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Codon Optimization for Alpha 1-Antitrypsin Disease

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Comments

Medical student Timothy Menz participated in this study as part of the Senior Scholars research program at the University of Massachusetts Medical School.

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Abstract

Alpha 1-antitrypsin deficiency is a genetic disorder caused by defective production of alpha 1-antitrypsin (AAT). Gene therapy approaches have been conducted in patients with AAT deficiency with successful AAT expression, but not to the therapeutic levels required to reduce the risk of emphysema. Codon optimization, a somewhat new and evolving technique, is used by many scientists to maximize protein expression in living organisms by altering translational and transcriptional efficiency as well as protein refolding. The purpose of this study was to develop single stranded and double stranded AAT gene constructs, test their protein expression in vitro, and compare with those levels expressed by the AAT construct that is currently in clinical trials. Three constructs were to be developed, yet only one construct was successfully cloned. This clone, optimized ds-CB-AAT, illustrated increased AAT protein expression as the transfection time increased. However, protein levels were appreciably lower in the optimized construct compared to the single stranded (long intron) AAT construct that is currently being administered in clinical trials. The data did not suggest that the optimized AAT construct does in fact express more AAT protein in vitro as expected. In order to achieve data that can be reproduced, the 2 remaining constructs need to be cloned and all of the isolated plasmid DNA should be prepared on the same scale to minimize any additional confounding variables.

Introduction

Background: Alpha-1 antitrypsin (AAT) is one of the primary circulating serum antiproteases in humans. It inhibits a variety of serine proteinases, with neutrophil elastase being one of the most physiologically important, as well as inhibiting a number of metallo-proteinases and other pro-inflammatory and pro-apoptotic molecules. AAT is normally produced within hepatocytes and macrophages, where hepatocyte-derived AAT forms the bulk of the physiologic reserve of AAT. AAT deficiency is a genetic disorder caused by defective production of this protein and deposition of increased abnormal AAT protein in liver cells. Gene therapy is an experimental technique that uses genes to treat or prevent disease. Gene therapy approaches have been conducted in patients with AAT deficiency with successful AAT expression, but not to the therapeutic levels required to reduce the risk of emphysema. Codon optimization, a somewhat new and evolving technique in the world of gene therapy, is used by many scientists to maximize protein expression in living organisms by altering translational and transcriptional efficiency as well as protein refolding. With this technique, it is thought higher AAT levels can be achieved.
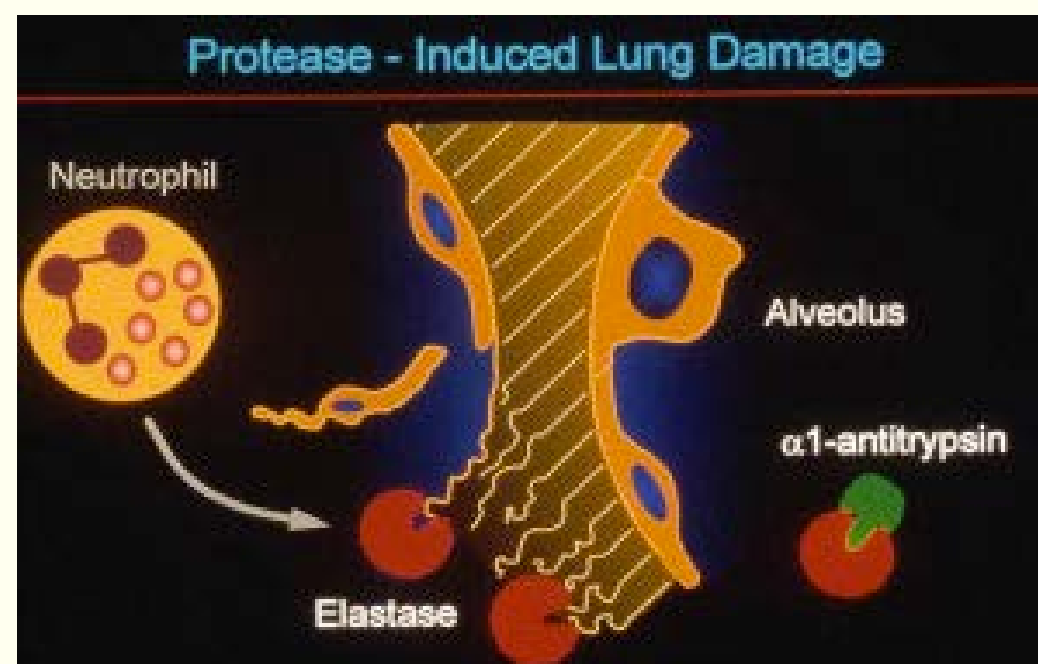
Figure 1. Protease-induced lung damage. With abnormally functioning alpha-1 antitrypsin (AAT), neutrophil elastase is constitutively expressed.
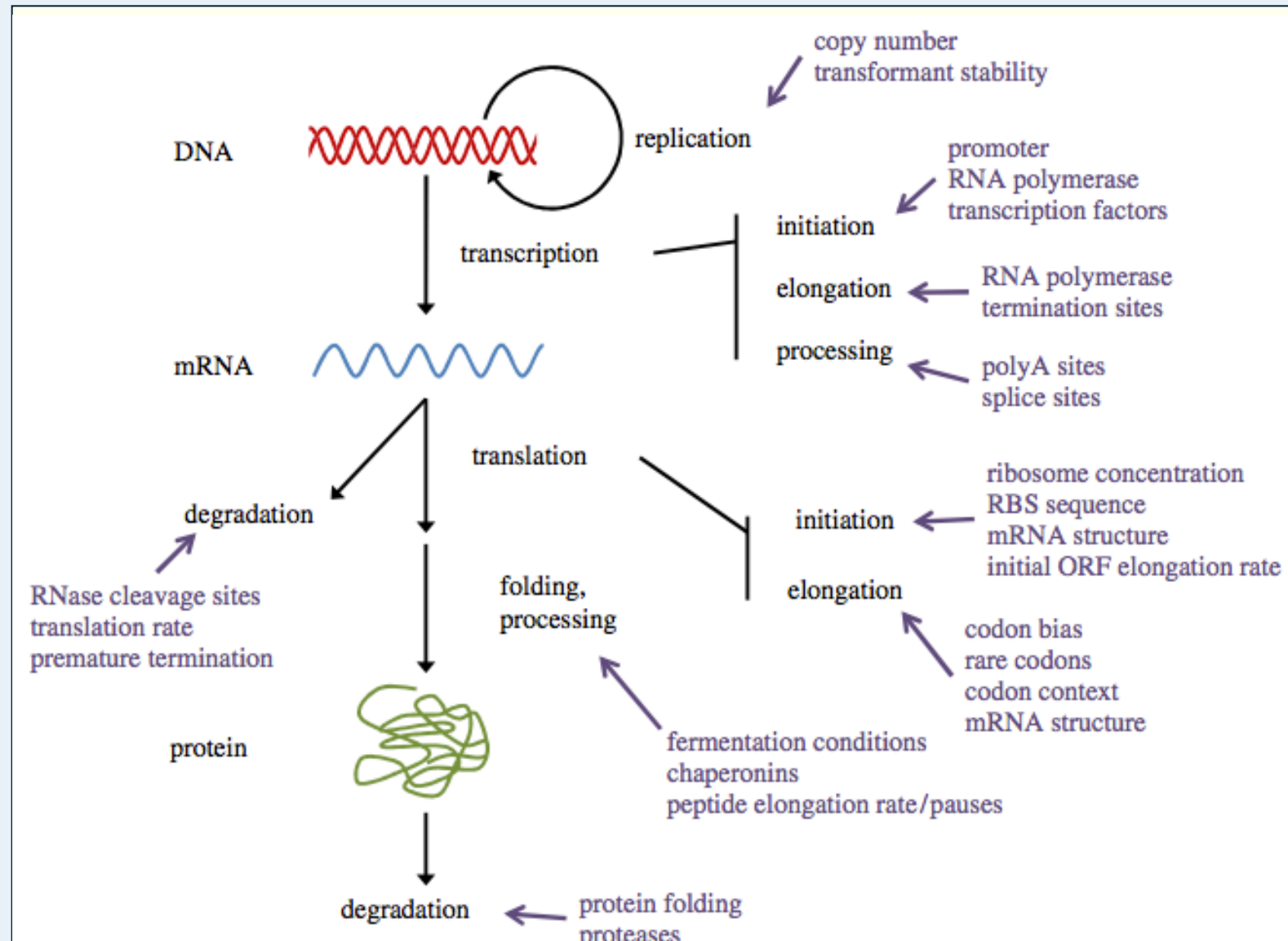
Figure 2. Factors influencing protein expression. Several factors that act along the path of expression from DNA to mRNA to protein are shown, any of which could be altered by or could affect the impact of gene design. RBS, ribosome-binding site.
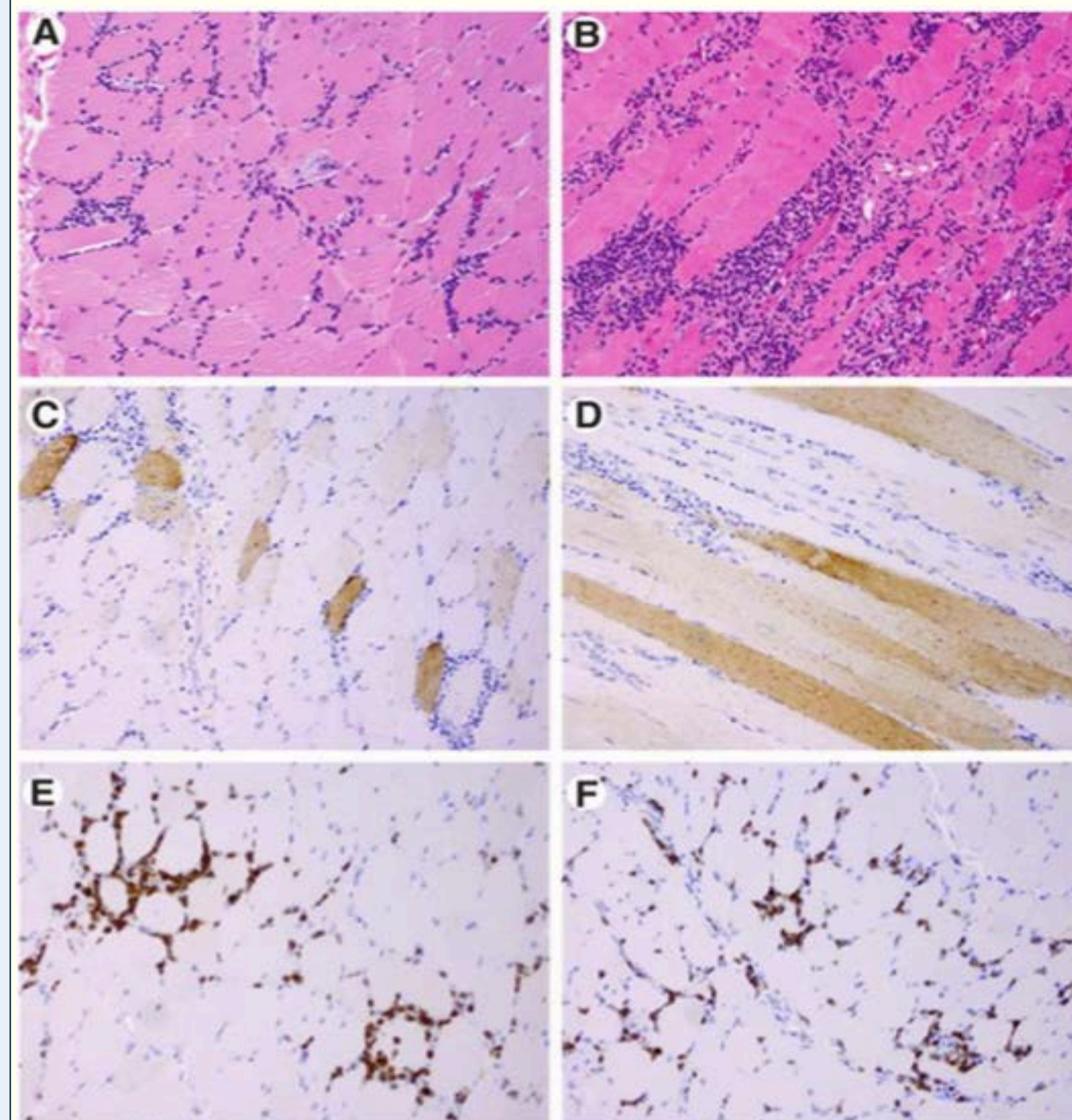
Figure 4. Histology and immunochemical study of skeletal muscle. (A) H&E-stained section showing moderate endomysial inflammatory reaction composed primarily of mononuclear cells. (B) H&E-stained section showing a marked endomysial inflammatory reaction. (C) Immunohistochemistry for AAT, showing granular reactivity in myofibers on cross-section. (D) Immunohistochemistry for AAT, showing granular activity in myofibers cut longitudinally. (E) Immunohistochemistry for CD3, showing a high proportion of T lymphocytes comprising the inflammatory infiltrate. (F) CD8-immunoreactive T cells comprise a significant subset of total lymphocytic infiltrate.

Materials and Methods

The gene construct that is currently being used in clinical trials is single stranded containing a long intron. The following constructs were to be cloned for comparing protein levels in vitro:

- Double stranded, self-complementary AAT (short intron)
- Double stranded, self-complementary, optimized AAT
- Single stranded optimized AAT

Each clone started with a DNA enzyme digestion of each insert and vector that then underwent ligation. Regular efficiency transformation was then carried out for bacterial replication. The plasmid DNA was then collected and isolated from each colony that formed on the agar plate. After enzymatically digesting the isolated DNA to test if the correct construct was obtained, the DNA was then transfected into HEK 293 cells using Lipofectamine. Serum was then collected after 24 and 48 hours as well as after cell lysis to test for AAT protein expression with human AAT ELISA, per protocol.
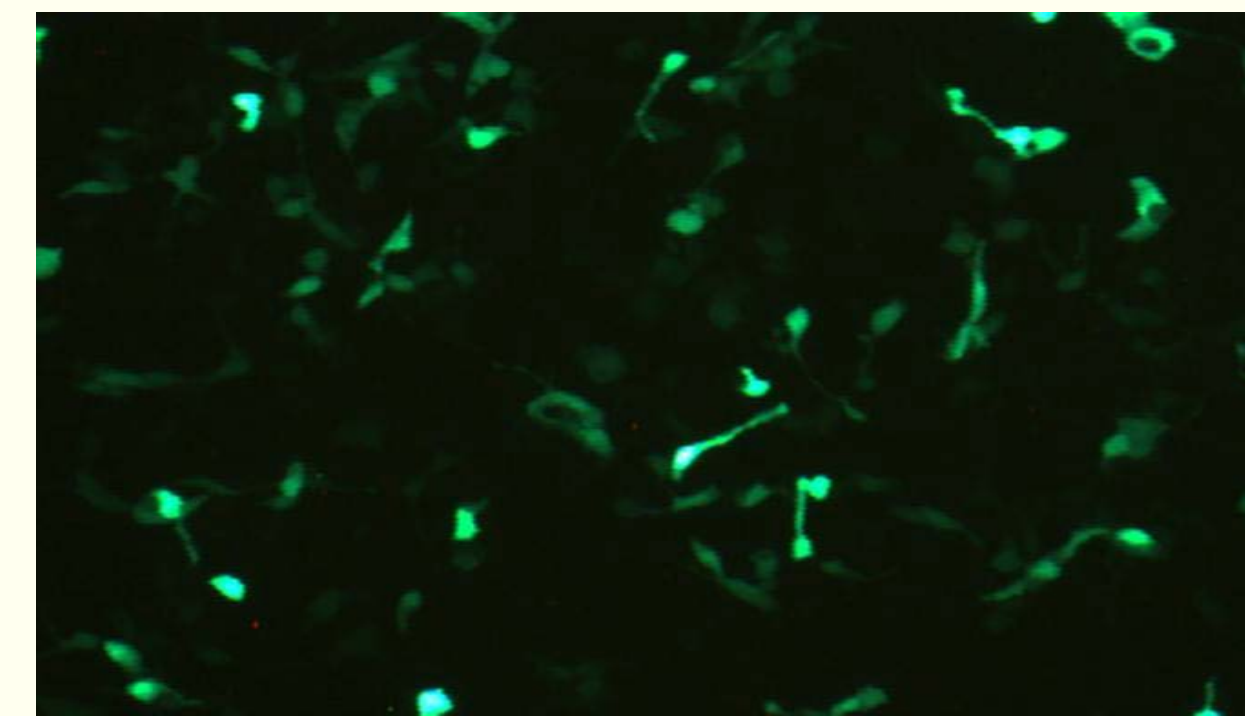
Figure 3. GFP transfection control. HEK 293 cells expressing green fluorescent protein (GFP) indicating successful transfection.

Results

HEK293 AAT Transfections

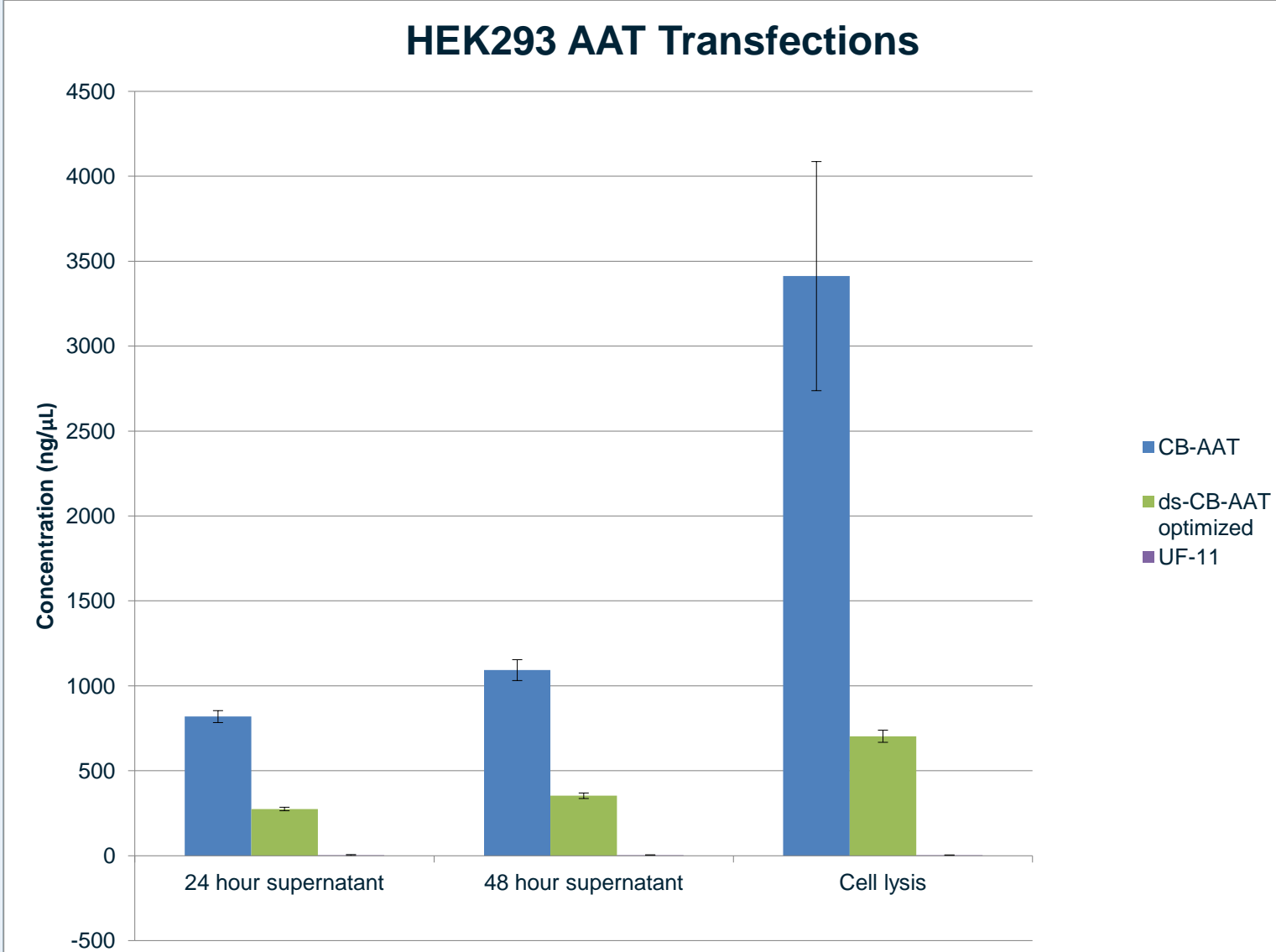Legend: CB-AAT, ds-CB-AAT optimized, UF-11

Figure 5. AAT protein expression in vitro. Supernatant from HEK 293 cells that were transfected with CB-AAT and optimized ds-CB-AAT constructs were collected and AAT levels were measured through ELISA.
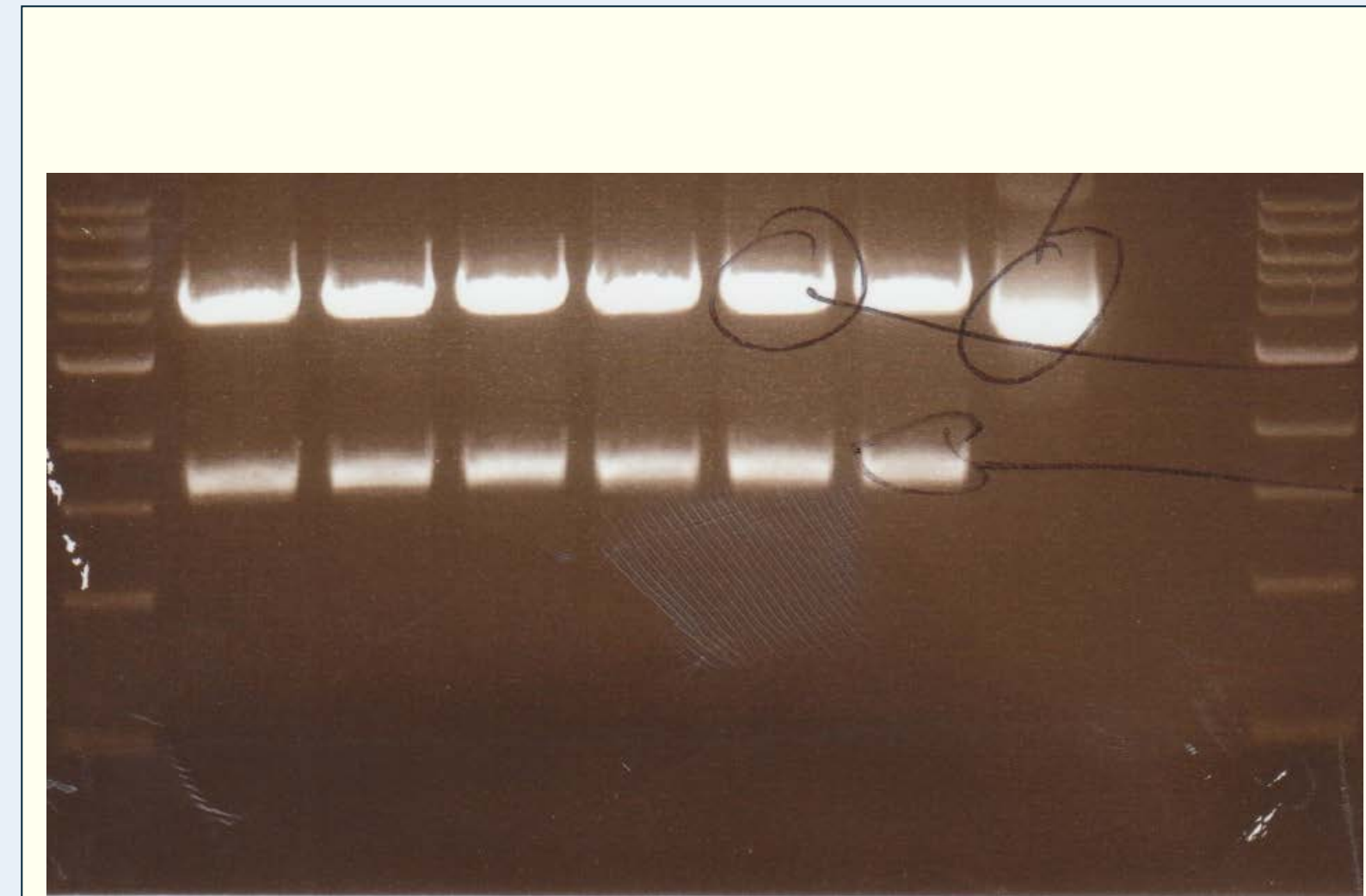
Figure 6. Gel electrophoresis of successful clone of optimized ds-CB-AAT. Optimized ds-CB-AAT was cut with XmnI enzyme that yielded the two desired bands of 4084bp and 1591bp with a 1Kb ladder for comparison.

Discussion

The results obtained are quite perplexing. It is under general knowledge that genetic cloning is a delicate and somewhat repetitious technique. That being said, it still does not explain why the single stranded AAT construct containing the long intron expressed more AAT than the double stranded, self-complementary optimized construct. One reason could be that the single stranded AAT was prepared and isolated from a larger plasmid preparation, which can account for a higher concentration of DNA. What is so striking is that the concentration for the cell lysis sample is almost 5 fold that of the optimized AAT. In order to fully elucidate the advantage of codon optimization, the 2 remaining constructs need to be cloned and all of the isolated plasmid DNA should be prepared on the same scale to minimize any other confounding variables.

Acknowledgements