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Chemotransformation of Bacterial Cells Without Heat-shock

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Abstract Transformation of DNA into microbial cells *via* heat-shock approach has been well established in the field of molecular biology for decades. Herein we described an unexpected finding that heat-shock may not play an essential role in the transformation process. This observation was verified *via* UV-Vis and fluorescence spectroscopies, and confocal microscopy images for various DNAs and bacterial cells. The non-heat-shock approach proposed in this study can be a convenient and beneficial modification for DNA transformation, especially for those laboratories lacking ice machine and heat-shock equipment.

Keywords Heat-shock; Chemotransformation; DNA; Bacteria

1 Introduction

Transformation of DNA into bacterial cells is among the fundamental technologies in the field of molecular biology, which has been utilized in numerous important bio-applications, such as amplification of plasmid DNA and over expression of exogenous recombinant proteins. Introduction of exogenous DNA into bacterial cells(*e.g.*, *Escherichia coli*) *via* chemical transformation approach has been established for many decades[1,2], where heat-shock has been thought to be the key step. Several other methods can also be used for transferring foreign genes into bacteria, such as electroporation[3,4], ultrasound[5], freeze-thaw[6,7], liposome[8], microwave[9], biolistic gun[10], tribos[11], and hydrogel exposure[12]. Nevertheless, most of these methods have shown obvious drawbacks, such as low efficiency, complicated procedure, and equipment-demanding. This far, chemotransformation(alternately called heat-shock approach) has been most widely used in molecular biological laboratories.

For chemotransformation, competent cells must be prepared beforehand using specific cations, such as calcium[13]. Several other cations may also work, *e.g.*, Mn^{2+}, Ba^{2+}, Sr^{2+}, Mg^{2+}, whereas other cations like Zn^{2+}, Cd^{2+}, Co^{2+}, or Mo^{2+} may not[14]. In fact, certain divalent cations, such as Cu^{2+}, Pd^{2+} and Ni^{2+}, and monovalent cations Rb^{+} and K^{+} can interact with DNA and thus might also be used to induce the DNA transformation[15—19]. These competent cells can be stored at a –80 °C freezer for long term use, making this approach very convenient. The details of the protocol concerning *E. coli* competent cell preparation and heat-shock transformation were also investigated. Briefly, the competent cells and DNAs were mixed and incubated on ice for 5 min, heat-shocked at 42 °C for 60 s, and then incubated again on ice for 3 min. Cells were directly plated on solid selective media and incubated at 37 °C till the colonies were visible(*ca*.14 h). During this transformation process, heat-shock treatment has been taken for granted as a crucial step in previous researches[1,2,20—22]. However, here we proposed a non-heat-shock approach based on an observation that heat-shock may be not a key step for the chemotransformation(Fig.S1, see the Electronic Supplementary Material of this paper).

2 Materials and Methods

2.1 Reagents and Instruments

Yeast extract, tryptone and sodium chloride(NaCl) were purchased from Beijing Chemical Reagent Company(Beijing, China). Agar and salmon sperm DNA(ssDNA) were obtained from DingGuoChangsheng Biotechnology(Beijing, China). 4,6-Diamidino-2-phenylin-dole(DAPI) was obtained from Tianjia Biological Technology(Changchun, China). Plasmid pLR631-HdeA, pLR631-GFP and Pacy-ACP-Duct-1 were built up in our own laboratory. Plasmid CFP-FKBP(CF) and Prset-B-mCherry were kind gifts from Tobias Meyer(Addgene plasmid #20160) and Gary Yellen(Addgene plasmid #32382), respectively. DH5α and TOP10 strains were obtained from Solarbio Technology(Beijing, China, Table S1, see the Electronic Supplementary Material of this paper). BL21-Gold(DE3) strain was purchased from Agilent Technologies(Beijing, China, Table S1). Double-distilled water(dH_2O) used in the experiments was obtained from a Milli-Q system(Millipore, Bedford, MA, USA). UV-visible absorption spectra were measured on a UV-3600 spectrophotometer(Shimadzu, Japan). Fluorescence spectra were measured on a 5301PC spectrophotometer (Shimadzu, Japan).

2.2 Preparation of *E. coli* Competent Cells

E. coli competent cells[DH5α, TOP10 and BL21-Gold

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(DE3)] were prepared *via* the classical $CaCl_2$ method. All supplies were subjected to high temperature sterilization before use. The detailed protocol is as follows. (1) Streaking of *E. coli* cells to fresh Luria-Bertani(LB) solid media, followed by 14 h incubation at 37 °C; (2) inoculation of *E. coli* colonies from LB plates into fresh LB liquid media, followed by shaking incubation at 37 °C for 16 h; (3) transferring 1 mL of the overnight cell culture into 100 mL of fresh LB liquid media, shaking and incubating at 37 °C till the value of OD_{600} reaches 0.6. The cells are then incubated on ice; (4) the precooled cells(30 mL) were further ice-bathed for 15 min, and then centrifuged at 4 °C (4000 r/min, 10 min); (5) removing the supernatant. The cell pellet is gently re-suspended using 20 mL of precooled $CaCl_2$(0.1 mol/L); (6) the resulting cell suspension was incubated on ice for 20 min，followed by centrifugation at 4 °C (4000 r/min, 10 min); (7) the supernatant was removed again and the cell pellet was gently re-suspended using 1 mL of precooled $CaCl_2$(0.1 mol/L); (8) the resulting cell suspension was incubated on ice for 30 min. Glycerol was added into the cell suspension to a final concentration of 15%—20%. The mixture was then divided into small aliquots and transferred to a –80 °C freezer for storage.

2.3 DAPI DNA Stain and Fluorescent Spectra

ssDNA(10 mg/mL, 20 μL) was added into DAPI(2.9 μmol/L, 480 μL) and then distilled H_2O(*ca*. 500 μL) was added into the mixture to make a final volume of 1 mL. The DNA-DAPI mixture was dialyzed against dH_2O for 24 h. The resulting DNA-DAPI complexes(200 μL) and competent cells(50 μL) were added into 1×PBS buffer and then divided into 6 aliquots. These aliquots were incubated at room temperature for different time(4, 10, 20, 30 and 40 min) and then centrifuged(11000*g*, 1 min). The supernatants were then subjected to the fluorescent spectroscopic analysis.

2.4 Process of the Classic Heat-shock Transformation or Non-heat-shock Approach

The competent cells(30 μL) were placed on ice for 5 min, then 2 μL(5 ng) of plasmids was added and mixed gently with cells. After 30 min incubation on ice, the mixture was transferred to a 42 °C water bath for 1 min. The resulting cell suspensions were then incubated on ice again for additional 3 min, followed by plating. These LB plates were incubated at 37 °C for 14 h.

For each non-heat-shock transformation, just thawed *E. coli* competent cells(30 μL) were gently mixed with 2 μL of plasmid DNAs, followed by either direct plating or plating after incubation at room temperature for indicated time(note: the ice bath and heat-shock steps were omitted for this approach), and then incubated at 37 °C for 14 h.

3 Results and Discussion

The transformation was initiated by DNA-cell interaction. This process can be monitored *via* UV-visible absorption and fluorescence spectra. To monitor DNA-cell binding, a simple centrifugation based system was applied[Fig.1(A)]. DNA(plasmid pLR631-HdeA, 100 ng/μL, 2 μL) and competent cells(DH5*α*, 30 μL) were mixed and incubated for indicated time followed by centrifugation to separate the unbound DNA from DNA-cell complexes. The DNA-cell combination process can be monitored at A_{260} to see the absorbance changes of the

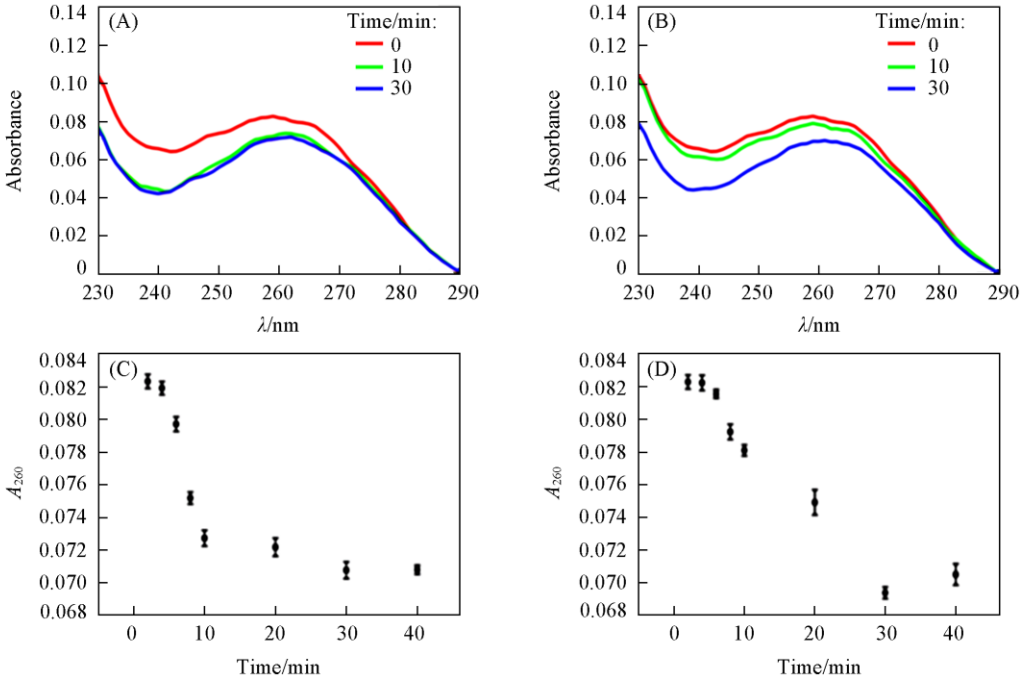
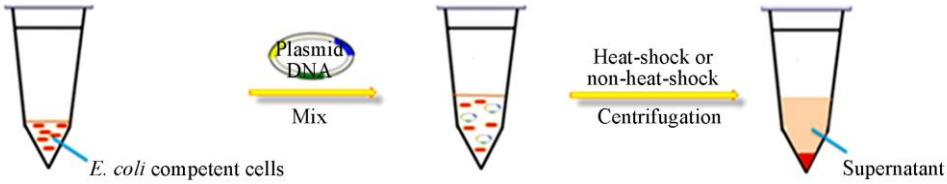


Fig.1 UV-Vis absorption spectra of supernatants of DNA-cell mixture from classic heat-shock transformation(A, C) and non-heat-shock transformation(B, D)

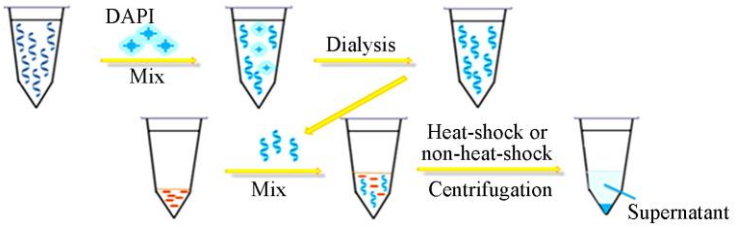
(A) and (B) Before centrifugation, DNA-cell mixtures were incubated for 0, 10 and 30 min, respectively; (C) and (D) DNA-cell mixtures were incubated for indicated time(*e.g.*, 2, 4, 6, 10, 20, 30 and 40 min) before A_{260} of the supernatants was measured. The error bars represent the standard deviation of three measurements.

supernatants because DNA has a characteristic absorption peak at 260 nm(A_{260})(Scheme 1). The heat-shock treatment might slightly facilitate the DNA-cell binding as shown by the rapid decrease of DNA concentration in the supernatant at the beginning[Fig.1(A) and (B)]. Nevertheless, the overall tendency of DNA-cell binding by these two approaches was very similar(Fig.1), suggesting heat-shock may be not required in this step.



Scheme 1 Schematic drawing for the preparation of the supernatants of DNA-cell mixtures

This process was verified further using fluorescence spectroscopy. DAPI is a routinely used fluorescent molecule that binds specifically to DNA and becomes more fluorescent when bound. Therefore, DAPI was used here as a fluorescent marker to label DNA. DAPI and DNA(salmon sperm DNA, ssDNA) were mixed proportionally as mentioned in Section **2**. The unbound DAPI was removed *via* 3500 Dalton cut-off dialysis(the molecular weight of DAPI is 277.3). The DAPI-DNA complexes were then mixed with *E. coli* competent cells(DH5α), followed by separation using centrifugation as mentioned above(Scheme 2). In both cases(heat-shock and non-heat-shock approaches), the content of DNA in the supernatant was reduced following the increase of incubation time, on the decrease of fluorescent signals(Fig.2). In particular, DNAs bound *E. coli* competent cells effectively in non-heat-shock route. This result is consistent with the data obtained by UV absorption spectroscopy(Fig.1), suggesting that heat-shock may be not critical for DNA-*E. coli* competent cell interaction.



Scheme 2 Schematic drawing for preparation of supernatants of DAPI-DNA-cell mixtures

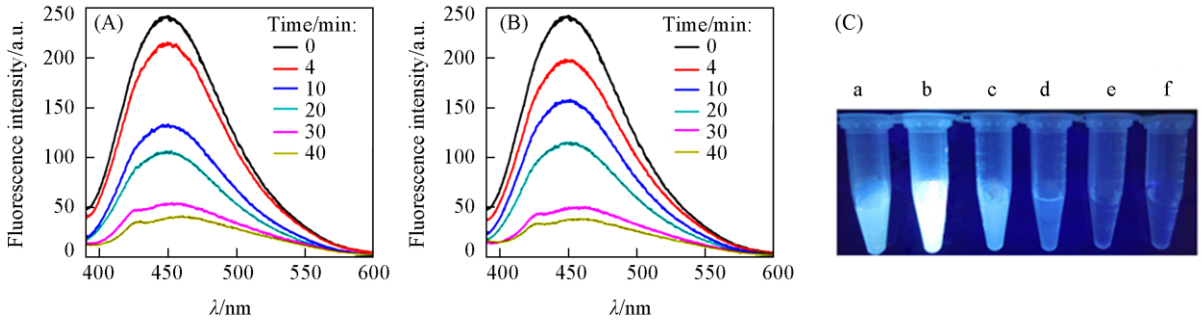


Fig.2 Fluorescence spectra(A, B) and image of the supernatants of DAPI-DNA-cell mixtures after incubation for indicated time(C)

(A) and (B) refer to heat-shock and non-heat-shock routes, respectively; (C) images show the following samples under UV irradiation: a. DAPI; b. DAPI+DNA; c. DAPI+DNA after dialysis; d—f. refer to the supernatants of DAPI-DNA-cell mixtures after incubation for indicated time of 0, 10, 30 min, respectively *via* non-heat-shock approach. 0, 10, and 30 min stand for the incubation time of DAPI-DNA-cell mixtures before centrifugation.

To investigate if the putative cell bound DNA can enter the cells when the heat-shock step is not applied, several plasmids[CFP-FKBP(CF), pLR631-GFP and Prset-B-mCherry][23,24] containing cDNA sencoding different fluorescent proteins(cyan, green and red fluorescent proteins) were transformed into *E. coli* strain[BL21-Gold(DE3): these *E.coli* cells contain a T7 polymerase, allowing the overexpression of recombinant protein under the control of T7 promoter](Table S1). This process was monitored visually using confocal fluorescence microscope(CFM). The plasmids and BL21-Gold(DE3) competent cells were mixed and immediately subjected to plating. All the plasmid DNAs can be readily taken up by the bacterial cells(Fig.S2—S4, see the Electronic Supplementary Material of this paper). These results suggest that non-heat-shock transformation is not only applicable to DH5α, but also to other *E. coli* strains, such as BL21-Gold(DE3). The colonies were inoculated into LB liquid media and cultured for 14 h. The cells were centrifuged and washed 3 times with 1×phosphate buffer saline(PBS buffer), and then subjected to CFM analysis. CFM images show that these fluorescent proteins have been all expressed, further hinting the heat-shock is not an essential step during the transformation process(Fig.3). Notably, *E. coli* cells containing Prset-B-mCherry plasmid show red colour even under visible light(Fig.S4, see the Electronic Supplementary Material of this paper), which also indicates the successful transformation.

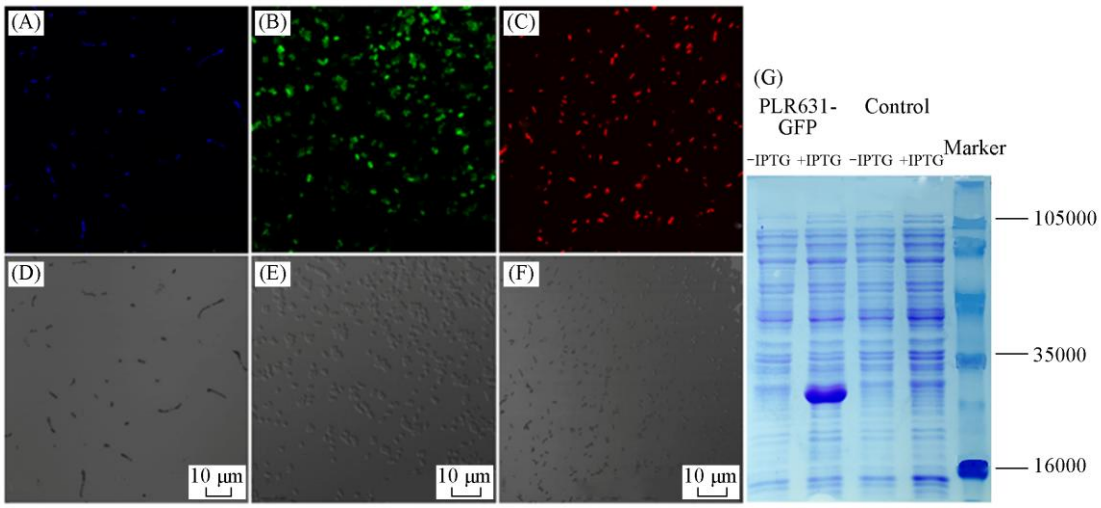


Fig.3 Laser scanning confocal fluorescence microscopy images of BL21-Gold(DE3) cells transformed with different plasmids(A—F) and SDS-PAGE of the cell extracts for the *E. coli* cells transformed with pLR631-GFP(G) *via* non-heat-shock approach

The following plasmids were used for transformation as indicated: (A) and (D) CFP-FKBP(CF); (B) and (E) pLR631-GFP; (C) and (F) Prset-B-mCherry. (A)—(C) and (D)—(F) refer to the fluorescent and bright-field images, respectively. (G) *E. coli* cells containing plain vector were served as control. The strong band localized at approximately 30000 corresponded to GFP protein.

Concerning classic heat-shock and non-heat-shock(this study) approaches, no clear difference can be found when commonly used *E. coli* DH5*α* was served as host cells and ampicillin selective marker containing plasmids were applied as transferring DNAs[Fig.4(A), Fig.S5(see the Electronic Supplementary Material of this paper)]. Although the non-heat-shock approach shows somewhat slightly less effective when another commonly used bacterial strain(TOP10 competent cells) was used as a host when compared to DH5*α*, nevertheless, the number of colonies is still comparable between the two approaches[Fig.4(B), Fig.S5]. Moreover, DH5*α* cells were also successfully transformed by plasmids containing other selection marker, such as Pacy-ACP-Duct-1(chloramphenicol marker) *via* the both approaches[Fig.4(C), Fig.S5], suggesting the non-heat-shock approach is applicable for various types of plasmid DNAs. Notably, the plasmids(Pacy-ACP-Duct-1) isolated from *E. coli* DH5*α* cells transformed *via* either classic heat-shock or non-heat-shock transformation are the same (Fig.5), which ruled out the possibility of contamination.

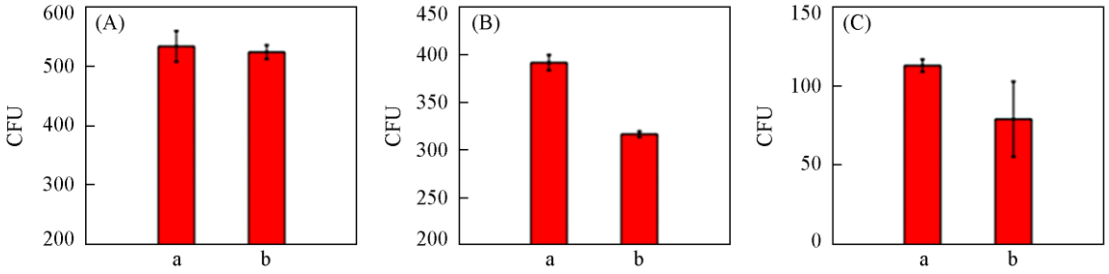


Fig.4 Efficiency comparison of classic heat-shock and non-heat shock transformation using histogram of colony forming units(CFU)

(A) DH5*α* cells transformed with pLR631-HdeA(on LB-ampicillin media); (B) TOP10 cells transformed with pLR631-HdeA(on LB-ampicillin media); (C) DH5*α* cells transformed with Pacy-ACP-Duct-1(on LB-chloramphenicol media). The error bars represent the standard deviation of three measurements from independent experiments. a. Heat-shock; b. non-heat-shock.

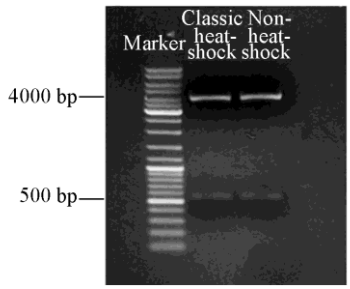


Fig.5 Agarose gel electrophoresis of plasmids(Pacy-ACP-Duct-1) isolated from DH5α cells transformed using either classic heat-shock or non-heat-shock approach

Indeed, the non-heat-shock approach worked well and showed comparable efficiency to the classic heat-shock approach when different amount of plasmid DNAs(pLR631-HdeA, 5.75, 11.5, 57.5 and 115 ng/μL) were applied[Fig.6, Fig.S6(see the Electronic Supplementary Material of this paper)]. Especially, when plasmid concentration approached 10 ng/μL, no statistically significant difference can be visualized between the heat-shock and the non-heat-shock approaches. Moreover, the time effect on non-heat-shock transformation was evaluated(Fig.7). When DNA-cell incubation time approached 0.5—1 h, the transformation efficiency of the non-heat-shock method was even slightly better than that of the classic heat-shock approach. It is believed that the physical

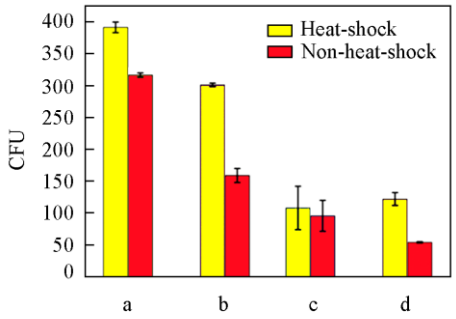


Fig.6 Quantitative illustration of different plasmid concentrations *via* heat-shock and non-heat-shock approaches

For each transformation was added 2 μL of plasmid(pLR631-HdeA) with indicated concentration into 30 μL of DH5*α* competent cells. The error bars represent the standard deviation of three measurements from independent experiments. a. 115 ng/μL; b. 57.5 ng/μL; c. 11.5 ng/μL; d. 5.75 ng/μL.

state of bacterial cell membrane can be changed during heat/cold shock[25], which may improve the uptake of xenogeneic DNA[26]. Our studies raised a question that these previous hypotheses might need to be reconsidered and verified. Importantly, the key heat-shock step of the well-recognized standard bacterial transformation protocol may be not as critical as previously thought. Currently we have not yet known the real mechanism for the DNA uptake by bacterial cells during non-heat-shock approach. However, two possibilities may play a role in this process: (1) suitable cation-DNA-cell interaction is sufficient to deliver the biomolecules into the bacteria; (2) bacterial cell membrane undergoes slight mechanical damage during the plating, which helps cells to take in DNA.

Taken together, transformation of different types of plasmid DNAs into different *E. coli* strains with different mixing time and DNA concentrations was compared and verified using both heat-shock and non-heat-shock approaches(Fig.8).

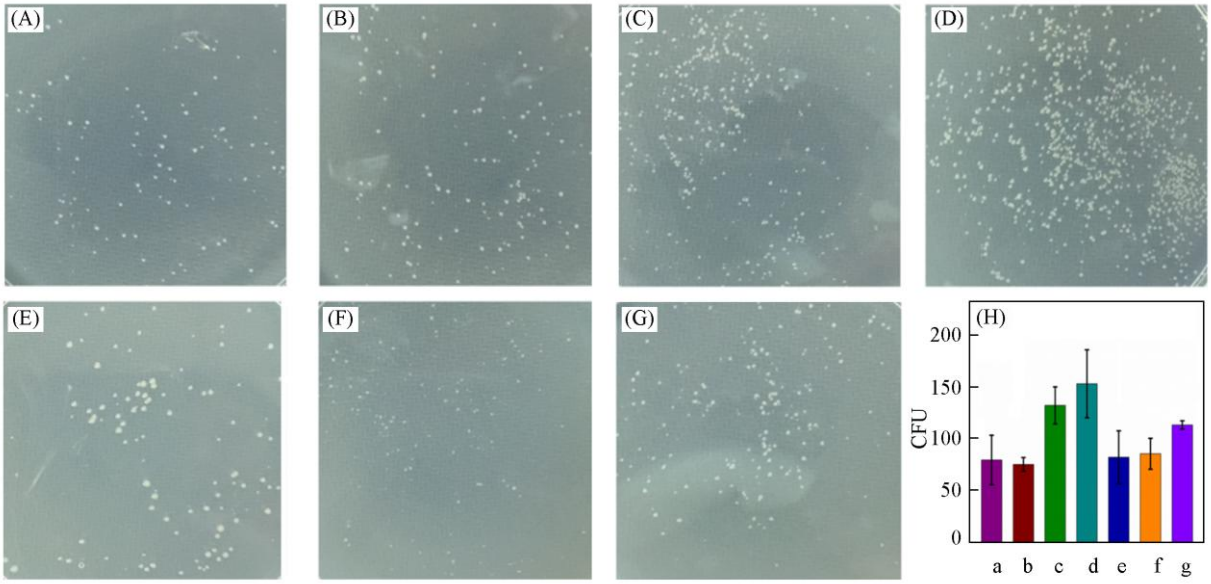


Fig.7 Time effect on non-heat-shock transformation

(A)—(F) Colonies on ampicillin contained LB plates(representative plates). Time/h: (A) 0; (B) 0.25; (C) 0.5; (D) 1; (E) 6; (F) 12. DNA(pLR631-HdeA, 2 μL) and competent cells(DH5α cells, 30 μL) were mixed for 0, 0.25, 0.5, 1, 6 and 12 h, respectively, before they were subjected to plating(note: 0 h stands for immediate plating after mixing); (G) by heat-shock transformation; (H) histogram of CFU. a. 0 h; b. 0.25 h; c. 0.5 h; d. 1 h; e. 6 h; f. 12 h; g. heat-shock transformation. The error bars represent the standard deviation of three measurements from independent experiments.

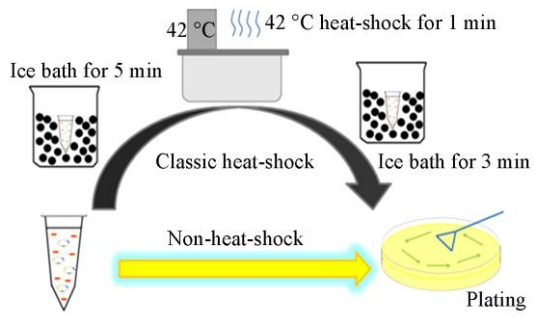


Fig.8 Summarized schematic presentation for the simplified procedure of the heat-shock(classic) and non-heat-shock(this study) approaches

Notably, there was no significant difference concerning the transformation efficiency of classic heat-shock and non-heat-shock methods. In fact, several other cations, such as Mn^{2+}, Ba^{2+}, Sr^{2+}, Mg^{2+}, Rb^{+} and K^{+} might be also function in the non-heat-shock approach for inducing DNA transformation into *E. coli* cells. With different cations, the transformation efficiency might vary depending on the specific interactions between the cations and DNA. Further elegantly designed experiments are needed to verify these issues.

4 Conclusions

DNA transformation is among the basic technologies in molecular biology, where heat-shock based chemotransformation plays an important role. However, based on the data obtained by UV-Vis, fluorescence spectroscopy and confocal microscopy, we described an unexpected finding that heat-shock do not play an essential role in the transformation process. This finding may in a way change the common view regarding bacterial transformation in the field of molecular biology, indicating that the mechanism for DNA chemotransformation requires re-examination. The omission of

the ice bath and heat shock steps implied a different transformation mechanism. Moreover, the non-heat-shock approach proposed in this study can be an alternative time-saving method for DNA transformation, especially for those laboratories lacking ice machine and heat-shock equipment.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s40242-017-6403-8.

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