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High-Efficiency Transformation of Yeast by Electroporation

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Undergraduate Thesis

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Abstract

This paper analyzes and optimizes efficiency in the yeast transformation protocol using electroporation. Three variables were tested for optimization, they are the following: DNA concentration, cell density and electroporation voltage. Further verification of transformation was done through the analyzation of HPLC data to ensure protein production. The results conclude that DNA concentration was the most important dependent variable for transformation efficiency. A voltage of 2000V is the optimal condition to achieve efficient yeast transformation. Cell density had no observational difference in transformation efficiency at the range of DNA concentration necessary for research use. Improving this efficiency of the yeast transformations helps to stream-line the discovery phase of new product development.

Introduction

The process of transforming yeast is a major molecular biology tool. Yeast are single-celled organisms that grow through a process known as fermentation. They have a well-defined genetic system and are the simplest eukaryotic organism. The yeast genome is approximately three times larger than that of E. coli and it is far more controllable than the genomes of more complex eukaryotes like humans (Ito H, Fukuda Y, Murata K, Kimura A, 1983). The term transformation refers to a yeast cell acquiring a new phenotypic trait due to its uptake of exogenous/foreign DNA carrying the genes for the trait (Johnston et. al, 1988). Transformation of yeast is efficient, although not as efficient as in E. coli. After transformation, yeast transformants can be used for amplification of plasmid DNA (Avery OT, MacLeod CM, McCarty M, 1995) or to produce proteins and enzymes at research and manufacturing scale. At Vestaron, a biopesticide company, yeast transformations play a crucial role in the research and development side of their commercial product. It is critical for research that the genes are transformed into yeast so it can then be fermented for product discovery and production. Improving the efficiency of transformation helps to stream-line the discovery phase of new product development.

This transformation protocol takes advantage of the process called electroporation. Electroporation is a simple process that involves an electrical pulse at an optimized voltage discharged through the cell (Russell, C., Jarvis, A., Yu, P. et al., 1993). This discharge lasts only a few milliseconds. It is important for forming temporary pores across the phospholipid bilayer of the membrane of the cell. In addition, it helps increase the membrane potential across the cell

membrane. These two conditions allow the charged molecules in DNA to be driven across the membrane through the formed pores (Shigekawa and Dower, 1988). This process is fast and easy which allows for large number of cells to be transfected.

Literature Review

Although the use of electroporation is desirable for transformations, there are conditions that must be optimized before the process of electroporation can be successful in yeast. It has been indicated in the past that the electroporation conditions differ for each yeast strain (Russell, C., Jarvis, A., Yu, P. et al., 1993). Most of the studies found in literature on optimizing electroporation yeast transformations were published over 20 years ago. The first studies completed on electroporating yeast, used a voltage range of 500-1000V on several different strains of yeast (Bolen, P.L., McCutchan, J.E., 1992). In comparison to that study the current protocol used at Vestaron, lacks data for testing higher voltages such as 2000-2500V. Another variable tested in the literature was the capacitance and resistance to change the pulse length. The pulse length represents the time required to dissipate the voltage to 37% of the peak (Son, Reuben S., et al., 2020). A study conducted by Russel Brown concluded that the capacitance was directly related to the pulse length; the higher the capacitance the longer the pulse length (Bolen, P.L., McCutchan, J.E., 1992). This longer pulse length showed higher transformation efficiency amongst the several different strains of yeast (Gášková, D., Sigler, K., Janderová, B., & Plášek, 1996). In another study, they concluded that voltage was the most important variable in their optimization of the electroporation procedure on yeast (Russell, C., Jarvis, A., Yu, P. et al., 1993). The Eppendorf electroporator, model 2510, currently being used at Vestaron does not

have the capability of changing the capacitance or resistance; only the voltage can be varied and therefore it is part of the optimization of the protocol.

Study Goal and Objectives

The study goal of this honors thesis project was to optimize efficiency in the yeast transformation protocol using electroporation. Efficiency, for this study, is defined as obtaining an optimal number of colonies per plate best suited for research use at Vestaron. This achieves a range in a minimum numbers of colonies on each plate up to as many where there is separation between them; the goal is not to obtain a maximum colony count. To achieve this, objectives were identified to test variables. This involved defining the variables to be tested and developing the tools to measure efficiency. Data was collected to determine the best conditions of the protocol.

Methods

A scientist from Vestaron, Lin Bao, developed the current yeast transformation protocol (Bao L, 2020). **Figure 1** represents the five main steps of this yeast transformation protocol. Step One was the linearization of the plasmid DNA that contains the gene of interest and Step Two incubates that gene with competent cells. During Step Three, an electrical pulse is discharged through the cells increasing the membrane potential. This allowed the charged molecules in the DNA to be driven across the membrane through formed pores. These cells then recovered for three hours in a specialized media. After recovery, the cells were washed and plated, growing at

30℃ for three days. Utilizing Lin's five step protocol, transformation efficiency was measured after the three-day growth period.

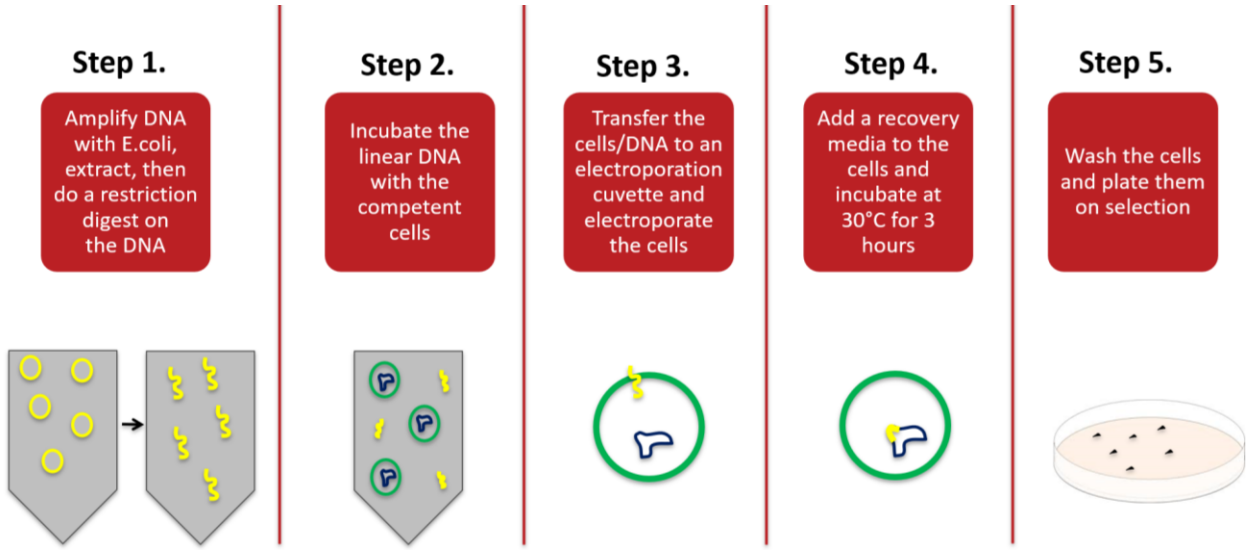

Figure 1. Visual representation of the five main steps in a yeast transformation using electroporation. The yellow circles represent the circular DNA from E.coli. The yellow twists represent the linearized DNA of E.coli after the restriction digest. The green circles represent the competent yeast cells and the blue in the middle represents the yeast genomic DNA. In step one the DNA gets amplified with E.coli, extracted and then a restriction digest is done on the DNA in order to make the DNA linear. Step two, the linear DNA gets incubated with the competent cells. In step three the mixture of cells and DNA are transferred to an electroporation cuvette and electroporated at a set voltage (2000V in current protocol). Step four, after the cells are electroporated they are then given a recovery media and are left to incubate at 30℃ for three hours. In the last step, step five, the cells are washed and plated on a selection plate. The cells grow at 30℃ for three days and are counted to collect data.

Figure 2 shows the variability between the number of colonies on each plate. Plate B shows a plate that has too many colonies to get an exact count for the entire plate. Plate A has a total 276 colonies on the entire plate with good separation between each plate. Due to this variability in colony count, the $1cm^2$ selection apparatus was created as a plate representative for achievable colony count (see **Figure 3**). In **Figure 2**, there are small and large colonies due to the nature of the selection marker, acetamide, indicative of more gene insertions (Haase A, personal communication, June 2020). If the cells were not transformed, they would not grow into

a large colony. These colonies were further verified for transformation by looking for protein production via high performance liquid chromatography (HPLC).

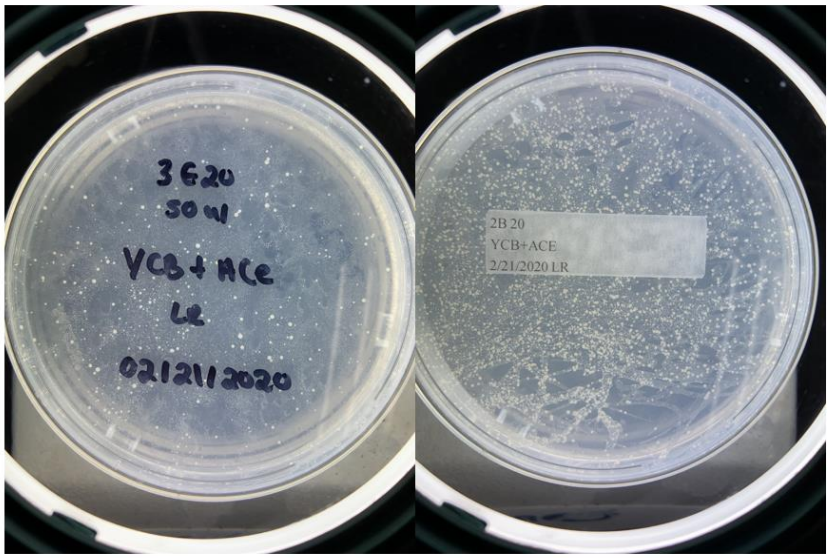

Figure 2: This figure represents the variability between transformed colony count on the YCB acetamide plates. Plate A has a total number of 276 colonies on the plate with good separation between each colony. Plat B has too many colonies to count and lacks separation between each colony.

Transformation efficiency is measured and referred to by the unit colony count per cm^2 per plate. The higher the colony count/cm^2, the higher the number of transformed cells. To measure transformation efficiency, a subset of colonies were counted in three different, small squares randomly distributed on each plate. **Figure 3** shows a representation of the apparatus used when measuring the colony counts. Colony counts were averaged to determine the colonies/cm^2/plate. The ideal number for optimal efficiency in this study is between 10-16 colonies/cm^2/plate.

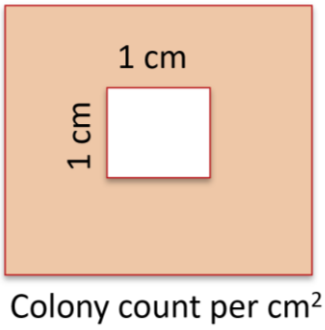

Figure 3: Shows a representation of the square cut out used to count colonies on the plate. Three squares were randomly distributed throughout each plate and the number of colonies were counted within each squared.

To determine efficiency of Lin's protocol, a multi-level framework study was designed with three variables of interest. They are the following: DNA concentration, cell density and electroporation voltage. For DNA concentration, it was hypothesized that the more DNA you have the more efficient the transformation will be. For cell density, it was hypothesized that the higher the cell density, the higher the number of transformed cells. For the third variable, it was hypothesized that 2000V would be optimal resulting in a high transformation efficiency (Bao L, 2020).

To test all three variables there were two strategies that were created. Strategy one tested the effects of DNA concentration and cell density on transformation efficiency. A matrix was designed to test both variables holding the voltage constant at the current standard, 2000V (Bao L ,2020). **Table 1** shows the matrix of conditions tested in strategy one. Five DNA concentrations were chosen based on the input received from the scientists at Vestaron (A. Haase, K. Schneider, L. Bao, personal communication, Feb. 2020). The DNA concentrations were: 30µg/ml, 60µg/ml, 100µg/ml, 300µg/ml, 600µg/ml. The standard cell density used was

$3x10^9$ cells/ml. That concentration was doubled and halved to make a total of three cell densities to test. These conditions were transformed, and colonies were counted after three days of plating.

1st Strategy: The effects of DNA concentration and cell density on transformation efficiency			
DNA Concentration (µg/ml)	Cell Density (Cells/mL)		
	$6x10^9$	$3x10^9$	$1.5x10^9$
30	30 , $6x10^9$	30 , $3x10^9$	30 , $1.5x10^9$
60	60 , $6x10^9$	60 , $3x10^9$	60 , $1.5x10^9$
100	100 , $6x10^9$	100 , $3x10^9$	100 , $1.5x10^9$
300	300 , $6x10^9$	300 , $3x10^9$	300 , $1.5x10^9$
600	600 , $6x10^9$	600 , $3x10^9$	600 , $1.5x10^9$

Table 1 : Represents the matrix used in strategy one to test the effects of DNA concentration and cell density on transformation efficiency. There were five DNA concentrations (µg/ml) tested with three cell densities (cells/ml). These were all electroporated at 2000V.

Based on results from strategy one a range of data was selected for the second strategy, a matrix of DNA concentration and voltage. Four different voltages were chosen to be tested: 1000V, 1500V, 2000V, 2500V. The electroporation machine maxed out at 2500V, so a voltage higher than that could not be tested. Another matrix was created to test these variables at the same time to optimize transformation efficiency.

2nd Strategy: The Effects of DNA concentration and Voltage			
Voltage (V)	DNA Concentration (µg/ml)		
	30	100	300
1000	30 , 1000 V	100, 1000 V	300, 1000 V
1500	30 , 1500 V	100, 1500 V	300, 1500 V
2000	30 , 2000 V	100, 2000 V	300, 2000 V
2500	30 , 2500 V	100, 2500 V	300, 2500 V

Table 2: Represents the matrix used in strategy two to test the effects of DNA concentration and voltage on transformation efficiency. There were three DNA concentrations (µg/ml) tested with four voltages (V). A cell density of $3x10^9$ (cells/ml) was used for all of these transformations.

Data and Results

The transformation efficiency was determined for all three variables at various conditions by counting the numbers of colonies/cm^2 (see **Figure 2**). The exact colony count per plate was not determined.

The first strategy, Error! Reference source not found., comparing DNA concentration showed a direct correlation, as the DNA concentration increased, so did the colonies/cm^2. There was no observed difference in the cell density on colony count, except the highest DNA concentration, 600 µg/ml. Although there were more colonies, it was not optimal because at the DNA concentration of 600 µg/ml there was a lack of separation between colonies making it difficult to pick colonies for further culturing. Separation of colonies is necessary for further down-stream screening. A DNA range of 100-300 µg/ml had optimal number of colonies on the

plate and separation between colonies for research use. A DNA concentration of 30-60 µg/ml still allows for transformation. However, it fell below the optimal colonies/cm^2 range.

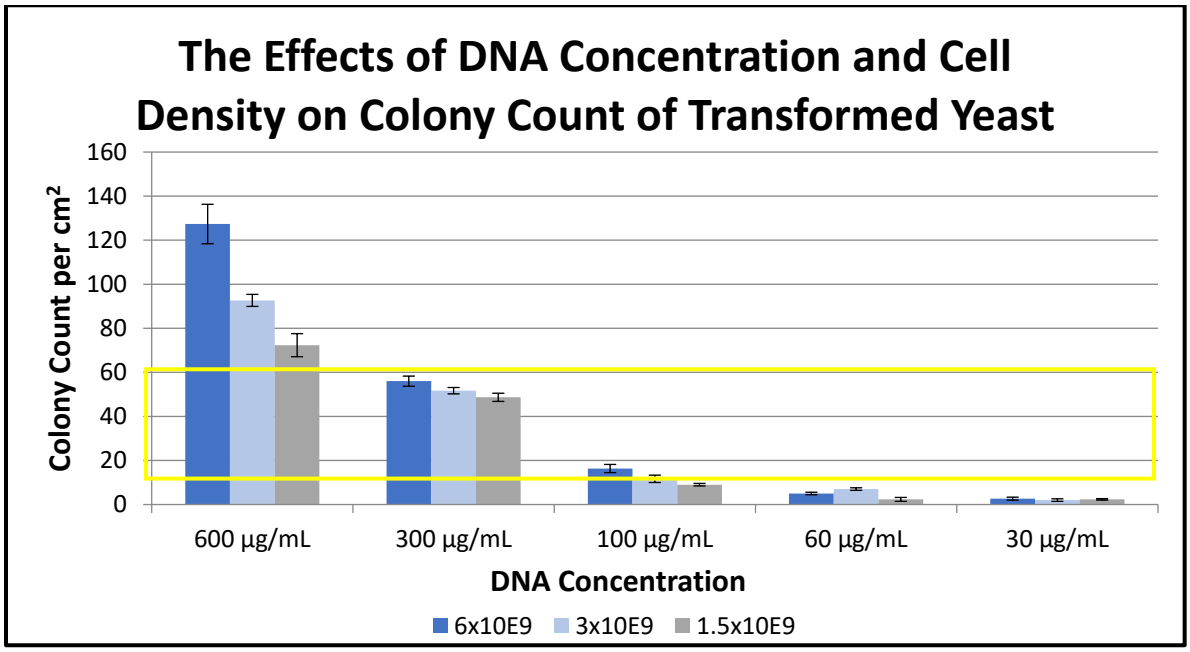

Figure 4:Represents the colony count/cm2 averaged from three different plates for each DNA concentration and cell density. As the DNA concentration increases the colony count/cm2 increases. There is no observed difference between each of the cell densities except at a concentration of 600 µg/ml. The error bars refer to the standard error. The box outlines the optimal range of colonies/cm².

There were observational differences between the varying voltages (see **Figure 5**) A cell density of 3x10^9 cells per mL was used for each of these samples due to no observed difference in the cell density on colony count (see **Figure 4**). There was no growth seen at 2500 V indicating that it may be detrimental to the yeast cell to go above 2000 V using the current apparatus. 2000V had the highest transformation efficiency and fell within the optimal

colonies/cm^2 at the two higher DNA concentrations. If the highest DNA concentration is available, 1500 V would work as well, but 1000 V is too low.

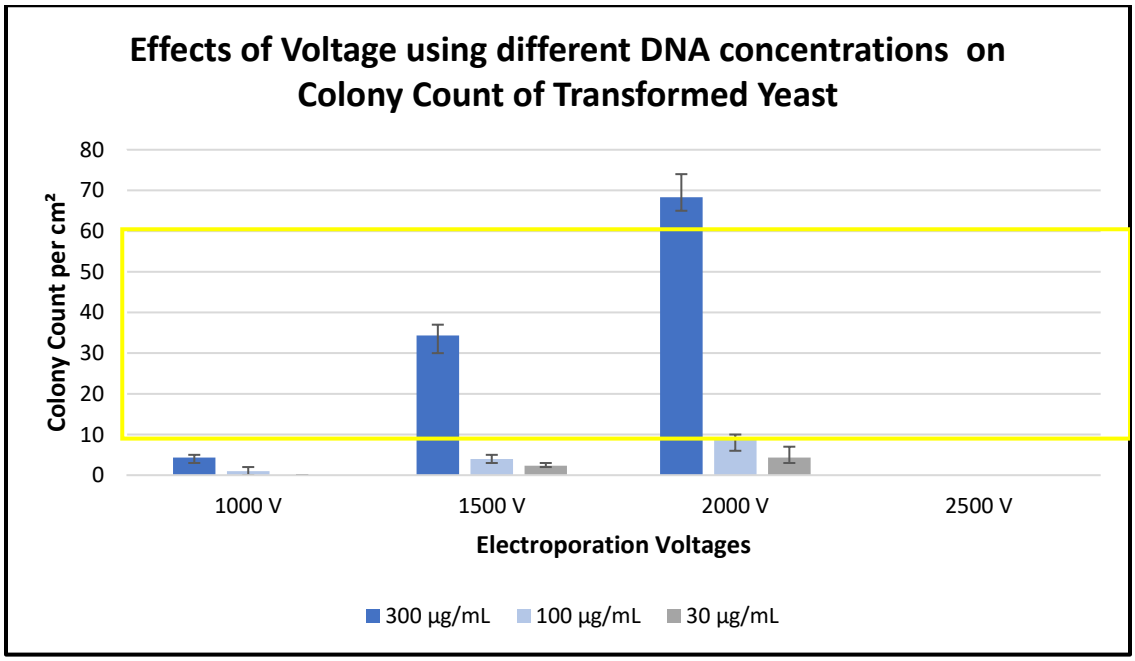

Figure 5: Represents the colony count per cm2 averaged from three different plates for each voltage. A competent cell density of 3x109 was used for each of these transformations. The error bars refer to the standard error. The yellow box outlines the optimal range of colonies/cm^2.

Colonies were selected from the plates in strategy 2 and cultured for protein production to verify transformation. The samples were verified by HPLC at both 1500V and 2000V at DNA concentrations of 100 and 300 µg/ml (see **Figure 6**).

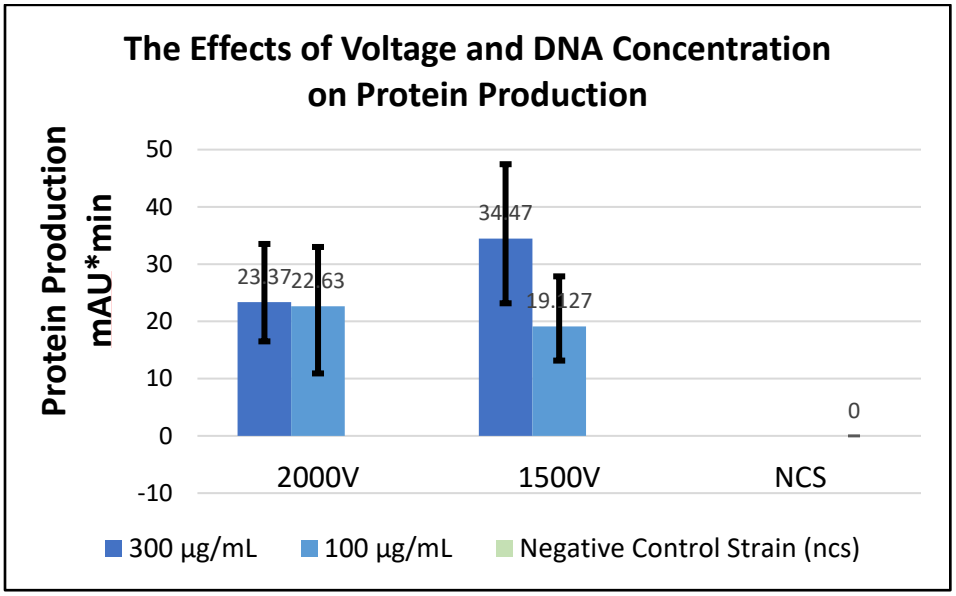

Figure 6: Represents the protein production for cells transformed at 1500V or 2000V and 100 or 300µg/ml DNA as a further verification of successful transformation. Negative control strain (NCS) is used to compare the protein production. Colonies were selected from the second strategy and cultured under Vestaron's standard protocol for each of these transformations. Each bar represents the average of four repetitions. The error bars refer to the standard error.

Conclusion

An efficient yeast transformation protocol using electroporation has been developed and verified. The variables to be tested were defined and the measurement tool to define transformation efficiency was developed. The data was collected and verified that the current protocol voltage of 2000V is the optimal condition to achieve efficient yeast transformation when a concentration of 100-300 µg/ml of DNA is used. A voltage higher or lower than 2000V negatively affects cell viability and lower than 1500 V does not yield enough positive transformants. DNA concentration was the most important dependent variable for transformation

efficiency. A range of 100-300 µg/ml is optimal in producing colonies for research use atboth 1500V and 2000V. Cell density with a range between $1.5x10^9$ and $6x10^9$ cells/mL showed no difference on transformation efficiency within the recommended DNA concentration range. Protein production was verified at both 1500V and 2000V at DNA concentrations of 100 and 300 µg/ml indicating successful transformation of the cells in addition to the growth on selection media.

Discussion and Suggestions for Further Research

There were three main limitations that were encountered during this project. One of the limitations was that the exact colony count on the plates was not obtained, meaning that there could be some variation of colony count. Another limitation is that only a narrow window of competent cell densities was tested, and it could be that more or less would be better. There was also a limitation with the electroporator instrument because it did not allow for changes in the conductance and capacitance, which could potentially further increase transformation efficiency (Shigekawa K, Dower WJ, 1988).

Futurer research plans are to adapt these methods to a high-throughput the yeast transformation protocol in a 96-well plate format. This project would use a different electroporator that can further optimize transformation efficiency allowing for more transformations at once.

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