Clark University
Clark Digital Commons

Undergraduate Student Academic Festivals | ClarkFEST Fall 2022

Oct 1st, 12:00 AM

The Effects of tRNA Methylation on Protein Synthesis in Dictyostelium discoideum

Chau Minh Duong
Clark University, cduong@clarku.edu

Follow this and additional works at: https://commons.clarku.edu/asdff

(2022). The Effects of tRNA Methylation on Protein Synthesis in Dictyostelium discoideum. Retrieved from https://commons.clarku.edu/asdff/clarkfestfall2022/Posters/32

This Open Access Event is brought to you for free and open access by the Student Works at Clark Digital Commons. It has been accepted for inclusion in Undergraduate Student Academic Festivals by an authorized administrator of Clark Digital Commons. For more information, please contact larobinson@clarku.edu, cstebbins@clarku.edu.

The Effects of tRNA Methylation on Protein Synthesis in *Dictyostelium discoideum*

Chau Duong '22, Professor Denis A. Larochelle

Biology Department - Summer Undergraduate Research Experience (SURE) Award Recipient

CLARK UNIVERSITY

Introduction

Epitranscriptomics describes RNA modifications leading to changes in gene expression. This is a potential research avenue in studying therapeutic targets for cancer. In this research project, we focus on studying how methylating transfer RNA (tRNA - which delivers amino acids during translation) will affect protein synthesis.

- Dnmt2/Trdmt1 (tRNA aspartic acid methyltransferase) in humans and DnmA in *Dictyostelium discoideum (D.discoideum)* are highly conserved, homologous enzymes that perform tRNA methylation (1,2). Structural analysis of human Dnmt2 reveals a binding site for SAM- a cofactor in the methyltransferase reaction.
- DnmA mutant cells (DnmA KO) showed abnormal cell morphologies: multiple sori, larger cell size, multiple centrosomes (3). Gene ontology revealed that this enzyme is also linked with other components regulating various cellular processes.
- Codon usage bias is the preference among synonymous codons in protein synthesis. This is a highly conserved phenomenon across eukaryotes. Codon usage bias can also affect gene expression by controlling translation efficiency.

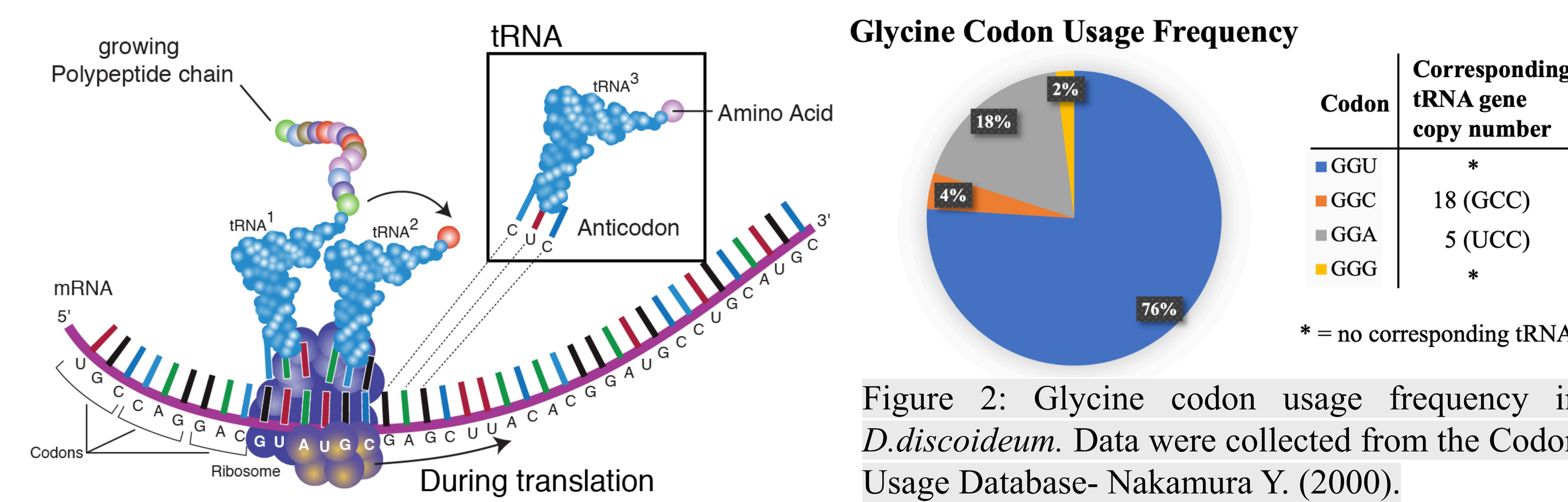
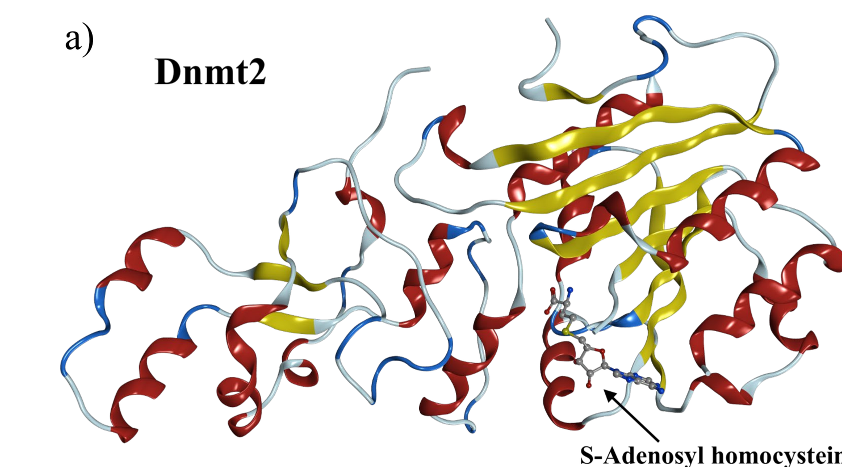


Figure 1: tRNA in the translation process (Source: NIH)

Figure 2: Glycine codon usage frequency in *D.discoideum*. Data were collected from the Codon Usage Database- Nakamura Y. (2000).



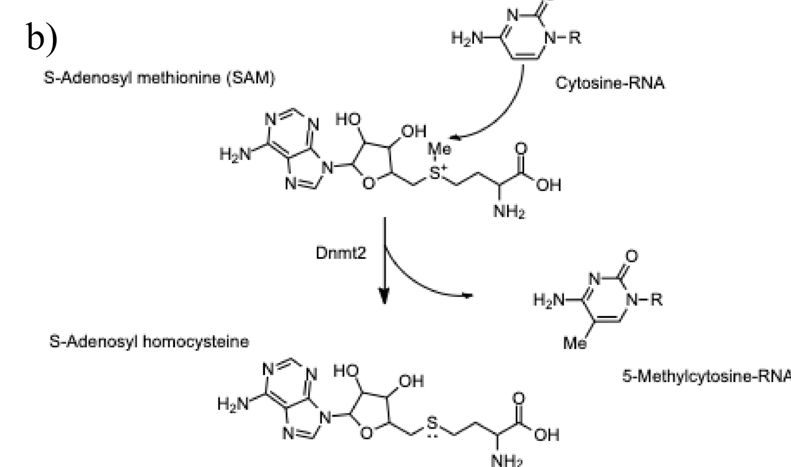


Figure 3: a) 3D Structure of human Dnmt2- (generated using MOE Program 2020, PDB-ID:1G55); b) Reaction mechanism of methylation of cytosine by Dnmt2 with SAM cofactor (generated with ChemDraw Program)

Research Questions

- **Does tRNA methylation by DnmA affect protein synthesis? If so, what are the molecular targets?**
- **What is the relationship between DnmA tRNA methylation activity and codon usage bias?**

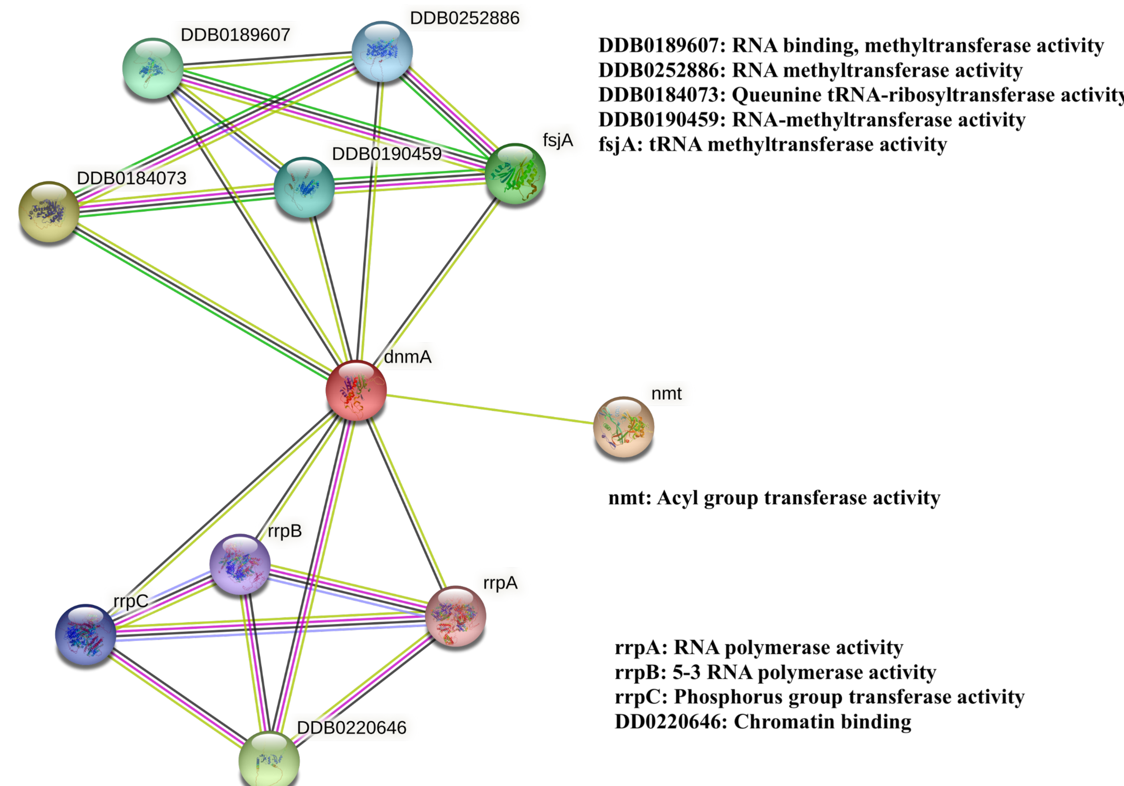


Figure 4: Gene ontology predictions of potential interactors of dnmA and their associated molecular functions. Data were obtained from STRING Database ver. 11.5

Methods

Vector Cloning

1. Generate 3 vector DNA constructs, each with a stretch of 8 **glycine** repeats of one of the 3 glycine codons (GGT, GGC, GGA)

- D-TOPO Reaction: Insert 8 glycine repeats of **GGT/GGC/GGA** codons into pENTR™/ D-TOPO vector
- LR Reaction: Insert D-TOPO vector contained glycine repeats into destination vectors to fuse the repeat with a **Green Fluorescence Protein (GFP) tag** or a **Red Fluorescence Protein (mRFP) tag**

2. Confirm the vector cloning by restriction digest, PCR, and Sanger gene sequencing

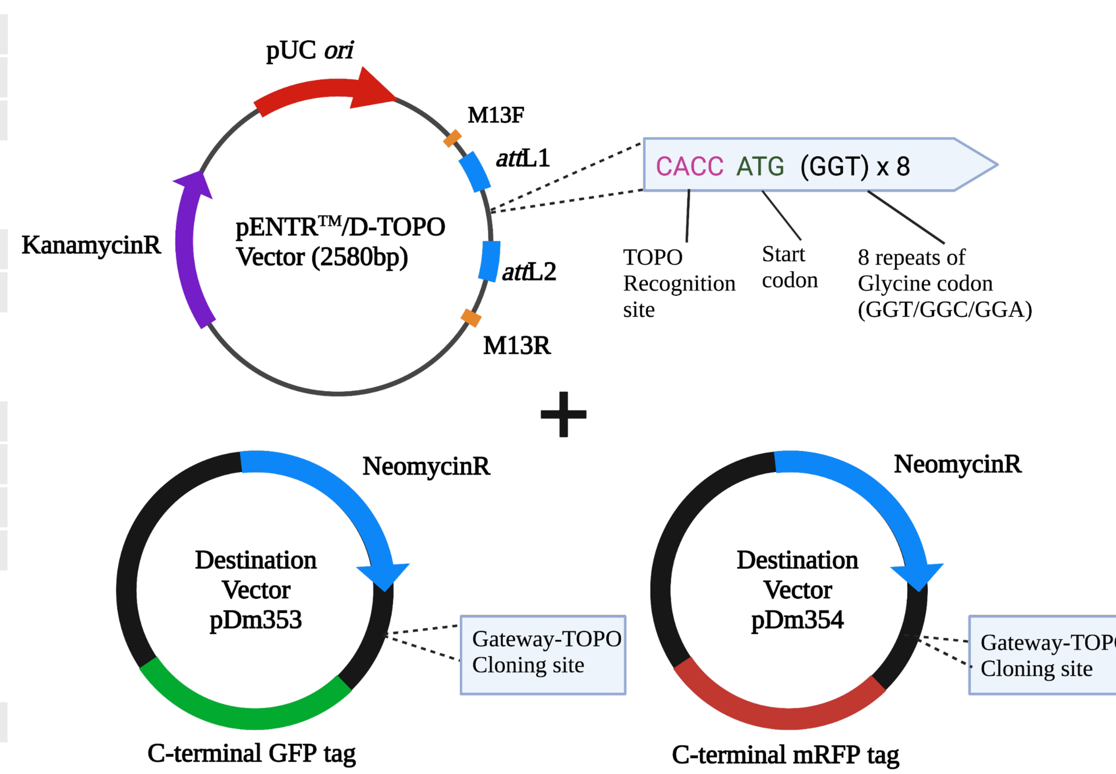
- Restriction digest with a single-cutter enzyme: EcoRV or ApaI.
- PCR with M13 primer and the insert primer to amplify the inserted sequence. The expected band on gel electrophoresis is **156bp**.
- Positive clones expressing the inserted band will be confirmed with gene sequencing.

Figure 5: Vector cloning map and codon repeat insert design. This working scheme includes:

1- inserting the DNA designed construct into the D-TOPO vector,

2- transferring the insert from D-TOPO to the destination vector containing the fluorescence tag using the LR reaction.

(Image was created using Biorender.com)



Protein Expression & Molecular Target Identification

1. Express DNA constructs in wild-type (AX4 strain) and DnmA-KO *D.discoideum* cell lines. Normal protein synthesis of the constructs will express GFP/mRFP. Cells will be observed under fluorescence microscopy and measured for fluorescence intensity using FACS (Fluorescence-activated cell sorting flow cytometry).

2. Using R programming to screen for **glycine-rich** protein targets from *D.discoideum* coding sequence database. If the effects of tRNA methylation are confirmed, we will investigate the potential molecular targets, including **glycine-rich** proteins.

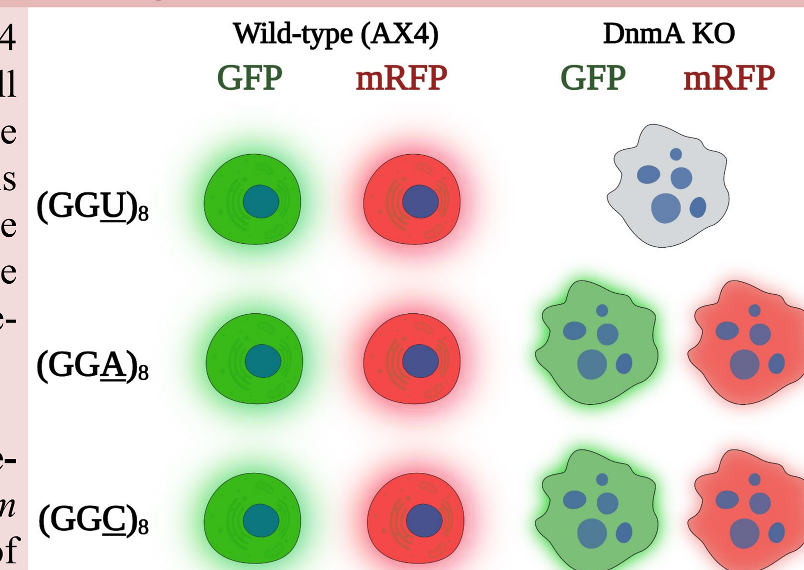


Figure 6: Predictions of protein expression in wild-type (AX4) and DnmA KO *D.discoideum* cells (Image was created using Biorender.com)

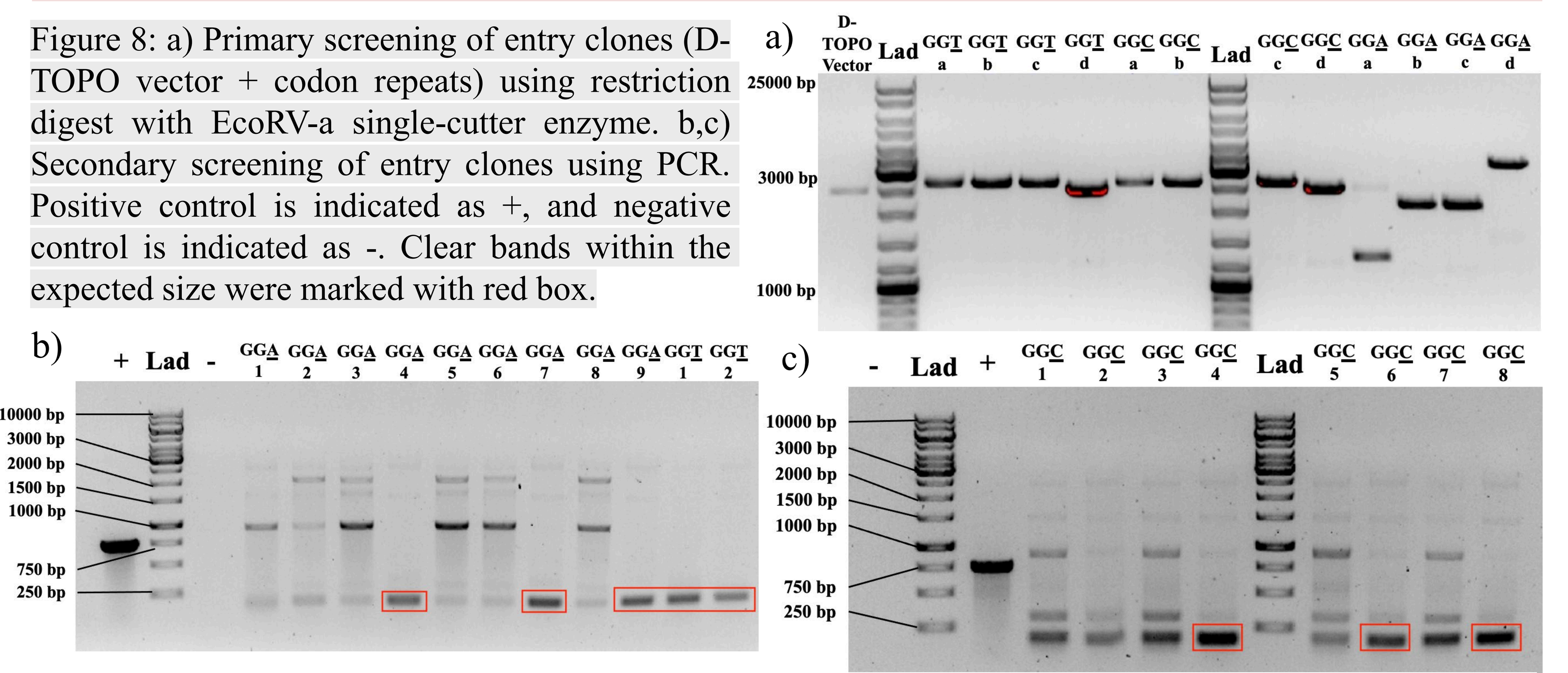
Order	Number of 10xG stretch	Sequence/Gene ID
561	1	DDB0307627|DDB_G0277479
646	1	DDB0214814|DDB_G0281545
1348	1	DDB0349171|DDB_G0288827
1627	1	DDB0220438|DDB_G0271870
2078	1	DDB0215358|DDB_G0277845
2344	1	DDB0238210|DDB_G0287951
2493	1	DDB0233625|DDB_G0284725
2534	1	DDB0305479|DDB_G0285487
2806	1	DDB0347280|DDB_G0294004
2873	1	DDB0233920|DDB_G0279657

Figure 7: Example of 10/34 results of glycine-rich proteins containing a stretch of 10X glycine obtained with R. The search includes a number order for the sequence, number of 10xG stretch and the sequence/gene ID.

Preliminary Results

- Successfully generated the designed DNA reporter constructs, each with 8 repeats of **GGT/GGC/GGA** and a fluorescent tag (Figure 9,10,11).
- The PCR results alone could not distinguish the orientation of the insert (Figure 8). The constructs were confirmed with Sanger sequencing.
- The first transfection showed no fluorescent signal in both the control and DnmA - KO cells.

Figure 8: a) Primary screening of entry clones (D-TOPO vector + codon repeats) using restriction digest with EcoRV-a single-cutter enzyme. b,c) Secondary screening of entry clones using PCR. Positive control is indicated as +, and negative control is indicated as -. Clear bands within the expected size were marked with red box.



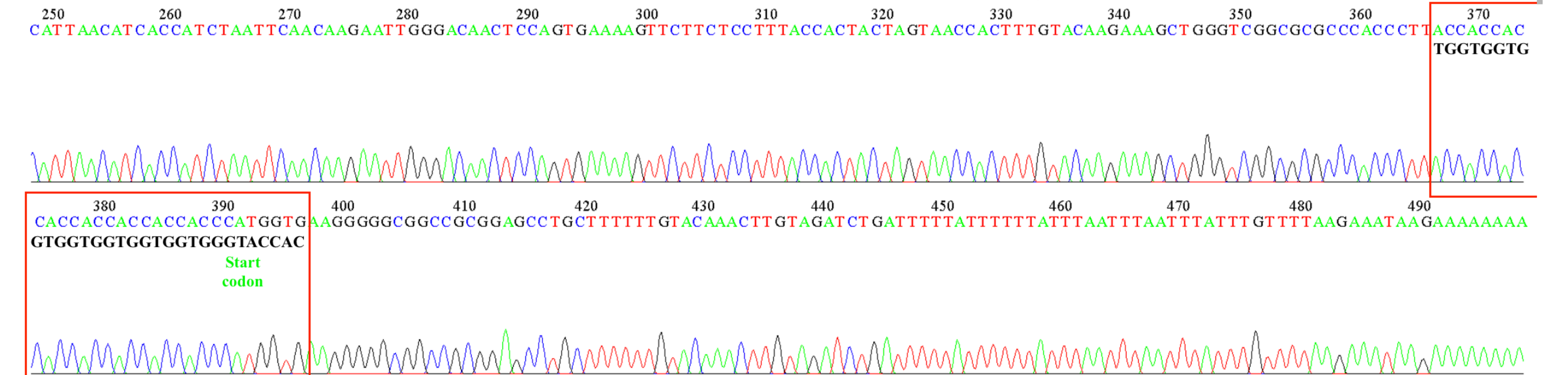


Figure 9: Gene sequencing chromatogram for **GGT**-repeat DNA fused with GFP tag.

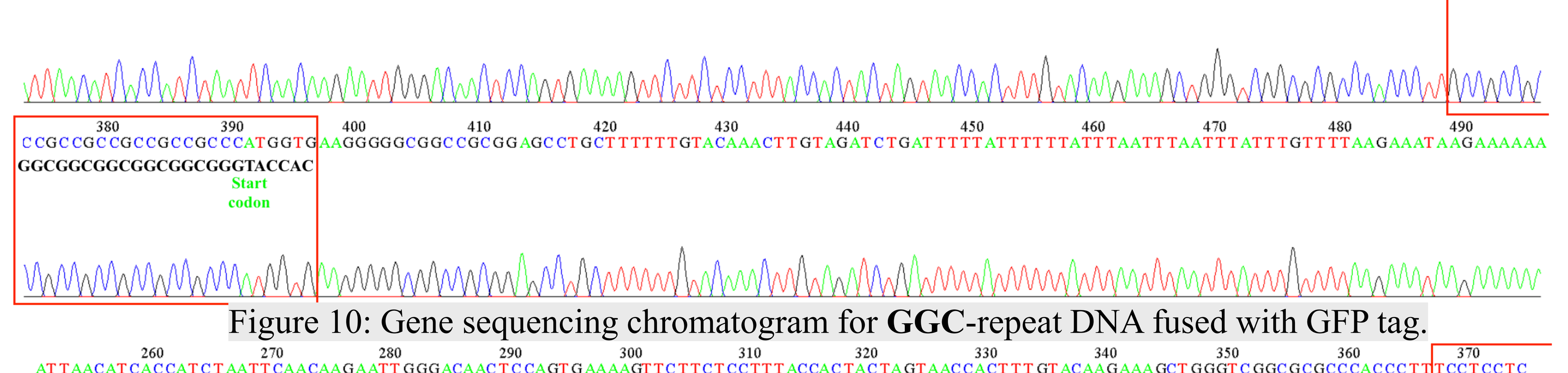


Figure 10: Gene sequencing chromatogram for **GGC**-repeat DNA fused with GFP tag.

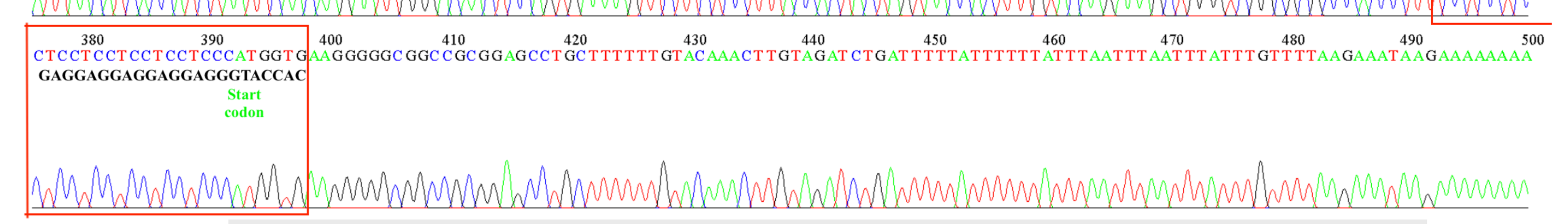


Figure 11: Gene sequencing chromatogram for **GGA**-repeat DNA fused with GFP tag.

Future Directions

- Optimize the transfection protocols.
- Observe the transfected cells with fluorescence microscopy and confirm the GFP expression with Western Blotting.
- Track the morphological and phenotypical differences (size, shape, growth rate) between AX4 and DnmA-KO cells.
- Assess the potential protein targets based on their genetic conservation and gene ontology .

Acknowledgements:

I would like to express my special thanks to Professor Denis A. Larochelle for research guidance and sponsorship in this research project. I also appreciate the help and advice from Professor Javier F. Tabima, and the former and current lab members: Zaza Gelashvili, Davin Tafuri, and Eun-Mi Jeong,. I want thank the Biology Department - Clark University for the Summer Undergraduate Research Experience (SURE) award. I would also like to acknowledge the Gustaf H. Carlson School of Chemistry and Biochemistry - Clark University for providing access to MOE (Molecular Operating Environment) Program.

References:

1. T. P. Jurkowski et al., Human DNMT2 methylates tRNA(Asp) molecules using a DNA methyltransferase-like catalytic mechanism. Rna 14, 1663-1670 (2008).
2. S. Müller et al., Target recognition, RNA methylation activity and transcriptional regulation of the *Dictyostelium discoideum* Dnmt2-homologue (DnmA). Nucleic Acids Res 41, 8615-8627 (2013).
3. Zaza Gelashvili, Determining the Effects of Methylation by DnmA *in Dictyostelium discoideum*. Master of Science Thesis (2020).
4. Nakamura, Y., Gojobori, T. and Ikemura, T, Codon usage tabulated from the international DNA sequence databases: status for the year 2000. Nucl. Acids Res. 28, 292. (2000)