Rapid Resuspension of Pelleted Bacterial Cells for Miniprep Plasmid DNA Isolation

Small-scale purification of plasmid DNA is commonly used in molecular biology procedures. Several protocols for rapid isolation of DNA have previously been published (1,2). Several companies have also developed reliable miniprep kits that have become the method of choice for most laboratories when preparing plasmid DNA. However, even when using a kit, these miniprep processes can be laborious and time-consuming, particularly when large numbers of minipreps are performed in parallel. For multiple plasmid preparations, the rate-limiting step of miniprep protocols is resuspension of the cell pellet. The standard technique is to pellet multiple bacterial cell cultures in 1.5-mL microcentrifuge tubes for 30 s to 10 min, depending on the volume of culture pelleted. After centrifugation, the cells are resuspended by vortex mixing. Complete resuspension of cells before lysis is critical to achieve a good yield. However, this process often requires 20 s to several minutes to achieve complete resuspension. We have devised a rapid protocol to completely resuspend the cell pellet in only 3–4 s without any negative effect on the quantity and quality of plasmid DNA, irrespective of size.

E. coli cells carrying plasmids of 3.0, 5.6 or 20 kb in size (pBluescript® [Stratagene, La Jolla, CA, USA] plus

### Table 1. Time Taken to Completely Resuspend Bacterial Cell Pellets

<table>
<thead>
<tr>
<th>Time of Cell Centrifugation</th>
<th>Volume of Culture</th>
<th>Vortex (mean)</th>
<th>Scraping Rack</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 s (4 samples)</td>
<td>1.5 mL</td>
<td>10–15 s (mean = 13.8 s)</td>
<td>3–4 s</td>
</tr>
<tr>
<td>2 min (5 samples)</td>
<td>1.5 mL</td>
<td>20–30 s (mean = 28 s)</td>
<td>3–4 s</td>
</tr>
<tr>
<td>2 min (30 s/spin) (5 samples)</td>
<td>6.0 mL</td>
<td>35–60 s (mean = 45 s)</td>
<td>3–4 s</td>
</tr>
<tr>
<td>9 min (3 min/spin) (7 samples)</td>
<td>4.5 mL</td>
<td>20 s–2 min (mean = 54 s)</td>
<td>3–4 s</td>
</tr>
</tbody>
</table>
insert) were grown to an optical density (OD) at 600 nm of 2.0 and harvested by centrifugation as recommended by Wizard™ Plus Minipreps DNA Purification System Kit (Promega, Madison, WI, USA) (3). The cells were pelleted by centrifugation for 30 s (1.5-mL cultures), 2 min (1.5-mL cultures), 2 min (6-mL cultures, 30 s per spin) to 9 min (4.5-mL cultures, 3 min per spin) in a 1.5-mL microcentrifuge tube (Catalog No. 05-407-10; Fisher Scientific, Pittsburgh, PA, USA) and resuspended in the Cell Resuspension Solution using a VWRbrand™ Vortex™ Genie 2 Mixer (VWR, Bridgeport, NJ, USA) or by scraping the base of the microcentrifuge tubes across the surface side with holes of a polypropylene microcentrifuge tube storage rack (Catalog No. 05-541; Fisher Scientific) back and forth 5 times. The resuspended cells were then lysed, and DNA was extracted according to the protocol of the Wizard kit. DNA samples were analyzed on a 0.8% agarose gel stained with ethidium bromide.

This rapid resuspension protocol resulted in identical yield and quality of DNA (Figure 1) but significantly reduced the time needed to fully resuspend the bacterial cells by at least 90% in most cases. All cells were resuspended in 3–4 s compared to the 30 s to 2 min required for resuspension by vortex mixing the cells (Table 1).

This method of resuspension is simple, rapid and should be applicable to any cell type or pellet. In just 3–4 s, we are routinely able to resuspend bacterial cell pellets that previously required 30 s to several minutes of vortex mixing. The amount and quality of DNA obtained by this method is the same as DNA obtained from the vortex resuspension. This resuspension method does not damage the rack, microcentrifuge tubes or DNA.

REFERENCES

method for plasmid miniprep from large numbers of clones for direct screening by size or restriction digestion. BioTechniques 18:214-217.


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Kui Shin Voo and Britta M. Jacobsen
Indiana University School of Medicine
Indianapolis, IN, USA

Extraction and Amplification of Mitochondrial DNA from Formalin-Fixed Deep-Sea Mollusks


The ability to extract, amplify and sequence DNA from various types of tissues including bone (4,6), minute larvae (7), formalin-fixed specimens (2,3) and dried museum specimens (1) has made it possible to address fundamental questions about evolution, phylogeny and the genetic structure of populations. A major problem in studying evolutionary patterns in the deep sea is the difficulty of obtaining amplifiable DNA from small macrofaunal organisms (usually about 1.0–5.0 mm). These species live at very low density, are collected from great depths (to 5000 m) and are typically fixed for 24–48 h in 10% buffered formalin and later transferred to 70% ethanol. The effects of fixation (3) and the very small amount of tissue in macrofaunal organisms have made it extremely difficult to obtain DNA sufficient for polymerase chain reaction (PCR). Though several methods have been published to extract DNA from formalin-fixed tissues (2,5,10), these have proved cumbersome with small amounts of tissue.

We describe methods to extract and amplify mtDNA from formalin-fixed deep-sea molluscan species by: (i) using a modification of a commercially available DNA extraction kit and (ii) developing species-specific primers. One of the greatest challenges we faced was extracting sufficient quantities of amplifiable DNA from minute amounts of tissue. Further, this material was extensively handled during the sorting process and prone to contamination.

To extract DNA, whole individuals were placed in microcentrifuge tubes with 200 µL of tissue lysis buffer ATL from the QIAamp® Tissue Extraction Kit (Qiagen, Chatsworth, CA, USA) and incubated for 24 h at 55°C. Then 5 µL of a 50 mg/mL solution of proteinase K and an additional 95 µL of lysis buffer were added and incubation continued at 55°C for another 72 h. The extraction then follows the manufacturer’s instructions, except that buffer AL and ethanol were increased from 200 to 300 µL. DNA was eluted with one 200-µL aliquot of 10 mM Tris-HCl, pH 8.0.

Developing species-specific internal primers and working with small (<300-bp) mtDNA fragments were crucial steps for consistent amplification of DNA from formalin-fixed specimens. Reactions with the universal 16S primers, 16sr (5′-CGC CTG TTT ATC AAA AAC AT-3′) and 16sb (5′-CTC CGG TTT GAA CTC AGA TC-3′) (8) resulted in the spurious amplification of human fragments. Because fresh material was not available for primer development, the following detailed strategy for targeting 16S rDNA was used: (i) a forward primer [Proto16F: 5′-A(A/T)-(A/G) (A/T)GA C(A/G)A GAA GAC CCT-3′] internal to 16sr was designed by aligning two protobranch bivalve species, Nucula proxima and Solemya...