from baculovirus DNA are usually removed by proteinase K digestion to avoid shearing of the DNA. The high solubility of polyhedra and ECV in GIT solution, and the increased transfection efficiency of DNA isolated by this method, suggest that these chaotropic agents dissociate proteins from DNA without shearing it. Chaotropic agents are also known to remove low molecular weight impurities such as polyamines (1) that are attached to DNA by salt linkage. Ammonium acetate prevents alcohol precipitation of low molecular weight impurities along with DNA.

This method should be very useful for isolating sufficient quantities of pure baculovirus DNA without proteinase K digestion and dialysis, even from a single infected larva or the virus propagated in a 35-mm plate.

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Long Primers for RAPD Mapping and Fingerprinting of Grape and Pear

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The random-amplified polymorphic DNA (RAPD) technique (10,12) has been widely used in plants for the construction of genetic maps in species such as *Arabidopsis* (9), bananas (4) and slash pine (7), and for genotype identification and taxonomic studies (2,5). RAPD markers are detected by the use of short oligonucleotides of arbitrary sequence as primers for the amplification of segments of the target genome. Generally, 10-mer primers with 50%-80% G+C content are preferred (8). However, complex banding patterns were also generated with primers as short as 5 bases (1). There are few reports on the use of long primers (over 12 bases) (10,11). The potential value of long primers (17-24 bases) for generating RAPD polymorphisms was investigated in this study. We compared the use of both short and long primers in RAPD assays of two plant species: grape and pear.

DNA was extracted from leaves according to the methods of Lodhi et al. (6) for grape and of Doyle and Doyle (3) for pear. RAPD amplification was performed in a reaction volume of 25 μ L containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2 mM MgCl₂, 0.1%

Triton® X-100, 120 μ M each dNTPs, 0.4 μ M primer, 100-200 ng genomic DNA and 0.5 unit of *Taq* DNA polymerase. Amplifications were performed on a PTC-100 thermal cycler (MJ Research, Watertown, MA, USA) for (i) 35 cycles of 30 s at 94°C, 1 min at 35°C and 1 min 45 s at 72°C, followed by an 8-min extension at 72°C for grape, or (ii) 40 cycles of 1 min at 94°C, 2 min at 35°C and 2 min at 72°C, followed by an 8-min extension at 72°C for pear. Primers (10-mers) were purchased from Operon Technologies (Alameda, CA, USA), National BioSciences (Plymouth, MN, USA), Genosys Biotechnologies (The Woodlands, TX, USA), University of British Columbia (Vancouver, B.C., Canada) and the New York State Center for Advanced Technology in Biotechnology (Cornell University, Ithaca, NY, USA). Long (17-mer to 24-mer) primers were provided by Dr. Sheng-Zhi Pang (Dept. of Plant Pathology, Cornell University, Geneva, NY, USA).

Typical gels displaying the amplification products generated from grape or pear DNA using long primers are shown in Figure 1. In general, long primers generated more DNA fragments, a wider range of DNA fragment sizes

Figure 1. RAPD profiles of grape DNA generated with 21-mer GY169 (CTAAGCTGCTTT-TGTTTGAGC) (Panel A) and pear DNA with the 18-mer GY107 (GTTCAGGGCTGTTT-ATAG) (Panel B). Each panel includes DNA from 9 individuals from the crosses Horizon x III. 547-1 (Panel A) and Bartlett x NY10353 (Panel B), respectively. Amplification products were separated by electrophoresis in 2% agarose gels (1% agarose/1% NuSieve® GTG® agarose; FMC BioProducts, Rockland, ME, USA), visualized by staining with ethidium bromide and photographed on a transilluminator using Polaroid Type 55 film (Cambridge, MA, USA).

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Table 1. Comparison of 10-Mer and 17-Mer to 24-Mer Primers in Genetic Mapping and Fingerprinting of Grape and Pear

	10-Mer Primers	17- to 24-Mer Primers
Pear Genetic Mapping Population		
No. of primers	52	7
Mean No. DNA fragments/primer	10.5	14.3*
Mean No. polymorphic fragments/primer	6.2	8.4*
Grape Genetic Mapping Population		
No. of primers	77 (310) ^a	13 (20)
Mean No. DNA fragments/primer	12.1 (5.9)	15.8* (14.9)
Mean No. polymorphic fragments/primer	4.8 (2.6)	6.7* (7.6)
Grape Variety Fingerprinting		
No. of primers	43	11
Mean No. DNA fragments/primer	5.6	11.8*
Mean No. polymorphic fragments/primer	3.0	4.5*

^aThe numbers in parentheses represent the combined data for primers tested on parents only, as well as those used with both the parent and the progeny populations. Numbers not in parentheses represent data from primers chosen for analysis with a progeny population.

*Significantly different at $P < 0.001$ according to a one-tailed t -test.

Table 2. The Effect of Primer Length (17 to 24 Bases) on Number of RAPD Fragments and Number of Polymorphic Fragments Per Primer

Primer Length (Bases)	No. of Primers Tested^a	Average Total No. of Fragments/Primer	Average No. of Polymorphic Fragments/Primer
17	3	12	6.7
18	9	18	8.8
19	3	12	4.3
21	3	13	6.7
22	1	17	7.0
24	1	18	7.0

^aThe data include results from primers used in grape and pear. Thirteen primers were used in grape and seven in pear (see Table 1), of which five were the same primers. Where the same primers were used in grape and pear, they were counted twice in the computation.

(typically 0.2–2 kb vs. 0.3–1.3 kb for 10-mer primers) and a greater number of polymorphic fragments per primer (Table 1). In grape genetic mapping studies, the number of polymorphic bands per 10-mer primer (4.8) is overestimated because the 77 primers used were the best among 310 surveyed, about 10% of which revealed few or no polymorphisms. When the data for all 310 primers were analyzed and compared with unselected long primers (Table 1), there was an even greater difference (2.9-fold) between the number of polymorphic bands generated with

short primers vs. long primers. Results from studies in pear genetic mapping and grape variety fingerprinting showed the same trend (Table 1). Data from these two studies were generated with 10-mer primers that had been selected from a larger group.

No significant correlation was observed between primer length and number of polymorphic fragments per primer within the long primer group (Table 2), suggesting that increasing primer length above 20 bases may not be an efficient method for exposing more polymorphism. Generally, 10-

Benchmarks

mer primers with 50%–80% G+C content are preferred (8) in RAPD analyses. As the length of a primer increases, the genomic target sites should decrease because there is less chance of finding perfect or near perfect homologies between the target sites and a longer primer. We observed, however, that long primers yielded more polymorphic bands than the short 10-mer primers, which is consistent with the observation on poplar (2). Long primers have also been used in genomic fingerprinting (10,11). However, no data were presented comparing RAPD results between 10-mer primers and longer primers (2,10,11). It is not clear exactly why the long primers produced more polymorphic bands. It should be pointed out, however, that the long primers used in this study have lower G+C content (from 29% to 55%) compared with commonly used 10-mer primers, and with long primers used by

Welsh and McClelland (10,11) (45%–61%) and Castiglione et al. (2) (45%–60%), yet they were able to generate more fragments than 10-mer primers. It is not clear in our study whether the increased number of bands produced by long primers was due to lower G+C content, primer length or a combination of both. Future experiments should be designed to determine the effects of primer length and G+C content upon RAPD polymorphisms in a variety of genera. If the generation of more fragments is due to primer length, one possibility could be that the extra bases at the 5'-end anneal to the templates in a way that either helps the 3' bases anneal to the template to start a new template-primer complex or stabilizes the unstable existing template-primer complex. Because most of the primers have less than 50% G+C content, intergenic or repetitive DNA regions may be preferentially targeted, which could be use-

ful in mapping telomere and centromere regions that are otherwise not as easily accessible with 10-mer primers. Sequencing of the amplified fragments or probing of the genomic DNA with the amplified fragments would help to determine the nature of amplified products and the mechanism(s) underlying the amplification.

The higher cost of synthesizing long primers can be justified by the greater number of polymorphic bands obtained. Long primers might also be available gratis from colleagues in a large research facility. To generate the same number of polymorphic bands, reactions with 10-mer primers cost up to three times as much in materials and labor as reactions with long primers.

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