a 4.3-kb vector. This plasmid differs from p14 in that pSFKIN has a poly(A) tail that is 31 bases long, and the nucleotide 5' to the poly(A) tail is T. This plasmid was purified by alkaline lysis (4). This purification method may be more representative of DNA provided to sequencing laboratories than CsCl banding. In Table 1, base 1 of the pSFKIN reference sequence starts 24 bases after the poly(T) of pSFKIN.

To generate the data in Table 1, alignments were performed using Macaw software (Version 2.0.5, National Center for Biotechnology Information; ftp://ncbi.nlm.nih.gov/pub/macaw/). Data from fluorescent sequencing runs were compared to reference sequences in 100-base increments. A gap in the sequence was scored as a miscall. An N that was reported by the sequencing software was also scored as a miscall. If more than five miscalls were observed in a reference block of 100 bases, subsequent miscalls were not determined. Signal strengths in Table 1 were copied from electropherograms.

Initial studies with oligonucleotide 17 investigated the effect of annealing temperature on the length of readable sequence (Table 1). Temperatures of 50° to 30°C were used in 5°C increments. When 32 pmol of oligonucleotide 17 were used with 500 ng of template, the least errors were achieved with 50°C. Even though the amount of primers is large, template or if longer sequencing runs are performed, larger amounts of template may be useful.

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**Rapid DNA Purification from Agarose Gels Using 96-Well Format, Centrifuge-Driven Filtration**

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The extraction of DNA from agarose gels is a basic technique in molecular genetics, and a number of suitable methods have been described. A number of informative reviews and comparisons of different methods have also been published (1–3,5). One of the most useful and quick methods is based on centrifugation-driven filtration, first described by Maxam and Gilbert in 1977 (4) and with many variations published by others thereafter. Unfortunately, to our knowledge, no technique is available that fulfills the requirements of being rapid, having high recovery and being economical for large number of samples.

We have developed a method for DNA extraction from agarose gel slices that is economical, rapid and suitable for processing many samples. After the gel slices are excised (minimizing the amount of agarose surrounding the fragment), they are delivered into individual wells of a 96-well filtration plate (Plate No. MAHV45; Millipore, Bedford, MA, USA). The membrane at the bottom of each well is a polyvinylidine fluoride filter with a 0.45-µm pore size and low protein and nucleic acid binding properties. The plate with agarose slices is frozen at -20°C; this forces out interstitial buffer, breaks up the gel structure and makes it less likely that larger DNA pieces get stuck in the gel matrix. The plate is thawed on ice or at room temperature until the gel pieces have the consistency of soft ice (i.e., they are not completely thawed and there are still ice crystals in the gel piece). Note that if thawing on ice, make sure the filter does not get wet from below. Each agarose piece is then crushed with a pipet tip against the wall of the well. This step is facilitated if the pieces are stuck to the wall from the beginning. After freezing and thawing, the filtration plate is placed on top of a Falcon® Model 3911 96-well Collection Plate (Becton Dickinson Labware, Bedford, MA, USA). Due to the centrifugal force, the collection plate has to be positioned in a support. We found that a common pipet tip rack (Corning Costar, Cambridge, MA, USA) offered a good fit and is sturdy enough to withstand compression. The three components are taped together into a convenient stack and centrifuged for 5–10 min at 1000×g in a Model GR4.22 Centrifuge (Jouan, Winchester, VA, USA).

The percent recovery at different centrifugation times was tested for 564- and 2027-bp DNA fragments that had been separated in a 1% Ultra Pure
Agarose gel (Life Technologies, Paisley, Scotland, UK) (Figure 1). The recovery was found to level off at about 45% for the smaller fragment after 5 min and at 40% for the longer fragments. In general, for polymerase chain reaction (PCR) products of sizes approximately 500 bp and separated in 1% agarose/TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) gels, we recommend 5 min of centrifugation at 1000×g. However, the centrifugation time may differ for other gel types, buffers and sizes of DNA.

In our hands, the method described has been shown to yield DNA of sufficient purity and ample yield for direct sequencing and cloning of PCR products, and it has become a routine method for generation of sequencing templates. Agarose contaminants that may interfere with subsequent enzymatic processes do not seem to affect the results using this procedure. Crushing agarose pieces is not strictly necessary if lower recovery rates are acceptable for further processing. Even higher recovery rates can be obtained if the crushed gel pieces are eluted with water or buffer before centrifugation. For small volumes, this can be done in the wells of the plate. Since this will yield a larger filtered volume, additional steps might be required for concentrating the volumes.

In summary, the 96-well filtration device, intended for different assay techniques, has proven extremely useful in purification of DNA from agarose gels. The method is suitable for processing a large number of samples, without the high cost associated with commercially available single-sample centrifugal extraction devices. When extracting 40–80 PCR products for direct sequencing, the price for purification can be reduced by about 90% as compared to commercially available single sample centrifugation devices. In addition, less time is required when using a 96-well plate format.

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