**COVER LETTER**

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Dr. Yekti Asih Purwestri, M.Si.

Editor-in-Chief

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Dear Dr. Yekti Asih Purwestri, M.Si.,

We are submitting an original article titled " Alternative Splicing Analysis of *Hd3a* Gene in Oil Palm (*Elaeis guineensis*)" for your consideration for publication in the Indonesian Journal of Biotechnology (IJBiotech). Alternative splicing is a complex process that contributes to the generation of diverse mRNA and protein isoforms. Despite their importance, many functions of alternative splicing genes remain poorly characterized. This study aims to investigate the best primer for analyzing alternative splicing *EgHd3a* mRNA specimens as initial steps for functional characterization.

Our study discovered that nucleotide variations at the 3'-end of the primers were observed to influence annealing specificity, with GC composition enhancing it. Furthermore, qPCR analysis reveals a strong correlation between Ct values and gene concentration for the isoforms, indicating reliable amplification. Although two isoforms, Hd3a-X2 and Hd3a-X3, exhibit slightly higher than acceptable PCR efficiency values, caution is advised thoroughly to avoid non-specific amplification. Despite the challenges posed by limited primer positioning due to alternative splicing, the chosen primer proved optimal for analysis. This study highlights the significance of considering alternative splicing in gene quantification experiments and provides insights into critical steps, methods, and quality control measures necessary for accurately detecting alternative splicing events, contributing to understanding of this complex biological process. This paper contains one table and three figures.

In addition, we believe that this manuscript meets the aims and scope of IJBiotech. The manuscript will appeal to the scientific community of quantitative genes analysis because it contributes valuable information on alternative splicing analysis methodologies. Therefore, we suggest Dr. Kurniawan Rudi Trijatmiko and Dr. Agus Budi Setiawan (more information are attached) as potential reviewer.

We confirm that this manuscript has not been published elsewhere and is not under consideration by any other journals. All authors have approved the manuscript and agree with its submission to the Indonesian Journal of Biotechnology. We declare that we have no conflict of interest to disclose.

Please direct all correspondence regarding this manuscript to me at encedarmo@apps.ipb.ac.id and aqwinpolosoro@apps.ipb.ac.id.

Sincerely,

Dr. Ence Darmo Jaya Supena

**Attachment 1.** Protentional reviewer

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