BioFeedback

The Biofeedback section accommodates letters to the editor, addenda, errata and other comments on previously published articles.

Cross-Contamination Limits the Use of Recycled Anion Exchange Resins for Preparing Plasmid DNA

BioTechniques 28:298 (February 2000)

DNA of high purity and quality is essential to most molecular biology studies and is easily obtained using silica-based resins or anion-exchange resins. These resins are used in many of the commercial kits available for DNA purification. Qiagen-tips® (Qiagen, Valencia, CA, USA) offer one of the most popular anion-exchange resin columns and is one that the manufacturer recommends discarding after a single use (2). Recently, a protocol to recycle these columns was reported, which used a high-salt solution to strip used columns for subsequent purifications (1). It was reported that Qiagen-tips could be reused up to 20 times without an apparent decrease in either their yield or purity. Different plasmids purified from the same column appeared to be free of contamination by restriction endonuclease analysis on agarose gels.

To test for the presence of occult contamination, we devised a more stringent method to assess the purity of multiple plasmid DNAs harvested from the same anion exchange column. Three different plasmids were selected, each containing unique inserts of different sizes that could be amplified with unique primer pairs using PCR. They were purified in succession over three days using the recycling protocol. On the first day, the first plasmid was purified using the manufacturer's maxi protocol. After purification, the column was rinsed with 20 mL of deionized water and stored at room temperature until the next day. On

Figure 1. PCR results of successive plasmid preparations using a single Qiagen-tip. PCR analysis (30 cycles) was conducted on each of the plasmid preparations using unique primers for each of the plasmid inserts. Products were resolved on 1% TBE agarose. A specific band corresponding to the expected size of plasmid 1's insert (600 bp) was observed in maxipreps 1, 2 and 3. (lanes 3, 4 and 5). PCR of preps 2 and 3 (lanes 9 and 10) showed specific amplification of the expected 1 kb insert from plasmid 2. A 1.4 kb product corresponding to plasmid 3's insert was obtained from prep 3. C₁, C₂ and C₃ are positive controls generated using independently purified known template. B is a PCR blank that was generated using no template. Molecular weight markers (Life Technologies, Gaithersburg, MD, USA).

the second day immediately before use, the column was washed 3 times with 20 mL of "renew" buffer (3 M NaCl, 0.15% Triton® X-100) and re-equilibrated as normal with the Qiagen equilibration buffer. A second plasmid was then purified according to the manufacturer's protocol. Again, the column was rinsed with 20 mL of deionized water and left overnight at room temperature. This process was repeated with the third plasmid. A diagnostic restriction enzyme digest was conducted on each purified plasmid and resolved on a gel as the original authors did. No contamination was visible with ethidium staining (data not shown).

PCR was conducted on each plasmid preparation using the three unique primer pairs for each of the three plasmid inserts. Unfortunately, we detected contamination from each of the previous DNA preparations in all subsequent preparations (Figure 1). In addition, there was a trend towards decreasing yield with subsequent preparations. We set out to see if our laboratory could apply the recycling protocol to our Qiagen columns in an effort to reduce costs. Because of the importance of clean DNA in molecular biology work, we used PCR to test more stringently the assertions made by the protocol's authors.

Regrettably, contamination was clearly evident in later preparations, thereby limiting its usefulness in applications that require a high degree of purity, such as functional studies. However, the recycling protocol may still be a useful alternative when a low level of contamination is tolerable.

REFERENCES

1. Chang, V.W., R. Wu and Y. Ho. 1999. Recycling of anion-exchange resins for plasmid DNA purification. *BioTechniques* 26:1056.
2. Qiagen. 1997. Qiagen® Plasmid Purification Handbook. Qiagen, Valencia, CA.

Address correspondence to Stephen P. Hebert, Department of Pediatrics, AS-312 MDCC, UCLA Medical Center, Los Angeles, CA 90024, USA. Internet: shebert@ucla.edu

Received 20 August 1999; accepted 23 November 1999.

Andrew Insoo Kim, Stephen P. Hebert and Christopher T. Denny
University of California at
Los Angeles
Los Angeles, CA, USA