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Temperature and Time Optimization of pGEM-T Plasmid Transformation in *Escherichia coli* JM109

Optimización de Temperatura y Tiempo de Transformación del Plásmido pGEM-T en *Escherichia coli* JM109

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SUMMARY

Introduction: *The widely used method for transformation was the heat shock method, which was influenced by the time and temperature of heat shock treatment. However, there was a lack of research on the optimal temperature for pGEM-T plasmid transformation in Escherichia coli JM109. This study uses the heat shock method to determine the optimum temperature and time for pGEM-T transformation in Escherichia coli JM109.*
Methods: *The research method used was a quasy experimental. The researched unit was the plasmid pGEM-T and Escherichia coli JM109 competent cells, with six treatment groups with temperature variations of 35°C and 42°C, for 30, 60, and 90 seconds and a controlled group that contained DNA insert. The transformed plasmids were cultured and then isolated to assess the intensity of the plasmid bands on agarose gel electrophoresis using ImageJ analysis.*
Results: *The results of the band width measurement obtained the largest value of 44.874-3.380 pixels in the 42°C- 60-second treatment group. At the same time, the results of measuring the intensity of the band obtained the highest value of 2.2235 in the 42°C-60-second treatment group.*
Conclusion: *The results indicate that the optimum condition for pGEM-T transformation in Escherichia coli JM109 was at 42°C for 60 seconds.*

Keywords: *Recombinant, technology, transformation, Escherichia coli JM109, imageJ.*

RESUMEN

Introducción: *El método ampliamente utilizado para la transformación fue el método de choque térmico, que estuvo influenciado por el tiempo y la temperatura del tratamiento de choque térmico. Sin embargo, existe falta de investigación sobre la temperatura óptima para la transformación del plásmido pGEM-T en Escherichia coli JM109. Este estudio tiene como objetivo determinar la temperatura y el tiempo óptimo para la transformación de pGEM-T en Escherichia coli JM109 utilizando el método de choque térmico.*
Métodos: *El método de investigación utilizado fue un cuasi experimental. La unidad investigada fue el plásmido pGEM-T y células competentes de Escherichia coli JM109, con 6 grupos de tratamiento con variaciones de temperatura de 35°C y 42°C por 30, 60 y 90 segundos y un grupo controlado que contiene inserto de ADN. Los plásmidos transformados se*

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cultivaron y luego se aislaron para evaluar la intensidad de las bandas de plásmidos en la electroforesis en gel de agarosa usando el análisis ImageJ.
Resultados: *Los resultados de la medición del ancho de banda obtuvieron el mayor valor de 44.874 y 3.380 píxeles en el grupo de tratamiento de 42 °C y 60 segundos. Mientras que los resultados de medir la intensidad de la banda obtuvieron el mayor valor de 2,2235 en el grupo de tratamiento a 42°C-60 segundos.*
Conclusión: *Los resultados indican que la condición óptima para la transformación de pGEM-T en Escherichia coli JM109 fue a 42 °C durante 60 segundos.*

Palabras clave: *Recombinante, tecnología, transformación, Escherichia coli JM109, imageJ.*

INTRODUCTION

Gene cloning introduces foreign DNA or genes to a host cell (bacteria, plant, or animal). This process begins by the insertion of the gene into the vector to form a recombinant DNA molecule. Vectors acted as vehicles for inserting genes into host cells to direct the DNA replication process and the genes' proper (protein) expression (1,2). The cloning process consisted of isolating the gene, preparing the target DNA, inserting the target DNA into the plasmid vector, transforming the plasmid into the host cell, and then the host cell produced protein (3). In the cloning stage above, there was a transformation process, namely inserting a plasmid containing the target gene into the host cell. The transformation is the bacterial ability to insert DNA into cells, and this ability is used for the cloning process (4).

Plasmids were used as cloning vectors by inserting target DNA into the plasmid to form recombinant DNA. Cells transformed with recombinant DNA plasmids were called recombinant bacteria (5). Plasmids were extrachromosomal molecules ranging from 1 kb to more than 200 kb, circular, double-stranded, and closed. The plasmid acted as an accessory genetic unit in bacteria but could replicate and pass on its properties separately from the bacterial chromosome (6). Recombinant plasmid transformation can be done using *Escherichia coli* (*E. coli*) host cells. *E. coli* bacteria can quickly replicate and grow with simple or unique media, making it easy to culture (7). Several strains of *E. coli* can be used for transformation, namely *E. coli* DH5a, *E. coli* BL21, *E. coli* JM109, and *E. coli* HB101. The JM109 strain was transformed because it has a high efficiency above 10^8 CFU/ug and sub-cloning efficiency above 10^7 CFU/μg, including white and blue selection (8). While other strains, such as HB101, are used for vectors that do not require white-blue selection, and BL21 (DE3) can be used as protein expression vectors under the control of the T7 promoter, such as the pET vector (9).

The transformation mechanism involves DNA and the cell surface, where the DNA will bind to the membrane surface and pass through the cell wall and membrane complex (10). The transformation can be carried out by several methods, namely heat shock and electroporation (7). The transformation method that is widely used is heat shock by heating at 42°C for 2 minutes so that the plasmid can enter the bacterial cell (4). This method has the principle that the plasmid will enter the bacterial cell by shocking the high temperature for a few seconds (11).

Giving cold and hot temperatures in the heat shock method can disrupt the complexity of the membrane. Low temperatures result in the release of lipids from the bacterial cell membrane. This repeated process results in the formation of pores on the surface of the plasma membrane and increases the transformation efficiency (12). After the pores in the cell wall are open, incubation is carried out with the plasmid, allowing the plasmid to enter the bacterial cell. This process is done by quickly moving the cells previously incubated on ice to a warm temperature of 42°C for 90 seconds. A heat shock incubation time that is too long can cause membrane damage and cell death. This process must be carried out quickly and precisely so that the cells are in shock. Rapid heating at 42°C creates a heat gradient that causes a current to flow into the cell, allowing the plasmid to enter the cell (4).

The transformation efficiency of the heat shock method is affected by the time and temperature of the heat shock treatment. Several studies have shown different temperatures and times depending on the type of plasmid used and the treatment used on competent cells. Some of the optimal temperatures and times are the pUC19 plasmid at 42°C for 30 seconds, and the

second optimal is the treatment at 37°C (13), the pRGEB32 plasmid at 55°C for 30 seconds (14), 42°C for 45 seconds (15), plasmid pUC19 at 35°C for 25 seconds (16). The optimum temperature and time will differ depending on the type of plasmid. However, there has been not enough research on the optimum temperature of pGEM-T plasmid transformation in Escherichia coli JM109. Based on the preceding description, this study aimed at optimizing the temperature and transformation time of pGEM-T using the heat shock method on *E. coli* JM109. The heat shock procedure was implemented with various time and temperature variables to ascertain the optimal temperature and time parameters for successfully transforming the pGEM-T plasmid into *E. coli* JM109 bacteria.

METHODS

Design Study

This research was conducted in the Biology Molecular Laboratory of Polytechnic of the Ministry of Health Bandung. This Study was a form of quasi experimental.

Samples

The researched unit was the pGEM-T plasmid and *E. coli* JM109 competent cells obtained from the Promega pGEM-T easy kit. Two temperature variations were used, namely 42° C and 35° C, and 3-time variations of 30, 60, and 90 seconds. Hence, the treatment groups in this study consisted of 6 treatment groups and a controlled group.

Data Collection

The data used in this study was primary data by conducting pGEM-T plasmid transformation experiments on *E. coli* JM109 with heat shock temperatures of 42° C and 35° C with variations in time of 30, 60, and 90 seconds. There were six treatment groups based on the variation in temperature and time. After the transformation process, the bacterial cells were cultured, and then grown colonies were observed through blue and white selection and then used. Electrophoresis was applied to assess the migration of the transformation results. The quality of the transformation results was evaluated from the bands formed and measured using the ImageJ software to measure the area and intensity of the plasmid bands. Bands were quantified using the ImageJ software by using the "analyze gels" menu, and then lanes were plotted to obtain band width graphs. Then, this graph was connected to obtain the Region of Interest (ROI) section, and the "magic wand tool" menu was selected to quantify the width of the plasmid band. To obtain the intensity value, the percentage of intensity needed to be obtained. This was done by using the "analyze gels" menu and selecting the "label peaks" option. Then, the percentage of intensity for each variation was divided by the control to produce the intensity value. Band intensity describes the optimality of the transformation using a specific temperature and time. A higher band width and intensity will indicate a higher level of optimality.

RESULTS

The experiment utilized the blue and white selection procedure to assess colony appearance. In the control group, white colonies indicated successful DNA insertion, while the treatment group showed blue colonies without DNA inserts. Figure 1 visually presents the distinct colony differentiation between the control and treatment groups. Table 1 quantitatively displays the number of blue colonies, with the treatment group at 42°C for 60 seconds showing the highest count and the group at 42°C for 90 seconds having the lowest count. Thus, it can be concluded that the 42°C for 60 seconds treatment group exhibited the most optimal colony growth.

The isolates obtained after plasmid isolation was then observed for migration using 0.4 % agarose gel electrophoresis. The corresponding results are presented in Figure 2. The treatment group that contained plasmids without DNA inserts migrated faster than the controlled contained DNA inserts. The results of plasmid migration are shown in Figure 2.

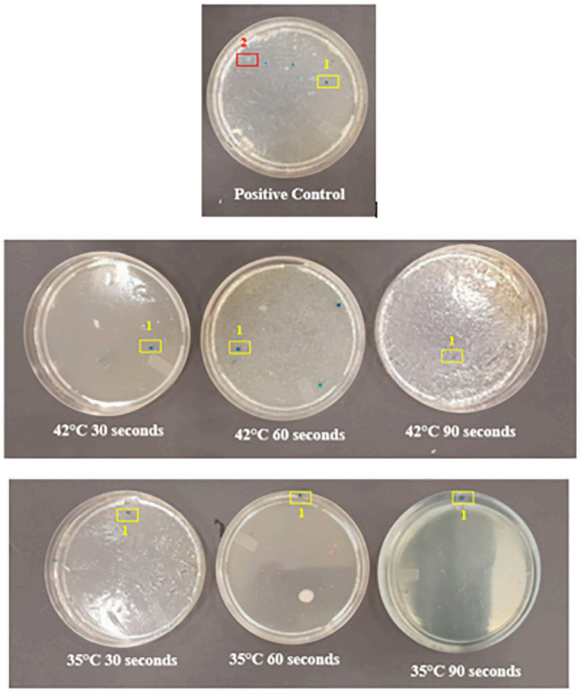

Figure 1. Blue and White Selection Results (1) Blue Colonies, (2) White Colonies.

The control group band was above the treatment group because the migration speed was slower than the treatment group band. This is due to the presence of a 542 bp DNA insert which increased the molecular weight of the plasmid. The migration bands formed were read using electrophoresis and UV transilluminators and then analyzed using ImageJ software. The data obtained from the analysis using ImageJ are in the form of band area and percent band intensity. The data was analyzed using ImageJ, as presented in Table 2.

Table 1. Number of Colonies of Treatment Group

No.	Treatment Group	Total White Colonies	Total Blue Colonies
1	Control	9	5
2	42°C 30 seconds	-	4
3	42°C 60 seconds	-	12
4	42°C 90 seconds	-	2
5	35°C 30 seconds	-	4
6	35°C 60 seconds	-	1
7	35°C 90 seconds	-	1

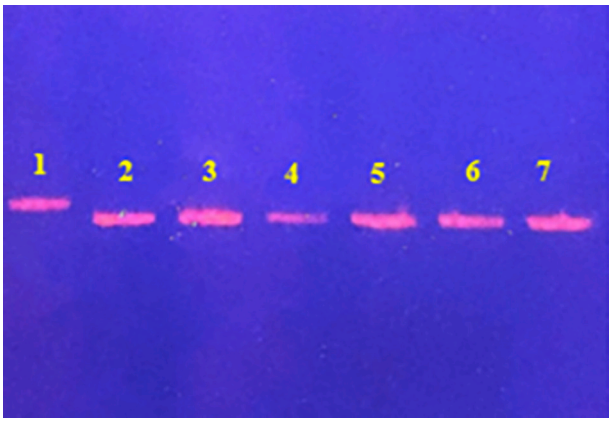

Figure 2. Electrophoresis results with UV transilluminator readings (1) Positive control, (2) Temperature 42°C 30 seconds, (3) Temperature 42°C 60 seconds (4) Temperature 42°C 90 seconds, (5) Temperature 35 °C 30 seconds, (6) Temperature 35°C 60 seconds, (7) Temperature 35°C 90 seconds.

The band's intensity was calculated by comparing the treatment group's intensity with the control groups. Descriptive statistical tests were conducted to determine the largest band width and band intensity. Table 3 presents the results,

Table 2. Results of Agarose Gel Analysis with ImageJ

No	Treatment group	Band Width (pixel)	% Intensity	Intensity
1	Control	20182.388	9.969	1
2	Temperature 42°C 30 seconds	32090.338	15.852	1.590129401
3	Temperature 42°C 60 seconds	44874.338	22.166	2.223492828
4	Temperature 42°C 90 seconds	12158.066	6.006	0.60246765
5	Temperature 35°C 30 seconds	36370.995	17.966	1.802186779
6	Temperature 35°C 60 seconds	26894.409	13.285	1.332631157
7	Temperature 35°C 90 seconds	26894.409	14.475	1.480188585

showing that the treatment group at 42°C for 60 seconds had the largest band width and highest intensity. The maximum band width recorded was 44874.3380 pixels, while the highest band intensity was 2.2235. In contrast, the treatment group at 42°C for 90 seconds had the smallest band width of 12158.0660 pixels and the lowest band intensity of 0.6025.

Table 3. Descriptive Test Results

	Total Treatment	Minimum	Maximum	Mean
Band Width	6	12158.0660	44874.3380	29880.425833
Band Intensity	6	0.6025	2.2235	1.505183

DISCUSSION

Blue-white screening offers a convenient and potent method for distinguishing bacterial colonies or phage plaques with a cloning vector carrying inserted DNA from those harboring empty vectors devoid of insert DNA. The approach relies on developing a blue pigment when beta-galactosidase facilitates the breakdown of the synthetic substrate X-gal through hydrolysis. Hydrolysis of X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) produces galactose and 5-bromo-4-chloro-3-hydroxyindole. *E. coli* colonies lacking DNA inserts will exhibit a blue colour due to the expression of beta-galactosidase. In contrast, colonies carrying DNA inserts will appear white as they lack the production of active enzymes (16). In the study, the control group yielded both blue and white colonies, indicating the presence or absence of DNA inserts. On the other hand, the treatment group produced solely blue colonies, signifying the absence of DNA inserts. The readings obtained using the UV transilluminator revealed bands in all treatment groups, which exhibited faster migration than the control group. Plasmids in the treatment group moved faster because the molecular weight was lighter without a DNA insert. Meanwhile, the positive control had slower migration due to the increased molecular size in the presence of a 542 bp DNA insert. This is because large DNA fragments migrate slower than smaller DNA fragments (17-19).

The obtained readings were analyzed using ImageJ to determine the largest band area and highest band intensity. Following the analysis, the data was acquired regarding band area and percentage of band intensity (20). The band intensity value was obtained by dividing the percentage of band intensity in the treatment group by the percent intensity of the control band. After calculating the intensity, the band intensity data were obtained for the control and each treatment group. Band width and band intensity indicates the DNA quantity, or in this case, the plasmid content within the isolate (21). Descriptive tests were applied to the band area and intensity to identify the largest value, indicating a higher number of plasmids and representing the most optimal treatment group.

The key to successful transformation lies in using chemically treated competent cells, such as $CaCl_2$ treated cells, which enhance the permeability of the *E. coli* cell membrane and facilitate the entry of plasmids into the cells. Previous studies have demonstrated that competent cells of *E. coli* strains BL21 (BE3), JM109, TG1, and HB101 exhibited optimal transformation efficiency when subjected to $CaCl_2$ treatment (22,23). In addition, recombinant plasmid DNA is needed to be transformed into cells. Plasmids usually encode antibiotic-resistant genes to differentiate them from non-recombinant bacterial cells so that *E. coli* that already contains recombinant plasmids will be resistant to antibiotics (24,25). This study used competent cells that had been added with $CaCl_2$ and was the most optimal treatment for competent cells (24). As for the recombinant plasmid, this study used the pGEM-T plasmid, which is resistant to the

antibiotic ampicillin and is observable from the blue and white selection results.

In the heat shock process involving temperature changes, *E. coli* cells responded to heat shock by inducing heat-shock protein (HSP) (26). This protein allowed cells to survive under stress conditions due to temperature (27). Heat shock protein acted as a chaperone which helped fold proteins in cells so that cells were not damaged when exposed to heat. The factor σ32 (sigma-32) controlled this heat shock response, encoded by the rpoH gene. Regulon σ32 could be classified as molecular chaperones and ATP-dependent proteases. Molecular chaperones, including DnaK-DnaJ-GrpE and GroEL-GroES, facilitated better folding of newly synthesized polypeptides and helped repair protein damage due to temperature (28). Heat shock proteases such as CIpP, Lon, and HfIB degraded misfolded proteins, which chaperones could not help (29). HSP chaperones and proteases played a role in protecting cells and protein folding under conditions of increased temperature (30).

DnaK-DnaJ-GrpE and GroEL-GroES were chaperones with the best characteristics found in *E. coli*. Other chaperones were Clp ATPases (ClpB, ClpX, and ClpY), Hsp90 HtpG homologs, and small Hsps (sHsps), IbpA, and IbpB (31). DnaK and GrpE were essential for the growth of *E. coli* at a temperature of around 43°C (32). GrpE allowed *E. coli* to grow at a maximum temperature of 43.5 °C (33). Meanwhile, ClpB was given a temperature of 42°C then grown at a temperature of 30°C, showing the results of growing at a maximum high temperature of 50°C. So, ClpB protected *E. coli* cells from lethal effects due to very high temperatures (31). ClpA could maintain cells up to 46°C, while the chaperone GroES30 could maintain cellular integrity and cell viability at 42-46°C. However, this chaperone could not maintain cell viability at 50°C, causing cell death (34).

This study employed heat shock treatments at 42°C and 35°C to determine the optimal temperature. The assessment was based on parameters such as colony count, band area, and intensity, with the optimal temperature identified as 42°C. At this temperature, the growth of *E. coli* was found to be favorable, primarily due to specific heat shock proteins, including DnaK, GrpE, ClpB, ClpA, and chaperone GroES30. Moreover, the presence of sHsps (small heat shock proteins) and Hsp90s (heat shock protein 90s) played a vital role in maintaining partially folded proteins in a conformation that can be reactivated through their interaction with Hsp70s (35).

It is essential to consider the temperature during the transformation process, as excessively high temperatures can result in cell death (4). At 42°C, forming larger and more membrane pores facilitates the entry of plasmids into bacterial cells. Heat shock induces the release of lipids from the outer membrane and reduces the potential of the inner membrane, allowing plasmids to enter *E. coli* bacteria (12). Moreover, heat shock proteins such as DnaK, GrpE, ClpB, ClpA, and chaperone GroES30 effectively protect *E. coli* cells (35,36). In this study, the treatment group subjected to heat shock at 42°C for 60 seconds demonstrated the highest number of colonies, the largest band area measuring 44874.3380 pixels, and the highest band intensity of 2.2235. Therefore, the treatment group at 42°C represents the optimum temperature for plasmid transformation.

The duration of the transformation process can significantly impact its efficiency. During the heat shock stage, the bacterial cell membrane opens its pores to facilitate the entry of the plasmid. If the duration is too short, the plasmid may not fully enter the *E. coli* cell, resulting in lower efficiency. On the other hand, if the pore opening time is too long, it can lead to membrane damage and cell death (4). In this study, the treatment group subjected to heat shock at 42°C for 60 seconds exhibited the highest number of colonies, the largest band area measuring 44874.3380 pixels, and the highest band intensity of 2.2235. Therefore, the 60-second transformation treatment group proved optimal for allowing the plasmid to enter *E. coli* cells without compromising viability.

CONCLUSIONS

The results indicate that the optimum temperature in this study was 42°C, and the optimal time was 60 seconds. This conclusion was drawn based on the highest number of colonies observed, which amounted to 12 colonies, the largest band

width recorded, measuring 44874.3380 pixels, and the highest intensity observed, reaching 2.2235. Researchers and laboratory technicians should consider this optimal condition to improve plasmid transformation experiments' efficiency and success rate. Further studies can explore variations in other parameters or combinations to optimize the transformation process and validate these findings in different *E. coli* strains or plasmids.

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