

DESIGN BRIEF

Degradation of viral NS5 polymerase mRNA from mosquito-borne diseases with engineered RNAi

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Reviewed on 9 May 2026; Accepted on 22 June 2026; Published on 26 October 2026

Mosquito-borne diseases result in 390 million infections annually, disproportionately affecting underprivileged regions. Existing treatments are often expensive and inaccessible in low and middle-income regions, leaving large populations without reliable protection. *Wolbachia pipientis* is a maternally inherited endosymbiont that reduces mosquito-vector competence by blocking virus replication and inducing cytoplasmic incompatibility. We engineer a *Wolbachia*-compatible expression plasmid to produce RNA targeting NS5 (Non-Structural 5) polymerase mRNA to form a dsRNA complex. This triggers Dicer-2-mediated cleavage of viral transcripts across three flaviviruses, with *Saccharomyces cerevisiae* serving as the proof-of-concept host for validating expression and binding of probe and target strands. First, we aligned NS5 polymerase coding sequences of Zika, Dengue, and Yellow Fever viruses to identify conserved regions across all three species, with candidate regions being evaluated using ViennaRNA's Web Services and Python API to minimize secondary structure formation and maximize binding probability. Our selected NS5 target sequence is used to produce a probe mRNA transcript in *S. cerevisiae* (strain INVSc1). Upon annealing to the viral mRNA target, the resulting dsRNA duplex serves as a Dicer-2 substrate, initiating RISC-mediated degradation of viral transcripts in a separate host organism complete with RNA interference (RNAi) pathways after validation of gene expression is finished in *S. cerevisiae*, as *S. cerevisiae* lacks RNAi machinery. We have engineered a superplasmid containing all three NS5 sequences and our probe strand that will be ordered from VectorBuilder. In the following months, lab work will include gene expression and validation of binding, which will involve RT-qPCR and northern blotting, to assess viral mRNA degradation in *S. cerevisiae*. After successful expression validation, transfer to a suitable RNAi host (*W. pipientis*) can potentially be performed and protein functionality can be assessed with western blotting and mass spectrometry. The integration of *Wolbachia*-based biocontrol with siRNA-mediated viral suppression offers a sustainable strategy for flavivirus suppression in the face of rising insecticide resistance and climate change.

Keywords: NS5 Degradation, mosquito-borne diseases, RNA interference, probe strand, RNAi



The authors were mentored by Alyssa Morrow, BioBuilder Educational Foundation, Boston, Massachusetts, United States of America; Catherine Sharer, Lambert High School, Suwanee, Georgia, United States of America. Please direct correspondence to: Catherine Sharer, f19723@forsythk12.org. This is an Open Access article, which was copyrighted by the authors and published by BioTreks in 2026. It is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

IN 2025 alone, the World Mosquito Program reported 3.6 million Dengue cases and over 1,900 deaths attributable to mosquito-borne diseases across dozens of countries (Jackson, 2025). Mosquito-borne diseases disproportionately affect poorer countries in tropical areas, such as countries in sub-Saharan Africa and South America (World Health Organization, 2024). Figure 1 shows the Center for Disease Control's estimate of the Dengue threat levels across the world in 2026, with a majority of the regions men-

tioned demonstrating low levels of development (CDC, 2026). This image does not imply endorsement by CDC, ATSDR, HHS or the United States Government and is available on the CDC's website free of charge.

No approved antiviral treatment exists for Dengue; current clinical management is limited to supportive care and analgesics, leaving severe cases with no targeted therapeutic option (CDC, 2024). Especially in severe cases, the lack of proper treatment presents itself as a significant issue.

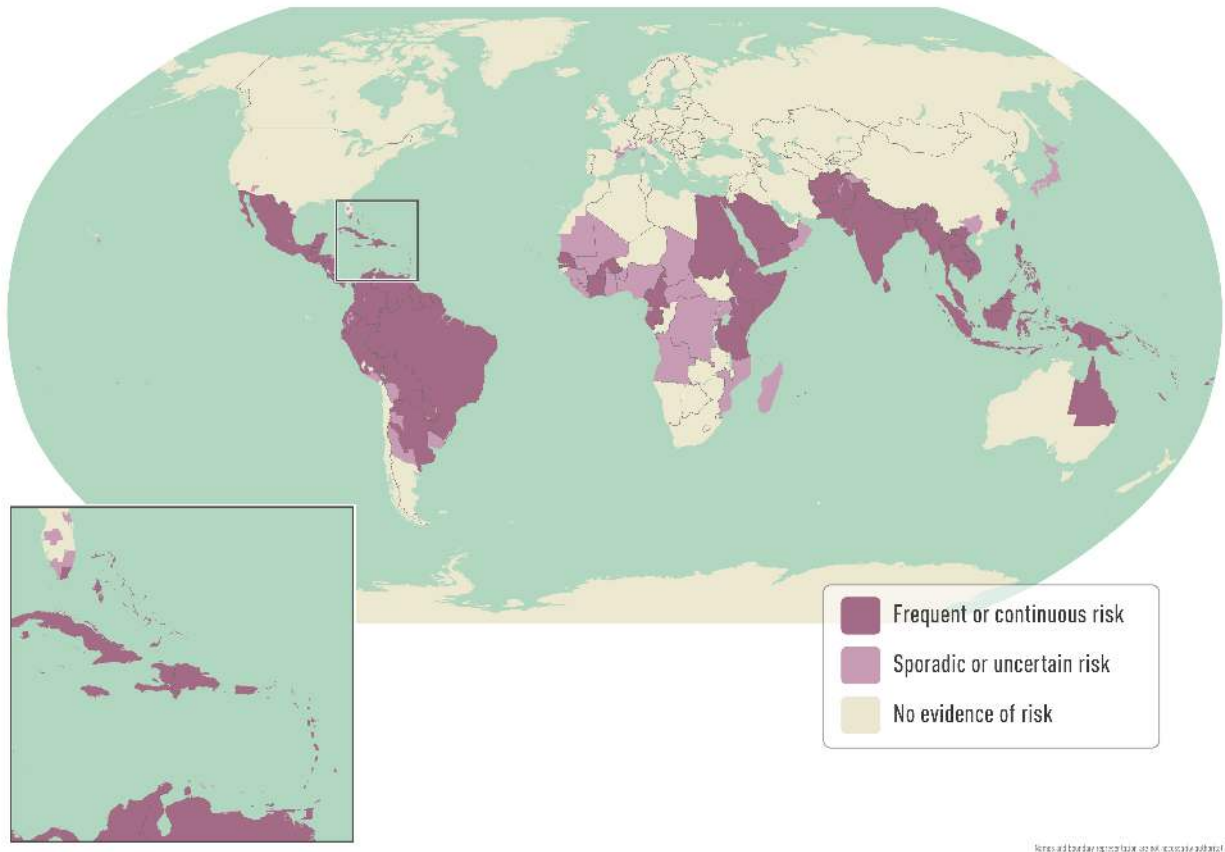


Figure 1. Risk of contracting Dengue around the world. This image shows the risk of contracting Dengue at a national scale of analysis. Countries where risk is higher are shaded darker. These regions are typically in less-developed regions such as East Africa, South America, and the Indian subcontinent. Source: U.S. Centers for Disease Prevention and Control (2026)

Much research has been ongoing about the use of *W. pipientis* to target mosquito-borne diseases. Currently, the World Mosquito Program engineers mosquitos with *Wolbachia* and attempts to release these mosquitos into the wild, as *Wolbachia* has been known to suppress mosquito-borne diseases such as Dengue and Zika when present in mosquitos (World Mosquito Program, 2011). However, surface-level vector control and symptom management fail to address viral replication directly. Hence, this design targets NS5 (Non-Structural 5) polymerase, the driver of flavivirus genome replication, across Dengue (DENV2), Zika (ZIKV), and Yellow Fever viruses. We set target sequences to require close identity across NS5 coding sequences, which we validated using Clustal Omega and Vienna RNA's Folding Kinetics Web Services. Further binding metrics such as binding energy were evaluated

using ViennaRNA's Python API, the specifics of which will be addressed in the following sections. Our design targets the mRNA coding for viral NS5 Polymerase, which is highly conserved among the aforementioned flaviruses, is responsible for the replication of viral strands, and shares no significant homology with the human or *S. cerevisiae* transcriptome, minimizing the risk of off-target silencing (El Sahili et al., 2017). This is a critical safety consideration given that NS5 homologs are absent from vertebrate genomes. Using an episomal plasmid that is inserted into *S. cerevisiae* (proof-of-concept for *W. pipientis*, an endosymbiotic bacterium in mosquitos), a probe strand that binds to viral NS5 mRNA will be expressed. This probe-target strand annealing produces a dsRNA complex recognized and cleaved by Dicer-2, initiating RISC assembly for targeted degradation of NS5 mRNA.

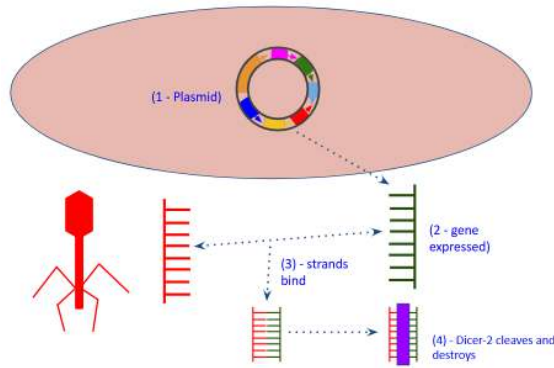


Figure 2. Comprehensive diagram of our *Wolbachia* system. This diagram shows our expression and binding proof-of-concept that occurs in yeast (Steps 1, 2, and 3). Step 4 will be performed in *Wolbachia* following validation of previous steps.

We predict that siRNA generated from this system will silence NS5 polymerase expression, directly halting genome replication of Dengue, Zika, and Yellow Fever viruses within the mosquito host. This system is designed to be hosted and expressed by *W. pipientis*, which is already known to suppress some viruses to a certain extent and provides its hosts—mosquitoes—with a comparative advantage against organisms without the endosymbiont. However, proof-of-concept validation is performed in *S. cerevisiae*, being an ideal model organism which supports the first step of RNA interference (RNAi): modeling expression and binding of target and probe genes as well as episomal plasmid maintenance, with *Escherichia coli* serving as the intermediate propagation host. Of course, yeast lacks insect-specific Dicer-2, R2D2, and AGO2-RISC, which require validation in host organisms complete with RNAi pathways. In this design, yeast is primarily used to validate expression of all genes prior to transformation into a potential host that comes with RNAi-specific proteins to implement in mosquitoes. Once lab validation of gene expression and binding has concluded, we plan on transforming our system into *W. pipientis*, thereby eliminating the limitation of yeast not having an inherent RNAi pathway.

Systems level

The system pictorially shown in Figure 2 is built around a yeast episomal shuttle plasmid (VectorBuilder pSC_Ex backbone), selected for its dual-origin replication, CUP1-inducible promoter, and URA3 selection marker compatible with *S. cerevisiae*. The plasmid encodes the antisense strand complementary to the conserved NS5 target sequence (5' → 3'), driven by

the CUP1 Cu²⁺ - inducible promoter, and is propagated in *E. coli* via ampicillin selection before yeast transformation. Using RNA polymerase, the antisense of our double-stranded DNA (3' to 5') will be transcribed to create an mRNA strand (5' → 3') identical to the sense of our double-stranded DNA, thus being highly complementary to the viral NS5 mRNA when aligned antiparallel (3' → 5'). This will be followed by the binding of the two highly complementary RNA strands, resulting in cleavage of the combined dsRNA strand by anticipating Dicer-2 and AGO2 proteins when extrapolated to an organism with these RNAi-specific proteins, as one limitation of *S. cerevisiae* is its lack of RNAi-specific proteins such as Dicer-2 and AGO2 (Duman-Scheel, 2019).

Device level

Plasmid assembly and propagation

Using VectorBuilder, we expanded upon a yeast episomal plasmid that contains a yeast inducible promoter that allows control over the expression of our probe gene. We plan on ordering this plasmid transformed into *E. coli* to propagate it using heat-shocking at 42°C and incubation at 37°C before transfer to *S. cerevisiae*. Direct transformation into yeast is feasible if there are stable plasmid yield and purity conditions. Once inside and propagated in yeast, we can perform selection to isolate the transformed bacteria.

RNA expression and protein function

Once our plasmid has been assembled and transformed into a yeast cell, using RNA polymerase, we will perform in vivo transcription of our custom gene of interest and model its interaction with viral NS5 mRNA. We hypothesize that—being highly complementary—the two strands will bind to create a double-stranded RNA. Of course, mere complementarity is insufficient to assess binding efficiency. Hence, we used ViennaRNA's Web Services and Python API to assess secondary structure formation and binding thermodynamics. Following validation of gene expression and binding in the lab, endogenous Dicer-2 recognizes the dsRNA duplex and processively cleaves it into ~21 nt siRNA fragments, which are loaded into RISC to guide sequence-specific degradation of NS5 mRNA, all of which will be attempted in *W. pipientis* following expression of genes.

Parts level

Sequence analysis/selection

The first stage of the design involves determining the correct portion of the NS5 mRNA sequence to target.

Analyzing the NS5 mRNA sequences of Yellow Fever (strain TN-96), Zika (strain MR-766), and Dengue virus Type-2 (strain Pecs/2003) from UniProt and GenBank, we identified four highly conserved regions, shown in Table 1. Their GenBank accession numbers are AF013417 (National Library of Medicine, 1998b), AF013415 (National Library of Medicine, 1998a), and AH013794 (National Library of Medicine, 2004) respectively.

With Clustal Omega, we chose four 21 nucleotide regions in which commonalities were at a maximum. Strand 1 had an average complementarity rate of 89% across all three viruses, while Strands 2, 3, and 4 all had an average complementarity rate of 81% across all three viruses (Madeira et al., 2024). Using ViennaRNA's Folding Kinetics Web Service, we modeled the folding path of each probe mRNA strand once it was transcribed from the corresponding DNA strand (Wuchty et al., 1999).

RNAsubopt analysis (Figure 3) identified Strand 1 as optimal (ViennaRNA, 2026a): it exhibited the fewest predicted secondary structures ($n = 6$) within an 8 kcal/mol window above the minimum free energy, indicating low conformational variance and high accessibility for mRNA base-pairing (Lorenz et al., 2011). With fewer possible structures, we have a probabilistically favorable system that can be implemented with the least possible variability. For context, Strand 2 exhibited 10 secondary structures within an 8 kcal/mol window (ViennaRNA, 2026b); Strand 3 exhibited 57 secondary structures within a 40 kcal/mol window (ViennaRNA, 2026c); and Strand 4 exhibited 7 secondary structures within a 40 kcal/mol window (ViennaRNA, 2026d).

After ensuring proper RNA structure of our selected probe strand, we modeled the interactions between the target NS5 mRNA and our custom probe strand, the central focus of this proof-of-concept, by first analyzing the thermodynamics of the binding. Using ViennaRNA's Python API Package, translating directly from their C-library documentation, we created a python script to model the binding structure and energy between the probe strand and each NS5 mRNA (Swaminathan et al., 2026), the code of which is shown in Figure 4. Specifically, we used the ViennaRNA python library and the RNA module from the main ViennaRNA Package (Bernhart et al., 2005). Running our code for each of the three interactions separately, we identified binding energies of -324.1 kcal/mol between Zika and the probe, -434.1 kcal/mol between Dengue and the probe, and -361.3 kcal/mol between Yellow Fever and the probe (Swaminathan et al., 2026). All code is stored in a public GitHub repository (see References). The negative energy values with high magnitudes indicate

an extremely stable RNA complex that forms spontaneously as well as the change in the energy state of the strands before and after binding. A significant drop in energy is suggestive of a much more stable state than that before binding, increasing the probability of strand binding (Chaurasiya et al., 2010).

Using this python script, we determined that there exists a thermodynamically stabler state (release of energy) for hydrogen bonds to form. The interaction between the probe and target strand is shown in Figure 5, along with stable hydrogen bonds (Pettersen et al., 2020). Figure 5 was created with UCSF ChimeraX, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH R01-GM129325 and the Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases. Both Figure 4 and Figure 5 indicate a stable dsRNA complex that is likely to form.

Plasmid design

As shown in the previous section, Strand 1 was identified as optimal across all tests including complementarity, secondary structure formation, energy range above MFE, and binding energy.

The next phase involves the design of our plasmid, which will be inserted into and propagated through *E. coli* before being transferred to *S. cerevisiae*. Our plasmid uses a yeast episomal plasmid from VectorBuilder (VectorBuilder, n.d.). The annotated plasmid map is shown in Figure 6.

This plasmid contains a CUP1 promoter, which triggers a high-level transcription of our gene of interest when in the presence of highly soluble Cu(II)SO_4 (VectorBuilder, n.d.). The ampicillin-resistant gene will ensure that our plasmid will be sustained when purifying our culture in *E. coli*. Next, the pUC origin of replication facilitates replication in *E. coli*, and following transfer to *S. cerevisiae*, the 2μ origin of replication permits maintenance in yeast, the host of our system. Furthermore, to isolate the specific yeast cells that have been transformed to host our plasmid, the orotidine-5'-phosphate decarboxylase (URA3) gene is expressed, which permits yeast transformant selection (VectorBuilder, n.d.). Finally, the CYC1 terminator will be employed to finish transcription and stabilize the expressed mRNA.

Safety

Our proposed system is designed with safety as a primary consideration during construction and testing. All recombinant DNA work will be conducted under BSL-1 conditions using non-pathogenic *E. coli* (DH5 α)

TABLE 1
SEQUENCE SELECTION USING NCBI DATABASES AND CLUSTAL OMEGA

Conserved GenBank Sequence (cDNA Sense 5' → 3')	Conserved GenBank Sequence (cDNA Antisense 3' → 5')	Target mRNA Expressed by Virus (RNA 5' → 3')	Reversed Probe mRNA Expressed by Plasmid (RNA 3' → 5')	Probe mRNA Expressed by Plasmid (RNA 5' → 3')	Sequence in Plasmid (cDNA)
ATGGGAAAGGTTA GGAAAGAC D: 10 0% - 631-651 Z: 9 5% - 673-693 Y: 7 1% - 679-699 Avg: 89%	TACCCTTTCCAATC CTTTCTG	AUGGGAAAGGUUA GGAAAGAC	UACCCUUUCCAUA CCUUUCUG	GUCUUUCCAUAAC UUUCCCAU	5' - GTCTTTCCTAA CCTTTCCCAT - 3' 3' ' - CAGAAAGGATTG GAAAGGGTA - 5'
GTTAGGAAAGACA CACAGGAG D: 81% - 640-660 Z: 100% - 684-704 Y: 62% - 690-710 Avg: 81% GGGAGTGGACAAG TTGTCACT D: 81% - 377-397 Z: 100% - 421-441 Y: 62% - 427-447 Avg: 81% GGATGGAGCAATT GGGAAGAA D: 76% - 674-694 Z: 100% - 718-738 Y: 67% - 724-744 Avg: 81%	CAATCCTTTCTGTG TGTCTC	GUUAGGAAAGACA CACAGGAC	CAAUCCUUUCUGU GUGUCCUC	CUCCUGUGUGUCU UUCCUAAC	5' - CTCCTGTGTGT CTTTCTTAAC - 3' 3' ' - GAGGACACACAG AAAGGATTG - 5'
	CCCTCACCTGTTCA ACAGTGA	GGGAGUGGACAAG UUGUCACU	CCCUCACCUGUUC AACAGUGA	AGUGACAACUUGU CCACUCCC	5' - AGTGACAACCTT GTCCACTCCC - 3' 3' ' - TCACTGTTGAAC AGGTGAGGG - 5'
	CCTACCTCGTTAAC CCTTCTT	GGAUGGAGCAAUU GGGAAGAA	CCUACCUCGUUAA CCCUUCUU	UUCUUCCCAAUUG CUCCAUC	5' - TTCTTCCCAAT TGCTCCATCC - 3' 3' ' - AAGAAGGGTTA ACGAGGTAGG - 5'

Note.—The table shows conserved cDNA sequences from GenBank converted to mRNA target strands, along with projected plasmid-expressed mRNA probe strands. Column 1 provides sequences with complementarity percentages and genomic coordinates (D Dengue, Z Zika, Y Yellow fever, Avg Average Complementarity). Columns 4 and 5 are mirror images of each other in order to synthesize an mRNA strand that, when aligned antiparallel to viral NS5 mRNA, will be complementary and ready to bind.

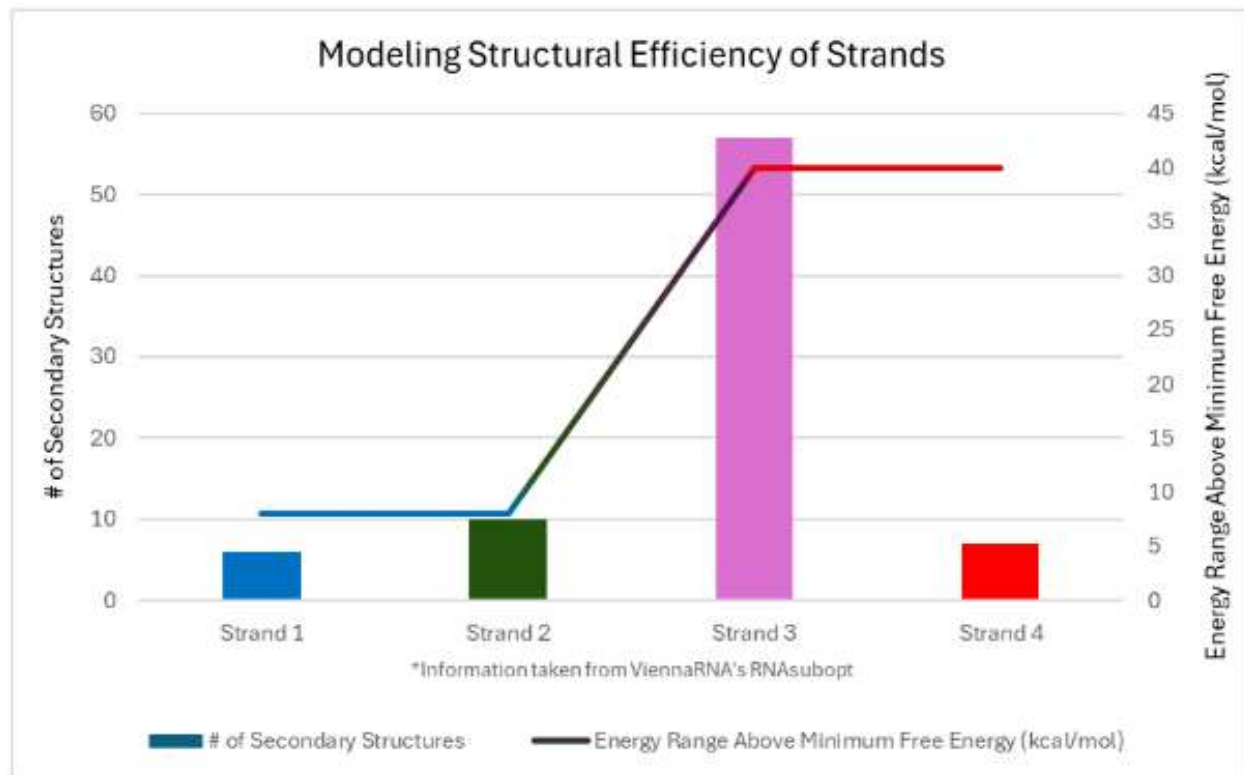


Figure 3. Statistics on strand efficiency from ViennaRNA's Folding Kinetics Web Service. Graph showing predicted number of secondary structures and energy range above minimum free energy for each strand (Hofacker, 1994).

```

1 import ViennaRNA
2 from ViennaRNA import RNA
3 # Two strands interacting
4 zika_ns5_mRNA_sequence = ""AAGCAAGGAGAGTTTCGGGAAAGCAAAAGGTAGCCGCGCCATCTGGTACATGTGGTTGGGAGCCAGATTCT
5 TGGAGTTTGAAGCCCTTGGATTCTTGAACGAGGACCATTGGATGGGAAGAGAAAACTCAGGAGGTGGAGT
6 CGAAGGGTTAGGATTGCAAGACTTGGATACATTCTAGAAAGAAATGAATCGGGCACCAGGAGGAAAGATG
7 TACGCAGATGACACTGCTGGCTGGGACACCCGCAATTAGTAAGTTTGATCTGGAGAAATGAAGCTCTGATTA
8 CCAACCAATGGAGGAAGGGCACAGAACTCTGGCGTTGGCCGTGATTAATACACATACCAAAACAAAGT
9 GGTGAAGGTTCTCAGACCAAGCTGAAGGAGGAAAAACAGTTATGGACATCATTTCAAGACAAGACCAAGAGA
10 GGGAGTGGACAAAGTTGTCACTTATGCTCTCAACACATTCACCAACTTGGTGGTGCAGCTTATCCGGAAACA
11 TGGAAAGCTGAGGAAGTGTAGAGATGCAAGACTTATGGTTGTTGAGGAAGCCAGAGAAAGTGACCAAGATG
12 GTTGCAAGCAATGGATGGGATAGACTCAACGAATGGCGGTCAAGTGGAGATGACTGCGTTGTGAAGCCA
13 ATCGATGATAGGTTTGACATGCCCTCAGGTTCTTGAATGACATGGGAAAAGTTAGGAAAGACACACAGG
14 AGTGGAAACCTCGACTGGATGGAGCAATTGGGAAGAAAGTCCCGTTCTGCTCCCACTTTCAACAAGCT
15 GTACCTCAAGGATGGGAGATCCATTGTGGTCCCTTGCCGCCACCAAGATGAAGTATTGGCCGAGCTCGC
16 GTCTACCAAGGGCAGGATGGAGCATCCGGGAGACTGCCTGTCTTGCAAAATCATATGCGCAGATGTGGC
17 AGCTCCTTTATTTCCACAGAAAGAGACCTTCGACTGATGGCTAATGCCATTTGCTCGGCTGTGCCAGTTGA
18 CTGGGTACCAACTGGGAGAACCACTGGTCAATCCATGGAAGGGAAGAA""
19
20 custom_made_probe_sequence = "GTCITTCCTAACCTTCCCAT"
21 combined_seq = zika_ns5_mRNA_sequence + "&" + custom_made_probe_sequence
22
23 # Create fold_compound for the duplex
24 fc_duplex = RNA.fold_compound(combined_seq)
25
26 # Compute interaction structure and energy
27 (duplex_struct, duplex_energy) = fc_duplex.mfe()
28
29 print(f"Duplex Structure: {duplex_struct}")
30 print(f"Binding Energy: {duplex_energy} kcal/mol")

```

Figure 4. Custom Python script modeling binding energy between two strands using ViennaRNA's Python package. This code shows the custom Python script created to model binding energy between two strands. This specific input shows the sequence for Zika alongside the probe sequence and is one of the three such inputs modeling interaction.

and *S. cerevisiae* strains, in compliance with NIH rDNA guidelines and institutional biosafety protocols. All experiments will be conducted under controlled lab settings to prevent accidents. Field deployment would require regulatory review under EPA FIFRA and WHO vector control guidelines, with controlled environmental release trials to assess ecological impact before any large-scale application.

Using *Wolbachia* to modify entire mosquito populations definitely has its risks, and careful measures need to be undertaken to limit ecological and environmental impact. For example, mutations in viral genomes and/or the probe strand sequences can cause unintended consequences and may result in viruses developing new forms of resistance (Duffy, 2018). Proper care must be taken when handling viral genomes to ensure that further mutations are not caused while testing that could potentially spread to other mosquitoes.

Furthermore, while handling infected mosquitoes outside of BSL-1 conditions, proper care and containment is required to ensure that local epidemics are not unintentionally caused.

Discussions

Our team's original plan was to create an RNAi system that could be implemented in *W. pipientis*. Due to its inaccessible nature in our position and the vast scope of our project, we started out by creating a proof-of-concept to first model the expression of our genes as well as the interaction that results between them. Creating a proof-of-concept and outlining our system proved effective, as we were able to perform quality research in identifying potential conserved strands to target and modeling efficiency. In that manner, we achieved our goal of outlining a viable solution to the problem that mosquito-borne diseases pose. We now

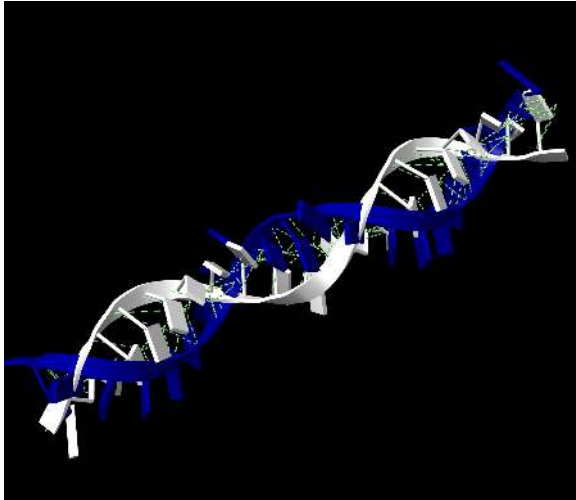


Figure 5. ChimeraX model showing interaction between probe and target strands. This ChimeraX model shows the interaction between the probe strand (white) and the target strand (dark blue) to create a dsRNA complex. The specific target shown here is Zika. Hydrogen bonds connecting the two strands are shown as light green dashed lines.

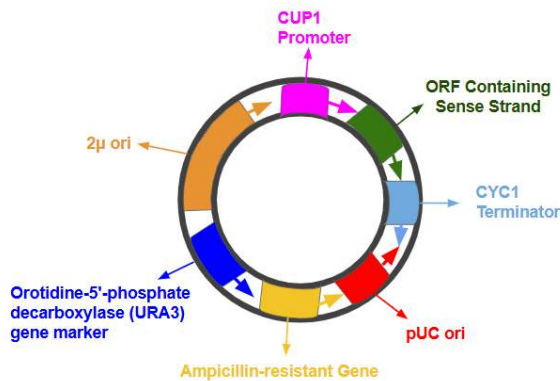


Figure 6. Episomal plasmid template from Vector-Builder. Image shows the yeast episomal base plasmid used for basic expression of the probe gene.

plan on performing the second stage of this project: validating our proof-of-concept in the lab.

Using *S. cerevisiae* as a proof-of-concept host certainly has its limitations, the main one being the lack of inherent RNAi-specific proteins. The most common way to surpass this is to transform *S. cerevisiae* with an episomal plasmid containing the genes to express Dicer-2 and AGO2 (Chen et al., 2020). However, with such a vast scope, the first step was to validate mere expression of target NS5 genes and custom probe genes as well as their binding. This step is extremely crucial and dictates whether an RNAi system can be im-

plemented in any organism to target mosquito-borne diseases. Research has been ongoing on inserting pure *Wolbachia* into mosquitoes, as their presence naturally defends mosquitoes against viruses like Zika and Dengue (Terradas et al., 2017). However, this project takes *Wolbachia*'s function a step further, analyzing how this intrinsic quality can be combined with the wonders of RNA interference to make mosquito-borne disease-targeting even more effective. Implementing our design in *Wolbachia* has the potential to target mosquito-borne diseases at a scale many times larger than what is currently being done.

Next Steps/Ongoing Labwork

Our goal was to use *Wolbachia* to target mosquito-borne diseases. However, the immediate experimental priority is validating probe strand expression via RT-qPCR and confirming dsRNA duplex formation through northern blotting in *S. cerevisiae*. Hence, we assembled all genes into one plasmid. To conserve resources, we built on the existing episomal plasmid that contains our custom insert to include the 3 NS5 genes of Yellow Fever, Zika, and Dengue, each with its own expression system. We plan to order this consolidated plasmid for sequential induction experiments, first confirming individual NS5 gene expression under GAL1, MET17, and AOX1, then co-inducing with the CUP1-driven probe strand and assessing binding efficiency by RT-qPCR and northern blotting.

This "superplasmid," shown in Figure 7, contains all the interacting pieces of DNA that, in the real world, would come from different sources (i.e., the virus, the yeast), but are being consolidated when testing.

Being a 12,154-nucleotide-long plasmid, our experimental plasmid contains four systems in one (Swaminathan et al., 2026). The first system (blue) consists of our probe gene, accompanied by a CUP1 promoter, which is induced in the presence of copper ions, and a CYC1 terminator. The second system is composed of the NS5 gene for Yellow Fever (strain TN-96 - red), expression of which is initiated by the GAL1 promoter in the presence of galactose; a Kozak sequence for stability in translation; as well as a CYC1 terminator identical to the previous system. The other two systems of Zika (strain MR-766 - green) and Dengue (strain Pecs/2003 - orange) are identical to the Yellow Fever system: they each contain a yeast inducible promoter, a common Kozak sequence, their respective NS5 gene insert, and a CYC1 terminator. However, Zika's system utilizes the MET17 promoter, induced in the presence of methionine, and Dengue's uses the AOX1 promoter, induced in the presence of methanol.

In the lab, we plan on expressing these genes both



Figure 7. Experimental superplasmid designed for in-lab expression of all genes. This superplasmid is designed using VectorBee software owned by VectorBuilder. We plan on ordering this plasmid from VectorBuilder transformed into *E. coli* and will express each system separately in the lab.

individually and together. Once gene expression has been validated in the lab, we hope to copy our system into *W. pipientis*, a different host organism that can be engineered to support RNAi pathways while naturally targeting mosquito-borne diseases, which would further the effectiveness of targeting viruses. First, we will install RNAi machinery and cause *W. pipientis* to express Dicer-2 and AGO2 proteins under constitutive promoters. Following this, we will transform our plasmid containing the probe gene and express it under an inducible promoter. This will be followed by validation of Dicer-2 and AGO2 protein functionality using mass spectrometry and western blotting. Ultimately,

we would like to collaborate with other researchers on finding a promising way to implement this RNAi system in *W. pipientis*, a naturally thriving bacterium in mosquitoes already working against mosquito-borne diseases. Using a self-sustaining bacterium such as *Wolbachia* reduces the need for multiple expensive clinical trials in the lab if our RNAi system can be implemented in it. However, further research is necessary as *Wolbachia* is extremely hard to culture outside of host cells and transforming *Wolbachia* poses a major challenge, but the combination of *Wolbachia*'s inherent antiviral qualities and a potential RNAi pathway can decrease mosquito-borne diseases by a much more sig-

nificant amount (Sucupira et al., 2020), and we hope to continue researching on the RNAi capabilities of *Wolbachia*.

Author contributions

RaS: Ideation, led the manuscript writing, analyzed literature for manuscript, designed the plasmid using VectorBuilder and VectorBee, wrote code for computational analysis, used ViennaRNA for sequence selection, created visual graphs and diagrams to represent software results and plasmid, presented work in video; RoS: Created idea, assisted with sequence aligning using Clustal Omega, assisted with plasmid designing; SR: Contributed to manuscript writing, analyzed literature for manuscript, created visuals using UCSF ChimeraX; SL: Assisted with manuscript writing, editing, and revision, assisted with video creation; NP: Assisted with initial abstract writing and editing; SM: Assisted with ideation, editing, and revision of paper; AA, CC, AJ: Assisted with writing the safety portion of manuscript and editing.

Acknowledgements

We express our gratitude to BioBuilder's partners, which include but are not limited to Ginkgo Bioworks, Carolina, BriteBound, New England BioLabs Inc., ucb, and the Ragon Institute. We are thankful to Dr. Alyssa Morrow for her expert guidance through the development of this project, Mrs. Catherine Sharer for her funding and continuous support, and the BioBuilder Educational Foundation for giving us the opportunity to present our research. Lastly, we would like to thank VectorBuilder Inc. for their continuous support in designing our plasmid for cloning, specifically Ms. Kathy Huang, Dr. Tautvydas Shuipys, and Dr. Micaela Ziraldo.

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