

## **Single nucleotide polymorphism of SSU rRNA gene among plasmodium knowlesi isolates of Sabah**

### **Abstract**

Background: The advent of PCR (Polymerase Chain Reaction) assays helped in correctly identifying *Plasmodium knowlesi*, which was previously misdiagnosed by microscopy as *Plasmodium malariae* in Sabah, Malaysia. The PCR-based diagnosis of *P. knowlesi* in Sabah is currently using a set of oligonucleotide primers namely Pmk8 and Pmk9 that target one of the parasite's small subunit rRNA (SSU rRNA) genes. PCR also helped in discovering a variant form of *P. malariae* which has a deletion of 19 bp and seven substitutions of base pairs in the target sequence of the small-subunit rRNA gene among isolates of Sichuan province of China and Thai Myanmar Border. The sequences of eight isolates identified as *P. knowlesi* in Kapit, Sarawek were not identical, showing within-species polymorphisms. Thus the possibility of variation in the DNA sequence of SSU rRNA gene of *P. knowlesi* isolates was expected. Aim & Objectives: To determine the within-species polymorphism of the fifth human malaria species among Sabah population in relation to geographical regions. Methods: The samples of *P. knowlesi* isolates, sent to the Sabah State Public Health Laboratory from the districts with *P. knowlesi* high prevalence, were included. In 10 samples, which gave positive in PCR with Pmk8 and Pmk9 primers in Nest 2 PCR, the Nest 1 PCR products were analysed by automated sequencer for DNA sequence to find out genetic variation of SSU rRNA of *P. knowlesi*. Results/Findings: All 10 samples showed SNPs (single nucleotide polymorphism) at 14 nucleotides when compared with the same gene of Standard Strain of *P. knowlesi*. The locations of SNPs were quite similar to the SNPs found in Kapit Division of Sarawek, Malaysia. No typical SNPs pattern in each geographical region could be identified. However, SNPs in each region have only 2-3 nucleotides in difference. Study Limitations: SSU rRNA gene is 2096 bp in length. However, Nest 1 product sequenced in this study is only 1622 bp in length that this study could not describe the variation in the sequence of the whole gene. Conclusion: There exists a somewhat similar regional pattern of Single Nucleotide Polymorphism in the sequence

of SSU rRNA gene of *P.knowlesi* isolates from Sabah, Malaysia. It is recommended to conduct further studies, involving the whole gene sequence and covering a larger amount of samples derived from different geographic regions of Sabah.