CLONING AND ISOLATION OF A DNA SEQUENCE FOR THE DEVELOPMENT OF A POLYMERASE CHAIN REACTION BASED DIAGNOSTIC ASSAY FOR *MYCOBACTERIUM TUBERCULOSIS*

by

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ABSTRACT

Tuberculosis continues to be a major disease worldwide, responsible for morbidity and mortality in humans. For control and/or eradication programs to be effective, one has to employ reliable and rapid methods for the detection of *Mycobacterium tuberculosis*. This study reports the cloning and isolation of a *M. tuberculosis* specific DNA sequence which appears to be suitable for the development of polymerase chain reaction (PCR) based assay for the rapid detection of *M. tuberculosis*.

Mycobacterium tuberculosis genomic DNA was partially cleaved with the restriction enzyme *Sau3A* I to obtain maximum number of fragments in the size range 0-9 kb. These fragments were cloned into the bacteriophage vector λ Zap and packaged *in vitro*.

To isolate sequences specific for *M. tuberculosis* an aliquot of the library (approximately 20,000 recombinants) was plated out, and the plaque lifts obtained were differentially screened with ^{32}P-labelled *M. tuberculosis* genomic DNA and a mixture of DNA from five organisms (*Staphylococcus epidermidis, Streptococcus viridans, Klebsiella* species, *Neisseria flavescens* and *Candida albicans*) commonly found in the respiratory tract.

Plaques giving strong autoradiographic signals with *M. tuberculosis* DNA were purified and the DNA isolated. Eight recombinant phages designated LZ 1 to 8 were selected for further analysis. Eight dot blots containing DNA of *M.tuberculosis* (2.0 μg), human (2.0 μg), 5 organisms commonly found in the respiratory tract (4.0 μg) and λ DNA (1.0 ng) were probed separately with the eight, ^{32}P-labelled, recombinant clones isolated

above. Only clone LZ 2 hybridized with *M. tuberculosis* DNA. This was selected for further analysis. Recombinant phage clone LZ 2 was then *in vivo* excised to yield the phagemid pLZ 2. The recombinant phagemid contained a DNA insert of ~ 3.5 kb. Currently sequencing of the cloned insert is in progress for the construction of two oligonucleotide primers that could be used for the development of a PCR based assay for the detection of *M.tuberculosis*.