A Novel Method of Constructing Short Synthetic Promoters Using Random Transcription Factor Binding Site Combinations

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While recent advances in gene therapy have reinforced the appeal for using Adeno-Associated Virus (AAV) for targeted gene delivery, the small genome delivery size limits the capabilities of AAV as a comprehensive delivery vector. One approach to increasing the AAV gene delivery capacity is to develop shorter promoters that maintain the high transcriptional strength of conventional promoters. However, it is challenging to shorten promoters without compromising transcriptional activity. This research presents a method of producing libraries of short promoters derived from the CMV-IE gene regulatory sequence by randomizing transcription factor binding sites (TFBS). These libraries have been constructed with a randomized design that express RNA barcodes, where each barcode is linked to a unique set of TFBS, and thus serves as surrogates. The randomized libraries are transfected into 293 and CHO-KI cell lines for characterization of delivery and expression, and were shown to decrease the size of the promoter. This research serves as a proof-of-principle for the rational design of new, shorter promoters to increase the delivery gene capacity of AAV. When applied to reducing the length of promoters, this technique maintains the high transcriptional activity from the standard CMV-IE promoter, and allows for an additional level of transcriptional specificity. This can also be used to customize promoters to cell-specific constraints using other gene regulatory sequences, such as Glial Fibrillary Acidic Protein (GFAP). Moreover, by increasing the capacity of viral vectors, these promoters add increased targeting to therapies for Human Cytomegalovirus (CMV), CF, and other genetically-related diseases.

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