

Cycle DNA sequencing with [α -³⁵S]dATP demonstrates polymorphism of a surface antigen in malaria parasites from Sri Lankan patients ☆

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Abstract

Structural diversity in a 45 kDa surface antigen on *Plasmodium falciparum* merozoites (termed GYMSSA, MSP-2 or MSA-2) and other candidate molecules for developing a malaria vaccine need to be investigated in parasites obtained directly from patients in different malaria endemic countries. A double-stranded DNA sequencing method suitable for this purpose, and also for studying diversity in genes of other haploid cells, is described. A first round polymerase chain reaction (PCR) on DNA isolated from blood was carried out with a primer containing the GCN4 binding site to amplify and subsequently purify the coding region of the MSA-2 gene on GCN4 coated tubes. A second round PCR with more internal primers incorporating M13 forward and reverse primer sequences was then performed. Cycle sequencing was done with unlabelled M13 primers and [α -³⁵S]dATP by the dideoxynucleotide procedure. Two different allelic forms of MSA-2 were identified in samples of *Plasmodium falciparum* from patients in Sri Lanka.

Keywords: Cycle sequencing; DNA sequence; Genetic diversity; Malaria; Membrane protein; PCR
