,proved method for high efficiency transformation of intact yeast cells

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An improved procedure for the transformation of LiAc treated yeast cells, using single stranded carrier DNA has been previously described (1). Here we describe further improvements and streamlining of this method, yielding reproducibly 10^6 transformants per microgram of vector DNA, a ten fold improvement, and reducing the time required by 1.5 hours. In addition, the method has been scaled down to various levels, and a colony procedure has been developed.

Incubating transformed yeast cells in liquid YPAD for one hour prior to plating on selective medium, increased the transformation efficiency about 7-fold (Table 1). Therefore, we investigated whether any of the incubation steps during transformation had an adverse effect and could be omitted or reduced. We found that it is not necessary to preincubate cells in TE/LiAc and that PEG/TE/LiAc can be added immediately after the cells are aliquoted into tubes containing vector and carrier DNA (see Figure 1). This shortened procedure resulted reproducibly in a transformation frequency of up to 1.2×10^6 colonies per μg of plasmid DNA (Table 1) and this frequency is not increased by post-incubation in YPAD. The volumes have also been scaled down compared to the original protocol (1). We now routinely regrow to 1×10^7 cells/ml in 50 μl and transform five 50 μl aliquots containing 1×10^8 cells. To transform many strains, 10 ml cultures can be harvested, washed, and resuspended in 50 ml of TE/LiAc; 25 μl samples of this suspension can be used for control as well as for the transformation, half the volumes of vector, carrier and PEG/TE/LiAc given in Figure 1 are added. This gave the same high transformation efficiency (Table 1).

Furthermore, the following colony procedure was developed: A large size colony, or better several medium size colonies (about 10^8 cells) are scraped off a YPAD plate, washed in 1 ml of sterile water in a microfuge tube, resuspended into 50 μl of 1×LiAc/TE and transformed as in steps 5 to 11 in Figure 1. This procedure which takes only about one hour gives up to 1.5×10^4 transformants per μg of vector DNA.

The high efficiency of the previously developed transformation method (1) made certain applications to some of the most important needs of current yeast molecular biology possible (2).

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Table 1. Influence of different procedure alterations on the transformation efficiency

Strain	Plasmid used	Protocol	Transformants/μg of vector DNA
LP2752-4B	YEplac195	As in Ref. 2	100,000
LP2752-4B	YEplac195	1 hr YPAD	690,000[a]
AB1380	YCplac33	1 hr YPAD	664,000[a]
CG379	YEplac195	−LiAc incub.	816,000
LP2752-4B	YEplac195	−LiAc incub.	1,108,000[b]
LP2752-4B	YEplac195	10 ml culture	813,000
LP2752-4B	YEplac195	colony procedure	15,000[a]

[a]Averages from two experiments. [b]Average from three experiments. The vectors used have been described (3). The strains used have been described (1) except for CG379 (MATα ade5 his7-2 leu2-3, 112 trp 1-289 ura3-52) which was kindly provided by Craig Giroux. The protocol is either a one hour post-transformation-treatment in liquid YPAD (1 hr YPAD) or omission of steps 8 and 11 from the published procedure (Fig. 1 Schiestl and Gietz 1989) with the regular 300 ml culture (−LiAc incub.) or scaled down to a 10 ml culture (10 ml culture) or scaled down to a single colony (colony procedure, see text).

Figure 1. Protocol for high efficiency transformation of intact yeast cells: 1. Inoculate cells into liquid YPAD medium and grow overnight to $1-2\times10^7$ cells/ml. 2. Dilute to 2×10^6 cells/ml in fresh, warm YPAD and regrow to 1×10^7 cells/ml. 3. Harvest cells and wash in sterile water, resuspend in 1.0 ml sterile water and transfer to 1.5 ml microfuge tubes and pellet cells. 4. Wash cells in 1.0 ml of TE/LiAc (made fresh from 10×filter sterile stocks: 10×TE [0.1 M Tris-HCl, 0.01 M EDTA, pH 7.5]; 10×LiAc [1 M LiAc pH 7.5, adjusted with diluted acetic acid]) and resuspend at 2×10^9 cells/ml in 1×TE/LiAc. 5. Mix 50 μl of yeast cell suspension with 1 μg transforming DNA and 50 μg of single stranded salmon sperm carrier DNA (as described in Ref. 1) in microfuge (Brinkman) tubes. 6. Add 300 μl sterile 40% PEG 4000 solution (40% PEG 4000, 1×TE, 1×LiAc, made fresh from sterile 50% PEG stock, and sterile 10×TE and 10×LiAc), mix thoroughly. 7. Incubate at 30°C with agitation for 30 min. 8. Heat shock in a 42°C waterbath for 15 minutes (Important). 9. Spin down in microfuge for 5 seconds. 10. Resuspend the cell pellet in 1.0 ml of 1×TE, dilute appropriately and plate on to selective medium.

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