Evaluating the Efficiency of CRISPR/Cas9-Mediated Homology-Directed Repair in Schizosaccharomyces pombe

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Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 is a powerful technology used for gene editing. While this method has opened many opportunities for scientific advancement, it also has the potential to create harmful consequences when used therapeutically in humans, leading to disease and triggering undesired cellular changes. This project is aimed at further evaluating the ability of CRISPR/Cas9 to introduce mutations that are various distances away from the Cas9 cut site using Schizosaccharomyces pombe as a model system. Specifically, this project uses the reporter gene ade6 and SpEDIT, a CRISPR/Cas9 system designed for use in fission yeast. Currently, the sequence of six clones has been obtained and aligned with the wild-type sequence to identify the mutations incorporated in each, revealing that five of the six clones underwent homology-directed repair (HDR) while one underwent non-homologous end-joining (NHEJ). This clone had a two-base-pair deletion at the cut site, resulting in a frameshift and a non-functional Ade6 protein. Of the clones that did undergo HDR, two had four identical mutations close to the cut site, and two had 11 identical mutations surrounding the cut site. The fifth clone had 33 mutations that reached the left end of the homologous repair fragment. Additional clones are being analyzed to determine the nature and location of mutations, which will reveal the frequency and distance of HDR events that CRISPR/Cas9 can accomplish. This will be useful in designing future gene-editing experiments, especially those targeted toward correcting mutations in the human genome.

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