Note

A Ten Minute Procedure for Transformation of *Saccharomyces cerevisiae*

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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 DK-KHr31 (*MATa ura3-52 leu2-3 leu2-112 trp1 his3 rad2-1*) were used as hosts. The circular plasmid YCp19³⁾ (pBR-322, *ARS1*, *CEN4*, *TRP1*, *URA3*) and the linear plasmid LYTC-2⁴⁾ (YCp19, *EcoRI* terminal restriction fragment from *Tetrahymena* macronuclear rDNA) were used as transforming DNA.

The following buffers were used. Tris buffer was 10 mM Tris-HCl (pH 7.5). Transformation buffer (TBT) was prepared by mixing 0.15 ml of triacetin with 1 ml of Tris buffer containing 0.2 M lithium thiocyanate, 1 mM magnesium acetate, and 0.2 mM calcium acetate.

The transformation procedure was done as follows: to an 1.5-ml Eppendorf tube, 115 μ l 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 μ l of Tris buffer was added and after centrifugation the cells were

Table I. TRANSFORMATION OF WHOLE YEAST CELLS BY THE 10 min TRANSFORMATION PROCEDURE

Strain ^c	Incubation temperature (°C)	DNA	
		YCp19 ^a (Transformants/ μ g)	LYTC-2 ^b (Transformants)
DKKAC2	42	568	59
	30	228	2
DKKHr31	42	1112	163
	30	348	26

^a 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 μ l was used in the transformation experiment.

^b Plasmid LYTC-2 was extracted from yeasts by the 10 min procedure⁶⁾; 30 μ l 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 μ g of DNA respectively.

Table II. TRANSFORMATION FREQUENCY BY DNA OF YCp19 ISOLATED FROM *E. coli* JM109⁷⁾ BY A RAPID ALKALI PROCEDURE⁸⁾

Strain	Transformants ^a	
	Fresh cells ^b	Stored cells ^b
DKKHAC2	329	83
DKKHr31	356	18

^a The DNA preparation was diluted 10 times in Tris buffer and then 10 μ l 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).

resuspended in 200 μ l of the same buffer. Fifty μ l samples were plated on SD medium⁵⁾ containing 50 μ g/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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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.

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