gel is negligible by this method. The transfer of the separated RNAs to the nylon membrane was nearly quantitative as shown in figure 1B, and no fluorescent bands could be seen in the gel after the transfer (data not shown). When the blotted membrane was probed with the cDNA described above, strong signals were observed on the autoradiograph (figure 1C). Furthermore, the blotted membrane was rehybridized subsequently with several different cDNA probes with no detectable loss of the signal (data not shown).

Thus the addition of ethidium bromide directly to the gel itself gave little background fluorescence in our experience and obviates the need for extensive staining and destaining procedures.

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A Plasmid Extraction Procedure on a Miniprep Scale

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The modification of the alkaline extraction (1) described here is simple and rapid enough to permit screening of many small samples with high yield and in a form pure enough for restriction enzyme digestion, transformation, subcloning, sequencing and nick translation. In this modification, the phenol extraction step has been removed to eliminate the risk of burns and toxic waste, and ammonium acetate has been substituted for sodium or potassium acetate to facilitate efficient separation of proteins, membranes and RNA from the plasmid DNA (2).

Protocol

1. Grow bacteria overnight at 30°C with vigorous aeration in 2 ml of LB medium in 10 ml glass tubes (3) containing the required antibiotic.
2. Harvest the bacterial cultures by low-speed centrifugation in microcentrifuge tubes (8000 rpm, 1 min) and resuspend in 200 µl of lysis buffer [50 mM glucose, 25 mM Tris-HCl (pH 8.0), 10 mM EDTA, 4 mg/ml lysozyme].
3. After 5 min at room temperature, add 400 µl of a freshly prepared alkaline solution (0.2 N NaOH, 1% SDS) and mix by inverting 3 to 6 times.
   Note: The suspension will become clear and viscous.
4. After 5 min on ice, add 300 µl of a 7.5-M ammonium acetate solution (pH 7.8 without adjustment) and mix the contents of the tube gently for a few seconds.
5. Maintain the tube at 0°C for 10 min to allow most of the protein, high molecular weight RNA and chromosomal DNA to precipitate.
6. Centrifuge for 3 min at 10,000 rpm.
7. Remove the clear supernate and transfer to a second tube.
8. Add 0.6 volumes of isopropanol (400 µl to 500 µl) and incubate at room temperature for 10 min.
9. Centrifuge at 15,000 rpm for 10 min.
10. Remove the supernate by aspiration and wash the pellet with 70% (v/v) ethanol.
11. Leave the tubes inverted on a tissue paper for 15 min at room temperature to dry.
12. Dissolve the pellet in 100 µl 10 mM Tris-HCl (pH 8.0), 1 mM EDTA.

continued on next page

Figure 1. Gel analysis of mini-prep plasmid DNA. Samples were prepared as described here and incubated for 1 h (lane 1) and 48 h (lane 2).

Note: The DNA solution is sometimes turbid. If it is, a short centrifugation (2 min, 8,000 rpm) should be performed. There is no need to discard the pellet, which does not interfere with further manipulations of the DNA.

13. If RNase digestion is desired, add 1 µl of a 1-mg/ml RNase solution to the 100 µl final volume. It is not necessary to incubate the mix; RNA will be digested during the restriction enzyme digestion.
    Note: Further DNA precipitation steps involving the addition of ammonium acetate and ethanol at a final concentration of 2.5 M and 70% (v/v), respectively, can be performed to remove more RNA, but are usually unnecessary.

A wide variety of plasmids and cosmids have been extracted using this method and digested with more than 80 different restriction enzymes. DNAs digested for 48 h did not show any exonucleolytic or random endonucleolytic degradation (figure 1). As much as a 10-µl aliquot of DNA can be digested in 20 µl in 1 h, and analysis of the reaction on a polyacrylamide gel allows visualization of fragments smaller than 100 bp. Eluted DNA fragments can be subcloned without the occurrence of artifacts, and the isolated DNA can be used directly for double-stranded DNA sequencing. A 20-µl aliquot is sufficient for sequencing ≥ 250 bp of the cloned fragment without appreciable background.

This simple, reliable and rapid method for processing minipreparations has been used for plasmid DNA up to 50 kb in size from *E. coli* cultures, and it has been used successfully in other laboratories. The purity and yield of the extracted DNA simplify analyses of recombinant DNA molecules.

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New Restriction Endonuclease for Genomic DNA: *Rsr* II

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Restriction endonucleases that cleave DNA infrequently are critical to genomic mapping strategies. The frequency of DNA cleavage by a restriction enzyme is determined partially by the size of its recognition sequence. The base composition of the target DNA, the occurrence of rare di- or trinucleotide sequences, and the methylation of nucleotides within the restriction endonuclease recognition sequence can also influence the average fragment size in a digest (1).

BRL's newest restriction enzyme, *Rsr* II, is suitable for genomic mapping strategies because the enzyme recognizes a heptanucleotide sequence, 5′-CG↓GA/TCCG-3′. It is a type II restriction endonuclease isolated from *Rhodopseudomonas sphaeroides* (2). *Rsr* II is especially useful for mapping mammalian genomes where the dinucleotide CpG is rare (3,4), and it is supplied at 20 units/µl to facilitate digestion of genomic DNA.

One unit of BRL's *Rsr* II is defined as the amount of enzyme required to digest 1 µg of λ DNA completely in 18 h at 37°C using 1X REact® 5 buffer and 1 mM DTT. Although most restriction endonuclease unit assays specify a 1-h incubation time, an 18-h incubation period is required for unit activity determinations of *Rsr* II. One of the five *Rsr* II recognition sequences in λ DNA is cleaved only upon extended incubation (>6 h), and the amount of time required for complete cleavage cannot be reduced by increasing the concentration of the enzyme in the reaction mixture. The antioxidant DTT is required for maximal enzyme activity in this extended digestion reaction.

In studies conducted at BRL the digestion of human lymphocyte genomic DNA with *Rsr* II is complete in 2 h under the following conditions:
5-10 µg genomic DNA
2 µl 10X REact® 5 Buffer
2 µl 10 mM DTT
10-20 units *Rsr* II
Distilled or deionized water to 20 µl

Due to the high purity of BRL's *Rsr* II, incubations can be safely extended to 18 h to ensure cleavage of all *Rsr* II sites.

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