



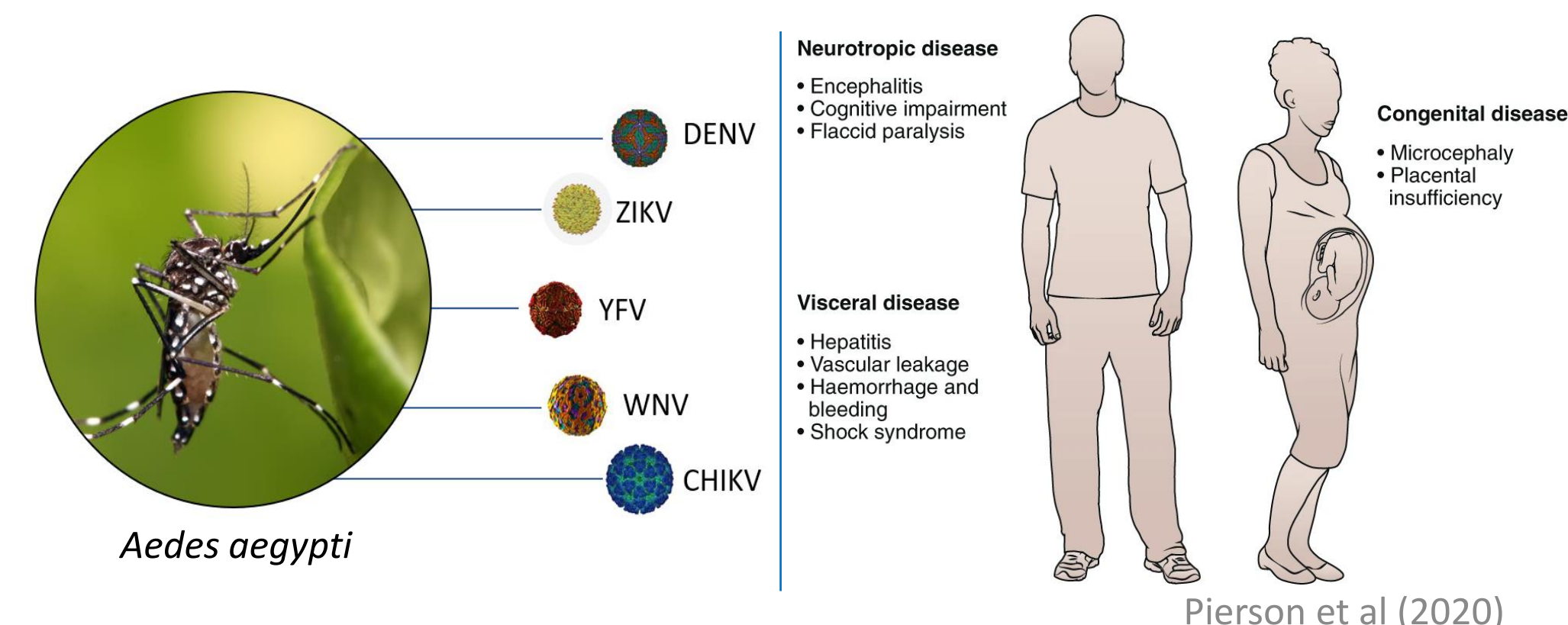
# A CRISPR-based strategy to disrupt Flavivirus transmission by Aedes Mosquitoes

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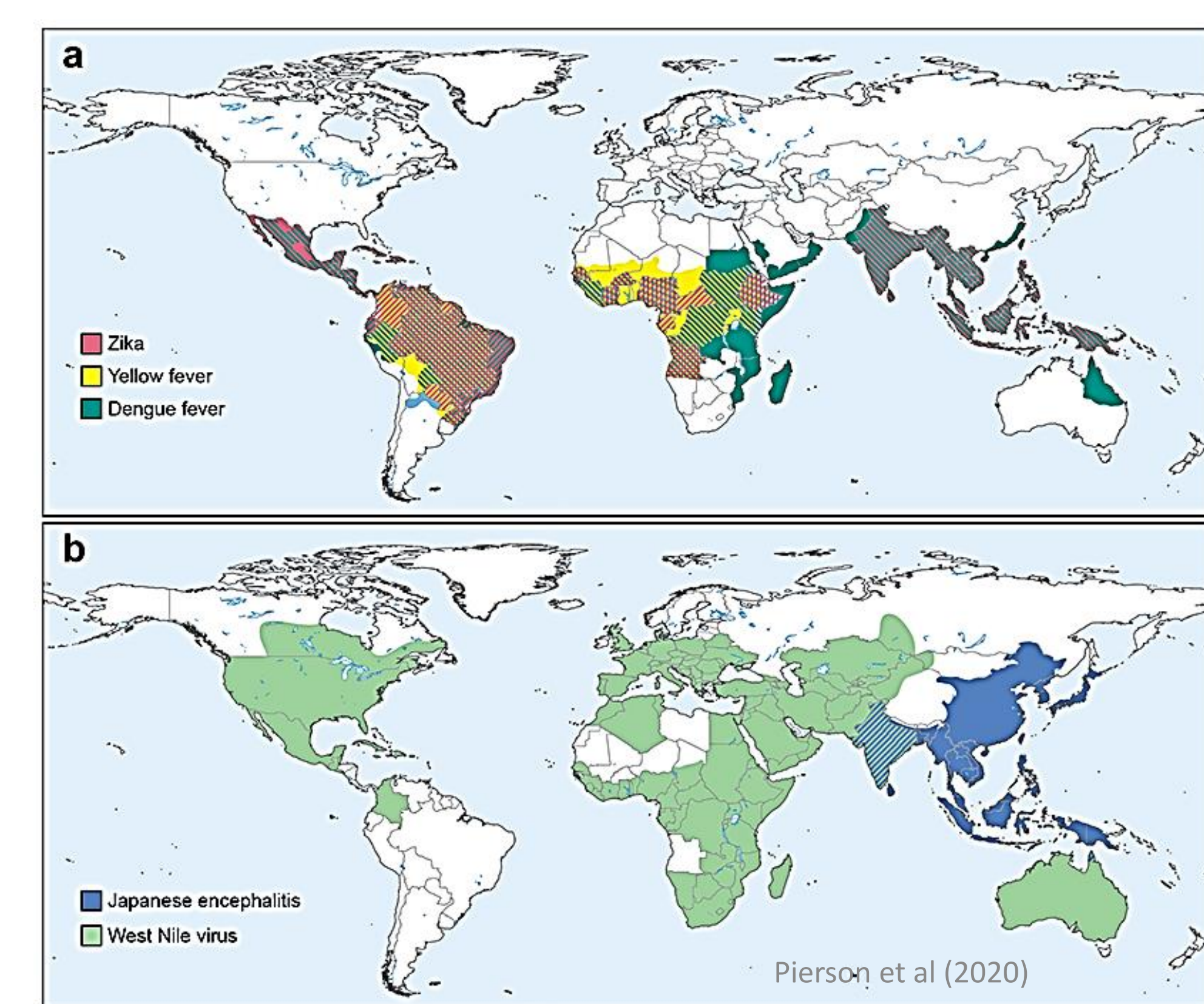
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## ABSTRACT

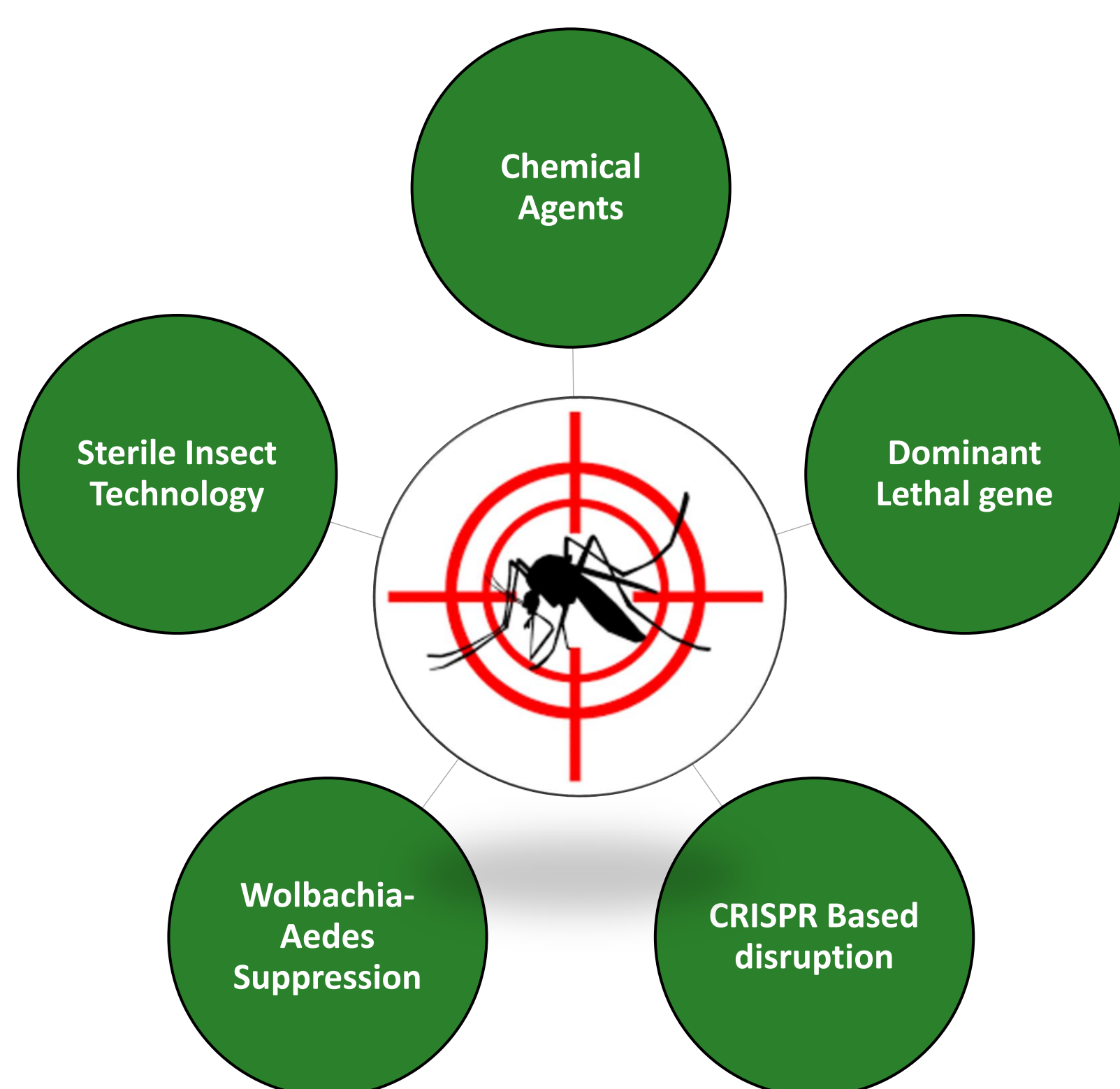
The Flavivirus genus comprises several human pathogenic viruses, such as DENV, YFV, ZIKV, JEV, and WNV, that can be vertically transmitted in arthropods (Mosquitoes). Several interventions that aim to crash the overall mosquito populations have been devised. But these, in the long run, can have serious ecological repercussions. This limitation calls for better and safer strategies backed by a fundamental understanding of flavivirus-host interactions. We wish to apply a Genome-wide CRISPR screen to decode Pan-flavivirus factors for the Aedes mosquito host.



## GLOBAL BURDEN

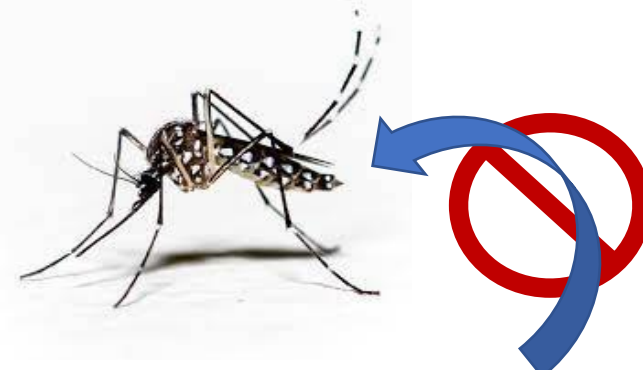


## KILLING THE VECTOR!

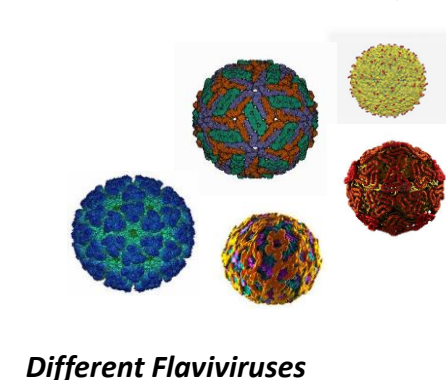


## HYPOTHESIS

- Flaviviruses share similar biology
- Have common mosquito vector (Aedes sp.)



Kill the message!  
Spare the messenger!



## OBJECTIVES

- Develop molecular tools and protocols for CRISPR-based genome editing & screening of cellular factors in Aedes sp. cell lines.
- Conduct an unbiased genome-wide CRISPR survival screen for DENV, ZIKV, JEV, and WNV to identify Pan-Flavivirus Aedes sp. host factors.

## WORKFLOW

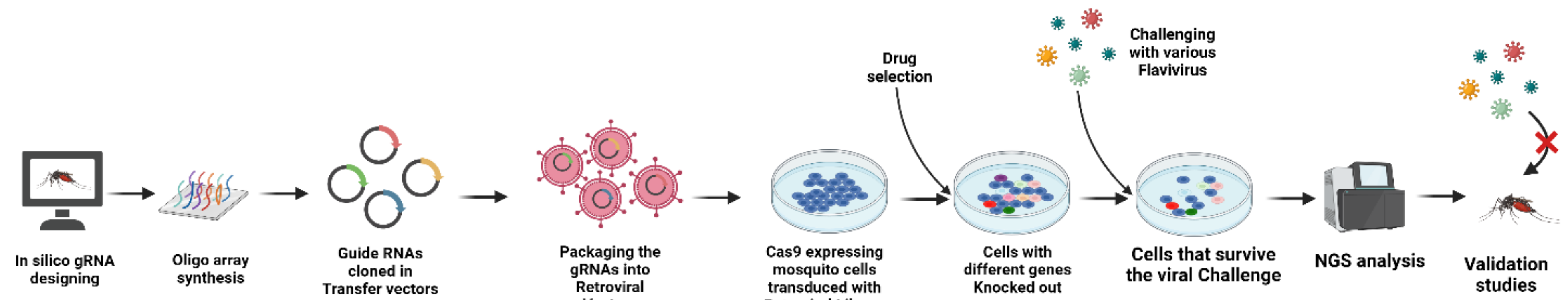


Fig1: Schematic to Perform Genome-Wide CRISPR KO Screen on Mosquito cells

## RESULTS AND CONCLUSIONS

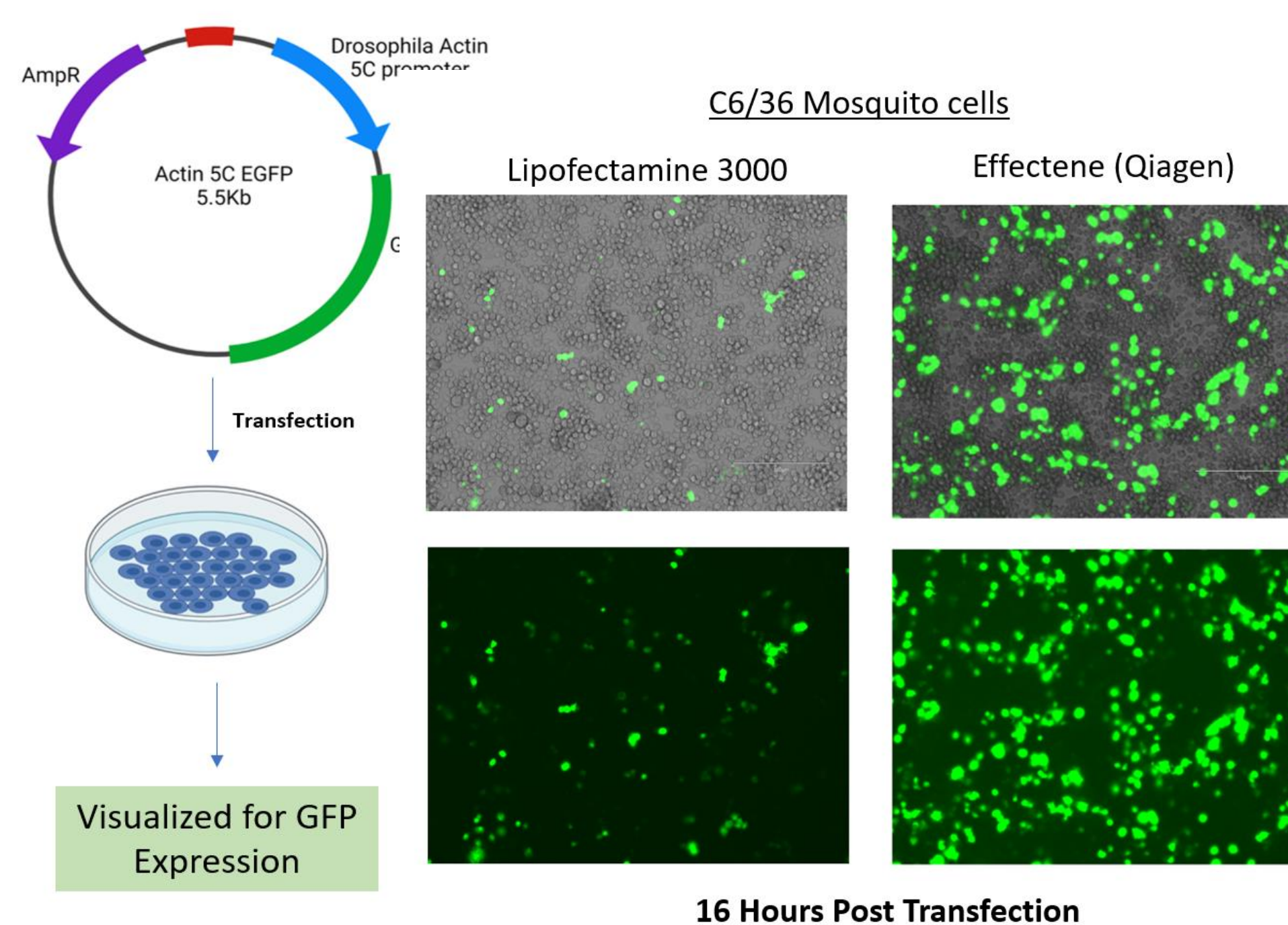


Fig 2: Optimizing gene delivery in Mosquito cells

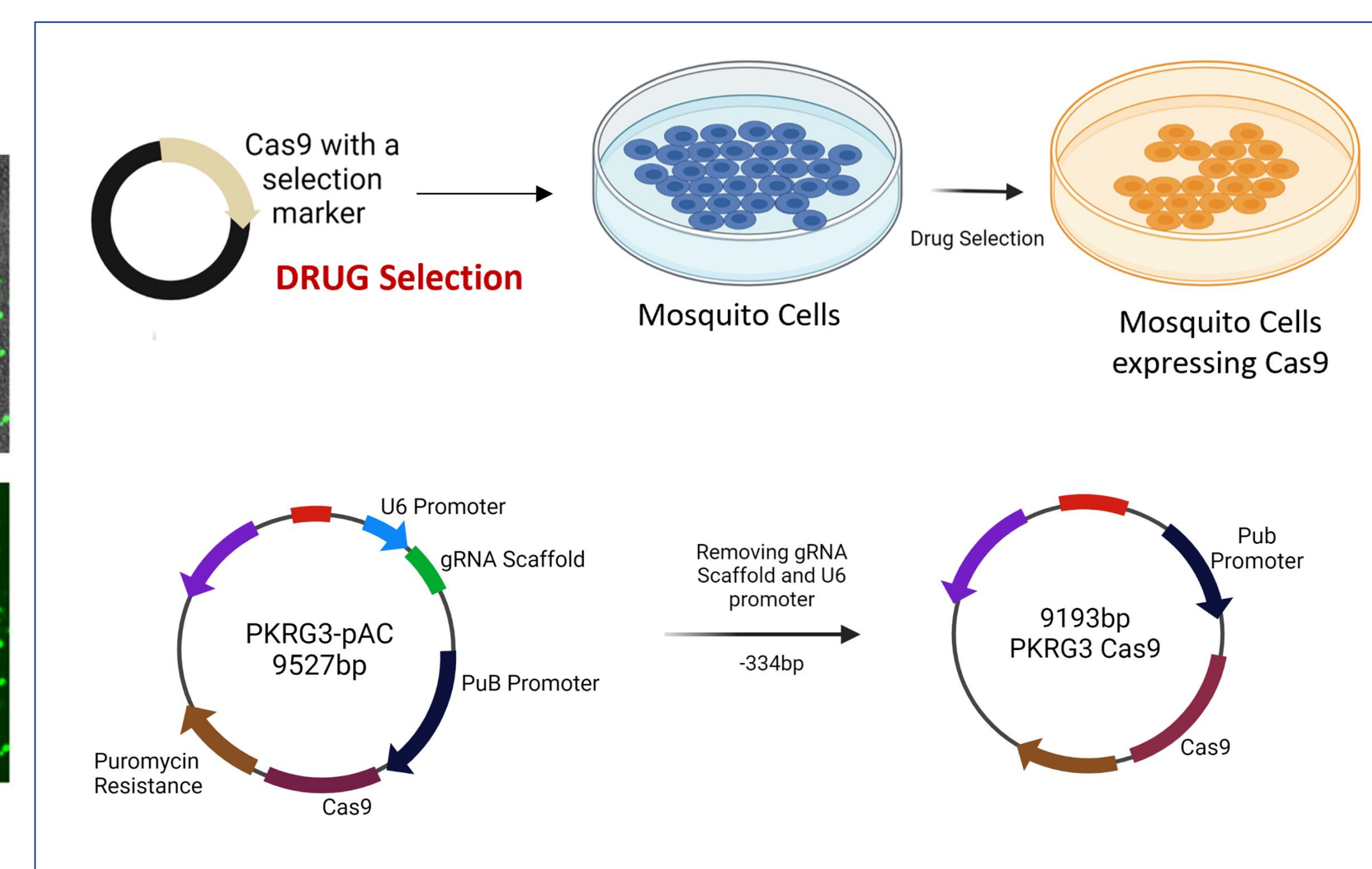


Fig 3: Making Plasmid system for Delivering Cas9 Into Mosquito Cells

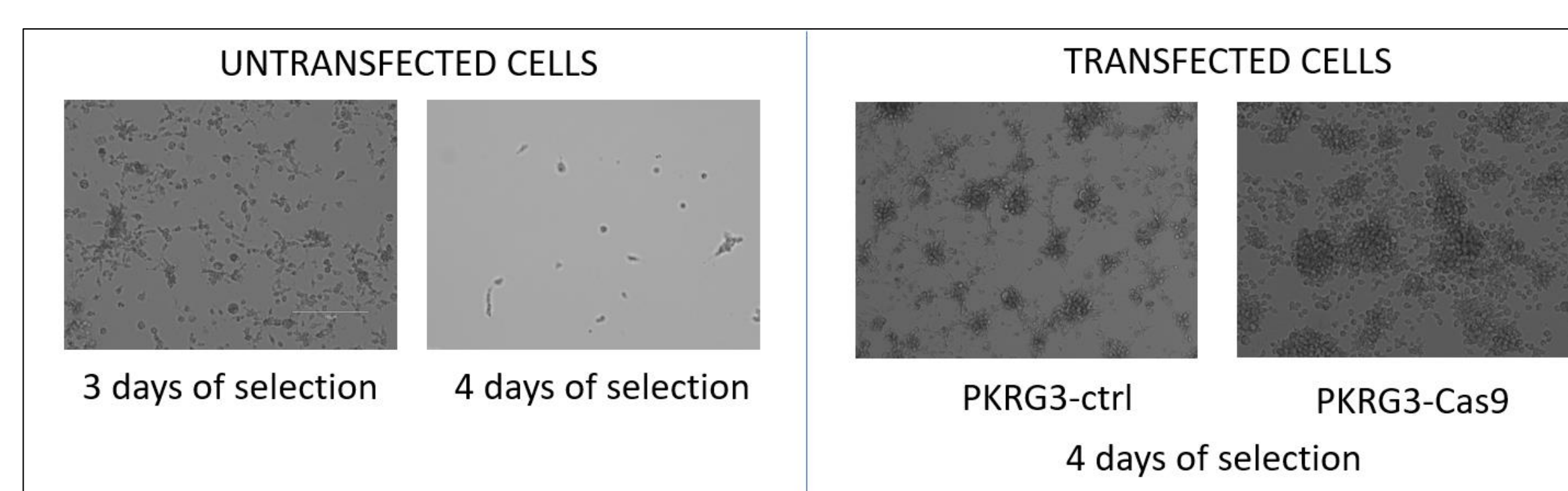


Fig 4: Mosquito cells surviving Drug selection

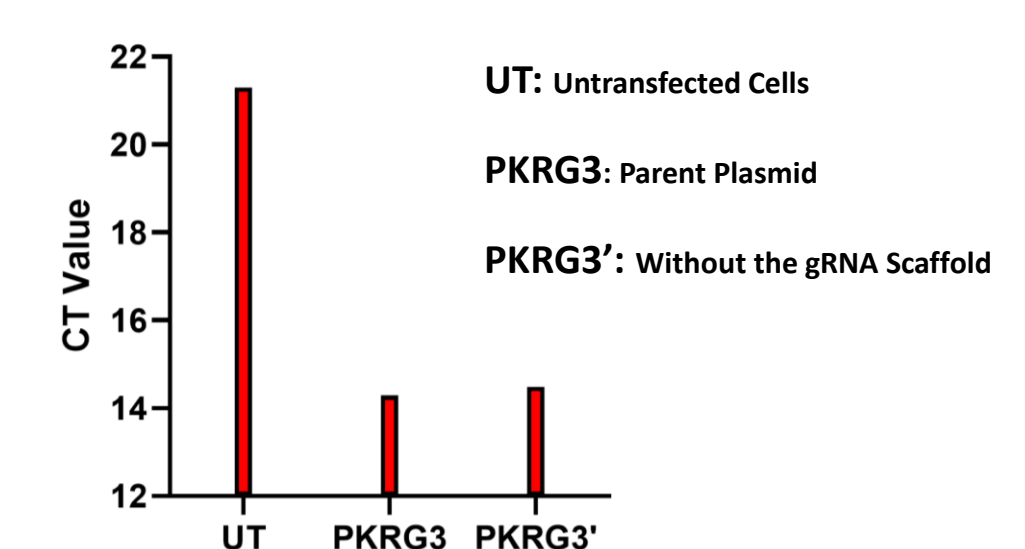


Fig 5: Cas9 Expression confirmed by RT-PCR

- Single vector system converted to Dual Vector system for delivery of Cas9 in mosquito cells (Fig 3)
- Cas9 Expression confirmed using RT-PCR and cell selection (Fig 4 & 5)
- Retroviruses can be used to deliver guide RNAs into mosquito cells (Fig 6 and 7)

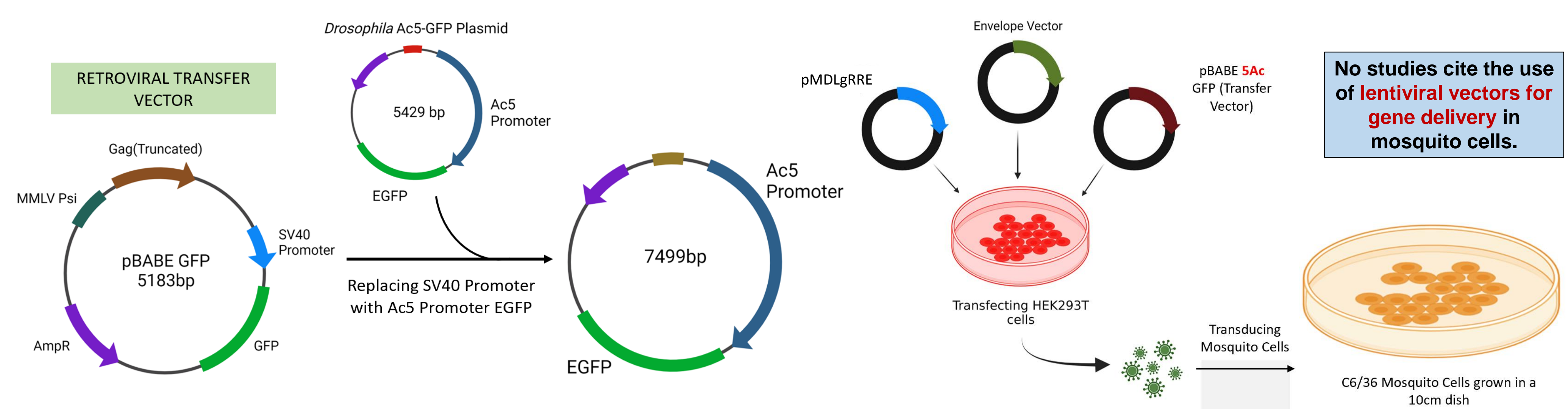


Fig 6: Engineering Retroviral transfer vector for delivering gRNAs into Mosquito cells

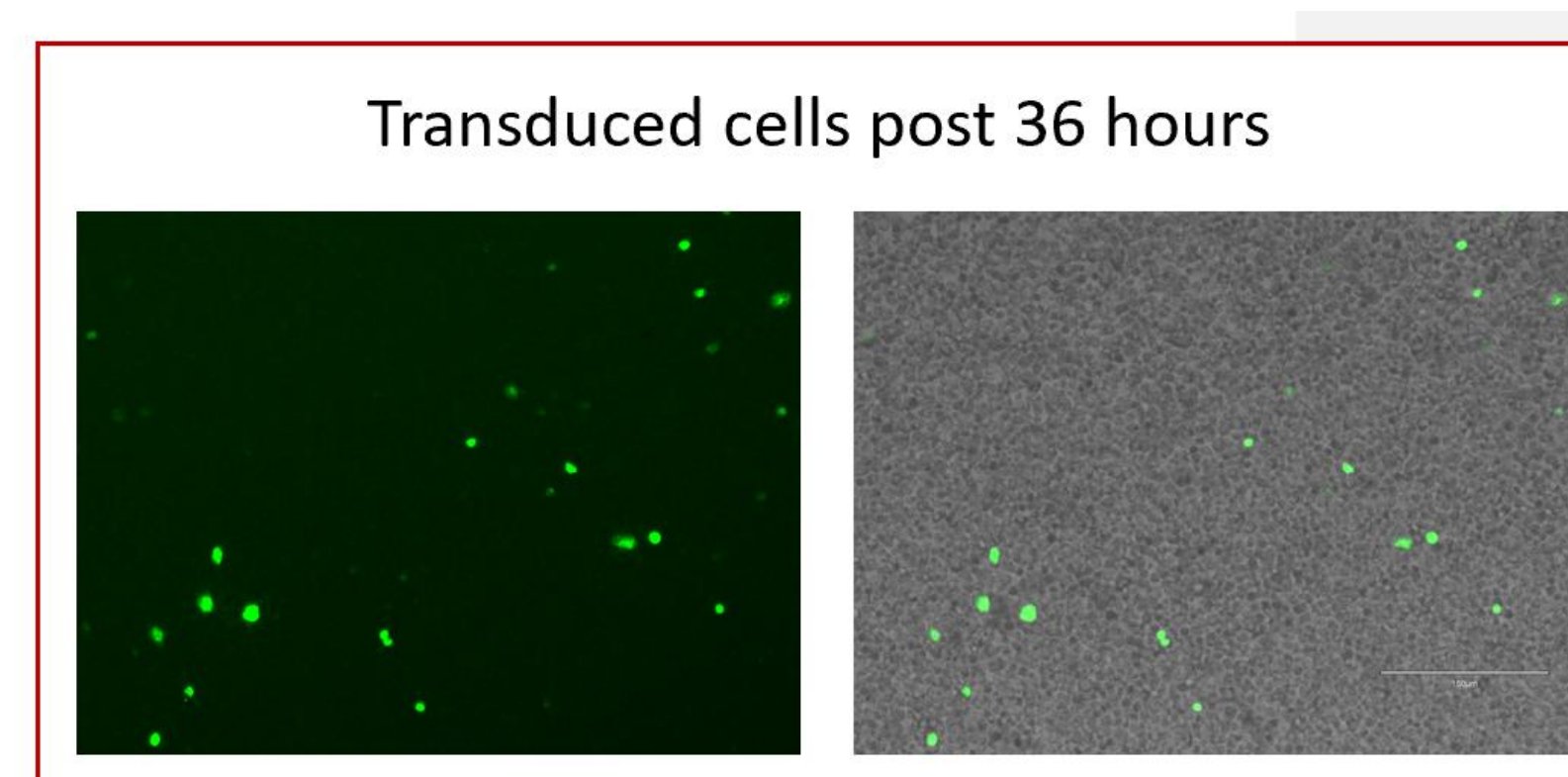


Fig 7: Retroviral transduction using pBABE 5Ac GFP

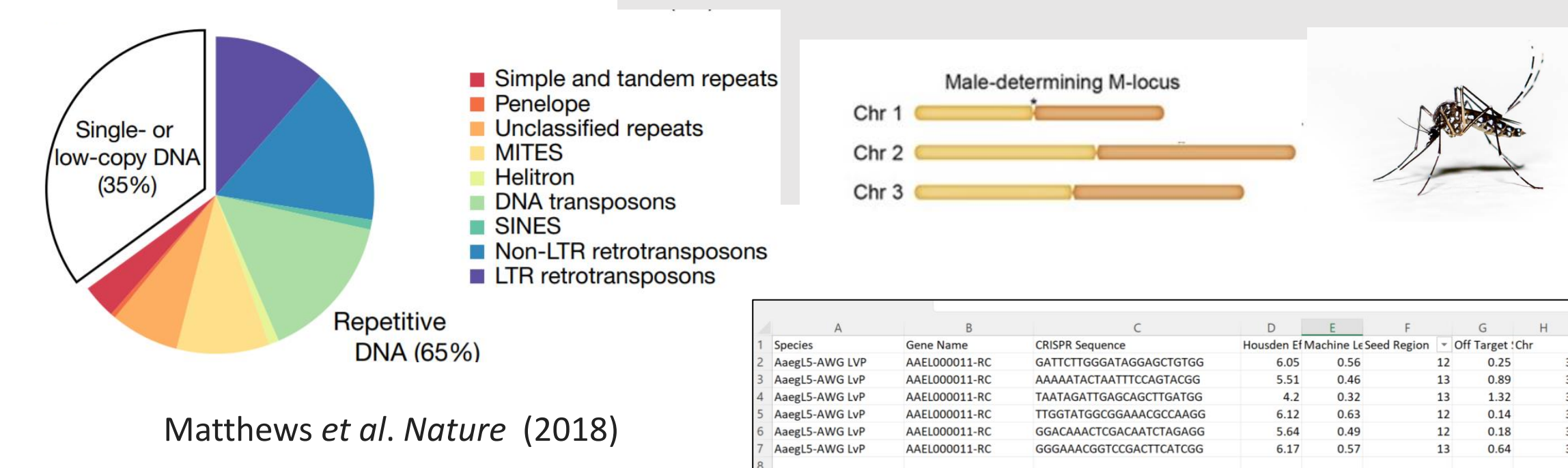


Fig 8: Designing gRNAs against Whole Genome of Aedes aegypti

## FUTURE DIRECTIONS

- Design and clone the gRNAs against the Aedes mosquito genome in the retroviral transfer vector.
- Perform a pilot CRISPR KO experiment and Flavivirus survival screen, followed by an Unbiased Whole Genome Knockout Screen.
- Validation and mechanistic studies.

## ACKNOWLEDGEMENTS

