The Arginine Methylation of P14ARF

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Introduction

P14ARF is a tumor suppressing protein that inhibits the growth of cancer cells by indirectly activating p53 which is a key tumor suppressing gene. P14ARF is mostly made up of the following four amino acids Arginine, Alanine, Glycine, and Leucine. This protein is important to deepen our understanding of cancer progression.

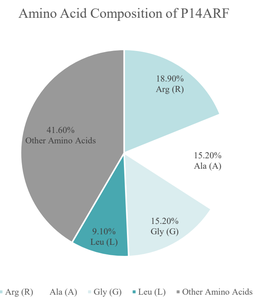

Figure 1: A pie chart that depicts the Amino Acid composition of p14ARF.

Goal

It is our goal to (1) express recombinant p14ARF protein via autoinduction in E. Coli expression system, (2) purify p14ARF via chromatography, and (3) characterize the posttranslational modification via arginine methylation. By doing so we can learn much deeper information that can help with the battle against cancer.

Methods

Autoinduction: A simple approach for protein expression that needs little user intervention after inoculation of the E. coli cultures.

Chromatography: This is based on His-tag affinity purification, and electrostatic separation.

Gel Electrophoresis: The method used to visualize the purity of proteins by molecular weight.

Methyltransferase Assay:
A bioluminescence-based assay that can be used to monitor the activities of SAM dependent methyltransferases (MTases) and can be used for all classes of substrate including arginines.

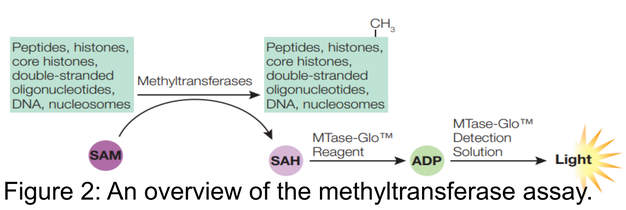

Figure 2: An overview of the methyltransferase assay.

Results

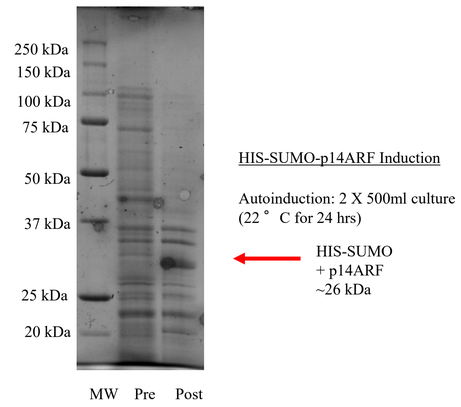

Figure 3: Shows the p14ARF protein production of the pre- and post-autoinduction of E. Coli culture.

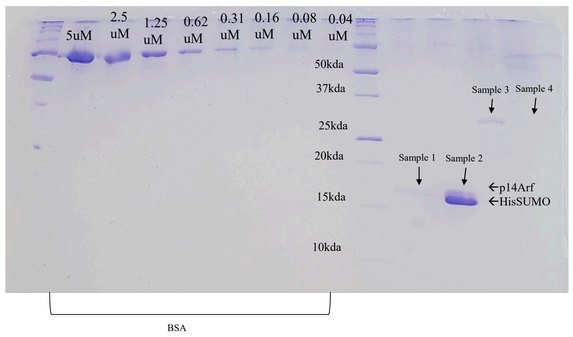

Figure 4: This gel electrophoresis was used to estimate the p14ARF concentrations by comparing to a gradient of BSA standard concentrations. Sample 1 is < 2 nM, Sample 2 ≈ 3.7 uM, Sample 3 ≈ 1 uM, Sample 4 < 40 nM.

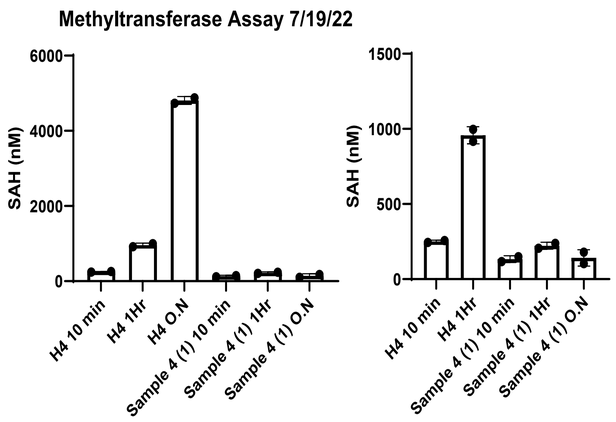

Figure 6: (Left) The graph shows the results of the methyltransferase assay with sample 4(1) with the positive control of histone H4 peptide (residues 1-20) at 10 uM, PRMT1 enzyme at 0.2 uM, and 10 uL of sample 4(1) added. (Right) The enlarged graph shows the weaker activities without the H4 Overnight sample to provide a closer look.

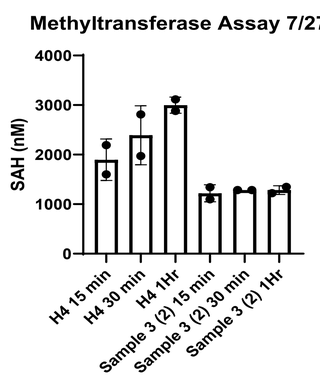

Figure 8: This graph shows the time dependent results of the methyltransferase assay with Sample 3(2) with 10 uL added, with the positive control of histone H4 peptide (residues 1-20) at 10 uM, PRMT1 enzyme at 1 uM.

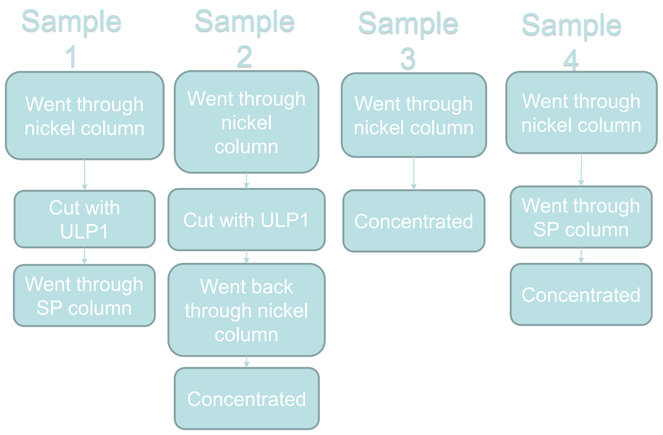

Table 1: Shows the flow chart of the four different purification processes of the different p14ARF samples (1-4) were acquired.

Methyltransferase Assay 7/15/22

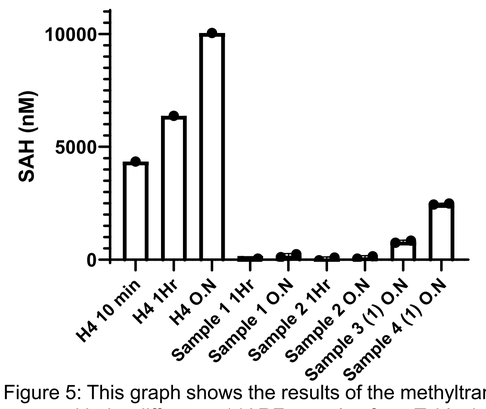

Figure 5: This graph shows the results of the methyltransferase assay with the different p14ARF samples from Table 1 with 10 uL used, with the positive control of histone H4 peptide (residues 1-20) at 10 uM, PRMT1 enzyme at 1 uM. The H4 sample was taken at 7/13/2022.

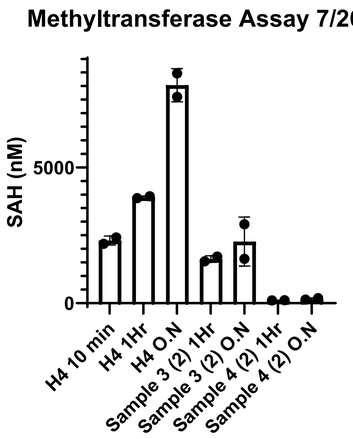

Figure 7: This graph shows the results of the methyltransferase assay with the different p14ARF samples from Table 1 with 10 uL added, with the positive control of histone H4 peptide (residues 1-20) at 10 uM, PRMT1 enzyme at 1 uM.

Conclusions

- Sample 3 which went through the nickel column, and was concentrated, and sample 4 which went through the nickel column, then the SP column, and was concentrated showed both showed high methylation activity compared to the others.
- Samples 3 and 4 are His-SUMO tagged.
- Sample 2 is cleaved but contains both p14ARF and His-SUMO. Sample 2 has very weak activity.
- In the future we plan to optimize the activity of the methylation of p14ARF.

References

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