FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

CELLEX

Expression and Purification of Cytotoxic Human Ribosomal Proteins in Bacterial Cell-Free Systems

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* Equal contribution

Introduction

- Ribosomal proteins (RPs) have functions outside of traditional translation including chromatin remodeling, tumor suppression and apoptosis, among others. Mutations in RPs can lead to diseases such as different cancers and Diamond-Blackfan anemia.[1,2]
- Cell-free expression systems (CFES) are promising avenues to express proteins that are otherwise toxic to normal cellular life.[3]
- MyTXTL (Arbor Biosciences) is a commercially available transcription-translation CFES we have used to emulate and create our own in-house cell free system.
- Prior work in our lab has shown that some ribosomal proteins from the small subunit, including RPS6 and RPS27, are cytotoxic to the host cell when heterologously expressed.

Objectives:

- Produce and purify cytotoxic ribosomal proteins which could not be expressed in whole-cell systems.
- Optimize protocols for best yield.

Methods

- Used pNH-Trxt-∅, pNH-TrxT-RPS6, pNH-TrxT-RPS10, pNH-TrxT-RPS12, and pNH-TrxT-RPS27 previously cloned in our laboratory.
- Utilized a plasmid miniprep kit to isolate the plasmid DNA from the E. coli cells and Monarch DNA clean up kit afterwards to store in DNAse free water to optimize the cell free reaction.
- Used premade in-house bacterial lysate system, in combination with Panox proprietary energy mix, to express ribosomal proteins utilizing transcription-translation CFES.
- Protein bands were separated using SDS-PAGE and analyzed by Western Blot with anti-His, anti-RPS6, and anti-GAPDH antibodies.
- Samples that utilized the in-house CFES and MyTXTL system were purified using Ni-NTA spin columns and analyzed by SDS-PAGE and Western Blot as described (**Figure 4**).

Results

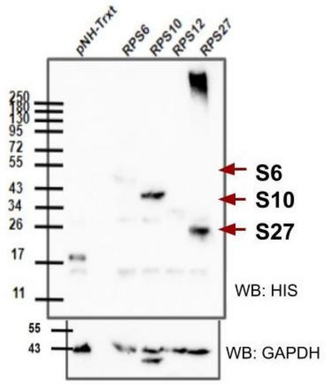


Figure 1: Expression of 6xHis-Trx-RPS6, 6xHis-Trx-RPS10, 6xHis-Trx-RPS12 and 6xHis-Trx-RPS27 in the commercial MyTXTL cell free expression system. A GAPDH probing is included as a loading control.[4]

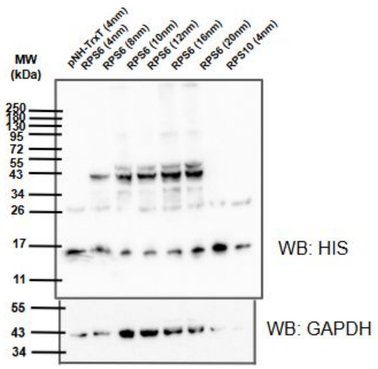


Figure 2: Titration of different concentrations of pNH-TrxT-RPS6 template plasmid from MyTXTL expression reactions.[4]

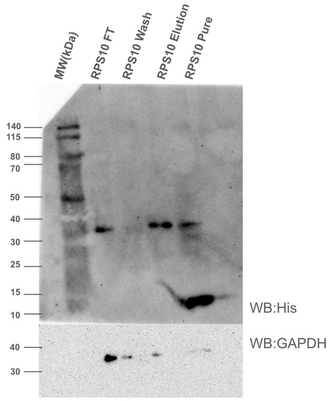


Figure 3: Ni-NTA spin column purification of CFES-produced 6xHis-Trx-RPS10.

Figure 4: Methods

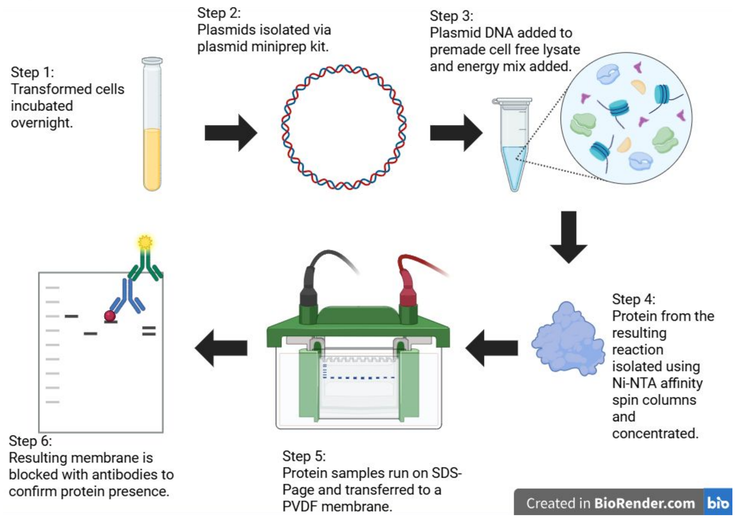
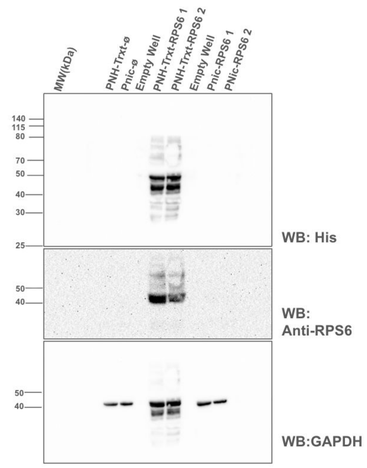


Figure 5: Western Blot analysis of 6xHis-Trx-RPS6 produced using in-house CFES.



Discussion

- These results demonstrate the ability of our in-house cell free system to produce different cytotoxic human ribosomal proteins comparable to the MyTXTL commercial system.
- The expressed protein products are abundant enough for purification via His-tag affinity chromatography and subsequent buffer-exchange concentration.
- The presence of additional bands recognized by the His antibody in the RPS6 expression indicates potential degradation or aggregation products that need further optimization.

Future Directions

- Express cytotoxic human ribosomal proteins using the bacterial CFES while optimizing parameters such as energy mix, template plasmid concentration, addition of RNAse and protease inhibitors, among others.
- Continue to optimize protein purification protocols using Ni-NTA spin columns.
- Combine purified ribosomal proteins with O-GlcNAc Transferase (OGT) enzyme and assay post-translational modification activity in vitro using Ni-NTA 96-well plates.
- Test other post-translational modification reactions using bacterial CFES.

References

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3. Lu, Y. (2017). Cell-free synthetic biology: Engineering in an open world. *Synthetic and systems biotechnology*, *2*(1), 23-27.
4. Murthy, M., Philip, J., Ryan, B., Heber, A., Aronsky, M., Spirito, C., & Zeidan, Q. (2024). Preparation and Application of In-House E. coli Cell-Free Protein Expression Kits. https://doi.org/10.13016/iqln-mu42

Acknowledgements

Financial support for this project was provided by the First-Year Innovation and Research Experience (FIRE) program at the University of Maryland. We thank the FIRE-HPI stream and the Ling Lab (Department of Cell Biology and Molecular Genetics) for generously providing equipment and reagents. We thank the organizers of Undergraduate Research Day for the opportunity to present our work.