

**OPTIMIZATION OF THE SONICATION ASSISTED
LiAc/SS-DNA/PEG TRANSFORMATION OF
*Saccharomyces cerevisiae****

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ABSTRACT

The yeast and *E. coli* shuttle vector pYES2 and *Saccharomyces scerevisiae* H158 were used. The influence of the sonication treatment on yeast LiAc/SS-DNA/PEG transformation protocol was studied, and four main factors that influenced the transformation efficiency were optimized. Using the optimized protocol, the recombinant plasmid pYES2-CBHI was transformed into H158. The results show that, sonication for 60s can increase the transformation efficiency to 1.1×10^3 / μg plasmid. But when the treatment lasted more than 60s, the efficiency decreased sharply. Analysis of the orthogonal experiment indicate that group 5 (sonication time 60 seconds, incubation 40 minutes, SS-DNA 150 μg , heat shock time 5 minutes) gave the best results . The main affecting factor was the quantity of the SS-DNA. The transformants of pYES2-CBHI were identified and the results indicate that the recombinant plasmid was transformed into the yeast cells with high efficiency.

Keywords: *Saccharomyces scerevisiae*; pYES2; transformation; sonication treatment

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1. INTRODUCTION

Several transformation systems of *Saccharomyces cerevisiae* have been established. Electroporation, embryo and whole cell transformation are the three main transformation systems for yeast. (Among these, the whole cell LiAc/SS-DNA/PEG protocol is the most applicable [1-5] although the transformation rate of this method is lower than that of electroporation transformation which retard the further application of it.) So, much effort had been made to improve the transformation efficiency of this method. Sonication treatment was thought to permit more DNA into the cell [6]. Trick *et al.* [7] used the sonication treatment in plant *Agrobacterium*-mediated transformation, and found that it increased the transformation efficiency of many crops.

In this paper, we studied the effect of sonic wave on yeast whole cell LiAc/SS-DNA/PEG transformation. The factors affecting the sonication assisted yeast transformation were optimized and the transformation efficiency was improved. A sonication assisted yeast transformation system was established and by using this system the pYES2-CBH I (yeast expression plasmid pYES2 carrying the cellobiohydrolase I cDNA gene from *Trichoderma viride*) was successfully transformed into *S. cerevisiae*.

2. MATERIALS AND METHODS

2.1 Strains and media

Escherichia coli DH5a was used in experiments which required a bacterial host, and *S. cerevisiae* H158 (his⁻, trp⁻, leu⁻, ura⁻) was used as yeast transformation host. Bacterial strains were grown at 37 °C in LB medium as described in Sambrook *et al.* [8]. *S. cerevisiae* H158 was grown aerobically at 30 °C in YPD (1 % yeast extract, 1.5 % peptone, 2 % glucose) and transformants were selected on SC-U medium (2 % glucose, 0.7 % yeast nitrogen base without amino acids, and appropriate amino acids lacking uracil).

2.2 Plasmids, molecular biology techniques

Yeast and *E. coli* shuttle vector pYES2 (Provided by Dr. BIAO Xiao-ming, State Key Laboratory of Microbial Technology, Shandong University) and pYES2-CBH I (pYES2 carrying CBH I cDNA gene from *Trichoderma viride*) were used for yeast transformation.

The normal *E. coli* molecular biology techniques were performed as described in Molecular Cloning: A Laboratory Manual [8] and the Yeast molecular biology techniques were according to Alison [9].

2.3. LiAc/SS-DNA/PEG transformation procedures

Transformation of yeast strain was performed according to the LiAc/SS-DNA/PEG method [3] with some modifications. Pick up 5 colonies of H158. Put into 50ml of YPD medium and shake overnight at 30 °C. Make sure that the OD₆₀₀ of the overnight culture reaches 1.0. Dilute culture to an OD₆₀₀ of 0.6 in 50ml of YPD medium and grow an additional 3 hours. Pellet the cells at 2500 rpm and resuspend the pellet in 40 ml 1X TE. Pellet the cells at 2500 rpm and resuspend the pellet in 2 ml of 1X LiAc/0.5X TE. Incubate the cells at 30 °C for 30-60 minutes. For each transformation, mix together 1 µg plasmid DNA and 100 µg denatured sheared salmon sperm DNA with 100 µl of the yeast suspension. Add 700 µl of 1X LiAc/40% PEG-3350/1X TE and mix well. Incubate solution at 30°C for 30 minutes. Add 88 µl DMSO, mix well, and heat shock at 42°C for 7 minutes. Centrifuge in a microcentrifuge for 10 seconds and remove supernatant. Resuspend the cell pellet in 1 ml 1X TE and re-pellet. Resuspend the cell pellet in 50-100 µl 1X TE and plate on a selective plate.

2.4 The growth curve of *Saccharomyces cerevisiae* H158

Inoculate a single clone of *Saccharomyces cerevisiae* H158 into YPD liquid medium and cultivated with shaking at 30 °C; When the OD₆₀₀ of the overnight culture reached 1.0, add 1% volume of the overnight culture to the new YPD liquid medium cultivated in the same condition, and measure the OD₆₀₀ at different time points with three replications [10].

2.5 Effect of sonication treatment on ratio of yeast transformation

In the transformation procedures, the yeast cells were divided into 8 groups, after the 30 min incubation step, each group was sonicated for 0s, 10s, 20s, 40s, 60s, 80s, 120s and 160s, respectively with three replications, and the transformation ratio was measured to study the influence of sonication treatment on the yeast transformation efficiency .

2.6 Experimental design for the Optimization of Sonication Assisted LiAc/SS-DNA/PEG yeast transformation

A L₉(3⁴) orthogonal experimental design was used to optimize the transformation, and four factors (Sonication treatment time, incubation period, quantity of the SS-DNA and duration of the heat shock) were chosen. A three-level, four-factor design was used. The coding of the levels of independent variables is shown in Table 1.

Table 1. Factors and levels of orthogonal experiment design $L_9(3^4)$

Levels	Sonication treatment	Incubation period	Quantity of	Duration of the
	time		the SS-DNA	heat shock
	A(s)	B(min)	C(μ g)	D(min)
1	50	30	50	5
2	60	40	100	7
3	70	60	150	9

2.7 Identification of yeast transformants

Yeast clone PCR and restriction enzymes double digestion (KpnI, SphI) were used to identify the yeast transformants of pYES2-CBHI. Methods followed the description of Alison *et al.* [9] and Madhu *et al.* [11]. Two primers were P9 (5'-tgaggcacagaaccaat -3') and R9 (5'-gccgcatctccagtgaaa -3').

3. RESULTS AND DISCUSSION

3.1 The growth curve of *Saccharomyces cerevisiae* H158

From the growth curve we can see that 0-7 h was the lag phase, 7-15 h was the logarithmic phase and longer than 15 h was stationary phase. At 12 h the $OD_{600}=1.0$ the yeast cell was in a proper condition for transformation (Figure 1).

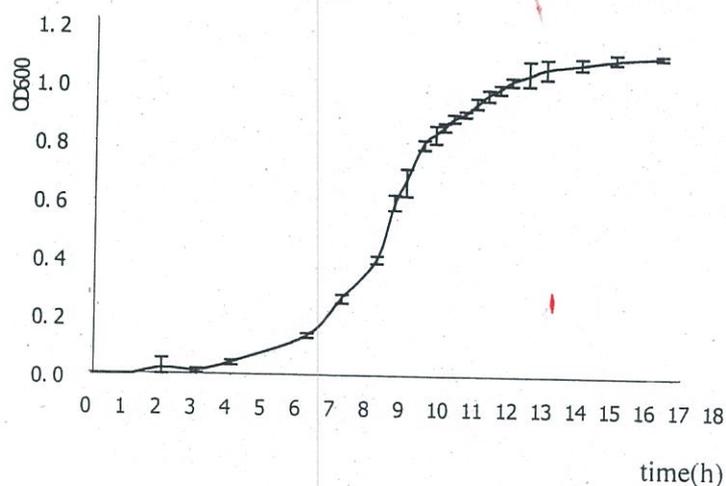


Figure 1. The growth curve of *Saccharomyces cerevisiae* H158

3.2 Effect of the sonication treatment on ratio of the yeast transformation

The best sonication treatment time was 60s, the transformation ratio was 1.1×10^3 transformants/ μg plasmid which was twice that of the untreated control. But when the treatment time was longer than 60s the transformation ratio decreased, and when the treatment time was 160s the transformation ratio was lower than that of the untreated control. This may be due to the sonic waves causing irreversible damage on the yeast cells (Figure 2).

From this result, we decided to use 50s, 60s and 70s sonication treatment levels for next step experiment.

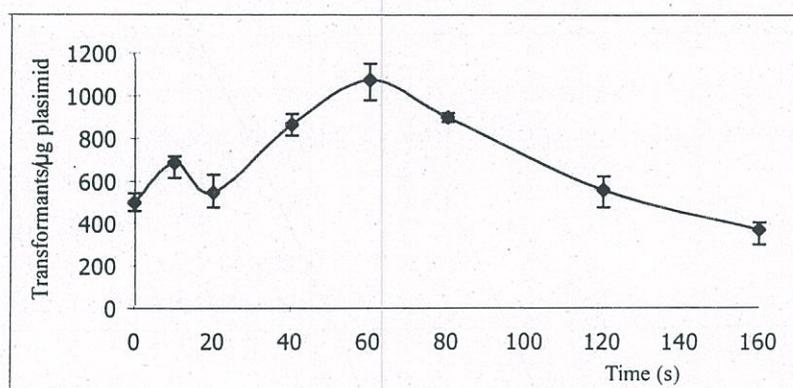


Figure 2. Effect of the sonication treatment on ratio of the yeast transformation

3.3 The results of orthogonal experiment

A $L_9(3^4)$ orthogonal design was used to optimize the yeast transformation. The treatments, the experimental data and range incidence analysis are shown in Table 2.

The largest k values of each factor were: factor A: $k_2=1400$; factor B: $k_2=1446$; factor C: $k_3=1536$; factor D: $k_1=1535$. These k values indicate that the group 5 (sonication treat time 60 seconds, incubation time 40 minutes, SS-DNA $150\mu\text{g}$, heat shock time 5 minutes) was the optimum group among the 9 groups.

The order of range incidence was $RD(852) > RA(699) > RB(587) > RC(583)$, so according to the range incidence analysis, the influence order of the four factors was the quantity of the SS-DNA > heat shock time > sonication treatment time > incubate time, and the quantity of the SS-DNA was the essential factor among them (Table 2).

Table 2. Result of the orthogonal test $L_9(3^4)$ of yeast transformation

No. of Exp.	A	B	C	D	Ratio of transformation (Transformants/ μg plasimid)			
	1	2	3	4	A	B	C	Avg. ^a
1	1	1	1	1	909	760	264	644
2	1	2	2	2	619	1175	832	876
3	1	3	3	3	472	1821	466	920
4	2	1	2	3	923	856	989	923
5	2	2	3	1	2667	2481	2851	2666
6	2	3	1	2	661	537	634	611
7	3	1	3	2	1036	1304	721	1021
8	3	2	1	3	874	787	727	796
9	3	3	2	1	982	1568	1332	1294
K1 ^b	2440	2588	2051	4604				
K2	4200	4338	3092	2507				
K3	3111	2825	4607	2639				
k1 ^c	813	863	684	1535				
k2	1400	1446	1031	836				
k3	1037	942	1536	880				
R ^d	587	583	852	699				
r ^e	3	4	1	2				

Note: ^aValues represent the means of three replicate samples.

^bThe total number of transformants of level 1 factor A. ^cThe average transformants number of level 1 of factor A. ^dRange incidence. ^eOrder of influence level.

3.4 Identification of yeast transformants

The pYES2-CBHI was transformed into *S. cerevisiae* H158 by using of this optimized yeast transformation system .

The transformants were verified by yeast clone PCR amplification. Product of Primers was 1.8 kb band. Samples (1, 2, 4 and 5) with this band were believed to be positive samples (Figure 3). Then the positive samples of yeast clone PCR were verified by double digestion using KpnI and SphI. The results are shown in Figure 4. The first band (5.9kb) represents the vector pYES2 while the second band (1.8kb) represents the CBHI cDNA of *T. viride*. This indicates that the pYES2-CBHI was transformed into *S. cerevisiae* H158 (Figure 4).

Establishing a sonication assisted yeast transformation system with high transformation efficiency establishes a good foundation for our next step which is to transform and express the cellulase cDNA genes from *Trichoderma viride*.



Figure 3. Results of yeast clone-PCR
 Note: 1, 2, 4, 5 positive samples
 (CBH I-cDNA)

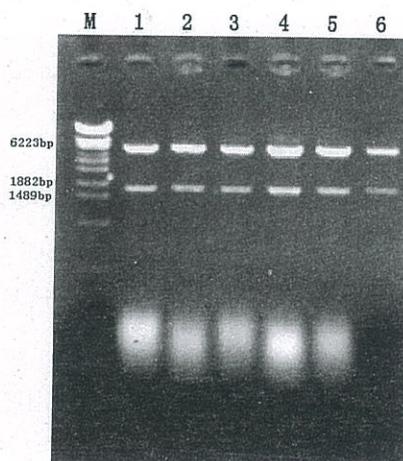


Figure 4. Results of the double digestion
 Note: The restriction enzymes were KpnI and SphI, two bands were 5.9kb for pYES2 and 1.8kb for CBH I-cDNA; 1-5 samples; 6 positive control.

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