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# Rapid DNA preparation for 2D gel analysis of replication intermediates

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Two-dimensional agarose gel electrophoresis (2D gel) analysis (1,2) is used extensively as a method to detect origins of replication. Standard DNA isolation procedures are time consuming and are often inadequate for the preservation of replication intermediates necessary for this analysis. The DNA isolation procedure that has been found most suitable for *Saccharomyces cerevisiae* (2,3) involves CsCl density gradient centrifugation, and can require three or more days of preparation time. Furthermore, we found it difficult to visualize CsCl-banded DNA when starting with small cell numbers (<4 × $10^9$ haploid cells). In this communication, we present a simplified method for the isolation of yeast genomic DNA for 2D gel analysis from smaller numbers of yeast cells. Using this method, 20–25 μg purified DNA, suitable for 2D gel analysis, can routinely be prepared from 50 ml cell culture in ~6 h. Modifications of this method should be suitable for 2D gel analysis in other organisms as well.

In this method, disruption of the cell wall is carried out using glass beads as described by Huberman *et al.* (2). We found that digestion of the cell wall with zymolyase often resulted in loss of detectable replication intermediates. DNA is then isolated using a Qiagen column. These two steps alone were sufficient to produce a typical bubble arc at the single copy chromosomal ARS1 locus when 7 μg DNA, from exponentially growing yeast cells, was subjected to neutral/neutral 2D gel analysis (Fig. 1A). The image was indistinguishable from a similar image produced using DNA prepared by the CsCl isolation method (Fig. 1B).

Exponentially growing haploid yeast cells (50 ml; 2 × $10^7$ cells/ml) were harvested by centrifugation in a 50 ml polypropylene centrifuge tube, washed once with 20–30 ml of ice-cold distilled water, and the pellet was resuspended in 0.8 ml ice-cold nuclear isolation buffer (NIB: 17% glycerol, 50 mM MOPS buffer, 150 mM potassium acetate, 2 mM magnesium chloride, 500 μM spermidine and 150 μM spermine; pH is adjusted to 7.2). An equal volume of acid-washed glass beads (Sigma, 425–600 μm diameter) was added, and the cell suspension was vigorously shaken with a VORTEX-GENIE (Scientific Industries model # K-550-G; our other vortex mixers did not provide sufficient power for efficient cell lysis) at maximum speed for 30 s, and quickly placed in ice water for another 30 s. This routine was repeated 12–20 times, until ≥90% of cells were broken (lysis monitored by phase contrast microscopy). Broken cells were removed with a Pasteur pipette to a 12 × 75 mm polypropylene culture tube, and the glass beads were rinsed twice with 0.8 ml

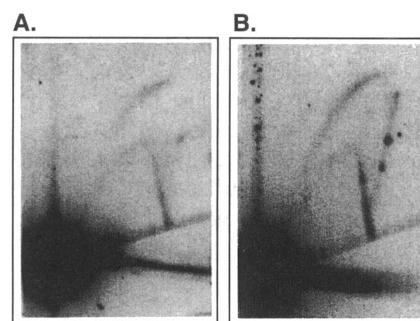


**Figure 1.** (**A**) DNA (7 μg) purified by the method described in this report was digested with *Nco*I and subjected to neutral/neutral 2D gel analysis by the method of Brewer and Fangman (1), hybridizing with the 5 kb *Nco*I fragment encompassing ARS1. (**B**) A similar gel is shown after isolation of DNA by the CsCl method (2). Images were collected with a Molecular Dynamics 425E PhosphorImager after a 24 h exposure to the imaging plate.

ice-cold NIB and transferred to the same tube (it does not matter if some glass beads get transferred). Broken cells were pelleted at 6500 *g* at 4°C for 10 min and the pellet was dissolved in 2 ml Qiagen Buffer G2 (800 mM GuHCl, 30 mM EDTA, 30 mM Tris–HCl, 5% Tween-20, 0.5% Triton X-100; pH 8.0) containing 200 μg RNase A/ml. It is important to generate a homogeneous lysate by gently inverting the tube ~20 times (do not vortex). The lysate was incubated at 37°C for 30 min, then 50 μl proteinase K (20 mg/ml) was added, and the lysate was incubated for an additional 60 min. After incubation, the lysate was centrifuged at 6500 *g* at 4°C for 10 min. The supernatant, which should be clear, was carefully removed to a 12 × 75 mm polypropylene culture tube. An equal volume of Qiagen Buffer QBT (750 mM NaCl, 50 mM MOPS, 15% ethanol, 0.15% Triton X-100, pH 7.0) was added to the supernatant. A Qiagen column (20/G) was equilibrated with 1 ml Buffer QBT, then the sample was loaded on the column and allowed to pass through by gravity flow. The column was washed 3 times with 1 ml Qiagen Buffer QC (1.0 M NaCl, 50 mM MOPS, 15% ethanol, pH 7.0). The DNA was eluted twice with 0.9 ml Qiagen Buffer QF (1.25 M NaCl, 50 mM Tris–HCl, 15% ethanol, pH 8.5) pre-warmed to 50°C. Isopropanol (1.26 ml; room temperature) was added to the sample, the solution was well mixed and divided into two 1.5 ml Eppendorf tubes. DNA was

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precipitated by centrifugation in a microfuge at full speed for 15 min at room temperature. The supernatants were discarded, 500 µl 70% ethanol (room temperature) was added to each tube, and the DNA pellets were dislodged into the 70% ethanol and pooled to one tube. DNA was again pelleted for 2 min in a microfuge at full speed and most of the 70% ethanol was discarded. The tube was again centrifuged at full speed for 3–5 s and all traces of 70% ethanol were removed. DNA was immediately resuspended in 40 µl TE (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) at room temperature for 1–2 h. If the DNA pellet is allowed to dry at this step, resuspension will be very difficult. Using this method we routinely obtain 20–25 µg DNA, with an $A_{260nm}/A_{280nm}$ ratio 1.7–1.8.

Qiagen mini preparation tip (Qiagen Genomic tip 20/G) was selected for this work since we wanted a method to isolate genomic DNA, starting with smaller numbers of cells. In principle, any of the genomic grade Qiagen tips should work (e.g. we have also used tip-500). One Qiagen genomic tip 20/G (designed for 20 µg DNA) costs just $4.00. The method can be scaled up to prepare larger quantities of DNA, if necessary, using larger columns (tip 100, $6.00 each; tip 500, $12.00 each)

Two additional steps are utilized by some investigators to increase the sensitivity of 2D gel analysis. One is to preserve replication intermediates by adding 0.1% sodium azide to the cells just before harvesting (3). The other is to enrich for replication intermediates using BND–cellulose (2). We have not employed either of these techniques to produce the data shown in Figure 1. However, there is no reason why these steps cannot be incorporated into the protocol described here.

Finally, we have successfully used modifications of this method for 2D gel analysis of genomic DNA from Chinese Hamster Cells (CHOC 400) and viral DNA from SV40 infected monkey cells.

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## REFERENCES

1 Brewer, B.J. and Fangman, W. L. (1987) *Cell* **51**, 463–471.
2 Huberman, J. A., Spotila, L. D., Nawotka, K. A., El-Assouli, S. M., and Davis, L. R. (1987) *Cell* **51**, 473–481.
3 Dubey, D., Zhu, J., Carlson, D. L., Sharma, K., and Huberman, J. A. (1994) *EMBO J.* **13**, 3638–3647.