

MANIPULATING TRANSPOSASE EXPRESSION IN Aedes aegypti

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While the number of insect species that have been successfully transformed through the use of a variety of transposable elements continues to increase, in many cases the efficiency of the transformation systems remains low. For mosquito species in particular, that are difficult to rear and maintain in large numbers, the low transformation efficiency is a limiting factor in the number of successful and meaningful experiments that can be completed in a reasonable time frame. This is particularly true for experiments designed to investigate promoter element activity, which require the generation of a large number of independent transgenic lines. We have chosen to focus on the expression of the transposase proteins as a means to increase the efficiency of the *Mos1* and *piggyBac* genetic transformation systems. Nuclear localization signal (NLS) - transposase fusion proteins were expressed in cultured cells and embryos to increase the delivery of transposase protein to the nucleus. Enhancer-promoter control elements were used to control the transcription of the transposases, such that the expression of these proteins could be increased through the co-expression of a transactivator protein. For the *Mos1* element only, mutagenesis was used to modify the inverted terminal repeats (ITRs) and the transposase coding sequence to determine if the activity of this system could be increased. Given the large number of variables to be tested in these experiments and the potentially small individual increases, we developed a quantitative PCR assay for measuring plasmid to plasmid transposition frequencies, replacing the previous system that required *E. coli* transformation and analysis of plasmid colonies.