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THE IMPORTANCE OF ISSR PRIMERS

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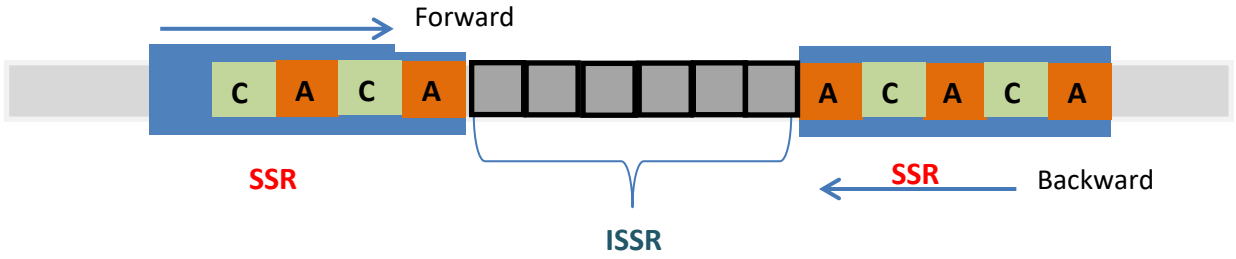
The genetic diversity of their populations must be considered while developing an approach for the protection of rare and endemic organisms. ISSR analysis (Inter Simple Sequence Repeats), is a PCR based molecular technique which uses primers typically 16 to 25 bp long and consists of tandem short 2-4 nucleotide repeats and is anchored at the 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences. This is one of the most popular methods for detecting genetic polymorphism in organisms that does not require knowledge of the nucleotide sequences of the genome. According to studies, ISSRs offer a potent, quick, easy, repeatable, and affordable way to access genetic variation among closely related cultivars, characterize accessions, and identify cultivars and varieties (Kumar et al., 2016).

ISSR are DNA fragments of about 100-3000bp located between two adjacent SSR repeats .



An ISSR primer is usually 16-25bp in length, comprising repeated DNA motifs 2-4bp each.

Types of ISSR Primers

Unanchored primer-Primer consist only of a repeated motif:

5’-anchored primer- Primer consist of a repeated motif with one or several non-motif nucleotides at the 5’-end

3’-anchored primer- Primer consist of a repeated motif with one or several non-motif nucleotides at the 3’-end

The markers generated by non-anchored primers are called single primer amplification reactions (SPARS) or microsatellite-primed PCR (MP-PCR).

The markers generated by anchored primers have been called inter-SSR PCR, anchored simple sequence repeats (ASSR), and anchored micro-satellite-primed PCR (AMP-PCR) or – amplification (ISA).

Modifications in ISSR Primers

Unanchored ISSR primers shows good band in gel when the motifs consist of tri or penta-nucleotides. But when they consist of dinucleotide motifs, smears will be formed on the band. Again in case of anchored ISSR primers, 5' and 3' anchored primersare used in both the ends of 5'anchored ISSR and 3'anchored ISSR respectively. To overcome such difficulties two modifications are made which are as follows –

- A 5' anchored SSR primer can be used in combination with a RAPD primer to yield markers termed as Randomly Amplified Microsatellite Polymorphism (RAMP or RAMPO; Wu *et al*. 1994)
- Another modification is termed as Selective Amplification of Microsatellite Polymorphic Loci (SAMPL), microsatellite based primers are combined with the AFLP primers in the AFLP procedure to yield markers that are regarded as improvement over SSRs

Advantages of ISSR Markers

- ISSR markers are more reproducible than RAPD
- These are easy to use, cheaper and have high throughput
- They yield multiple polymorphic loci
- A prior knowledge of the template DNA sequence is not required
- Generally, ISSR markers are dominant, but the use of a larger 50 anchored primer can yield co dominant ISSR marker.

Disadvantages of ISSR Markers

- They aren't highly reproducible
- Some primers generate poorly reproducible band patterns
- Dominant markers

Designing of ISSR Markers

ISSR are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16-18bp). About 10-60 fragments from multiple loci are generated simultaneously, separated by gel electrophoresis and scored as the presence or absence of fragments of particular sites.

Application of ISSR markers

- Studying genetic identity
- Studying parentage
- Clone and strain identification
- Taxonomic studies of closely related species
- Gene mapping studies

Conclusion

The abundant microsatellites that are present throughout an organisms genome are the target of the ISSR primers (Wang et al. 1994). Due of the higher annealing temperature and lengthier primer sequences, these markers have been shown to be more repeatable than RAPD markers and generally display higher degrees of polymorphism (Qian et al. 2001). Like SSRs, they don't necessitate prior understanding of flanking sequences. They are also less expensive, and easier to utilize than AFLPs (Reddy et al. 2002).

References

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