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Rapid preparation of megabase plant DNA from nuclei in agarose plugs and microbeads

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Conventional methods for preparation of megabase plant DNA require a protoplast preparation step (1–5). Preparation of protoplasts is tedious and time-consuming. In contrast isolation of nuclei from leaf tissue is simple. Many researchers have attempted to prepare megabase DNA from nuclei (e.g., 3–6), but no success has been reported. As an alternative approach Guidet and Langridge (6, 7) described a method to prepare megabase DNA by directly embedding ground tissue in agarose plugs. This method, however, is not in general use for large DNA cloning, and cannot be used to prepare DNA in agarose microbeads. Here we describe a simple and rapid method for preparation of megabase DNA by isolating nuclei directly from whole plants or leaf tissue and embedding them in either agarose plugs or microbeads. The microbeads preparation is advantageous for pipetting and enzymatic manipulations (8–10).

Plant nuclei were isolated by grinding whole plants (*Arabidopsis*) or leaf tissue (rice) in liquid nitrogen, suspending the powder in nuclei isolation buffer and filtering through nylon meshes. We used a nuclei isolation buffer modified from previous reports (11–13) and pelleted the nuclei directly in this buffer without resort to percol gradients. For preparing DNA in agarose microbeads, we found that adding cold mineral oil to the agarose–mineral oil emulsion resulted in quick chilling, enhancing microbead size uniformity.

The majority of *Arabidopsis* and rice DNA prepared by this method in agarose plugs or microbeads was larger than 2.5 Mbp, beyond the resolving power of our CHEF gel electrophoresis conditions (Figure 1). This demonstrates that most of the nuclei were maintained intact during the isolation. In contrast, when nuclei were isolated by homogenizing plant tissue in isolation buffer using a blender, a large number of nuclei were broken and no DNA of desired size could be obtained (data not shown). About 200–300 μg DNA could be obtained from 20 g *Arabidopsis* whole plants or 25 g rice leaves. In comparison to protoplast-based methods, this method is very simple and rapid, requiring only 1.5 h for all manipulations from intact plant tissue through the start of lysis. Since megabase DNA could be isolated reliably from both *Arabidopsis* and rice, this method should be readily used for other dicot and monocot species.

The megabase DNA is amenable to digestion with both frequent and rare cutting restriction enzymes (Figure 2A). Digestion of *Arabidopsis* DNA with *Sal*I and *Sma*I produced a majority of fragments smaller than 200 kb, while digestion with *Not*I yielded much larger fragments. This is in agreement with a previous report (1). Hybridization of the digested DNA with DNA probes produced distinct band patterns (Figure 2B). In addition, digestion proceeded more rapidly for DNA prepared in microbeads.

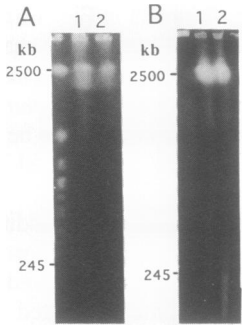

Figure 1. CHEF gel electrophoresis of megabase DNA prepared from *Arabidopsis* (**A**) and rice (**B**). The gels (1% agarose/0.5×TBE) were run with a CHEF-DRII apparatus (Biorad) at 200 volts and 14°C with switching times of 70 sec for 15 h and 100 sec for 9 h. Lanes 1, 2 in each panel indicate megabase DNA prepared in agarose plugs and microbeads, respectively. The margin lane is yeast (*Saccharomyces cerevisiae*) chromosome size standards. Method: Three-week old *Arabidopsis* whole plants (20 g) or 2-week old rice leaves (25 g) were ground to a fine powder in liquid nitrogen with a mortar and pestle, and the powder was transferred to a beaker. After the liquid nitrogen had evaporated completely, 200 ml of ice-cold nuclei isolation buffer (10 mM Tris–HCl pH 9.5, 10 mM EDTA, 100 mM KCl, 0.5 M sucrose, 4 mM spermidine, 1.0 mM spermine, 0.1% mercaptoethanol) was added to the powder and mixed. The homogenate was filtered on ice sequentially through one layer each of 250, 90, 50, and 20 μm nylon mesh. One-twentieth volume of isolation buffer supplemented with 10% Triton X-100 was added to the filtrate (this step is optional but promotes chloroplast lysis). The nuclei were pelleted at 4°C (in 50-ml tubes) at 4000 rpm (2000 g) for 10 min and resuspended with isolation buffer to a final volume of 1–1.2 ml. The nuclei were heated at 42–45°C for 1–2 min, then mixed gently with an equal volume of 1.4% low-melting agarose (45°C) prepared in 10 mM Tris–HCl pH 9.5 and 10 mM EDTA. The mixture was poured into a mold to form plugs. For preparing DNA in agarose microbeads, the preheated nuclei were mixed in a 30-ml flask with an equal volume of 1.2% low-melting agarose (45°C). Based on the agarose–nuclei combined volume, 1.5 volumes of pre-warmed (45°C) mineral oil were added and the flask was swirled vigorously for 10–15 seconds. Immediately another 1.5 volumes of ice cold mineral oil were added to the emulsion and the flask was quickly swirled in an ice water bath for 2 minutes. The microbeads were collected by brief centrifugation. The agarose plugs and microbeads were treated with lysis buffer (1% sarkosyl, 0.25 M EDTA pH 8.0 and 0.2 mg/ml proteinase K) at 50°C for one day with one change of lysis buffer.

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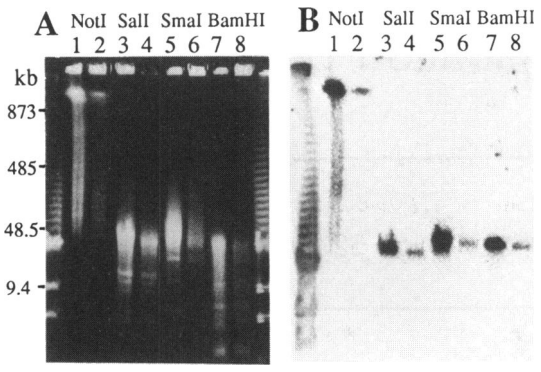

Figure 2. Restriction digestion (**A**) and hybridization analysis (**B**) of *Arabidopsis* DNA. The DNA prepared in agarose plugs (lanes 1, 3, 5, 7) and microbeads (lanes 2, 4, 6, 8) was digested with restriction enzymes as indicated and separated by CHEF gel electrophoresis running at 200 volts and 14°C with switching times ramped from 30 sec to 90 sec over 18 h. The margin lane is multimers plus *Hin*dIII-digest of lambda DNA. Prior to digestion the agarose plugs and microbeads were washed at 50°C for 1 h in several volumes of 10 mM Tris−HCl pH 8.0, 10 mM EDTA containing 1 mM phenylmethylsulfonyl fluoride (PMSF), then in several changes of TE buffer. The DNA samples (40 μl, 3−5 μg DNA) were equilibrated for 30 min in 400 μl of the appropriate digestion buffer supplemented with 8 mM spermidine. Digestion was performed in 120 μl volumes with 50 units of restriction enzyme at the recommended temperature for 4−5 h (for microbeads) or overnight (for plugs). After CHEF gel electrophoresis, the gel was irradiated at 254 nm for 2 min and the DNA was depurinated in 0.25 N HCl for 25 min. DNA fragments were transferred overnight to Hybond-N^+ membrane by alkaline blotting. Southern blot analysis of the digests with an RFLP probe (17A6T, unpublished) is shown in **B**.

We have used *Arabidopsis* and rice DNAs prepared by this method to construct P1 libraries. The DNA was partially digested with *Sau*3AI (for *Arabidopsis*) or *Mbo*I (for rice), separated on low- melting agarose gels, and fragments of 75−100 kb were recovered (data not shown). The DNA was released from the gel by digestion with agarase. Using DNA prepared by this way, we have constructed *Arabidopsis* and rice P1 libraries (unpublished work).

The method described here provides a simple and quick approach to megabase DNA preparation from plants facilitating long-range physical mapping and large DNA cloning in plant genome research programs.

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