Single-Guide RNA Optimization With COTER

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CRISPR-Cas9 has emerged as a catalyst in treating genetic diseases such as sickle cell disease and transfusion-dependent beta thalassemia. Multiplex CRISPR-Cas9 is a subsystem of CRISPR editing that encodes multiple guide RNA sequences into a single CRISPR array, which is imperative for high precision in polygenic traits. However, the selection and optimization of the Cas9-directing guide RNAs (gRNAs) remains one of the largest obstacles in gene therapy today, and the presence of multiple gRNAs in a CRISPR array increases the number of optimal candidates exponentially. There are currently few computational technologies for gRNA array optimization, and in-vivo methods remain costly and inefficient. This research proposes a novel gRNA array optimization tool that utilizes a hybrid deep learning architecture with a fusion module. OMEGA uses bidirectional long short-term memory combined with a gated recurrent unit to enable chromatin context, significantly improving optimization accuracy. In addition, a genetic algorithm is employed to combinatorially optimize gRNA arrays. This hybrid was trained on data from 16 experimental and functionally validated gRNA datasets, optimizing gRNA arrays after extensive hyperparameter tuning and backpropagation. To enable researchers' discretion, the model allows for a user-defined protospacer adjacent motif, bulges, and cell-based computational modeling. After training, OMEGA successfully exceeded industry standards in four key benchmarks: on-target efficiency, off-target prediction, minimum free energy, and convergence time, all while maintaining ideal GC content. OMEGA is a promising computational tool for optimal gRNA array selection, leading to higher gene targeting accuracy and off-target effect reduction.

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