A-06: Preliminary studies towards the development of a PCR based assay for *Mycobacterium tuberculosis*

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Tuberculosis continues to be a major disease responsible for human morbidity and mortality worldwide. In the diagnosis of tuberculosis, procedures based on the polymerase chain reaction (PCR) for the detection of *M.tuberculosis* has many advantages over conventional methods. We have previously evaluated and established a PCR based method which amplifies a 245 bp fragment of the repetitive insertion element IS 986. Because of recent reports indicating the absence of the insertion element IS 986 in some isolates, studies were initiated towards the cloning and isolation of a DNA sequence for the development of an alternative PCR based assay for *Mycobacterium tuberculosis*.

A virulent strain of *M.tuberculosis* was isolated from a clinical sample using standard procedures. After isolation and further identification, large scale liquid cultures were grown & *Mycobacterium* DNA was isolated.

Microorganisms commonly found in the respiratory tract (*Staphylococcus epidermidis, Streptococcus viridans. Candida albicans, Klebsiella* sp and *Neisseria* sp.) were cultured and the DNA extracted.

M.tuberculosis DNA isolated above was partially cleaved with *Sau* 3A to yield maximum number of fragments in the size range 0-9Kb. The fragments were then cloned in the vector Zap and packaged *in vitro* according to manufacturers instructions (Stratagene Inc. USA). The above library was amplified, titred and stored over chloroform at 4^0C. An aliquot of the amplified library (approximately 20,000 recombinant) was plated out and the plaque lifts obtained were screened with ^{32}P labelled total *M.tuberculosis* DNA. Plaques giving strong signals on autoradiography were plaque purified and the DNA isolated. Nine recombinant phages designated LZM 1 to 9 were selected for further analysis. Nine dot blots containing 1.0μg of human. *M.tuberculosis* and a mixture of *Staphylococcus epidermidis, Streptococcus viridans, Candida albicans,Klebsiella* sp and *Neisseria* sp. DNA were screened separately with ^{32}P labelled LZM 1 to 9. A single recombinant phage LZM 2 which hybridized strongly to *M.tuberculosis* DNA only, was selected for further analysis. LZM 2 was then *in vivo* excised to yield the plasmid pLZM 2. The plasmid is currently being sequenced.

Screening of the library constructed in the vector λ ZAP with ^{32}P labelled *M.tuberculosis* DNA resulted in the isolation of among others, 9 strongly hybridizing recombinant phages. Of these the clone designated LZM 2 which hybridized to *M.tuberculosis* DNA only, was *in vivo* excised to yield pLZM 2. pLZM 2 is currently being sequenced.

A clone containing a repetitive DNA element from the genome of *M.tuberculosis* was isolated. It was found to be specific for *M.tuberculosis* as it did not cross hybridize with human, *Staphylococcus epidermidis, Streptococcus viridans, Candida albicans, Klebsiella* sp and *Neisseria* sp DNA.

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