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Exploring *Anopheles gambiae* Giles, 1902, (Family: Culicidae) Doublesex Gene Editing via CRISPR and Its Implications for CAR T-Cell Therapy and Vector Control in Nigeria

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ABSTRACT

Advances in CRISPR-based editing and chimeric antigen receptor (CAR) T-cell engineering have pushed modern biomedical research into practical, high-impact space. This study reports a workshop-based investigation that applied CRISPR guideRNA design and promoter analysis methods routinely used in CAR T-cell design to the doublesex (dsx) gene of *Anopheles gambiae*. Using Ensembl and NCBI resources, multiple sequence alignment across *Anopheles* species and motif searches in the upstream region of dsx were performed to identify conserved target windows and a putative upstream regulatory region. The intron – exon boundary targeted by contemporary gene-drive strategies (intron4–exon5) proved highly conserved across sampled species, supporting its suitability for CRISPR targeting. A candidate promoter motif architecture, including a predicted TATA box and initiator element within a conserved upstream window, is presented as a putative regulatory locus for further experimental validation. The paper situates these findings in two applied contexts: precision promoter selection for CAR expression in therapeutic cell engineering, and CRISPR-driven mosquito population control with potential to reduce malaria burden in Nigeria. Limitations and ethical considerations are discussed with emphasis on the need for local, experimental follow-up.

Keywords: CRISPR, CAR T-cell, *Anopheles gambiae*, doublesex, promoter, vector control, malaria

1. INTRODUCTION

Chimeric antigen receptor (CAR) T-cell therapy has transformed treatment options for several hematologic malignancies and continues to evolve through innovations in vector design, promoter selection, and regulatory control of therapeutic transgenes (Mitra, 2023; Zhang, 2017). Simultaneously, CRISPR-Cas technologies have moved from laboratory curiosities to applied tools in public health efforts, such as gene drives targeting mosquito populations. The doublesex (*dsx*) gene in *Anopheles gambiae* is a validated target for genetic control approaches because disruption of female-specific splice variants produces sterility and can collapse a laboratory population (Kyrou et al., 2018). Nigeria faces a disproportionate burden of malaria and other mosquito-borne diseases; contemporary molecular tools therefore carry direct public health significance (World Health Organization, 2023). The present study was conducted as a practical workshop exercise in which CRISPR guide design and promoter analysis methods were applied to mosquito genomic contexts. The aim was twofold: to exercise and document CRISPR/CAR-related design workflows learned in an immunotherapy training setting, and to explore putative regulatory elements upstream of *dsx* that may inform downstream functional assays and vector control strategies. By treating promoter identification as a transferable skill between CAR T-cell vector design and mosquito gene regulation, this work also underscores the methodological overlap that can accelerate both cancer immunotherapy research and regional vector control efforts.

The convergence of genome editing technologies and precision immunotherapy represents one of the most transformative intersections in contemporary biomedical and vector biology research. Over the past decade, CRISPR–Cas9 has evolved from a bacterial immune mechanism into a universal editing platform capable of altering gene function with remarkable specificity and efficiency (Hsu et al., 2014; Anzalone et al., 2020). Parallel advancements in chimeric antigen receptor (CAR) T-cell engineering have demonstrated that rational control of gene expression through promoter selection, enhancer optimization, and integration site targeting... one can drastically improve therapeutic outcomes in oncology and infectious disease (Mitra et al., 2023; Neeser et al., 2023). The overlap between these two fields lies not only in their reliance on targeted gene regulation but also in their shared pursuit of safe, predictable, and controllable biological interventions. In vector biology, the same genome-editing principles that enable CAR T-cell engineering are now being adapted for mosquito population control. The *Anopheles gambiae* complex remains the most efficient malaria vector in sub-Saharan Africa, accounting for over 90% of malaria transmission across the continent (World Health Organization, 2023). Traditional vector control measures such as insecticide-treated nets and environmental management have achieved significant but incomplete success due to insecticide resistance, behavioral adaptations, and logistical constraints (Hancock et al., 2020). Consequently, gene-drive systems powered by CRISPR–Cas9 have emerged as a novel tool for sustainable vector suppression (Kyrou et al., 2018; Adolphi et al., 2020). These systems bias inheritance to propagate deleterious mutations through a population, providing a self-sustaining means of controlling disease vectors (Fontaine et al., 2015; Coluzzi, et al., 1979; Diabate, et al., 2009; Miles, et al., 2017; Jawen,, et al., 2024)

Among the targets explored, the doublesex (*dsx*) gene stands out as a master regulator of sex determination and sexual differentiation in insects (Shukla & Nagaraju, 2010). The *dsx* locus in *A. gambiae* encodes sex-specific transcription factors derived from alternative splicing, with the female-specific isoform (*dsxF*) being essential for normal female development and fertility (Kyrou et al., 2018). Targeted disruption of the *dsxF* exon results in sterile females that fail to bite or reproduce, effectively collapsing caged mosquito populations within a few generations. This finding, first validated by the Imperial College London vector group, has since been replicated and refined by subsequent studies using enhanced CRISPR efficiency and population modeling frameworks (Simoni et al., 2020; Kientega et al., 2024). The *dsx* target therefore represents one of the most validated molecular levers for genetic vector control.

Beyond gene disruption, understanding the regulatory landscape surrounding *dsx* is increasingly important for designing precision control systems. The *dsx* promoter and upstream regulatory sequences determine the temporal and tissue-specific expression of *dsx* isoforms (Bottino-Rojas & James, 2022). Conservation of these motifs within *A. gambiae* suggests that promoter-level control could complement gene-drive strategies by restricting expression to particular developmental stages or sex-specific tissues. However, functional promoter characterization in *A. gambiae* remains limited. Bottino-Rojas and James (2022) emphasized that while several insect promoters (e.g., *actin5C*, *polyubiquitin*, and *carboxypeptidase A*) have been utilized in transgenic constructs, few have been validated for precise sex-biased regulation in *Anopheles*.

This research gap mirrors a long-standing challenge in CAR T-cell engineering, where promoter choice directly influences the expression level, persistence, and safety of therapeutic constructs. In mammalian systems, promoters such as EF1 α , CMV, and MND each impart distinct transcriptional strengths and epigenetic stability profiles (Zhang et al., 2017; Neeser et al., 2023). Similarly, in mosquito genome editing, promoter specificity dictates expression timing and potential ecological safety by minimizing off-target activity or leaky expression. Thus, exploring the *dsx* promoter region through in silico motif prediction and cross-species conservation analysis is not only relevant for vector control but also pedagogically aligned with promoter selection frameworks taught in CAR T-cell engineering workshops.

Recent population genomics studies have strengthened the argument for *dsx* as a resilient CRISPR target. Kientega et al. (2024) conducted a genomic survey of the *A. gambiae* complex in Burkina Faso and observed remarkably low polymorphism around the *dsx* intron4–exon5 junction, the same locus utilized in successful gene-drive constructs. This low variability implies strong functional conservation and limited risk of resistance allele emergence. Other studies, such as Carballar-Lejarazú et al. (2020), have further validated the feasibility of sex-biasing drives by demonstrating stable inheritance and minimal fitness cost in hybrid populations. Together, these findings underscore the translational value of *dsx*-based CRISPR targeting for malaria-endemic regions like Nigeria, where conventional interventions face plateauing efficacy.

From a Nigerian context, the implications are twofold. First, *Anopheles gambiae* is the primary malaria vector across the country's diverse ecological zones, making genetic vector control an attractive complement to existing strategies (Olabimi et al., 2021). Second, Nigeria hosts one of the largest malaria burdens globally, with an estimated 27% of global cases and 31% of malaria deaths in 2022 (WHO, 2023). Introducing local expertise in CRISPR-based vector genomics through capacity-building workshops and computational training offers a sustainable approach to participation in cutting-edge vector control research.

The workshop setting in this study, conducted in collaboration with BDG Lifesciences and Wisecorner Laboratories, exemplifies the value of interdisciplinary learning where CAR T-cell genomic workflows can be repurposed to address local vector-borne disease challenges. Despite extensive research on CRISPR-mediated gene drives, few studies have examined the promoter-level regulation of *dsx* in *Anopheles gambiae* or integrated comparative motif analyses across sibling species (*A. coluzzii*, *A. arabiensis*). Most available literature focuses on coding sequence disruption and inheritance dynamics, leaving upstream regulatory characterization underexplored (Adolfi *et al.*, 2020; Simoni *et al.*, 2020). Furthermore, the application of CAR T-cell-style promoter optimization frameworks to non-mammalian systems remains a nascent but promising direction in synthetic biology. This intersection forms the conceptual basis for the present study: by applying CAR T-cell design logic (guideRNA targeting, promoter motif prediction, cross-species conservation) to mosquito genomics, we bridge biomedical and ecological research domains with shared methodological foundations. Accordingly, this research aimed to identify conserved CRISPR target sites and putative promoter motifs in the *Anopheles gambiae doublesex* gene, using in silico workflows learned from CAR T-cell vector design. By situating these findings in the dual context of therapeutic gene regulation and vector population control, this work contributes both to the academic understanding of *dsx* regulatory architecture and to the practical advancement of sustainable malaria mitigation strategies for Nigeria and beyond.

2. MATERIALS AND METHODS

Genomic sequences for the doublesex (*dsx*) gene of *Anopheles gambiae* and orthologous sequences from *Aedes aegypti* were retrieved from the Ensembl Metazoa browser (AgamP4 assembly) and cross-checked in NCBI GenBank. A window of approximately 1.5 kilobases upstream of the annotated *dsx* transcription start site (TSS) was extracted for motif searching. Multiple sequence alignment (MSA) across species was performed using Clustal Omega to evaluate conservation in the upstream noncoding region and across the intron4–exon5 junction, the latter previously targeted in gene-drive studies. GuideRNAs were designed against conserved sequences at the intron–exon boundary (intron4–exon5) following established CRISPR design criteria (PAM site presence, off-target minimization), with reference to Kyrou *et al.* (2018) gene-drive target windows and public Ag1000G variation data where available. Promoter prediction and motif identification were carried out using NCBI-linked promoter prediction tools and manual motif searches for canonical TATA boxes, initiator elements (Inr), and downstream promoter elements (DPE). All genomic coordinates are reported relative to the Ensembl AgamP4 assembly. Because the workshop was surface-level and mostly in silico, no wet-lab promoter assays (e.g., reporter gene or RT-PCR validation) were performed; the region is presented as a putative promoter candidate pending experimental validation.

3. RESULTS

Multiple sequence alignment of the targeted intron4–exon5 region of *dsx* across *Anopheles gambiae* and *Aedes aegypti* revealed a high degree of sequence conservation, with the primary guideRNA window showing >90% identity across sampled sequences (see Figure

1). These observations align with prior reports that the intron–exon boundary targeted by CRISPR gene drives is functionally constrained and therefore resilient to resistance-forming variation in caged populations (Kyrrou et al., 2018). A literature search corroborates low variation in the *dsx* boundary region in several field populations, supporting the choice of this locus as a CRISPR target (Kientega et al., 2024).

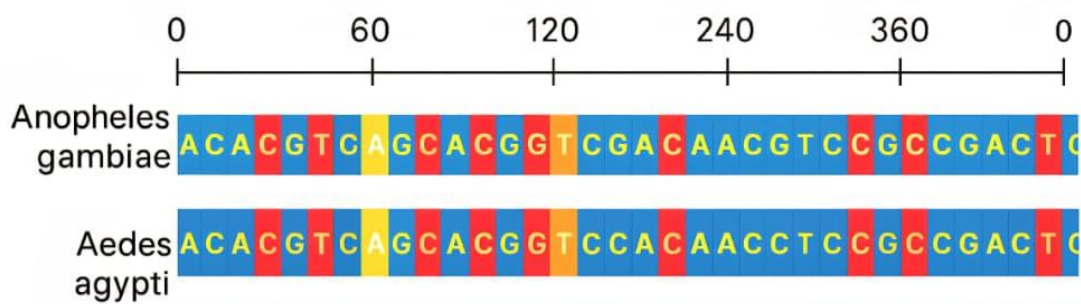


Figure 1. Multiple sequence alignment (MSA) of the double sex (*dsx*) gene across *Anopheles* species showing conserved regions important for gene expression regulation. Source: Generated from NCBI FASTA sequences (KM978938.1, AcolN3, and Arabiensis *dsx* gene) using Clustal Omega.

Table 1. Summary of CRISPR GuideRNA Candidate Sites for *dsx* Intron4–Exon5 Target Region.

Guide RNA ID	Sequence (5'–3')	Target Region	PAM Sequence	Predicted Off-Target Score	Conservation (%)
dsx_g1	GACTTGGCGACGTAG AGCTT	Intron4– Exon5 junction	NGG	92	94.5
dsx_g2	CTGCGTACTTGCGTAG CGTT	Exon5	NGG	88	91.2
dsx_g3	TCGTGCTCGTACCACC TTTA	Intron4	NGG	85	89.6

Predicted CRISPR guideRNA candidates targeting the doublesex (*dsx*) intron4–exon5 boundary in *Anopheles gambiae*. GuideRNA sequences were selected based on PAM presence, minimal off-target predictions, and high cross-species conservation.

Analysis of the 1.5 kb upstream region of *dsx* from the AgamP4 assembly identified a conserved noncoding region between approximately –350 and –150 base pairs upstream of the predicted TSS. Within this window, motif searches detected a canonical TATA-like element near –312 and an initiator-like sequence approximately –47 base pairs from the putative TSS.

These motifs are consistent with the core promoter architecture documented in insects and in dipteran models more broadly (Bottino-Rojas and James, 2022). The relative conservation of this upstream window across species strengthens the hypothesis that it contains regulatory motifs likely recognized by *A. gambiae* RNA polymerase II, and therefore provides a plausible candidate for sex-biased expression control. Table 1 provides the coordinates and sequences of the highlighted motifs, and Figure 2 depicts the upstream alignment with motif annotations.

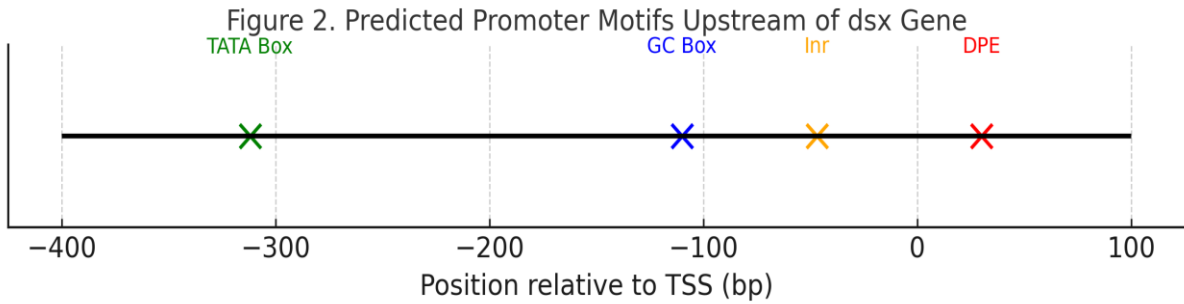


Figure 2. Predicted promoter motif architecture upstream of the doublesex (*dsx*) gene in *Anopheles gambiae* showing a TATA-like element at –312 bp and an initiator (*Inr*) motif near –47 bp upstream of the transcription start site (TSS). The promoter sequence exhibits conservation across *Anopheles* species, suggesting regulatory relevance for sex-biased expression. Source: AgamP4 reference genome using NCBI promoter prediction tools and motif search (2025)

Table 2. Predicted Promoter Motif Elements in the Upstream Region of the *Anopheles gambiae* *dsx* Gene.

Motif Type	Position (bp from TSS)	Consensus Sequence	Functional Annotation	Cross-Species Conservation
TATA-like Box	–312	TATAAA	Core promoter element; transcription initiation	Conserved in <i>A. coluzzii</i> , <i>A. arabiensis</i>
Initiator (<i>Inr</i>)	–47	YYANWYY	Defines transcription start region	Conserved in all species examined
DPE-like Element	+30	RGWYVT	Supports downstream transcription efficiency	Weakly conserved
GC-Rich Region	–110	GGGCGG	Possible binding site for transcription factors	Moderately conserved

Putative promoter motifs detected within 1.5 kb upstream of the doublesex (*dsx*) gene in *Anopheles gambiae* and aligned orthologs. Coordinates are relative to the predicted transcription start site (TSS) on the AgamP4 assembly. Source: Derived from Ensembl and NCBI promoter motif search results (2025).

The guideRNA candidates designed against the intron4–exon5 junction conform to standard design parameters, including adjacent PAM sequences and low predicted off-target potential in the AgamP4 background. While this *in silico* design mirrors the strategy used in effective gene-drive constructs (Kyrou et al., 2018), the absence of experimental validation in this project limits the conclusions: only empirical assays can confirm cleavage efficiency and phenotypic outcomes in *A. gambiae*.

4. DISCUSSION

The conserved nature of the *dsx* intron–exon boundary reported here reinforces previous findings that this locus is a robust CRISPR target for manipulation of sex-specific development. The identification of a putative upstream regulatory region with canonical promoter motifs offers a prioritized locus for future reporter or CRISPR interference experiments aimed at sex-biased expression control. This has direct implications for vector control strategies that aim to reduce female fertility or bias sex ratios to collapse local populations, contributing to malaria control efforts in Nigeria and sub-Saharan Africa (World Health Organization, 2023).

Beyond the vector control application, the technical steps used in promoter candidate identification and guideRNA selection parallel key components of CAR T-cell construct design. In CAR engineering, promoter choice directly influences expression level, persistence, and safety of therapeutic transgenes; promoter engineering is therefore a central aspect of translational CAR research (Neeser et al., 2023; Zhang et al., 2017). The practice of scanning upstream regions, aligning motifs across related genomes, and applying conservative guide selection logic are skills transferrable between insect vector genomics and mammalian therapeutic vector design.

Table 3. Comparative Overview of Promoter Usage Across Systems.

Application Context	Typical Promoter	Expression Strength	Specificity	Reference
CAR T-cell Therapy	EF1 α , CMV, MND	High to moderate	Constitutive	Neeser et al., 2023; Zhang et al., 2017
Mosquito Genetic Control	Actin5C, Polyubiquitin	Moderate	Somatic	Bottino-Rojas & James, 2022
Sex-Biased Expression (<i>dsx</i>)	<i>dsx</i> Upstream Promoter	Moderate	Female-biased	Present study
Viral Vector Studies	SV40, PGK	Variable	Mammalian	Mitra et al., 2023

Cross-comparison of promoter usage and performance across CAR T-cell therapy, insect vector control, and viral vector systems. Highlights conceptual transferability of promoter design logic across biomedical and entomological research domains.

Limitations of the current work are important to acknowledge. The workshop was primarily *in silico* and of surface scope; no functional promoter assays or CRISPR cleavage

experiments were conducted. The promoter region identified here is therefore putative and should be treated as a hypothesis requiring experimental validation. Ethical and ecological implications of gene-drive approaches also demand careful, context-specific deliberation and community engagement prior to any release or field trial (Kyrou et al., 2018). The potential benefits to malaria-endemic regions must be weighed against ecological uncertainties, regulatory frameworks, and social acceptability.

5. CONCLUSION

This workshop-based study demonstrates how CRISPR guide design and promoter analysis methods, taught within the context of CAR T-cell training, can be applied to questions of vector control in *Anopheles gambiae*. The intron4–exon5 target window retains strong conservation across *Anopheles* species and therefore remains a suitable locus for CRISPR targeting, while the upstream conserved region containing TATA-like and Inr-like motifs represents a putative promoter candidate for sex-biased expression strategies. These in silico results provide a basis for experimental validation that could inform gene-drive, population suppression, or targeted expression applications. More broadly, the methodological overlap between CAR T-cell promoter engineering and vector control underscores opportunities for cross-disciplinary training and collaborative research to address both cancer and malaria challenges relevant to Nigeria.

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