

# Highlighting Molecular Biology as a Focal Point for Mosquito Research

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## Editorial

Molecular biology underpins many of the key technologies that have driven the scientific advances made in the last 40 years of genetic research. Mosquitoes and the pathogens they transmit have been studied in detail for more than 100 years, most typically through the lenses of ecology, immunology, vaccinology, and vector biology. Molecular biology remains an underutilized discipline with respect to informing and developing novel, specific approaches to prevent the spread of mosquito-borne disease. This Methods Collection, entitled “Molecular Biology in Mosquito Midguts and Salivary Glands”, highlights key methods applied to mosquito vector biology problems that span many facets of molecular biology and multiple developmental time points. The growth of research efforts in mosquito molecular biology research is essential and should facilitate progress toward the completion of complex tasks such as genome plasticity evaluation, gene drive applications, multi-omics analysis, and the genome-wide investigation of gene function within *in vivo*, tissue-specific contexts. As molecular biology efforts in mosquitoes expand, the medical relevance of mosquito research interests for patients with mosquito-borne diseases will increase due to translational advances resulting from

the acquisition of additional context-specific, basic research information.

Chiu et al. provide a clear, detailed explanation of how to dissect and conduct immunostaining on larval mosquito salivary glands (SGs)<sup>1</sup>. Historically, SGs have been regarded as a difficult tissue to dissect *en masse*, thus limiting the scope of analysis. Following various refinements in adult mosquito SG studies<sup>2,3,4</sup>, this research group developed a powerful means to view the adult tissue’s larval precursor<sup>1</sup> and further confirmed that the method can be applied across multiple *Anopheles* species. Future uses for this technique include the evaluation of molecular, genetic, and drug strategies aimed at disabling mosquito SG development or function.

Kojin et al. deliver a succinct method for indel detection by high-resolution melt analysis following the gene editing of *Aedes aegypti* mosquitoes<sup>5</sup>. Importantly, the assay does not require sacrificing the mosquito<sup>5</sup>. This facilitates broad application of the technique to the generation of any mutant mosquito strain, as the same individuals remain available for subsequent genetic crosses and/or phenotypic assays.

Kumpitak et al. 2021 offer a clever strategy for the detection of *Plasmodium* sporozoites in *Anopheles* mosquitoes using an ELISA<sup>6</sup>. This method has distinct advantages when compared to microscopy, and it can be applied broadly across parasite subtypes. This method has been used to study the dynamicity of infection by mosquitoes across seasons and across malaria vector species.

Taracena et al. document highly impactful advances in feeding dsRNA-producing bacteria to *Anopheles* mosquitoes for robust gene knockdown, even in a tissue with low levels of endogenous RNAi activity—the salivary glands<sup>7</sup>. This technique was consistently effective across the candidate target genes tested, the results were valid across multiple assay readouts, and the method required little expertise and minimal effort. With this powerful method now available, a host of researchers should be fully equipped to begin reverse genetics studies of mosquito gene function.

Erlank et al. carefully describe the application of a standard membrane-feeding assay to *Anopheles* mosquitoes for the detection and screening of *Plasmodium falciparum* midgut oocysts following small-molecule treatment and staining with mercurochrome<sup>8</sup>. In their study, much attention is given to the considerations needed for success, the limitations of a low feeding rate, as well as the broad applicability of this assay<sup>8</sup>.

This methods collection showcases the many opportunities available to apply molecular biology approaches in cutting-edge, medically relevant ways to support the development of the next generation of vector control measures. The articles within are most relevant for novice researchers and those who have recently joined the field of vector biology from other areas. It is the author's hope that these articles facilitate the

planning of new experiments and approaches aimed at better understanding vector biology and preventing the transmission of mosquito-borne diseases. There is great potential for the expansion of mosquito molecular biology approaches and their uses, so this collection may also serve as a beacon to draw the field's attention and interest to this area. With articles discussing topics such as the validation of mosquito gene editing and effective approaches for RNAi, these studies will remain highly relevant moving forward.

## Disclosures

The author has nothing to disclose.

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