# I. INTRODUCTION

The Flavivirus Dengue virus (DENV) is an etiological agent of dengue fever, a major disease burden in tropical countries commonly manifesting as acute febrile illness or hemorrhagic fever in severe cases (Wang et al., 2020). Between January and July 2023, there have been over three million cases of dengue fever being reported globally (European Centre for Disease Prevention and Control, 2023). The vectors, *Aedes aegypti* and *Aedes albopictus*, are well-distributed in tropical regions, underlying the endemicity of DENV in Indonesia (Kraemer et al., 2015; Sasmono et al., 2020). The high prevalence of DENV in Indonesia is evident by more than 143,000 confirmed cases in 2022 (Kementerian Kesehatan Republik Indonesia, 2023). The highest rate of infection in Indonesia was reported to be in Denpasar and Makassar (Utama et al., 2019). Moreover, the highest mortality rate has been reported to be 1,598 in 2016, increasing the challenge to manage the disease (Hendra et al., 2020).

The disease burden is further increased by the genetic diversity of DENV. The DENV genome is positive-sense single stranded RNA and can be categorized into four antigenically different serotypes: DENV-1, -2, -3, and -4 with only 60-75% of amino acid homology (Guzman & Harris, 2015; Martinez et al., 2020), all of which have been found to circulate around Indonesia (Yohan et al., 2018a). These differences in the four serotypes may affect the replication rate and transmission of the virus (Datu et al., 2023). In addition, the high mutation rate of DENV gives rise to different genotypes, further resulting in the diversity of pathogenicity. A study done by Sasmono et al. (2015) found that DENV-1 and -2 have a higher replication rate *in-vitro* using mammalian Vero76 cell line compared to the other two serotypes, with DENV-1 genotype I replicating faster up to a tenfold than genotype IV. The rapid replication abilities of certain serotypes may contribute to their rapid transmission in local communities, which can also be argued to have a role in disease severity due to the high secretion of non-structural protein 1 (NS1) (Guzman & Harris, 2015; Niu et al., 2020).

Studying the genetic diversity of four DENV serotypes is critical to elucidate the dynamics of DENV infection, such as the transmission capabilities within hosts in specific areas. Other than that, genotyping methods are commonly performed for genomic surveillance and epidemiological studies to assess possibilities of predominant genotype shifting circulating in certain population, the expansion of circulating serotype (Cruz et al., 2016; Santiago et al., 2019), and for analysis of genetic variants in correlation to vaccine development and diagnostic targets (Ko et al., 2020; Rodriguez-Manzano et al., 2018).

It is not only crucial to perform molecular-based research to observe viral genotypic changes, but also to conduct viral isolation methods for the identification of viral replication behavior, which may function as a tool to study phenotypic effects of mutations in DENV biological characterization. Being the gold standard for detection and confirmation of infectious viruses in samples (Kennedy, 2005), DENV propagation in cell culture is able to serve as a qualitative evidence of infection through the observation of cytopathic effects (CPE), such as cell rounding and deformation (Storch et al., 2000; Upadhyay, 2022). In addition, viral propagation is essential to purify viral particles and increase viral titer for downstream analysis (Pavel et al., 2020).

With that being said, the objective of this research is to perform genotyping for clinical samples through Sanger sequencing, aiming to support genomic surveillance and vaccine development specific to the circulating DENV Indonesian strain. In addition, this research aims to conduct viral propagation techniques for the detection and isolation of DENV reference strain. It is then hypothesized that the Indonesian strain will have a close genetic distance with strains from surrounding regions and the reference strains will be successfully isolated.