Synthetic mRNA-Mediated GFP and Nluc Expression with Messenger Max

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Abstract

Synthetic mRNA transfection is an effective method for delivering engineered mRNAs directly into the cytoplasm, where they are translated into proteins without the risk of genomic integration. Optimal transfection efficiency is typically achieved when cells reach 80–90% confluence, as insufficient cell density reduces protein production. This study was conducted at the Wise Center, Mississippi State University (USA), under the supervision of Dr. Amelia Woolums. Synthetic mRNAs encoding GFP and nanoluciferase (Nluc) were introduced into bovine kidney cells using the Invitrogen™ MessengerMAX™ lipid-based transfection reagent. To assess gene expression, protein synthesis was measured by quantifying Nluc using the NanoGlow Assay, with luminescence detected by an ELISA reader. In parallel, total protein concentration was determined using the BCA assay in both cell lysates and transfection media. These analyses, performed at 24 h and 48 h post-transfection, enabled calculation of the ratio of Nluc protein concentration (NanoGlow) relative to total protein concentration (BCA). The results demonstrated a higher percentage of RLU/µg at 48 h compared to 24 h, reflecting the extended time available for protein synthesis. Whereas Nluc expression was quantified by luminescence assays, GFP-mRNA expression was evaluated using inverted microscopy to visualize green fluorescence in the cytoplasm of transfected cells. Consistently, fluorescence intensity was higher at 48 h post-transfection compared to 24 h, further confirming enhanced protein expression over time. In conclusion, mRNA transfection is a reliable and efficient method for time-dependent protein expression with broad applications in biomedical research and therapeutic development.

References

[1] K. McCormick, J. Moreno Herrero, H. Haas, S. Fattah, A. Heise, F. J. O'Brien, and S. A. Cryan. Optimizing the Delivery of mRNA to Mesenchymal Stem Cells for Tissue Engineering Applications. Molecular Pharmaceutics 2024 21 (4), 1662-1676.

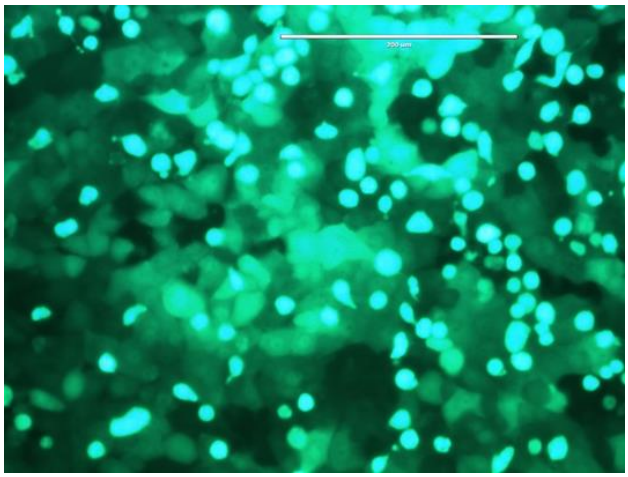

Figure 1: GFP expression in bovine kidney cells 48 hours post-transfection with GFP-mRNA, showing cytoplasmic localization. Image captured using inverted microscopy.