CLONING AND PARTIAL CHARACTERIZATION OF REPETITIVE SEQUENCES FROM PLASMODIUM VIVAX

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In recent times DNA probes have made great strides in providing rapid, accurate and sensitive methods for the detection of parasites[1]. An ideal probe would be a cloned highly repetitive sequence which is specific and hence could differentiate between closely related species. The present studies were initiated to develop a DNA probe for Plasmodium vivax.

Plasmodium vivax infected human erythrocytes were concentrated by Percoll gradient centrifugation and the DNA extracted[2]. The DNA was randomly sheared and fragments in the size range 3.0-8.0 killo bases were recovered from a low melting agarose gel. Following protection of EcoRl restriction sites within the fragments by methylation, the fragments were bluntended with T_4 polymerase and Klenow fragment of E.coli polymerase. ^{32}P-Labelled EcoRl synthetic linkers were then ligated onto bluntended fragments using T_4 DNA ligase. EcoRl cohesive ends were generated by digestion with excess EcoRl. The excess EcoRl linkers were separated on a precalibrated Sepharose CL 4B column. The purified fragments were then ligated to dephosphorylated, EcoRl cleaved λgtll vector arms[3].

The phage DNA was packaged in vitro and plated out on protease deficient bacterial host E.coli Y 1090. The amplified library was then subjected to differential screening by Plaque hybridization using $^{4}\alpha$-^{32}P-dCTP nick translated total P.vivax DNA and human placental DNA. After 3-4 cycles of screening several putative clones giving strong signals with P.vivax DNA have been isolated.

Southern hybridization experiments with EcoRl cleaved P.vivax DNA and ^{32}P-labelled recombinant phage DNA as probes, indicate that the clones contain parasite specific DNA fragments.

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