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Construction of a Lentiviral Vector Expressing EGFP-tagged human CFTR for Testing Gene Therapy in Cystic Fibrosis Animal Models

Cystic fibrosis (CF) is a genetic disorder, caused by mutations in the CFTR gene that encodes CFTR, a chloride channel. CF affects 1/~3000 live births in the United States. Clinically, adult patients suffer mostly from lung complications, characterized by chronic bacterial infection, persistent neutrophilic inflammation and mucopurulent airway obstruction. While treatments to improve the quality of life and lifespan of afflicted people are practiced, there is no cure for this disorder. As CF is caused by gene mutations, gene therapy presents a potentially wide-spread and life-long treatment. To test this concept, we aimed to construct a lentiviral vector designed to express eGFP-tagged human CFTR, which will allow us to trace and evaluate gene transfer. First, human CFTR (hCFTR) cDNA was amplified via PCR from a carrying plasmid with a set of primers that have tail sequences homologous to a lentiviral vector plasmid that expresses eGFP driven by the EF1α promoter. The hCFTR amplicon is annealed to the lentiviral vector plasmid and extended via PCR so that hCFTR gene is fused in frame with the eGFP gene at its C-terminus. After a heat-shock transformation of *E. coli* competent cells, overnight culture gave rise to hundreds of colonies, which were screened via phenol extraction and gel electrophoresis. The colonies of the correct size were obtained. Currently, we are doing plasmid preparation and sequencing to verify the correctness of the expected eGFP-hCFTR lentiviral plasmid. Once it is confirmed, a large preparation of the plasmid will be produced and used for transfection of a lentiviral producing cell line to produce the needed lentiviral vector for CFTR gene transfer to CF cell lines and CF animals.