Comparing the ability of two PCR based techniques, RAPD and ISSR, to detect low levels of genetic diversity

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Abstract

Researching the genetic structure of small plant communities is often difficult because of population-wide low levels of genetic diversity. The PCR based technique RAPD is popular not only because it detects low levels of genetic diversity but also because it is relatively easy to learn and economical. Initial reports suggest that ISSR, a similar technique that uses a different type of primer, is more sensitive to detecting low levels of genetic diversity while still having the benefits of ease and cost. Two species under study using RAPD, Viola conspersa and Platanthera leucophaea, were demonstrating extremely low levels of genetic diversity, having problems with reproducibility between replicates and bands in the negative control. Samples were subsequently analyzed using ISSR. The results from our comparison of the two techniques indicate that ISSR does better without additional technical problems. Because of the similarities between the two methods, switching from RAPD to ISSR only requires purchasing new primers and re-optimizing PCR parameters.

Background

Similarities between RAPD and ISSR

RAPD (random amplified polymorphic DNA) and ISSR (inter-simple sequence repeat) are both PCR (polymerase chain reaction) based techniques for molecular analysis. Since their primers are not designed based on a known target sequence, they are considered arbitrary or random primers. This means the time-consuming process of primer design is not necessary. They are single primer reactions that produce multilocus dominant markers. Because they are dominant markers, heterozygotes and homozygotes are indistinguishable at any particular locus potentially underestimating the amount of genetic diversity. Markers are visualized on electrophoretic gels as bands that are scored as present or absent. DNA analysis is the same for both techniques. RAPD and ISSR are known for their speed, economy, technical simplicity and frequency of polymorphisms.

Differences in primers

ISSR primers are derived from an arbitrary nucleotide sequence of dinucleotide repeats with a 3’ or 5’ anchoring sequence of a few nucleotides to prevent strand-slip (12-22 bp). These nucleotide repeats are based on the ubiquitous presence of simple sequence repeats (SSRs) aka microsatellites, simple tandem repeats that are distributed throughout genomes. PCR reactions amplify the sequence between two SSRs. Because of these abundant and rapidly evolving SSR regions, ISSR amplification has the potential to reveal much larger numbers of polymorphic fragments per primer than RAPD which have primers made up of short 10bp oligonucleotides.

Longer primers, ISSR’s 14-22 bp vs. RAPD’s 10, can have higher annealing temperatures. For our studies, ISSR annealing temperatures were between 44 and 49 degrees Celsius while for RAPD the annealing temperature is 36 degrees Celsius. Higher annealing temperatures mean more stringent primer annealing conditions, which leads to greater consistency. Additionally, the number of denaturation/ annealing extension cycles is lower for ISSR (55 cycles) than for RAPD (45 cycles). Greater cycle numbers increase the odds of imperfect anneals. These imperfect anneals create inconsistent bands.

Other studies

A number of studies have used both ISSR and RAPD, finding that ISSR produces more information with fewer primers than RAPD (table below). Only the Lanham and Brennan (1989) study found a lower percent of polymorphic bands using ISSR, though the number of polymorphic bands was still higher with less than half the number of primers. Importantly, the average polymorphism per primer was 2.0 for RAPD and 6.5 for ISSR. Qian et al. (2001) found that ISSR bands were less reproducible. Nagoaka and Ogihara (1997), not giving numbers, found several times more information with ISSR primers than with RAPD because of higher percentage of polymorphic bands. They also found that RAPD produced less reliable bands than ISSR.

<table>
<thead>
<tr>
<th>ISSR</th>
<th>RAPD</th>
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<tbody>
<tr>
<td>No. of primers</td>
<td>8</td>
</tr>
<tr>
<td>No. of bands</td>
<td>15</td>
</tr>
<tr>
<td>No. of bands</td>
<td>31</td>
</tr>
<tr>
<td>No. of polymorphic bands</td>
<td>22</td>
</tr>
<tr>
<td>No. of bands used</td>
<td>11</td>
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</tbody>
</table>

It took twice as many RAPD primers to generate approximately the same number of bands. More important is the number of usable bands, i.e. those that were consistent between replicates and not present in the negative control. Over twice as many RAPD bands were dropped. Interestingly, not only does ISSR provide more polymorphic bands per primer, given the number of haplotypes per polymorphic band, ISSR provides more information per polymorphic band. Additionally, with each primer a few samples are never resolved and are thus dropped from statistical analysis. Therefore fewer samples are needed, the less samples are dropped. Seven samples were dropped from ISSR analysis because they could not be resolved compared to 24 samples for RAPD.

Conclusion

It took less ISSR primers to get more information. Fewer primers means less time, less DNA used, less supplies and thus expenses, and fewer samples not included in analysis. The increased amount of information was due to more usable bands and most importantly, more differentiated haplotypes.

Because RAPD and ISSR use the same DNA, equipment and statistical analysis, converting from RAPD to ISSR is painless. It only requires purchasing new primers then undergoing the same optimization process. Additionally, studies already started with RAPD can be finished with ISSR and their data combined for statistical analysis (Esselman et al. 1999, Lanham et al. 2000, Hollingsworth et al. 1998). Although ISSR is not perfect, consistency and negative controls still need to be checked, these problems are reduced and sensitivity is increased.

Literature Cited


see other posters for Platanthera leucophaea and Viola conspersa research