Log-scale improvement in protein yield with vankyrin-enhanced transfer vector

Bruce Webb[1,2], Angela Martin[1], Ali Maldonado[2] and Angelika Fath-Goodin[1]

[1]ParaTechs Corporation, 1122 Oak Hill Drive, Lexington, KY 40505; Phone (859) 317-9213; Fax (859) 257-2489; www.paratechs.com
[2] Entomology Department, University of Kentucky, Lexington, KY 40546

Abstract

The viral ankyrins, or vankyrins, are genes derived from polydnaviruses that significantly delay death and lysis of baculovirus-infected cells while enhancing recombinant protein production. ParaTechs' vankyrin-enhanced, or VE-technology is an innovative approach to markedly enhance the BEVS by overcoming one of the main limitations of that expression system.

Vankyrin genes were initially used to develop transformed cell lines, the VE-CL cells (a.k.a. SuperSf9 cells), which are used with conventional, existing recombinant baculoviruses to enhance protein production for research and commercial purposes. Recently, we have constructed and evaluated a transfer vector (pAcVE1) that co-expresses the vankyrin gene with a gene of interest. We have found that co-expression of the vankyrin gene consistently and significantly enhanced protein expression relative to comparable virus. This enhancement is observed in all cell lines which have been tested. ParaTechs continues to increase the number of proteins expressed using this vankyrin-enhanced baculovirus, or VE-BEVS, with results supporting the general utility and compatibility of VE-BEVS with many of the current baculovirus technological advancements.

ParaTechs' vankyrin-enhanced transfer vector

- **Polyhedrin-locus based transfer vector**
- **Contains vankyrin gene under the control of the p10 promoter for enhanced protein production**
- **Gene of interest under the control of the polyhedrin promoter**
- **Compatible with BEVS that utilizes homologues recombination**

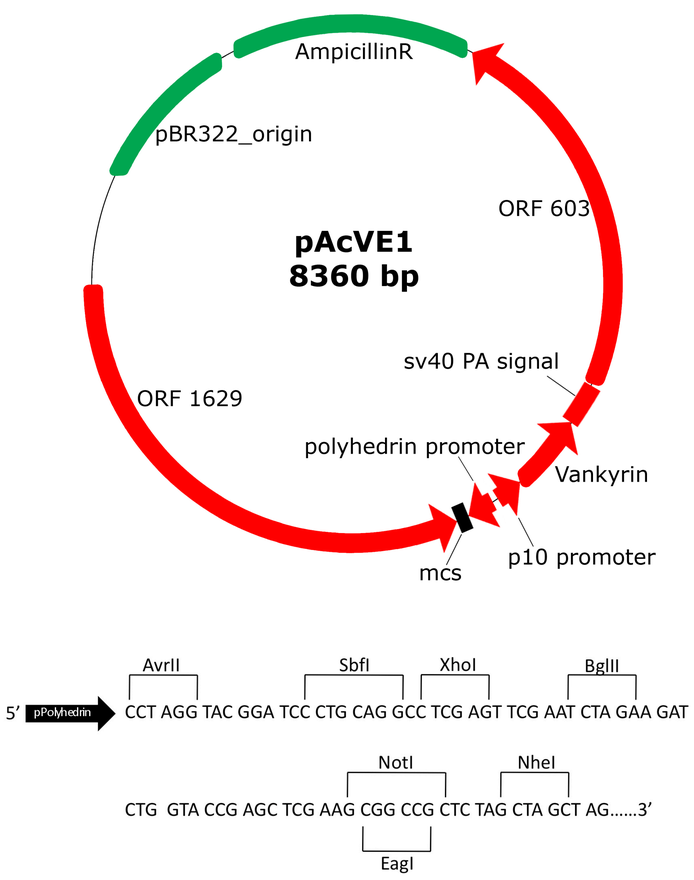

Figure 1. pAcVE1 vector map and multiple cloning site

Enhanced protein expression from pAcVE1

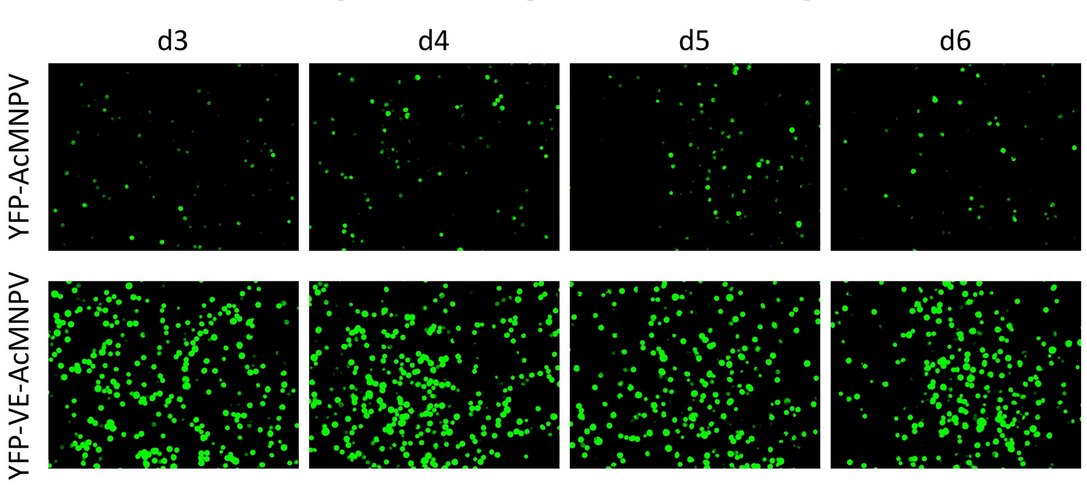

Figure 2. Yellow Fluorescent Protein (YFP) expression is enhanced when expressed from ParaTechs' pAcVE1. Top row: Fluorescent microscope images of insect Sf9 cells infected with conventional BEVS expressing YFP (YFP-AcMNPV) at an MOI of 5. **Bottom row:** Fluorescent microscope images of insect Sf9 cells infected with ParaTechs' VE-BEVS co-expressing the vankyrin protein and YFP (YFP-VE-AcMNPV) at an MOI of 5. Pictures were taken 3-5 days post infection (10x magnification). (Note: All pictures were taken at the same exposure time. All cells infected with the YFP control vector showed YFP expression as well but the exposure time was too low to pick it up with the camera.)

Synergistic effect on protein expression when ParaTechs' VE-CL03 cells are infected with pAcVE1

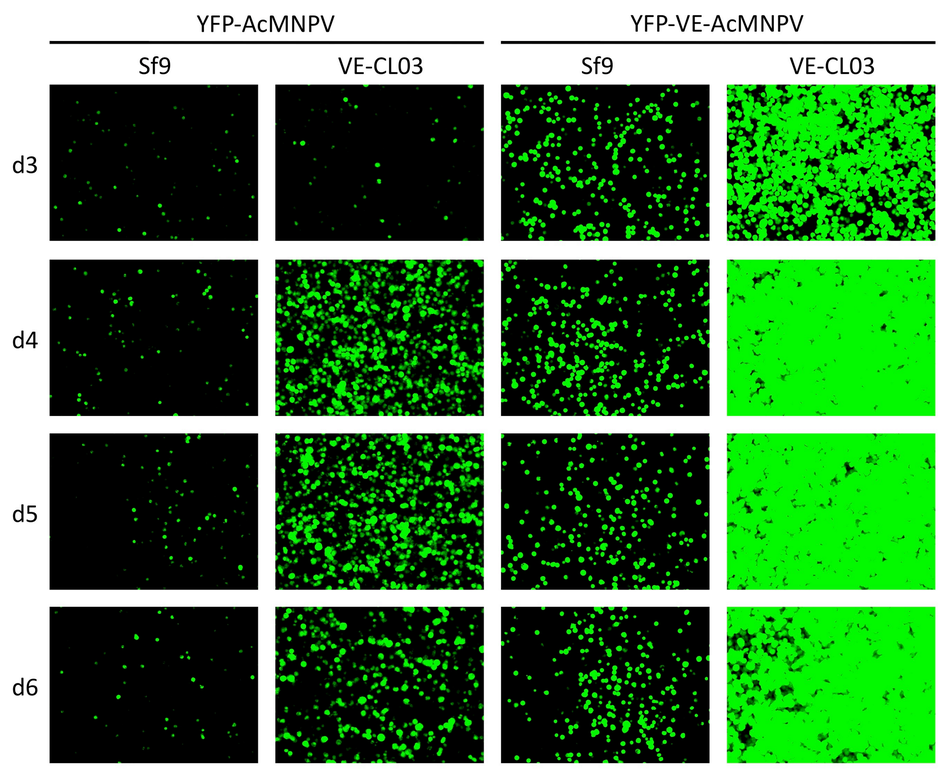

Figure 3. YFP expression from pAcVE1 is further increased in ParaTechs' VE-CL03 cells. Sf9 or VE-CL03 cells were either infected with a conventional BEVS expressing YFP (YFP-AcMNPV) or with ParaTechs' VE-BEVS co-expressing the vankyrin protein and YFP (YFP-VE-AcMNPV) at an MOI of 5. Pictures were taken 3-5 days post infection (10x magnification).