Targeting Susceptibility in Mutations in the Cell Cycle: Knockout of the Ade2 Gene Using CRISPR

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Using different inhibitors to stop the cell cycle in different phases, we will see significant differences in DNA damage. In S-phase, we find DNA synthesizing, whereas in M-phase, DNA has condensed into their chromosomal structures. By doing so, we will be able to conclude in which stage of the cell cycle is the cell most vulnerable to mutations and translate it into CRISPR editing to see if the same results are shown. The procedure involves dividing the yeast cells in two groups for each trial and, within those groups, three experimental procedures in which the cells will be treated with cell cycle inhibitors [DMSO (negative control), hydroxyurea (arrests the S-phase), and nocodazole (arrests the M-phase)]. One half will be the used to measure the DNA damage susceptibility via comet assay. The other half will be used to measure the CRISPR editing efficiency. Data will be collected by the application ImageJ which will allow us to precisely measure the density and lengths of each gel streak which should correlate with the DNA damage and translate into the yeast red colony count. For precise analyzation, we ran a total of 5 trials to find averages and standard deviation of our data. After conducting trials and analyzing data, we see that the cell is most susceptible to DNA alterations in the S-phase. The trials treated with Hyd. showed longer and denser streaks, which is an indicator of mutations occuring, and that directly translated into gene editing using CRISPR since they also produced a higher yield of red colonies compared to the rest of the trials with different treatments. Although we were able to gather significant data after 5 trials, future trials should still be conducted.

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