The Promises and Challenges of SSR Marker Analysis

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What is an SSR marker?

- A region of DNA containing a stretch of repetitive sequence amplified by primers outside the repeat stretch to yield a PCR product from one site in the genome
- Simple repeat CACACACA
- Compound repeat AT$_7$AG$_5$
- Imperfect repeat CACAGCACA
How are SSR markers made?

- Microsatellite sequence is identified either from a library or publicly available sequence information
- Flanking primers outside the repeat are identified specific for one genomic location
- Primers are synthesized with colored labels attached and microsatellite is amplified by PCR

Different genotypes can be distinguished by the size of the PCR product
Utility of SSR’s

- Highly polymorphic
- Highly reproducible
- Codominant
- Have multiple alleles

Applications

- Identify parents and progeny for MAS
- Identify the origin of germplasm
- Determine the phylogenetic relationship of rice accessions
- Tag candidate genes for positional cloning
Steps in SSR analysis

- Harvest plant tissue
- Isolate DNA
- PCR Amplification of SSR’s
- Dilute PCR products
- Run on acrylamide gel or automated sequencer
- Analyze data

DNA isolation

Parameters to consider
- Decide on scale
- Quality needed
- Always include controls

Recommended procedures
- Dellaporta technique
- CTAB
- Rapid method-
  Biotechniques
  34:820-826
- Commercial kits
PCR

- Pick good primers and lose the bad ones
- Alter annealing temp if you are getting nonspecific bands
- Set up PCR away from where DNA isolation is performed and always have a blank and other appropriate controls

Suggestions for high-throughput

- Use Standardized conditions
  1. Use DNA of equal concentration—we dilute to 5ng per µl for all reaction. If quantitation is unfeasible Dilute DNA the same amount uniformly
  2. Use the same concentration of PCR reactants for all primers
  3. Have standard sets of primers that can be multiplexed
Use Robotics

Electrophoresis

- Gels vs Automated
- What device to pick?
- Properly maintain your device
Data Analysis

- Data Analysis can be tricky. Hire someone with an in-depth knowledge of genetics and computers.
- SSRs often stutter and PCR can produce artifacts—be aware of this.

SSR Stutter
Stutter Problems

Plus A Artifact
Data Analysis

- Data Analysis can be tricky. Hire someone with an in-depth knowledge of genetics and computers.
- SSRs often stutter and PCR can produce artifacts—be aware of this.
- Sometimes an SSR can be missized.
### Missized peak

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- **182bp**
- **183bp**
- **180bp**
- **181bp**
Data Analysis

- Data Analysis can be tricky. Hire someone with an in-depth knowledge of genetics and computers.
- SSRs often stutter and PCR can produce artifacts. Be aware of this.
- Sometimes an SSR can be missized.
- Some SSR markers amplify other regions of the genome be able to determine which is the real one.

Extra peak 166bp

Extra peak 180bp

213 bp
Data Storage

- Automated sequencers create large data files
- 3 years = 40 GB
- Get a server and back it up weekly

Recommendations for mapping

- Run parents first
- Have good chromosome coverage
- Bulked Segregant Analysis?
- Include parents and a blank in every PCR
Recommendations for Genotyping

- Use easy to analyze markers
- Include some known controls

Cultivar Identification

- Include parents
- Select SSRs with several alleles
SNPs-future markers for MAS

- Often inside the gene of interest
- Less artifacts
- Harder to make

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