DMSO-enhanced whole cell yeast transformation

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Submitted August 22, 1991

The transformation efficiency of *Saccharomyces cerevisiae* by the lithium acetate (LiOAc) method (1) is low compared with spheroplast (2) and electroporation (3) protocols. It is, however, quick and simple, making it ideal when a high efficiency of transformation is not essential. Dimethyl sulfoxide (DMSO) has previously been shown to enhance transformation of prokaryotes (4) and eukaryotes (5). We report a DMSO-modified version of an existing yeast LiOAc transformation method (6) that is quicker, and more efficient than the original method. The method below gives optimal transformation efficiency for *S.cerevisiae* JRY188 (**MAT**α *leu*2−3, 112, *his*3Δ1, *trp*-289, *ura*3−52, *sir*3−8):

1. A stationary culture of JRY188 grown in YEPD is used to inoculate 100 ml of YEPD in a Pyrex flask.
2. Cells are grown at 30°C with shaking (200 rpm) until a cell density of $1-4\times10^7$ is reached. Yeast are transferred to four sterile 30 ml plastic disposable tubes and centrifuged at 4000 rpm for 2 minutes at room temperature.
3. The pellets are washed in 10 ml total of LiOAc solution (0.1 M LiOAc, 10 mM Tris HCl (pH 8.0) 1 mM EDTA) and pooled into a single tube. The suspension is centrifuged as before then resuspended by adding 1 ml of LiOAc solution and gently shaking by hand.
4. 100 μl of yeast suspension is added to a 1.5 ml centrifuge tube containing 10 μl of transforming DNA. The contents are mixed gently then left at room temperature for 5 mins.
5. 280 μl of PEG4000 solution (50% PEG4000, (Fisons) in LiOAc solution) is added, the contents mixed by inverting 4−6 times, then the tube is placed at 30°C for 45 mins without shaking.
6. DMSO is added (43 μl) to give an approximate 10% (v/v) DMSO solution in the tube. The contents are mixed by inversion then heat shocked at 42°C for 5 mins.
7. Cells are spun at 12 000 g just long enough to pellet (usually 3−5 seconds), washed in dH_2O, spun for 3 seconds, then resuspended in 1 ml dH_2O. The *LEU2* gene of a 9.2 kb derivative of YEp351 (7) was used to transform JRY188 (1 μg per assay). Transformed cells (100 μl) were plated on SD media supplemented with histidine, uracil and tryptophan (8), and transformants scored after 3 days at 30°C. Efficiency was calculated as the number of *LEU2* transformants per μg DNA. Results shown (Table 1) are the average of four separate tubes per method.

The importance of Li^+ in this protocol was assessed by transforming JRY188 in the absence if Li^+. This was done by washing cells in TE buffer, and using PEG4000 dissolved in TE. The efficiency in the absence of LiOAc ($-Li^+$) is more than halved, but is still significantly greater than the control. This technique may therefore prove useful with yeast strains that transform poorly with the LiOAc or Ca^{2+} (9) methods, as DMSO improves transformation in the absence of any cations. Carrier DNA did not enhance any of the transformation methods described; this may be due to non-denatured sheared calf thymus DNA being used (10).

Transformation of another strain of *S.cerevisiae* with a dominant marker ($G418^{res}$) has been demonstrated using the above protocol; a 90 min incubation in YEPD (8) is necessary, prior to selection for $G418^{res}$. A derivative of the centromeric plasmid YCp50 was used as transforming DNA. Similar enhancement of transformation was observed although optimal efficiency was achieved at a slightly different DMSO concentration. This is indicative of strain specificity with yeast whole cell transformation. It also demonstrates that the above protocol works for other strains and large centromeric plasmids. This may prove to be a quick, technically simple, and efficient method of whole cell yeast transformation.

ACKNOWLEDGEMENTS

We thank SERC and Whitbread plc for financial support, and Dr C. Hadfield for providing a yeast $G418^{res}$ marker (11).

REFERENCES

1. Ito,H., Fukuda,Y., Murata,K. and Kimura,J. (1983) *J. Bacteriol.* **153**, 163−168.
2. Beggs,J.D. (1978) *Nature* (London) **275**, 104−108.
3. Becker,D.M. and Guarente,L. (1991) *Methods Enzymol.* **194**, 182−187.
4. Chung,C.T. and Miller,R.H. (1988) *Nucl. Acids Res.* **16**, 3580.
5. Lopata,M.A., Cleveland,D.W. and Sollner-Webb,B. (1984) *Nucl. Acids Res.* **12**, 5707−5717.
6. Ausubel,F.M., Brent,R., Kingston,R.E., Moore,D.D., Seidmann,J.G., Smith,J.A. and Struhl,K. (1989) *Current Protocol in Molecular Biology*. vol. 2, Wiley-Interscience, New York.
7. Hill,J.E., Myers,A.M., Koermer,J. and Tzagoloff,A. (1986) *Yeast* **2**, 163−167.
8. Sherman,F. (1991) *Methods Enzymol.* **194**, 14−15.
9. Bruschi,C.V., Comer,A.R. and Howe,G.A. (1987) *Yeast* **3**, 131−138.
10. Schiestl,R.H. and Geitz,R.D. (1989) *Curr. Genet.* **16**, 339−346.
11. Hadfield,C., Jordan,B.E., Mount,R.C., Pretorius,G.H.J. and Burak,E. (1990) *Curr. Genet.* **18**, 303−313.

Table 1. Comparison of transformation method

METHOD	EFFICIENCY	ENHANCEMENT
NORMAL	1589 ± 338	−
DMSO ($-Li^+$)	18970 ± 1340	× 11.9
DMSO ($+Li^+$)	41070 ± 6700	× 25.8