A Highly Efficient Protocol for Transformation of *Saccharomyces cerevisiae* and *Pichia pastoris* by Electroporation*

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Abstract: Electroporation is widely used for highly efficient transformation of *Saccharomyces cerevisiae*. As many strains degenerate over time or over environmental exposure, their electroporation efficiencies could drop markedly by 2 to 3 orders of magnitude. Here we describe a modified electroporation procedure which included single stranded carrier DNA for electroporation as well as a post-electroporation growth period in YPD medium, which gave rise to 13 fold enhancement in transformations than an optimized lithium acetate (LiAc) and dithiothreitol (DTT) Pretreatment protocol previously reported. An enhancement of 114 fold on *Pichia pastoris* transformations has been observed using the modified procedure. The modified method enhanced the transformation efficiencies of some degenerated *S. cerevisiae* strains by nearly 100 fold. It will enable molecular manipulations in all kinds of *S. cerevisiae* and *P. pastoris* strains by allowing propagations of all sorts of DNA libraries made from minute samples.

Key words: electroporation; *Saccharomyces cerevisiae*; single stranded carrier DNA; post-electroporation growth; *Pichia pastoris*

一种高效的酿酒酵母和毕赤酵母电击转化法

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摘 要：电击转化由于其高效率而被广泛地应用于酿酒酵母的转化过程中。但是由于菌种退化或是暴露于污染的环境，很多菌种的转化效率会显著地下降2~4个数量级。探索了一种改进的电击转化方法，它借助于单链载体DNA和转化后在YPD培养基中的复苏过程，可以使转化效率比之前曾报道过的已经最优化的用醋酸锂和二硫苏糖醇进行预处理的转化方法效率提高13倍。在毕赤酵母中使用这一改进的方法，可以使转化效率提高高达114倍，在一些已经退化的酿酒酵母菌株中，此方法也可以提高转化效率将近100倍。这一方法将为几乎所有酿酒酵母和毕赤酵母的分子操作提供极大的便利。

关键词：电击转化；酿酒酵母；单链载体DNA；转化后复苏；毕赤酵母
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Saccharomyce cerevisiae is the workhorse of eukaryotic genetics and molecular biology. It is also widely used for high throughput screening in drug development. Electroporation is regarded as one of the most efficient transformation techniques for *S. cerevisiae*, particularly in the propagation of cDNA libraries, genomic libraries and random DNA libraries. However, we frequently encountered problems of yeast strain degeneration over time or over environmental exposure, which resulted in sharp decline of electroporation frequency in routine experiments. Pretreatment of *S. cerevisiae* cells with lithium acetate (LiAc) and dithiothreitol (DTT) enhanced the frequency of transformation by electroporation[1], but the efficiencies still varied with strains, and changed over time. Although the LiAc/single stranded carrier DNA/PEG mediated method was reported to be highly efficient[2-3], it did not work very well in our hands.

The yeast *Pichia pastoris* is widely used as a host for the expression of heterologous proteins. Most vectors must integrate into the *Pichia* chromosome, an inherently inefficient process[4], hence transformation efficiencies were very low[5-6]. *Pichia* is usually transformed by electroporation and requires DNA in the microgram range. The optimized lithium acetate (LiAc) and dithiothreitol (DTT) Pretreatment protocol has been adapted to *P. pastoris* and yielded good results[7]. However, as *Pichia* strains degenerate over time, transformation efficiencies drop substantially.

Single-stranded carrier DNA has been previously reported to be capable of enhancing transformations[2]. As electroporations produce membrane fracture and holes, it is hypothesized that an 1 hour post electroporation reviving growth in an isotonic medium may heal these cells and substantially raise transformation efficiency. This has been shown previously[8]. Here we present data in the investigations for a highly efficient electroporation procedure for *S. cerevisiae* and *P. pastoris*.

1 Materials and methods

1.1 Strains and plasmids

S. cerevisiae strain GY2050 (*his*3Δ200 *ura*3Δ0*trp*1Δ63 *leu*2Δ0) was provided by Dr. Greg Prelich (Albert Einstein College of Medicine, NY). *S. cerevisiae* strain INVSc1 (*MATa/MATa his*3 −Δ1/*his*3 −Δ1 *leu2/leu2 trp*1 −289/*trp*1 −289 *ura*3 −52/*ura*3 −52) and *P. pastoris* strain GS115 (*His*4) were products of Invitrogen (Carlsbad, California). *S. cerevisiae* S150 −2B (*MAT*a *leu*2 −3, 112 *ura*3 −52 *trp*1 −289 *his*3 −Δ1) was provided by Dr. Derek Jamieson (Heriot-Watt University, United Kingdom). The procedure was developed using the 6 kb *E. coli* and yeast shuttle vector pYES2/CT plasmid (Invitrogen, Carlsbad, California) as the input DNA. *Pichia* plasmid pICZαA was also a product of Invitrogen (Carlsbad, California). Plasmid DNA was prepared with the Spin Miniprep (Minipreparation) Kit (ACT · Gene, China) as per manufacture's instruction and quantitated on a DU® 530 DNA/Protein Analyzer (Beckman, Fullerton, CA).

1.2 Media and single stranded DNA

The YPD medium consisted of 2.0% (w/v) Peptone, 1.0% (w/v) yeast extract, 2.0% dextrose. *S. cerevisiae* Ura^+ transformants were selected on synthetic medium containing 0.67% Yeast Nitrogen Base without amino acids (DIFCO Laboratories, Sparks, Maryland) supplemented with 1.0% glucose, 1 mol/L sorbitol, and 20 mg/L of leucine, histidine and tryptophan each as well as 2% agar. YPDS plates contained 1 mol/L sorbitol and 100 μg/mL Zeocin in addition to YPD.

Single stranded Salmon sperm carrier DNA (Sigma-Aldrich, Saint Louis, MO) was prepared by weighing out DNA and placing it in a boiling water bath for at least 5 min followed by a quick cooling in ice water slurry. Carrier DNA can be frozen after boiling and used 3 or 4 times, and re-boiled for 1 min for subsequent uses. The ssDNA is used at a concentration of 20 μg/μL.

1.3 Electroporation procedure

S. cerevisiae competent cell preparation was as described[1]. Briefly, Both *S. cerevisiae* and *P. pastoris* were propagated overnight to stationary phase. 100 μL of the culture were inoculated into 200 mL of YPD broth and grown overnight at 30 ℃ with shaking. Yeast cells were harvested at an A_{600} nm of 1.0 ~ 1.5. The cells were pelleted by centrifugation (2 500 × g, 5 min), suspended in 25 mL of 0.1 mol/L lithium acetate, 10 mmol/L dithiothreitol, 10 mmol/L Tris-HCl, pH 7.5, 1 mmol/L EDTA (LiAc/DTT/TE) and incubated at 25 ℃ for 1 h. The cells were centrifuged at 2 500 × g for 5 min at 4 ℃ and the pellet was suspended in 50 mL ice-cold water. This step was repeated and the pellet was then suspended in 10 mL of ice-cold 1 mol/L sorbitol followed by centrifugation. Subsequently the pellet was suspended in 800 μL of 1 mol/L sorbitol which yielded 1 200 μL of cell suspension in total. Twenty μg of single stranded carrier DNA and 15 ng plasmid DNA in no more than 10 μL were added to 80 μL of cell suspension corresponding to around 5×10^8 cells, which was transferred to an electroporation cuvette and electroporated at 9.0 kilovolts/cm on an Eppendorf 2 510 electroporator (Eppendorf AG, Hamburg, Germany) with a built-in time constant of 5 ms. Immediately, 650 μL cold 1 mol/L sorbitol was added and the contents of the cuvette were gently mixed, and transferred to 1.5 mL Eppendorf tubes. 650 μL 2 × YPD and 1 mol/L sorbitol were then added to the mixture, and incubated at 30 ℃ for 1 hour with gentle shaking. The cell suspensions were washed once with sterile water and concentrated by spinning at 400 × g for 2 min. An aliquot was spread on plates. Ura^+ transformants were selected on synthetic medium containing 0.67% Yeast Nitrogen Base without amino acids supplemented with 1.0% glucose, 1 mol/L sorbitol, and 20 mg/L of leucine, histidine and tryptophan each, and 2% agar. Incubation was carried out at 30 ℃, and *S. cerevisiae* colonies were counted after 2 ~ 3 days.

P. pastoris cells electroporated with 10 ng pICZαA linearized with *Sac*II were directly plated on YPDS/zeocin plates without washing, and incubated for 3 ~ 4 days at 30 ℃.

2 Results and discussion

The preparation of competent cells was essentially as described[1]. Modifications were conducted at 3 levels, and all included a lithium acetate (LiAc) and dithiothreitol (DTT) pretreatment step. Initially an hour of post-electroporation growth in YPD/1 mol/L sorbitol was included. The next modification was the addition of single stranded carrier DNA into cell suspensions prior to electroporation. The final optimization included an incubation of cell and DNA mixture in refrigerator for 5 minute before pulsing. The refrigerator usually has a temperature of 6 ~ 8 ℃.

The results of an electroporation experiment are shown in Fig. 1. A post-electroporation growth in YPD had a clear effect on transformations of *S. cerevisiae* with shuttle vector pYES2/CT, resulting in 3.5 fold enhancement over lithium acetate (LiAc) and dithiothreitol (DTT) pretreatment along. The presence of single stranded carrier DNA for electroporation had an effective role in the improvement of electroporation efficiency, and resulting in about 4 fold enhancement. Although occasionally 2 – 3 fold increase in transformation frequencies was obtained with a 5 minute incubation in the refrigerator immediately before electroporation, only marginal effect was observed in Fig. 1. Overall, a 13 fold enhancement was clear in Fig. 1 with inclusion of ssDNA and post-electroporation growth prior to plating. Untransformed *S. cerevisiae* strain failed to grow. We observed near 100 fold enhancement over lithium acetate (LiAc) and dithiothreitol (DTT) pretreatment along in electroporations of some degenerated strains of *S. cerevisiae* S150 – 2B and INVsc1, to a level of 10^5 transformants/μg (data not shown).

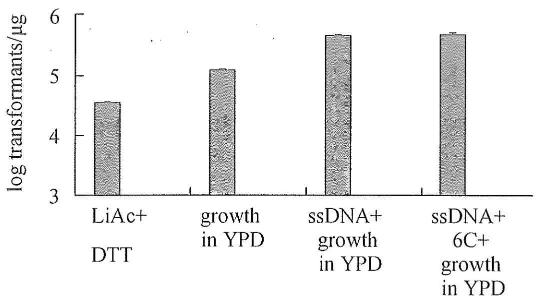

Fig. 1 Logarithm of transformants of a 6 kb plasmid pYES2/CT electroporated into *S. cerevisiae* GY2050 cells via different treatments. (LiAc + DTT), pretreatment with LiAc + DTT. (Growth in YPD), an hour of post-electroporation growth in YPD/1 M sorbital was conducted in addition to a LiAc + DTT pretreatment. (ssDNA + Growth in YPD), ssDNA was added to cell suspensions prior to electroporation in addition to pretreatment and growth in YPD. (ssDNA + 6C + Growth in YPD), DNA and cell mixture were incubated in a refrigerator for 5 minutes before pulsing in addition to pretreatment, growth in YPD and inclusion of ssDNA. Transformation efficiencies are shown with vertical bar in average value of triplicate electroporations with one standard deviation. Standard deviations were calculated using Microsoft Excel 2003.

Linearization of pICZαA at site of the multiple cloning region by *Sac*II was performed before electroporation of *P. pastoris*. Marked enhancement in transformation by inclusion of ssDNA and an hour of post-electroporation growth prior to plating, over lithium acetate (LiAc) and dithiothreitol (DTT) pretreatment along[7], is evident from Fig. 2, and an 114 fold increase was recorded. It has been reported that linearization using different restriction enzymes may have 30 fold variations in transformation frequencies, and *Sac*I was the most efficient among tested[7].

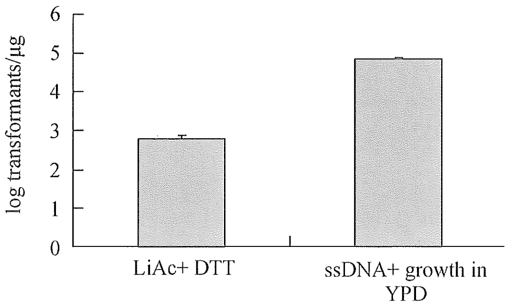

Fig. 2 Logarithm of transformants of a 3.6 kb plasmid pICZαA into *P. pastoris* GS115 cells via different treatments. (LiAc + DTT), pretreatment with LiAc + DTT. (ssDNA + Growth in YPD), ssDNA was added to cell suspensions prior to electroporation in addition to pretreatment with LiAc and DTT and growth in YPD. Transformation efficiencies are shown with vertical bar in average value of duplicate electroporations with one standard deviation. Standard deviations were calculated using Microsoft Excel 2003.

The enhancement of ssDNA was attributed to its higher binding affinity to the cell wall than double stranded DNA. Thus the titration of the ds-transforming DNA by absorption to the cell wall was inhibited, and it increased the entry of transforming DNA molecules into the cell[9].

In summary, inclusion of ssDNA and a post-electroporation growth period markedly enhanced the transformations of *S. cerevisiae* and *P. pastoris*. The 5 minute incubation in the refrigerator prior to electroporation is optional as it was effective in some transformations.

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References:

[1] THOMPSON J R, REGISTER E, CUROTTO J. An improved protocol for the preparation of yeast cells for transformation by electroporation[J]. Yeast, 1998, 14(6): 565-571.

[2] SCHIESTL R H, GIETZ R D. High efficiency transformation of intact yeast cells using single stranded nucleic acids as carrier[J]. Curr Genet, 1989, 16:339-346.

[3] GIETZ R D, WOODS R A. Transformation of yeast by the LiAc/ss carrier DNA/PEG method[J]. Methods Enzymol, 2002, 350: 87-96.

[4] STRUHL K, STINCHCOMB D T, SCHERER S. High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules[J]. Proc Natl Acad Sci USA, 1979, 76:1035-1039.

[5] CREGG J M, RUSSELL K A. *Pichia* protocols[M]. Totawa: Humana Press, 1998: 27-39.

[6] ROSENFELD S. Use of *Pichia pastoris* for expression of recombinant proteins[J]. Methods Enzymol, 1999, 306: 154-169.

[7] WU S, LETCHWORTH G J. High efficiency transformation by electroporation of *Pichia pastoris* pretreated with lithium acetate and dithiothreitol [J]. Biotechniques, 2004, 36(1):152-154.

[8] QIN YUJING, JIN JIANLING, BAO XIAOMING. Transformation of *S. Cerevisiae*. by electroporation[J]. Journal of Shangdong University, 1999, 34(2): 236-240.

[9] GIETZ R D, SCHIESTL R H, WILLEMS A R. Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure[J]. Yeast, 1995, 11: 355-360.