Rapid colony transformation of *Saccharomyces cerevisiae*

Rohan Baker
Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

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Transformation of *Saccharomyces cerevisiae* with recombinant plasmids is a standard procedure in yeast molecular biology. One commonly used method for preparing competent yeast cells involves treatment with lithium acetate (LiOAc), or other alkali cations, as described by Ito *et al.* (1). The following modification of this procedure enables the rapid preparation of competent cells directly from colonies grown and stored on solid media, and is especially useful when a large number of yeast strains must be transformed, such as re-screening candidate mutant strains.

When preparing competent yeast cells by the LiOAc procedure (1, 2), it was noticed that although maximum transformation efficiency was achieved with exponentially growing cultures, the growth stage of the culture did not greatly affect the resulting transformation efficiency. In their original report, Ito *et al.* (1) quantified the effect of growth stage on transformation efficiency (using Cs^+ as the alkali cation) and observed that competent cells prepared from stationary phase cultures were only two-fold less efficient than those prepared from exponentially growing cells (1). It therefore seemed reasonable that, for situations not requiring maximum transformation efficiency, yeast colonies grown and stored on solid media may provide a suitable source of cells for transformation. Using this premise, the following protocol was developed. Yeast strain BWG1−7a (3) was grown for 2 days at 30°C on rich media (YPD/agar; ref. 4) to yield colonies 2−3 mm in diameter. Two colonies were picked with a sterile toothpick and suspended in 0.5 ml of TEL buffer (0.1 M LiOAc, 10 mM Tris−HCl (pH 7.5), 1 mM EDTA) in a microfuge tube. After 1 min at room temperature, cells were collected by centrifugation (10 sec, 12,000×*g*), resuspended in 0.2 mL of TEL buffer, and incubated at 30°C for 15 min with gentle agitation (taped to a roller drum). Five μl of a plasmid miniprep was added and incubation was continued for 15 min at 30°C without agitation; 0.7 ml of 40% (w/v) polyethylene glycol 4000 in TEL buffer was then added and vortexed. After standing for 15 min at 30°C, the mixture was heated at 42°C for 5 min, cells were collected by centrifugation, resuspended in 1.0 ml distilled water, and an aliquot was plated onto selective medium (4). This procedure yielded approximately 4,000 transformants per μg of DNA, using 2 μ circle-based plasmids (5) (Table 1). CEN-based plasmids yielded similar transformation efficiencies. The addition of randomly sheared carrier DNA with the miniprep DNA increased transformation efficiency approximately three-fold (Table 1), but may be omitted when the aim is merely to obtain transformants. The effect of the age of the colonies on the plate was investigated by storing plates at 4°C and preparing competent cells as described above after either one or four weeks. As shown in Table 1, the transformation efficiency (without carrier DNA) decreased with the length of storage at 4°C, but even with colonies stored for 4 weeks, bona fide transformants were obtained from 5 μl of miniprep DNA. The procedure is simple to scale up when one needs to transform the same strain with several different plasmids. For example, twenty colonies may be suspended in 1.2 ml TEL buffer in one microfuge tube, carried through the first 30°C incubation, and then divided into ten aliquots for transformation. Competent cells can also be prepared from colonies grown on selective media so that strains already carrying one plasmid can be transformed with a second plasmid. Variation in transformation efficiency with different yeast strains was observed using this protocol, but transformants were always obtained. If necessary, the number of colonies and the amount of DNA per transformation can be increased.

This method has several advantages over the standard LiOAc procedure, including the time saved by not having to grow a culture to exponential phase, and the ability to prepare competent cells at short notice and in small amounts from plates stored for several weeks at 4°C. Thus in situations where maximum transformation efficiency is not required, and especially where large numbers of strains need to be transformed, this method should be generally applicable.

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REFERENCES

1. Ito,H., Fukuda,Y., Murata,K. and Kimura,A. (1983) *J. Bacteriol.* **153**, 163−168.
2. Stearns,T., Hong,M. and Botstein,D. (1990) *Methods Enzymol.* **185**, 280−297.
3. Guarente,L., Yocum,R.R. and Gifford,R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7410−7414.
4. Sherman,F., Fink,G. and Hicks,J. (1981) *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
5. Gietz,R.D. and Sugino,A. (1988) *Gene* **74**, 527−534.

Table 1. Comparison of transformation efficiency

Protocol[a]	Efficiency[b]
Standard LiOAc (1,2)	18,000
Colony Method (CM)	4,000
CM + carrier[c]	11,000
CM, 1 week/4°C	155
CM, 4 weeks/4°C	60

[a]the plasmid used was YEplac195 (2 μ, *URA3*; ref. 5)
[b]number of URA$^+$ transformants per μg plasmid DNA
[c]50 μg of sheared salmon sperm DNA