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A rapid and reliable DNA preparation method for screening a large number of yeast clones by polymerase chain reaction

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Yeast is an ideal host system for studying exogenous eukaryotic gene expression and for studying gene structures from recombinant plasmid DNAs and yeast artificial chromosomes (YACs). Many yeast expression studies may also require the altering of the genome itself by homologous recombination. All these studies require the screening for positive transformants and the most popularly used method of screening is PCR. As some gene replacement experiments have a low success rate (as low as 4%) (1), it becomes necessary to screen a large number of clones. Hundreds and thousands of transformants, e.g.1200 YAC clones/day/lab as reported by Khristich *et al.* (2), are screened by PCR in many laboratories.

Conventionally, PCR is carried out with purified DNAs, i.e. plasmids (3), YACs (4) or genomic DNAs (5) from yeast. The purification of DNAs involves either the use of glass beads to disrupt intact cells or the use of lytic enzymes to digest cell walls, followed by numerous inconvenient steps of organic solvent extraction and alcohol precipitation. These purification methods are time-consuming (2–5 h for $\leq$24 samples) and require a significant amount of starting culture. With a generation time of 100–300 min, single colonies of yeast require an additional 1–3 days to grow to the late log phase in 1–15 ml culture to provide sufficient amounts of cells for manipulation during the purification. A previous report showed that fresh yeast colonies can be used directly, without prior DNA purification, for PCR to obtain a 1.1-kb genomic PCR product (6). However, another group reported that direct PCR with fresh yeast colonies could not even detect products amplifiable from plasmid DNAs (7). In fact, plasmid DNAs are easier to isolate than genomic DNAs and plasmid DNAs can be used in higher molar concentrations for PCR. Some researchers have success in boiling yeast colonies in a small volume of water for 5–10 min, and using either the boiled cells directly or the supernatant from a brief spinning of the boiled cells as a plasmid or YAC source for PCR (unpublished results and personal communications). Our repeated trials using these two methods could not consistently yield genomic PCR products ranging from 0.3 to 3 kb in size.

Here we present a very rapid method for the preparation of plasmid, YAC and genomic DNAs from yeast to be used for PCR screening. This method is designed to overcome the disadvantages of boiling and direct methods and at the same time to remain as simple as possible. As the thick yeast cell wall may have contributed to the difficulty of directly using yeast cells for PCR in contrast to the relative ease of direct use of bacteria (7–9) for PCR, we introduce a single incubation solution (5 min) to make spheroplasts prior to PCR. This new method does not require the culturing of yeast cells for 1–3 days (although cells from liquid culture may be used with equal efficiency) and alleviates the need for purified plasmid, YAC or genomic DNA. In addition, the use of organic solvent extractions and even routine manipulations, e.g. ethanol precipitations, boiling baths and centrifugations are avoided.

An average-size yeast colony (0.5–2 mm) or a cell pellet from a liquid culture is touched with a sterile disposable pipette tip. The cells do not need to be washed in water or buffers as is required by many other methods for spheroplast preparation. The tip is then thoroughly rinsed with 2–10 µl of the incubation solution by pipetting the solution up and down three to five times. The incubation solution is comprised of 1.2 M sorbitol, 100 mM sodium phosphate, pH 7.4 and 2.5 mg/ml Zymolyase (ICN Canada), an enzyme often used to transform intact yeast cells into spheroplasts. Aliquots of this enzyme incubation solution can be stored at –20°C and remain active for at least 6 months. The resulting enzyme/cell mixture solution is then incubated at 37°C for 5 min. At the end of this incubation, 0.2–5 µl of the mixture solution can then be used for each 100 µl of PCR reaction. The remaining sample (spheroplasts containing DNAs) can be stored at –20°C for repeated use. The results obtained by using this method are shown in Figure 1. For plasmid PCR products, this method works as efficiently as the boiling method (lane 2 versus lane 1). For amplification of YAC products, this method routinely works better than the boiling method (lane 4 versus lane 3) in terms of the final productivity of PCR reactions. For genomic DNA amplification this method consistently yielded the expected PCR products with high yield (~1 µg/5 µl finished PCR reaction, lanes 11, 12 and 13). Neither the boiling method (lanes 6 and 7) nor the direct method (lanes 8, 9 and 10) yielded any observable

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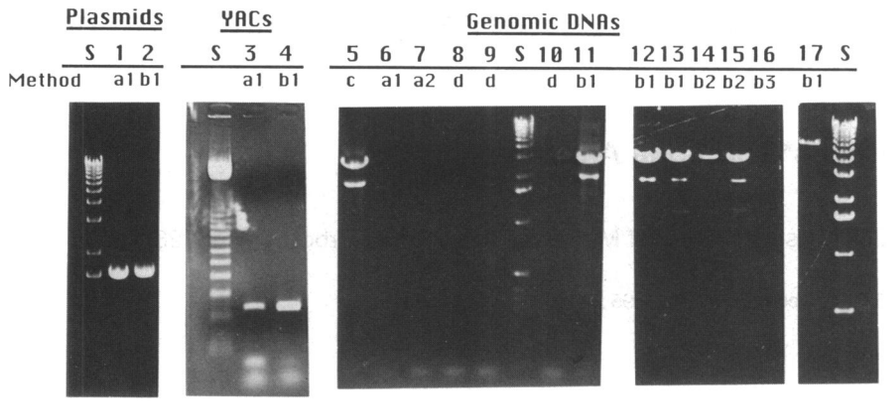

Figure 1. Comparison of different methods of preparation of yeast plasmid, YAC and genomic DNA for PCR. Template DNAs from yeast were isolated by the following methods: (**a1**) the boiling method with the use of the supernatant; (**a2**) the boiling method with the direct use of boiled cells; (**b1**) the rapid method (spheroplasts) presented in this paper; (**b2**) use of pre-lysed spheroplasts; (**b3**) use of the supernatant from pre-lysed spheroplasts; (**c**) conventional DNA purification by the method of Strathern and Higgins (3); (**d**) the direct method. For plasmid PCR (the left panel), a primer from the *Escherichia coli bla* gene and another primer from the *LEU2* gene of the vector pJJ250 were used to amplify a product of 1.7 kb, using DNAs prepared by method **a1** (lane 1) or method **b1** (lane 2). For YACs (the middle panel), a pair of primers yielding an expected product of 250 bp in size were used. The templates were prepared by method **a1** (lane 3) or method **b1** (lane 4). For genomic DNAs (the right panel), PCR was performed using a pair of primers binding to a wild-type copy and an inserted disrupted copy of the yeast pyruvate dehydrogenase $E_1\beta$ gene, giving a 3.2 kb band and a 2 kb band. Genomic DNAs were prepared by method **c** (lane 5), method **a1** (lane 6), method **a2** (lane 7), method **d** (lanes 8, 9 and 10) and method **b1** (lanes 11, 12 and 13). In lanes 14 and 15, method **b2** was used. In lane 16, method **b3** was used. Lane 17 (method **b1**) shows a 6.2 kb genomic PCR product using a pair of primers binding to a region of yeast genome containing the gene coding for the pyruvate dehydrogenase E1β subunit. Lane S stands for molecular standards, 1 kb ladder for plasmid and genomic DNAs, or 100 bp ladder for YACs.

amounts of genomic PCR products. Amplification of genomic PCR products as large as 6.2 kb with high yield (lane 17) was also attained with this method.

It has been found that the intact cells, a colony on a plate or liquid cultures which were stored at 4°C for even 3 months, still gave satisfactory PCR products when using this method. The disruption of spheroplasts by adding 15 µl of TE, pH 7.6 to 4 µl of spheroplasts prior to PCR was performed to observe whether or not releasing DNAs from spheroplasts is beneficial. The cellular disruption was confirmed by viewing under a microscope. It was observed that the disruption of spheroplasts prior to PCR did not improve the results (lanes 14 and 15 versus lanes 11, 12 and 13). Presumably after the spheroplasts were added to the mixture of PCR components, the spheroplasts would be sufficiently disrupted in the setting of a PCR reaction–a hypotonic buffer and a high-temperature environment; therefore, it was not necessary to pre-lyse the spheroplasts to release DNAs. Interestingly, if TE-lysed spheroplasts were spun and the supernatant used for PCR, no genomic PCR products were obtained (lane 16). A simple explanation for this observation cannot be formulated. It was possible that genomic DNA adhered tightly to proteins and was removed by centrifugation, leaving insufficient quantities of genomic DNA in the supernatant. Indeed, when the pellet, instead of the supernatant, from the lysed spheroplasts was used for PCR, expected bands were obtained (data not shown).

In summary, the method presented here for preparing yeast DNA for PCR is rapid (5 min), simple (one solution), and allows reliable PCR amplification from plasmids, YACs and genomic DNAs from yeast. This method simplifies PCR screening and saves both labor and time, and therefore makes PCR screening more convenient and cost-effective.

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REFERENCES

1 Manivasakam, P., Weber, S.C., McElver, J. and Schiestl, R.H. (1995) *Nucleic Acids Res.*, **23**, 2799–2800.
2 Khristich, J.V., Bailis, J., Diggle, K., Rodkins, A., Romo, A., Quackenbush, J. and Econs, G.A. (1994) *Biotechniques*, **17**, 498–501.
3 Holm, C., Meeks-Wagner, D.W., Fangman, W.L. and Botstein, D. (1986) *Gene*, **42**, 169–173.
4 Green, E.D. and Olson, M.V. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 1213–1217.
5 Strathern, J.N. and Higgins, D.R. (1991) In Guthrie, C. and Fink, G. (eds) Guide to Yeast Genetics and Molecular Biology. *Methods in Enzymology*. Academic Press, pp. 319–328.
6 Sathe, G.M., O'Brien, S., McLaughlin, M.M., Watson, F. and Livi, G.P. (1991) *Nucleic Acids Res.*, **19**, 4745.
7 Ward, A. C. (1992) *Biotechniques*, **13**, 350.
8 Güssow, D. and Clackson, T. (1989) *Nucleic Acids Res.* **17**, 4000.
9 Joshi, A.K., Baichwal, V. and Ames, F.-L. *Biotechniques*, **10**, 42–44.