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A comparative analysis of mRNA codon optimization methods

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The significance of mRNA as a therapeutic modality lies in its ability of rapidly producing a customized treatment for a specific disease. A major challenge when developing mRNA-based therapeutics is the structural instability of the mRNA molecule, because unstable mRNA often leads to reduced therapeutic effects due to molecular degradation. Codon optimization is needed to increase the stability of mRNA by preventing hydrolysis of the ribose-OH site by forming a double-stranded secondary structure. The structural stability can be measured by minimum free energy (MFE); therefore, computationally minimizing MFE is the most important goal in mRNA codon optimization. In this study, we compared widely used optimization tools such as RiboTree, iCodon, mRNAid, and LinearDesign. ICodon and mRNAid demonstrated high computational speed, but relatively low optimization performance. On the contrary, RiboTree and LinearDesign resulted in high MFE optimization performance, but RiboTree took a long computation time, and LinearDesign did not store intermediate optimization results. To evaluate the above methods, we used the enhanced green florescence protein (eGFP) as a benchmark sequence. As a result, LinearDesign achieved the lowest MFE of 440 while RiboTree did the MFE of 412. Interestingly, in the perspective of secondary structure, the mRNA optimized by RiboTree tends to retain the original wildtype structure, while that from LinearDesign predominantly forms a more linear shape. This structural difference may be attributed to the distinct characteristics of their basis algorithms, Monte Carlo Tree Search (MCTS) for RiboTree and Probabilistic Context-Free Grammar (SCFG) for LinearDesign. Consequently, additional investigation will be needed since these structural variations may have impact on immunogenicity or stability.