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Genome-Wide Mining of Potential Guide RNAs for Streptococcus pyogenes and Neisseria meningitides CRISPR-Cas Systems for Genome Engineering

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Abstract : Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (Cas) system can facilitate targeted genome editing in organisms. Dual or single guide RNA (gRNA) can program the Cas9 nuclease to cut target DNA in particular areas; thus, introducing concise mutations either via error-prone non-homologous end-joining repairing or via incorporating foreign DNAs by homologous recombination between donor DNA and target area. In spite of high demand of such promising technology, developing a well-organized procedure in order for reliable mining of potential target sites for gRNAs in large genomic data is still challenging. Hence, we aimed to perform high-throughput detection of target sites by specific PAMs for not only common Streptococcus pyogenes (SpCas9) but also for Neisseria meningitides (NmCas9) CRISPR-Cas systems. Previous research confirmed the successful application of such RNA-guided Cas9 orthologs for effective gene targeting and subsequently genome manipulation. However, Cas9 orthologs need their particular PAM sequence for DNA cleavage activity. Activity levels are based on the sequence of the protospacer and specific combinations of favorable PAM bases. Therefore, based on the specific length and sequence of PAM followed by a constant length of the target site for the two orthogonals of Cas9 protein, we created a reliable procedure to explore possible gRNA sequences. To mine CRISPR target sites, four different searching modes of sgRNA binding to target DNA strand were applied. These searching modes are as follows i) coding strand searching, ii) anti-coding strand searching, iii) both strand searching, and iv) paired-gRNA searching. Finally, a complete list of all potential gRNAs along with their locations, strands, and PAMs sequence orientation can be provided for both SpCas9 as well as another potential Cas9 ortholog (NmCas9). The artificial design of potential gRNAs in a genome of interest can accelerate functional genomic studies. Consequently, the application of such novel genome editing tool (CRISPR/Cas technology) will enhance by presenting increased versatility and efficiency.

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