Enhanced Recombinant Protein Production by ParaTechs' Vankyrin-Enhanced Baculovirus Expression Technology

Angelika Fath-Goodin[1,2], Jeremy Kroemer[1], Roland Hilgarth[1], and Bruce Webb[1,2]

[1]ParaTechs Corporation, University of Kentucky, A-205 ASTeCC Building, Lexington, KY 40546; www.paratechs.com; Phone (859)-433-5293; Fax (859)-257-2489
[2]University of Kentucky, Department of Entomology, Lexington, KY 40546

Abstract

The baculovirus expression vector system (BEVS) is a powerful and versatile eukaryotic protein expression system. As a lytic viral expression system, the BEVS is limited by death and lysis of infected cells which precludes protein expression and requires repetitive infection cycles. This results in decreased productivity levels and higher production costs to generate recombinant proteins. ParaTechs Corp. has identified a gene family (*vankyrins*) from an insect virus that significantly delays death and lysis of baculovirus infected cells while enhancing recombinant protein production.

ParaTechs' *Vankyrin*-Enhanced BEVS (**VE-BEVS**) increased recombinant protein production up to **15-fold** when yellow fluorescent protein or VHv1.1, a secreted insect virus protein, were coexpressed with the vankyrin protein from a dual BEVS (VE-BEVS). When monoclonal Sf9 insect cell lines stably expressing vankyrin protein (**VE-CL-01, 02, 03**) were used to provide the protein activity in trans, a **5-fold** increase in intracellular protein production and up to **14-fold** increase in secreted protein production was obtained. As with VE-BEVS, an increase in cell viability and prolonged protein expression post-infection was also observed with VE cell lines. ParaTechs' enhanced VE-Sf9 cells are commercially available. ParaTechs' is also offering a **VE monoclonal cell transformation service** of customer provided cell lines. Furthermore, VE transfer vectors, VE baculovirus DNA and additional VE insect cell lines are in late stages of development with expected availability in spring 2008.

ParaTechs' VE-BEVS Technology

❶ Enhancement of the BEVS by Co-expressing ParaTechs' Vankyrin Protein from a Dual Expression Vector (VE-BEVS)

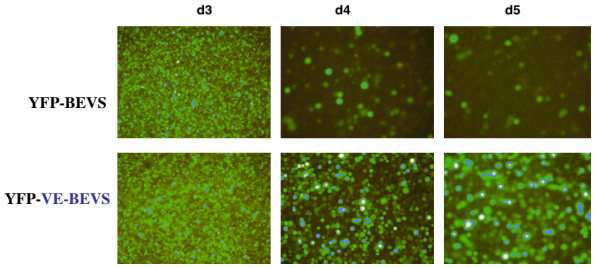

Figure 1. Yellow Fluorescent Protein (YFP) expression is enhanced when expressed from ParaTechs' VE-BEVS. Top row: Fluorescent microscopic images of insect Sf9 cells infected with conventional BEVS expressing YFP. Bottom row: Fluorescent microscopic images of Sf9 cells infected with ParaTechs' **VE-BEVS** co-expressing the vankyrin protein and YFP. Pictures were taken 3-5 days post infection (20x magnification).

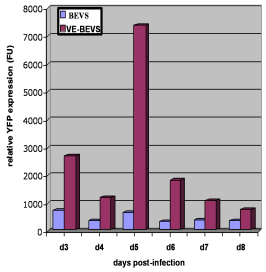

Figure 2. YFP expression is up to 15-fold increased when expressed in VE-BEVS. Quantification of YFP expression in Sf9 cells infected with recombinant YFP BEVS (blue bars) or recombinant YFP-**VE-BEVS** (red bars) was performed by fluorometry.

ParaTechs' VE-Sf9 Cell Lines

❷ Enhancement of the BEVS by Co-expressing ParaTechs' Vankyrin Protein from Stably Transformed Insect Sf9 Cells

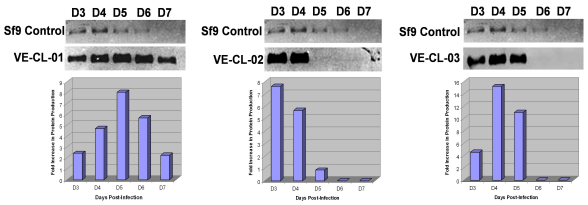

Figure 3. ParaTechs' VE cells enhance recombinant protein production up to 14-fold. Sf9 cells and ParaTechs' **VE-Sf9 cell lines (VE-CL 01, 02, and 03)** were infected with recombinant baculovirus expressing secreted VHv1.1. Samples were collected after 3-7 d and analyzed by fluorescent Western blotting (top). Protein production was quantified using a fluoroskan imaging system (bottom).

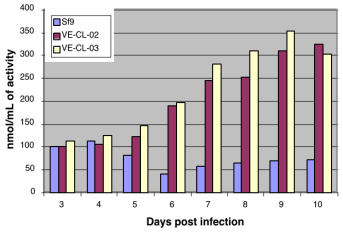

Figure 4. Phospolipase A (PLA) activity is enhanced and prolonged in ParaTechs' monoclonal VE-Sf9 cells. Blue bars show PLA activity of conventional Sf9 insect cells, magenta and yellow bars show PLA activity of the **VE-CL-02** and **VE-CL-03** cell lines infected with PLA-expressing baculovirus at 3-10d p.i..

❸ Protein Production Capacity is Enhanced Further when VE-BEVS is Combined with VE-Sf9 cells

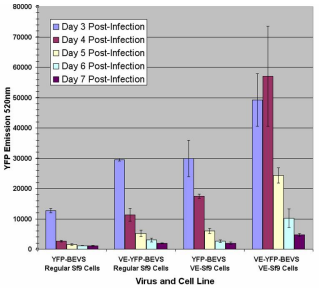

Figure 5. YFP Expression is enhanced the greatest by the presence of the vankyrin protein in both the recombinant BEVS and stably transformed Sf9 cells. An up to 21.5 fold (d4 p.i.) increase in YFP production is observed when monoclonal **VE-Sf9 cells** were infected with **VE-YFP-BEVS**. Error bars represents the mean ± SD of three independent experiments.

ParaTechs' Monoclonal Cell Line Service

❹ Monoclonal VE-Customer Insect Cells Exhibit Significant Enhancement of Recombinant Protein Production from BEVS

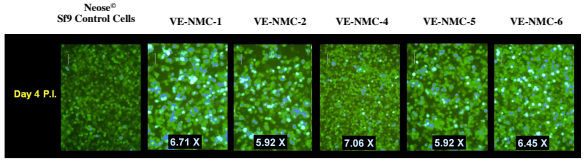

Figure 6. YFP expression is up to 7-fold enhanced at 4d p.i. in VE-Neose® Monoclonal Cells (VE-NMC) transformed with ParaTechs' VE Technology and selected for optimal protein production. Boxed numbers represent quantified fold increase versus untransformed custom Neose® cells.

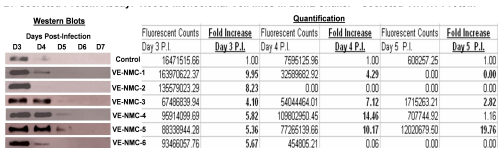

Figure 7. Secreted Protein Production is up to 15-fold enhanced at 3-7d p.i. in custom VE-NMC lines. Samples were collected after 3-7 d and analyzed by fluorescent Western blotting. Protein production was quantified using a fluoroskan imaging system.

Summary

- **ParaTechs' VE technology is an enhancement of existing BEVS technology that markedly improves protein expression levels while reducing the cost of labor and materials.**
- **Expression of ParaTechs' vankyrin proteins from VE-BEVS results in an up to 15-fold improvement of recombinant protein production.**
- **Expression of ParaTechs' vankyrin proteins from a stably transformed monoclonal VE-cell lines results in an up to 14-fold improvement of recombinant protein production.**
- **No redesign of the BEVS is necessary when ParaTechs' monoclonal VE cell lines are used for infection. The VE-technology was tested in Expression Systems' ESF 921 and Invitrogen's SF900 serum-free medium and similar results were obtained.**
- **Please visit www.paratechs.com for more details**

ParaTechs Products and Services

- **ParaTechs' VE-CL-01, 02, and 03 Cell Lines are Commercialy Available**
- **ParaTechs' VE-Monoclonal Cell Line Transformation Service is Available Upon Request**
- **ParaTechs' VE-Transfer Vectors and Linearized VE-*Ac*MNPV DNA are Under Development**

Acknowledgements

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