JBrowse2 as a tool to visualize highly pathogenic, transmissible or therapy-resistant mutations in Dengue viruses against vaccine strains

The Dengue virus is the most widely-spread viral disease in humans that is transmitted by mosquitoes (da Silva & Harris, 2018). As climate change impacts the habitat of these mosquitoes, Dengue becomes an increasingly large global threat (Mills & Donnelly, 2024). The WHO has reported over 12 million cases in 2024 so far, an increase of more than 140% from 2023 (PAHO/WHO, 2024). Infection can lead to life-threatening conditions such as Dengue haemorrhagic fever (DHF) and Dengue shock syndrome (DSS) (Kalayanarooj, 2011). Dengue has four main serotypes: DENV-1, DENV-2, DENV-3 and DENV-4, which separately crossed into humans over the last centuries and evolved further (Vasilakis *et al.*, 2011). As these serotypes evolve and vaccine development catches up, the need arises for a customizable, genome-wide visualization tool to compare strains.

When analyzing the dengue virus genome using JBrowse2, our main goal was to build a database that would allow us to draw conclusions between mutations and virus characteristics. These conclusions would be useful for identifying mutations linked to increased virulence or resistance to treatment, providing valuable insights for disease management in future outbreaks. Additionally, such insights could guide the development of targeted antiviral therapies and future vaccine development.

To do this, we chose to look at the alignments between different strains within the four dengue serotypes, using bowtie and JBrowse2's alignment tracks (Langmead *et al.*, 2009). This software, generally used for aligning sequencing reads, proves useful as well in comparing entire genomes. Among the different strains, one of the strains for each serotype was the genome of the virus from which a vaccine was developed. Alignment visualization of such data would help researchers compare the genome sequences side-by-side, allowing for identification of conserved regions and mutations. By highlighting differences between protein domains, it becomes possible to pinpoint specific genetic changes associated with altered virus characteristics. We also aligned the strain used to produce the Dengvaxia vaccine to each serotype, as identified by Thomas & Yoon (2018). This vaccine, which targets the E-protein and prM-protein, is the only licensed vaccine to date (Torres-Flores *et al.*, 2022). Researchers can thus compare the alignment of the provided genomes or genomes they sequence themselves to the E- and PrM-proteins of the vaccine strains, to assess the impact of mutations with certain genotypic and epidemiological consequences on vaccine efficacy and herd immunity. We provide modified GFF files of the reference genomes, sourced from NCBI genbank, that reveal the sites of these protein-coding regions, as they were previously masked by the annotation for a poly gene. The exact sites remain the same as in the original annotations. We assume that the sites for these proteins have not majorly changed, as major recombination within the same serotype strains is rare (Stica *et al.,* 2022). As an additional safeguard, the JBrowse2 browser shows empty alignment tracks when too many significant relocations have occurred.

For each of the serotypes, we looked at four genomes in total. One of the genomes was used as a reference. This genome was found on the NCBI Genomes section and had accompanying annotation files. The other four genomes were sourced from the NCBI nucleotide database. The selections for the three different outbreak strains for each serotype are described in detail below:

For the DENV-1 serotype genomes, we selected the 2016-2017 dengue virus 1 outbreak that occurred in New Caledonia that was more severe than the 2013-2014 outbreak, despite the newer strain having reduced replicative fitness due to four amino acid substitutions (Inizer & Minier *et al.,* 2021). Then an outbreak in Xishuangbanna Dai in 2019, with increased severity was observed despite amino

acid mutations occurring in only one of six strains, with no recombination detected among them. These mutations can potentially be identified as non-contributors to the virus's pathogenicity (Li *et al.,* 2022). Finally, Guangzhou has experienced severe dengue outbreaks linked to the highly virulent DENV 1 genotype V, which is associated with severe clinical cases (Leng *et al .,* 2024). Studying this strain could reveal specific mutations contributing to its virulence, offering insights for improved disease management.

For the DENV-2 serotype genomes, we selected the cosmopolitan genotype of DENV-2 is the most widespread and has caused severe outbreaks in Malaysia and other Asian countries. Alignment information can help identify common mutations among its highly variable strains, linking them to the genotype's high transmissibility (Yenamandra et al., 2021). An outbreak in Malaysia of this strain presented with significant warning signs and severe dengue. Its severity highlights the importance of further research to better understand its characteristics and inform public health efforts (Jiang et al., 2022). Lastly, another cosmopolitan strain introduced in Brazil in 2021, is notable for a case where a patient exhibited DENV-2 symptoms but initially tested negative in an antibody panel. Comparing its genome to the reference could help identify genes coding for diagnostic antibodies, improving test accuracy (Iani et al., 2024)

For the DENV-3 serotype genomes, we selected the 2013 outbreak in Nicaragua containing a mutation, V91A, in the NS4B protein, which causes a resistance to medicinal prophylactic mosnodenvir (Bouzidi *et al.*, 2024). We annotated it in the provided annotations. The same mutation was also found in strains sequenced in the Florida 2022-2023 breakout. We included a strain from this outbreak without the mutation (Jones *et al.*, 2024). Note that this sequencing result contains areas of undetermined nucleotides, which show up as black regions. The outbreak was relatively large as Dengue has only minimally been endemic in the United States. Studying this strain is important in the light of changing climate conditions globally. In Senegal in 2018, DENV-3 became increasingly prolific, where only DENV-2 dominated before. These strains show 22 significant mutations in the E-protein, targeted by vaccines (Dieng *et al.*, 2023).

For the DENV-4 serotype genomes, we selected an outbreak in Indonesia in 2019, a serotype usually known for its low transmission and spread. The strain had a positive selection pressure on mutations in non-structural protein 5 (NS5), a common target for novel vaccines (Wardhani *et al.*, 2022). We also included a strain from Paraguay in 2020, which caused its strongest epidemic yet with seven new mutations, as well as an exceptionally low detection rate by NS1 rapid tests (Rojas *et al.*, 2024). Lastly, we compare a strain from Cambodia (2010) and French Polynesia (2009), as the vaccine only produced neutralizing antibodies against the former, caused by four mutations in the E-protein (Gallichotte *et al.*, 2022)

The visualization we provide sits between offering detailed information on specific mutations, and providing a genome-level overview on commonly mutating areas. Whereas both these approaches are valuable to pursue independently, we believe that providing this middle ground allows researchers to customize to their needs. An avenue of future research could be to add Multiple Sequence Alignment (MSA) to improve scalability when more genomes are added, as well as to add protein structure visualizations. This can aid vaccine development and assess the impact of modifications on antibody-dependant enhancement, an important mechanism in Dengue pathogenesis (da Silva & Harris, 2018; Khan *et al.*, 2023; Sawant, Patil & Kurle, 2023).

References

Bouzidi, H. S., Sen, S., Piorkowski, G., & others. (2024). Genomic surveillance reveals a dengue 2 virus epidemic lineage with a marked decrease in sensitivity to Mosnodenvir. *Nature Communications, 15*, 8667. https://doi.org/10.1038/s41467-024-52819-z

Dieng, I., Balde, D., Talla, C., Camara, D., Barry, M. A., Sagne, S. N., & others. (2023). Molecular evolution of dengue virus 3 in Senegal between 2009 and 2022: Dispersal patterns and implications for prevention and therapeutic countermeasures. *Vaccines (Basel), 11*(10), 1537. https://doi.org/10.3390/vaccines11101537

Gallichotte, E. N., Henein, S., Nivarthi, U., Munteanu, A., Baric, R., & de Silva, A. M. (2022). Vaccine-induced antibodies to contemporary strains of dengue virus type 4 show a mechanistic correlate of protective immunity. *Cell Reports*, 110930. https://doi.org/10.1016/j.celrep.2022.110930

Iani, F. C. de M., de Campos, G. M., Adelino, T. E. R., da Silva, A. S., Kashima, S., Alcantara, L. C. J., Sampaio, S. C., Giovanetti, M., Elias, M. C., & Slavov, S. N. (2024). Metagenomic Analysis for Diagnosis of Hemorrhagic Fever in Minas Gerais, Brazil. Microorganisms, 12(4), 769. https://doi.org/10.3390/microorganisms12040769

Inizan, C., Minier, M., Prot, M., O'Connor, O., Forfait, C., Laumond, S., Marois, I., Biron, A., Gourinat, A.-C., Goujart, M.-A., Descloux, E., Sakuntabhai, A., Tarantola, A., Simon-Lorière, E., & Dupont-Rouzeyrol, M. (2021). Viral evolution sustains a dengue outbreak of enhanced severity. Emerging Microbes & Infections, 10(1), 536–544. https://doi.org/10.1080/22221751.2021.1899057

Jiang, L., Liu, Y., Su, W., Cao, Y., Jing, Q., Wu, X., & Yang, Z. (2022). Circulation of genotypes of dengue virus serotype 2 in Guangzhou over a period of 20 years. Virology Journal, 19(1). https://doi.org/10.1186/s12985-022-01773-7

Jones, F. K., Morrison, A. M., Santiago, G. A., Rysava, K., Zimler, R. A., Heberlein, L. A., & others.; Florida Department of Health Bureau of Public Health Laboratory Team. (2024). Introduction and spread of dengue virus 3, Florida, USA, May 2022–April 2023. *Emerging Infectious Diseases, 30*(2), 376–379. https://doi.org/10.3201/eid3002.231451

Kalayanarooj, S. (2011). Clinical manifestations and management of dengue/DHF/DSS. *Tropical Medicine and Health, 39*(4 Suppl), S83–S87. https://doi.org/10.2149/tmh.2011-S10

Khan, M. B., Yang, Z. S., Lin, C. Y., Hsu, M. C., Urbina, A. N., Assavalapsakul, W., Wang, W. H., Chen, Y. H., & Wang, S. F. (2023). Dengue overview: An updated systematic review. *Journal of Infection and Public Health, 16*(10), 1625–1642. https://doi.org/10.1016/j.jiph.2023.09.011

Langmead, B., Trapnell, C., Pop, M., & Salzberg, S. L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology, 10*(3), R25. https://doi.org/10.1186/gb-2009-10-3-r25

Leng, X., Zhao, L., Liao, L., Jin, K., Feng, J., & Zhang, F. (2024). Genotype of dengue virus serotype 1 in relation to severe dengue in Guangzhou, China. Journal of Medical Virology, 96(5). https://doi.org/10.1002/jmv.29635

Li, D., Long, M., Li, T., Shu, Y., Shan, X., Zhang, J., Ma, D., Long, S., Wang, X., Jia, F., Pan, Y., Chen, J., Liu, P., & Sun, Q. (2022). The whole‐genome sequencing of prevalent DENV‐1 strains during the largest dengue virus outbreak in Xishuangbanna Dai autonomous prefecture in 2019. Journal of Medical Virology. https://doi.org/10.1002/jmv.28115

Mills, C., & Donnelly, C. A. (2024). Climate-based modelling and forecasting of dengue in three endemic departments of Peru. *PLoS Neglected Tropical Diseases, 18*(12), e0012596. https://doi.org/10.1371/journal.pntd.0012596

Pan American Health Organization & World Health Organization. (n.d.). *Dengue indicators*. Retrieved December 4, 2024, from https://www3.paho.org/data/index.php/en/mnu-topics/indicadores-dengue-en.html

Rojas, A., Shen, J., Cardozo, F., Bernal, C., Caballero, O., Ping, S., Key, A., Haider, A., de Guillén, Y., & Langjahr, P. (2024). Characterization of dengue virus 4 cases in Paraguay, 2019–2020. *Viruses, 16*(2), 181. https://doi.org/10.3390/v16020181

Sawant, J., Patil, A., & Kurle, S. (2023). A review: Understanding molecular mechanisms of antibody-dependent enhancement in viral infections. *Vaccines, 11*(7), 1240. https://doi.org/10.3390/vaccines11071240

de Silva, A. M., & Harris, E. (2018). Which dengue vaccine approach is the most promising, and should we be concerned about enhanced disease after vaccination? The path to a dengue vaccine: Learning from human natural dengue infection studies and vaccine trials. *Cold Spring Harbor Perspectives in Biology, 10*(6), a029371. https://doi.org/10.1101/cshperspect.a029371

Stica, C. J., Barrero, R. A., Murray, R. Z., Devine, G. J., Phillips, M. J., & Frentiu, F. D. (2022). Global Evolutionary History and Dynamics of Dengue Viruses Inferred from Whole Genome Sequences. Viruses, 14(4), 703. https://doi.org/10.3390/v14040703

Torres-Flores, J. M., Reyes-Sandoval, A., & Salazar, M. I. (2022). Dengue vaccines: An update. *BioDrugs, 36*(3), 325–336. https://doi.org/10.1007/s40259-022-00522-5

Thomas, S. J., & Yoon, I. K. (2019). A review of Dengvaxia®: Development to deployment. *Human Vaccines & Immunotherapeutics, 15*(10), 2295–2314. https://doi.org/10.1080/21645515.2019.1658506

Vasilakis, N., Cardosa, J., Hanley, K. A., Holmes, E. C., & Weaver, S. C. (2011). Fever from the forest: Prospects for the continued emergence of sylvatic dengue virus and its impact on public health. *Nature Reviews Microbiology, 9*(7), 532–541. https://doi.org/10.1038/nrmicro2595

Wardhani, P., Yohan, B., Tanzilia, M., & others. (2023). Genetic characterization of dengue virus 4 complete genomes from East Java, Indonesia. *Virus Genes, 59*(1), 36–44. https://doi.org/10.1007/s11262-022-01942-4

Yenamandra, S. P., Koo, C., Chiang, S., Lim, H. S. J., Yeo, Z. Y., Ng, L. C., & Hapuarachchi, H. C. (2021). Evolution, heterogeneity and global dispersal of cosmopolitan genotype of Dengue virus type 2. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-92783-y