FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

CELLEX

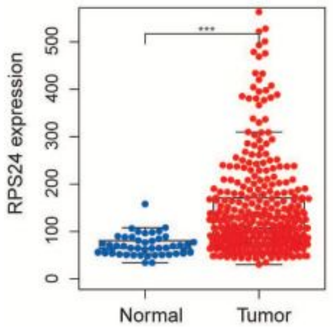
Cloning and Expression of Human RPS24 into *E.coli* and the HEK293 Cell Line

Katherine Merrifield, Sofia Apgar, Jessica Whitney, Vicky Argueta, and Quira Zeidan

Introduction

- The 40s Ribosomal Protein 24 (RPS24) is mainly involved in ribosome assembly and translation. Recently, it has been linked to the regulation of cell growth, proliferation and tumorigenesis.[1]
- **Objectives:**
  - Express and purify human RPS24 in bacteria to study its biochemical characteristics *in vitro*
  - Express human RPS24 in a human embryonic kidney cell line (HEK293) to investigate the effects of cell stress on post-translational modifications and tumor proliferation.

Figure 1:
RPS24 mRNA Expression[2]



Methods

- Used PCR to amplify the human RPS24 coding sequence from pcDNA3.1+CDYK and linearize expression vectors, pNH-TrxT (adds a 6xHis-Trx tag) and pNIC28-Bsa4 (adds a 6xHis tag).
- Gel electrophoresis was done and the desired DNA fragments were excised.
- Inserted RPS24 gene into the expression vectors through In-Fusion Cloning, then the recombinant plasmids were transformed into BL21 *E.coli* cells.
- A bacterial growth curve was done to find the optimal time for induction. IPTG was used to induce RPS24 protein expression which was subsequently measured using SDS-PAGE.

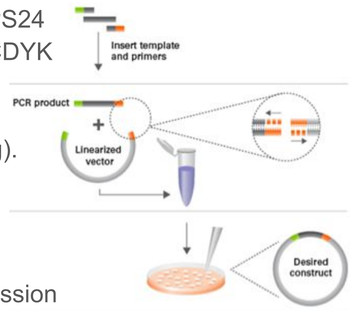


Figure 2: *The In-Fusion Cloning workflow*[3]

Results

Plasmid Maps:

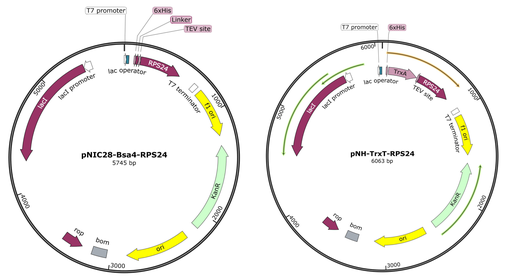


Figure 3: The two recombinant plasmids after the RPS24 gene was inserted.

Plasmid Chromatographs:

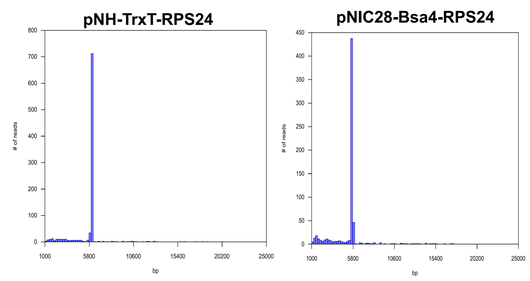
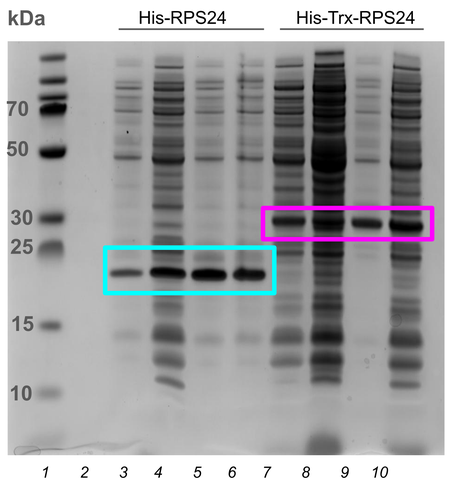


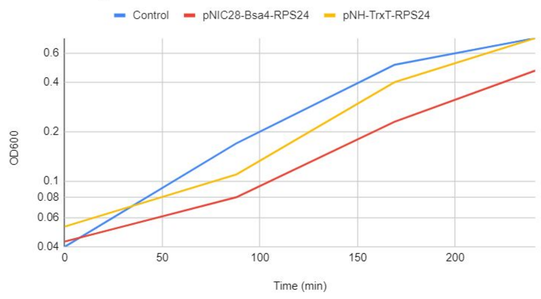
Figure 4: The single, prominent peak indicates pure DNA in the plasmid minipreps (important for DNA sequencing).

SDS-PAGE Gel:



Lanes 3, 5, 7, and 9 are pre-induction, lane 2 is empty

Figure 6: *E. coli* transformed with either expression vectors had protein expression after IPTG induction. Lanes three through six show His-RPS24 which was expected to be 16.263 kDa and appears around 20 kDa on the gel. Lanes seven through ten correspond to His-Trx-RPS24 which was expected to be 28.070 kDa and appears around 28 kDa on the gel.



Cell Density as a Function of Time:

Figure 5: Bacterial growth curve of transformed BL21 *E.coli* was measured relative to absorbance at 600nm and used to optimize time of induction with IPTG. This ensured that the presence of the exogenous vectors did not inhibit binary fission.

Discussion

- SDS-PAGE exhibited thick bands of protein at the predicted molecular weights for both RPS24 bacterial expression vectors
- This indicates successful RPS24 protein expression in *E.coli* cells transformed with either expression vector following induction
- Leaky expression was observed in *E.coli* cells transformed with either expression vector pre induction, however, this has been documented in expression systems using the T7 promoter.

Future Directions

- RPS24 will be overexpressed in the HEK293 cell line to assess effects on cell growth and viability.
- Immunoprecipitation will be done under stress conditions to investigate protein interactions and post translational modifications
- These experiments will be replicated in cancer cell lines for medical relevance

References

1. Wang, Y. et Al, RPS24 knockdown inhibits colorectal cancer cell migration and proliferation in vitro,Gene, Volume 571, Issue 2,2015, Pages 286-291,ISSN0378-1119 https://doi.org/10.1016/j.gene.2015.06.084.(https://www.sciencedirect.com/science/article/pii/S0378111915008124)
2. Li, H. et Al, (2023). RPS24 Is Associated with a Poor Prognosis and Immune Infiltration in Hepatocellular Carcinoma. *International journal of molecular sciences*, *24*(1), 806. https://doi.org/10.3390/ijms24010806
3. *The In-Fusion Cloning workflow*. [Image]. (n.d.). Takarabio.com. https://www.takarabio.com/learning-centers/cloning/applications-and-technical-notes/efficient-multiple-fragment-cloning

Acknowledgements

Financial support for this project was provided by The First-Year Innovation and Research Experience program at the University of Maryland. Thanks to the FIRE-HPI lab and the Ling lab (CBMG) for their generosity with equipment and reagents. We would like to thank the organizers of Undergraduate Research day for the opportunity to present our work. Additional thanks to Audrey Heber and Blaise Ryan.