p36C: an improved baculovirus expression vector for producing high levels of mature recombinant proteins

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The insect/baculovirus expression system has proved particularly useful in recent years for the production of recombinant proteins which are authentically processed, folded and post-translationally modified. In this system, the foreign gene is cloned under control of the strong polyhedrin promoter, contained within an expression vector, and is then re-introduced, via homologous recombination events, back into the genome of the insect virus Autographa californica nuclear polyhedrosis virus (AcNPV) (see ref 1 for procedures).Although there are currently a wide range of baculovirus expression vectors available, the vectors pAc373 and pAc360 have been used extensively for the production of either mature or fusion recombinant proteins respectively (2). In these vectors there is a Bam H1 cloning site at position -8 (pAc373) or +33 (pAc360) relative to the natural polyhedrin ATG translation initiation codon (see figure). The length of intact polyhedrin leader present appears to be very important for optimal expression from the polyhedrin promoter (3) and, in this respect, since pAc373 lacks 8 bases of the natural leader prior to the insertional cloning site, it is likely to be sub-optimal for expression.In order to derive an improved vector suitable for expression of mature proteins, an oligonucleotide (GAATAATCCGGGATATTTA) was used on an M13 single strand sense DNA template, derived from pAc360, to specifically mutate the polyhedrin ATG translation initiation codon to a nonfunctional ATC (the underlined G in the oligonucleotide is responsible for this event). This produced the baculovirus expression vector p36C which now has the entire polyhedrin leader intact, the first 11 amino acids of the polyhedrin N-terminus converted to an extended leader and a unique Bam H1 cloning site at +33 (see figure). Foreign genes cloned into this site will produce mature proteins, and in direct comparative examples between pAc373 and p36C for the expression of three different recombinant proteins (CMV immediate early protein, HIV reverse transcriptase and Ha-ras p21), in each case, p36C produced about five-fold higher levels of protein.

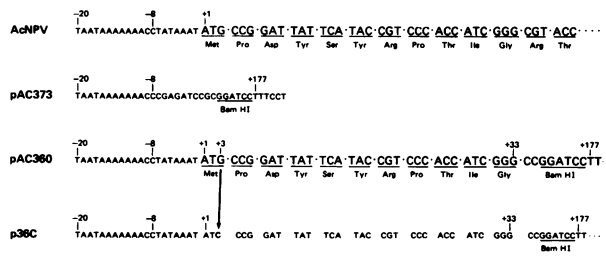

Fig: DNA sequence around the 5' end of the polyhedrin gene for the wild type virus (AcNPV) and the three baculovirus expression vectors. The Bam H1 insertional cloning site for foreign genes is underlined.

References: 1.Summers, M.D. and Smith, G.E. (1987) Texas Agricultural Experiment Station Bulletin No. 1555. 2. Luckow, V.A. and Summers, M.D. (1988) Biotechnology 6, 47-55. 3. Matsuura, Y. et al. (1987) J.Gen.Virol 68, 1233-1250.