hybridization probes or sequenced to design PCR primers. These markers are useful in screening YAC or bacterial artificial chromosome (BAC) libraries to develop contigs and to characterize YACs for chimerism by mapping the ends in backcross panels or radiation hybrid panels.

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Isolation of pcDNAI-Based Mammalian Expression Vectors from Escherichia coli Strain MC1061/P3


Expression of cloned DNA sequences in mammalian systems is becoming an increasingly important tool in all areas of biological and biochemical research. This process allows one to determine what features of the cloned sequence are important for proper function in mammalian systems and to dissect these regions on a molecular level. Many mammalian expression vectors are currently available, each with its own advantages and limitations. The mammalian expression vector pcDNAI (Invitrogen, Carlsbad, CA, USA) and its derivatives have been widely used for cloning, expression and analysis of genes in higher eukaryotes—especially when plasmid rescue is required. pcDNAI contains the supF gene rather than a selectable antibiotic resistance gene, a feature that keeps this vector small enough to be useful for expression library construction. The supF gene encodes a tRNA that suppresses amber mutations. Thus, pcDNAI must be propagated in a host that carries an amber mutation in an antibiotic resistance gene. Although pcDNAI has been superseded by a derivative that contains the ampicillin-resistance gene (pcDNAI.1/Amp), a variety of cDNA li-

Figure 1. Comparison of DNA integrity by gel electrophoresis. DNA samples isolated from overnight cultures as indicated were run on a 0.8% agarose gel in 1X TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) in the presence of 1 µg EdBr/mL at 90 V (ca. 50 mA) for 45–60 min at room temperature. Photographs of the gels were taken using Polaroid® Type 57 Film (Polaroid, Cambridge, MA, USA) under UV illumination and reproduced using UMax VistaScan Version 2.28 (UMax Data Systems, Hsin Chu, Taiwan, ROC) and Adobe® Photoshop® (Adobe Systems, San Jose, CA, USA). The migration of marker DNA (lambda DNA cut with HindIII) is shown for each gel. Lanes 1 and 2, 1/25th of total pcDNAI isolated from 1.5 mL of an overnight culture by alkaline lysis (4); lanes 3 and 4, 1/25th of total pcDNAI isolated from 1.5 mL of an overnight culture by alkaline lysis (4); lanes 5 and 6, entire pcDNAI sample isolated from a 1.5 mL of an overnight culture by direct lysis in PCI; lanes 7 and 8, 1/30th of the total pcDNAI-βgal isolated from 1.2 mL of an overnight culture by alkaline lysis (4) with PCI extraction step added; lane 9, 1/30th of the total pcDNAI-βgal isolated from 1.2 mL of an overnight culture by direct lysis in PCI; lanes 10 through 12, isolated from 1.2 mL of an overnight culture using a N.S.A.P. MiniPrep Kit; lanes 10 and 11, 0.1 µg Prep. No. 8 pcDNAI DNA; lane 12, 0.3 µg Prep. No. 8 pcDNAI DNA. The DNA samples in lanes 1, 3, 5 and 11 were digested with BamHI to linearize pcDNAI. The DNA samples in lanes 2, 4, 6 and 12 were digested with PstI to produce 3.1- and 0.9-kb fragments from pcDNAI. The DNA in lane 10 was undigested, indicating that the DNA preparation yielded primarily covalently closed circular (supercoiled) DNA.

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Table 1. Comparing Effects of Growth Media, Antibiotic Selection and Preparation Methods on Yields of pcDNAI

<table>
<thead>
<tr>
<th>Prep. No.</th>
<th>Growth Mediuma</th>
<th>Antibioticb (µg/mL)</th>
<th>Prep. Chloramphenicolc</th>
<th>PCI Extractione</th>
<th>Yieldf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LB</td>
<td>100 (Amp)</td>
<td>AL (+)</td>
<td>-</td>
<td>0.109</td>
</tr>
<tr>
<td>2</td>
<td>K</td>
<td>15 (Tet)</td>
<td>SL (+)</td>
<td>-</td>
<td>0.058</td>
</tr>
<tr>
<td>3</td>
<td>LB</td>
<td>100 (Amp)</td>
<td>AL (+)</td>
<td>+</td>
<td>0.164</td>
</tr>
<tr>
<td>4</td>
<td>LB</td>
<td>50 (Amp)</td>
<td>AL (+)</td>
<td>-</td>
<td>0.161</td>
</tr>
<tr>
<td>5</td>
<td>LB</td>
<td>25 (Amp)</td>
<td>AL (+)</td>
<td>+</td>
<td>0.081</td>
</tr>
<tr>
<td>6</td>
<td>IB</td>
<td>50 (Amp)</td>
<td>AL (+)</td>
<td>+</td>
<td>0.686</td>
</tr>
<tr>
<td>7</td>
<td>LB</td>
<td>25 (Amp)</td>
<td>AL (-)</td>
<td>+</td>
<td>1.995</td>
</tr>
<tr>
<td>8</td>
<td>TB</td>
<td>25 (Amp)</td>
<td>AL (-)</td>
<td>+</td>
<td>4.450</td>
</tr>
</tbody>
</table>

aLB Medium, prepared as described (4); K, K Medium (4); IB, A medium formulation containing 4 g Bacto® Tryptone, 2.5 g Bacto Yeast Extract (both from Difco Laboratories, Detroit, MI, USA) and 2.5 g NaCl per 500 mL total volume; TB, Terrific Broth (4).
bAmp, ampicillin; tet, tetracycline.
cAL, alkaline lysis procedure (4); SL, sarkosyl lysis procedure (1). All plasmid preparation protocols include cesium chloride-EtdBr gradient purification to isolate supercoiled DNA, removal of the EtdBr by butanol extraction and removal of the cesium salt by ethanol precipitation (6).
d+, 200 g/mL chloramphenicol were added to the culture when the optical density at 600 nm reached 0.5, and an overnight incubation followed. -, Cultures were grown to saturation overnight with no chloramphenicol added.
e+, The DNA sample was PCI-extracted after a butanol extraction step; -, the DNA sample was not extracted with PCI.
fYield values represent mg/500 mL.

The degradation problem is most likely because of high nuclease activity of the required host Escherichia coli host strain MC1061/P3 [Genotype: F- delta (ara-leu) delta (lacIPOZY) chi-74 araD139 galE15 galK16 hisD20 lambda- (P3: kanR amp(Am) tet(Am))]. MC1061/P3 is required for the selection of pcDNAI because it carries the P3 episome that has two antibiotic resistance genes with amber mutations, and pcDNAI contains the supF gene encoding the tRNA required to suppress amber mutations.

Because we were interested in using a pcDNAI expression library in E. coli host strain MC1061/P3, we sought suitable plasmid preparation methods. Plasmid pcDNAI that was isolated [by the alkaline lysis miniprep protocol previously described (4)] from overnight cultures of E. coli strain MC1061/P3 (transformed with pcDNAI), which were grown in LB medium with ampicillin and tetracycline, often showed signs of degradation that are seen as smearing in Figure 1, lanes 1 and 2. Alternatively, we lysed the bacteria directly by extraction with phenol:chloroform:isoamyl alcohol (PCI; 25:24:1). This procedure did not alleviate the degradation problems and resulted in very low yields of intact plasmid (see Figure 1, lanes 5 and 6). However, the addition of a PCI extraction step before the final resuspension of the DNA in the alkaline lysis miniprep procedure did significantly decrease the degradation of the DNA and increased the yield of intact plasmid (Figure 1; lanes 3, 4, 7 and 8). However, the PCI extraction step did not always completely eliminate degradation of plasmid DNA (Figure 1, lanes 7 and 8).

However, use of the S.N.A.P.™ MiniPrep Kit (Invitrogen) according to the manufacturer’s directions consistently yielded intact pcDNAI plasmid (Figure 1, lane 9) in a highly purified form. pcDNAI-based plasmid DNA isolated using the S.N.A.P. MiniPrep Kit has been used successfully in polymerase chain reactions (PCRs), for both manual and automated sequencing and in mammalian cell transfections (B. Waldman, unpublished observations). In parallel experiments, it appears to work at least as well as cesium chloride-ethidium bromide (EtdBr) gradient-pu-
rified plasmid preparations (data not shown). The S.N.A.P. MiniPrep Kit procedure yields intact plasmid DNA, does not require the use of noxious reagents (e.g., PCI) and overall is more rapid than the traditional alkaline lysis miniprep procedure. Using the S.N.A.P. MiniPrep Kit, we have been able to obtain as much as 10 µg of pcDNAI plasmid from 1.5 mL of a saturated culture.

Although small quantities of plasmid DNA were sufficient for certain applications, we needed large amounts (>200 µg per experiment) of intact DNA from pcDNAI and its derivatives for some of our studies. We found that the yields of these plasmids using large-scale alkaline lysis (4) or sarkosyl lysis (1) protocols followed by cesium chloride-EDTA gradient purification (4) were very poor (e.g., 40–160 µg/500 mL culture). A more productive method of large-scale plasmid preparation was desirable.

Because the reversion rates for the ampicillin (Am) and tetracycline (Tet) genes on the P3 episome of E. coli strain MC1061/P3 plasmid yield. Therefore, all large-scale cultures were started from a single colony that was isolated by co-selection on LB plates containing both 25 µg ampicillin and 7.5 µg tetracycline per mL, a procedure that should keep the reversion rate below 1%.

Several variations and combinations of procedures and techniques were tested, and representative results are presented in Table 1. The most productive procedure tested was the modification of the alkaline lysis protocol as indicated in Table 1, Prep. No. 8. This procedure incorporates: (i) growth to saturation in Terrific Broth medium, (ii) selection with low amounts of both ampicillin and tetracycline, (iii) omission of any chloramphenicol amplification step and (iv) includes an extraction with PCI before the final ethanol-precipitation step. This procedure routinely yields ≥2 mg of plasmid DNA/500 mL culture. The DNA isolated has an A260/A280 ratio of 1.8 or higher and appears to be intact upon analysis by agarose gel electrophoresis (Figure 1, lanes 10–12).

Treatment with chloramphenicol not only did not increase the plasmid yield, but in fact caused a decreased yield of pcDNAI produced in E. coli strain MC1061/P3 (compare Prep. Nos. 5 and 7 in Table 1). We also found that, when used together, relatively low levels of the antibiotics ampicillin and tetracycline (25 and 7.5 µg/mL, respectively) were sufficient for selection of bacteria transformed with pcDNAI in either liquid or solid medium cultures (data not shown). In addition, these modified protocols (both small- and large-scale) have also been used successfully for the production of derivatives of pcDNAI containing DNA fragments (from 0.5 to ca. 4 kb) inserted into the pcDNAI multiple cloning site. (see Figure 1, lane 9; other data not shown).

In summary, we have found that use of the S.N.A.P. MiniPrep Kit and large-scale plasmid isolation protocols described here have routinely given very good yields of intact pcDNAI-based plasmid DNA. The modified alkaline lysis miniprep method described usually gave good yields of intact DNA, but lacked the consistency of the S.N.A.P. MiniPrep Kit. Although other DNA preparation kits that use the same DNA binding principles as the S.N.A.P. MiniPrep Kit were not tested they might also provide consistent and efficient results. Our findings should be of interest to investigators working with pcDNAI and its derivatives amplified in E. coli strain MC1061/P3 and may be applicable to other vectors that are difficult to produce.

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