Construction of a Tetracycline-Inducible System for Controlled Gene Expression in Crithidia fasciculata

GSURC 2013

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Introduction: Trypanosomes are unicellular eukaryotes that cause human diseases for which there are effective, safe treatments. Development of novel therapeutic agents requires greater understanding of the cellular physiology unique to trypanosomes. We are developing a tetracycline-inducible, gene expression system to selectively investigate functions of various proteins using *Crithidia fasciculata*, a non-pathogenic trypanosomatid that can be cultured in the absence of its mosquito host.

Methods: Vectors for the delivery of the T7 RNA polymerase gene, and the tetracycline repressor were designed for incorporation into the *C. fasciculata* genome. Each vector also includes an antibiotic resistance gene and UTR fragments necessary for transcriptional regulation. Each vector is being built using the following general protocol for each genetic element: design of primers to amplify the gene by PCR, optimization of PCR to determine the annealing temperature for each primer set, sub-cloning, sequencing to validate the identity of each fragment, and ligation into pBlueScript II. Following vector construction, we will transfect *C. fasciculata.*

Results: Six gene fragments were selected for construction of the T7 RNA polymerase vector (CfT7RNApol), and four target genes were used to develop the Tet-repressor vector (CfTetR) Sequencing is complete for one element (*C. fasciculata* 18S rDNA fragment) of CfT7RNApol vector. The remaining genes are at different stages in the cloning process.

Conclusion: Once we complete vector construction, the two vectors will be utilized to transfect *C. fasciculata.* Antibiotic resistance genes will allow for drug-enabled selection in the transformed cells. We will then be able to introduce a third vector carrying a gene under the control of tetracycline in our modified *C. fasciculata* line, and we anticipate usage of this system to over-express proteins as well as double-stranded RNA for RNAi.