

High efficiency site-specific genetic engineering of the mosquito genome.

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Abstract

The development of transgenic insect technology has the potential to combat a broad range of vector-borne diseases and insect pests. Current technologies involve transposable genetic elements, as represented by the *P*-element paradigm in *Drosophila*. However, phylogenetic restriction of this system resulted in a search for alternative transposon vectors that offered broader functionality in non-drosophilid insects. Four such transposons have now been developed into vector-helper systems for insect transgenesis, including *piggyBac*, *Hermes*, *mariner-Mos1* and *Minos*. These have been used successfully to integrate transgenes into the genomes of numerous insect species, including Diptera, Lepidoptera, Coleoptera and Hymenoptera. However, such transformation vectors are inefficient, have limited carrying capacity and can give rise to position effects, insertional mutagenesis and instability.

As an alternative approach, we have investigated two site-specific integration mechanisms in *Aedes aegypti*. One was a modification of the Cre/*lox* system from phage P1 and the other a viral integrase system from the *Streptomyces* phage ϕ C31. First, *piggyBac* transformation was used to introducing appropriate target sites into the genome, creating multiple independent 'docking' strains. These were subsequently tested for site-specific integration of transgenes at the docking site. We found that the modified Cre/*lox* system consistently failed to produce stable germline transformants. However, the ϕ C31 system was highly successful, increasing integration efficiency by up to 524% compared to conventional transformation. These gains result partly from the intrinsic efficiency of ϕ C31 integrase and partly from the doubling of survival that is seen when embryos and larvae are no longer exposed to *piggyBac* transposase.

The ability to efficiently target transgenes to specific chromosomal locations and potentially to integrate very large transgenes has broad applicability, with interest to research on many medically and economically important species. Site-specific integration should circumvent many of the limitations associated with transposable elements, particularly position effects on transgene expression and insertional mutagenesis. Site-specificity would also facilitate true comparative transgene analyses, such as those required for promoter optimization.

In the future, regulatory concerns over the deployment of transgenic insects in biocontrol programmes will require demonstrable transgene stability. With transposon technology, there is a small (perhaps theoretical) risk that the transposon cassette might be remobilized by an identical or closely related endogenous transposase. Such transgenes might be compromised in their expression if they move to a new genomic location or represent an opportunity for horizontal transfer to non-target species. We propose that *piggyBac*-mediated integration of an *attP* docking site followed by post-integration stabilization would generate a universal docking strain capable of ϕ C31-mediated integration of any desired transgene but incapable of remobilization. Such a mechanism may come to represent the paradigm for second-generation transgenic technologies in insects of medical and economic importance.