It's Shocking!

A comparison between transformation rates of electroporation and heat shock

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Introduction

What is the most efficient way to transplant DNA into a bacterial cell? There are several methods to do bacterial transformations. Bacterial transformation is the placement of a designer gene in bacterial cells so that the bacteria replicate the desired gene. The two most utilized methods are heath shock and electroporation. Heat shock uses water temperature to open cells so that transformation can occur. Electroporation uses electricity to shock the cells open so that transformation can occur (Chang, Dower, Hanahan, et. all). A third method, the alkali method, is no longer generally used by the scientific community and was not a method we chose to employ in this experiment. While popular in the 80s, the Alkali method has mostly been discarded because of the harsh chemicals required for the process, the difficulty of the experiment, and the inefficiency of the results (Vogt). Investigating the efficiency of the two most common methods will hopefully determine the best method for laboratory use. Finding the best method will help labs cut costs and work as efficiently as possible.

Having more than one method raises many other questions. Which method is the most efficient for successful transformation? Which method is more reliable? Which method is more cost effective? Which method utilizes labor most effectively? Taking all of these questions into consideration leads back to the initial investigative question.

During any experiment where there will be an analysis of pros and cons, cost becomes a factor in the final decision. In this analysis, cost can be defined in several ways. The financial cost of heat shock is significantly low, as it can be done in water baths instead of a dry bath (which is still relatively inexpensive at only $300-$400). The financial cost of electroporation is significantly higher, as the only way to do it is with an electroporator, which costs around $1,800. There are other costs to consider that are not entirely financially based. What other financial costs are involved for other materials and procedures? Performing these different methods and analyzing the results will help to answer each of these questions and determine which technique is most efficient and should be used as a standard for laboratories.

To complete this experiment, we worked with several different key methods: competent cells, heat shock, electroporation, and serial dilutions. Competent cells are good, clean cells free of bacterial excrement and other contaminants, which are ideal for working with in many experimental situations because they make transformations more efficient. It's more efficient because only the best harvested cells are being used. Making competent cells helps make transformations more efficient because the only things utilized are the bacteria (Sambrook).

Serial dilutions were utilized because they act as an important verification of results. By diluting the original transformations, one would expect to see proportional growth on each diluted plates. If the dilutions are unsuccessful, it could point to a problem within the original transformation process.

Methods

To begin the experiment agar plates were made using LB nutrient agar, ampicillin (amp), and arabanose (ara). The nutrient agar is used to gel the plates and as a small food source, the ampicillin acts as an antibiotic, eliminating all other bacteria that may contaminate the plates due to human error, and the arabanose is used as a food source for the growing bacteria. LB plates were used to culture the E. Coli starter plate. LB/amp/ara plates were used for viewing transformations. E. Coli HB101 from BioRad was used for the entirety of the experiment. For the experiment, the pGLO plasmid (from BioRad) was used. The plasmid will fluoresce if transformations are successful.

Once E. Coli colonies were cultured, competent cells were made. To do this a suspended cell culture was made. Two flasks were filled with 50ml of LB-broth (each) and several colonies of E. Coli HB101 from the starter plate were added using a sterile inoculation loop. Finally, the flasks were placed on a rocker in an incubator at 37°C for 24 hours. Using such a large quantity ensured that there would be enough cells to work with throughout the entire experiment, and excess should any part

need to be repeated.

An electroporation curvette, distilled water, and 10 tubes *(labeled: 80ul E. Coli HB101 Comp. Cells)* were stored at 20°C overnight. Keeping the tubes on ice, each was filled with 80ul of the suspended cell culture (see Table 1). Once filled, they were transferred to the centrifuge and spun for 5 minutes at 6,000rpms**.** After pouring off all supernatant and re-suspending the cells in 2ml of ice-cold distilled water, the centrifuge process was repeated for the same time and speed. Centrifuging was repeated, all supernatant was poured off, and cells were then re-suspended in 1ml of ice-cold distilled water. After completion, all supernatant was poured off and the cells were re-suspended in 80ul of ice-cold 10% glycerol solution (see Table 2). Tubes were then placed at -20°C until ready for use. Next, set up was done for the heat shock, electroporation, and control procedures by labeling 3 tubes (*Heat 1:1, Electro 1:1, Control 1:1*). In each tube, 40ul of competent cells and 2ul of re-hydrated pGLO plasmid was added (see Table 3).

Tube	Content
1	80ul of suspended cell culture
2	80ul of suspended cell culture
3	80ul of suspended cell culture
4	80ul of suspended cell culture
5	80ul of suspended cell culture
6	80ul of suspended cell culture
7	80ul of suspended cell culture
8	80ul of suspended cell culture
9	80ul of suspended cell culture
10	80ul of suspended cell culture

Table 1

Centrifuge Step	rpms	Time (minutes)	Pour off supernatant	Add	Final Product
1	6,000	5	pour off supernatant	2ml ice-cold distilled water	2ml suspended cells
2	6,000	5	pour off supernatant	1ml ice-cold distilled water	1ml suspended cells
3	6,000	5	pour of supernatant	80ul ice-cold 10% glycerol solution	80ul suspended cells

Table 2

Heat 1:1	*Electro 1:1*	*Control 1:1*
40ul Competent cells + 2ul re-hydrated pGLO plasmid	40ul Competent cells + 2ul re-hydrated pGLO plasmid	40ul Competent cells + 2ul re-hydrated pGLO plasmid

Table 3

To perform the heat shock method, the *Heat* tube was placed in a dry bath at 42°C for 50 seconds, then removed and placed in an ice bath for 2 minutes. After the ice bath 250ul of LB-broth was immediately added and the tube was incubated at room temperature (37°C) for 10 minutes. After incubation, 30ul of the solution was pipetted onto an LB/amp/ara plate and spread evenly with an inoculation loop.

To perform the electroporation method, the contents of the electroporation tube (42ul) were placed into the chilled electroporation curvette, the curvette was placed in the electroporator, the machine was set to Ec2, and then run. After the cycle 40ul of LB-broth was immediately added and the tube was incubated at 37°C for 1 hour. After incubation, 30ul of the solution was pipetted onto an LB/amp/ara plate and spread evenly with an inoculation loop.

To perform the control, 40ul of LB-Broth was added to the original control tube and 30ul of the solution was pipetted onto an LB/amp/ara plate and spread evenly with an inoculation loop.

After each method, serial dilutions of 1:10 (3ul: 30ul), 1:100 (3ul: 300ul), and 1:1000 (1.5ul: 1,500ul) were performed in individual tubes and 30ul of each dilution for each method were plated on LB/amp/ara plates. At the conclusion of all plating, we placed the 12 plates in an incubator at 37°C for 24 hours (see Table 4).

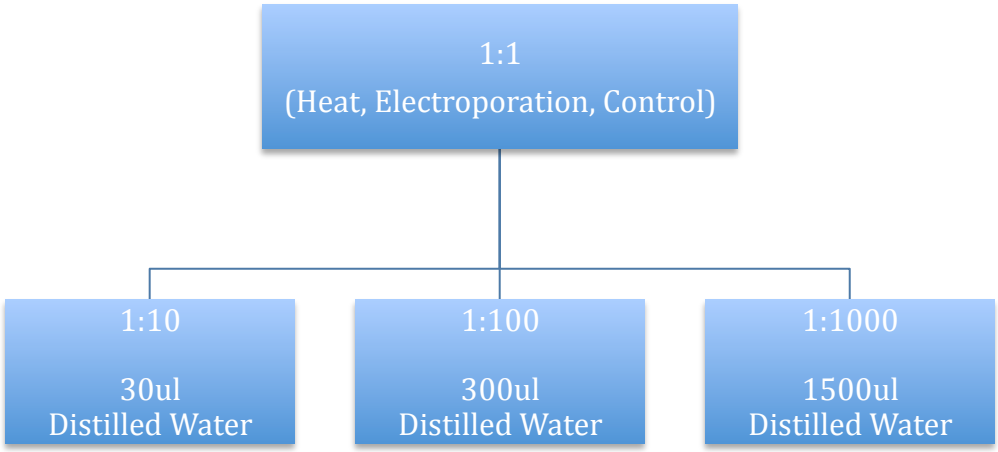

Table 4

Results

Each plate was analyzed and the following conclusions were drawn: heat plates *1:1, 1:10, 1:100,* and *1:1000* grew no colonies; electroporation plate *1:1* grew 37 colonies, plate *1:10* grew 3 colonies, plate *1:100* grew 1 colony, and plate *1:1000* grew no colonies; control plate *1:1* grew 1 colony, and plates *1:10, 1:100,* and *1:1000* grew no colonies (see Table 5).

Plates	Heat	Electroporation	Control
1:1	0	37	1
1:10	0	3	0
1:100	0	1	0
1:1000	0	0	0

*Each 0 represents no growth

Table 5

To analyze the results, we used a transformation efficiency calculator (CITATION). Use of the following information enabled transformation calculations: concentration of DNA (in ug/ul) was 0.08; volume of DNA added to transformation mix (in ul) was 2; volume of total transformation reaction (in ul) was 42; volume actually plated from solution was 30ul; number of colonies from plate varied with each method. This information gave the following efficiency calculations: heat efficiency is 0 transformants/ugDNA; electroporation efficiency is 323.75 transformants/ugDNA; control efficiency is 8.75 transformants/ugDNA (see Table 6).

Measurements	**Heat**	**Electroporation**	**Control**
Concentration of DNA (ug/ul)	0.08	0.08	0.08
Volume of DNA added to transformation mix (ul)	2	2	2
Volume of total transformation reaction (ul)	42	42	42
Volume actually plated from solution (ul)	30	30	30
Number of colonies from plate	0	37	1
Transformation Efficiency (Transfromants/ugDNA)	0	323.75	8.75

*Calculations do not work with dilutions

Table 6

The heat shock method was unsuccessful and yielded no results. This was extremely surprising as the method is very standard and has always yielded results in the past. In order to verify, the heat procedure was repeated twice more, each time with no results. After a third attempt in which cultured E. Coli was mixed with LB-broth, without creating competent cells, but maintaining the remainder of the previous heat shock procedure. Each plate was analyzed and the following conclusions were drawn: heat II plate *1:1* grew 11 colonies, plate *1:10* grew 2 colonies, and plates *1:100* and *1:1000* grew no colonies (see Table 7). Analyzing transformation efficiency using the same measurements as the previous methods gave an efficiency rating of 96.25 transformants/ugDNA (see Table 8).

Plates	**Heat**
1:1	0
1:10	0
1:100	0
1:1000	0

*Each 0 represents no growth

Table 7

Measurements	**Heat**
Concentration of DNA (ug/ul)	0.08
Volume of DNA added to transformation mix (ul)	2
Volume of total transformation reaction (ul)	42
Volume actually plated from solution (ul)	30
Number of colonies from plate	11
Transformation Efficiency (Transfromants/ugDNA)	96.25

*Calculations do not work with dilutions

Table 8

Discussion

Despite the success of the altered heat procedure, the calculations still suggest that electroporation is the most mathematically efficient method to use. The final results were interesting to say the least. The heat shock methods has been performed many times in the past with measurable success, so why didn't it work this time? The conclusion that was reached was that the key to this mystery lies in the competent cell procedure. The heat shock protocol is designed to work best with dirty cells. At first thought it would stand to reason that if it works with dirty cells, then it would work with clean cells as well. However, after this experiment the belief is that this is not the case after all. A key component of making competent cells is the 10% glycerol solution, which we believe was the key negative effect on the original heat shock procedure. Taking this into consideration, we had to reevaluate the merits of the heat shock protocol under these circumstances. Competent cell production is a standard procedure in many labs. Making competent cells ensures that you are working with the best cells you can harvest. They will give you the best results and are, generally speaking, the easiest to work with. Because many labs use the competent cell method and the heat shock method is apparently ineffective when using competent cells, the heat shock method is by far the least effective.

Besides the unexpected results from the heat shock procedure, there was also a surprising occurrence during electroporation. After starting the cycle there was an electrical arc, something that is not supposed to happen during the cycle and can (and usually does) negatively affect the results. However, despite the arc, the electroporation plates grew very well. Because the procedure is so violent, the conclusion was reached that even when things don't go exactly planned, there is enough reaction to substantiate the process and the cost of the machine. Despite the procedure not going exactly as planned, there were still results, making it not only the most mathematically efficient method, but also the most reliable procedure for transformation. There was hesitancy at first to hypothesize that electroporation was the most cost effective, the high price of the machine weighing heavily in the considerations. However, after running the heat shock process three additional times after the original experiment, the overall costs of both run ever closer to each other. Even though the equipment for electroporation is more expensive, the amount of labor, time, and other resources saved is large and, in the long run, it is much more 'cost' and results efficient to run electroporation. This information is especially prevalent in large labs that repeat the process many times a day.

Works Cited

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