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Optimizing heat-shock conditions for competent cell preparation: A study on temperature and duration variability

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Abstract
The efficacy of bacterial transformation is essential in molecular biology, especially for cloning, gene expression, and recombinant protein synthesis. This study aims to optimize heat shock parameters, that is, temperature and duration, to improve transformation efficiency in *Escherichia coli* DH5α utilizing the significant binary vector pBI121 (~14.7 kb), extensively employed in plant biotechnology. Competent cells were generated via a modified calcium chloride technique and exposed to nine heat shock combinations: three temperatures (37℃, 42℃, and 47℃) and three durations (30, 45, and 60 seconds). The success of transformation was assessed by measuring colony-forming units (CFU) per microliter. The ideal setting was determined to be 47℃ for 60 seconds, resulting in the highest transformation efficiency (125.6 CFU/µl), whereas the lowest efficiency (4.8 CFU/µl) was observed at 42℃ for 60 seconds. The findings suggest that bigger plasmids require more powerful heat shock to permeate the bacterial barrier efficiently. Although hampered by the absence of biological duplicates and plasmid quantification, this study addresses a critical methodological need for laboratories handling large plasmid vectors. It enables further procedure refinement for plant genetic engineering.

Keywords: Competent cells, DH5α, pBI121, heat shock, transformation efficiency, large plasmid

Introduction
A fundamental method in molecular biology, genetic transformation enables the insertion of foreign genes into host organisms for scientific, medical, or commercial applications. Bacterial transformation, especially in *Escherichia coli*, has emerged as a standard transformation technique for recombinant DNA technology [1, 2]. In order to provide temporary membrane permeability, this procedure entails the uptake of plasmid DNA, which is frequently facilitated by chemical treatments and thermal stress [3].
The calcium chloride-mediated heat shock technique remains the gold standard for producing competent E. coli cells, especially strains like DH5α [4]. Key alterations like reca1 and endA1 contribute to plasmid stability and increased yield during propagation [5]. While this approach is extensively used, the literature demonstrates significant diversity in recommended heat shock parameters, notably concerning plasmid size [6, 7].
The binary vector pBI121, roughly 14.7 kilobase pairs long, is widely utilized in Agrobacterium-mediated plant transformation [16]. Despite its widespread usage in plant biotechnology, optimizing transformation conditions for pBI121 in E. coli DH5α has received little attention [8]. Most published procedures are designed for small plasmids (e.g., pUC19) and do not consider the steric hindrance and membrane translocation problems that larger constructs provide [9].
This study aims to systematically evaluate transformation efficiency for the DH5α-pBI121 system over a matrix of temperatures and durations. By determining the ideal circumstances for massive plasmid absorption, it fills a crucial gap in molecular cloning procedures and offers useful information to scientists working with intricate constructs in plant and microbial biotechnology [10, 11].

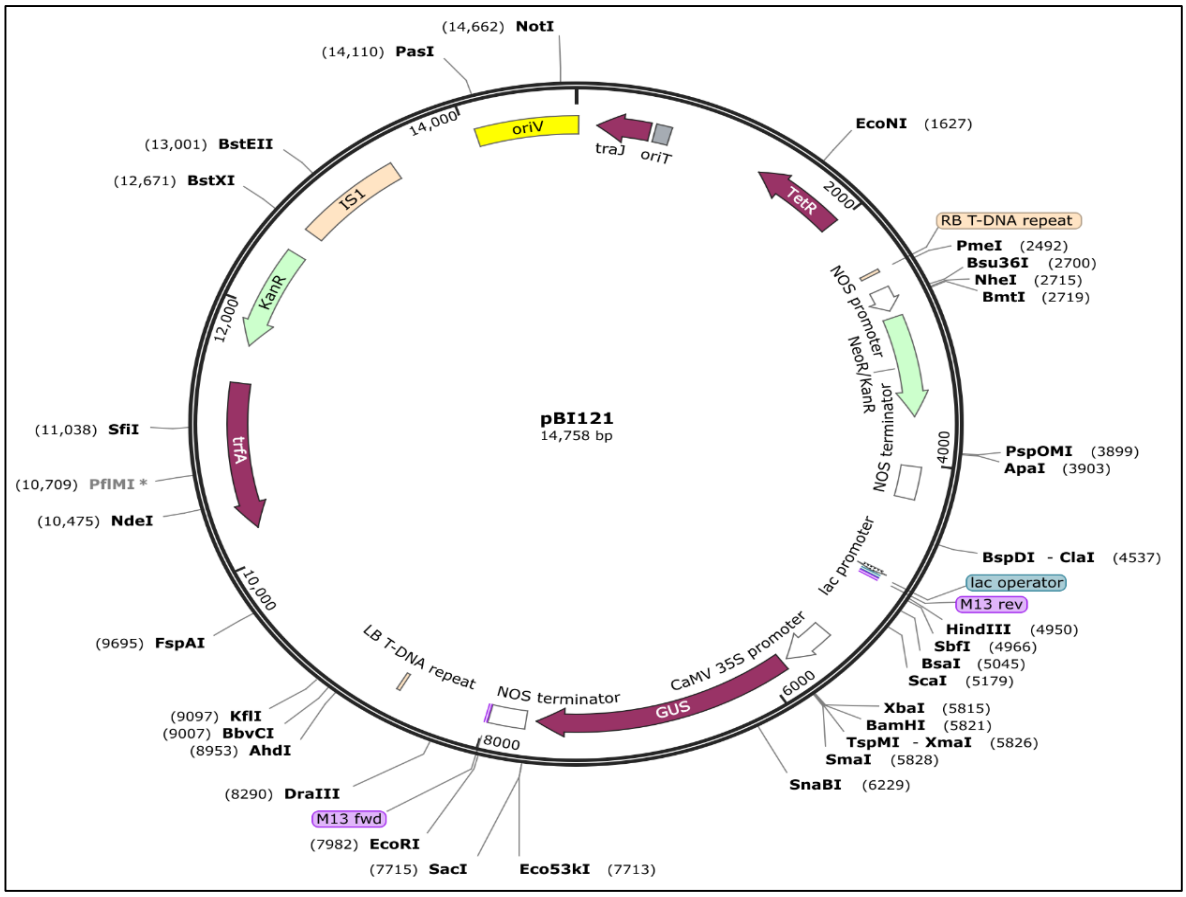

Fig 1: pBI121, Binary Agrobacterium Vector with a GUS Reporter Gene for Plant Transformation, SnapGene

Materials and Methods

Bacterial Strain and Plasmid

The laboratory strain *Escherichia coli* DH5α was chosen for its genetic changes that improve transformation efficiency and plasmid stability. The plasmid utilized was pBI121 (~14.7 kb), a binary vector commonly used in plant genetic engineering [16]. It was diluted 1:20 in sterile distilled water to make a working stock.

Preparation of Media and Reagents

Tryptone (10 g/l), yeast extract (5 g/l), and NaCl (10 g/l) were the typical ingredients used to make Luria-Bertani (LB) broth. The pH was adjusted to 7.0, and the broth was autoclaved for 15 minutes at 121℃ to sterilize it [8]. 15 g/l of agar was added before autoclaving to create LB agar after cooling to about 50℃. Plates were poured under aseptic conditions.

50% glycerol and a calcium chloride solution (0.1 M) were made and sterilized independently. Every reagent that was utilized was of analytical grade.

Preparation of Competent Cells

A modified calcium chloride technique was used to create competent cells using Hanahan's methodology [7]. After being inoculated into 5 ml of LB broth, a single colony of E. coli DH5α was cultured for the entire night at 37℃ and 180 rpm. The overnight culture was grown to the mid-log phase (OD_{600} = 0.4-0.6) after diluting 1:100 into fresh LB. After centrifuging the cells for 10 minutes at 4℃ at 3000 rpm, they were resuspended in 0.1 M $CaCl_2$, allowed to sit on ice for 30 minutes, and then centrifuged again. The finished pellet was aliquoted, resuspended in 50% glycerol and $CaCl_2$ solution, and kept at -20℃ [10].

Transformation Procedure

Each transformation reaction comprised 100 µl of competent cells and 10 µl of plasmid solution. The mixture was incubated on ice for 15 minutes to promote plasmid binding to the membrane surface [7, 11].

Heat Shock Treatment

Transformation tubes underwent nine distinct combinations of temperature and duration (Table 1) derived from modified protocols of established heat shock studies [6, 10]. The tubes were heated in a water bath for a short duration and subsequently placed on ice for 5 minutes.

Table 1: Temperature duration combinations

Temperature (℃)	Duration (s)
37	30
37	45
37	60
42	30
42	45
42	60
47	30
47	45
47	60

Recovery and Plating

After heat shock, 1 ml of LB broth was given to the cells, and they were cultured at 37℃ for 1-3 hours to allow antibiotic resistance genes to be expressed. After a brief centrifugation, cells were plated onto LB agar. Due to a lack of antibiotics, the transformation was determined visually through colony counts.

Colony Counting
Colonies were manually counted after overnight incubation at 37℃ to determine CFU/µl. No replicates were performed, and counts indicate individual observations.

Controls
Negative controls (cells without plasmid DNA or heat shock) proved the absence of background colonies and validated successful transformation events.

Results
Transformation efficiency across conditions
Transformation efficiency differed considerably among the nine heat shock combinations of temperature and duration (Table 2). At 47℃ for 60 seconds, the maximum efficiency of 125.6 CFU/µl was recorded. The lowest efficiency, however, was obtained at 42℃ for 60 seconds (4.8 CFU/µl). These results corroborate earlier research showing that exposure duration and heat intensity both affect transformation efficiency.

Table 2: Transformation Efficiency of *E. coli* DH5α under different heat shock conditions

Temperature (℃)	Duration (s)	Colony Count	Transformation Efficiency CFU/µl)
37	30	28	28.0
37	45	18	18.0
37	60	35	35.0
42	30	46	46.0
42	45	11	11.0
42	60	4	4.8
47	30	92	92.0
47	45	117	117.0
47	60	125	125.6

Optimal heat shock conditions
Transformation efficiency improved with both temperature and duration. All 47℃ treatments had much higher efficiency than their equivalents at 37℃ or 42℃. This pattern indicates a temperature threshold above which the bacterial membrane becomes more permeable to big plasmids such as pBI121, enabling DNA uptake.

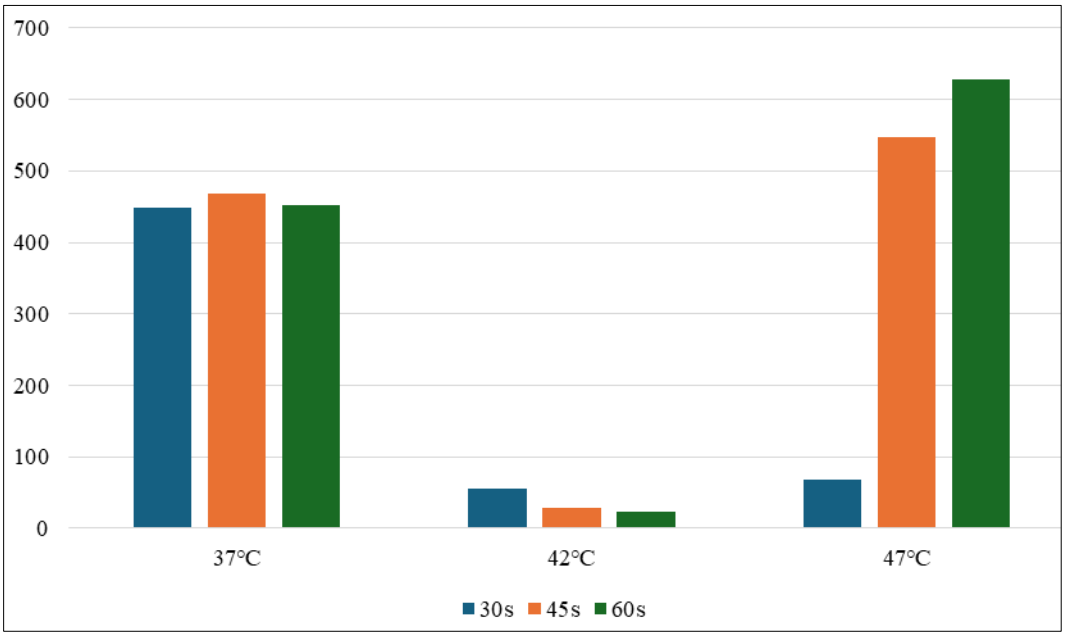

Fig 2: Bar graph of colony counts under different heat-shock conditions

Colony Morphology and Controls
E. coli DH5α colonies had a spherical, off-white, and well-isolated appearance. Negative controls (plasmid deleted or heat shock skipped) exhibited no growth, indicating that colonies were the product of successful transformation processes.

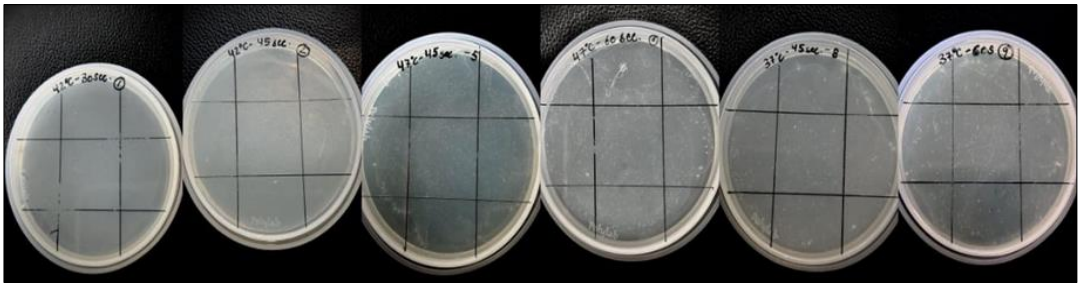

Fig 3: LB agar plates showing colonies under various conditions

Discussion
This work examined the impact of heat shock parameters (temperature and duration) on the transformation efficiency of E. coli DH5α using the binary vector pBI121, a large (~14.7 kb) plasmid often used in plant biotechnology [16]. The data show that transformation efficiency strongly depends on heat shock strength and exposure length, with the best results obtained at 47℃ for 60 seconds.

Effects of Temperature and Duration

The increase in transformation efficiency at higher temperatures and longer durations complements recent observations that greater heat shock enhances membrane permeability and facilitates plasmid entry [6, 10]. The maximal efficiency reported at 47℃ for 60 seconds (125.6 CFU/µl) demonstrates that more extensive thermal treatment is particularly favorable for larger plasmids, which experience steric and membrane transport problems not faced by smaller vectors.

Interestingly, poorer effectiveness at 42℃ for 60 seconds (4.8 CFU/µl) may be attributed to prolonged inadequate heat exposure, leading to diminished membrane fluidity or cellular stress. Similar inconsistencies were seen in prior research when the duration of exposure was not matched correctly to the temperature threshold [6, 10].

Comparison with Previous Literature

Small plasmids (such as pUC19, less than 5 kb) are best suited for the traditional heat shock technique, which involves applying 42℃ for 30 to 90 seconds [7, 10]. However, larger constructs like pBI121, which need more intensive circumstances to facilitate entry, may not always be amenable to these conditions. This study expands on previous research by directly testing a big plasmid under a matrix of heat settings and quantifying the results using CFU estimation, a rarely systematized method in transformation techniques involving high-molecular-weight plasmids.

Methodological Limitations

While the transformation methodology produced useful results, it was limited in scope. Manual colony counting caused observer bias, and transformation was carried out without technical or biological replicates, reducing statistical power. The plasmid solution was utilized without re-quantification or integrity testing, which could affect repeatability.

Notably, no positive control was included, such as transformation with a tiny plasmid like pUC19. This limits the comparative evaluation of competent cell quality and baseline transformation efficiency. Despite these limitations, the findings provide a good foundation for improving techniques that involve big plasmids in typical molecular biology operations.

Application and Relevance

This work offers a simple, repeatable method for increasing the transformation efficiency of big plasmids, particularly those used in plant genetic engineering. This heat shock optimization directly applies to researchers dealing with Agrobacterium binary vectors or other high-molecular-weight constructs.

Conclusion

This study indicated that adjusting heat shock parameters considerably improves the transformation efficiency of E. coli DH5α, particularly when working with big plasmids such as pBI121 (~14.7 kb). Among the nine combinations evaluated, 47℃ for 60 seconds emerged as the most effective condition, resulting in a 125.6 CFU/µl transformation efficiency.

These findings demonstrate that the thermal stress necessary for successful transformation is not universal but varies with plasmid characteristics, including size and cellular physiology. By overcoming a frequent restriction in traditional transformation techniques, our study offers an accessible and efficient approach for researchers doing molecular cloning with bigger constructs, notably in plant biotechnology and genetic engineering contexts.

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References

1. Madigan MT, Bender KS, Buckley DH, Sattley WM, Stahl DA. Brock Biology of Microorganisms. 15th ed. Pearson, 2019.
2. Johnston C, Martin B, Fichant G, Polard P, Claverys JP. Bacterial transformation: Distribution, shared mechanisms and divergent control. Nat Rev Microbiol. 2014;12(3):181-196.
3. Griffith F. The significance of pneumococcal types. J Hyg (Lond). 1928;27(2):113-159.
4. Avery OT, MacLeod CM, McCarty M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J Exp Med. 1944;70(2):137-158.
5. Lederberg J, Tatum EL. Gene recombination in E. coli. Nature. 1946;158(4016):558.
6. Lederberg J. Cell genetics and hereditary symbiosis. Physiol Rev. 1952;32(4):403-430.
7. Hanahan D. Studies on transformation of Escherichia coli with plasmids. J Mol Biol. 1983;166(4):557-580.
8. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. 3rd Ed, Cold Spring Harbor Laboratory Press, 2001.
9. Chen I, Christie PJ, Dubnau D. The ins and outs of DNA transfer in bacteria. Science. 2005;310(5733):1456-1460.
10. Inoue H, Nojima H, Okayama H. High efficiency transformation of *E. coli* with plasmids. Gene. 1990;96(1):23-28.
11. Green MR, Sambrook J. Molecular Cloning: A Laboratory Manual. 4th Ed, Cold Spring Harbor Laboratory Press, 2012.
12. Taylor RG, Walker DC, McInnes RR. E. coli host strains significantly affect the quality of small-scale plasmid DNA preparations used for sequencing. Nucleic Acids Res. 1993;21(7):1677-1678.
13. Cameron DE, Bashor CJ, Collins JJ. A brief history of synthetic biology. Nat Rev Microbiol. 2014;12(5):381-390.
14. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816-821.
15. Nielson AA, Der BS, Shin J, Vaidyanathan P, Paralanov V, Strychalski EA, *et al.* Genetic circuit design automation. Science. 2016;352(6281):aac7341.
16. Jefferson RA, Kavanagh TA, Bevan MW. GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion

marker in higher plants. EMBO J. 1987;6(13):3901-3907.
17. Hellens RP, Edwards EA, Leyland NR, Bean S, Mullineaux PM. pGreen: A versatile and flexible binary Ti vector for Agrobacterium mediated plant transformation. Plant Mol Biol. 2000;42(6):819-832.
18. Das S, Dash HR. Handbook of Metal-Microbe Interactions and Bioremediation. CRC Press, 2014.
19. Domingues S, Harms K, Fricke WF, Johnsen PJ, Salva DGJ, Nielsen KM. Natural transformation facilitates transfer of transposons, integrons, and gene cassettes between bacterial species. Proc Natl Acad Sci., USA. 2012;109(33):13480-13485.