DEVELOPMENT OF THE SCREENING SYSTEM FOR TRANSPOSABLE ELEMENTS

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Prokaryotic CRISPR-Cas systems are responsible for the adaptive immunity against foreign DNA, which is based on guide RNA-dependent DNA or RNA nuclease activity. Nowadays, these systems are adapted and widely used for genome editing; however, they are inefficient, prone to off-target activity, and require PAM sequences that may greatly limit possible targets in the genome. In addition, gene editing with Cas proteins relies on homologous recombination repair, which is tied to active cell division and, therefore, is not applicable to some cell types [1]. All these limitations of CRISPR-Cas systems raise demand for novel genome editing tools. Recent studies and bioinformatic analysis show that Cas12 nucleases may have evolved from TnpB – proteins encoded by IS200/IS605 family insertion sequences (IS) [2]. IS, as well as plasmids, bacteriophages, and transposons, are mobile genetic elements (MGE) that are abundant in both prokaryotes and eukaryotes and play an important role in the evolution and expression of the host genome [3]. The wide variety and evolutionary proximity of IS to Cas proteins make IS a potential source of novel genome editing tools. This study aimed to develop a screening system for transposable elements. Based on the previously described work [4,5], we constructed vectors, which contain an active transposase and an antibiotic resistance gene-carrying minimal transposable element (mini-Tn). We conducted experiments to select bacterial strains that are most suitable for transposition assay, optimized bacterial conjugation conditions, and determined the best conditions for transposase expression. These results contribute to the development of a robust IS screening system, which will facilitate the detection of new IS that could lead to novel genome editing tools.

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