UC Irvine
UC Irvine Previously Published Works

Title
Mosquito Transposon-Mediated Transgenesis.

Permalink
https://escholarship.org/uc/item/0vw5f1vp

Authors
Bottino-Rojas, Vanessa
James, Anthony A

Publication Date
2023-10-10

DOI
10.1101/pdb.top107687

Copyright Information
This work is made available under the terms of a Creative Commons Attribution License, availalbe at https://creativecommons.org/licenses/by/4.0/

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Downloaded from http://cshprotocols.cshlp.org/ at CALIF-IRVINE on October 13, 2023 - Published by
Cold Spring Harbor Laboratory Press

Topic Introduction

Mosquito Transposon-Mediated Transgenesis

Vanessa Bottino-Rojas[1] and Anthony A. James[1,2,3]

[1]*Department of Microbiology and Molecular Genetics, University of California, Irvine, California 92697-4500, USA;* [2]*Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92697-3900, USA*

Transposon-mediated transgenesis of mosquito vectors of disease pathogens followed the early success of transgenesis in the vinegar fly, *Drosophila melanogaster*. The *P* transposable element used in *Drosophila* does not function canonically in mosquitoes, and repeatable, routine transgenesis in mosquitoes was not accomplished until new transposable elements were discovered and validated. A number of distinct transposons were subsequently identified that mediate the introduction of exogenous DNA in a stable and heritable manner in mosquito species, including members of the genera *Aedes*, *Anopheles*, and *Culex*. The most versatile element, *piggyBac*, is functional in all of these mosquito genera, as well as in many other insects in diverse orders, and has been used extensively outside the class. Transposon-mediated transgenesis of recessive and dominant marker genes and reporter systems has been used to define functional fragments of gene control sequences, introduce exogenous DNA encoding products beneficial to medical interests, and act as "enhancer traps" to identify endogenous genes with specific expression characteristics.

Cold Spring Harbor Protocols
www.cshprotocols.org
CSH PROTOCOLS

BACKGROUND

Following the discovery that the *P* element could be used to efficiently transform *Drosophila melanogaster* (Rubin and Spradling 1982), concerted efforts were made to develop transposon-based transgenic technologies for a wide variety of arthropod species of agricultural and public health significance. Early efforts in mosquitoes focused unproductively on trying to adapt the *P* element to several species (Miller et al. 1987: McGrane et al. 1988; Morris et al. 1989), but it was not until the discovery of a number of new elements that success was finally achieved. "Plasmid mobility assays" were used to determine whether or not a particular element *could* be mobilized (excise and insert) in the germline of a specific insect (O'Brochta and Handler 1988). These assays clearly showed that despite its power in *D. melanogaster*, the *P* element could not transform the vast majority of other, non-drosophilid species in which it was tested. Efforts were made to use the *P* element by identifying host factors necessary for activity in *D. melanogaster* (Rio and Rubin 1988; Handler et al. 1993), but the parallel discovery and testing of new elements ultimately led to their adoption.

Work in mosquitoes has focused on Class II transposable elements, which mobilize through a DNA intermediate and can result in either conservative (no increase in element copy number) or replicative (results in an increase in copy number of the element) transposition (Atkinson and James 2002). Class II elements have variable-length terminal repeat DNA sequences, which are most often inverted (designated inverted terminal repeats [ITRs] or terminal inverted repeats [TIRs]). These

[3]Correspondence: aajames@uci.edu
From the Mosquitoes collection, edited by Laura B. Duvall and Benjamin J. Matthews.
© 2023 Cold Spring Harbor Laboratory Press
Advanced Online Article. Cite this introduction as *Cold Spring Harb Protoc*; doi:10.1101/pdb.top107687

Downloaded from http://cshprotocols.cshlp.org/ at CALIF-IRVINE on October 13, 2023 - Published by
Cold Spring Harbor Laboratory Press

repeats flank an open reading frame encoding a transposase that recognizes the ITRs and a conserved target site 2–8 bp in length and catalyzes the mobility of the element from one site in a plasmid or chromosome to another (Fig. 1). Following successful testing in mobility assays, the elements *Mos1 mariner*, *Minos*, and *Hermes* (Franz and Savakis 1991; Medhora et al. 1991; Warren et al. 1994) were adapted to transform mosquito species (Table 1; Sarkar et al. 1997; Coates et al. 1998; Jasinskiene et al. 1998; Catteruccia et al. 2000a,b; Allen et al. 2001). Molecular characterization of the baculovirus insertion element IFP2 using mobility assays in insect cells set the groundwork for the element now known as *piggyBac* to be applied widely across mosquito species (Fraser et al. 1995; Elick et al. 1997). Currently, *piggyBac* is sufficiently versatile to be useful across species from yeast to mammals (Yusa 2015) and is now the element used predominantly for transposon-mediated transgenesis of all the major mosquito vectors (Table 1).

Transposon-mediated mosquito transgenesis experiments have focused primarily on defining functional fragments of gene control sequences (promoters, 5′- and 3′-end DNAs, introns), introducing exogenous DNA encoding products beneficial to mitigating pathogen transmission, modifying expression profiles of endogenously derived genes, and introducing “enhancer traps” to identify endogenous genes with specific expression characteristics. Enhancer traps based on *piggyBac* were used to identify genes with potentially useful expression profiles (O’Brochta et al. 2012; Reid et al. 2018). Functional promoter analysis studies were biased at the outset by looking at genes that were expressed in mosquito tissues important for their role as pathogen vectors. This bias was expected to reduce potential transgenesis-related fitness costs by restricting the expression of transgenes to the infection-relevant sex, developmental stage, and mosquito body compartments in which the pathogens are found. To date, transgenesis technologies have identified gene sequences that allow stage-, tissue-, and sex-specific expression of dominant marker and reporter genes, and DNA sequences whose products interfere with viral and protozoan pathogen development and transmission (Table 1). Future studies could identify effective and robust regulatory sequences that are uniquely activated in response to pathogen infection in order to minimize even further any fitness issues associated with transgenesis. Additionally, reporter strains for binary expression systems (e.g., QF2) can be generated easily through transposon-mediated transgenesis and are useful for the characterization of neural circuits and neuronal activity visualization (Lynd and Lycett 2012; Riabinina et al. 2016).

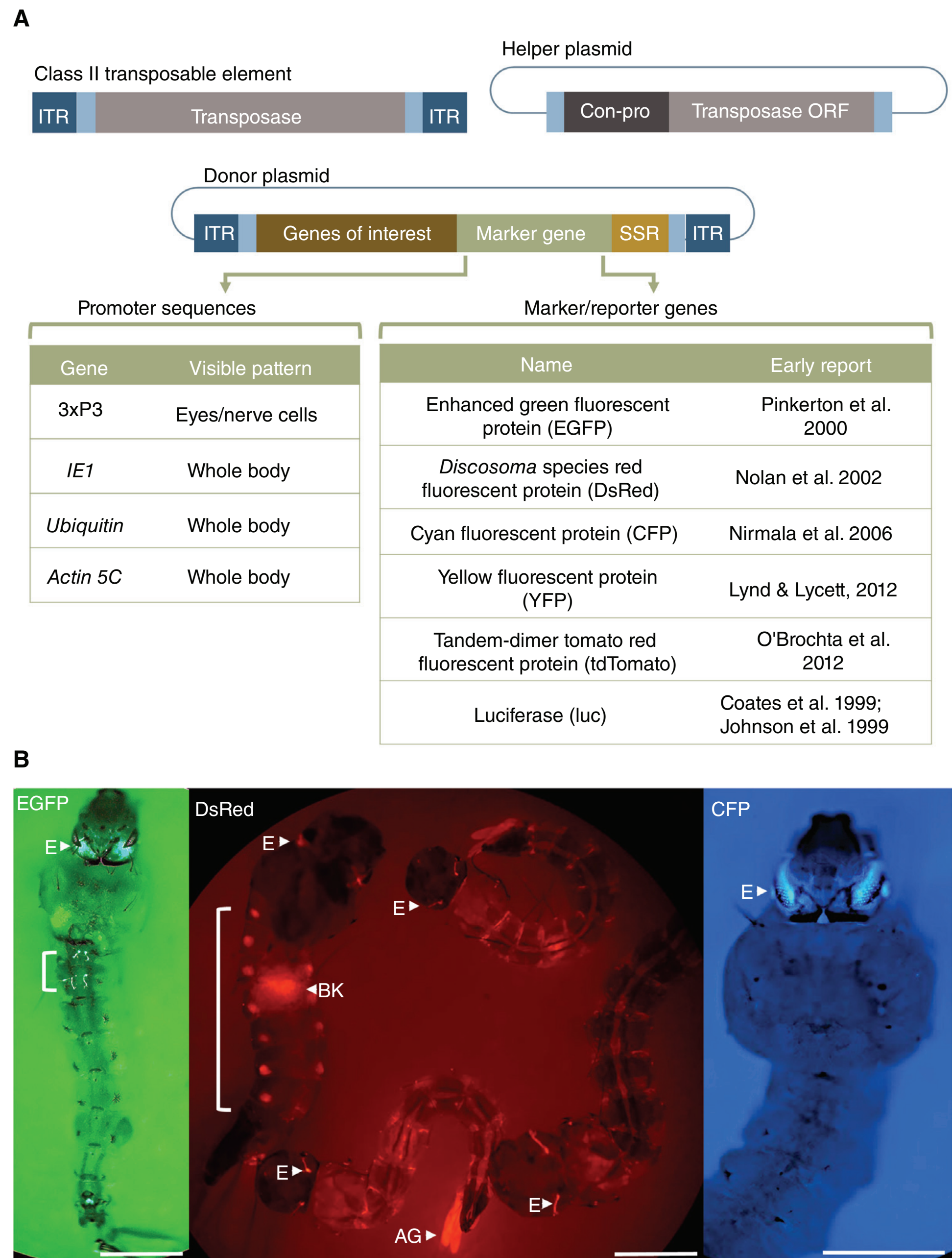
Cold Spring Harbor Protocols
www.cshprotocols.org

EXPERIMENTAL DESIGN CONSIDERATIONS

Element choice is significant for transposon-based experiments. Although all elements have a preferred target nucleotide sequence for integration, these vary in size and complexity (Table 1). Both *Minos* and *Mos1* recognize the dinucleotide TA, which occurs frequently enough in the genome to make integrations of these elements appear random. *piggyBac* and *Hermes* recognize longer target sequences, four (TTAA) and eight bases (GTNCAGAC) in length, respectively, but these sequences also occur frequently enough in the genome to appear to be distributed randomly. In our experience with *Aedes aegypti*, *Mos1* tends to insert in fewer copy numbers in the genome than *piggyBac*, making post-transformation characterization of the transgenes easier to manage (V Bottino-Rojas and AA James, unpubl.). However, *Anopheles* transgenesis is almost exclusively performed with *piggyBac*. Elements can exhibit noncanonical and unstable integration and postintegration behavior, making it important to accurately characterize the insertion copy number, structure, and stability in individual transgenic lines (Jasinskiene et al. 2000; Adelman et al. 2004; Palavesam et al. 2013).

Transposon-mediated transgenesis is usually performed with a binary system of donor and helper plasmids (Fig. 1). The donor plasmid contains ITRs flanking the transgene, whereas the helper plasmid transiently expresses the transposase enzyme, which catalyzes element excision from the donor plasmid and insertion into the host genome (Fraser et al. 1996). Our associated protocol provides detailed experimental considerations (see Protocol: **Generating and Validating Transgenic Mosquitoes with Transposon-Mediated Transgenesis** [Bottino-Rojas and James 2023]). Plasmid

Downloaded from http://cshprotocols.cshlp.org/ at CALIF-IRVINE on October 13, 2023 - Published by
Cold Spring Harbor Laboratory Press



Gene	Visible pattern
3xP3	Eyes/nerve cells
IE1	Whole body
Ubiquitin	Whole body
Actin 5C	Whole body

Name	Early report
Enhanced green fluorescent protein (EGFP)	Pinkerton et al. 2000
Discosoma species red fluorescent protein (DsRed)	Nolan et al. 2002
Cyan fluorescent protein (CFP)	Nirmala et al. 2006
Yellow fluorescent protein (YFP)	Lynd & Lycett, 2012
Tandem-dimer tomato red fluorescent protein (tdTomato)	O'Brochta et al. 2012
Luciferase (luc)	Coates et al. 1999; Johnson et al. 1999

FIGURE 1. General organization and use of Class II transposable elements. (*A*) Schematic representations of a Class II transposable element and engineered helper and donor plasmids. Active Class II transposable elements have inverted terminal repeated (ITR) DNA sequences flanking a functional transposase gene complete with promoter and non-transcribed control DNA (Transposase). The helper plasmid (Helper) has the transposase open reading frame (ORF) under the control of a constitutive promoter (Con-pro). The donor plasmid (Donor) contains ITRs flanking genes of interest, a dominant marker gene along with control sequences to allow visible screening of transgenic animals, and in some cases sites for site-specific recombination (SSR). Both donor and helper DNA sequences are cloned into bacterial plasmids (thin lines) that allow production and purification for use in embryo microinjections or other DNA-introduction technologies. (*Table*) Examples of promoter sequences and marker/reporter genes along with early- or first-use citations in mosquitoes. (*B*) Examples of transposon-mediated transgenic mixed-instar *Anopheles stephensi* mosquito larvae marked with 3xP3-fluorescence genes. All larvae show transgene-mediated fluorescence in their eyes (E). Some larvae exhibit additional transgene-specific fluorescence in the segmented nervous tissue (vertical brackets), and anal gills (AG). The food bolus in the gut can produce background (BK) fluorescence. Horizontal bars, ~1 mm. (EGFP) Enhanced green fluorescent protein, (CFP) cyan fluorescent protein, (DsRed) *Discosoma* species red fluorescent protein.

Cold Spring Harbor Protocols
www.cshprotocols.org
CSH PROTOCOLS

Downloaded from http://cshprotocols.cshlp.org/ at CALIF-IRVINE on October 13, 2023 - Published by Cold Spring Harbor Laboratory Press

TABLE 1. Transposable elements (A) and common promoters (B) used in mosquito transgenesis

A

Transposable elements	Origin	Family	Size (kb)[a]	Recognition site	Species[b]	Reference(s)[c]
Hermes	*Musca domestica*	hAT	2.7	GTNCAGAC	*Aeae, Cuqu*	Jasinskiene et al. 1998; Allen et al. 2001
Mos1	*Drosophila mauritiana*	mariner	1.3	TA	*Aeae*	Coates et al. 1998
Minos	*Drosophila hydei*	TC1-like	1.8	TA	*Anst*	Catteruccia et al. 2000b
piggyBac	*Trichoplusia ni* baculovirus	Sleeping beauty	2.5	TTAA	*Aeae, Aeal, Aefl, Anal, Anga, Anst, Ansi*	Kokoza et al. 2001; Grossman et al. 2001; Nolan et al. 2002; Perera et al. 2002; Rodrigues et al. 2006; Labbé et al. 2010; Liu et al. 2021

B

Promoters: Gene	Origin	Expression pattern	Reference(s)[c]
Heat-shock protein 70 (hsp70)	*Drosophila melanogaster; Aedes aegypti*	Ubiquitous; constitutive/heat-inducible	Morris et al. 1991; Carpenetti et al. 2012
Heat-shock protein 82 (Hsp82)	*Drosophila pseudoobscura*	Ubiquitous; constitutive	Coates et al. 1998
Act88f	*D. melanogaster*	Flight muscles	Allen and Christensen 2004
Actin5C	*D. melanogaster*	Ubiquitous; constitutive	Pinkerton et al. 2000
Actin4	*Ae. aegypti*	Female-specific; flight muscles	Fu et al. 2010
Ubiquitin (L40 and PUb)	*Ae. aegypti*	Ubiquitous; constitutive	Anderson et al. 2010
D7-related (D7r)	*Anopheles gambiae*	Salivary glands	Lombardo et al. 2005
Antiplatelet protein (aapp)	*Anopheles stephensi*	Female-specific; salivary glands; blood-meal inducible	Yoshida and Watanabe 2006
Apyrase	*Ae. aegypti; An. gambiae*	Female-specific; salivary glands	Coates et al. 1999; Lombardo et al. 2005
Aegyptin (30k a and 30k b)	*Ae. aegypti*	Female-specific; salivary glands	Mathur et al. 2010
Vitellogenin	*Ae. aegypti; An. stephensi; An. gambiae*	Female-specific; fat body (hemolymph); blood-meal inducible	Kokoza et al. 2000; Nirmala et al. 2006; Chen et al. 2007
Carboxypeptidase	*An. gambiae*	Female-specific; midgut; blood-meal inducible	Moreira et al. 2000;
G12	*An. gambiae*	Female-specific; midgut; blood-meal inducible	Nolan et al. 2011;
Peritrophin	*An. gambiae*	Female-specific; midgut; constitutive	Abraham et al. 2005
Antryp1	*An. gambiae*	Female-specific; midgut; blood-meal inducible	Nolan et al. 2011
Vasa	*An. gambiae*	Gonads	Papathanos et al. 2009
nanos	*Ae. aegypti*	Female-specific; ovaries/embryos	Adelman et al. 2007
exuperantia	*Ae. aegypti*	Female-specific; ovaries; blood-meal inducible	Akbari et al. 2013
bZip1	*Ae. aegypti*	Female-specific; ovaries/embryos	Kojin et al. 2020
β2 tubulin	*An. gambiae; Ae. aegypti*	Male-specific; testes	Catteruccia et al. 2005; Smith et al. 2007

[a]Approximate size in kilobase pairs (kb).
[b]Mosquito species transformed: (*Aeae*) *Ae. aegypti*, (*Aeal*) *Aedes albopictus*, (*Aefl*) *Aedes fluviatilis*, (*Anal*) *Anopheles albimanus*, (*Anga*) *An. gambiae*, (*Ansi*) *Anopheles sinensis*, (*Anst*) *An. stephensi*, (*Cuqu*) *Culex quinquefasciatus*.
[c]First reported use in mosquito species using transposon-mediated transgenesis.

Downloaded from http://cshprotocols.cshlp.org/ at CALIF-IRVINE on October 13, 2023 - Published by Cold Spring Harbor Laboratory Press

cloning allows the production and purification of large amounts of DNA before experimental usage. The minimal donor plasmid design contains the ITRs flanking a marker gene and genes of interest. Target sequences for site-specific recombination (SSR) also can be included that allow manipulation of the transgene insert once it is integrated into the genome. DNA-insulating sequences can be added just inside the ITRs to help mitigate insertion-site effects (Carballar-Lejarazú et al. 2013). Fully functional mosquito-derived introns have been used to modify gene-of-interest configurations when needed (Franz et al. 2006). Other components of the genetic toolbox (e.g., Gal4-UAS systems, SSR docking sites) are discussed elsewhere (see Topic Introduction: **Genetic Toolbox Approaches in Mosquitoes** [Riabinina et al. 2022]). The exact order of the genes in the element depends on the purpose of the experiment. The success rate of transposase-mediated mobilization from a plasmid into a mosquito genome is likely to depend on the size of the DNA being inserted, but a meta-analysis of the published work showed that most elements can efficiently insert 10–15 kb of DNA (Gregory et al. 2016).

Helper plasmids contain the transposase open reading frame (ORF) under the control of a constitutive promoter cloned into a plasmid. Heat-shock protein gene promoters (*hsp70* from *D. melanogaster* and *hsp82* from *Drosophila pseudoobscura*; Table 1) are active constitutively in mosquito embryos and have been used successfully in many transgenesis experiments. Helper plasmids will not replicate in the insect embryo and are expected to be lost by dilution as the injected animals progress through development.

Purified transposases also have been used in place of a helper plasmid (Coates et al. 2000). Hyperactive transposase proteins were shown to improve transformation rates in nonmosquito species, and transposase mRNAs can be injected when helper plasmids are not readily available (Otte et al. 2018).

A key contribution to mosquito transgenic technology was the discovery and application of dominant-acting fluorescent proteins that can be assayed in live animals and are visible when expressed from a single copy of their encoding gene (Fig. 1; Handler and Harrell 2001). Genes encoding proteins that fluoresce in green, red, and blue wavelengths were adapted quickly as marker genes in mosquitoes and have been used extensively. These gene products also can be used as reporter sequences in both qualitative and quantitative assays. Indeed, characterization of specific gene control DNA (promoters, 5′- and 3′-end untranslated regions [UTRs]) has been performed successfully for many genes by linking the predicted control elements to one of the fluorescent protein–encoding reading frames and scoring activity in live animals under fluorescence microscopy (Table 1).

Cold Spring Harbor Protocols www.cshprotocols.org CSH PROTOCOLS

DISCUSSION

Transposon-mediated generation of genetically engineered mosquito vectors of disease has revolutionized both basic and applied studies of these insects. The application of CRISPR–Cas technologies to mosquito transgenesis is likely to make obsolete some aspects of transposon-based technologies except in circumstances where quasi-random insertion generation is desirable (enhancer traps, random "docking" sites for SSR, insertion-site impact analyses) (see Topic Introduction: **Design and Validation of Guide RNAs for CRISPR/Cas9 Genome Editing in Mosquitoes** [Lo and Matthews 2023]; Amenya et al. 2010; O'Brochta et al. 2012; Carballar-Lejarazú et al. 2013). Therefore, efficient and routine methods for transposon-mediated germline transgenesis and genomic analyses provide tools for investigations where its unique, remobilizable, insertional mutagenesis features are needed.

ACKNOWLEDGMENTS

Funding was provided by the University of California Irvine Malaria Initiative. A.A.J. is a Donald Bren Professor at the University of California, Irvine. We are grateful to Kiona Parker for providing images of transgenic *An. stephensi* larvae.

Downloaded from http://cshprotocols.cshlp.org/ at CALIF-IRVINE on October 13, 2023 - Published by
Cold Spring Harbor Laboratory Press

REFERENCES

Abraham EG, Donnelly-Doman M, Fujioka H, Ghosh A, Moreira L, Jacobs-Lorena M. 2005. Driving midgut-specific expression and secretion of a foreign protein in transgenic mosquitoes with *AgAper1* regulatory elements. *Insect Mol Biol* **14:** 271–279. doi:10.1111/j.1365-2583.2004.00557.x

Adelman ZN, Jasinskiene N, Vally KJ, Peek C, Travanty EA, Olson KE, Brown SE, Stephens JL, Knudson DL, Coates CJ, et al. 2004. Formation and loss of large, unstable tandem arrays of the *piggyBac* transposable element in the yellow fever mosquito, *Aedes aegypti*. *Transgenic Res* **5:** 411–425. doi:10.1007/s11248-004-6067-2

Adelman ZN, Jasinskiene N, Onal S, Juhn J, Ashikyan A, Salampessy M, MacCauley T, James AA. 2007. *nanos* gene control DNA mediates developmentally regulated transposition in the yellow fever mosquito *Aedes aegypti*. *Proc Natl Acad Sci* **104:** 9970–9975. doi:10.1073/pnas.0701515104

Akbari OS, Antoshechkin I, Amrhein H, Williams B, Diloreto R, Sandler J, Hay B. 2013. The developmental transcriptome of the mosquito *Aedes aegypti*, an invasive species and major arbovirus vector. *G3 (Bethesda)* **3:** 1493–1509. doi:10.1534/g3.113.006742

Allen ML, Christensen BM. 2004. Flight muscle-specific expression of *act88F*: GFP in transgenic *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol Int* **53:** 307–314. doi:10.1016/j.parint.2004.04.002

Allen ML, O'Bhochta DA, Atkinson PW, Levesque CS. 2001. Stable, germline transformation of *Culex quinquefasciatus* (Diptera: Culicidae). *J Med Entomol* **38:** 701–710. doi:10.1603/0022-2585-38.5.701

Amenya DA, Bonizzoni M, Isaacs AT, Jasinskiene N, Chen H, Marinotti O, Yan G, James AA. 2010. Comparative fitness assessment of *Anopheles stephensi* transgenic lines receptive to site-specific integration. *Insect Mol Biol* **19:** 263–269. doi:10.1111/j.1365-2583.2009.00986.x

Anderson MAE, Gross TL, Myles KM, Adelman ZN. 2010. Validation of novel promoter sequences derived from two endogenous ubiquitin genes in transgenic *Aedes aegypti*. *Insect Mol Biol* **19:** 441–449. doi:10.1111/j.1365-2583.2010.01005.x

Atkinson PW, James AA. 2002. Germline transformants spreading out to many insect species. *Adv Genet* **47:** 49–86. doi:10.1016/S0065-2660(02)47002-2

Bottino-Rojas T, James AA. 2023. Generating and validating transgenic mosquitoes with transposon-mediated transgenesis. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot108194

Carballar-Lejarazú R, Jasinskiene N, James AA. 2013. Exogenous gypsy insulator sequences modulate transgene expression in the malaria vector mosquito, *Anopheles stephensi*. *Proc Natl Acad Sci* **110:** 7176–7181. doi:10.1073/pnas.1304722110

Carpenetti TLG, Aryan A, Myles KM, Adelman ZN. 2012. Robust heat-inducible gene expression by two endogenous *hsp70*-derived promoters in transgenic *Aedes aegypti*. *Insect Mol Biol* **21:** 97–106. doi:10.1111/j.1365-2583.2011.01116.x

Catteruccia F, Nolan T, Blass C, Muller HM, Crisanti A, Kafatos FC, Loukeris TG. 2000a. Toward *Anopheles* transformation: Minos element activity in anopheline cells and embryos. *Proc Natl Acad Sci* **97:** 2157–2162. doi:10.1073/pnas.040568397

Catteruccia F, Nolan T, Loukeris TG, Blass C, Savakis C, Kafatos FC, Crisanti A. 2000b. Stable germline transformation of the malaria mosquito *Anopheles stephensi*. *Nature* **405:** 959–962. doi:10.1038/35016096

Catteruccia F, Benton JP, Crisanti A. 2005. An *Anopheles* transgenic sexing strain for vector control. *Nat Biotechnol* **23**: 1414–1417. doi:10.1038/nbt1152

Chen X, Marinotti O, Whitman L, Jasinskiene N, Romans P, James AA. 2007. The *Anopheles gambiae* vitellogenin gene (*VGT2*) promoter directs persistent accumulation of a reporter gene product in transgenic *Anopheles stephensi* following multiple blood meals. *Am J Trop Med Hygiene* **76:** 1118–1124. doi:10.4269/ajtmh.2007.76.1118

Coates CJ, Jasinskiene N, Miyashiro L, James AA. 1998. *Mariner* transposition and transformation of the yellow fever mosquito, *Aedes aegypti*. *Proc Natl Acad Sci* **95:** 3743–3747. doi:10.1073/pnas.95.7.3748

Coates CJ, Jasinskiene N, Pott GB, James AA. 1999. Promoter-directed expression of recombinant fire-fly luciferase in the salivary glands of *Hermes*-transformed *Aedes aegypti*. *Gene* **226:** 317–325. doi:10.1016/S0378-1119(98)00557-5

Coates CJ, Jasinskiene N, Morgan D, Tosi LR, Beverley SM, James AA. 2000. Purified *mariner* (*Mos1*) transposase catalyzes the integration of marked elements into the germ-line of the yellow fever mosquito, *Aedes aegypti*. *Insect Biochem Mol Biol* **30:** 1003–1008. doi:10.1016/S0965-1748(00)00110-7

Elick TA, Lobo N, Fraser MJ Jr. 1997. Analysis of the *cis*-acting DNA elements required for *piggyBac* transposable element excision. *Mol Gen Genet* **255:** 605–610. doi:10.1007/s004380050534

Franz G, Savakis C. 1991. Minos, a new transposable element from *Drosophila hydei*, is a member of the Tc1-like family of transposons. *Nucl Acids Res* **19:** 6646. doi:10.1093/nar/19.23.6646

Franz AWE, Sanchez-Vargas I, Adelman ZN, Blair CD, Beaty BJ, James AA, Olson KE. 2006. Engineering RNA interference-based resistance to dengue virus type-2 in genetically-modified *Aedes aegypti*. *Proc Natl Acad Sci* **103:** 4198–4203. doi:10.1073/pnas.0600479103

Fraser MJ, Cary L, Boonvisudhi K, Wang HG. 1995. Assay for movement of Lepidopteran transposon IFP2 in insect cells using a baculovirus genome as a target DNA. *Virology* **211:** 397–407. doi:10.1006/viro.1995.1422

Fraser MJ, Ciszczon T, Elick T, Bauser C. 1996. Precise excision of TTAA-specific lepidopteran transposons *piggyBac* (IFP2) and *tagalong* (TFP3) from the baculovirus genome in cell lines from two species of Lepidoptera. *Insect Mol Biol* **5:** 141–151. doi:10.1111/j.1365-2583.1996.tb00048.x

Fu G, Lees R, Aw D, Jin L, Gray P, Berendonk TU, White-Cooper H, Scaife S, Phuc HK, Marinotti O, et al. 2010. Female-specific flightless phenotype for mosquito control. *Proc Natl Acad Sci* **107:** 4550–4554. doi:10.1073/pnas.1000251107

Gregory M, Alphey L, Morrison NI, Shimeld SM. 2016. Insect transformation with *piggyBac*: getting the number of injections just right. *Insect Mol Biol* **25:** 259–271. doi:10.1111/imb.12220

Grossman GL, Rafferty CS, Clayton JR, Stevens TK, Mukabayire O, Benedict MQ. 2001. Germline transformation of the malaria vector, *Anopheles gambiae*, with the *piggyBac* transposable element. *Insect Mol Biol* **10:** 597–604. doi:10.1046/j.0962-1075.2001.00299.x

Handler AM, Harrell RA. 2001. Polyubiquitin-regulated DsRed marker for transgenic insects. *Biotechniques* **31:** 820. 824-8.

Handler AM, Gomez SP, O'Brochta DA. 1993. A functional analysis of the *P*-element gene-transfer vector in insects. *Arch Insect Biochem Physiol* **22:** 373–384. doi:10.1002/arch.940220306

Jasinskiene N, Coates CJ, Benedict MQ, Cornel AJ, Rafferty CS, James AA, Collins FH. 1998. Stable transformation of the yellow fever mosquito, *Aedes aegypti*, using the *Hermes* element from the housefly. *Proc Natl Acad Sci* **95:** 3748–3751. doi:10.1073/pnas.95.7.3743

Jasinskiene N, Coates CJ, James AA. 2000. Structure of *Hermes* integrations in the germline of the yellow fever mosquito, *Aedes aegypti*. *Insect Mol Biol* **9:** 11–18. doi:10.1046/j.1365-2583.2000.00153.x

Johnson BW, Olson KE, Allen-Miura T, Carlson JO, Coates CJ, Jasinskiene N, James AA, Beaty BJ, Higgs S. 1999. Inhibition of luciferase expression in transgenic *Aedes aegypti* mosquitoes by Sindbis virus expression of antisense luciferase RNA. *Proc Natl Acad Sci* **96:** 13399–13403. doi:10.1073/pnas.96.23.13399

Kojin BB, Biedler JK, Tu Z, Adelman ZN. 2020. Characterization of a female germline and early zygote promoter from the transcription factor bZip1 in the dengue mosquito *Aedes aegypti*. *Parasit Vectors* **13:** 1–11. doi:10.1186/s13071-020-04216-w

Kokoza V, Ahmed A, Cho WL, Jasinskiene N, James AA, Raikhel A. 2000. Engineering blood meal-activated systemic immunity in the yellow fever mosquito, *Aedes aegypti*. *Proc Natl Acad Sci* **97:** 9144–9149. doi:10.1073/pnas.160258197

Kokoza V, Ahmed A, Wimmer EA, Raikhel AS. 2001. Efficient transformation of the yellow fever mosquito *Aedes aegypti* using the *piggyBac* transposable element vector pBac [3xP3-EGFP afm]. *Insect Biochem Mol Biol* **31:** 1137–1143. doi:10.1016/S0965-1748(01)00120-5

Labbé GM, Nimmo DD, Alphey L. 2010. *piggybac*- and *PhiC31*-mediated genetic transformation of the Asian tiger mosquito, *Aedes albopictus* (Skuse). *PLoS Negl Trop Dis* **4:** e788. doi:10.1371/journal.pntd.0000788

Liu JG, Qiao L, Zhang JJ, Chen B, He ZB. 2021. *piggyBac*-mediated germline transformation of the malaria mosquito *Anopheles sinensis* (Diptera: Culicidae). *Insect Sci* **28:** 1202–1206. doi:10.1111/1744-7917.12836

Cold Spring Harbor Protocols
www.cshprotocols.org

Downloaded from http://cshprotocols.cshlp.org/ at CALIF-IRVINE on October 13, 2023 - Published by Cold Spring Harbor Laboratory Press

Lo IHY, Matthews B. 2023. Design and validation of guide RNAs for CRISPR/Cas9 genome editing in mosquitoes. *Cold Spring Harb Protoc* doi:10.1101/pdb.top107688

Lombardo F, Nolan T, Lycett G, Lanfrancotti A, Stich N, Catteruccia F, Louis C, Coluzzi M, Arcà B. 2005. An *Anopheles gambiae* salivary gland promoter analysis in *Drosophila melanogaster* and *Anopheles stephensi*. *Insect Mol Biol* **14:** 207–216. doi:10.1111/j.1365-2583.2004.00549.x

Lynd A, Lycett GJ. 2012. Development of the bi-partite Gal4-UAS system in the African malaria mosquito, *Anopheles gambiae*. *PLoS ONE* **7:** e31552. doi:10.1371/journal.pone.0031552

Mathur G, Sanchez-Vargas I, Alvarez D, Olson KE, Marinotti O, James AA. 2010. Transgene-mediated suppression of dengue viruses in the salivary glands of the yellow fever mosquito, *Aedes aegypti*. *Insect Mol Biol* **19:** 753–763. doi:10.1111/j.1365-2583.2010.01032.x

McGrane V, Carlson JO, Miller BR, Beaty BJ. 1988. Microinjection of DNA into *Aedes triseriatus* ova and detection of integration. *Am J Trop Med Hyg* **39:** 502–510. doi:10.4269/ajtmh.1988.39.502

Medhora M, Maruyama K, Hartl DL. 1991. Molecular and functional analysis of the mariner mutator element Mos1 in *Drosophila*. *Genetics* **128:** 311–318. doi:10.1093/genetics/128.2.311

Miller LH, Sakai RK, Romans P, Gwadz RW, Kantoff P, Coon HG. 1987. Stable integration and expression of a bacterial gene in the mosquito *Anopheles gambiae*. *Science* **237:** 779–781. doi:10.1126/science.3039658

Moreira LA, Edwards MJ, Adhami F, Jasinskiene N, James AA, Jacobs-Lorena M. 2000. Robust gut-specific gene expression in transgenic *Aedes aegypti* mosquitoes. *Proc Natl Acad Sci* **97:** 10895–10898. doi:10.1073/pnas.97.20.10895

Morris AC, Eggelston P, Crampton JM. 1989. Genetic transformation of the mosquito *Aedes aegypti* by micro-injection of DNA. *Med Vet Entomol* **3:** 1–7. doi:10.1111/j.1365-2915.1989.tb00467.x

Morris AC, Schaub TL, James AA. 1991. FLP-mediated recombination in the vector mosquito, *Aedes aegypti*. *Nucl Acids Res* **19:** 5895–5900. doi:10.1093/nar/19.21.5895

Nirmala X, Marinotti O, Sandoval JM, Phin S, Gakhar S, Jasinskiene N, James AA. 2006. Functional characterization of the promoter of the vitellogenin gene, *AsVg1*, of the malaria vector, *Anopheles stephensi*. *Insect Biochem Molec Biol* **36:** 694–700. doi:10.1016/j.ibmb.2006.05.011

Nolan T, Bower TM, Brown AE, Crisanti A, Catteruccia F. 2002. *piggyBac*-mediated germline transformation of the malaria mosquito *Anopheles stephensi* using the red fluorescent protein dsRED as a selectable marker. *J Biol Chem* **277:** 8759–8762. doi:10.1074/jbc.C100766200

Nolan T, Petris E, Müller H-M, Cronin A, Catteruccia F, Crisanti A. 2011. Analysis of two novel midgut-specific promoters driving transgene expression in *Anopheles stephensi* mosquitoes. *PLoS ONE* **6:** e16471. doi:10.1371/journal.pone.0016471

O'Brochta DA, Handler AM. 1988. Mobility of *P* elements in drosophilids and nondrosophilids. *Proc Natl Acad Sci* **85:** 6052–6056. doi:10.1073/pnas.85.16.6052

O'Brochta DA, Pilitt KL, Harrell RA II, Aluvihare C, Alford RT. 2012. Gal4-based enhancer-trapping in the malaria mosquito *Anopheles stephensi*. *G3 (Bethesda)* **2:** 1305–1315. doi:10.1534/g3.112.003582

Otte M, Netschitailo O, Kaftanoglu O, Wang Y, Page RE Jr, Beye M. 2018. Improving genetic transformation rates in honeybees. *Sci Rep* **8:** 16534. doi:10.1038/s41598-018-34724-w

Palavesam A, Esnault C, O'Brochta DA. 2013. Post-integration silencing of *piggyBac* transposable elements in *Aedes aegypti*. *PLoS ONE* **8:** e68454. doi:10.1371/journal.pone.0068454

Papathanos PA, Windbichler N, Menichelli M, Burt A, Crisanti A. 2009. The vasa regulatory region mediates germline expression and maternal transmission of proteins in the malaria mosquito *Anopheles gambiae*: a versatile tool for genetic control strategies. *BMC Mol Biol* **10:** 1–13. doi:10.1186/1471-2199-10-65

Perera OP, Harrell RA II, Handler AM. 2002. Germline transformation of the South American malaria vector, *Anopheles albimanus*, with a *piggyBac/EGFP* transposon vector is routine and highly efficient. *Insect Mol Biol* **11:** 291–297. doi:10.1046/j.1365-2583.2002.00336.x

Pinkerton AC, Michel K, O'Brochta DA, Atkinson PW. 2000. Green fluorescent protein as a genetic marker in transgenic *Aedes aegypti*. *Insect Mol Biol* **9:** 1–10. doi:10.1046/j.1365-2583.2000.00133.x

Reid W, Pilitt K, Alford R, Cervantes-Medina A, Yu H, Aluvihare C, Harrell R, O'Brochta DA. 2018. An *Anopheles stephensi* promoter-trap: augmenting genome annotation and functional genomics. *G3 (Bethesda)* **8:** 3119–3130. doi:10.1534/g3.118.200347

Riabinina O, Task D, Marr E, Lin C-C, Alford R, O'Brochta DA, Potter CJ. 2016. Organization of olfactory centres in the malaria mosquito *Anopheles gambiae*. *Nat Commun* **7:** 13010. doi:10.1038/ncomms13010

Riabinina O, Quinn M, Whitehead JP. 2022. Genetic toolbox approaches in mosquitoes. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot107691

Rio DC, Rubin GM. 1988. Identification and purification of a *Drosophila* protein that binds to the terminal 31-base-pair inverted repeats of the P transposable element. *Proc Natl Acad Sci* **85:** 8929–8933. doi:10.1073/pnas.85.23.8929

Rodrigues FG, Oliveira SB, Rocha BC, Moreira LA. 2006. Germline transformation of *Aedes fluviatilis* (Diptera: Culicidae) with the *piggyBac* transposable element. *Mem Inst Oswaldo Cruz* **101:** 755–777. doi:10.1590/S0074-02762006000700008

Rubin GM, Spradling AC. 1982. Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218:** 348–353. doi:10.1126/science.6289436

Sarkar A, Yardley K, Atkinson PW, James AA, O'Brochta DA. 1997. Transposition of the *Hermes* element in embryos of the vector mosquito, *Aedes aegypti*. *Insect Mol Biol* **27:** 359–363. doi:10.1016/S0965-1748(97)00018-0

Smith RC, Walter MF, Hice RH, O'Brochta DA, Atkinson PW. 2007. Testis-specific expression of the β2 tubulin promoter of *Aedes aegypti* and its application as a genetic sex-separation marker. *Insect Mol Biol* **16:** 61–71. doi:10.1111/j.1365-2583.2006.00701.x

Warren WD, Atkinson PW, O'Brochta DA. 1994. The Hermes transposable element from the house fly, *Musca domestica*, is a short inverted repeat-type element of the *hobo*, *Ac*, and *Tam3* (*hAT*) element family. *Genet Res* **64:** 87–97. doi:10.1017/S0016672300032699

Yoshida S, Watanabe H. 2006. Robust salivary gland-specific transgene expression *in Anopheles stephensi* mosquito. *Insect Mol Biol* **15:** 403–410. doi:10.1111/j.1365-2583.2006.00645.x

Yusa K. 2015. *piggyBac* transposon. *Microbiol Spectr* **3:** MDNA3-0028-2014. doi:10.1128/microbiolspec.MDNA3-0028-2014

Cold Spring Harbor Protocols www.cshprotocols.org CSH PROTOCOLS

Downloaded from http://cshprotocols.cshlp.org/ at CALIF-IRVINE on October 13, 2023 - Published by
Cold Spring Harbor Laboratory Press

Cold Spring Harbor Protocols

Mosquito Transposon-Mediated Transgenesis

Vanessa Bottino-Rojas and Anthony A. James

Cold Spring Harb Protoc; doi: 10.1101/pdb.top107687; published online October 10, 2023

Email Alerting Service Receive free email alerts when new articles cite this article - **click here.**

Subject Categories Browse articles on similar topics from *Cold Spring Harbor Protocols.*

DNA Delivery/Gene Transfer (354 articles)
Fluorescent Proteins (263 articles)
Genetics, general (393 articles)
Mosquitoes (109 articles)
Mosquitoes: A Laboratory Manual (110 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
http://cshprotocols.cshlp.org/subscriptions

© 2023 Cold Spring Harbor Laboratory Press