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High-Throughput Artificial Guide RNA Sequence Design for Type I, II and III CRISPR/Cas-Mediated Genome Editing

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Abstract : A huge revolution has emerged in genome engineering by the discovery of CRISPR (clustered regularly interspaced palindromic repeats) and CRISPR-associated system genes (Cas) in bacteria. The function of type II Streptococcus pyogenes (Sp) CRISPR/Cas9 system has been confirmed in various species. Other S. thermophilus (St) CRISPR-Cas systems, CRISPR1-Cas and CRISPR3-Cas, have been also reported for preventing phage infection. The CRISPR1-Cas system interferes by cleaving foreign dsDNA entering the cell in a length-specific and orientation-dependant manner. The S. thermophilus CRISPR3-Cas system also acts by cleaving phage dsDNA genomes at the same specific position inside the targeted protospacer as observed in the CRISPR1-Cas system. It is worth mentioning, for the effective DNA cleavage activity, RNA-guided Cas9 orthologs require their own specific PAM (protospacer adjacent motif) sequences. 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 target site for the three orthogonals of Cas9 protein, a well-organized procedure will be required for high-throughput and accurate mining of possible target sites in a large genomic dataset. Consequently, we created a reliable procedure to explore potential gRNA sequences for type I (Streptococcus thermophiles), II (Streptococcus pyogenes), and III (Streptococcus thermophiles) CRISPR/Cas systems. 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. The output of such procedure highlights the power of comparative genome mining for different CRISPR/Cas systems. This could yield a repertoire of Cas9 variants with expanded capabilities of gRNA design, and will pave the way for further advance genome and epigenome engineering.

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