

# Whole-genome Sequencing of *Plasmodium falciparum* from Low-density dried Blood spot samples

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Whole-genome sequencing (WGS) may be a comprehensive technique for analyzing entire genomes. Genomic info has been instrumental in distinguishing transmissible disorders, characterizing the mutations that drive cancer progression, and following sickness outbreaks. speedily dropping sequencing prices and therefore the ability to supply massive volumes of knowledge with today's sequencers build whole-genome sequencing a robust tool for genetic science analysis.

WGS data provide a complete picture of a pathogen genome and are revolutionizing molecular epidemiology. The availability of WGS data from field collected malaria samples has increased in recent years, paralleling the development of more sensitive, faster, and lower-cost sequencing technologies. Until recently, generating these sequence data required collecting large volumes of venous blood, depleting leukocytes at the time of sample collection, and storing in the field—tasks that were often difficult to complete in resource-limited settings. DBS collection, on the other hand, necessitates small sample volumes that are easily stored and transported. However, the low volumes of blood present in DBS frequently result in low quality and quantity of parasite DNA, especially when compared to the sample's overwhelming proportion of human DNA. Several studies have used various enrichment methods to address these challenges, including non-selective whole genome amplification (WGA), hybrid selection, and enzymatic digestion of host DNA using the Using restriction enzymes

from the MspJI family and selective whole genome amplification (sWGA). Recently, the collection of leukocyte-depleted dried erythrocyte spots has shown promise in terms of providing parasite-enriched samples for WGS. For example, two recent studies that used two different sets of sWGA primers to perform sWGA on clinical samples reported an average 55 percent of reads mapped to the *P. falciparum* genome with 48 percent of the genome covered at 5 in 24 samples with an average parasite density of 73,6. of 73,601 parasites per  $\mu\text{L}$ . Oyola et al., on the other hand, established a parasitaemia threshold of 0.03 percent ( $> 1200$  parasites/L) to obtain at least 50% of the genome at 5 in clinical samples. A similar level of coverage was reported in a *P. vivax*-specific sWGA, emphasising the importance of improving these protocols for DBS samples. Standardized comparison and optimization of different DBS DNA extraction methods; identification of optimal sWGA primer sets that provide higher yield with less bias; and performing enzymatic cleavage of human DNA prior to sWGA could all potentially improve sWGA efficiency. Four DNA extraction methods were used in this study, followed by either enzymatic cleavage of human DNA or no cleavage and amplification with WGA or sWGA with various primer sets were compared The combination of conditions was compared in standardised samples extracted from DBS with a range of low parasite densities, and the resulting WGS data were compared in terms of parasite genome coverage, dropout rate, and concordance of called variants.

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