Utilizing Plasmid DNA Hydrogels for Cell-Free Protein Production

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Introduction

Proteins, vital to numerous biological processes, are essential components in biomedical research and therapeutics. The development of cell-free protein synthesis (CFPS) has become a valuable approach for quickly producing proteins outside of living cells, involving both transcription and translation (**Fig. 2**) processes. However, traditional CFPS methods face challenges in low efficiency, batch-to-batch variability, and high costs during these transcription and translation steps.

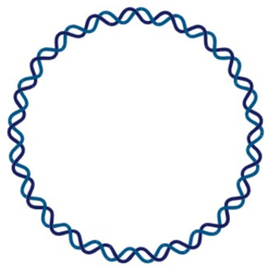

Figure 1. *Structure of relaxed pDNA*

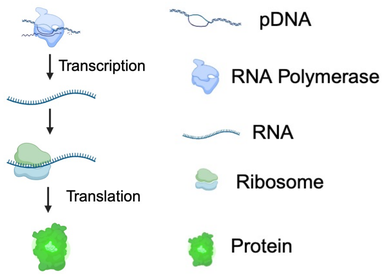

Figure 2. *Overview of the process of transcription and translation.*

These limitations are addressed by utilizing plasmid DNA (pDNA) hydrogels as substrates for CFPS. pDNA (Fig. 1) is used to produce proteins, and the reusability of pDNA hydrogels enhances the efficiency and sustainability of CFPS processes.

Methods

To obtain large amounts of pDNA, there's a need for a scalable and standardized method applicable across academic labs. Adapting the large-scale industrial protocols to cater to academic environments enables mass production of pDNA. Through this approach, we have achieved DNA samples with concentrations up to 116 mg/mL and have successfully purified plasmids.

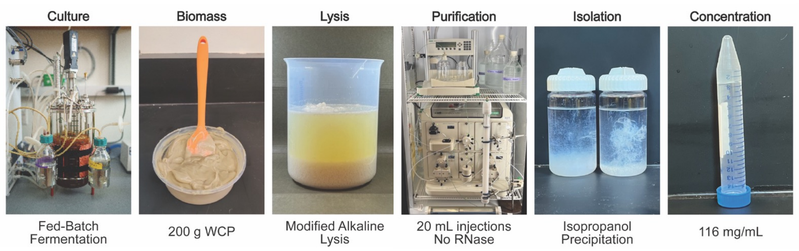

Figure 3. *Overview of bulk pDNA production and purification. Cultures are cultivated in a 7 L bioreactor through fed-batch fermentation. pDNA is purified through the process of alkaline lysis and anion exchange chromatography for higher concentrations.*

pDNA Production & Purification

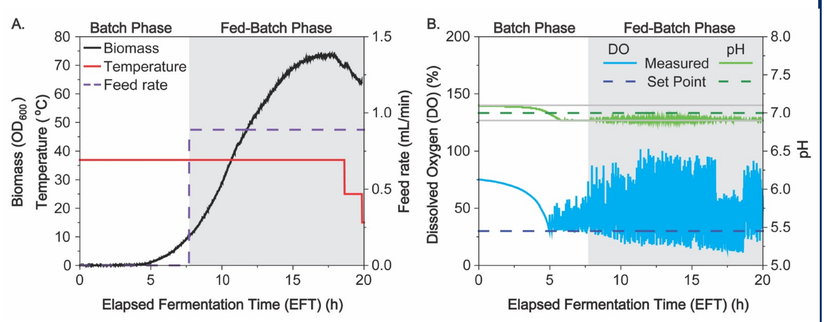

Figure 4. *A) Elapsed fermentation time depicting biomass, temperature and feed rate and B) Elapsed fermentation time of dissolved oxygen (DO) and pH monitored during fermentation.*

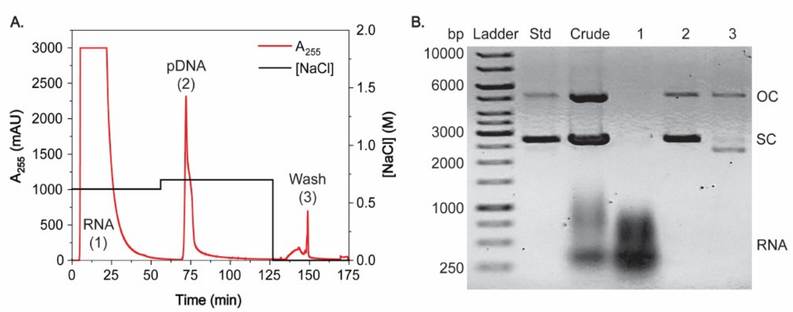

Figure 5. *A) Chromatogram illustrating purification of pDNA through anion exchange chromatography, indicating the elution points for RNA, pDNA, and washing phases and B) Analysis of purification through gel electrophoresis.*

Trial Run of CFPS

The process of CFPS involves the preparation of cell extract through cell lysis, causing the opening of the cells to remove unwanted debris and DNA. Nucleotides, RNA polymerase, and cofactors are added to the cell extract. pDNA for the desired protein is also included, and the target protein produced is purified from the cell-free system.

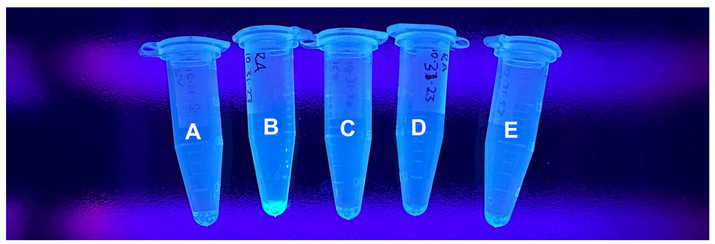

Figure 6. *CFPS of protein eYFP from pUCP20T-eYFP using myTXTL Sigma 70 Master Mix Kit. A) negative control, B) positive control, C-E) three pUCP20T-eYFP trials.*

Future Work

We will focus on purifying pUCBB-pT7-eGFP to optimize solution CFS with myTXTL T7 expression kit (**Fig. 7**). We will then begin trials on chemically cross-linked pDNA hydrogels (**Fig. 8**).

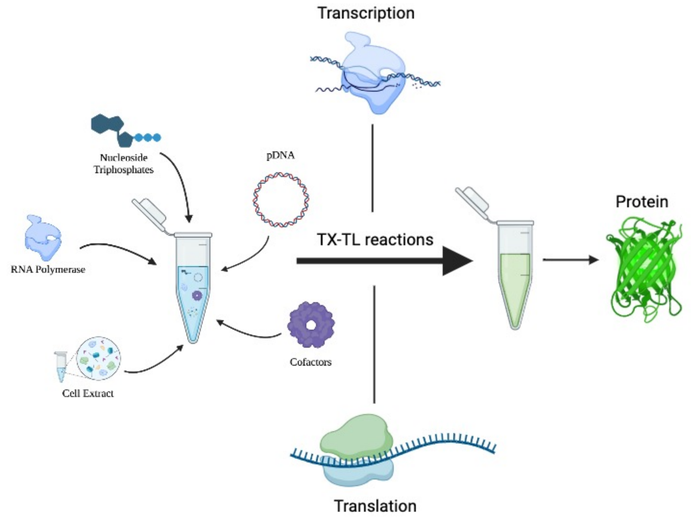

Figure 7. *Successful workflow of CFPS of eGFP from pUCP20T-eYFP using myTXTL Sigma 70 Master Mix Kit.*

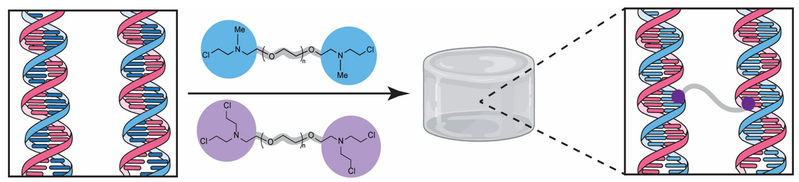

Figure 8. *Formation of chemically cross-linked pDNA hydrogels using bisfunctionalized PEG cross-linkers.*

References

Arbor Biosciences. *Arbor Biosciences* **2019**; 1, 1-31.
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Acknowledgments

We would like to thank the UNH Chemistry Department, Shaina Hughes, Lola Fadairo, Tran Truong, Patrick Strobel, Matt Currier, Nick Pierini, Samuel Ashooh, Carmelina Minico, and Aylin Aykanat.

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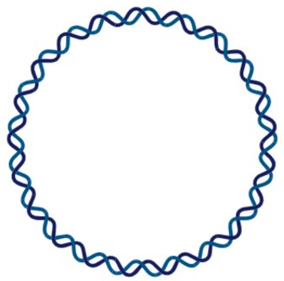

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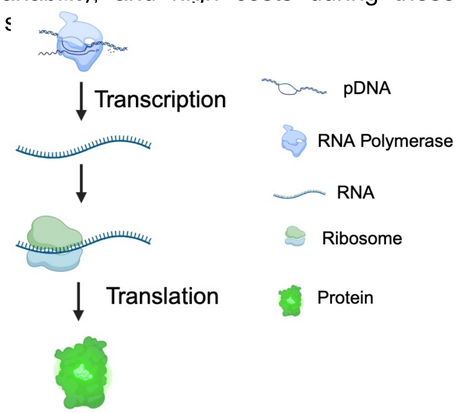

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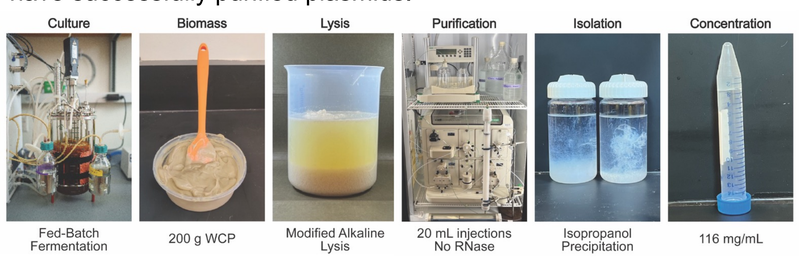

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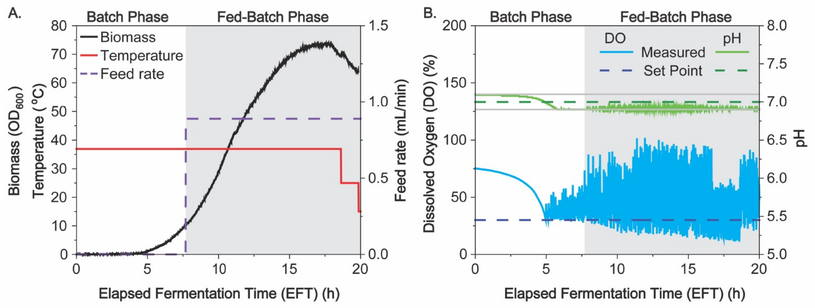

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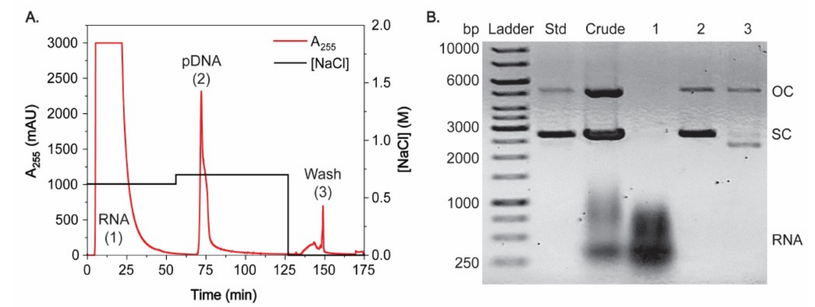

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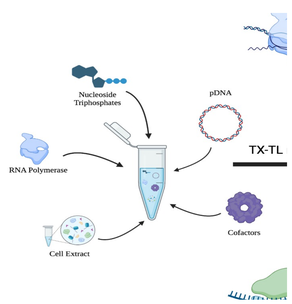


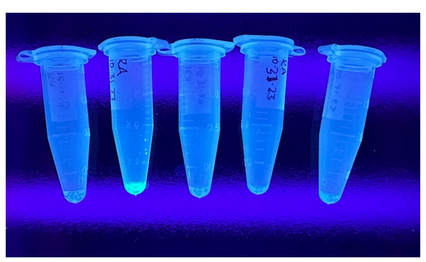
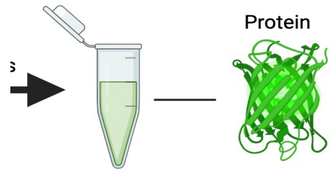


Figure 5. *myTXTL reaction containing a positive control template shows the green fluorescence of protein under UV light.*

Future Work



Optimization of solution CFPS

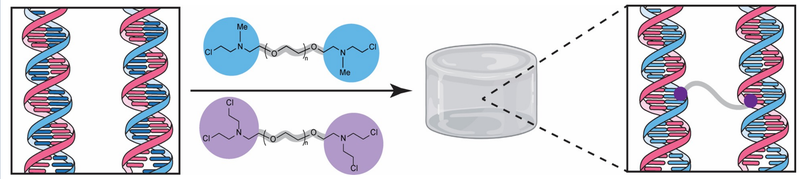


Figure X. *Formation of chemically cross-linked pDNA using bisfunctionalized PEG cross-linkers.*

-use the T7 promoter kit with plasmid pUCBB-pT7-eGFP
-test out if it works
-make hydrogels

-show figures of would happen if is actually worked

Figure X.

Figure X.

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