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Categorizing the Unintended Side Effects of Genetic Transformation

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In a genetic transformation, exogenous DNA is introduced into cells to target a specific genomic sequence. Anecdotal cases have suggested that transformations may cause unintended collateral mutations outside the targeted region, but this has never been investigated systematically. Here, I analyze these collateral mutations using *Cryptococcus neoformans*, a pathogenic fungus responsible for approximately 625,000 deaths each year. Bioinformatics software was used with the genome sequence data of 29 independently transformed strains to identify single nucleotide polymorphisms (SNPs), small insertions/deletions (indels), large structural variants (SVs), and copy-number variants (CNVs); 30 untransformed control wildtype strains were also sequenced. A total of 26 SNPs and indels were found in the transformed strains and not the wildtype strains, but 10 are false-positives due to direct effects of genetic transformation, mononucleotide runs, or low sequencing coverage relative to the coverage across the genome. The remaining 16 variants have sufficient coverage, substantial evidence supporting an alternate allele to the reference genome, and almost always occur in only one strain. Meanwhile, only four true variants are naturally segregating in the control strains. These data indicate side effects of genetic transformations are rare. Eleven variants occur in exons, seven of which cause missense mutations in proteins, suggesting the side effects may be to compensate for targeted gene deletions or to improve fitness in growth medium. No SVs or CNVs caused by genetic transformation were found. Additionally, I have discovered an unpublished region of divergence in the genome of our freezer stock, *C. neoformans* KN99, from the common *C. neoformans* H99 reference genome, despite being closely related. Consequently, a reference genome has been generated for the KN99 strain. These results show that genetic transformations are safe in their common laboratory use and provide resources for other researchers that use *C. neoformans* KN99 as their stock strain.