RNase-Free Purification of pDNA using Anion Exchange Chromatography

Madigan Jennison-Henderson, April Weeks, Tinotenda Duche, Somkene Alakwe , Wynter Paiva, Nathan Oldenhuis

madigan.jennison-henderson@unh.edu | natolab.com

Introduction

- To truly probe polymer structure-property relationships, bulk production of a polymer with a high degree of control over topological state is needed.
- Double stranded DNA (dsDNA) is a polymer widely studied in life sciences due to its role in genetic information, Reliable and predictable manipulation of dsDNA chains through enzymes and small molecules has been studied extensively (*Fig.* 1).
- The reliable manipulation of dsDNA chains can be exploited to probe structure-property relationships in DNA hydrogels if large quantities of dsDNA.
- Current methods for dsDNA synthesis are wasteful, sequence limited, costly and time-consuming. To utilize DNA as a generic commodity polymer there is a need for new methodologies to purify it on scale at a low cost
- Advancements in bioreactor-based production of pDNA for vaccines has resulted in gram scale quantities of dsDNA, but purification methods are still costly and time-consuming.
- Purification using anion exchange chromatography is a promising route, but many protocols rely on RNase A to separate pDNA from RNA which would not be practical on a large scale

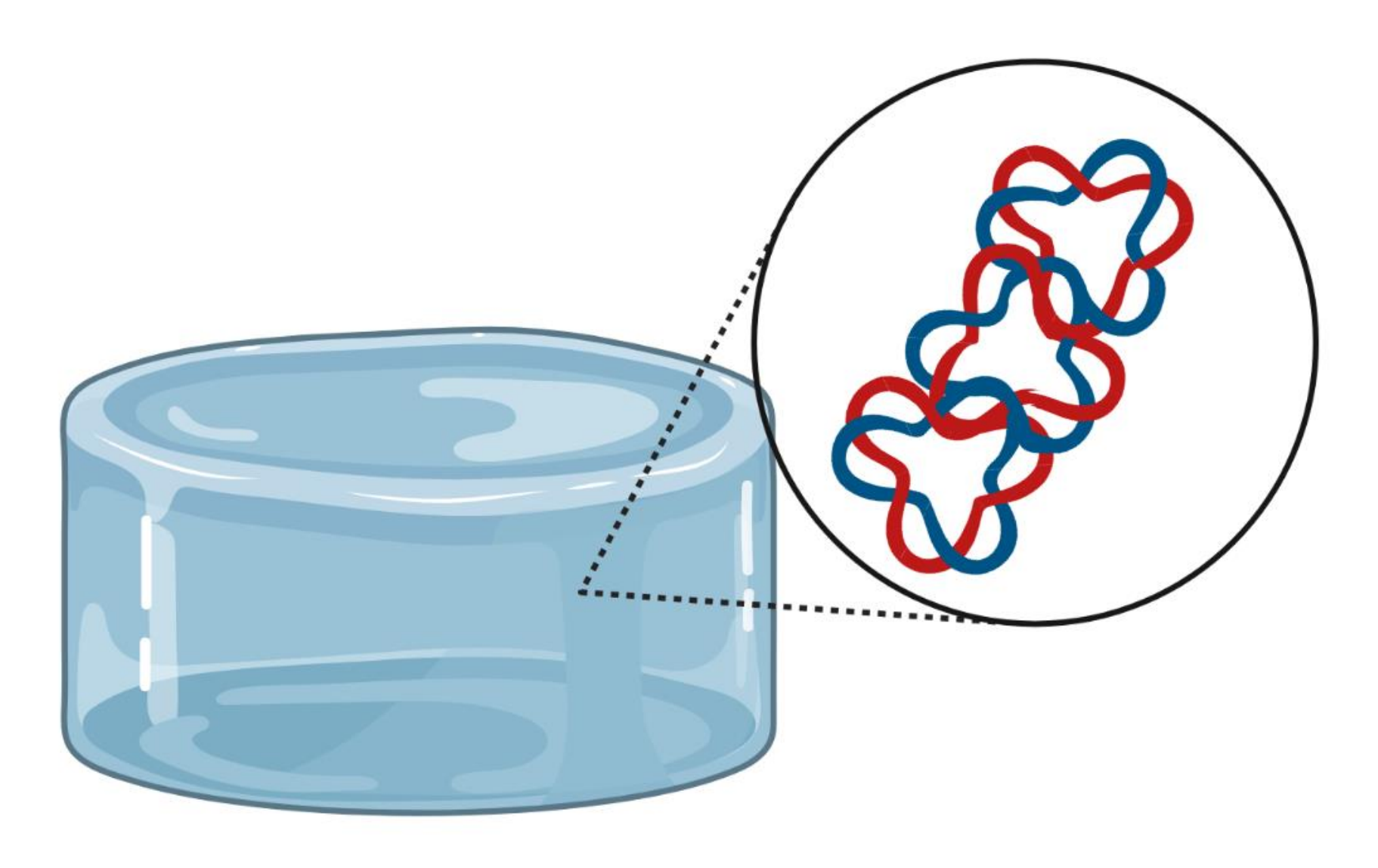

Figure 1 *Creation of a DNA hydrogel as a potential use for purified pDNA.*

DNA hydrogels may be enzymatically altered for study of topological control of polymers.

- An optimized procedure (*Fig. 2*) for the purification of pDNA that eliminates the need for RNase completely has been developed to combat this issue.

Methods Overview

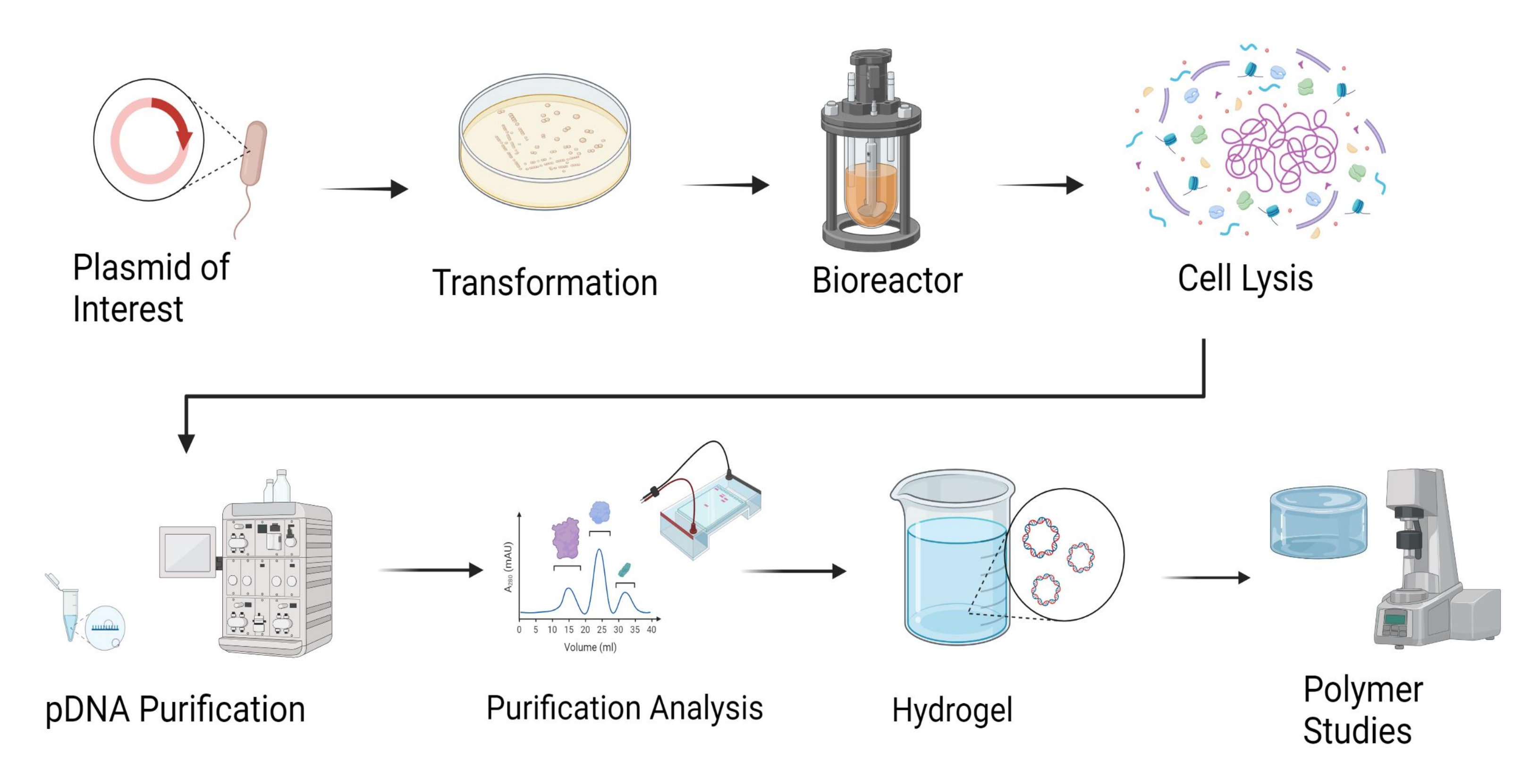

Figure 2 *Overview of methods for pDNA purification process.*

Experimental

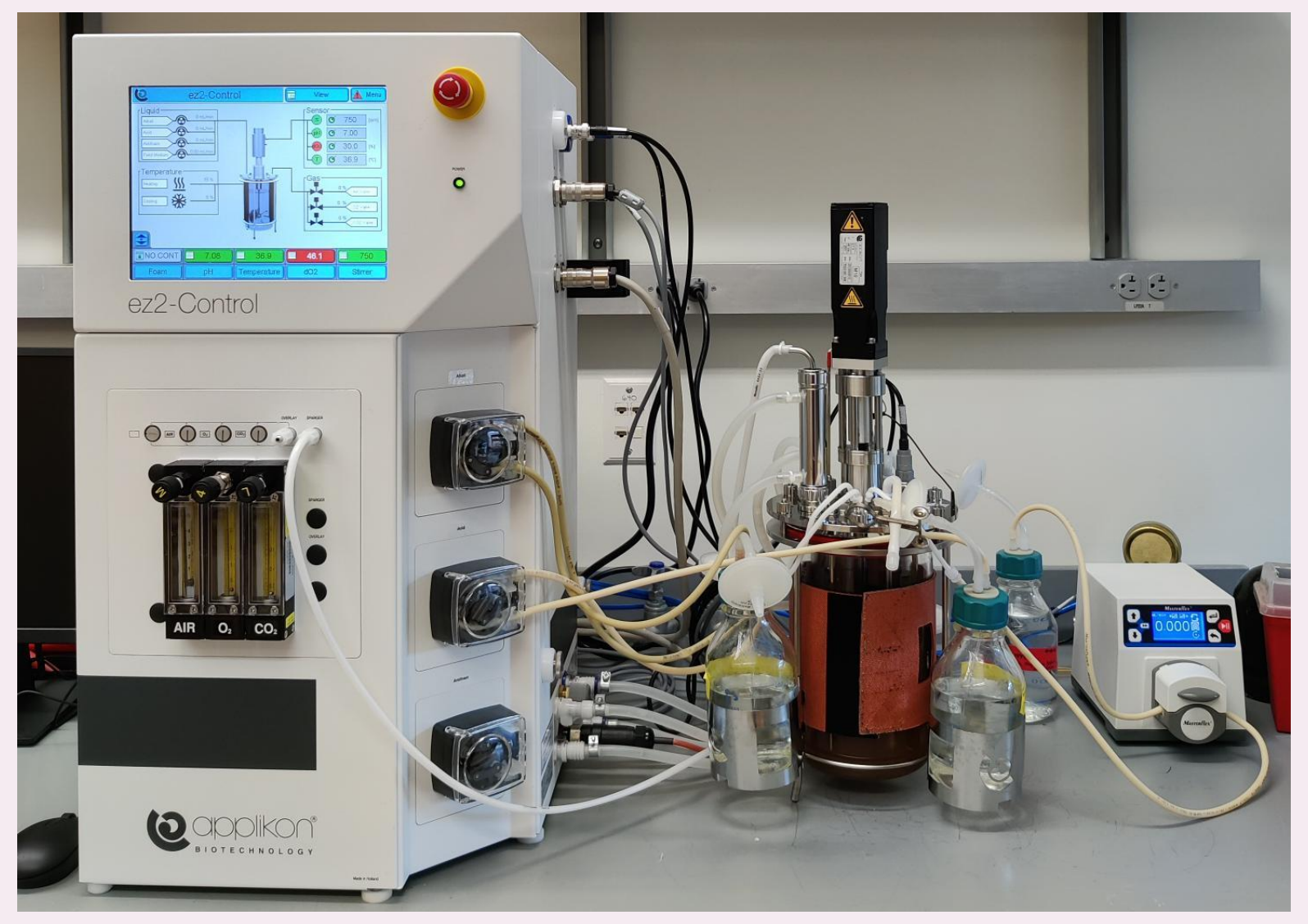

Figure 3 *Benchtop bioreactor with a 3L vessel.*

- Cells containing the target plasmid (pUCP20T-eYFP) are grown using a benchtop bioreactor (*Fig. 3*).
- Cells are grown in a selective growth medium and various parameters (pH, dissolved oxygen, temperature, etc.) are observed and manipulated.
- Harvested cells are resuspended and a highly alkaline solution is added to the mixture to alter the pH and crack open cell membranes.
- pDNA and RNA are separated from other cellular debris, the alkaline mixture is neutralized (*Fig. 4*), and RNA and pDNA remain in solution while cellular debris becomes solid.
- The mixture is centrifuged, pelleted debris is disposed of, and supernatant is sterile filtered through different pore sizes.

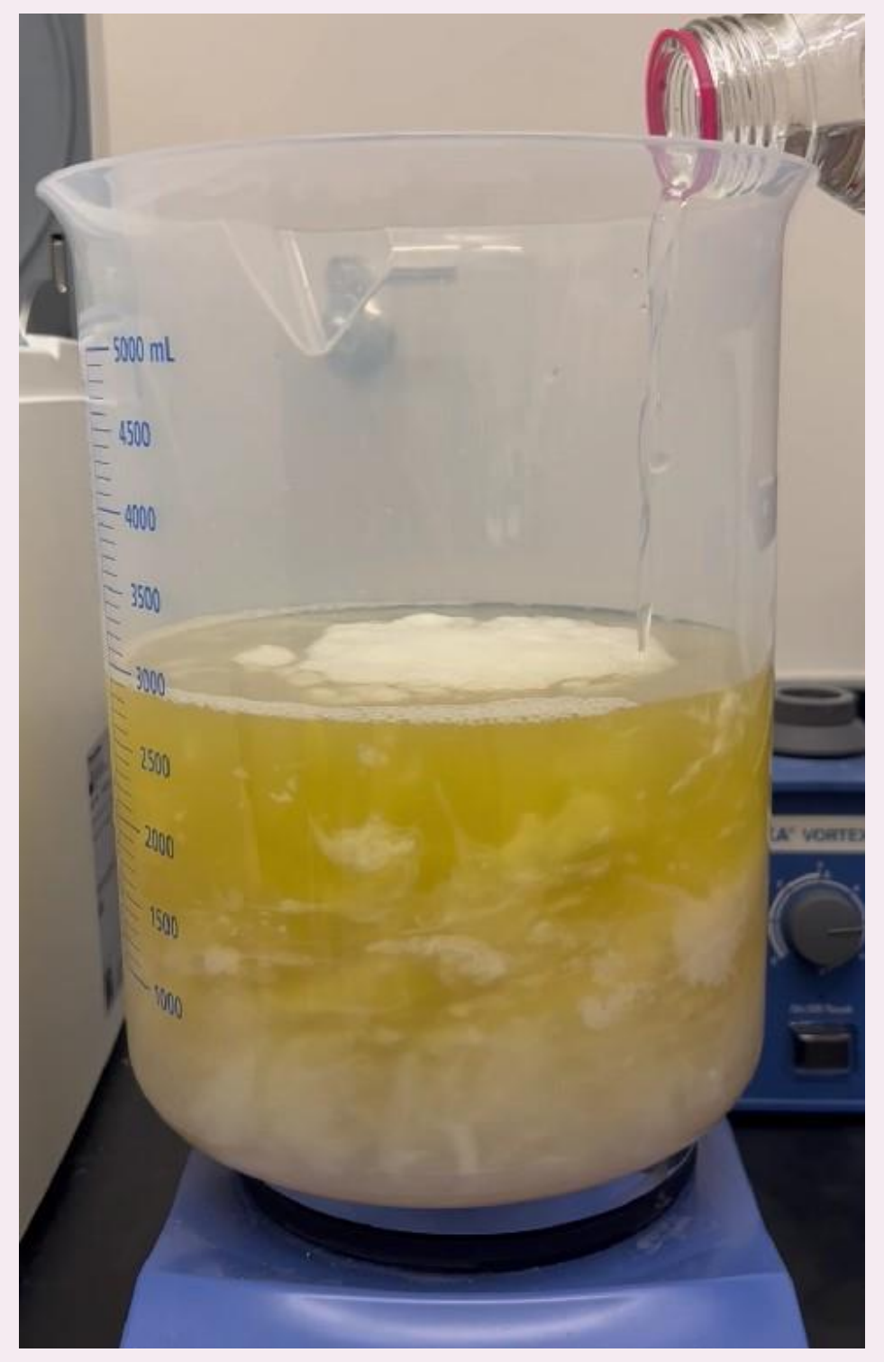

Figure 4 *Neutralization of harvested cells*

Figure 5 *FPLC instrument with Anion Exchange Column*

- Fast Protein Liquid Chromatography (FPLC) instruments (*Fig. 5*) are designed and traditionally used for purification and analysis of proteins. This procedure utilized an FPLC for purification of pDNA from RNA.
- Chromatographical "peaks" are formed by a stepwise increase of the ionic strength of solution, generated through increasing the concentration of sodium chloride (NaCl) buffer flowing through the column and triggering elution of bound molecules(*Fig. 6*).
- Elution order is based on net negative charge of molecules bound to the column.
- Following pDNA elution, the column is washed with sodium hydroxide (NaOH) to remove any molecules still bound to the column.

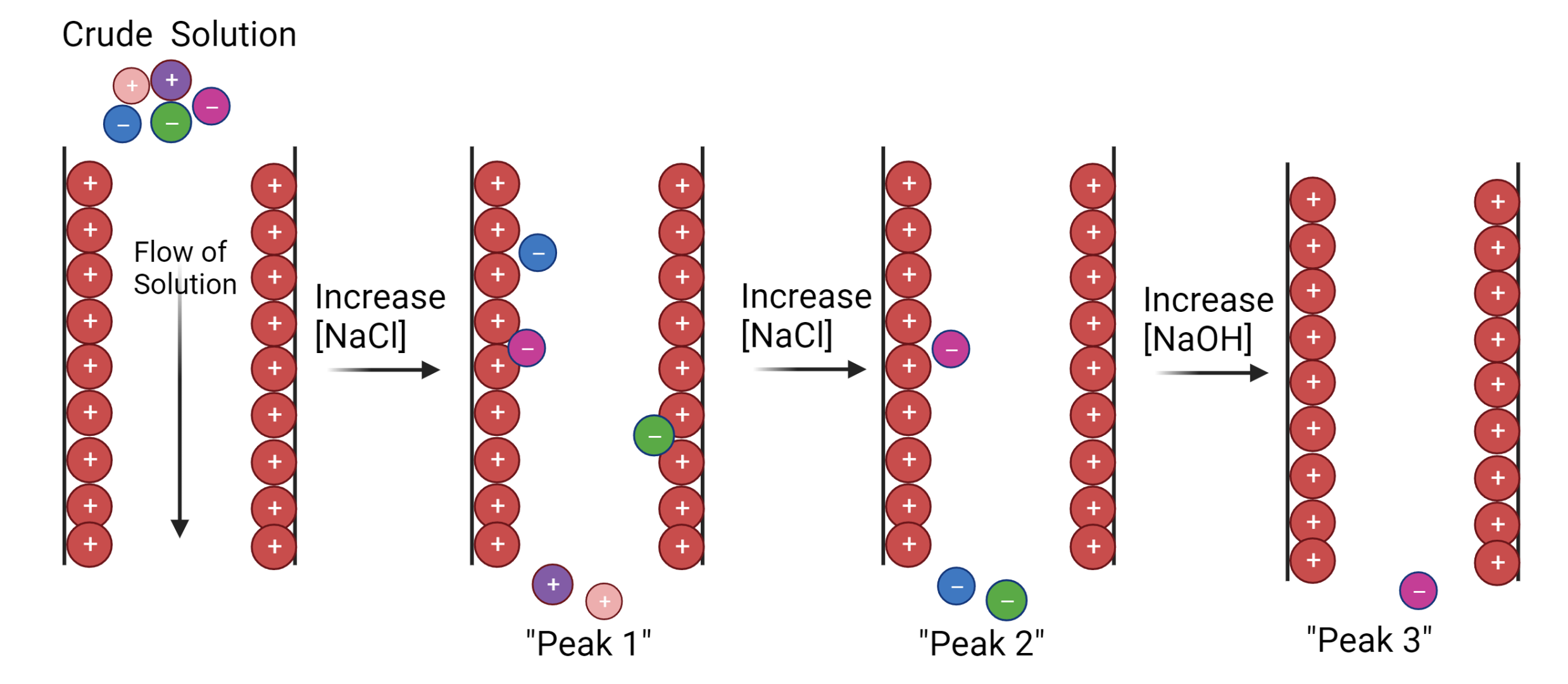

Figure 6 *Overview of mechanism for anion exchange chromatography*

Results

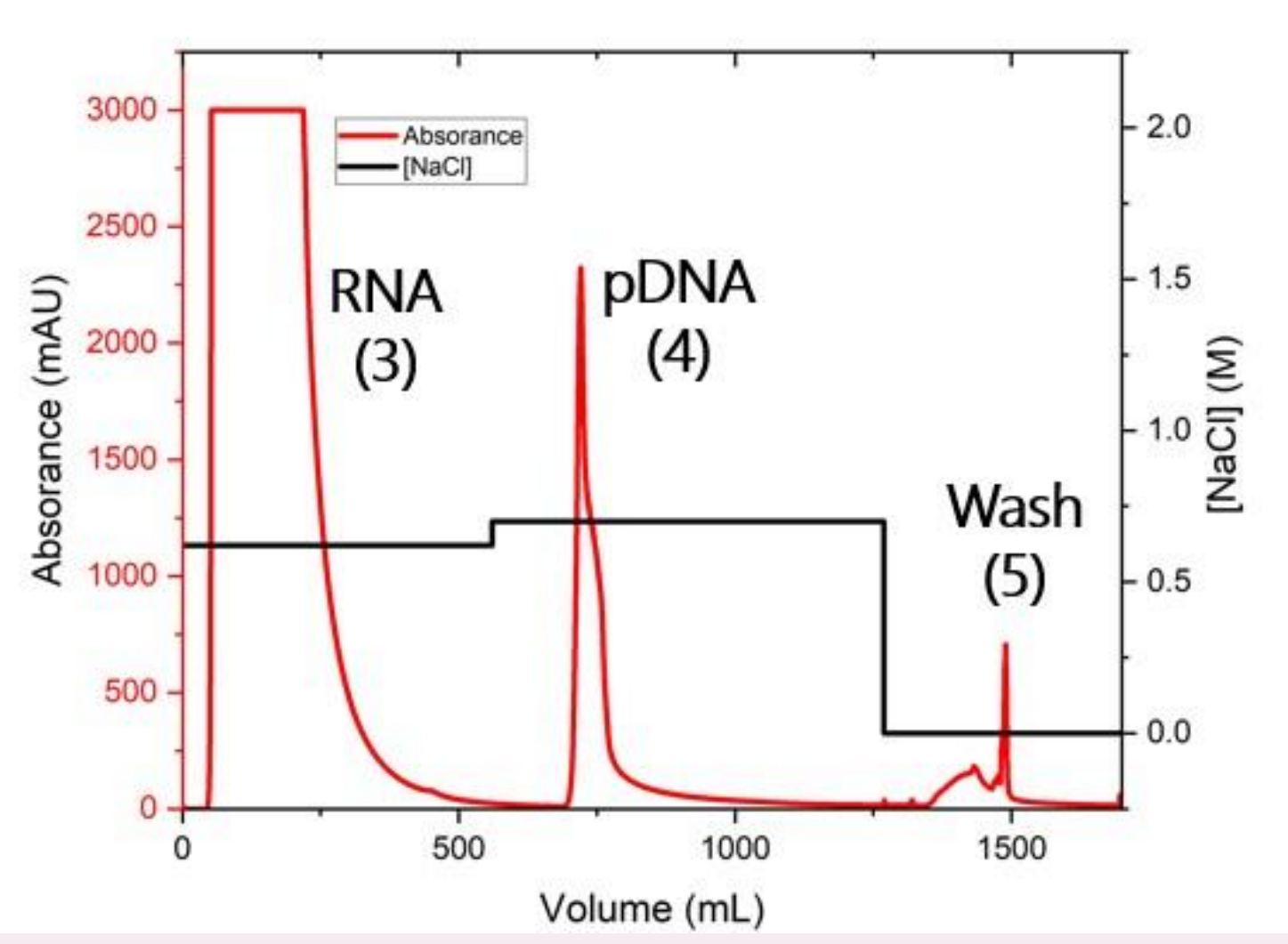

Figure 8 *Chromatogram depicting elution order of RNA and pDNA using a TE buffer with NaCl to increase ionic strength of solution.*

- "Peak 1" elution with a maximum absorbance of 3000 mAU.
- "Peak 2" indicates elution with a maximum absorbance of ~2300 mAU.
- "Peak 3" indicates NaOH wash of column with maximum absorbance of 750 mAU.
- pDNA percent yield 70.6% .

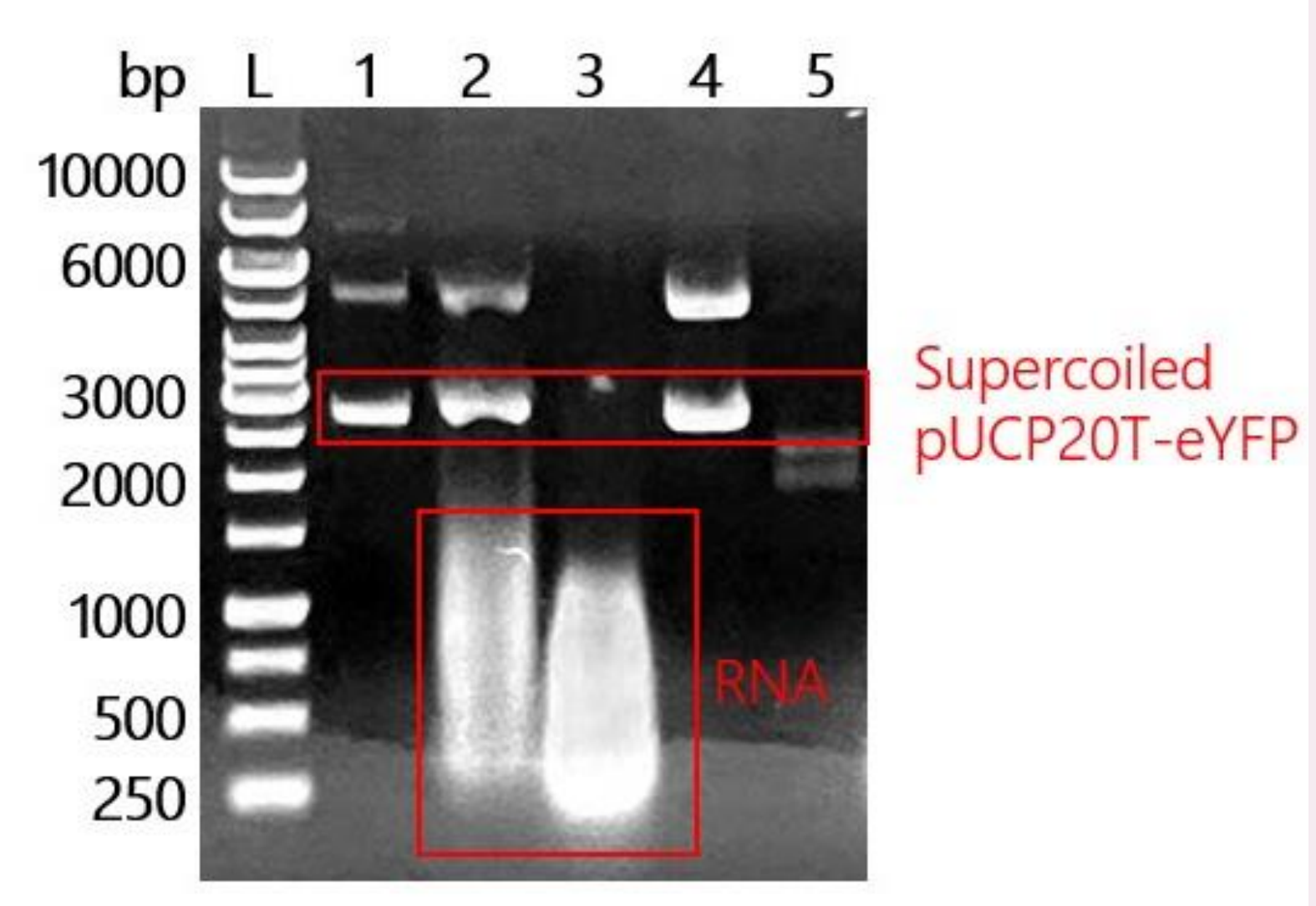

Figure 9 ***Agarose gel assessing purity of pDNA post-purification process using 10µL of sample at 75 volts**. Lanes denote (L) Ladder, (1) Standard Purified pUCP20T-eYFP, (2) Crude pUC20T-eYFP, (3) "Peak 1" RNA Elution, (4)"Peak 2" pDNA Elution, (5) "Peak 3" NaOH Wash.*

- Minimal banding at 3000 bp in (3)
- Band 3000 bp in (4)
- Lack of banding <2000 b in (4)

Discussion and Conclusions

- Sterile filtering of crude solution is a time intensive process with room for improvement. We are exploring the replacement of sterile filtering with tangential flow filtration to overcome this obstacle.
- pDNA can be successfully purified without the addition of RNase to the pre-suspension buffer.
- Findings demonstrate that it is possible to utilize pDNA as a biomaterial precursor that is cost-effective, sustainable, and scalable for materials science research.

Future Work

- Continued optimization of both bioreactor-based fermentation procedures and purification procedures to increase plasmid yield, generating a greater quantity of biomaterial precursors.
- Studying impact of storage conditions for maintaining plasmid integrity of highly concentrated samples of pDNA over extended periods of time.

References

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Acknowledgements

We would like to thank the following people: Zahraa Albeshir, Samuel Ashooh, Aylin Aykanat, Matt Currier, Lola Fadairo, Jason Gomez, Shaina Hughes, Carmelina Minico, Nick Pierini, Pat Strobel, Tran Truong.

NH BioMade is funded by NSF Award #IIA 1755371 administered by NH EPSCoR at UNH