

Research article

Transformation of *Saccharomyces cerevisiae* by pET plasmid using lithium acetate

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ABSTRACT

The recombinant pET 16b was propagated and amplified in *E. coli* using heat shock method, then plated and incubated on luria agar containing ampicillin (100µg/ml) for 18 hours at 37°C. One colony was picked and mini-culture was made. Transformed *E. coli* was cultured on luria broth containing ampicillin. After incubation 18 h at 37°C the recombinant plasmid was extracted using QIAprep Spin Miniprep Kit from transformed *E. coli* and transferred to *Saccharomyces cerevisiae* using lithium acetate/SS carrier DNA/PEG. The positive transformed clones were grown on selective media lacking of tryptophan. The plasmid was extracted using the same extraction kit with some modification and the concentration of obtained DNA was 70.8 ng/µl measured by Nanodrop. In this study, the recombinant pET 16b that amplified in *E. coli* was transformed in *S. cerevisiae* by lithium acetate method.

Keywords: Transformation, *S. cerevisiae*, plasmid.

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INTRODUCTION

Saccharomyces cerevisiae is the yeast that has several properties to establish as an important tool to express foreign proteins for many researches for industrial and medical use. It is a suitable host organism for the high-level production of secreted and soluble cytosolic proteins [1]. The expression of proteins in yeast is a good alternative to prokaryotic and higher eukaryotic expression system. Yeast cells have many advantages of proteins production in microbes such as growth speed, easy genetic manipulation and low cost media, while it has some attributes of higher eukaryotic systems [2].

Most yeast vectors have to be propagated and amplified firstly in *E. coli* to facilitate cloning and for that reason, it contain an *E. coli* replication origin and ampicillin selectable marker [3]. Chemical treatment method is the most commonly used. It involves temporary destabilization of the cell wall to allow the entry of DNA and likely to an endocytosis-like mechanism [4]. Many species of yeasts can be transformed by exogenous DNA in the environment. There are several methods, which have been developed to facilitate the transformation at high frequency in the lab [5]. Some of them include treating with enzymes to degrade their cell walls and yielding sp-



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heroplasts. The cells will be very fragile and they can take up foreign DNA at a high rate [6]. Another one involves there exposing intact yeast cells to alkali cations such as, cesium or lithium allows the cells to take up plasmid DNA [7]. Later protocols involve using lithium acetate, polyethylene glycol, and single-stranded DNA. In these protocols, the single-stranded DNA preferentially binds to the yeast cell wall to prevent the plasmid DNA from doing so and leaving it available for transformation [8]. Other methods use electroporation, formation of transient holes in the cell membranes using electric shock; this will allow DNA to enter the cell. Enzymatic digestion or agitation with glass beads can also be used to transform yeast cells [9]. Different yeasts can take up foreign DNA with different efficiencies. Also, most transformation protocols developed for baker's yeast, *S. cerevisiae* and thus it may not be optimal for other species. Even within the same species, different strains have different transformation efficiencies [10]. On the other hand a wide range of vectors are available for various requirements for insertion, deletion, alteration and expression of genes in yeast. Most plasmids used are the shuttle vectors which contain sequences permitting them to be selected and propagated in *E. coli*, thus allowing for amplification and subsequent alteration *in vitro*. The most common yeast vectors contain an origin of replication (*ori*), to promote high copy-number maintenance in *E. coli*, and selectable antibiotic markers like, β -lactamase gene (*AmpR*), and sometime to tetracycline-resistance gene, (*TetR*), conferring resistance to ampicillin and tetracycline respectively. In addition, yeast genetics and all yeast vectors contain markers, which allow selection of transformants containing the desired plasmid. The most commonly used yeast markers are URA3, HIS3, LEU2, TRP1 and LYS2, which are specific auxotrophic mutations in yeast [11]. In current study, we used the lithium acetate method to transfer the recombinant plasmid of *E. coli* to yeast.

MATERIALS AND METHODS

The recombinant Plasmid pET 16b in which RSV M gene (gene coding for RSV matrix protein) was extracted from transformed bacteria using QIAprep Spin Miniprep (Qiagen/USA), and the procedure was done according to manufacture's instructions [12]. The bacterial cells pellet was resuspended in 250 μ l buffer P1, the lysis buffer, and transferred to a microcentrifuge tube. Buffer P2 (sodium hydroxide) (250 μ l) was added and the tube inverted 4–6 times gently. Buffer N3 (guanidine hydrochloride and acetic acid) (350 μ l) was added and the tube was inverted gently 4–6 times. The tube was centrifuged for 10 min at 13,000 rpm in a microcentrifuge. A compact white pellet was formed. The supernatant was applied from step 3 to the QIAprep spin column by pipetting. The tube was centrifuged for 60 seconds at 8000 rpm and the flow-through was discarded. The QIAprep spin column was washed by

adding 0.75 ml of buffer PE (guanidine hydrochloride and isopropanol) and centrifuged for 60 second at 8000 rpm. The flow-through was discarded and centrifuged for an additional 1 min to remove residual wash buffer. The QIAprep column was placed in 1.5 ml microcentrifuge tube. To elute DNA, 30 μ l of buffer EB (10 mM Tris·Cl, pH 8.5) was added to the center of each QIAprep spin column, left stand for 1 min and centrifuged for 1 min at 8000 rpm. The DNA samples were measured by Nanodrop.

The yeast strain, *S. cerevisiae*, AH109, Genotype: MATa, *trp1-901*, *leu2-3, 112*, *ura3-52*, *his3-200*, James et al. (1996); *gal4 Δ* , *gal80 Δ* , LYS2: GAL1UAS-GAL1TATA-HIS3, A. Holtz, unpublished GAL2UAS-GAL2TATA-ADE2, URA3: MEL1UAS-MEL1 TATA-*lacZ* was inoculated into 5 ml of liquid medium (2X yeast extract peptone dextrose) and incubated overnight on a shaker incubator at 30°C and 250 rpm until O.D. at 600 became more than 1.5. The overnight culture (15 μ l) was added onto yeast extract peptone dextrose agar plat and incubated at 28°C for 4 days. The tube of carrier DNA was heated in a boiling water bath for 5 min and then it was chilled in ice/water. A colony of yeast from the yeast extract peptone dextrose plate was scraped and the cells were suspended in 1 ml of sterile water in a 1.5 ml micro centrifuge tube. Pellet the cells at 15,000 rpm in a micro centrifuge for 30 seconds and discard the supernatant. The following components of the transformation Mixture were added to the cell pellet in the order, PEG 3500 50% w/v (240 μ l), LiAc 1.0 M (36 μ l), Boiled SS-Carrier DNA (2 mg/ml) (50 μ l), Plasmid DNA (0.1 to 1 μ g) plus water (34 μ l), the total volume was 360 μ l. The tube was incubated in a water bath at 42°C for 40 to 60 min. The mixture was microcentrifuged at top speed for 30 sec and the transformation mixture was removed with a micropipettor. Sterile water (1 ml) was added into the tube, the cells were resuspended by stirring with a micropipette tip, and then vortex mixing vigorously was done. The suspension (100 μ l) was put onto plates of stringency medium out off tryptophan Minimal Synthetic Dropout medium; comprised of a nitrogen base, a carbon source (galactose) and was incubated at 30°C for 5-6 days and the transformants was isolated [13].

The Plasmid was extracted using QIAprep Spin Miniprep kit with some modification including vortex with glass beads for (3-5) min and the rest steps were done as the same as those done in extraction the plasmid from *E. coli*. The extracted plasmid was transferred into a competent *E. coli* BL21 which was cultured on Luria Bertani (LB) agar plate containing ampicillin to verify the transformation of the plasmid into yeast.

RESULTS AND DISCUSSION

Transformation was done successfully in yeast as there were colonies of transformed yeast on stringency medium out off tryptophan (**Fig. 1**) and the extracted plasmid concentration was 70.8 ng/ μ l measured by

nanodrop spectrophotometer (Fig. 2). The plasmid was successfully transferred into a competent *E. coli* as there were colonies appeared on LB agar (+ampicillin).

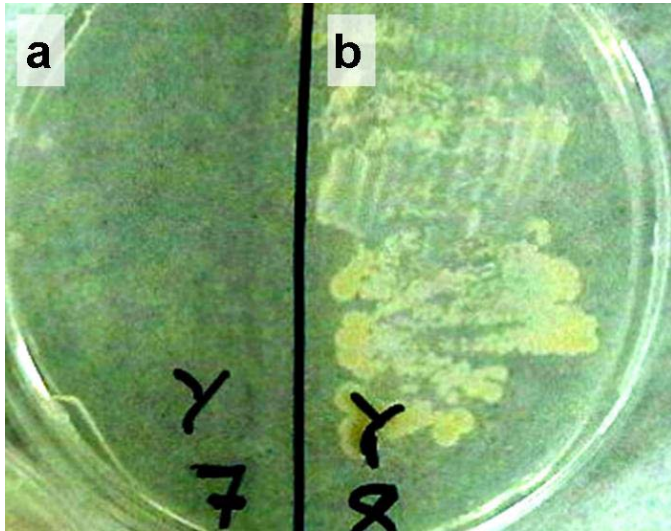


Fig. 1 Growth of *S. cerevisiae* on stringency medium drops out of tryptophan. a, control; b, test.

Our study is comparable to previous studies used similar technique to transform *S. cerevisiae*. Mohamed (2009) and McPhee *et al.* (2011) reported that yeast *S. cerevisiae* has several properties, which have established it as an important tool in the expression of foreign proteins for research, industrial and medical use (12-14). Kawai *et al.* (2010) found that yeast is a suitable host organism for high-level production of secreted as well as soluble cytosolic proteins. They stated that expression of proteins in yeast is a common alternative to prokaryotic and higher eukaryotic expression and yeast cells offer many of the advantages of producing proteins in microbes including growth speed, easy genetic manipulation, low cost media and also yeast offering some of the attributes of higher eukaryotic systems such as post translational modifications [15].

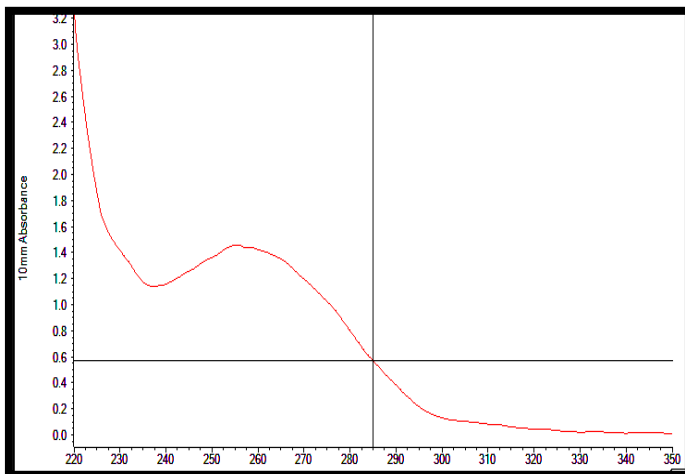


Fig. 2 DNA concentration of extracted plasmid from *S. cerevisiae* by QIAprep Spin Miniprep.

This study agrees with many studies, which stated that most yeast vectors can be propagated and amplified in *E. coli* to facilitate cloning and they also contain an *E. coli* replication origin and ampicillin marker and *S. cerevisiae* strains have been described that increase yield of secreted proteins, improve the performance of certain affinity tags and also reduce proteolysis. Our study is also comparable to many studies used chemical treatment methods to transform yeast using this technique which an endocytosis-like mechanism [16].

The expression system used in this study is designed to produce many copies of a desired protein within the host cell. This vector contains all of the genetic coding that necessary to produce the protein, like a promoter appropriate to the host cell, a sequence for terminates transcription, and a sequence that codes for ribosome binding [17]. This expression is now widely used because of its ability to mass-produce proteins and the specificity involved in the T7 promoter which only binds T7 RNA polymerase, also the design of this system allows for the easy manipulation of how much of the desired protein is expressed and when the expression occurs [18]. In this study, we propagated the plasmid in *E. coli* and then transfer the plasmid into *S. cerevisiae*, which can be used for protein production or for further studies.

Conflict of interest

The authors declare that they have no conflict of interests.

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