Spoligotyping

Ocimum Biosolutions

High-Throughput – 43 samples/ run

No expensive equipment
Simple Protocol

Cost-Effective

Quick Results

Alternative for typing Southern blotting when rapid results required.

Level of differentiation higher for strains with less than 5 copies compared to IS6110 fingerprinting.

Preferred method to type M. bovis strains, which usually contain only one or two copies.

PCR-Based Method to Simultaneously Detect and Type Mycobacterium tuberculosis Complex

The Spoligotyping Principle is based on DNA polymorphism present at one particular chromosomal locus, the "Direct Repeat" (DR) region, which is uniquely present in Mycobacterium tuberculosis complex bacteria.

This locus was first described by Hermans et al. who sequenced this region in Mycobacterium bovis BCG, the strain used worldwide to vaccinate against tuberculosis.

The DR region in M. bovis BCG consists of directly repeated sequences of 36 base pairs, which are interspersed by non-repetitive DNA spacers, each 35 to 41 base pairs in length.

The number of copies of the DR sequence in M. bovis BCG was determined to be 49. In other M. tuberculosis complex strains the number of DR elements was found to vary significantly. The vast majority of the Mycobacterium tuberculosis strains contain one or more IS6110 elements in the DR region. (Fig 1)

In contrast to the DRs, the spacers are usually present only once in the DR region, but occasionally some are found twice, either separated by one or by several DR's and other spacers. One DR and its neighboring non-repetitive spacer is termed "Direct Variant Repeat" (DVR).

When the DR regions of several strains were compared, it was observed that the order of the spacers is about the same in all strains, but deletions and/or insertions of spacers and DR's occur. (Figure 2)

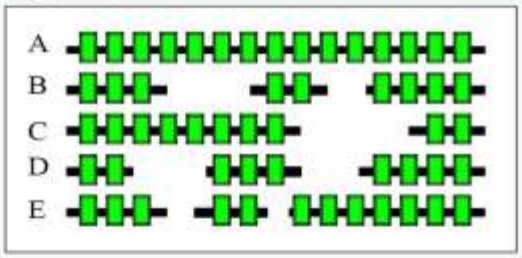

Fig 2: Schematic representation of the polymorphism in DR regions of different M. tuberculosis complex strains. Blocks of DVR are missing in one strain when compared to another. The spacer order remains about the same.

By spoligotyping one can detect the presence or absence of spacers of known sequence. The first step in the method is to amplify the DR region of a given strain by PCR. The primers used are based on the sequence of the DR, and allow the amplification of the spacer(s) between the DR targets (Fig. 3; Turn overleaf).

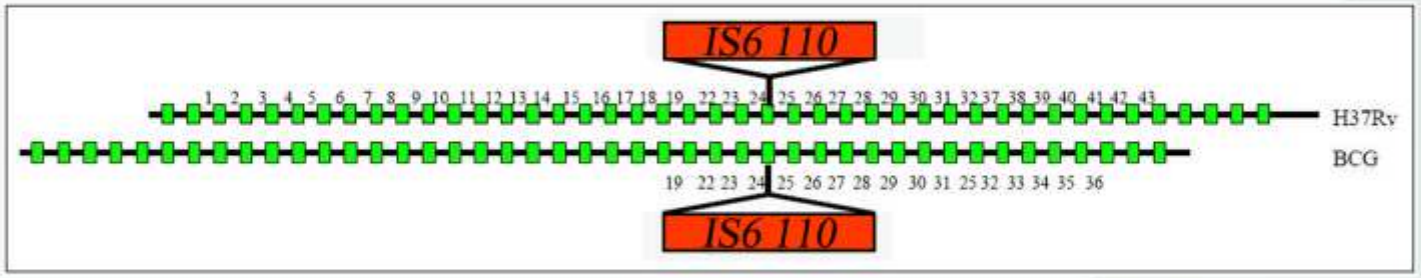

Figure 1: Structure of the DR locus in the genome of M. tuberculosis H37Rv and M. bovis BCG P3. The green rectangles depict the 36 bp Direct Repeat (DR).

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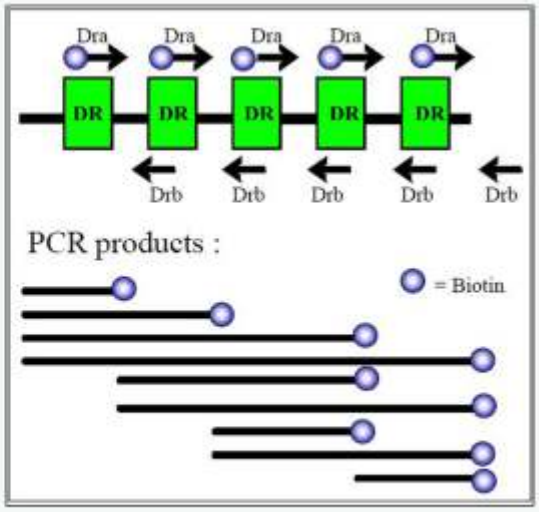
Figure 3: Principle of the in vitro amplification of DNA within the DR region of M. tuberculosis complex bacteria. The use of the 2 primers, a and b, for in vitro amplification, will lead to the amplification of any spacer or a stretch of neighbouring spacers and DRs.

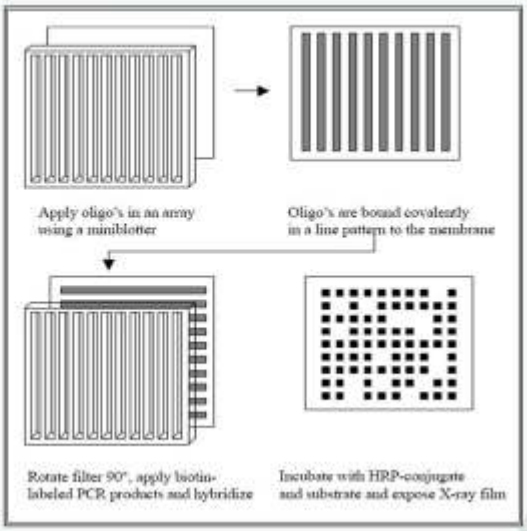


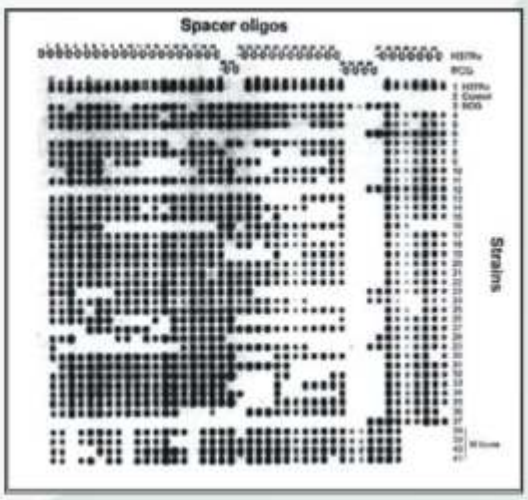
The obtained PCR products differ in length because of two reasons. First, the product contains several spacers and the DR's in between if the primers anneal to DR's not next to each other. Second, the product itself can act as a primer, and become elongated with one or more DVRs. Therefore, the PCR product provides no reliable information about spacer order or total length of the DR region. A biotin labeled reverse primer is used, so that all the reverse strands synthesized are biotin labeled.

An example of the result of the spoligotyping method used to analyze a variety of clinical isolates

Oligonucleotides derived from the known spacers in the DR cluster are covalently linked to an activated membrane in parallel lines. PCR products are hybridized perpendicular to the oligo lines. After hybridization the membrane is incubated in streptavidin peroxidase, which binds to the biotin label on the PCR products. Detection of hybridization signals is optimized by the enhanced chemiluminescence (ECL) detection system (of course any biotin-detection method can be used when optimized). The peroxidase present on the streptavidine catalyzes a reaction resulting in the emission of light which can be detected by autoradiography of the membrane. We refer to this type of blot as reversed line blot (Figure 4).

Figure 4 Overview of the spoligotyping method.





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