seen here might not be extrapolated to other subcloning protocols, as they have been compared only with the pCON-based plasmids. Since this method has produced favorable outcomes in the production of other plasmids, however, it appears to have a relatively broad usefulness. It may be most useful for difficult recombinations such as those using large plasmids. Of interest, the recombination yields described here and using the standard method appeared lower than those using other plasmids. This may be specific to the input sequences, especially those originating from HSV-1, which contain high GC content and homologous regions. As the probability of a mixture of plasmid fragments being successfully ligated into a functional circular plasmid is further inversely dependent on the number of fragments, including the selective restriction enzymes in the initial digest mixture may increase the relative yield of intended recombinants.

This method cannot necessarily be applied to all recombinations because it presumes the existence of unique restriction sites in the unwanted sequence of both the donor plasmid of the insert and the excised portion of the acceptor plasmid. However, this requirement is frequently fulfilled, particularly when enzymes having multiple sites in the unwanted sequence are considered. Another option is to use restriction enzyme-producing fragments, that when ligated, result in a novel sequence that is not cleaved by the initial enzymes. Finally, if frequent repetition of similar recombinations using one or both of the same sources is expected, the sources can be designed to include unique sites using techniques such as site-directed mutagenesis.

REFERENCES

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DNA Extraction Method for Screening Yeast Clones by PCR

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ABSTRACT
A simple procedure for isolating yeast DNA suitable for use as a template for PCR amplification is described. SDS treatment alone is sufficient for extraction of chromosomal DNA from yeast cells. Cells of a yeast colony are suspended in a small volume (about 20 μL) of a 0.25% SDS solution, mixed vigorously and centrifuged. The supernatant can be directly used as a template after dilution to give an SDS concentration of less than 0.01% in the final PCR mixture.

INTRODUCTION
Screening of yeast clones for a sequence of interest is a routine step in yeast DNA manipulations. To obtain insertions in a gene of interest, it is necessary to screen several candidates to identify one with the desired modification. This screen can be performed using Southern blot hybridization, but the method is time consuming and requires expensive reagents. In contrast, PCR is a simple, fast and inexpensive method for this purpose. The use of yeast DNA as a PCR template can be prepared by several methods involving either enzymatic digestion of the cell wall (8) or mechanical disruption by glass beads (1,3). For screening many transformants by PCR, simple DNA isolation procedures have been reported such as the direct use of yeast cells (9,10), alkali extraction (12), glass bead disruption (6) and enzymatic digestion (2,7). In our experience, however, some of these methods were not reproducible (13) and others were not efficient for screening large numbers of yeast clones. Here, we describe a rapid and reliable procedure for isolating yeast DNA that can be used directly for PCR.
MATERIALS AND METHODS

Isolation of Chromosomal DNA from Yeast Cells

Yeast strains used were Saccharomyces cerevisiae W303-1A (MATa ade2-1 can1-100 ura3-1 leu2-3,112 trp1-1 his3-11,15) and Kluyveromyces lactis IFO 1267. Cells were grown on YPD plates (1% yeast extract, 2% bac-
topeptone, 2% glucose and 2% agar).

For the development of the DNA isolation procedures, S. cerevisiae cells (3.8 × 108) were suspended in 200 µL of either Zymolyase solution (60 U/mL in water; Seikagaku, Tokyo, Japan) or deionized water; the suspensions in Zymolyase were incubated for 30 min at 37°C. The suspensions were treated with either phenol/chloroform [1:1 (vol/vol)], SDS or both. For the phenol/chloroform treatments, 200 µL of phenol/chloroform were added. For the SDS treatments, 20 µL of 10% (wt/vol) SDS solution were added. For the SDS and phenol/chloroform treatments, 20 µL of 10% SDS were added and mixed vigorously, and then 200 µL of pheno-

Table 1. Primer Sequences

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDR5-HP1</td>
<td>5'-TTCACTGAACCTTGGTTGCATAAC-3'</td>
</tr>
<tr>
<td>PDR5c-CL1</td>
<td>5'-ATGCGTTTCAAAAATAATCGATC-3'</td>
</tr>
<tr>
<td>CUP1-1</td>
<td>5'-ATGGAGCTGATCCATTACCGACATT-3'</td>
</tr>
<tr>
<td>CUP1-2</td>
<td>5'-GTAGCGGCGGCAGTCTTTATGTGATGTGATTGATGAT-3'</td>
</tr>
<tr>
<td>SSK1-1</td>
<td>5'-TAGCTTGTGATCCACTGCT-3'</td>
</tr>
<tr>
<td>SSK1-2</td>
<td>5'-TCCCTCTACAGTGACAACCA-3'</td>
</tr>
<tr>
<td>CHR3-A</td>
<td>5'-GTCGCGGATTAGAGCAA-3'</td>
</tr>
<tr>
<td>CHR3-B</td>
<td>5'-GGTAGCTCGGCTGTGAGA-3'</td>
</tr>
<tr>
<td>CHR3-1</td>
<td>5'-GTGAGACGTAGCTCTGG-3'</td>
</tr>
<tr>
<td>CHR3-2</td>
<td>5'-CGTCTGATTCATGGCTGT-3'</td>
</tr>
<tr>
<td>KLURA3-1</td>
<td>5'-CGGATCCATGGTCTGTGGTTGTC-3'</td>
</tr>
<tr>
<td>KLURA3-2</td>
<td>5'-CGGATCCATCCATGGTGATGAGACATC-3'</td>
</tr>
</tbody>
</table>

a Italics indicate added sequences containing restriction sites.

Table 2. Quick SDS Extraction Protocol

1. Prepare microtubes containing 20 µL or less 0.25% (wt/vol) SDS.
2. Use sterile toothpicks to transfer yeast cells (about 107) into the tubes with brief mixing. Mix and centrifuge briefly.
3. Add 1 µL supernatant into tubes containing a PCR mixture. The final SDS concentration should be less than 0.01%. The addition of Triton X-100 to a PCR mixture supports efficient amplification in the presence of SDS.
4. Run the PCR program.

PCR Amplification

PCR was performed in an automatic thermal cycler (Progene; Techne Ltd., Cambridge, England, UK) using 1× buffers recommended by the polymerase suppliers. Primers used are listed in Table 1. PCR using KOD and KOD Dash systems (TOYOBO Co., Ltd., Osaka, Japan) was carried out in a total
volume of 25 µL, including 1 µL cell extract, 2.5 µL 2 mM dNTP mixture (dGTP, dATP, dTTP and dCTP), 2.5 µL each primer (0.1 µg/µL) and 1.25 U DNA polymerase. The KOD DNA polymerase is derived from Pyrococcus kodaakensis KOD1 strain (11), and the KOD Dash system is programmed for long PCR. To the 25 µL reaction using AmpliTaq® DNA polymerase (PE Biosystems, Foster City, CA, USA) and Taq Plus® Long PCR system (Stratagene, La Jolla, CA, USA), 2.0 µL of the dNTP mixture and 1.25 U DNA polymerase were used. The standard KOD Dash PCR protocol, which was used for PDR5 primers, included an initial incubation for 1 min at 94°C, followed by 30 cycles of denaturation for 20 s at 94°C, annealing for 2 s at 50°C and elongation for 2 min at 74°C. For the CUP1 primers, the elongation time was changed to 30 s. For the SSK1 primers, the annealing temperature was changed to 55°C. For the CHR3 primers, the annealing temperature was changed to 52°C and the elongation time to 30 s. For the KLURA3 primers, the annealing temperature was changed to 53°C and the elongation time to 1 min. PCR amplifications by AmpliTaq and Taq Plus DNA polymerases involved an initial incubation of 2 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at 45°C and 2 min at 72°C, and a final incubation at 72°C for 5 min. Five microliters of each amplification mixture were loaded on a 1% agarose gel for electrophoretic analysis.

RESULTS AND DISCUSSION

Several procedures for extraction of chromosomal DNA from S. cerevisiae were compared. The requirement for Zymolyase treatment was tested, and single treatments with SDS or phenol/chloroform treatments were compared to a combined treatment, as described in Materials and Methods. The most efficient chromosomal DNA extraction was obtained with Zymolyase treatment, followed by the combined SDS and phenol/chloroform steps (Figure 1). However, SDS alone, without Zymolyase treatment, was sufficient for chromosomal DNA extraction.

Next, we examined the inhibition of PCR amplifications by SDS. PCR was done in various SDS concentrations using purified chromosomal DNA as a template (Figure 2). The inhibitory effect of SDS varied with the PCR reagents used. This may be due to the constituents of the buffers and characteristics of the polymerases used. It is known that nonionic detergents neutralize the inhibitory effect of SDS on PCR (4,5).

The effect of Triton® X-100 on PCR amplification was tested (Figure 2). Efficient PCR amplifications were detected even in 0.02% SDS when the mixtures contained 1% and 5% Triton X-100.

Yeast DNA extracted by 1% SDS alone could be used for PCR amplification after at least a 100-fold dilution in the PCR mixture (data not shown). However, greater dilutions of the extracts might result in insufficient quantities of template DNA. Therefore, we examined the efficiency of chromosomal DNA extraction using various concentrations of SDS (Figure 3). The amount of chromosomal DNA extracted showed an inverse correlation with SDS concentration. One microliter of the cell extracts was directly used for KOD Dash PCR with or without 1% Triton X-100 (Figure 3). Amplification of a 500 bp DNA fragment, which is the same fragment in Figure 2, was detected without

Figure 1. Amount of chromosomal DNA extracted by various treatments. S. cerevisiae cells (3.8×10^9) were suspended in either 200 µL of 60 U/mL Zymolyase or deionized water. The cell suspensions in Zymolyase were incubated for 30 min at 37°C. For single treatments, either 200 µL phenol/chloroform (P/C) or 20 µL 10% SDS were added. For the double treatment (SDS/P/C), 20 µL 10% SDS were added and mixed, and then 200 µL phenol/chloroform were added. All samples were mixed vigorously for 30 s and centrifuged for 2 min at 9500×g. Thirty microliters of the supernatants were subjected to electrophoresis on a 1% agarose gel.

Figure 2. Effect of SDS and Triton X-100 concentrations on PCR. PCR mixtures contained the indicated concentrations of SDS, using purified chromosomal DNA as a template, the CHR3-A and CHR3-B primers and either KOD, KOD Dash, AmpliTaq or Taq Plus PCR systems (left panel). PCR mixtures contained 0.02% SDS and the indicated concentrations of Triton X-100, using the same systems (right panel). PCR yielded a 500 bp amplification product.

Figure 3. DNA extraction by various concentrations of SDS and its direct use in PCR. Yeast cells (1.0×10^8) were suspended in 50 µL distilled water. Five microliters of SDS solution, of various concentrations, were added to give the final SDS concentrations indicated. The cell suspensions were mixed vigorously and centrifuged, and 20 µL supernatant were subjected to agarose gel electrophoresis (upper panel). PCR amplification using the CHR3-A/B and the PDR5 primers were carried out in a 25 µL KOD Dash PCR mixture with or without 1% Triton X-100. One microliter of the supernatant was used (lower panel). Sizes of the PCR products are given on the right (lower panel).
Triton X-100 from extracts using 0.5% SDS. This suggests that components extracted from yeast cells may neutralize the inhibitory effect of SDS. The addition of Triton X-100 enabled amplification at higher concentrations of SDS. The results showed that the inhibitory effect of SDS and amplification efficiency will vary with PCR settings, and concentrations of SDS may need to be optimized in a different experimental setting. The most efficient amplification for 4.8 kb DNA fragment was observed in extracts using 0.25% SDS. This procedure allows direct transfer of the cell extract into the reaction tube without prior dilution, which simplifies the procedure. This method was tested for both S. cerevisiae and K. lactis, using the simplified approach of picking cells from yeast colonies (about 10^7 cells) with a sterile toothpick, transferring them into a microtube containing 20 µL of 0.25% SDS and mixing. The cell suspensions were vigorously mixed and centrifuged for 30 s. One microliter of the supernatant was added to a final 25 µL PCR mixture, and either KOD Dash or Taq DNA polymerases were used for amplification with a variety of primers. All combinations tested with DNA from colonies of S. cerevisiae and K. lactis showed amplification (Figure 4). The simple extraction method presented here (Table 2) will therefore be useful for screening large numbers of yeast colonies and may be applicable to other yeast strains and fungi.

REFERENCES


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