

Spoligotyping for the Detection of Mycobacterium Complex Bacteria

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Tuberculosis (TB) is a life threatening contagious disease caused by the bacteria *M. tuberculosis* that primarily affects lungs. Other mycobacteria from the *M. tuberculosis* complex such as *M. bovis*, *M. africanum*, *M. canetti* and *M. microti*, can also cause tuberculosis, but these species do not usually infect healthy adults. Approximately, one third of the world's population is infected with *M. tuberculosis*. Every year, about 8 million new cases are reported and 3 million deaths occur due to this dreaded infection. If left untreated, this disease can be fatal. For this reason, accurate and early diagnosis of tuberculosis is important for effective management of the disease.

Several traditional methods for the diagnosis of tuberculosis are available which include tuberculin skin test, culture and sputum smear microscopy. However, there are several limitations of these traditional techniques used for diagnosis. One of the most important drawbacks of the tuberculin test is that prior vaccination with BCG may result in a false-positive result even many years afterwards. Culture method, which is more sensitive, is time consuming and takes 6-8 weeks for the results. Furthermore, the suspected cases might be missed due to this delay. Staining of infective organism by Zeihl-Neelsen technique in sputum has both the problems of sensitivity and specificity.

Advances in molecular medicine and understanding of the genetic structure of mycobacteria has provided us with many new techniques/tools that can be used for confirmation of identity of isolates from clinical samples and study the epidemiology of tuberculosis. PCR based methods are available as a diagnostic and confirmatory test for tuberculosis and are expected to detect as low as 1-10 organisms, but its use in surveillance and epidemiological studies might not be possible. On the other hand, molecular typing methods such as IS6110-RFLP and spoligotyping can be used for identification of outbreaks and can facilitate contact tracing of tuberculosis. IS6110-RFLP is considered a gold standard for the molecular typing of *M. tuberculosis* due to its high discriminative power and reproducibility. However, this technique requires large amount of DNA, and is therefore restricted to the mycobacterial cultures which take around 20-40 days to obtain sufficient DNA needed. Moreover, this technique is also technically demanding, expensive and requires sophisticated analysis software for result analysis.

Spoligotyping (spacer oligonucleotide typing) is a novel method that can be simultaneously used for detection as well as typing of the *M. tuberculosis* complex. This is a PCR-based method, which depends on the amplification of of a highly polymorphic Direct Repeat (DR) locus in *M. tuberculosis* genome. The DR region in *M. bovis* BCG contains direct repeat sequences of 36 bp, which is interspersed by the non-repetitive DNA spacers of 35-41 bp in length. In other *M. tuberculosis* complex strains, the number of DR elements vary significantly and majority of the *M. tuberculosis* strains contain one or more IS6110 elements in DR region (Figure 1).

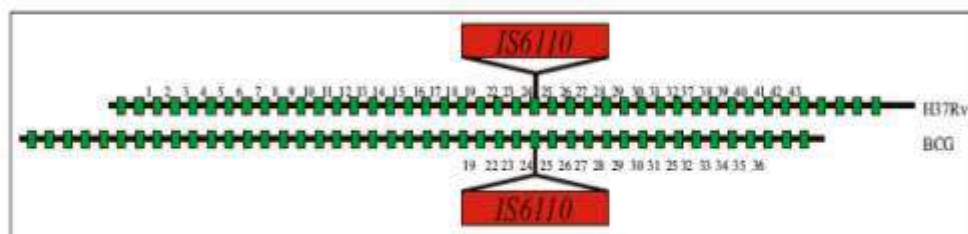


Figure 1: Structure of the DR locus in the genome of *M. tuberculosis* H37Rv and *M. bovis* BCG P3 strain. The green rectangle depicts 36 bp Direct Repeats(DR).

One DR and its neighboring non repetitive spacer is termed as 'Direct Variant Repeat' (DVR). Spoligotyping can be used for detecting the presence or absence 43 spacers of known sequences in DR region by hybridizing the PCR amplified spacer DNA (Figure 2) to the set of immobilized oligonucleotides, representing each of the unique spacer sequences (Figure 3).

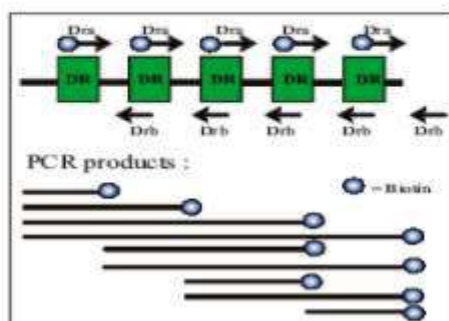


Figure 2: Principle of in vitro amplification of DNA within DR region of *M. tuberculosis* complex bacteria

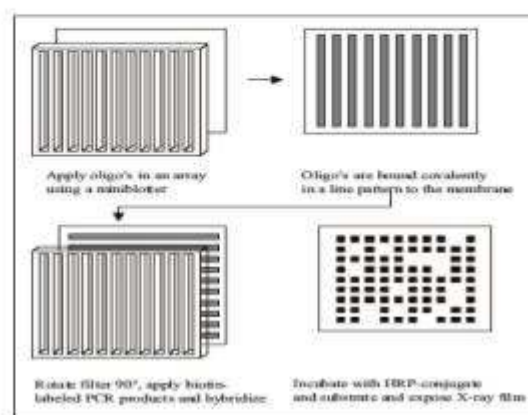


Figure 3: Overview of Spoligotyping method

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This technique is highly useful for clinical samples as it can detect the causative bacteria and is capable of providing epidemiological information on strain identities. The specificity and sensitivity of this technique has been found to be 98% and 96% respectively with the clinical samples.

Ocimum Biosolutions offers a spoligotyping kit for detection of the *M. tuberculosis* complex. This kit contains one membrane, positive control 1 (*M. tuberculosis* strain H37Rv), positive control 2 (*M. bovis* BCG P3), Primer DRa (biotinylated) and Primer DRb.

References:

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