Large Scale Enzyme Production for Topologically Controlled dsDNA Hydrogels

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dsDNA as a Polymer

Double stranded DNA (dsDNA) is a water soluble, semiflexible polymer that comes in many sizes, making it a viable option for biomaterials, such as hydrogels (***Fig. 1***). Current sources of dsDNA are incompatible with the scale necessary for bulk material production. To address this, we developed a simple, scalable and unified method that produces gram-scale quantities of plasmid DNA (pDNA) for use as a generic polymer.[1]

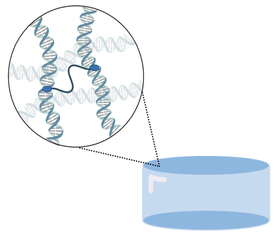

Figure 1. *Depiction of a hydrogel made from dsDNA.*

This work aims to build a scalable enzyme toolbox for precise and selective control of DNA topology, focusing on restriction enzymes for site-specific cleavage (***Fig. 2A***) and ligases for backbone repair (***Fig. 2B***).[2]

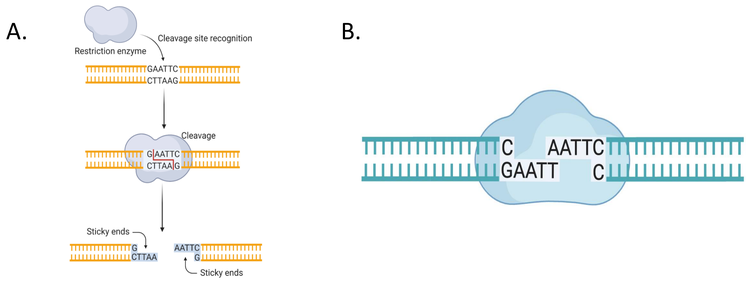

Figure 2. *A) Depiction of how restriction enzyme EcoRI works; B) Depiction of a DNA ligase.*

Purifying EcoRI-RTX via Calcium Precipitation

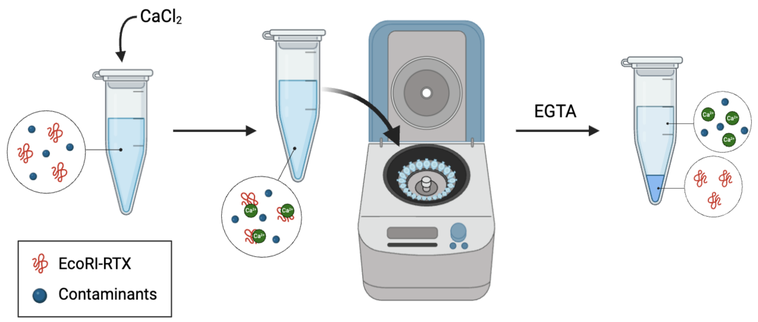

Figure 3. *Depiction of EcoRI-RTX purification via calcium precipitation. Following transformation, cell growth, and chemical lysis, $CaCl_2$ is added to lysates to a final concentration of 50 mM to precipitate EcoRI-RTX. The RTX tag appended to the enzyme makes this possible. It is then washed with 50 mM Tris and 50 mM Tris containing EGTA, a chelating agent, to remove the calcium ions from solution. The pellet is then resolubilized and stored at -20°C.*[3]

Purifying p50-Ligase via IMAC

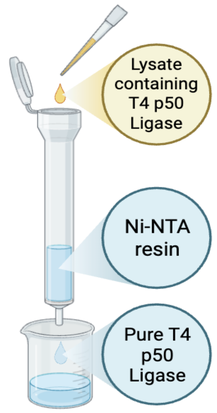

Figure 4. *Depiction of the purification method for p50-ligase (a T4 DNA ligase appended to a p50 DNA binding domain) via Immobilized Metal Affinity Chromatography (IMAC). Following transformation, cell growth, protein induction, and chemical lysis, lysates are washed and eluted on a gravity flow column containing Ni-NTA IMAC resin. Purification is analyzed via SDS-PAGE.*[4]

Cell-Free Protein Synthesis

Cell-Free Protein Synthesis (CFPS) utilizes extracted cell machinery combined with a DNA template and exogenous substrate to produce protein without the use of cells (***Fig 5***).[5] With CFPS, the process of protein production is shortened by days, without the necessity for bacterial transformation and culture. Proteins produced can be further purified via chromatography without the need for cell lysis.

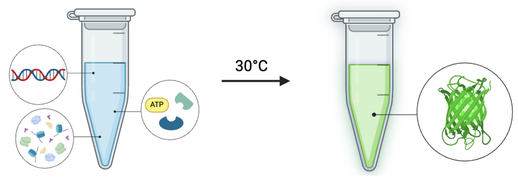

Figure 5. *Overview of a CFPS reaction. BL21(DE3) cell extract is combined with cofactors, energy components, and a DNA template. The reaction is incubated at 30°C for at least 1 hour. Production of fluorescent proteins can be analyzed via UV-Vis.*

CFPS Reaction

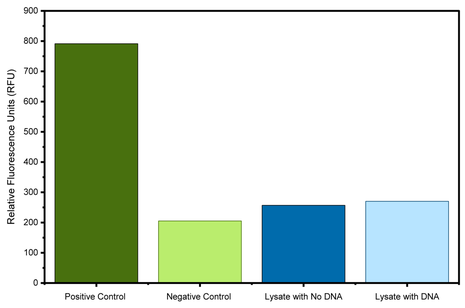

Figure 6. *Lysates produced from BL21(DE3) cells supported transcriptional activity, as confirmed by control reactions. However, low yield in experimental samples suggests limited compatibility between lysate and CFPS system under current conditions.*

Analysis of Purification

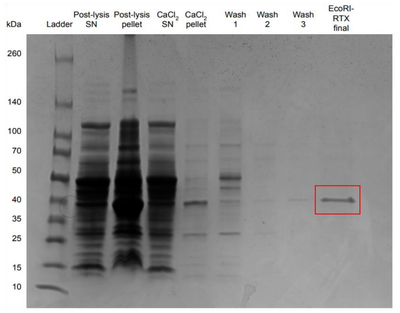

Figure 7. *SDS-PAGE analysis of EcoRI-RTX purification. The red box denotes EcoRI-RTX. The protein appears to be present in the final lane, indicating effective purification.*

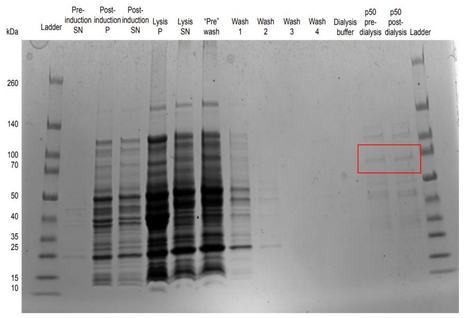

Figure 8. *SDS-PAGE analysis of most recent p50-ligase (denoted by red box) purification. Based on the appearance of multiple bands in the final lane, the purification was unsuccessful.*

Conclusions and Future Work

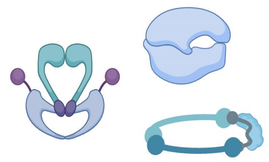

Figure 10. *A depiction of gyrase, RNA polymerase II, and condensin.*

Calcium precipitation of EcoRI-RTX produced function enzyme but still requires further optimization, along with IMAC purification of p50-ligase. CFPS enabled rapid protein production but showed limited compatibility under current conditions. Future work will focus on improving purification methods and yields and expanding the enzyme toolbox for dsDNA hydrogel systems.

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