

Molecular cloning and sequencing of the attachment site and integrase gene of the temperate mycobacteriophage FRAT1

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We previously reported the isolation of a temperate mycobacteriophage (FRAT1) growing onto *M.smegmatis* and *M.bovis*-BCG (1).

Southern blotting of restricted phage DNA as compared to genomic DNA of lysogenic *M.smegmatis* bacteria showed that a *ClaI* 11 kbp DNA fragment was disrupted during the process of integration into the bacterial genome.

We constructed a set of pJRD184 (2) derived plasmids containing the kanamycin resistance gene from TN903 (3) and overlapping pieces of the 11 kbp *ClaI* DNA fragment.

These plasmids, unable to replicate into mycobacteria, were tested for their ability to generate kanamycin resistant colonies of *M.smegmatis* and *M.bovis*-BCG after electroporation. Transformants were obtained only when at least a *BamHI* 1400 bp fragment of the phage DNA was present on the transfer plasmid. Stable and site specific integration of one of them (pNIV2173) into the *M.smegmatis* (results not shown) and *M.bovis*-BCG (Figure 1) genome was demonstrated by Southern blotting. The sequence of the *BamHI* fragment and flanking regions showed that it contained the attachment site (attP) and the integrase gene of the phage flanked by potential transcription terminators.

The sequence reported here showed about 80% identity to the homologous region of mycobacteriophage L5 reported by Hong Lee *et al.* (4) and constitutes the basis for designing mycobacterial integration vectors.

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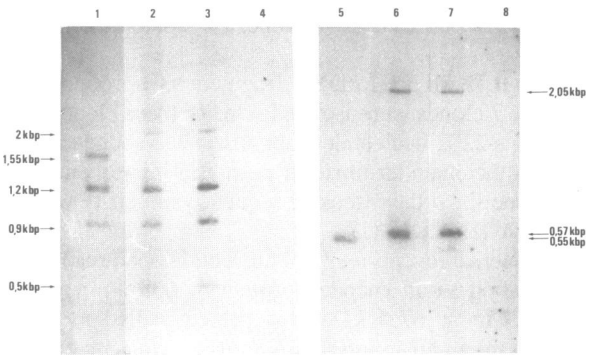

Figure 1. Southern blot analysis of genomic DNA from recombinant *M.bovis*-BCG. Lanes 1 to 4: DNA restricted by *SalI* probed by the *ScaI-EcoRI* 3000 bp fragment of phage DNA. Lane 1: Transfer plasmid DNA; lanes 2 and 3: genomic DNA from independant recombinants; Lane 4: genomic DNA from untransformed strain. Lanes 5 to 8: Same samples restricted by *NruI* probed by the *NruI* 550 bp fragment of phage DNA.