FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

CELLEX

Expression of Human RPS17 and RPS23 in *E. Coli* Bacterial Cells

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Introduction

Ribosomal proteins are proteins that along with rRNA make up the ribosomal subunits involved in the translation of proteins. RPS17 and RPS23 are ribosomal proteins that are part of the small ribosomal subunit responsible for translation, stabilization, assembly, and folding. RPS17 mutations are connected to Diamond-Blackfan anemia (DBA)[1], which is anemia that causes deficiency in the production of red blood cells in bone marrow. DBA patients have a mutation in the translation start codon of RPS17 which changes thymine to guanine[2]. RPS23 is overexpressed in both early- and late-stage colorectal carcinoma[3], and high expression of RPS23 is unfavorable in renal cancer and liver cancer (prognostic)[4].

Objectives

- Clone and express human RPS17 and RPS23 in bacteria with two different plasmids to purify and explore the effects of over and under expression.
- Study how the mechanisms of RPS17 and RPS23 and how the overexpression of them affect the development of diseases.

Methods

- Bacterial Transformation
  - Transformed pcDNA3.1(c)DYK in DH5 alpha cells to test cell resistance to Carbenicillin
- PCR & Gel Electrophoresis
  - Linearized pNH-TrxT and pNIC28-Bsa4 plasmids through PCR and separated the amplified fragment and linearized vector through gel electrophoresis
- Plasmid minipreps to isolate plasmid DNA
- In-Fusion Cloning
  - Incubated the purified PCR fragment and PCR linearized vector together with assembly mix
- Bacterial colony creation (transformation) to plate RPS17 and RPS23 in bacterial E. coli cells
- Sequence analysis to confirm DNA sequence of clones
- IPTG Induction
  - Induced expression of T7 RNA polymerase from *lac*UV5 promoter
- Bacterial growth curve of E. coli cells with and without plasmids to assess fitness
- SDS-PAGE
  - Allowed proteins to separate and used Coomassie staining to visualize the proteins and confirm the gene expression
- Western Blot
  - Visualized the protein using HRP to confirm RPS17 and RPS23 gene expression

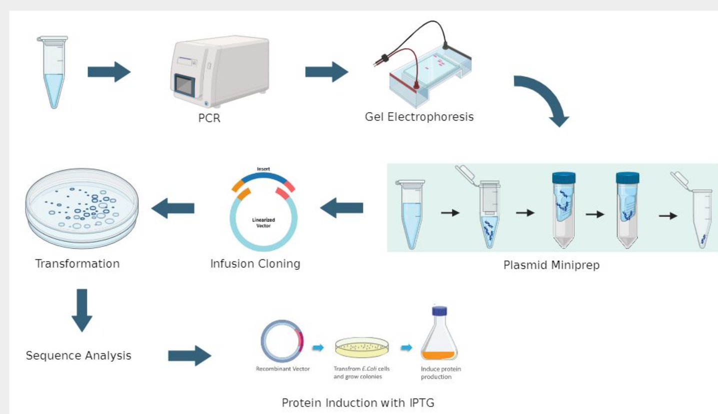
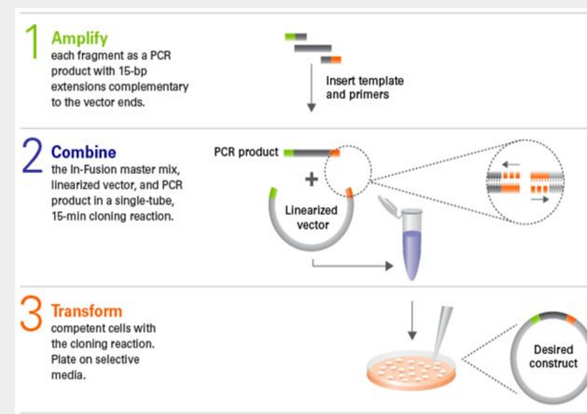


Figure 1: Diagram of techniques employed (Image generated on BioRender)

Figure 2: Infographic of In-fusion cloning of PCR products into stellar competent cells[5]



Results

Figure 3: Plasmid maps of pNH-TrxT-RPS17 and pNIC28-Bsa4-RPS17 confirmed with 100% sequence match

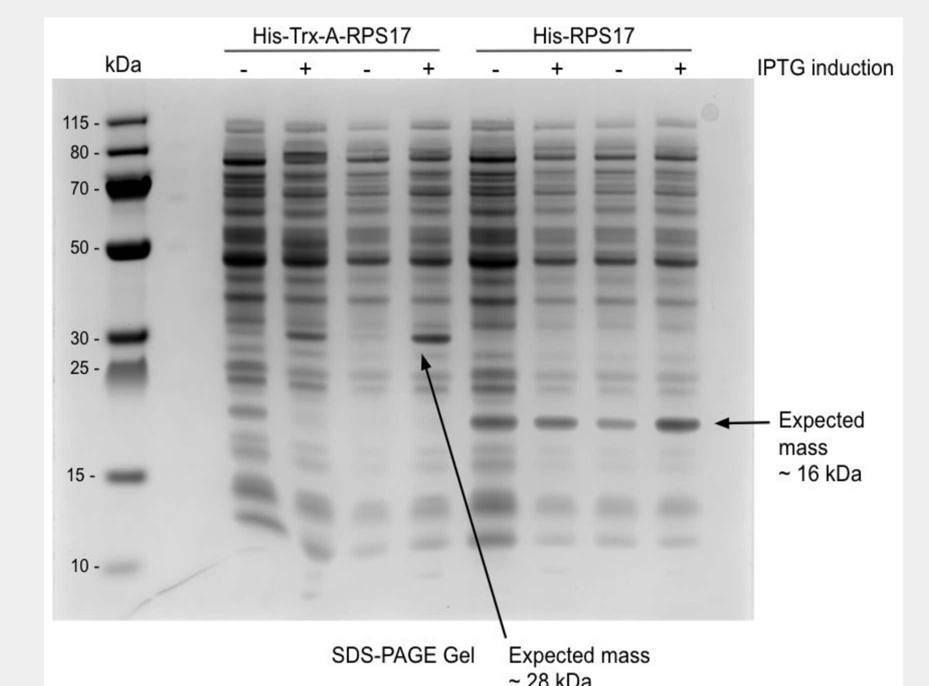


Figure 4: SDS-PAGE of pNH-TrxT-RPS17 and pNIC28-Bsa4-RPS17 transformants before and after induction with IPTG

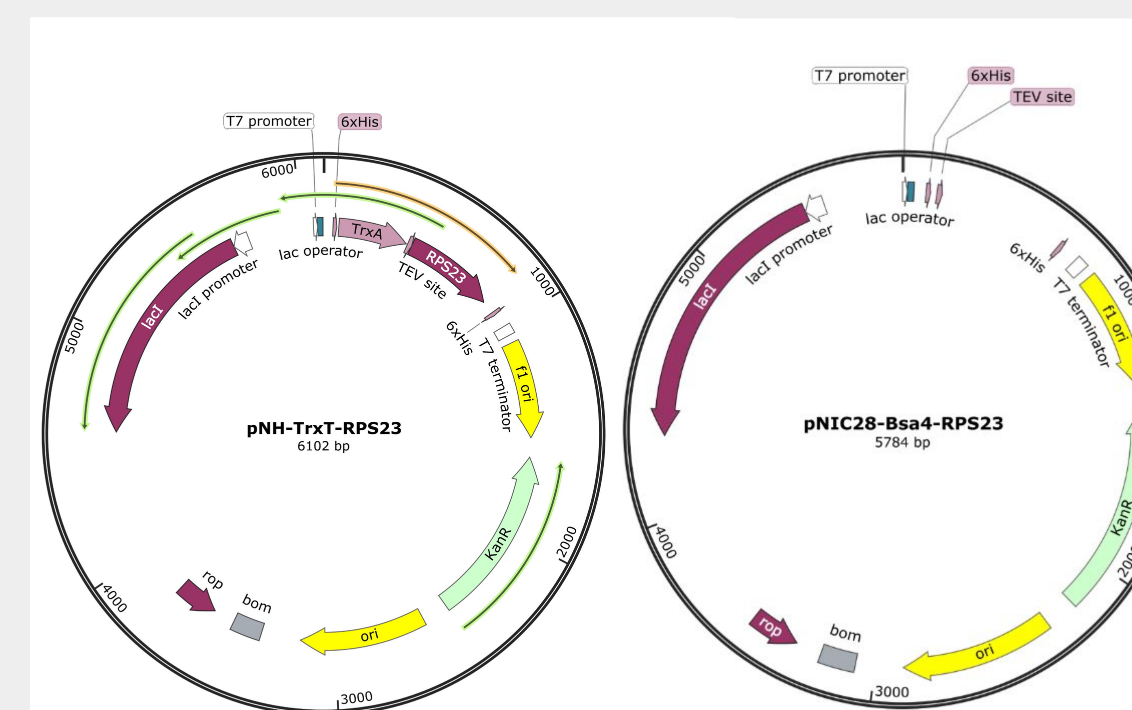


Figure 5: Plasmid maps of pNH-TrxT-RPS23 and pNIC28-Bsa4-RPS23 confirmed with 100% sequence match

Figure 6: SDS-PAGE of pNIC28-Bsa4-RPS23 and pNH-TrxT-RPS23 transformants before and after induction with IPTG

Discussion

- RPS17 and RPS23 were successfully cloned as shown in **Figure 3** and **Figure 5**
- Successfully expressed fusion proteins of RPS17 and RPS23 via IPTG induction, also seen in SDS-PAGE Gel **Figure 4** and **Figure 6**
- pNIC-RPS17 showed leaky expression without the induction of IPTG in **Figure 6**
  - pNH-TrxT is a more efficient vector for selectively expressing this protein

Future Directions

We aim to continue learning more about the structure and extraribosomal functions of RPS17 and RPS23, as well as their biochemical interactions and potential roles in disease. Further experiments will include affinity-based chromatography with a Ni+ column to purify the protein and use it as a substrate for in vitro assays. We will proceed with protein expression trials in HEK293T cells to observe the effects of overexpression and underexpression of RPS17 and RPS23 in vivo. We hope to relate these results to the functionality of the transfected RPS17 and its mutated variant which is connected to DBA as well as uncontrolled growth in RPS23, which can lead to cancer.

References

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