Temperature Manipulation of Small-Scale Growth Cultures for Increased pDNA Yield

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Introduction

Optimizing biological equipment is often a complex and repetitive process. Much of the relevant research focuses on the optimization of larger bioreactors which often require significant product volumes and tend to be cost-inefficient. This research project aims to address this challenge by scaling down to utilize small-scale (20 mL) bioreactors – Pioreactors©. Using this smaller scale enables more efficient optimization practices, both in terms of time and cost.[3] These Pioreactors© share many common capabilities of a typical bioreactor set up. Similarities include temperature monitoring, agitation, media waste management, and optical density measurements.[1]

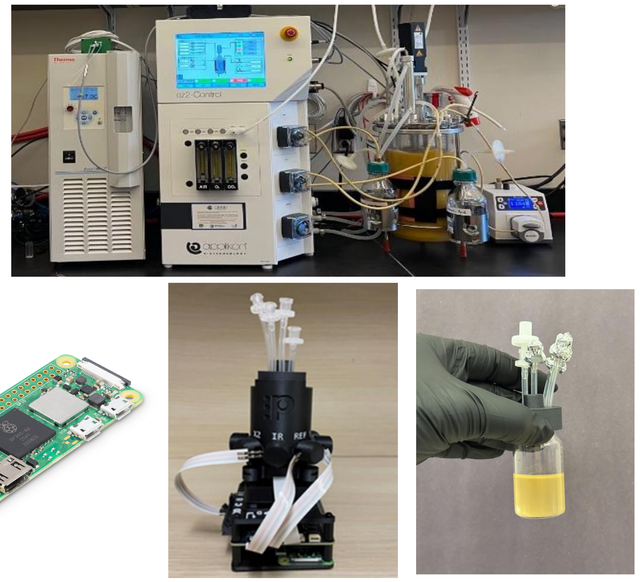

Figure 1. *Size comparison of 7 L bioreactor to 20 mL Pioreactor©*

Pioreactors are small-scale bioreactors with functionality adjacent to industrial size bioreactors. They have small computer systems known as Raspberry Pi and a reaction vessel that is 20 mL in size. To establish a baseline, a common plasmid of EcoRI-RTX was utilized as the control. EcoRI is a type II restriction enzyme (RE) known for its application in recombinant DNA Technology.[2] This category of RE is highly commercialized and easily accessible. For experimentation, the use of pUCP20t-pEYFP, a temperature-adjustable plasmid, is the focus. pUC plasmids have a unique characteristic during growth that increase pDNA production based on the temperature it is grown.[4] The goal of this project will be to exploit this property of the pUC plasmid to further utilize in the refinement of our inoculation and growth protocol for our 7 L bioreactor.

Experimental Design

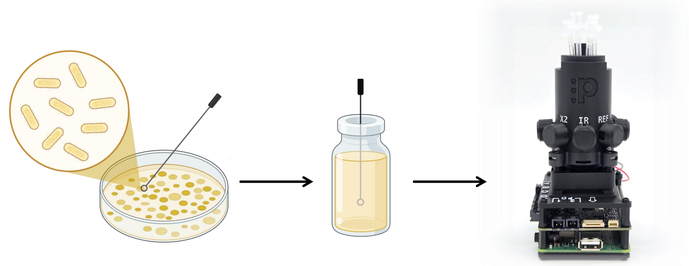

Figure 3. *Inoculation of Pioreactor© vial with transformed bacteria*

Pioreactor cultures are run at a set temperature and RPM. A bacterial transformation utilizing a select plasmid is conducted with E. coli DH5α competent cells. Sterilized Luria Broth (LB) media is inoculated with 1000x ampicillin, and a single colony of the transformed bacteria is then transferred into the Pioreactor© where growth conditions are determined and executed. After growth, cells undergo a MiniPrep procedure to purify DNA and determine dsDNA concentration.

Current Work

Overnight culture results with EcoRI-RTX/DH5α

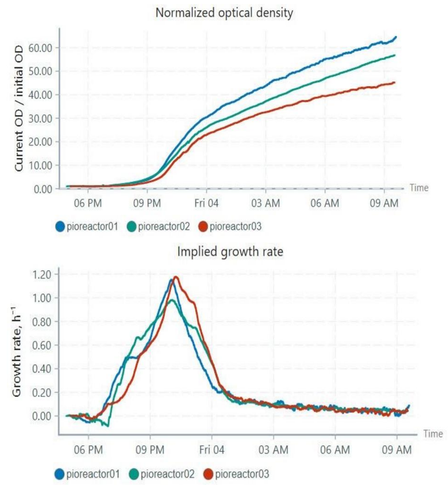

Figure 4: *nOD and implied growth rate results of 16 h overnight culture run at 37°C for EcoRI-RTX/DH5α*

Sample	Ng/uL	A260/A280	A260/A230
1	88.0	1.96	2.24
2	68.7	1.93	2.15
3	82.3	1.95	2.21

Table 1: *dsDNA concentration and purity of EcoRI-RTX/DH5α*

Overnight culture results with pUC20t-EYFP/DH5α

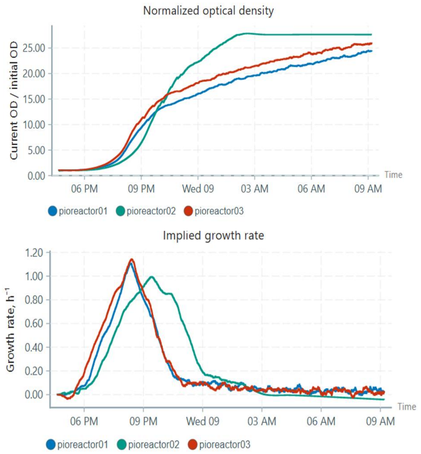

Figure 5: *nOD and implied growth rate results of 16 h overnight culture run at 37°C for pUC20t-EYFP/DH5α*

Sample	Ng/uL	A260/A280	A260/A230
1	207.7	1.91	2.29
2	100.4	1.95	2.17
3	224.2	1.92	2.30

Table 2: *dsDNA concentration and purity of pUC20t-EYFP/DH5α*

Acknowledgments

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OD 600 Readings

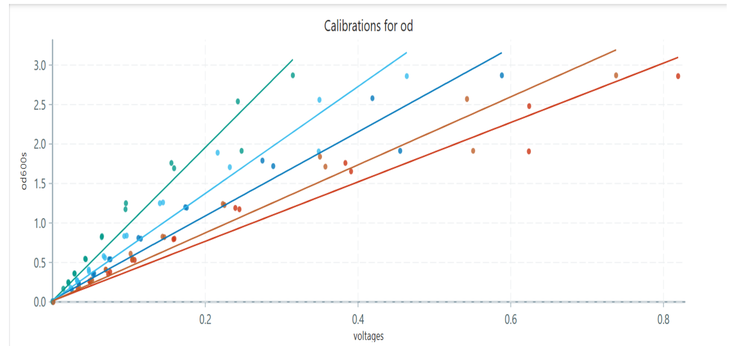

Figure 6: *OD600 calibration graphs for Pioreactor© 1-3, 5-6*

OD600 measurements explain the turbidity of a solution which is then used for estimation of concentration of cells within a liquid sample. A calibration map is utilized within each Pioreactor© run to act as a foundation for each reading that is administered.

Troubleshooting

Relatively new biological equipment comes with endless trials of determining correct protocols and experimentation. The Pioreactors© have had lots of issues in their startup process including calibration errors, root-coding issues, heating and stirring stopping in the middle of a 16 hour cultures, etc. Conversations with the CEO of Pioreactor© and participation on their forum has been beneficial in this process.

Future Work

One goal of this work is to capitalize on the unique properties of pUC plasmids to optimize dsDNA yield for use in our 7 L benchtop bioreactor. To achieve this goal, a temperature automation protocol will be written in Python to be utilized in the Pioreactor© setup. The automation will be based off the exponential growth phase of the pUC plasmid, beginning at a temperature of 30 °C. Once growth begins to increase exponentially – indicated via a target OD reading – the Pioreactor will increase heating to 42 °C.

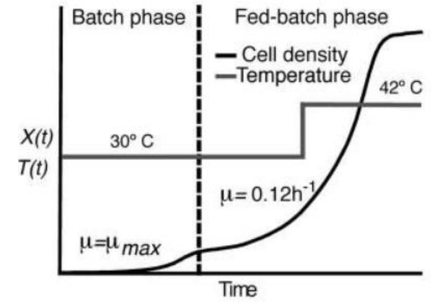

Figure 7: *Temperature change from 30 °C to 42 °C during exponential growth phase of pUC plasmid* [4]

During growth of pUC containing cells at 30 °C, the cell mass is increasing with reduced focus on pDNA production. During exponential growth, the temperature is increased to 42 °C shifting the growth focus to pDNA production. This is due to a mutation in the RNA II promoter in pUC plasmids that permits this characteristic. This protocol has not been administered in the 7 L bioreactor due to expensive test conditions on a scale of that magnitude. Pioreactor© use negates these costs due to its small scale, providing a useful approach to experimentation and refinement of procedure.

References

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(2) Loenen, W. A. M.; Dryden, D. T. F.; Raleigh, E. A.; Wilson, G. G.; Murray, N. E. 2014, 42, 3–19.
(3) Delavar, M. A.; Wang, J. 2022; pp 195–245.
(4) Nelson, J.; Rodriguez, S.; Finlayson, N.; Williams, J.; Carnes, A. 2013, 9 (10), 2211–2215.