

## **Characterization of a Potential Tyramine Receptor in the Malpighian Tubules of *Drosophila melanogaster***

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The Malpighian tubules function as a major part of the *Drosophila* excretory system and, as such, are responsible for initial production of urine. The tubules are comprised of an outer cell layer which surrounds the inner fluid called the lumen. This cell layer is made up of two cell types which are termed the principal and stellate cells. We have previously shown that the Malpighian tubules respond to an application of extracellular tyramine by increasing chloride conductance. This results in a characteristic voltage decrease which has been measured using sharp electrode electrophysiology. It has been proposed that tyramine binds to a g-protein coupled receptor which induces a cascade to increase chloride conductance. Preliminary recordings performed on tubules from mutants indicate that the *CG7431* gene encodes the tyramine receptor. In addition, it is thought that this receptor is located in the stellate cells. It is our desire to establish the validity of these two statements.

In order to locate the tyramine receptor it is necessary to create flies with rescued *CG7431* gene expression in both the principal and the stellate cells separately. Therefore, a *UAS-CG7431* transgene is being constructed which will be inserted into mutant *CG7431* flies. These flies can be crossed with two lines of Gal4 flies, one which will activate expression of the *UAS-CG7431* transgene in the principal cells and the other in the stellate cells. As such, the location of the expression of the *CG7431* gene can be controlled for use in electrophysiology tests. In order to create the *UAS-CG7431* construct, it is necessary to insert the *CG7431* gene into the pUAST vector. To do so, the *CG7431* gene was first amplified in two separate segments using PCR. Next, TOPO cloning was used to insert both sections of the gene into plasmids. Ligation of each piece of the *CG7431* gene into the TOPO vector was confirmed using restriction digests and sequencing. Once both sections of the gene had been inserted into vectors, they were ligated together into one TOPO vector. The ligation was again verified using restriction digests. The complete gene can now be excised from the TOPO vector and inserted into a pUAST transformation vector.