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MICROSATELLITE MARKER AND ITS UTILITY

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INTRODUCTION

A **microsatellite** is a tract of repetitive DNA in which certain DNA motifs (ranging in length from 2–5 base pairs) are repeated, typically 5–50 times. **Microsatellites** are used in population genetics to measure levels of relatedness between subspecies groups and individuals.

Microsatellites:-

- The term microsatellite was first coined **by Litt and Luty** (1989) **.**
- Microsatellites are **simple repeats.**
- Motifs consisting of **1 to 6 base pairs.**
- The mutation rate of this type of genetic marker has been estimated to be between **10^{-2} and 10^{-4}** per generation.
- They are inherited in a Mendelian fashion as codominant markers. This microsatellite markers co-segregate in to the offsprings during F_1 development . So, we can use these markers to screen the F_1 plants.
- Flanking region is highly conserved in related species.

Classification of microsatellites:-

- **Based on the arrangement of nucleotides in the repeat motifs-**

- Perfect – GAGAGAGGAGAGAGAGAGA
- Imperfect-CACACACACA---CACACACA---CACACACA
- Compound - CACACACACACACA CATACATACATA CATACATACATA

Based on the number of nucleotides per repeat-

- Mononucleotide- AAAAAAAAA
- Dinucleotide- GTGTGTGTGTGT
- Trinucleotide- CTGCTGCTGCTG
- Tetranucleotide- ACTCACTCACTCACTC
- Pentanucleotide- AAATTAAATTAAATTAAATT
- Hexanucleotide- CTTTAACTTTAACTTTAACTTTAA

Based on location of SSRs in the genome

- Nuclear (nuSSRs) – present in nuclear genome.
- Chloroplastic (cpSSRs) – present in chloroplast genome.
- Mitochondrial (mtSSRs) – present in mitochondrial genome.

Advantages of microsatellites as genetic markers

- **Locus-specific** (in contrast to multi-locus markers such as mini-satellites or RAPDs).
- **Codominant** in nature**.**
- **PCR-based**.
- Highly **polymorphic in nature**.

Definition of dominant and codominant marker

Dominant marker:-

A marker is called dominant if only one form of the trait (which is targeted to be marked) is associated with the marker, whereas the other form of the trait is not associated with any marker. Such markers cannot discriminate between heterozygote and homozygote marker allele.

R M1 R M1 R M1 S

Homo1 Homo2 Hetero

Monomorphic phenotype

M1 M1 M1-

R M1 S

If only one form of the trait (which is targeted to be marked) is associated with the marker whereas the other form of the trait has no marker

It can not discriminate between heterozygote and homozygote individuals

Co-dominant marker:-

A marker is designated as co-dominant if both forms of the trait (which is targeted to be marked) are associated with the marker. It can discriminate between heterozygote and homozygote marker allele.

Genesis of Co-dominant markers

R M1 S M2

If both forms of the trait (which is targeted to be marked) is associated with the marker

It can discriminate between heterozygote and homozygote individuals

R M1 R M1 R M1 S M2

Homo1 Homo2 Hetero

Polymorphic phenotype

M1M1 M2M2 M1M2

- **Simple sequence repeat (SSR) marker:-**

Microsatellites or Simple sequence repeats (SSRs) provide fairly comprehensive genomic coverage. They are amenable to automation, they have locus identity and they are multi-allelic. Many agronomic and quality traits show quantitative inheritance and the genes determining these traits have been quantified using Quantitative trait locus (QTL) tools. SSR markers have wide applicability for genetic analysis in crop improvement strategies. They are widely used in plants because of their abundance, hyper-variability, and suitability for high throughput analysis.

- **Principles of SSR:-**

Length of the repeats are not same in all the genotypes, it varies. Designed SSR primers can bind to its complementary sequence in different locations of the genome and in PCR it amplifies products of different length. After electrophoresis we can easily separate the product of different sizes and we can distinguish the varieties named 1, 2, 3 in the figure.

- **Types of SSR**

(A) Genic SSR

- Quickly obtained by electronic sorting
- Transcribed region of genome
- Putative function known
- Mostly concentrated in gene rich region

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(B) Genomic SSR

- Transcribed and non transcribed region

Comparative advantages of genic SSR over genomic SSR

Genomic SSRs(Limitations)	Genic SSRs (Advantages)
High time and cost	Quickly obtained by electronic sorting
Transcribed and non transcribed region of genome	Transcribed region of genome
Function not known	Putative function known
Less transferable	More transferable
Clustered near centromeres	Mostly concentrated in genomic region

Mutation mechanisms and mutation rates in Microsatellite markers

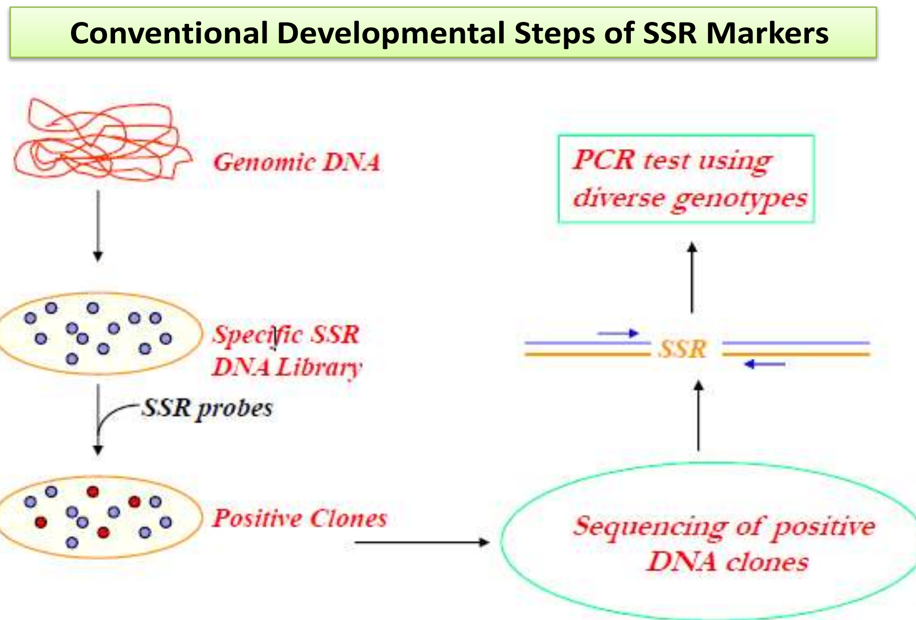
Unlike point mutations, which affect only a single nucleotide, microsatellite mutations lead to the gain or loss of an entire repeat unit, and sometimes two or more repeats simultaneously. Thus, the mutation rate at microsatellite loci is expected to differ from other mutation rates, such as base substitution rates. The actual cause of mutations in microsatellites is debated. One proposed cause of such length changes is replication slippage, caused by mismatches between DNA strands while being replicated during meiosis. DNA polymerase, the enzyme responsible for reading DNA during replication, can slip while moving along the template strand and continue at the wrong nucleotide. DNA polymerase slippage is more likely to occur when a repetitive sequence (such as CGCGCG) is replicated. Because microsatellites consist of such repetitive sequences, DNA polymerase may make errors at a higher rate in these sequence regions. Several studies have found evidence that slippage is the cause of microsatellite mutations. Typically, slippage in each microsatellite occurs about once per 1,000 generations. Thus, slippage changes in repetitive DNA are three orders of magnitude more common than point mutations in other parts of the genome. Most slippage results in a change of just one repeat unit, and slippage rates vary for different allele lengths and repeat unit sizes. and within different species. If there is a large size difference between individual alleles, then there may be increased instability during recombination at meiosis. Another possible cause of microsatellite mutations are point mutations, where only one nucleotide is incorrectly copied during replication. A study comparing human and

primate genomes found that most changes in repeat number in short microsatellites appear due to point mutations rather than slippage.

❖ Microsatellite marker development

✍ Two general strategies are used to identify and create microsatellite markers

1) Constructing and screening genomic libraries with probes complementary to microsatellite sequences
2) Searching for sequences containing microsatellites within databases



Couch *et al.*,1997

Abundance of DNA markers discovered and developed in rice.

Crop	Genome Size	RFLP	RAPD	AFLP	SSR	SNP
Rice	415-460	3553	133	1062	12992	5418373

www.ncbi.nlm.nih.gov , Gramene web browser (http://www.gramene.org)

Recommended websites for microsatellite markers

Gramene web browser http://www.gramene.org	Gramene is a data resource for comparative genome analysis in cereals: rice, wheat *etc*. It provides comprehensive and in-depth information regarding markers used for mapping plant species such as RAPD, SSR, AFLP and RFLP.
MSU rice genome annotation projct http://rice.plantbiology.msu.edu	This website provides genome of rice and annotation of the 12 rice chromosomes

Miah *et al.*,[18] *2013*

Invention of Microsatellite markers enhances the process of crop improvement. Among the microsatellite markers SSR markers are the most exploited ones, which are used extensively in the modern plant breeding process.

Use of microsatellite markers in crop breeding

- Analysis of genetic diversity.
- Population genetic structure.
- Genome mapping, Linkage mapping, QTL mapping.
- Germplasm conservation.
- Crop breeding programme - variety development, genetic purity identification.
- Association mapping

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