FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

CELLEX

Using site-directed mutagenesis to engineer human ribosomal protein variants

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Introduction

- Ribosomal Proteins (RPs) are the subunits that make up the ribosome
- Although the function of the assembled ribosome is known, the extraribosomal functions of the individual proteins are understudied
- Following translation, the eukaryotic cell modifies ribosomal proteins with Post-Translational Modifications such as glycosylation
- **Objectives:**
  - Create plasmid constructs encoding ribosomal proteins using a Site-Directed Mutagenesis (SDM) protocol
  - Express modified RPS3A with mutations in bacterial cells
  - Express RPS6 in HEK-293 cells to carry out fluorescence and fluorescent microscopy assays

Methods

- 2 plasmids contained the coding sequence for RPS6 with an autofluorescence protein (mTurquoise) one of them also containing a nuclear export signal (NES) sequence and RPS3A
- 2 PCRs with modified primers for SDM
  - A single nucleotide erroneously inserted into the RPS6 plasmid was removed
  - Primers were mutated at 2 Serines determined to be glycosylation sites on the RPS3A plasmid
- RPS6 plasmid constructs were transfected into HEK-293 cells. Cells were plated on a 96 well plate and analyzed using a fluorescence plate reader to confirm the chimeric protein was fluorescing. Arbitrary fluorescence values for mTurq-RPS6 fusion proteins plotted using excel (Figure 8), 10 assays were carried out for each plasmid. A standard curve was calculated using 1:2 dilutions of 10 μM Fluorescein blue dye with PBS
- The RPS3A plasmids were transformed into BL21 E.Coli bacteria and expression was induced and identified with an SDS-PAGE followed by a Coomassie blue stain

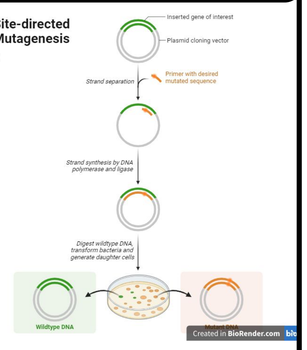


Figure 1: Diagram of SDM

Results

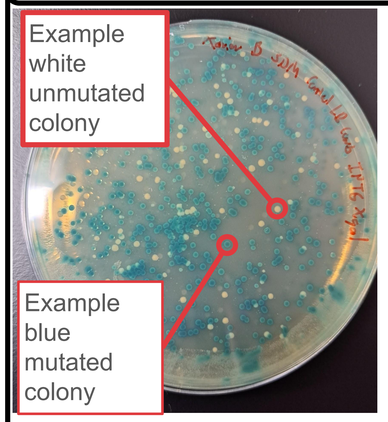


Figure 2: Control plate for SDM. Blue colonies are mutated and successfully expressing lacZ. Efficiency is > 80%

RPS3A Substitution Results

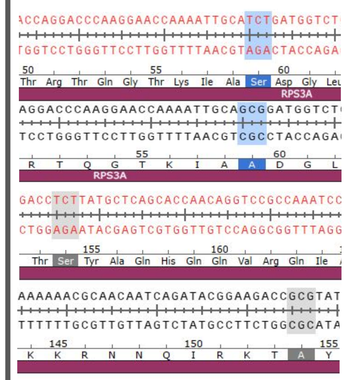


Figure 3: Sequencing results showing the substitution of Alanine for Threonine in RPS3A

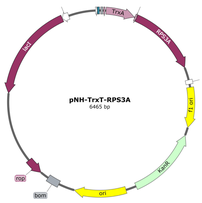


Figure 4: pNH-Trxt RPS3A plasmid map

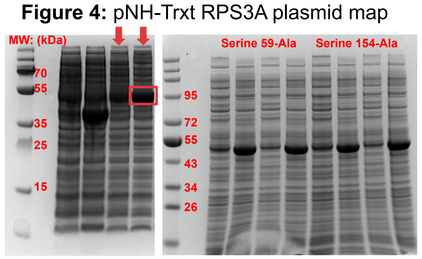


Figure 5: Coomassie stained gels showing wild type RPS3A and mutated RPS3A extracted from BL21

RPS6 Fusion Protein Results

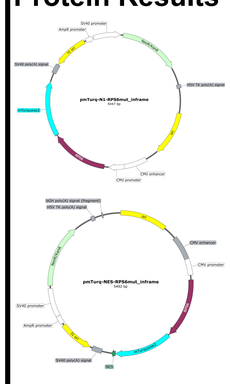


Figure 6: pmTurq-N1-RPS6(top) and pmTurq-NES-RPS6 (bottom) plasmid maps

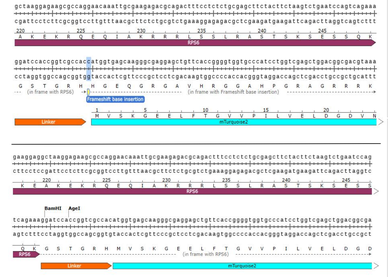


Figure 7: Sequencing results of pmTurq-N1-RPS6 before(top, out of frame) and after(bottom, in frame) SDM. Nucleotide targeted for deletion highlighted in blue. SDM removed the same nucleotide in pmTurq-NES-RPS6 plasmid sequence

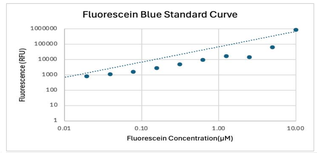


Figure 8: Fluorescein standard curve measured in 96 well plate with a linear fit of Y=68228x, R^2=0.8127

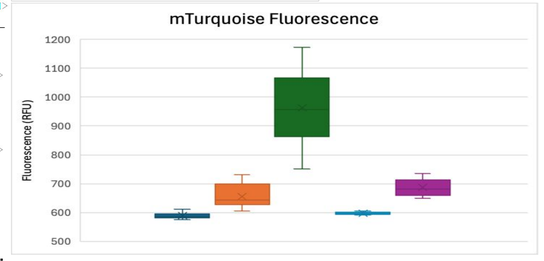


Figure 9: Results from 96 well plate fluorescence assay of HEK-293 cells transfected with the following plasmids, Blue: No transfection, Orange: N1, Green: N1⦰, Cyan: NES, Purple: NES⦰ measured at GFP optimal wavelength

Discussion

- Transformation efficiency of the site directed mutagenesis protocol was seen to be over 80%
- Sequencing of the plasmids generated using the SDM protocol indicated successful modification of the desired sites
- Fluorescence patterns indicated the successful creation of a chimeric RPS6 protein with the reading frame restored
- SDS-PAGE analysis of protein extracted from BL21 cultured with mutated RPS3A plasmids indicates successful inducible expression
- mTurquoise-RPS6-NES fusion protein showed fluorescence at around the same levels as the untransfected cells, this could mean that proteasomes in the cytoplasm are degrading the protein, possibly because it is toxic to the cell

Future Directions

- Proven method to repair mistakes that could arise in other plasmids with other proteins or test other post-translational modification sites
- Double RPS3A Mutant will be created
- Modified RPS3A proteins will be used for detecting post-translational modifications in in-vitro assays
- New chimeric RPS6 proteins generated will be used to identify the location of RPS6 in the cell
- Methodology can be applied to other Ribosomal Proteins
- Express mTurquoise-RPS6-NES in cell lines along with proteasome inhibitors to observe RPS6 activity in the cytoplasm

References

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