Production of SPF30 Tudor domain in E. coli as binder for Dimethylated Arginine

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Introduction

The project that was assigned to me by my P.I. was a project in which I was given the task to clone a gene using the plasmid vector, "pET-28a-c(+)" that would be absorbed by a bacteria. In turn, the gene would be replicated through the bacteria's reproduction growth. Eventually, the gene that was "pasted" into the plasmid will eventually be expressed as the protein SPF30. While within the plasmid, the SPF30 would be linked to a histone tag, which will assist in the process of extracting the protein from the plasmid for further use where it will assist in binding methylated arginine.

Figure 1: The figure to the left is the protein produced by the plasmid pET-28a(+). SPF 30 is specifically found in homo sapiens and is an RNA binding protein

Figure 2: The figure to the right is a Nanodrop that measures the absorption and calculates the concentration of proteins and nucleic acids , using mathematical algorithms.

Objective & Impact of Professor's Research

Professor Graham's lab pursues experimental and computational systems biology approaches to develop quantitative models of cancer and other human diseases. The lab draws biology, statistics, and engineering to build data-driven, predictive models of tumor phenotypes using quantitative data generated in-house. The genetic events that drive human disease must be implemented at the protein level. Using mass spectrometry, they are identifying and quantifying proteins and their post-translational modifications in cell lines, mouse models and human patient samples.

Materials and Methods

Through the seven weeks of the SHINE program journey, I was exposed to a plethora of fascinating molecular biology and analytical chemistry knowledge and techniques. The production of SPF30 has several steps, including bacterial cell culture, gene cloning, protein extraction, protein purification, and electrophoresis analysis. I managed to clone the plasmid containing the target gene of the SPF30 Tudor domain into E. coli bacteria. Since the plasmid has an antibiotic resistance gene, the bacteria that failed to obtain the plasmids was discarded from the cell culture by using Kanamycin. Adding IPTG activated the lac operon of plasmid and induced SPF30 gene expression inside the bacteria. The protein was extracted via the Ni-NTA column through the polyhistidine (His) tag added to the SPF30 protein sequence on the plasmid. Then we ran electrophoresis analysis to admit protein expression inside the bacteria. Alongside my project, I was familiarized with statistics concepts and R programming applications in biological research.

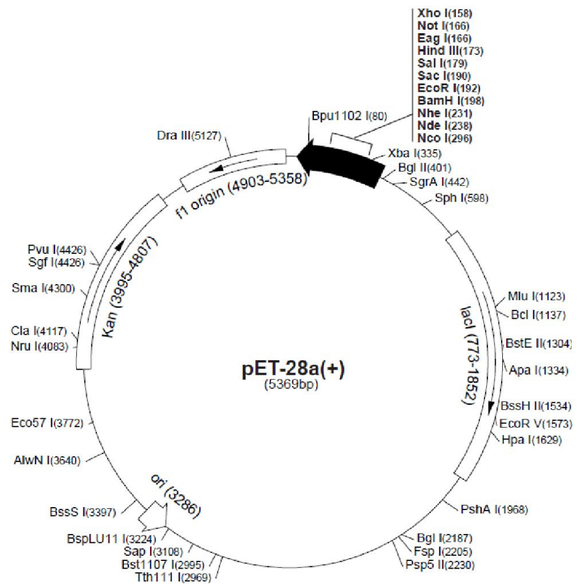

Figure 3: The figure above is the plasmid pET-28a(+) which is a nonviral vector that we used for bacterial expression and is resistant to the antibiotic kanamycin. pET-28a(+) 's tag is the histone tag.

Discussion and Conclusions

The outcome that followed the process of producing the SPF30 will impact the future projects of my lab. It has been reported that SPF30 has a binding affinity to Dimethylated Arginines. Arginine residues are post-translationally modified to include methyl groups, resulting in monomethyl arginine and symmetric and Asymmetric dimethyl arginine. Methylation of arginine residues is involved in the regulation of fundamental cellular processes, including transcription, RNA processing, signal transduction cascades, and the DNA damage responses, which are all contributed to the cancer cell proliferation and aging processes. Dr. Graham's lab is trying to develop new binders that can enrich methylated arginine residues to enable the study of them with mass spectrometry techniques. Production of this Tudor domain will pave the way for further research on methylation profile of proteins through mass spectrometry for cancer and aging research.

How This Relates to Your STEM Coursework

SHINE had great impact on my overall STEM coursework as it gave me great exposure to a type of study that I had never previously been exposed toor have interest in. Prior to SHINE I did not have much interest in pursuing a STEM field in the future. However, with my exposure in Dr. Graham's Chemical Engineering Lab, I soon gained a true interest in pursuing a STEM field. This came into fruition through the knowledge that I absorbed when seeing the work that my mentor carried out in the lab and the impact his work had on society. I had no clear idea on what exactly a scientist did in a lab, but soon came to learn and appreciate the complex work that the Graham lab carried out.

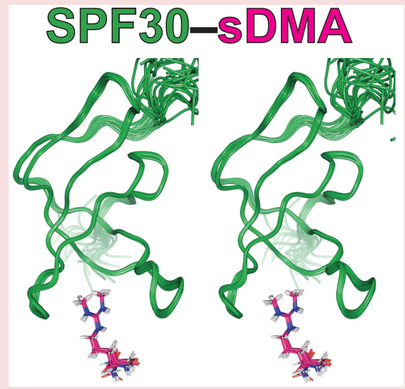

Figure 4: structure ensembles of SPF30 bound to symmetric DiMethylated Arginine.

Advice for Future SHINE Students

My advice for any future SHINE students or those who have interest in being part of the SHINE program would be to enjoy the most out of the limited time you have of this experience as although initially seven weeks may sound like a long time, in reality it is not and goes by extremely fast. I would also advise them to take as much advantage as possible from this great and unique opportunity as not every student has the chance to encounter SHINE and be involved with the program. Another very important piece of advice to give would be to ensure that you do have an impact on the lab you are in and its work and research and although you may not directly work in the lab's general research as in my case, remind yourself that you still contribute in some shape of form.

Acknowledgements

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