pean populations. Frond samples were obtained from three sites in Glamorgan (Wales), one from Cornwall (England) and four from Southern Europe (Spain and Italy). PCR conditions were the following: 1 cycle of 94°C for 5 min, 60°C for 1 min and 72°C for 1 min followed by 35 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min and then 1 cycle of 94°C for 1 min, 60°C for 1 min and 72°C for 5 min. Clear DNA fragment patterns were obtained (Figure 2), and differences were scorable using this PCR application, which is known to be more sensitive to DNA purity than standard PCR, confirming the high quality of our template DNA.

The extraction technique described is thus applicable to a wide range of fern species and provides sufficient quality DNA for some of the important PCR-based applications needed for population studies. Although we are not using random-amplified polymorphic DNA (RAPD) analysis in our laboratory, the DNA quality required for this procedure is comparable to that needed for inter-SSR PCR, which is a similar technique, and we anticipate that our extraction procedure could also be of use for isolating RAPD markers. The extraction method described could be of use to workers who want to analyze other fern populations, including those from threatened fern species such as *Cystopteris fragilis* and taxonomically complex genera such as *Dryopteris* and also very variable species such as *Cystopteris fragilis*.

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**Rapid, Economical Filter Alternative to Chromosomal DNA Centrifugation in the Alkaline Lysis Plasmid Protocol**

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One of the more widely used techniques for preparing plasmid DNA from bacteria is the alkaline lysis method (1,4) in which bacterial cells are treated successively with (i) a lysozyme solution to degrade the cell wall, (ii) an alkaline sodium dodecyl sulfate (SDS) solution to disrupt the cells and denature chromosomal DNA and (iii) a high-salt, low-pH solution to neutralize the sample and selectively precipitate the bacterial chromosomal DNA and proteins, which excludes the plasmid DNA. The chromosomal DNA is removed by two successive high-speed centrifugation steps, and the plasmid is then isolated from the supernatant by one of several techniques (4). With the introduction of DNA affinity resins in column formats for purification, the chromosomal sedimentation step became the most time-consuming (ca. 1.5 h). Further streamlining came with the replacement of the successive centrifugations with a simple filter step (QIAfilter™ Cartridges; Qiagen, Valencia, CA, USA). While convenient, the filters may represent a 15% or more increase in cost per purification, and occasionally there may be fewer filters than samples, necessitating that some samples be centrifuged.

We have found that an efficient filter can be simply and inexpensively assembled from common laboratory items: a syringe and glass wool. We assessed the quality of plasmid DNA obtained by removal of chromosomal DNA with the original centrifugation method, a commercial filter or an in-laboratory syringe filter, by the criterion of restriction enzyme digestion and transfection efficiency. Bacteria (XL1-Blue; Stratagene, La Jolla, CA, USA) harboring a eukaryotic expression plasmid for green fluorescent protein (pEGFP-N1; Invitrogen, Carlsbad, CA, USA) were grown overnight in 100 mL
of LB medium containing 100 µg/mL ampicillin. Thirty milliliters were aliquoted into three 50-mL tubes, and the pelleted bacteria were processed by the alkaline lysis procedure with the QIAGEN-tip 100 version of the QIAGEN® Plasmid Kit (Qiagen). Briefly, cells are resuspended in 4 mL Buffer P1 (50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 µg/mL RNase A), and 4 mL of Buffer P2 (200 mM NaOH, 1% SDS) are added. The solution is held at room temperature for 5 min, and then 4 mL of Buffer P3 (3.0 M potassium acetate, pH 5.5) are added. After addition of the high-salt solution, the precipitated DNA, protein and cellular debris were removed in one of three ways: (i) centrifugation, (ii) QIAfilter cartridge and (iii) in-laboratory syringe filter. Sample No. 1 was placed on ice for 10 min and then centrifuged in a SS-34 rotor (Sorvall, Newtown, CT, USA) at 13,000 rpm (ca. 20,000x g) for 30 min at 4°C. The supernatant was subjected to a second centrifugation under the same conditions. The final supernatant was then applied to a QIAGEN-tip 100 column and processed according to the manufacturer’s protocol. Sample No. 2 was poured into a QIAfilter cartridge to which a supplied luer block was fitted to prevent sample flow-through, left
Therefore, DNA (a sensitive measure of DNA quality) was found to be critical. It is important that the lysate not leak from the syringe during the 10-min period between application and expulsion. We found that the tip caps supplied with the syringe are not sufficient to prevent sample flow-through; thus, we used spare luer blocks that are supplied with the QIAGEN kit. However, Parafilm® wrapped around the syringe tip caps is also an effective plug. Sample No. 3 was applied to the in-laboratory filter and treated the same as the sample for the commercial filter. DNA was eluted from the QIAGEN-tip 100 with 5 mL of elution buffer (Buffer QF), precipitated with 0.7 vol of isopropanol, washed with 70% ethanol and resuspended in 100 µL of TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

The DNA yield and optical density (OD) ratio for samples Nos. 1 through 3, respectively, was 203 µg/1.89, 202 µg/1.8 and 223 µg/1.76. The DNA was digested with AvaII, NcoI or no enzyme, and was run on a 1% agarose gel stained with ethidium bromide (Figure 1). DNA from the three methods gave a similar ratio of relaxed to covalently closed circular forms (Figure 1, lanes 2, 5 and 8), and there was no observable difference in digestion efficiency with AvaII (Figure 1, lanes 3, 6 and 9) or NcoI (Figure 1, lanes 4, 7 and 10). Thus, the three methods produced similar yields and equivalent purities as assessed by absorption, and the DNA was of indistinguishable physical characteristics and digestibility.

It has been suggested that transfection efficiency of eukaryotic cells is negatively affected by contaminants in some DNA preparations (5) and thus is a sensitive measure of DNA quality. Therefore, DNA (3 µg) isolated by the three methods was used to transfect duplicate dishes of secondary chicken embryo fibroblasts (CEF) or human 293 cells. CEFs were transfected by the DEAE-dextran method (3), while the calcium phosphate procedure (2) was used for 293 cells. Twenty-four hours later, six independent fields from various areas of the plates were observed by fluorescence microscopy, and the number of green cells per field was scored (Figure 2). Each DNA gave similar numbers of green CEFs. Method No. 1 DNA gave somewhat fewer green 293 cells, but there was no difference between the two filter methods (Nos. 2 and 3). Thus, using two different cell types and transfections protocols, the data show that the DNA produced by the filter methods is as good as, if not better, than the DNA produced by method No. 1, and that the in-laboratory filter performed as well as the commercial version.

We have used the in-laboratory filters with a number of different plasmids, both high- and low-copy number, with consistent success. The DNA also supports long reads from automated DNA sequencing (data not shown). The filter step is much faster and simpler than centrifugation, and the in-laboratory filters represent an equivalent and economical alternative to commercial filters. While the timesavings can be appreciated by all, the cost saving might be particularly important in a teaching setting or where the added expense of commercial filters is prohibitive. In this regard, while we have used the in-laboratory filters in combination with the QIAGEN tip-100, they should be equally useful for other variations of the alkaline lysis method (4).

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