IN SILICO PREDICTION AND VALIDATION OF TnpB AND Cas12a OFF-TARGETS IN THE HUMAN GENOME

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The ability to modify a living organism's genome in a targeted way allows precise changes in phenotype, revolutionizing life science research and providing treatments for genetic diseases that act on the problem at its core [1]. CRISPR-Cas nucleases are the most widely used genome editing tools to date, known for their high efficiency and easy programming. These nucleases use RNA as a guide, recognizing the target via Watson-Crick base pairing and creating double-stranded breaks in DNA, which are then sealed by the cell's intrinsic repair mechanisms, resulting in random insertions or deletions that can inactivate the gene, or precise alterations if a repair template is provided [2]. However, their large size is incompatible with adeno-associated virus vectors widely used in gene therapy for *in vivo* delivery of functional genes into target cells [3]. In 2021, transposon-encoded TnpB proteins were identified as compact RNA-guided nucleases, about 3 times smaller than Cas9 or Cas12a, making it an appealing candidate for use in genome editing [4].

Being guided by RNA makes TnpB and Cas nucleases versatile, but it also means that DNA sequences similar to the target site might also be edited because of partial RNA-DNA complementarity. Off-target editing can be detrimental, especially if coding or regulatory sequences are modified, making deliberate RNA design and nuclease choice crucial. There are certain tendencies of RNA-DNA mismatch toleration for each nuclease that allow the prediction of potential off-target sites based on sequence similarity. Although bioinformatic tools for specificity analysis are powerful, the identified sites won't necessarily be modified inside a living cell, largely depending on chromatin accessibility. Because of this, further experimental validation is needed [5].

This study investigates the potential for off-target activity of TnpB and Cas12a nucleases in human cells. Multiple target sequences were examined, including one shared between both nucleases, revealing evidence of unintended editing. These findings offer preliminary insights into the specificity of selected nucleases, highlighting the importance of target site selection to minimize unintended off-target cleavage.

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