DEVELOPMENT OF CELL-BASED SCREENING SYSTEM FOR TnpB GENOME EDITING TOOLS

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The discovery of CRISPR-Cas systems revolutionized genome editing, making the development of treatments for genetic diseases more achievable than ever. Currently, Cas9 and Cas12a are the most widely applied nucleases due to their high efficiency in double-strand DNA break induction and specificity in target sequence recognition. However, the large size of these nucleases is incompatible with the limited packaging capacity of adeno-associated viruses that are commonly used in *in vivo* therapy, creating a demand for smaller nucleases.

Recent studies have shown that Cas12 nucleases are derived from TnpB proteins encoded in the bacterial IS200/IS605 family insertion sequences [1]. TnpB also exhibit RNA-guided nuclease activity and are smaller ($\sim$400 amino acids) than Cas12 family nucleases (500–1500 amino acids), and therefore are attractive candidates to be used for genome editing. The widely studied TnpB from *Deinococcus radiodurans* (ISDra2) can cleave genomic DNA in eukaryotic cells, but demonstrates lower activity compared to modern genome-editing tools [2]. Therefore, TnpB protein engineering is an appealing strategy to increase its activity, which in turn demands an effective screening system.

In this study, we aimed to develop a cell-based system to identify the active TnpB variants. We demonstrate the feasibility of detecting TnpB nuclease activity in a cellular context and outline the principles of a cell-based platform for screening TnpB variants.

In the future, such cell-based screening could be applied to large-scale studies of protein evolution and to the development of other nuclease properties, such as target specificity or recognition of TAM sequences.

[1] G. Sasnauskas et al., “TnpB structure reveals minimal functional core of Cas12 nuclease family,” Nature, vol. 616, no. 7956, Art. no. 7956, Apr. 2023, doi: 10.1038/s41586-023-05826-x

[2] T. Karvelis et al., “Transposon-associated TnpB is a programmable RNA-guided DNA endonuclease,” Nature, vol. 599, no. 7886, pp. 692–696, Nov. 2021, doi: 10.1038/s41586-021-04058-1