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# Inadvertent Platelet Transfusion from Mpox-Infected Donor to Recipient, Thailand, 2023

## Appendix

### Gene Amplification and DNA Sequencing

The DNA helicase and Schlafen protein gene sequences were amplified using semi-nested RT-PCR with the primers specified in Appendix Table, resulting in amplicon lengths of 315 bp and 314 bp, respectively. The first-round PCR were performed using PerfectTaq MasterMix PCR system, according to the manufacturer's instructions (5 PRIME, Darmstadt, Germany). The amplification conditions for PCR involved 40 cycles with the following parameters: denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, and extension at 68 °C for 1 minute 45 seconds. A final extension step at 68 °C for 5 minutes was included. In the second-round PCR reaction, 1 µL of the first-round reaction served as a template, using second-round primers and PerfectTaq MasterMix (5 PRIME, Darmstadt, Germany) in accordance with the manufacturer's instructions. Subsequently, 40 cycles were conducted with the following conditions: denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, and extension at 72 °C for 90 seconds, followed by a final extension at 72 °C for 5 minutes. The sequencing and product amplification were performed simultaneously in both the forward and reverse directions at First BASE Laboratories Sdn Bhd (Selangor Darul Ehsan, Malaysia).

**Appendix Table.** Primers used for conventional real-time PCR assays

Target region	Primers	Sequence (5'-3')	Position	Strand
DNA helicase	F129088	CACTCCAGCACCCGCAGAG	129088-129107	Sense
	F129182	ATGTCACTATTAAAGATGGAGTAT	129182-129206	Sense
	R129496	AGTGAAGAGTGATGTATAGAGG	129474-129496	Antisense
Schlafen protein	F167584	TTACTACTGTAGACGTGCATGG	167584-167606	Sense
	F167644	TGCTTCCGATTCCAATCTGG	167644-167665	Sense
	R167957	AAGACATGCTCCCATAGTCTTC	167935-167957	Antisense



**Appendix Figure.** Phylogenetic analysis of the DNA helicase gene region (position 123,565–123,898 nt). The phylogenetic tree was constructed using the neighbor-joining method with 1,000 bootstrap replicates and implemented in MEGA version 7 ([www.megasoftware.net](http://www.megasoftware.net)). Evolutionary distances were computed using the maximum composite likelihood method. Bootstrap values >75 are shown. Scale bar represents substitutions per site.