the desired fragment. Unfortunately, partial digests are difficult to control and are not feasible when the number of internal sites is high. A second solution would be to amplify the desired region with PCR using a primer set that flanks the terminal EcoRI and HindIII sites. In this case, a unique restriction site would be incorporated into the downstream primer in such a way that HindIII digestion would not be required for the cloning of the PCR product. Typically, this approach results in the addition of a portion of the primer sequence to the end (the HindIII-end in this case) of the desired DNA fragment. This would be problematic if, for example, one was trying to reconstitute an open reading frame (after subcloning of the PCR product into a vector) that ran through the terminal HindIII site.

Here, we present a class of restriction enzymes, referred to as the BbvI class, that permits specific cleavage of DNA at an otherwise non-unique restriction site. A partial list of such enzymes is shown in Table 1. All of the BbvI class enzymes cut DNA outside of their recognition sequences. As illustrated in Figure 1B, the proximity of the BbvI recognition sequence to the terminal HindIII restriction site allows digestion with BbvI equivalent to that with HindIII. Note that the recognition sequence of BbvI and other enzymes of its class are non-palindromic. Thus, depending on the orientation of the recognition sequence within the primer, they are useful when incorporated into either the upstream or downstream primer for PCR. The non-palindromic nature of these recognition sequences also avoids problems associated with the inclusion of possible secondary structures into primers. The recognition sequences of most of the BbvI class enzymes are five or six nucleotides. With many such enzymes from which to choose, it should not be difficult to find one that is unique to the region of interest. The only obvious limitation of the BbvI enzyme class is that it does not include any enzymes that give a four-nucleotide 3’ overhang or a two-nucleotide 5’ overhang. Thus, these enzymes are not viable alternatives for cleavage of the recognition sites of PstI (5’-CT-GCA-GG), Clal (5’-ATCGAT) and related enzymes.

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Fluorescein PAGE
Analysis of Microsatellite-Primed PCR: A Fast and Efficient Approach for Genomic Fingerprinting

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A simple but efficient DNA fingerprinting method is described. Microsatellite-anchored primers were labeled with fluorescein Cy5 and used for PCR amplification. The amplified fragments were detected on a denaturing polyacrylamide gel using an ALFexpress™ (Amersham Pharmacia Biotech, Uppsala, Sweden) automated DNA sequencer with the fragment option. High levels of polymorphism were detected at the intraspecific level, comparable with those detected with AFLP.

Microsatellite-primed PCR involves amplification of DNA using a single primer composed of a microsatellite sequence with 1–3 degenerate nucleotides anchored at the 3’ or 5’ end. The amplified fragments are termed inter-simple sequence repeats (ISSRs). As a new source of genetic markers, ISSR analysis combines advantages of RAPD (that is, no need for prior DNA sequence information) and the “standard” microsatellite analysis (that is, high-stringency annealing and thus more reproducible banding patterns). This PCR-based marker system has been applied to reveal intra- and interspecific polymorphisms from a variety of eukaryotic taxa (2,4). However, the number of loci detected using this method is usually much smaller than that detected with AFLP, although PAGE combined with silver staining can increase the resolution of the amplification products. Here, we describe a new microsatellite-primed PCR method, in which Cy5 labeled primers were used for each PCR. The amplification products were separated in DNA sequencing gels, and the data were acquired with an ALFexpress automated DNA sequencer.

Genomic DNA from nine accessions of sweet potato (Ipomoea batatas (L) Lam.) was used in this study. Microsatellite primers UBC807 (AG)₇T, UBC888 BDB(CA)₂ and UBC891 HVH (TG)₇ [abbreviations for degenerative base positions: B = (C, G and T), D = (A, G and T), H = (A, C and T) and V = (A, C and G)] were purchased from the Oligonucleotide Synthesis Laboratory, Nucleic Acid-Protein Service Unit, University of British Columbia, Vancouver, BC, Canada. The primers were 5’-end labeled with fluorescein Cy5 (Amersham Pharmacia Biotech, Little Chalfont, UK). Amplification was carried out in a total volume of 25 μL containing 10 ng template DNA, 30 ng labeled primers, 0.1 mM dNTPs, 2 mM MgCl₂, 0.75 U Taq DNA polymerase (Promega, Madison, WI, USA) and 1× PCR buffer containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0) and 0.1% Triton® X-100. PCR was performed using the following cycle profile: 1 cycle at 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, 51.5°C for 45 s and 72°C for 1.5 min; and a final 7-min extension at 72°C. Following PCR, the products were mixed with an equal volume of formamide dye, and 7 μL were loaded on a 6% polyacrylamide sequencing gel. Data acquisition was performed with an ALFexpress automated sequencer using the fragment option. Results were analyzed by the software package DNA Fragment Manager version 1.2 (Amersham Pharmacia Biotech). A binary data matrix recording the presence or absence of the resulting bands was analyzed using PAUP* 4.0 (3) based on parsimony.

With two primer pairs HVH(TG)₇ + (AG)₃T and BDB(CA)₂ + (AG)₃T, the
total number of amplified fragments in the range of 50–500 bp were 36–52 and 31–49, respectively, in each of the nine sweet potato accessions (Table 1). Comparing the banding patterns obtained with single versus double primers, the number of fragments amplified using a pair of primers was much higher than the number amplified with a single primer (Table 2), and many of the fragments amplified with a pair of primers were shorter than those amplified with a single primer (Figure 1). The band profile generated with double primers was not simply a summary of the products obtained by single primers. Many short fragments, most less than 250 bp, were unique products of the double-primer PCR. This result indicates that the occurrence of two different microsatellites in adjacent and inverse orientation is much higher than that of two identical microsatellites. In addition, there may be target-site competition between the primers, as is usually observed with RAPDs.

Nearly all the amplified fragments were distinctively resolved and analyzed with the aid of an automated DNA sequencer and computer software. Compared with other detection methods, such as ethidium bromide or silver staining, the resolution and detection of the amplified fragments were greatly increased. A data set of 166 characters was constructed for the fragments generated with primer pair HVH(TG)\textsuperscript{7} + (AG)\textsuperscript{8}T and BDB(CA)\textsuperscript{7} + (AG)\textsuperscript{8}T in the nine accessions studied, with most fragments ranging from 100–450 bp. Among the characters, 113 are parsimony informative for intraspecific phylogeny analysis (cladograms not shown). The numbers of polymorphisms detected using this method are comparable with those revealed with fluorescein AFLP (unpublished data).

However, in comparison with AFLP in genetic analysis of plant germplasm, our method has several advantages, such as its simplicity, rapidity and economy. For example, multilocus fingerprints of individual accessions can be obtained within a few hours. Because of

<table>
<thead>
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<th>Accession</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Polymorphic Fragments</th>
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</thead>
<tbody>
<tr>
<td>HVH(TG)\textsuperscript{7} + (AG)\textsuperscript{8}T</td>
<td>45</td>
<td>52</td>
<td>44</td>
<td>45</td>
<td>36</td>
<td>41</td>
<td>38</td>
<td>41</td>
<td>42</td>
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<tr>
<td>BDB(CA)\textsuperscript{7} + (AG)\textsuperscript{8}T</td>
<td>45</td>
<td>47</td>
<td>49</td>
<td>43</td>
<td>40</td>
<td>34</td>
<td>35</td>
<td>33</td>
<td>31</td>
<td>53%</td>
</tr>
</tbody>
</table>

Table 1. The Total Number of Fragments (50–500 bp) Amplified by Primer Pairs HVH(TG)\textsuperscript{7} + (AG)\textsuperscript{8}T and BDB(CA)\textsuperscript{7} + (AG)\textsuperscript{8}T in Each of Nine Accessions of Sweet Potato (I. batatas) Accessions 1–9

Figure 1. DNA fragments generated with double and single Cy5-labeled microsatellite primers. The band image was taken from the ALFExpress automated DNA sequencer using Pharmacia DNA fragment manager version 1.2. Lane 1, 50–500 bp Cy5 size marker; lanes 2–11, fingerprints of sweet potato accessions 1–9 detected with primer pair BDB(CA)\textsuperscript{7} + (AG)\textsuperscript{8}T (lanes 10 and 11 are duplicates of the same accession 9); lanes 12–20, fingerprints of sweet potato accessions 1–9 detected with primer pair HVH(TG)\textsuperscript{7} + (AG)\textsuperscript{8}T; lanes 21–25, fingerprints of sweet potato accessions 1–5 detected with a single primer (AG)\textsuperscript{8}T; lanes 26–30, fingerprints of sweet potato accessions 1–5 detected with a single primer BDB(CA)\textsuperscript{7}; and lanes 31–35, fingerprints of sweet potato accessions 1–5 detected with a single primer HVH(TG)\textsuperscript{7}. 

Benchmarks
its efficiency, the method described here is also preferable to some previously reported ISSR detection methods (1,4). The increased value in the number of bands resolved may make the present approach more appealing and thus increase its use in genetic analysis. We have successfully used the method in the phylogenetic analysis of 27 accessions of seven Amaranthus species, and the relationships resolved among the intra- and interspecific accessions are largely consistent with those revealed based on AFLP data (Xu and Sun, unpublished data).

REFERENCES


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Use of Dialysis to Prepare Bacterial DNA Suitable as PCR Template

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The availability of reagent kits for PCR amplification of selected DNA regions has made this technique routine in research, diagnostic and educational laboratories. Nevertheless, successful amplification can be elusive in specific instances.

In a multiphasic characterization of 81 new isolates and 10 stock strains (3) of Caulobacter spp., a major problem was encountered in PCR amplification of putative 16S rRNA genes for analyzing similar restriction fragment length patterns. Specifically, although InstaGene™ (Bio-Rad Laboratories, Hercules, CA, USA) supernatants prepared from broth-cultivated cells were successfully amplified, preparations from cells cultivated on agar- or agarose-containing media were unsuitable as templates for Tag-catalyzed gene amplification. Of several remedies tested, dialysis of the DNA preparations against TE buffer was effective in making the preparations suitable as templates.

The bacterial isolates were maintained on slants of PyCM medium containing media were unsuitable as templates.

Figure 1. Agarose gel electrophoresis of PCR products amplified from DNA extracted from agar-cultivated cells and dialyzed for zero, one or three days, as indicated by the numbers. Lanes 1–4, C. leidyi strain CB37; lane 5, Δ DNA-BseEII digestion (Sigma); lanes 6–9, C. crescentus strain CB2.