Harnessing pDNA Hydrogels for Cell-Free mRNA Synthesis

Rachel A. Achong,[a] Tinotenda Duche,[a] Madigan Jennison-Henderson,[a] Wynter Paiva,[a] Nathan J. Oldenhuis[a]
[a]Department of Chemistry, University of New Hampshire, 23 Academic Way, Durham, NH 03824
rachel.achong@unh.edu || natolab.com

Introduction

The demand for better techniques to mass-produce mRNA was amplified through the publicized development of the SARS-CoV-2 (COVID-19) vaccine. However, the mRNA production requires a difficult to separate plasmid DNA (pDNA) template due to its similar chemical properties and composition[1]. Incorporating the pDNA template into a chemically cross-linked hydrogel for use in cell-free synthesis would keep it out of solution, simplifying the subsequent purification (**Fig. 1**). Unfortunately, current production and purification processes do not meet the supply demand needed to make pDNA hydrogels.

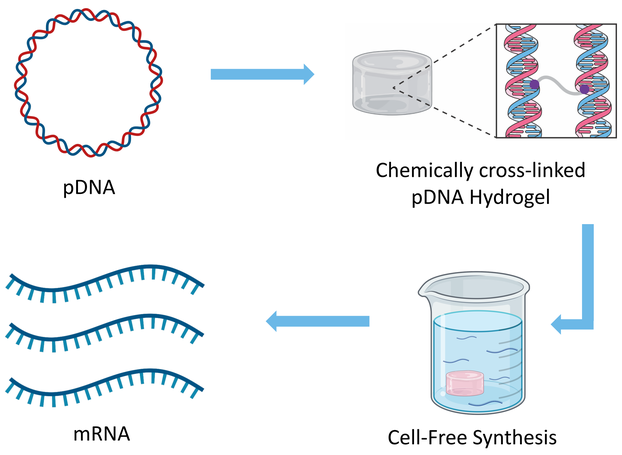

Figure 1. *Workflow: from pDNA to hydrogel to cell-free mRNA synthesis*

Methods

To obtain large quantities of pDNA, a scalable production and purification process was developed (**Fig. 2**). This was crafted through a combination of academic and industry protocols[2-6]. This procedure has been utilized to purify three different plasmids without modification: pUC19 (2.686 kbp), pUCP20T-eYFP (5.045 kbp), and pPIC11 (11 kbp).

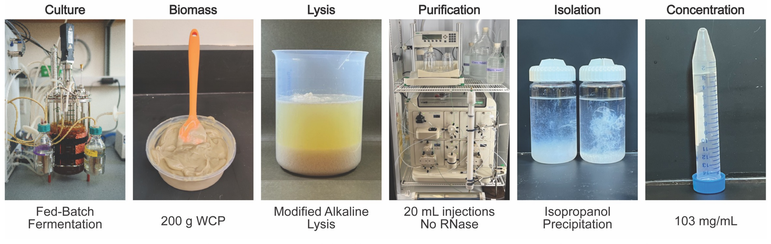

Figure 2. *Experimental overview of bulk production and purification of pDNA. DH5α Escherichia coli cells containing the pDNA of interest were grown in a 7 L bioreactor, isolated through scaled up alkaline lysis, and purified through anion-exchange chromatography to generate solutions with a concentration of 100 mg/mL pDNA.*

pDNA Production and Purification

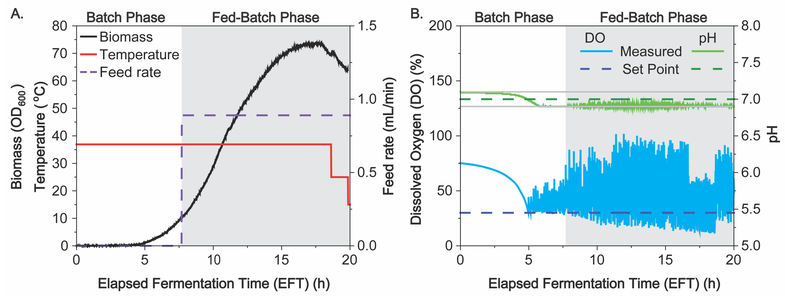

Figure 3. *A) Time course of biomass, temperature, and feed rate and B) Time course of dissolved oxygen and pH during fed-batch fermentation. Cultures grow approximately 24 h, producing 150 – 200 mg pDNA / L culture. 100 – 400 g wet cell paste (WCP) is produced each run and stored at – 80℃*

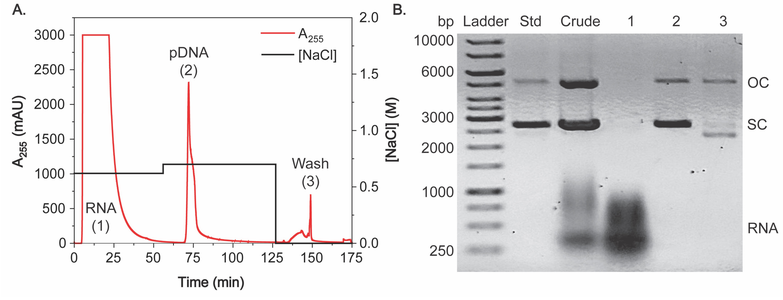

Figure 4. *A) Chromatogram representing the different elution points of RNA, pDNA, and wash for pDNA purification through anion-exchange chromatography using a 193.9 mL column packed with Q-Sepharose using an optimized NaCl gradient. B) Analysis of Purification fractions through gel electrophoresis. This purification process yields ~70% yields.*

Initial Cell-Free Synthesis Trial

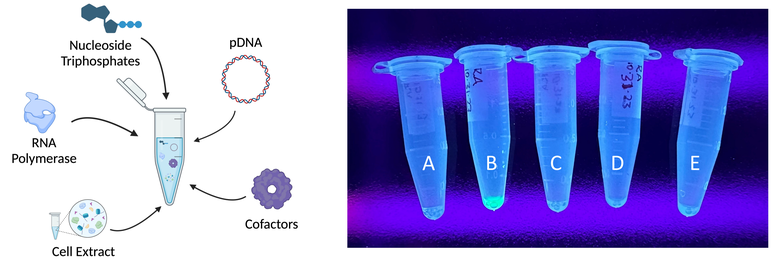

Figure 5. *Cell-Free Protein Synthesis of eYFP from pUCP20T-eYFP [7] using myTXTL Sigma 70 Master Mix Kit. A) negative control, B) positive control, C-E) three pUCP20T-eYFP trials.*

Future Directions

We will shift to purifying pUCBB-pT7-eGFP. It has a T7 promoter, which is compatible with the myTXTL Sigma 70 Master Mix Kit, and encodes eGFP, which will allow us to easily visualize the accessibility of our plasmid without the need to quantify mRNA (**Fig. 6**). Once we confirm our plasmid can be used for cell-free synthesis in solution, we will begin trials with chemically cross-linked pUCBB-pT7-eGFP hydrogels (**Fig. 7**).

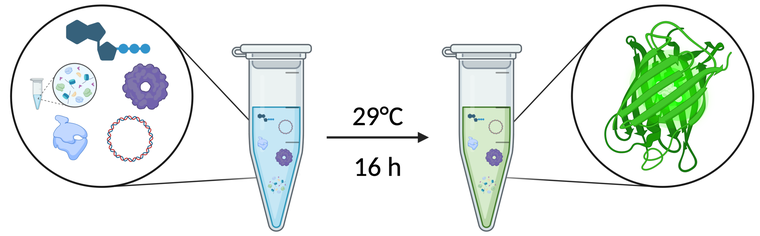

Figure 6. *Successful workflow of Cell-Free Protein Synthesis of eGFP from pUCBB-pT7-eGFP using myTXTL Sigma 70 Master Mix Kit*

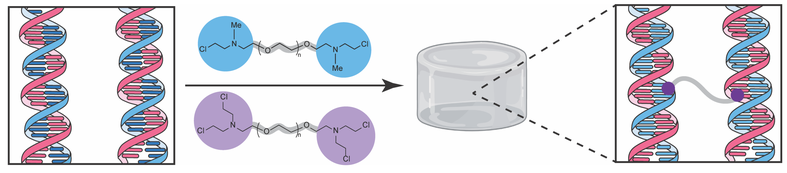

Figure 7. *Formation of chemically cross-linked pDNA hydrogels using bisfunctionalized PEG cross-linkers.*

Acknowledgements

We would like to thank the following people: Carmelina Minico, Matthew Currier, Shaina Hughes, Lola Fadairo, Nick Pierini, Tran Truong, Brynna Hone, Sam Ashooh, Aylin Aykanat, and Amy Michaud. This research is funded by NIH P20GM113131, NSF IIA NSF1757371, UNH Core Pilot, and NSF CAREER 2340569 grants

References

1. Rosa et al. *Vaccine* **2021**, 39 (16), 2190-2200
2. Laib et al. *Macromolecules* **2006**, 39 (12), 4115-4119
3. DNA vaccines: methods and protocol, **2014**
4. Urthaler et al. *J. Biotechnol*. **2007**, 128, 132-149
5. *Cytotechnology* **2005**, 35 (3), 165-173
6. Prazeres et al. *J. Chromatography A* **1998**, *806*, 31-45
7. Arbor Biosciences. myTXTL Cell-Free Expression Handbook **2019**