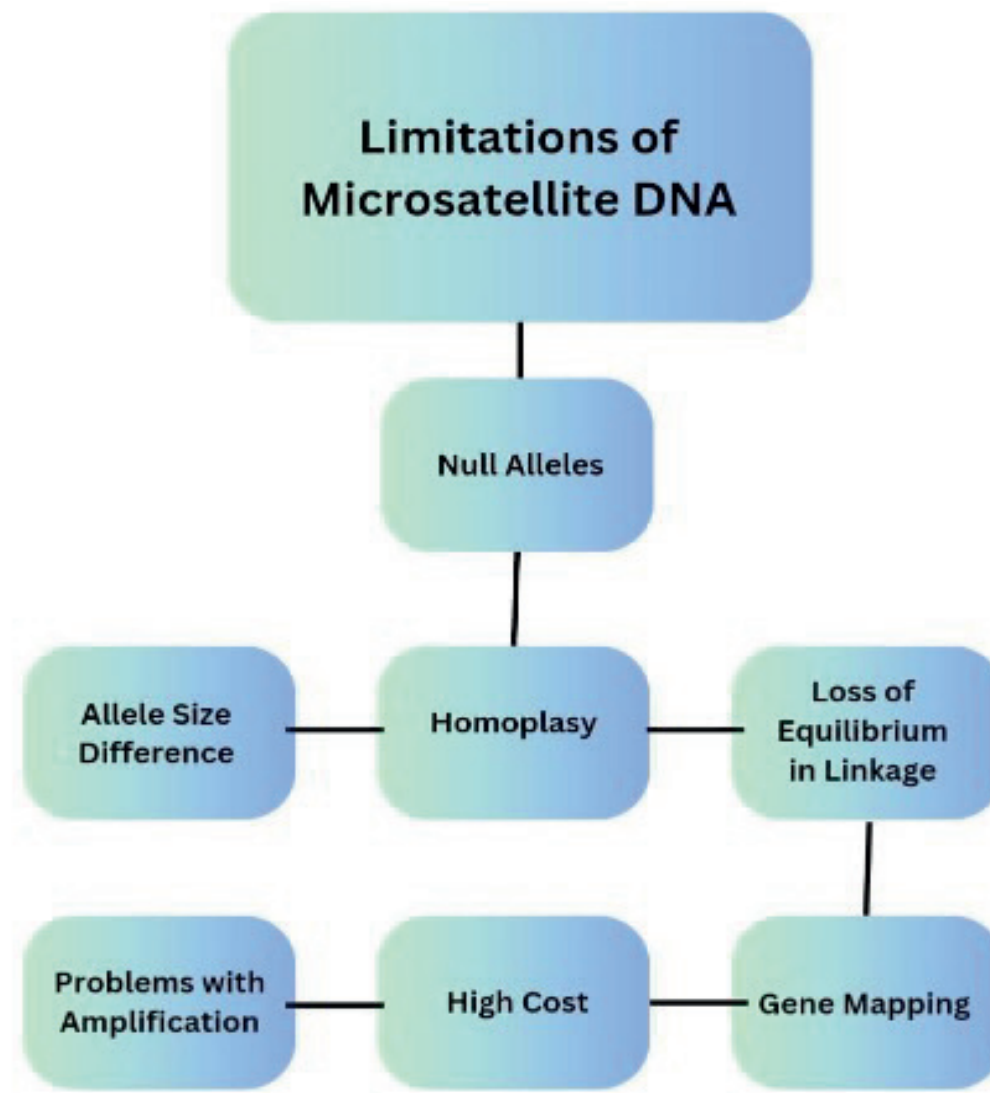


Fig.4. Flow- Sheet diagram showing Microsatellite DNA Markers Limitation

Limitations of Microsatellite Markers:

10.1. Null alleles:

Recognition of heterozygotes and amplification of locus can be prohibited by mutation or deletions of the locus in the annealing primer site and conduct to incorrect assessment of segregation rates and frequency of allele. This issue can probably be settled by primer redesign.

- An additional main barrier in the employment of microsatellite markers is null allele's

presence, which are alleles that fail to amplify during PCR reactions (O'Reilly et al., 1995).

10.2. Allele Size Difference:

Provided with alleles of a microsatellite locus that have slight dissimilarities in size between them (like a locus that is constituted by repeats of di-nucleotide as small as 2 base pair) can appear as a outcome of slippage of polymerase in the time of replication.

10.3. Homoplasmy:

Jarne & Lagoda described the homoplastic alleles as alleles that don't share the same ancestral origin but share the same length (Jarne & Lagoda 1996). Furthermore homoplastic alleles can share both sequence and same length, yet have divergent evolutionary histories (Anmarkrud et al., 2008). Unfortunately; homoplasmy can direct the true genetic divergence to underestimations in between the populations, because it is overlooked. The identification of evolutionary history difference may only possible by the use of pedigree that is documented mutations, while sequence difference can be detected by sequencing.

- The existence of homoplasmy can generate bias into genetic analyses of natural populations; consequently impediment is produced in efforts to identify conservation units (Curtu et al., 2004).

10.4. Loss of Equilibrium in Linkage:

Extensive breeding and sub-structuring of population mainly results in non-random distribution of alleles in a population (Weising et al., 2005). Particularly for paternal exclusion and population studies it is significantly causing troublesome. The issues can be identified using several techniques such as offspring analysis and computer programs.

10.5. Gene mapping:

The presence of fragments of unknown DNA makes the use of microsatellite detrimental in gene mapping (Mojekwu et al., 2012). Moreover, when utilizing primers produced from one taxon to investigate phylogeny, they may not be useful on all the taxa whose genotype is being analyzed.

10.6. High cost:

Furthermore pitfall of microsatellites usage involve high cost that is associated with species-specific primers production, as well as the labor-intensive procedure that demands considerable personnel time for primer development (Telles et al., 2010).

10.7. Problems with amplification:

Portion of the genome with an adequately prominent mutation rate that multiple versions (alleles) occurs in a designated population, must be determined in order to discover a functional DNA marker locus. Furthermore, this area should be located next to a low mutation rate, extend of DNA that can tie up PCR primers in nearly all individuals of the species, approaching nearly 100%. Few individuals have alleles that maybe unsuccessful to amplify at all or few individuals have one amplified allele only (Paetkau & Strobeck 1995).

The effect of the error fluctuates following the usage of the data. Usually, homoplasmy is not an important issue in population genetic analyses (Estoup et al., 2002), with the exclusion of markers that are hyper-mutable and the slippage rate they exhibit is high (Anmarkrud et al., 2008). The rate of inaccurate paternity restriction surpasses 20% even with error rates per allele as minimal as 0.01, demonstrating that even little errors can have a significant impact (Hoffman & Amos2005).For genotyping errors there are a great deal of informatics tool that have been listed, are considered for such errors (listed by Pompanon et al., 2005).The error rates are analyzed by a protocol that is proposed by these authors, which should be employed to ensure the dependability

of published genotyping research.

Conclusion:

Taking everything into account, this review provides comprehensive details on the role & potential of microsatellite DNA markers for monitoring genetic diversity in fishes using. Measuring genetic diversity helps in the understanding and effective management of fish stock. Microsatellite markers are based on DNA sequence polymorphisms and are pivotal in determining the genetic variation/diversity in fishes. Microsatellite loci are widely distributed in eukaryotic genomes, and each locus has a unique DNA sequence consisting of single DNA and DNA repeat motifs. Microsatellites are reliable, cost-effective and easy to isolate and these attributes make them valuable and suitable genetic markers for performing various functions, including Parentage and Pedigree Analysis, Conservation of Fish Stocks, Gene Tagging/mapping, determination of genetic diversity within and among population, DNA Fingerprinting, population genetics, and developing efficient strategies for fish conservation. These markers can provide essential information and are more efficient than mtDNA or allozymes type markers. Thus, provide an effective alternative over other markers for keeping track of the sources of mixed populations of migrating fish specimens, identifying Quantitative Trait Loci and genome mapping, which necessitate a large number of loci.

Preserving genetic diversity is essential to ensure that the stocked fish attain their maximum potential, making the species to get adapted to their changing environment. Genetic Variation results from mutational changes, migration, selection and breeding strategies. Both genetic variants intraspecific and interspecific exist. Genetic markers monitor and manage the genetic diversity of an organism. In addition, PCR products must be limited to 220 bp to reduce selectivity and vague alleles. Working of microsatellites involves Polymerase chain Reaction amplification of loci flanking repetitive sequences by using fluorescent primers. The microsatellite genotyping system mostly involves developing primer sequences that are specific to the targeted microsatellites, amplifying the microsatellites to generate enough material for analysis, and confirming the presence of polymorphism by conducting assays to detect variations between samples.

The main limitation of microsatellites is the presence of null alleles that fail to amplify. Non-random allele distribution in a population and presence of unknown DNA fragments can cause troublesome. Furthermore, species-specific primers production associated with high cost is another limitation. Microsatellite markers provide opportunities for different types of research to identify and track genetic variation within and among fish species and provide better information for conservation strategies. Simple sequence repeats are the most preferred markers in fisheries and aquaculture for monitoring the genetic diversity of stocks breeding programs, as they along with other genetic tools and technologies can be integrated for developing efficient strategies for the conservation and rehabilitation of genetic diversity in fish. Microsatellites can be the most unusual candidates for novel and unique marker systems that may be useful and can potentially replace allozymes markers for monitoring genetic diversity in fish.

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