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Targeted Gene Deletion using Intrinsic Deletion Machinery in *Tetrahymena thermophila*

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The ciliate *Tetrahymena thermophila* eliminates nearly one third of its genome from the developing somatic nucleus. The goal of this work was to optimize the targeted deletion of genes by exploiting this intrinsic DNA deletion machinery, a method known as “co-deletion.” Generating knockouts is an important strategy for determining the function of genes, and co-deletion had the potential to generate knockouts more easily or to knock out multiple related genes at once. Co-deletion occurs when a DNA sequence normally retained in the developing somatic genome is imbedded within an eliminated sequence and the chimeric sequence is introduced into developing *Tetrahymena* cells. The genomic copies homologous to the imbedded sequences are induced to undergo deletion. To attempt to induce co-deletion of multiple genes efficiently, I used Gateway-mediated recombination vectors that contained a recombination cassette flanked by internal elimination sequences into which cloned genes of interest could be inserted. These vectors were transformed into *Tetrahymena* using electroporation and biolistic transformation protocols to determine whether one or both methods promoted co-deletion. Experimental conditions were altered to optimize the efficiency of the transformation, as quantified by the number of drug resistant cell lines generated, and to increase the efficiency of gene deletion, which was demonstrated through PCR analyses and verification of deleted regions by DNA sequencing. I was able to induce co-deletion of several genes, but this method does not appear useful, for several reasons. Deletions occur only in the somatic genome, so they are not heritable. Phenotypic analysis of one gene appeared to indicate that for this particular gene, deletion from the somatic genome alone is insufficient to stop expression during development, when the gene is expressed. Results indicate that co-deletion is not an easier or more efficient method than standard homologous recombination to generate gene knockouts.