

## **Hexaplex PCR Detection System for identification of five human plasmodium species with an internal control**

### **Abstract**

Malaria remains one of the major killers of humankind and persists to threaten the lives of more than one-third of the world's population. Given that human malaria can now be caused by five species of Plasmodium, i.e., Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale, and the recently included Plasmodium knowlesi, there is a critical need not only to augment global health efforts in malaria control but also, more importantly, to develop a rapid, accurate, species-sensitive/species-specific, and economically effective diagnostic method for malaria caused by these five species. Therefore, in the present study, a straightforward single-step hexaplex PCR system targeting five human Plasmodium 18S small-subunit rRNAs (ssu rRNAs) was designed, and the system successfully detected all five human malaria parasites. In addition, this system enables the differentiation of single infection as well as mixed infections up to the two-species level. This assay was validated with 50 randomly blinded test and 184 clinical samples suspected to indicate malaria. This hexaplex PCR system is not only an ideal alternative for routine malaria diagnosis in laboratories with conventional PCR machines but also adds value to diagnoses when there is a lack of an experienced microscopist or/and when the parasite morphology is confusing. Indeed, this system will definitely enhance the accuracy and accelerate the speed in the diagnosis of malaria, as well as improve the efficacy of malaria treatment and control, in addition to providing reliable data from epidemiological surveillance studies.