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Development of a bioinformatic tool to predict M1GS ribozyme target sites in RNA and demonstration of M1GS-mediated downregulation of nucleolar ribosomal RNA in a human cancer cell line

Ribonuclease P (RNase P) is a ribozyme conserved across all domains of life and plays a central role in the maturation of the 5′ end of transfer RNA (tRNA). Unlike most nucleases, RNase P recognises precursor tRNA based on structural features rather than sequence, a property that has been exploited to engineer RNase P for selective targeting and cleavage of diverse RNA molecules as gene inactivation strategies. One such approach, termed M1GS, involves coupling the *E.coli* M1 RNA to a short guide sequence that is complementary to the target RNA, enabling specific recognition and cleavage. Despite its simplicity and versatility, the M1GS approach has remained underutilised compared with other gene inactivation tools, partly due to the requirement for prior knowledge of the features that make a site suitable for M1GS targeting. To overcome this limitation and enable broader application, we developed a user-friendly Python-based bioinformatic tool that predicts potential M1GS target sites for any RNA of interest. The tool accepts DNA or RNA sequences as input and evaluates them using criteria derived from known M1GS targeting requirements. We demonstrate its utility by predicting M1GS target sites for human nucleolar 28S rRNA, a ribosomal RNA often upregulated in cancers. Customised M1GS constructs designed from these predictions successfully downregulated 28S rRNA in a human lung cancer cell line, highlighting the potential of M1GS-mediated rRNA cleavage as an anticancer modality. Additionally, an RNA mimic of GFP called squash was tagged to M1GS to visualise its localisation in human cancer cells, providing further insights into its intracellular behaviour. The tool was also validated by predicting M1GS target sites for previously studied RNAs, with predictions consistent with reported results.

Authors: Prof. MOHANNATH, Gireesha (Birla Institute of Technology and Science-Pilani, Hyderabad Campus); PRIYADARSHINI, Neha (JAIN University)

Co-authors: Mr POIYAMOZHI, Harikrishnan (Birla Institute of Technology and Science-Pilani, Hyderabad Campus); Mr PUPPALA, Navinchandra Venkatarama (Birla Institute of Technology and Science-Pilani, Hyderabad Campus); Prof. BISWAS, Swati (Birla Institute of Technology and Science-Pilani, Hyderabad Campus)

Presenter: PRIYADARSHINI, Neha (JAIN University)

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