Purification of Nucleic Acids for Biophysical Characterization

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Introduction

Plasmid DNA can be used as a **molecular standard** for validating biophysical instruments. This project focuses on producing high-purity DNA from E. *coli* using a combination of selective growth, lysis, and chromatography-based purification.

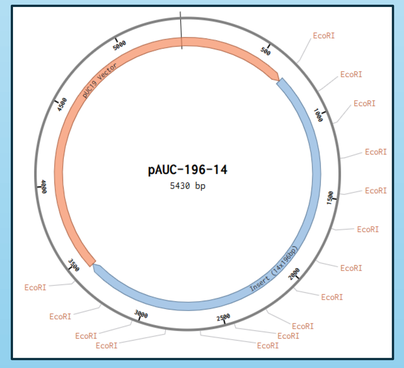


Figure 1. Map of the pAUC-196-14 **plasmid** displaying **EcoRI** enzyme cut sites used to generate 196 base-pair (bp) and 2696 bp linear DNA fragments. The plasmid consists of a 2744 bp insert and a 2696 bp pUC19 backbone.

Preparation of Plasmid DNA

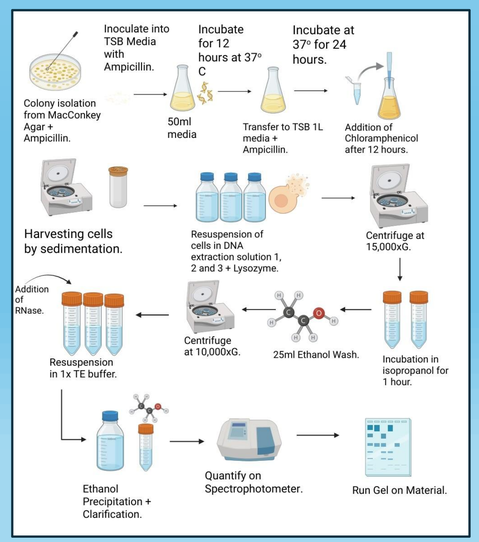


Figure 2. Overview of the **plasmid DNA preparation workflow.**

Size Exclusion Chromatography (SEC)

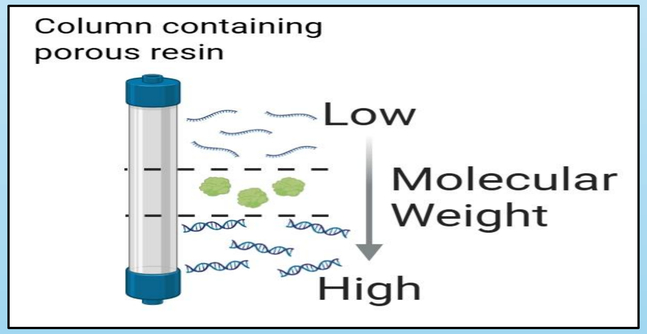


Figure 7. **Illustration of Size Exclusion Chromatography**, where larger molecules elute earlier than smaller ones due to resin pore exclusion.

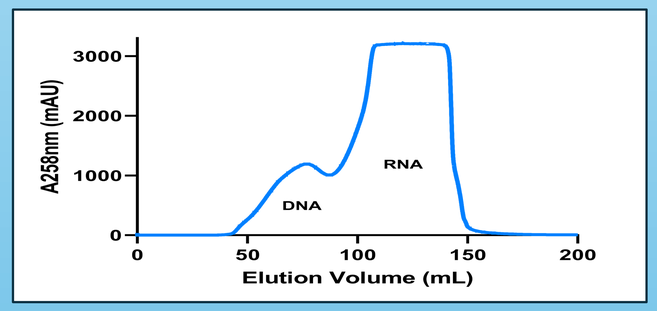


Figure 8. **SEC profile** with larger plasmid DNA eluting early and smaller RNA eluting later.

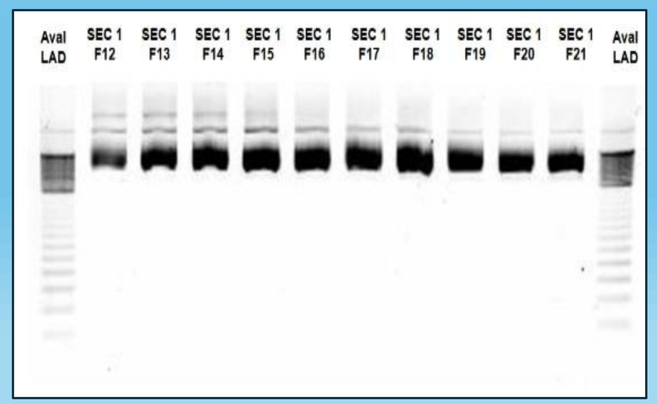


Figure 9. **Agarose gel** of SEC fractions F12-F21 showing plasmid DNA recovery.

Anion Exchange Chromatography (AEX)

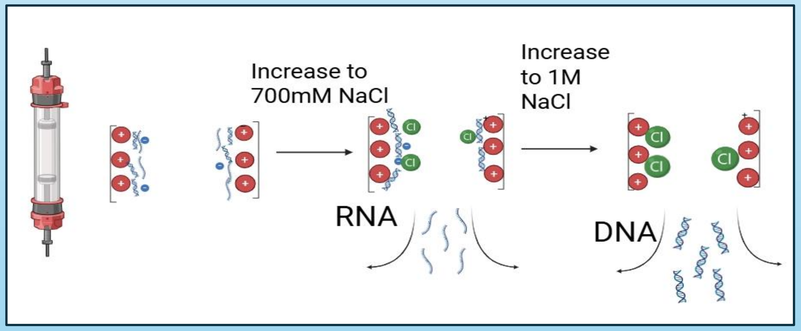


Figure 11. Diagram of Anion Exchange Chromatography showing RNA and DNA separation by salt elution.

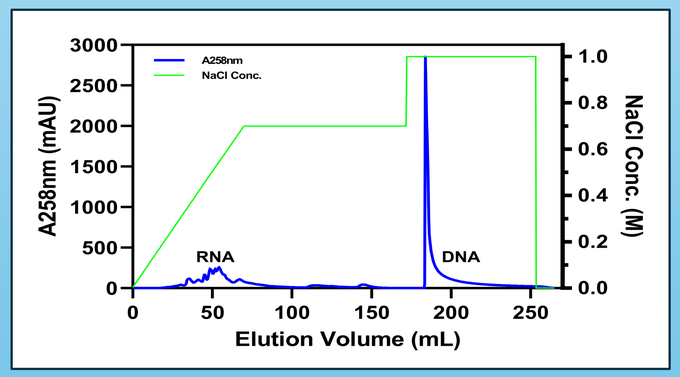


Figure 12. **Anion Exchange Chromatogram** with purified plasmid DNA eluting as the later sharp peak.

Future Directions

- Ensure scalability and reproducibility of DNA fragment production.
- Determine shelf-life or stability of the fragments over time.
- Analyze the fragments in a multi-lab AUC study to ensure consistency and reproducibility.
- Utilize orthogonal methods such as Size Exclusion Chromatography coupled with Multi-Angle Light Scattering (SEC-MALS), DNA sequencing and Gel Electrophoresis to validate DNA fragment measurements.

References

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