Note

A Ten Minute Procedure for Transformation of Saccharomyces cerevisiae

David Keszenman-Pereyra* and Kotaro Hieda

Biophysics Laboratory, Department of Physics, Rikkyo University, Nishi-Ikebukuro 3-34-1, Toshimaku, Tokyo 171, Japan

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Current procedures for DNA-mediated transformation of Saccharomyces cerevisiae use batch cultures as a source of recipient cells and more than 2 hr of manipulations.1) Recently, we reported a procedure that uses cells growing on solid agar media.2) The advantages of this procedure are multifold: a) it is speedy as there is no need of batch cultures; b) it is cheap and technically simple to perform; c) it is applicable to a variety of yeast strains; d) yeast cells derived from plates stored at 4°C can be used although they are less transformable than fresh cells.

Here we describe a simplification of our transformation procedure in which the incubation was reduced from 60~120 min to only 5 min. We assayed conditions which allowed the recovery of transformants for a variety of strains and DNA preparations. All manipulations can be done in approximately 10 min.

The S. cerevisiae strains DKKHAC2 (MATa ura3-52 leu2-3 leu2-112 lys2-801 trp1 his3 ade2-1 RAD+) and DKKHr31 (MATa ura3-52 leu2-3 leu2-112 trp1 his3 rad2-l) were used as hosts. The circular plasmid YCp19 (pBR-322, AR51, CEN4, TRP1, URA3) and the linear plasmid LYTC-2 (YCp19, EcoRI terminal restriction fragment from Tetrahymena macronuclear rDNA) were used as transforming DNA.

The transformation procedure was done as follows: to an 1.5-ml Eppendorf tube, 115 μl of TBT, a loopful of yeast cells from a YPAD5) agar plate incubated for 4 days at 30°C, DNA preparation, and 4 volumes of PEG solution (70 g of polyethylene glycol-4000 in 100 ml of Tris buffer) were added, vortexed, and incubated for 5 min at either 30°C or 42°C in a water bath. Then, 800 μl of Tris buffer was added and after centrifugation the cells were resuspended in 200 μl of the same buffer. Fifty μl samples were plated on SD medium5) containing 50 μg/ml of histidine, adenine, leucine, tryptophan, and lysine for the selection of URA+ transformants.

The results presented in Tables I and II demonstrate that whole cells simply incubated for 5 min in presence of lithium thiocyanate, divalent cations (Ca2+, Mg2+), triacetin and PEG, were effectively transformed by DNA prepared by different procedures. The yield of transformants was markedly influenced by the temperature; incu-

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Table I. Transformation of Whole Yeast Cells by the 10 min Transformation Procedure

<table>
<thead>
<tr>
<th>Strain</th>
<th>Incubation temperature (°C)</th>
<th>YCp19 DNA (Transformants/μg)</th>
<th>LYTC-2 DNA (Transformants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DKKHAC2</td>
<td>42</td>
<td>568</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>228</td>
<td>2</td>
</tr>
<tr>
<td>DKKHr31</td>
<td>42</td>
<td>1112</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>348</td>
<td>26</td>
</tr>
</tbody>
</table>

* Closed circular DNA of plasmid YCp19 was isolated from the E. coli strain JM1097 and purified by CsCl2-ethidium bromide density gradient centrifugation as described.2) Stock DNA solutions were diluted in Tris buffer and 10 μl was used in the transformation experiment.

b Plasmid LYTC-2 was extracted from yeasts by the 10 min procedure6); 30 μl of the aqueous layer was used in the transformation experiment.

c Two other strains (DKKHAS12, DKKHAS16; both MATa ura3-52) were poorly transformed by DNA of YCp19 at 42°C giving 8 and 20 transformants per μg of DNA respectively.

Table II. Transformation Frequency by DNA of YCp19 Isolated from E. coli JM1097 by a Rapid Alkali Procedure8

<table>
<thead>
<tr>
<th>Strain</th>
<th>Fresh cells</th>
<th>Stored cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>DKKHAC2</td>
<td>329</td>
<td>83</td>
</tr>
<tr>
<td>DKKHr31</td>
<td>356</td>
<td>18</td>
</tr>
</tbody>
</table>

a The DNA preparation was diluted 10 times in Tris buffer and then 10 μl was used in the transformation experiment.

b Yeast cells were incubated in YPAD plates5) at 30°C for 4 days (fresh) and then kept at 4°C for 30 days (stored).

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* To whom correspondence should be addressed.
bation at 42°C increased the transformation frequencies 2~3 fold with highly purified DNA of plasmid YCp19 and 7~30 fold with plasmid LYTC-2 extracted from yeasts by a rapid procedure6) (Table I). The transformation efficiency varied also with the DNA-host strain combination (Tables I and II). Table II shows that a high number of transformants was recovered with DNA isolated from *Escherichia coli* JM1097) by a rapid alkali extraction procedure.8) Yeast cells kept for 30 days at 4°C were also transformed by crude plasmid DNA although with less efficiency (Table II).

To our knowledge, the procedure described here is the simplest so far reported for *S. cerevisiae* and it is potentially suitable for routine transformations of strains without the necessity of costly equipment, reagents, and time.

References