In silico Design of sgRNAs for CRISPR/Cas9 Mediated Gene Mutageneis of Arundo donax to Optimize Biofuels

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The need for reliable renewable energies has grown, expanding research into biofuel opportunities. However current feedstocks use agricultural land, causing significant impacts. This research addresses a critical gap in the exploration & optimization of Arundo Donax— an invasive species— as a viable biofuel feedstock. This study focuses on two key genetic targets: the Flowering Locus T (FT) gene to increase biomass production by suppressing flowering, and the DWARF 53 (D53) gene to mitigate invasiveness. In this study, guide RNAs (gRNAs) were designed in-silico for CRISPR/Cas9 mediated mutagenesis of both target genes for optimization. Utilizing recent chromosome assembly data, this research functionally annotated the genome to extract target genes, then validated target genes through NCBI blasts against homologous species such as rice and maize. Subsequently, 3 gRNAs were designed for each gene chosen to ensure a wide range of options. A systematic scoring approach was adapted from established AI scoring models to predict the efficiency of each gRNA to ensure the selection of a successful gRNA for experimental trials. Validation against experimentally successful rice gRNAs demonstrated high correlation, suggesting the reliability of this scoring approach. For each gene the respective gRNA sequences were identified as the most optimal gRNAs: FT-1 (5'-CCCACTAGTCGTAGGACATG-3'); FT-2 (5'-CATCACAACAGCGTCGCTGA-3'); D53 (5'-CGTCGATGGCGAAGCTACAG-3'). Finally, computational modeling of CRISPR-Cas9 edits predicted successful frameshift mutations that would effectively disrupt gene function. There is a calculated prediction of a 55% increase in biomass with a 5% margin of error and a significant decrease in branching for Arundo Donax, exceeding industry standards.

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