A-29 Further studies towards the development of a PCR based assay for *Mycobacterium tuberculosis*

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Procedures based on the Polymerase chain reaction (PCR) for the detection of *Mycobacterium tuberculosis* have many advantages over conventional methods. The cloning of a recombinant clone (pLZM2) from a genomic library constructed in the vector λ Zap was previously reported. The subcloning and partial sequencing of the above clone for the development of a PCR based assay for *M. tuberculosis*, is reported here.

4 subclones designated pLZM 21, 22, 23 and 24 were derived from pLZM 2. pLZM2 then partially sequenced and sequences obtained were computer analyzed using either DNA Strider 1.2 Gene Jockey or Gene works 2.45

sequence analysis programmes. The sequence data bases (Gene bank 88, UEMBL 42 and Swiss-prot 31) were searched with the available sequences using Gene works 2.45.

DNA sequences obtained from 5' region of pLZM 2 were found to be homologous (94%) to the insertion element IS *1081* characterized previously from *Mycobacterium bovis.* DNA sequences obtained from the 3' region of pLZM 2 did not reveal any significant homologies to previously characterized sequences. Therefore sequences from the 3' region were used to design 2 pairs of amplification primers (5'GGCATACCAGGAACATCG3' and 5'GGACCTCCAAAGACTTCG3', 5'GGCATACCAGGAACATCG3' and 5'TGATTGTTGTCGCACAGTTCGC3') for a PCR based assay for *M.tuberculosis.*