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Category: Predoc
Name: Uday Baliga
PI: David Dean
Title: Improved multi-plasmid delivery using PEG-linked bis-PNAs
Abstract: Controlled delivery of multiple genes to a single cell is critical to many research approaches that impact the lung. These include viral reverse genetics for respiratory virus vaccine production, generation of induced pluripotent stem cells (iPSCs), and expression of multiple genes in pathways or for multi-gene therapies. iPSCs are being developed for therapeutic treatment of lung diseases including CF and AAT deficiency by transplanting gene-corrected iPSCs. Viral reverse genetics is being developed for vaccines against such respiratory pathogens as influenza. Multi-gene expression and interactions are used to understand complex pathways such as lung development and to treat complex diseases such as asthma. While methods for delivering multiple genes are available, they have limitations that hinder their utility. Multi-gene expressing plasmids are exceedingly large, difficult to produce, and may contain multiple similar or identical cloning sites reducing the ease of manipulation by recombinant DNA technologies. Usage of IRES sequences for promoter-independent expression results in variable expression and using multiple viral vectors increases potential for off-target integration and recombination. An alternative to these strategies, co-transfection, the delivery of multiple plasmids to an individual cell, uses any plasmid, allows each to use independent, identical promoters, and is not limited by copy number or DNA modification techniques. The use of the same promoter in each plasmid expressing a single gene ensures similar levels of transgene expression. However, co-transfection is limited by the fact that as the number of different distinct plasmids that are co-transfected into cells increases, the number of cells receiving at least one of each different plasmid decreases, leading to a very small fraction of transfected cells that receive and express all the different desired DNAs. There are currently no methods to improve or control this delivery. The goal of my project is to improve the controlled delivery of multiple plasmids. Peptide nucleic acids (PNAs) are DNA analogs that present bases in the same orientation, geometry, and spacing as DNA, but which have a polyamide backbone. Triplex-forming PNAs (bis-PNAs) can be targeted to specific unique polypurine tracts of DNA that can be cloned into any plasmid. Bis-PNA targeting has both high specificity and avidity. By linking two of these bis-PNAs (each binding a different sequence) with a polyethylene glycol (PEG) spacer, plasmids carrying these target sites can be linked in a highly stable complex. We term these linked bis-PNAs as “bi-bis-PNAs”. I hypothesize that bi-bis-PNAs can link plasmids together and that the linked plasmids can be delivered by normal transfection methods but at higher co-transfection efficiencies than unlinked plasmids. Linked plasmid delivery relies on a single delivery event rather than multiple delivery events needing to occur simultaneously. I show preliminary data on co-transfection efficiencies of multiple different fluorescent reporter plasmids without linking and improved co-transfection efficiency by linking using spectral flow cytometry and fluorescence microscopy.