

Expressing Human Ribosomal Proteins S20 and S26 in Modified *E.coli* (BL21) Cells

Erika Quenano*, Jennifer Owen*, Maia Lee*, Andrea Ngouba, Hannah Zahniser, Talah Tafesh, Stacie Ofoegbu, Louis Weber, and Quira Zeidan
*Equal Contribution

INTRODUCTION

- Ribosomes are responsible for making proteins for the cell and are made up of 2 subunits, a large subunit and a small subunit
- The subunits are made up of ribosomal proteins (RPs), which are difficult to study since they are known to be insoluble outside the context of the ribosome
- The mutations in individual RPs are known to cause disruptions in translation, as well as the production of red blood cells which can cause a variety of diseases including Diamond-Blackfan Anemia (DBA) and different cancers¹
- RPs are modified with myriad post-translational modifications (PTMs), which may impact ribosome structure, subunit assembly, translation, and extra-ribosomal functions²
- The expression of human RPs in bacteria, followed by their purification via affinity chromatography, can assist in generating unmodified substrates for enzymatic assays aimed at investigating target sites for PTMs

Research Objective

- To clone human RPS20 and RPS26 into bacterial expression vectors pNH-TrxT and pNIC28-Bsa4 to evaluate the solubility of these resulting fusion proteins. pNH-TrxT has a His-Thioredoxin tag which has been proposed to improve solubility of RPs³

RESULTS

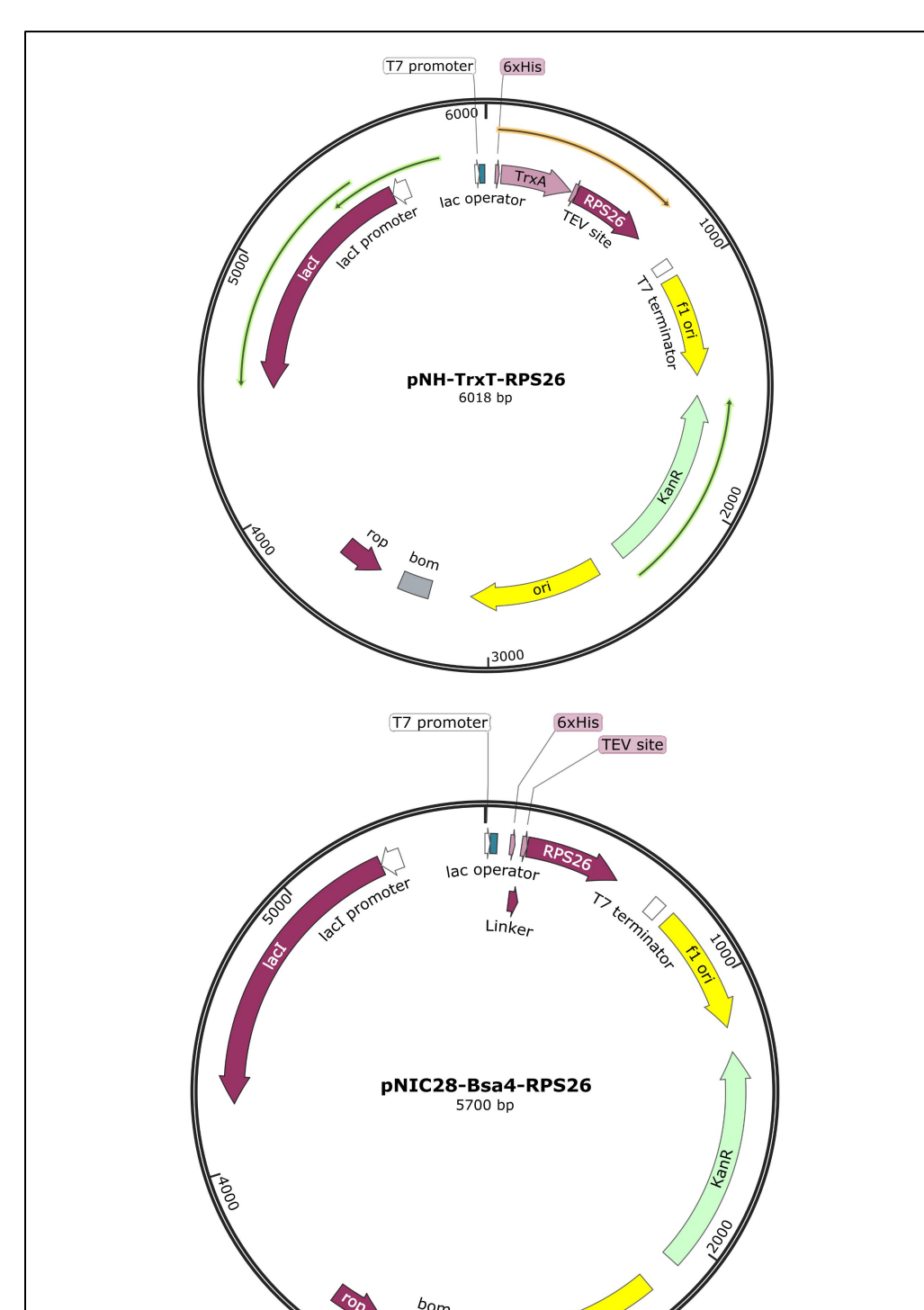


FIGURE 3a: SnapGene plasmid maps of successful human RPS26 cloning into pNH-TrxT and pNIC28-Bsa4 bacterial expression plasmids

pNIC28-Bsa4-RPS26 and pNH-TrxT-RPS26 IPTG Induction Protein Gel

Expected Molecular Weight:
pNH-TrxT-RPS26 = ~25 kDa
pNIC28-Bsa4-RPS26 = ~14 kDa

Observed Molecular Weight:
pNH-TrxT-RPS26 = ~27.5 kDa
pNIC28-Bsa4-RPS26 = ~17.5 kDa

Key
- = Pre-induction → Greater protein expression
+ = Post-induction

FIGURE 4: SDS-PAGE gel electrophoresis with Coomassie staining of pNIC28-Bsa4-RPS26 and pNH-TrxT-RPS26 protein expression in BL21 *E. coli* before and after overnight induction with IPTG (0.5mM) at 18°C

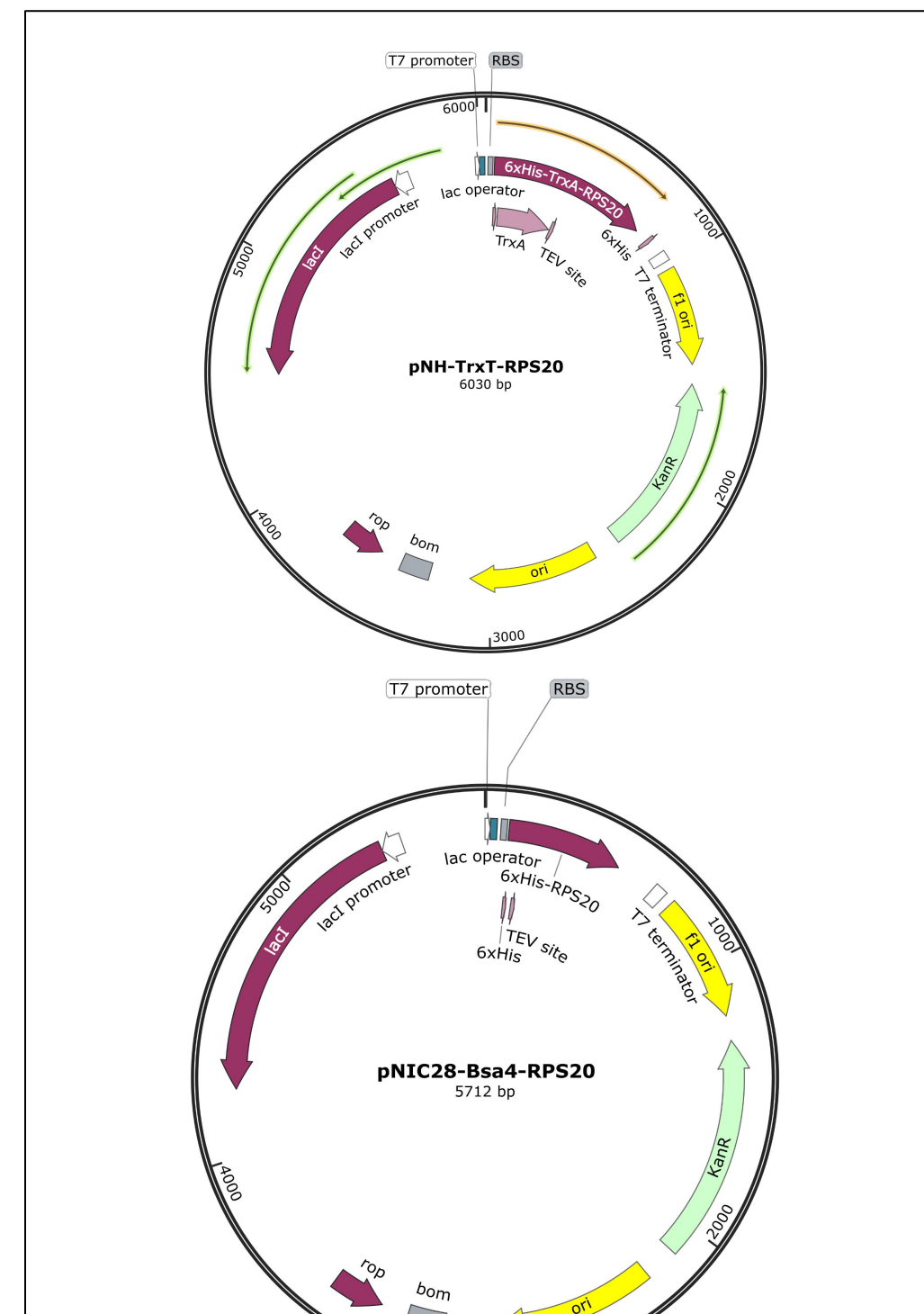
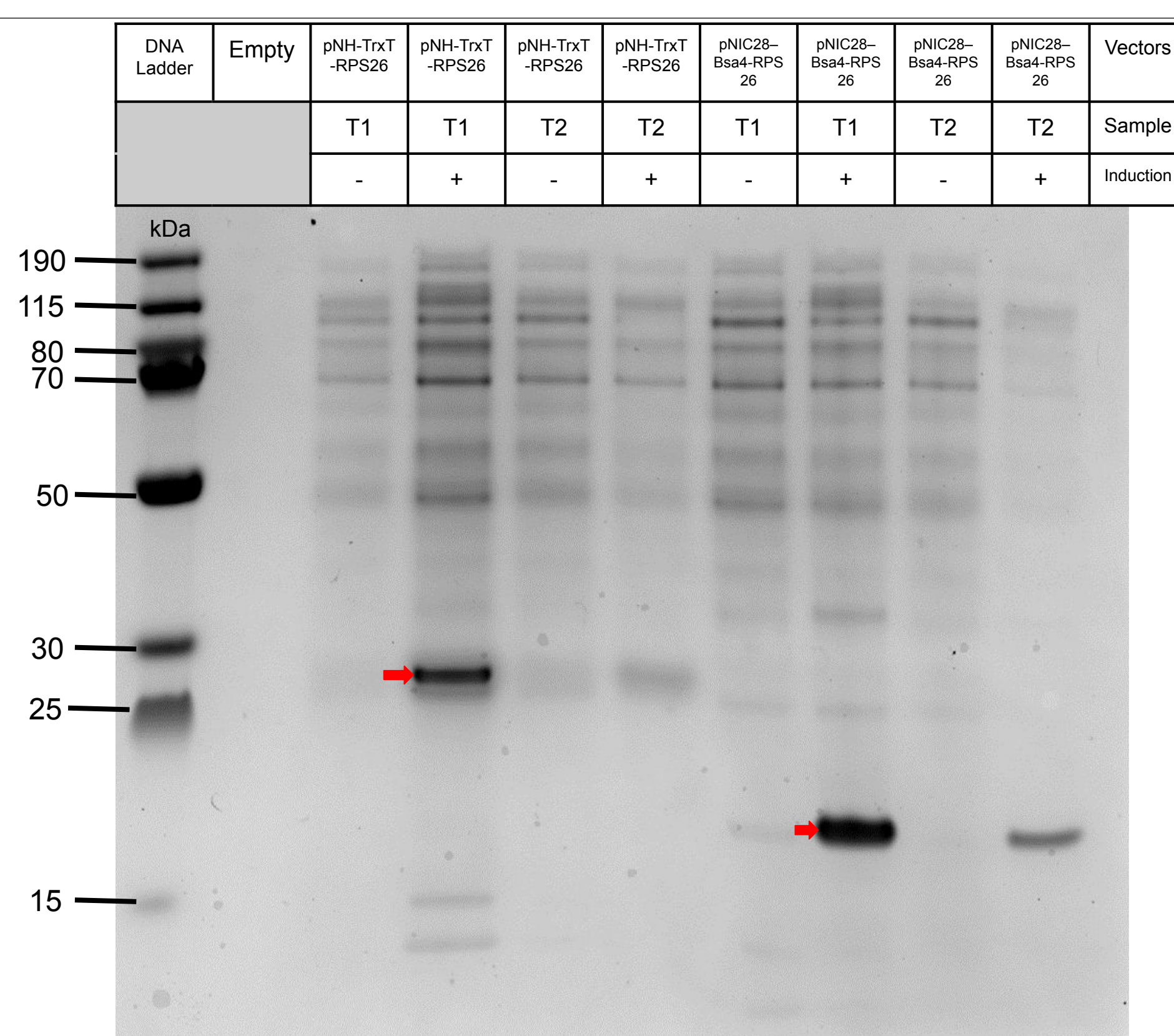


FIGURE 3b: SnapGene plasmid maps of successful human RPS20 cloning into pNH-TrxT and pNIC28-Bsa4 bacterial expression plasmids

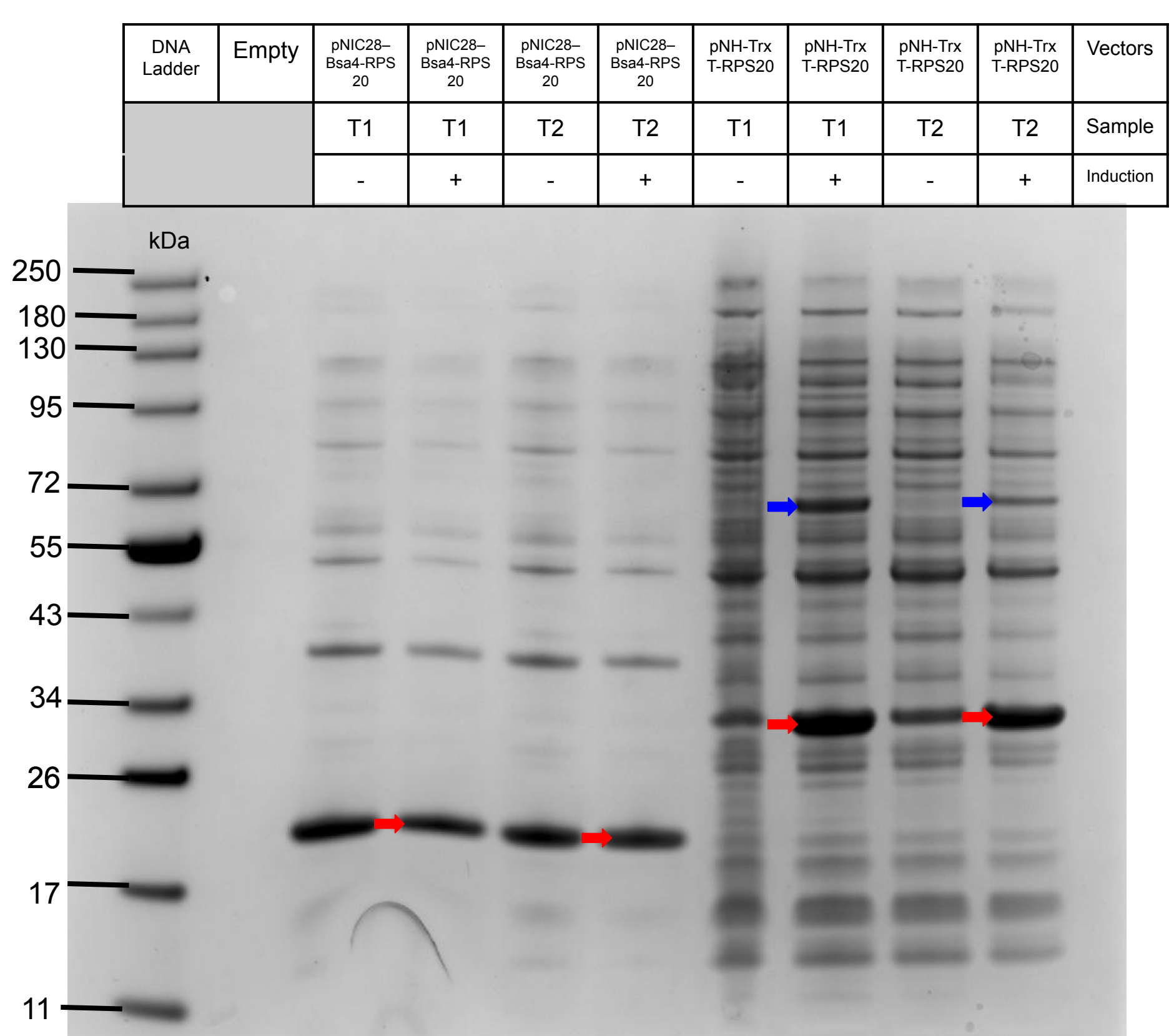
pNIC28-Bsa4-RPS20 and pNH-TrxT-RPS20 IPTG Induction Protein Gel

Expected Molecular Weight:
pNH-TrxT-RPS20 = ~27 kDa
pNIC28-Bsa4-RPS20 = ~16 kDa

Observed Molecular Weight:
pNH-TrxT-RPS20 = ~30 kDa
pNIC28-Bsa4-RPS20 = ~22 kDa

Key
- = Pre-induction → Non-target proteins that were induced in pNH-TrxT-RPS20
+ = Post-induction → Induced RPS20

FIGURE 5: SDS-PAGE gel electrophoresis with Coomassie staining of pNIC28-Bsa4-RPS20 and pNH-TrxT-RPS20 protein expression in BL21 *E.coli* before and after a one hour induction with IPTG (0.5mM) at 37°C. Bands that consisted of non-induced and induced conditions reveal leaky expression, and unexpected bands were also included in the pNH vector



METHODS

- Transformed cloned plasmids of pNIC28-Bsa4-RPS20, pNIC28-Bsa4-RPS26, pNH-TrxT-RPS20, and pNH-TrxT-RPS26 into DH5 alpha competent *E. coli* cells
- Used PCR to amplify the human RPS20 and RPS26 genes, isolated DNA fragments through gel electrophoresis
- Inserted PCR products into linearized bacterial expression vectors pNIC28-Bsa4 and pNH-TrxT
- Transformed new cloned plasmids into BL21 *E. coli* cells
- Determined optimal induction conditions for *E. coli* cells using spectrophotometry to obtain bacterial growth curves
- Induced cells with IPTG (0.5 mM) at various temperatures and times post-inductions to determine optimal expression
- Lysed BL21 cells and separated by denaturing gel electrophoresis (SDS-PAGE) to measure protein expression
- Gel stained with Coomassie Blue
- In parallel experiments, RPS20 and RPS26 cloned into pcDNA3.1-CDYK were transfected into human embryonic kidney (HEK293T) cells with FuGene reagent
- Lysed HEK293T cells and separated by denaturing gel electrophoresis (SDS-PAGE) to measure to protein expression
- Analyzed results using Western blot with a secondary antibody conjugated to HRP

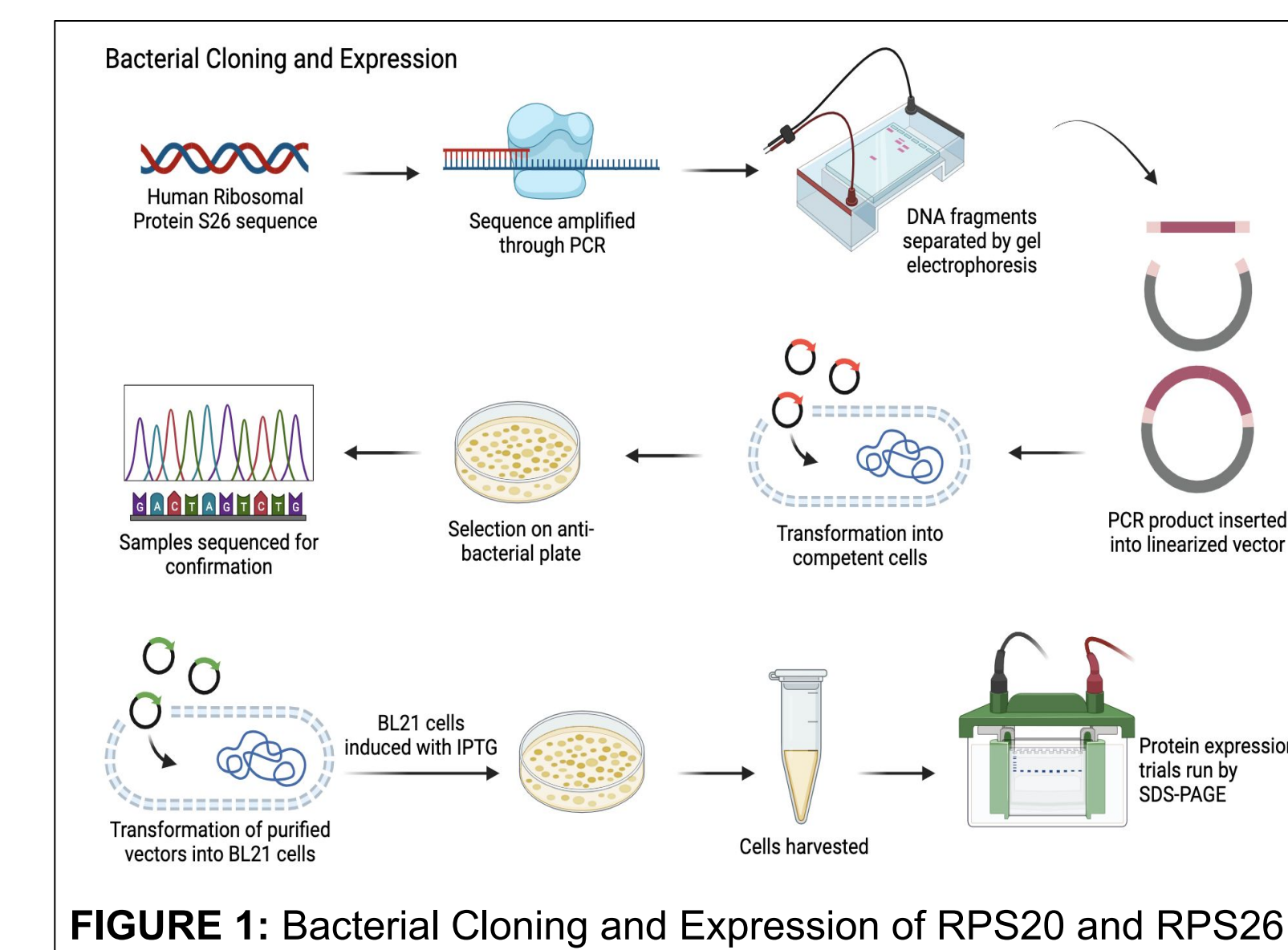


FIGURE 1: Bacterial Cloning and Expression of RPS20 and RPS26

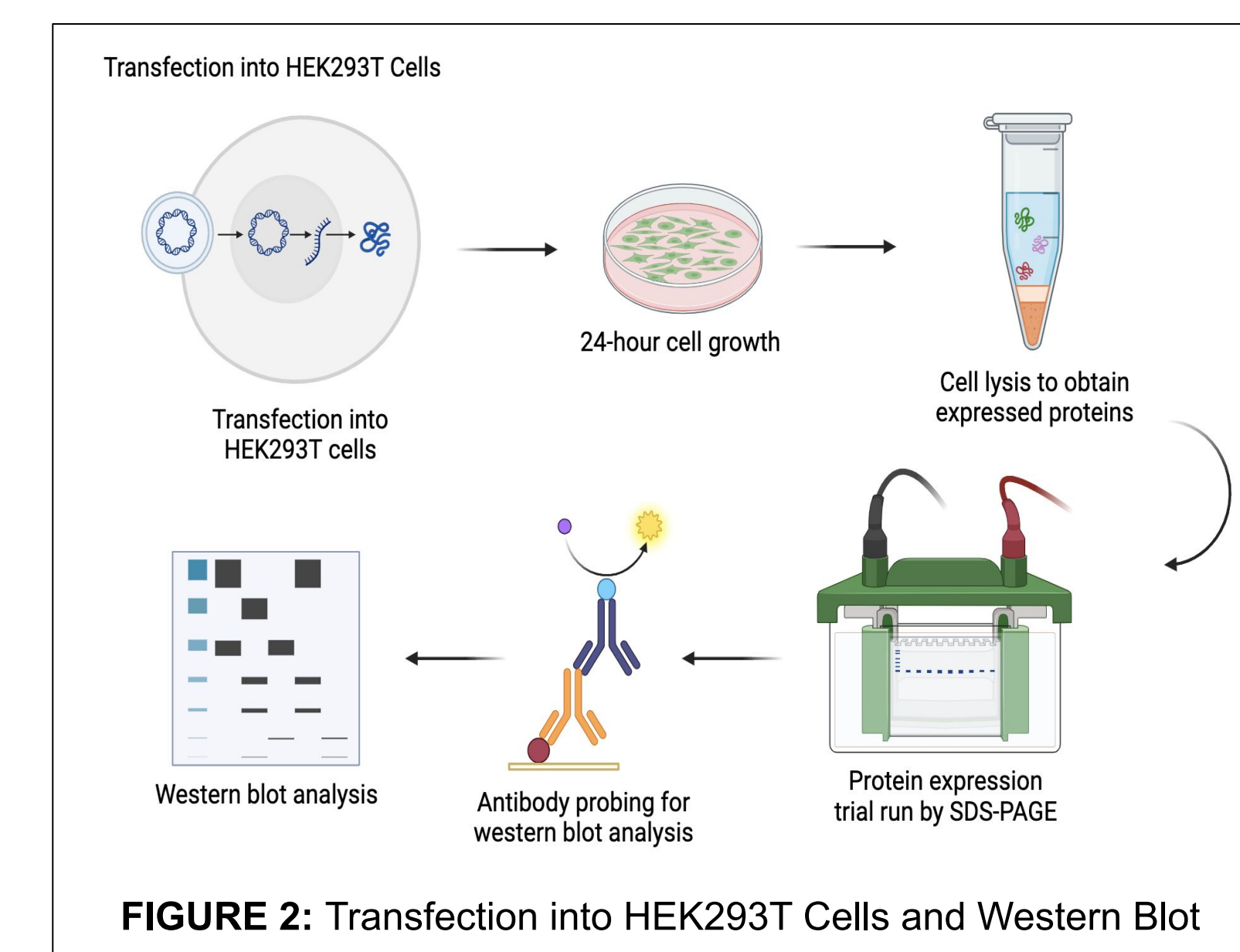


FIGURE 2: Transfection into HEK293T Cells and Western Blot

CONCLUSIONS

- We successfully cloned and expressed human RPS26 in *E. coli* cells after induction with IPTG
- Optimal IPTG induction conditions were 18°C overnight for RPS26
- We are still determining optimal conditions for expression of RPS20 from the bacterial expression vectors used
- RPS20 showed leaky expression at 37°C, where non-induced vectors still produced target proteins
- Expression of RPS20 from the pNH-TrxT vector revealed a protein closer to the expected molecular weight

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FUTURE DIRECTIONS

- Affinity purification of fusion protein His-TrxA-RPS20 and His-TrxA-RPS26 to purify RPs and analyze their PTMs via *in vitro* enzymatic assays
- Further map targets sites of modification of RPS20 and RPS26 by mass spectrometry
- Express RPS26 and RPS20 in HEK293T cells for immunoprecipitation to study PTMs and protein interactions
- Investigate the roles of RPS20 and RPS26 in various diseases such as carcinoma and Diamond Blackfan Anemia