Benchmarks

protocol for DNA isolation is outlined in Table 1.

This protocol was used to isolate phage DNA from two different libraries and phage vectors. The first was a λEMBL3 genomic library of the house fly, *Musca domestica*. The second was a λZAP® (Stratagene, La Jolla, CA, USA) cDNA library of *Drosophila virilis*. An aliquot of 2 µL resuspended DNA was used per individual restriction digest. As shown in Figure 1, this small volume was sufficient for restriction mapping and Southern hybridization.

The effect of glycerol during cell-phage growth was compared. Identical 50-mL cultures were prepared for inoculation with phage isolated from the same purified plaque. One flask contained LB with 0.3% glycerol as described above, and the second contained LB without glycerol. Following lysis and DNA isolation, total DNA yield was compared. Total DNA yield from the glycerol-supplemented LB was 1.0–2.0 mg while the yield for LB only was 0.125–0.25 mg. Thus, glycerol seems to have a dramatic effect on the DNA yield from this protocol.

REFERENCES

1. Donovan, J., X. Lu and L. Nagarajan. 1993. Rapid purification of bacteriophage λ DNA. BioTechniques *15*:602-604.
2. Kernodle, S.P., R.E. Cannon and J.G. Scandalios. 1993. Rapid and simple phage DNA isolation. BioTechniques *14*:360-362.
3. Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. CSH Laboratory Press, Cold Spring Harbor, NY.
4. Yamamoto, K., B. Alberts, R. Benzinger, L. Lawhorne and G. Treiber. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology *40*:734-740.

Address correspondence to S.H. Lee, Dept. of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA. Internet: lees@ccit.arizona.edu

Received 18 February 1997; accepted 14 April 1997.

Seung Hoon Lee and Jonathan B. Clark
University of Arizona
Tucson, AZ, USA

Efficient Recovery of Plasmid DNA from *Erwinia herbicola* with High Nuclease Activity

BioTechniques 23:600-603 (October 1997)

An efficient method is described for the isolation and purification of plasmid DNA from *Erwinia herbicola* (a bacterium with high nuclease activity), for which none of the standard methods yielded plasmid DNA in desired amounts. The procedure adopted here is based on elimination of the endogenous nuclease activity using guanidinium thiocyanate. The DNA preparation does not need a separate purification step. The method precludes the use of RNase, protease and phenol. The plasmid DNA preparation obtained by this method is of high purity suitable for restriction digestion, cloning, hybridization, preparation of DNA probes and sequencing.

E. herbicola (Catalog No. 21998; ATCC, Rockville, MD, USA) is an industrially important organism because of its ability to produce keto-sugar acids as intermediates of ascorbic acid. As a result, the organism is a good candidate for genetic manipulations. We studied the organism for the presence of plasmid DNA using several standard methods from Sambrook et al. (11) such as the alkaline lysis method (2), the sodium dodecyl sulfate (SDS) lysis method (6), the boiling method (7) etc. The gel picture revealed that the bacteria harbored the plasmid DNA, but the yields were invariably very low, and the isolated DNA was susceptible to degradation within a day, even when preserved at -20°C. Apparently, it shows that the organism possesses high endogenous nuclease activity.

Various other methods described for the isolation of plasmid DNA from prokaryotic organisms were also tried (1,3–5,9). However, plasmid DNA could not be recovered easily and efficiently in reasonably large quantities from this bacteria. Several modifications in the known standard procedures were also unable to increase plasmid DNA yield or inhibit its degradation.

Guanidinium thiocyanate, a strong protein denaturant, has been success-