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Mini Review Article

Microsatellite Markers in Fisheries

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Abstract

Simple Sequence Repeats (SSR) or microsatellites are the genetic markers which are highly abundant and evenly distributed in eukaryotic genome. They have become the ideal markers for a wide range of population genetic, conservation, and evolutionary biology applications. Microsatellites are highly polymorphic because they have many alleles that are highly variable among individuals. Polymorphism is produced by having various numbers of tandem repeat motifs, which results in size variation that may be observed using PCR using pairs of locus-specific flanking primers and electrophoresis of the amplified product.

Keywords: Polymorphism; Locus-Specific Flanking Primers; PCR; Electrophoresis

Introduction

A molecular marker is a DNA sequence used to "mark" or track a particular location (locus) on a particular chromosome, i.e., marker gene [15]. Molecular markers are categorized under two types: protein and DNA markers. DNA markers are most preferred and advantageous over protein markers. DNA markers can be found in repetitive and non-coding sequences, independent of environment, developmental stage or expression and most of them are selectively neutral. The four types of DNA markers are RFLP (Restriction Fragment Length Polymorphisms), RAPD (Randomly Amplified Polymorphic DNA), Microsatellite or Simple Sequence Repeats (SSRs), AFLP (Amplified Fragment Length Polymorphisms). Molecular markers are further classified into two categories such as Type I and Type II markers. Type I markers are associated with genes of known function e.g., Allozyme markers and RFLP. Type II are the markers with genomic segments of unknown function e.g., RAPD, AFLP and microsatellite markers [15]. Amidst all the molecular markers, microsatellites have recently become a very common marker type in a wide range of genomic studies [9].

Molecular markers are useful in many aspects of aquaculture. The development and application of DNA marker technologies already underway in other areas such as molecular systematic, population genetics, evolutionary biology, molecular ecology, conservation genetics and seafood safety monitoring will certainly impact the aquaculture industry in unexpected ways [1].

Microsatellite makers

Microsatellites are multiple copies of tandemly organized simple sequence repeats (SSRs) ranging in size from 1 to 6 base pairs [8,21]. Microsatellites are widely distributed across the eukaryotic genome [20] and referred as "simple sequence repeats" (SSR) [21] and "short tandem repeat" (STR) [3,7,23]. They have been discovered in gene coding regions, introns, and non-gene sequences [10]. Microsatellites mutate at a rate of 10^{-3} to 10^{-4} per generation

makes them a fastest evolving markers, which is 10 times faster than point mutations [5] and found to exist as frequently as once every 10 kb in all fish species investigated to date [24]. Microsatellites are inherited as codominant markers in a Mendelian method, i.e., both alleles in a heterozygote individual are expressed in the analysis [12]. Microsatellite markers have high PIC (Polymorphic Information Content) value i.e., PIC refers to the value of a marker for detecting polymorphism in a population. In addition to their abundance, even genomic distribution; small locus size, and high polymorphism, microsatellite markers provide this advantage. The DNA polymerase slippage and/or uneven chromosomal recombination processes made SSR highly polymorphic and reproducible.

Despite the advantages of microsatellite markers, main constraint is the presence of null alleles. Null alleles occur when the primer binding regions of the SSR locus gets mutated but not in the microsatellite DNA itself. Mostly the locus exhibiting null alleles are discarded. Another important disadvantage of microsatellite markers is shutter or shadow bands. Shutter bands occur due to slipped strands impairing during PCR [21] or incomplete denaturation of amplification products [17].

Identification and development of microsatellite markers

Different bioinformatics tools/algorithms are used to identify SSR markers such as MISA, SSR Locator, SSRIT, MSATCOMMANDER and GMA To which are available on a web interface and provide an appropriate way to identify microsatellite markers [19]. The SSR markers were discovered using cDNA cloning and sequencing. The database contains a significant number of expressed sequence tags (EST)-SSRs for fish species [11]. Microsatellite loci are conservative in their flanking regions and can persist fast rate of evolution. Primers developed for a species from the flanking regions of a microsatellite locus can be used to amplify the same locus in other related species [25]. Alleles at microsatellite loci can be amplified from tiny quantities of genomic DNA using the polymerase chain reaction [18], but each microsatellite locus must be identified and its flanking region sequenced before PCR primers can be designed. Individual alleles at a locus have varied numbers of tandem repeats and can thus be distinguished using electrophoresis (typically PAGE) based on their size. Different numbers of repetition units distinguish different alleles at a locus. The alleles are isolated and correctly sized as one or two bands on a polyacrylamide gel, and they are used to measure genetic changes within and between species populations [15]. In genetic studies of linkage in families and linkage disequilibrium stand dies in populations, five alleles with different repeat length microsatellites can be employed as markers. They also have taken fit allowing the use of minute or degraded DNA [16].

Application of microsatellites in fisheries and aquaculture

Microsatellites are useful molecular markers in Fisheries and Aquaculture for genome mapping studies and Identification of Quantitative Trait Loci (QTL) [13,14]. SSR loci with very high number of alleles per locus makes them useful for parent-offspring identification in mixed population and more suited for population genetics and phylogeny [4,15]. Microsatellite markers exhibits high variability of all genetic markers makes them very useful for conservation of biodiversity and effective population size [17], inbreeding [22], studies of kinship and behavioral [2], gene flow analysis [6].

Conclusion

The best marker systems for a given program must be chosen on a case-by-case basis and will be determined by a variety of factors, including the availability of technological platforms, marker development costs, species transferability, information content, and simplicity of documentation. Microsatellites are highly effective genetic markers for study of genetic variation among closely related species, determining fish stock structure and pedigree analysis. Microsatellite markers analysis provides effective information on developing conservation strategies for fisheries and aquaculture management. This, in addition to the other technologies, Captive breeding and sperm cryopreservation can be combined into a package for genetic diversity conservation and restoration of fish populations in their natural habitats.

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