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Effects of DNA size on cell transfection

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Gene therapy requires the delivery of nucleic acid to replace, regulate, or correct genes to treat diseases. Our laboratory developed minimized nonviral vectors called minivectors. Minivectors are devoid of bacterial and viral sequences, and are unlikely to integrate or be silenced. Published data show that minivectors transfect hard-to-transfect cell types, including primary cells without causing toxicity. During transfection, DNA vectors need to enter the cell, translocate to the nucleus, and be expressed to mediate an effect. Whereas minivectors get into cells extremely well, the observed minivector-mediated knockdown was less efficient than when equal moles of plasmid were transfected. The author hypothesized that smaller vectors get expressed less efficiently than larger ones. To test how vector length affects transfection, it was created DNA vectors, from 383 to 4,556 bp long, expressing anti-GFP-shRNA. Transfection efficiency by using electroporation and measuring GFP-knockdown was tested. Electroporation tests how vector length affects nuclear localization and expression without the variable of cell entry. Fluorescence was quantified using microscopy and flow cytometry. The smallest vector showed the least knockdown, while the largest three vectors showed similar knockdowns. To test directly whether these differences in GFP-knockdown are a consequence of RNA polymerase inhibition, *in vitro* transcription is quantitatively measured. Because clinical application vary, understanding vector size-effects on transfection is necessary for calculating the amount of vector needed to elicit the appropriate expression level for any given application. The goal of this project is to find optimal minivector size for nonviral gene therapy vectors to maximize expression while minimizing toxicity.

Biography

Benjamin Hornstein is a PhD candidate in the Department of Molecular Virology and Microbiology at Baylor College of Medicine. He graduated from Brandeis University in 2011 with a BS/MS in Biochemistry. While at Brandeis, he worked in the laboratory of Dr. Susan Lovett studying regulation mechanisms of E. coli replication. He currently works in the laboratory of Dr. Lynn Zechiedrich working on developing new gene therapy techniques for HIV-1.

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