

An ORF coding for a putative 406aa Integrase was identified in the phage PIS136 which is related to the Tyrosine Recombinases or Phage Integrase family. The protein has the conserved tetrad of R-Y-R-Y in the C-terminus and all the conserved boxes and patches that are characteristic of all Integrases. Integrase of PIS136 (Int^{PIS136}) has a very high homology to Integrases of Conjugative Transposon Tn916, phages Mx8, mv4 and putative prophage integrase of *S. coelicolor*. The N-terminus has a Serine residue at the 9th position which is unusual for Integrases. Though potential junction fragments were identified, neither a putative attachment site nor a target for integration could be identified. Putative *E. coli* IHF binding sites were also identified around the *integrase* gene.

A strong secondary structure (cruciform structure) present immediately downstream to the dCTP deaminase gene may be a potential transcriptional terminator for late genes or a potential *attP* site and probably responsible for the instability of all potential *attP* related clones. In the present study, a putative dCTP deaminase gene and a gene for α -subunit of DNA Polymerase III were also identified.

Though Int^{PIS136} expression was toxic to *E. coli* cells, enough protein was expressed and formed inclusion bodies. Proper refolding of Int^{PIS136} could not be achieved by the methods used in the present study. The protein has a strong tendency to aggregate and was bound to *E. coli* DNA. This binding was so strong that 3M salt also did not lead to complete dissociation. Expression of the native protein was achieved by using *E. coli* chaperones GroEL-GroES but the concentration of the purified protein was very less. Int^{PIS136} was also expressed as a MBP-fusion protein which also was found to aggregate at lower salt concentrations.

Expression of Int^{PIS136} in presence of pKY206 and pLysS resulted in the loss of GroEL expression from pKY206. Attempts to understand the loss of GroEL expression resulted in the isolation of a recombinant plasmid pM15. Analysis of pM15 showed that instead of pKY206 and pLysS, it consists of two copies of pACYC184 and one copy of pINT22b. Both pKY206 and pLysS are pACYC184 based vectors, therefore, the loss of *groEL-groES* from pKY206 and T7 *lysozyme* from pLysS and then homologous recombination between the two backbones would lead to the original pACYC184.

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In a DNA resolving gel, pM15 showed three species named pR1, pR2 and pR3. Thorough analysis of pR1 and pR2 showed that pR2 was derived from pR1. The recombinant pR1 was of ~15.8Kb and had lost *groEL-groES* and *17 lysozyme* but had intact *integrase* gene and *lacI*. The recombinant pR2 (~11.5Kb) was identical to pR1 except that it had lost one copy of pACYC184. The Tyrosine351 mutant of Int^{PIS136} did not generate pM15 which clearly showed that the Tyrosine351 is a functional nucleophile of PIS136 Integrase.

The part played by RecA in the observed process of recombination could have been studied by using an expression host deleted for *recA*. Unfortunately, Int^{PIS136} did not express in JM109(DE3) and so the extent of the role of RecA in the formation of the plasmid pR1 could not be defined.

DNA-Protein interaction reaction with the PIS136 cruciform structure and the purified His-tagged protein resulted in the DNA getting trapped in the wells. Treatment with SDS removed the trapping of DNA in the wells and a probable DNA-Protein complex could be detected.

The C-terminal region of the *int* gene (*intSS* fragment) showed sufficiently strong interaction with the MBPInt^{PIS136} crude protein and was detected as a shifted band on a non-denaturing gel. The fragment has 180bp of the *int* gene with an extra 12bp from the *int22br-Hin* primer used for amplification of *int* gene and 4bp from the plasmid. Binding was lost when the 16bp extra sequence was removed by restriction digestion. Binding could not be restored even by providing a 72bp sequence flanking the *integrase* gene in PIS136 genome. The result suggested that the 16bp sequence was essential for binding. Interestingly, this 16bp sequence is neither a part of the *integrase* gene nor is present anywhere near the gene but was necessary to form a stable secondary structure with the C-terminus of *integrase* gene.

It is quite possible that Int^{PIS136} may require a secondary structure rather than large specific DNA sequence, which may help it to maintain the host range and mutational properties of phage PIS136. Finally, it may be said that Integrase of PIS136 is a strong DNA binding protein which forms co-integrates, resolves the co-integrate and probably recognizes a DNA structure rather than a specific sequence. Due to these considerations, the wild-type Int^{PIS136} is not suitable as a molecular tool.