Sreenidhi Sankararaman – Thomas Jefferson High School for Science and Technology

Determination of the Firefly Luciferase mRNA Concentration and Encapsulation Efficiency in Protein Nanoparticles Using RiboGreen Assay

under the direction of
Dr. Michael Buschmann, M.D. Ph.D.
Volgeneau School of Engineering, George Mason University

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Abstract

Messenger RNA (mRNA) has been used as a powerful therapeutic for infectious diseases through protein gene therapy carriers. Novel delivery systems, such as protein nanoparticles and reagents, are aimed at protecting mRNA from degradation. To quantify mRNA and to find its Encapsulation Efficiency, RiboGreen, a proprietary fluorescent RNA binding dye, was used for the detection and quantitation of RNA. The purpose of this study was to optimize a Ribogreen Assay for determination of Fluc mRNA Encapsulation Efficiency in nanoparticles, including Lipofectamine Messenger Max (LFMM) protein nanocomplexes and solid Lipid Nanoparticles (LNPs). In the study, the effects of assay conditions associated with detergent (Triton) extraction of the mRNA were analyzed.

The study found that increasing the volume of LFMM to the weight of the mRNA ratio from 1.5 to 6 ul/ug resulted in complete encapsulation of the mRNA. The initial 25 percent underestimate of free mRNA without LFMM (3.5 vs 4.7) was found to be due to poor replicates in the standard curve and differing conditions in the sample vs standard (OptiMEM, Triton). By using identical conditions in the sample and standard and ensuring duplicate repeatability (5 percent), the 25 percent underestimate was reduced to 6 percent. The optimal carrier condition range, in terms of carrier type and volume to weight ratio of carrier to contents, was determined. The further influence of laboratory interferents needs to be completed. Future applications include finding the exact optimal setting (vector type, reagent components, and ratio) for the absolute highest encapsulation for varied protein carriers.