Chapter 1

Introduction

1.1 Background

Dengue remains an escalating global threat, formally classified as a Grade 3 emergency by the World Health Organization (WHO) in 2019. This classification reflects the relentless rise of dengue cases and associated mortality over recent years spreading across more than 100 endemic countries (World Health Organization, 2019). In 2023 alone, dengue virus (DENV) accounted for over 6.5 million infections and 7,000 deaths. Within a year, these figures more than doubled, reaching 14 million reported cases and 9,990 deaths by late 2024. Yet these numbers only capture what is visible, highlighting a persistent and underlooked global emergency (Haider et al., 2025). Dengue is a mosquito-borne viral disease that is further distinguished into 4 serotypes namely DENV-1, DENV-2, DENV-3, and DENV-4 which can infect humans (Pourzangiabadi et al., 2024).

Among all these serotypes, DENV-2 is frequently associated with the most severe clinical outcomes and large-scale outbreaks. The infection of dengue often results in mild or asymptomatic cases. However, DENV-2 is more frequently associated with secondary infections leading to higher risk of severe dengue including hemorrhagic manifestations, plasma leakage and shock. This serotype has also been linked to greater viral replication rates, increased viremia and more aggressive host immune responses (Vicente et al., 2016). Moreover, this strain is responsible for Taiwan's deadliest epidemic in 2015 and continues to re-emerge, particularly during periods of increased rainfall (Tsai et al., 2023). Thus far, clinical management remains entirely supportive, emphasizing hydration and pain control while approved dengue-specific antiviral therapies remain unavailable (Obi et al., 2021)

Despite decades of research, there is currently no FDA-approved antiviral drug available for the treatment of dengue virus. The development of novel therapeutics is a notoriously protracted and

resource-intensive endeavor, typically requiring 10 to 15 years of preclinical and clinical evaluation prior to market approval. This timeline is further exacerbated by exceptionally high attrition rates during drug discovery. In dengue research, late-stage drug failures are frequently driven by the presence of four antigenically distinct serotypes, as well as specific biological hurdles such as a narrow therapeutic window post-symptom onset and the rapid emergence of drug-resistant viral mutants (Lee et al., 2023). To mitigate these high failure rates and accelerate the discovery timeline, there is an urgent need for screening methods that are highly scalable and capable of high-throughput execution (Arias-Arias et al., 2021). However, the current gold standard for antiviral screening remains the plaque assay, whose prolonged incubation period and labor-intensive workflow render it poorly suited for high-throughput applications. Alternative approaches, such as nucleic acid-based detection systems, often fail to distinguish infectious from non-infectious viral particles, further emphasizing the deficit in ideal screening technologies (Praditya et al., 2025).

Recently, reporter-expressing viruses have emerged as promising tools for high-throughput antiviral screening. These systems incorporate fluorescent or bioluminescent reporters into viral genomes, enabling real-time monitoring and rapid quantification of viral replication (Liu et al., 2024). For instance, an eGFP-based Japanese encephalitis virus (JEV) reporter system enabled automated quantification of GFP-positive cells using high-content imaging platforms, demonstrating its suitability for large-scale antiviral screening applications (Zhang et al., 2020). Similarly, luciferase-based reporter systems have demonstrated enhanced assay sensitivity and rapid signal detection in Zika and Chikugunya Viruses studies (Legros et al., 2025; Xu et al., 2023). Collectively, these findings highlight the potential of reporter-based viral systems as scalable platforms for antiviral screening applications. However, application of these systems in dengue virus research still requires further optimization to ensure assay stability, reproducibility, and scalability for high-throughput screening. Therefore, this study aims to optimize a fluorescence-based DENV-2 EGFP infectious clone system as a potential platform for rapid and scalable antiviral screening.

1.2 Objective

The main objective of this thesis is to optimize and evaluate a fluorescence-based DENV-2 EGFP infectious clone system for high-throughput antiviral screening applications. With sub-objectives including:

1. To generate and amplify the DV-2 eGFP infectious virus stock
2. To optimize infection conditions for the DV-2 eGFP reporter system
3. To evaluate the applicability of the DV-2 eGFP system for antiviral screening

1.3. Hypothesis

The hypothesis of this thesis is that the optimization of the DV-2 eGFP reporter system will enable reliable real-time monitoring of viral replication and facilitate quantitative evaluation of antiviral activity in high-throughput screening applications.