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“Microprep” Method for Rapidly Isolating Plasmid DNAs for Restriction Enzyme Analysis

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Screening of plasmid cDNA libraries (1,2) can result in hundreds of positive recombinant clones containing varying lengths of 5′ sequence information. Determining which plasmid contains the longest cDNA insert from a large population of heterogenous plasmids can be accomplished by either polymerase chain reaction (PCR) amplification of the 5′ end using forward vector and backward gene-specific primers, or by sequence or restriction enzyme analysis of purified miniprep plasmid DNA. Unfortunately, PCR amplification of the 5′ end frequently fails. For large numbers of recombinant clones, commercial miniprep kits can be prohibitively expensive, and most ‘rapid’ miniprep procedures involve multiple steps and reagents that prove to be tedious and time-consuming. To overcome these obstacles, we have developed a rapid and cost-effective microprep procedure using microliters, rather than milliliters, of bacterial culture for rapidly isolating plasmid DNA that is suitable for restriction enzyme analysis.

The procedure is described in Table 1. Figure 1 shows an analytical gel with typical results.

Use of the procedure to prepare DNA for screening saves use of the larger miniprep or maxiprep procedure for preparing only the desired clones. Agarose gel analysis of restriction enzyme digested microprep DNA results in ethidium bromide (EtBr)-stained bands that are easily resolved from high-molecular-weight bacterial, chromosomal DNA and small-molecular-weight bacterial RNA (Figure 1). SalI and NotI restriction endonuclease digested microprep DNA prepared from DH10B™ cells (Life Technologies, Gaithersburg, MD, USA) equally well in both 1× Buffer H (Boehringer Mannheim, Indianapolis, IN, USA) and 2× Universal Buffer (Stratagene, La Jolla, CA, USA). However, we have not checked the impact of using other bacteria, restriction enzymes or buffers on microprep DNA quality. It would be prudent for an investigator to run a pilot microprep analysis with their particular bacterial strain(s) and restriction digestion conditions before scaling-up the microprep procedure.

In summary, we have developed a streamlined, microscale approach for isolating plasmid DNA from overnight

![Figure 1. Restriction enzyme analysis of multiple heterogenous plasmid cDNAs isolated by the microprep method.](image)
Table 1. Microprep Procedure

1. Transformed _Escherichia coli_ are spread on an LB agar dish with the appropriate antibiotic and, after incubating overnight, individual colonies are picked and used to inoculate 5 mL of LB plus antibiotic in numbered tubes, which are then incubated at 37°C overnight with shaking at 250 rpm.

2. A 500-µL aliquot of overnight culture is transferred to a 1.5-mL microcentrifuge tube, and the bacteria are pelleted by spinning 1 min in a microcentrifuge at 14,000 x g.

3. The LB is completely removed and discarded and the bacterial pellet resuspended in 19 µL H₂O plus 1 µL 10 mg/mL RNase A by vigorous vortex mixing.

4. The plasmid DNA is liberated from the bacteria by boiling the tubes 1 min.

5. Bacterial debris is pelleted by spinning the tubes for 1 min in a microcentrifuge.

6. Enough supernatant is then removed to a fresh 1.5-mL microcentrifuge tube containing premixed restriction enzyme buffer and restriction enzymes (≤1 U per reaction) to obtain a final total volume of 20 µL.

7. After a 1-h incubation, 5–10 µL of the restriction digests can be analyzed using agarose gel electrophoresis.

8. Once recombinant plasmids containing the largest cDNA inserts are identified from the agarose gels, the corresponding positive overnight LB cultures are then retrieved and used for purifying plasmid DNAs using a standard miniprep procedure or kit.

This amount of bacterial suspension was determined to be optimal for efficient subsequent restriction enzyme digestion and visualization on an agarose gel.

Simple Version of “Megaprimer” PCR for Site-Directed Mutagenesis

Site-directed mutagenesis (SDM) is used to introduce a defined mutation into a target DNA of known sequence to study gene expression and protein structure/function relationship. In the past few years, numerous methods have been developed for achieving SDM, including several based on polymerase chain reaction (PCR) amplification (10). Among the PCR-based SDM methods, two of them are particularly appealing because they are simple and efficient: (i) “overlap-extension” PCR (5) and (ii) “megaprimer” PCR (6,13).

The overlap-extension PCR method requires three PCR amplifications and...