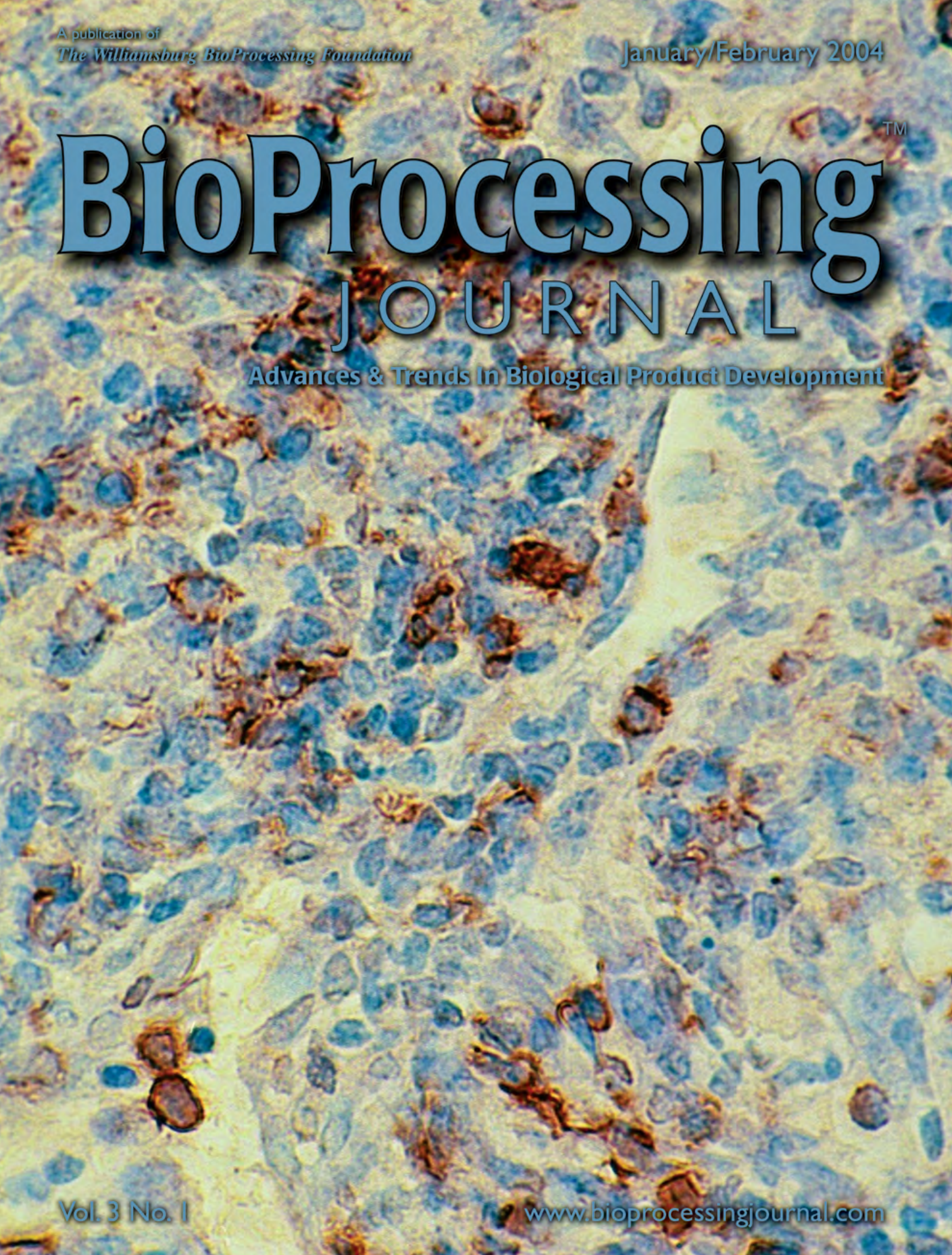
A publication of
The Williamsburg BioProcessing Foundation

January/February 2004

BioProcessing™ JOURNAL

Advances & Trends In Biological Product Development



Vol. 3 No. 1

www.bioprocessingjournal.com

Development of a High-Throughput Protein Expression Strategy

BY DOUGLAS A. AUSTEN, JOHN R. FULGHUM, FAN LU, RICHARD A. PETRILLO, and STEPHEN P. CHAMBERS

At the onset of modern-day biotechnology, products typically fell into two distinct categories, the traditional high volume, low value products (e.g. beer and industrial enzymes) that had come to characterize the biotechnology industry, and low volume, high cost products. Recombinant proteins, the result of technological advances in molecular biology, have come to typify these latter products.

Recombinant protein therapeutics have been hugely successful, potentially outstripping production capacity and continue to drive much of the biotechnology.[1,2] Meanwhile, many recombinant proteins, those characterized as research tools and reagents, are governed by a price-volume relationship typical of industrial enzymes.[3] In a competitive environment, they are fast becoming commodities — price sensitive, packaged as kits, coupled to instrumentation, and relying on heavy marketing and brand recognition. Ominously, the advantage protein therapeutics have enjoyed with patent protection and regulatory constraints on production is being threatened as patents expire and competition from generics increases.[4]

The publication of the human genome and the abundance of potential protein products have only added to the need for efficient protein production strategies. In addition, the expensive investment in the technology for high-throughput inhibitor screening and automation in protein crystallography requires a constant and predictable supply of protein to obtain a return on that investment. The widely held perception

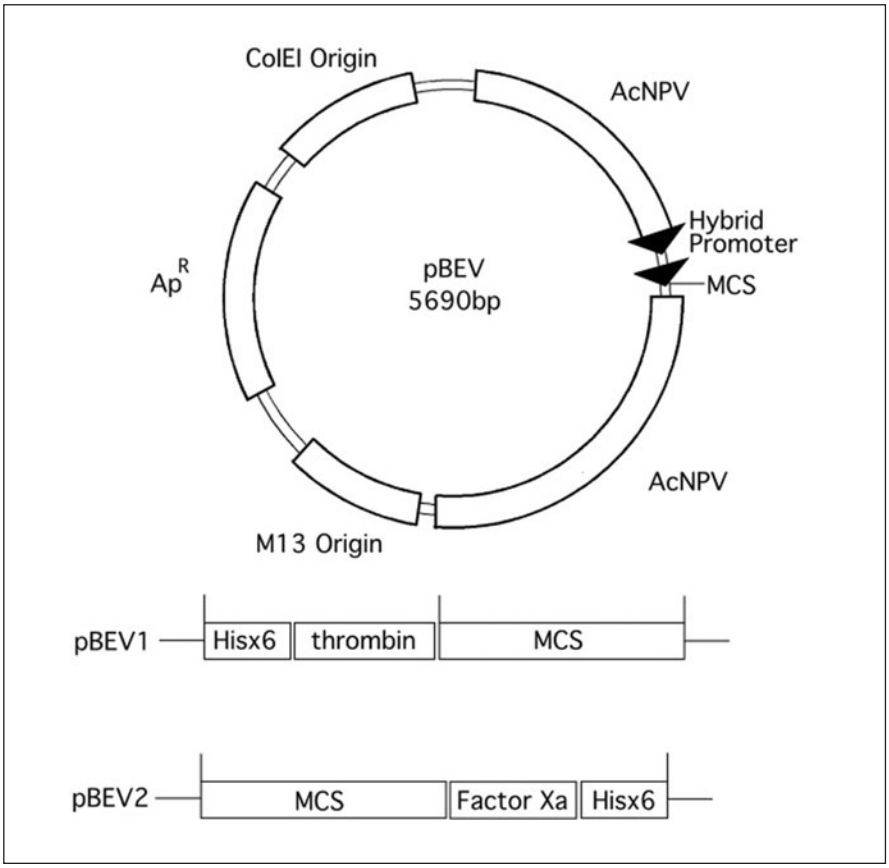


Fig 1. pBEV expression vector and cloning region of pBEV1 and pBEV2

The vector contains: *polh T7lac* promoter regions, multiple cloning sites (MCS), flanking *Autographa californica* nuclear polyhedrosis viral (AcNPV) region for recombination, the ColE1 origin of replication derived from the high copy number cloning vector pUC, the M13 origin for preparation of single strand DNA for mutagenesis, and the β-lactamase gene for selection. It also contains amino and carboxyl-terminal His-tag cloning regions and the accompanying proteolytic cleavage sites of pBEV1 and pBEV2, respectively.

Douglas A. Austen is a staff investigator; John Fulghum is a staff investigator, Fan Lu, M.S. is an investigator, Richard Petrillo is an investigator; and ***Stephen P. Chambers, Ph.D.*** *(stephen_chambers@vrtx.com) is principal investigator and head of gene expression, Gene Expression Group, Vertex Pharmaceuticals Inc., Cambridge, MA.*

of protein expression as the principal bottleneck in protein characterization and structure determination has only been aggravated by the increases in emerging targets, potential protein therapeutics, technological advances, and changing economics.[5]

These conditions have provided the impetus for changes in the way protein expression and production is performed. Aided by advances in cloning, expression, and purification a number of high-throughput strategies have been developed, changing the way proteins are produced.

We describe our strategy to provide a diverse set of recombinant proteins for both enzymatic and structural characterization as part of an overall chemogenomic program of drug discovery.[6] To meet the demands of high-throughput protein expression, with a premium on reliability and quick delivery, many conventions and widely held tenets have been discarded to produce a simple and efficient process.

Materials and Methods

Cells, Viruses, and Vectors

BL21 [F^-, *omp*T, *hsd*S_B (r_B^-, m_B^-) *gal*, *dcm*] (DE3) pLysS, (Stratagene, La Jolla, CA) was used for all *Escherichia coli* recombinant expression. The insect cell line *Spodoptera frugiperda* (Sf9) was used for the generation of recombinant viral stocks while *Trichoplusia ni* (High-5) was used exclusively for the expression of recombinant proteins. The insect cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and Invitrogen (Carlsbad, CA), respectively. Monolayer culture of Sf9 cells was maintained in TNM-FH medium supplemented with 10% fetal bovine serum in a T-flask at 27° C. The suspension culture of Sf9 cells was grown in SF900-II protein-free, serum-free medium in a shaker flask at 27° C. High-5 cell culture in suspension and monolayer were maintained in Excel-405 (JRH Biosciences, Lenexa, KS) at 27° C. Also, we believe importantly, each cell line was routinely started from an early-passage frozen stock every six months to ensure cell viability and maintain productivity. Cells are initiated as monolayers then split to suspension once they have obtained normal doubling times and growth conditions. *Autographa californica* nuclear polyhedrosis virus (AcNPV) and linear viral DNA were obtained from Pharmingen (San Diego, CA). All the proteins described in this work were produced using pBEV (Fig. 1), and expressed as His-tagged proteins in either *E. coli* or insect cells.[7]

Expression in Deep-Well Blocks

Expression was initiated with freshly transformed *E. coli* BL21(DE3) pLysS, in a 24-well block, aseptically sealed with AirPore™ tape sheets (Qiagen, Valencia, CA) and grown overnight in a HiGro™ incubator-shaker (Genomic Solutions, Ann Arbor, MI) at 37° C in 5 ml Brain Heart Infusion (BHI) media (Becton Dickinson & Company, Sparks, MD), supplemented with 100 µg/ml carbenicillin and 35 µg/ml chloramphenicol. Overnight cultures were subsequently pelleted at 2,000*g* for 5 mins using a micro-titer plate centrifuge and re-suspended in 1 ml BHI media. 5 ml of fresh BHI media was inoculated with 20 µl of re-suspended overnight culture and grown at 37° C for 3–4 hrs in the 24-well block. Expression was induced at mid-log phase ($A_{600nm} \approx 1$) with the addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Cells were harvested 6–8 hrs after induction by centrifugation at 2,000*g* for 5 mins. High-5 insect cells used in expression were grown to a density of 2.0 x 10^6 cells/ml in a Fernbach flask. Expression in a 24-well block was then initiated by infecting 2.5 ml of the aforementioned cells with a high-titer baculovirus with a multiplicity of infection (MOI) of 2.5 pfu/cell. Cells were then grown in serum-free EX-CELL™ 405 with L-glutamine at 27° C for 48–60 hrs following infection and harvested by centrifugation at 2,000*g* for 5 mins. Cells were harvested at 70–80% viability, measured using a Cedex analysis system (Innovatis GmbH, Bielefeld, Germany).

Automated Purification

Purification was carried out with nickel-nitrilotriacetic acid (Ni-NTA) magnetic agarose beads (5% suspension) using the BioRobot 3000 automated liquid handling system (Qiagen, Valencia, CA). A protocol adapted from the manufacturer's manual was used for the purification of cultures grown in the 24-well blocks. Following expression, the cell pellets were re-suspended in 400 µl lysis buffer; 10 mM Tris-HCL (pH 8.0), 50 mM NaH_2PO_4, 100 mM NaCl, 20% glycerol, 0.25% Tween-20, and 10 mM imidazole. Lysis in the presence of 0.1% benzonase solution (Novagen,

Table 1. Comparison of post-translational processing and yields of proteins between *E. coli* and Insect cell expression systems.[20]

	E. coli	Insect Cells
Proteolytic Cleavage	+/-	+
Glycosylation	-	+
Secretion	+/-	+
Folding	+/-	+
Phosphorylation	-	+
Acylation	-	+
Amidation	-	+
Yield (%) Based on dry weight	1–5	30

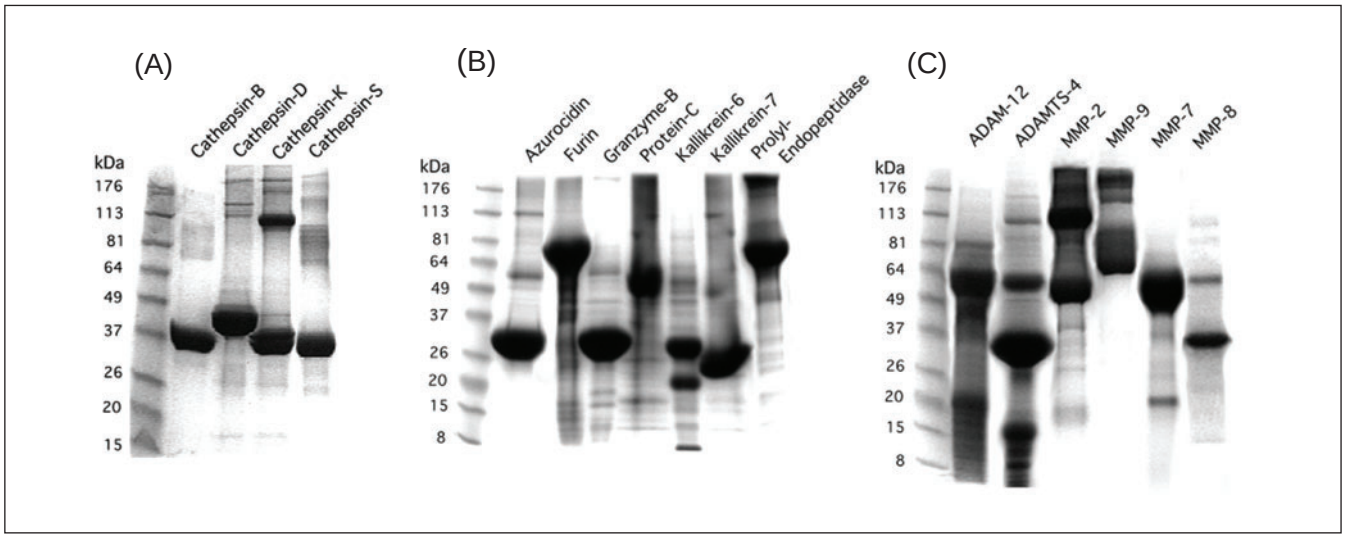


Fig 2. Proteases expressed and secreted into the media from insect cells

(A) Cysteine proteases including full-length cathepsin-B, -D, -K, -S; (B) serine proteases including azurocidin, furin, granzyme-B, human protein-C, kallikrein-6, -7 and proyl endopeptidase: (C) metalloproteases including matrix metalloprotease-7, -8, -9 and truncated ADAM-12 (1-705) and ADAMTS-4 (1-510) minus their trans-membrane domains, expressed and secreted from insect cells and purified from media with Ni-NTA affinity resin.

Madison, WI) was completed by disruption using a deep-well cup horn sonicator (4 x 1 min bursts) (Misonix Inc., Farmingdale, NY). Cells were divided into soluble and insoluble fractions by centrifugation at 6,000*g* for 5 mins; the latter insoluble (pellet) fraction was then solubilized in 400 µl lysis buffer containing 8 M urea. The 400-µl fractions to be purified were transferred 200 µl at a time to a 96-well micro-titer plate containing 20 µl Ni-NTA magnetic-agarose beads, mixed for 1 min, placed on a 96-well magnet for 1 min, and the supernatant discarded before the remaining 200 µl was added. The beads were washed with 200 µl lysis buffer and the His-tagged proteins eluted with 35 µl of lysis buffer containing 1 M imidazole after placing the micro-titer plate on the magnet for 1 min. Purified fractions were analyzed and quantified by SDS-PAGE or Western blot.

Large-Scale Production

E. coli BL21(DE3) pLysS freshly transformed with recombinant pBEV was grown at 37° C in a 10-L fermentor (B.Braun Biotech Inc., Allentown, PA), in complex media, supplemented with antibiotics for maintenance. When the cell density reached an A_{600nm} = 3–4, the temperature of the culture was rapidly reduced to 30° C and expression induced with 1 mM IPTG. Cells were maintained at 30° C for a further 4 hrs, harvested by centrifugation, flash frozen, and stored at –70° C prior to purification and analysis.

The majority of insect cell production was performed in a Cellbag®-20 (10-L working volume) using a Wave Bioreactor® (Wave Biotech, Bridgewater, NJ).[8] Adapting the protocol from the manufacturer, High-5 cells were grown at 27° C with a rocking speed of 15 rocks/minute (rpm) at an angle of 8.5°, and with an air flow rate of 0.1 L/min to a target density of 2 x 10^6 cell/ml. At this point, cells were infected with virus at an MOI of 2.5. During infection, rocking speed was increased to 20 rpm with the air flow rate raised to 2 L/min. Infection lasted approximately 72 hrs, during which time the cell viability was monitored, using the Cedex analysis system. The culture was then harvested when cell viability was within the 70–80% range. Cell pellets or media were flash frozen at –70° C until ready for use.

Protein analysis

The analysis of protein expression from large-scale protein production was performed on 500 mg of the cell pellet resuspended in 10 ml of lysis buffer, chilled on ice, and sonicated using a micro-tip probe (Misonix Inc., Farmingdale, NY) with a single 0.5 min pulse. Following centrifugation at 30,000*g* for 30 min, the supernatant was added directly to 350 µl of pre-equilibrated Ni-NTA resin, batch incubated for 2 hrs at 4° C, and washed with 100-fold column volume with lysis buffer. His-tagged protein was then eluted from the column using 5 x 150 µl of lysis buffer with 200 mM imidazole. The proteins were analyzed following isolation by SDS-PAGE and stained with Coomassie blue.

Proteins secreted from insect cells were analyzed from 10 ml of media concentrated using ammonium sulfate precipitation and or centrifugal filter device with a 10-kDa molecular weight cut-off (Millipore, Bedford, MA). Concentrated proteins were separated by SDS-PAGE and analyzed by staining with Coomassie blue or Western blot.

Discussion

Expression Hosts

The choice of host for expression is a critical factor in determining the quality and quantity of protein produced. We chose to build our expression platform around *E. coli* and insect cells, two of the most commonly used hosts for the production of recombinant proteins. Our decision to pursue two expression sys-

tems was a compromise, based principally on the impracticality of pursuing every possible expression host available.

The bacterial host, *E. coli*, was an early vehicle for much of the development of recombinant DNA technology and has a long history of successful production of heterologous proteins.[9] This prokaryotic expression system is simple to manipulate and cultivate, providing a rapid method for producing recombinant proteins. Although successful *E. coli* expression results in over-expression, the bacterium lacks the post-translational processing machinery of eukaryotic cells, so the proteins produced are often insoluble and inactive.[10] A eukaryotic host would complement any deficiencies produced with *E. coli* expression, providing a favorable host environment for the expression of complex heterologous proteins.

Baculovirus-mediated insect cells, more complicated manipulatively in comparison to *E. coli*, was chosen as the host of our secondary expression system. This decision was based on our own previous successful experience with this expression system, following difficulties producing proteins in *E. coli*.[11,12] Insect cell expression has a formidable track record of producing authentic protein in biologically active form and was an ideal host for the expression of diverse proteins ranging from simple enzymes to complex membrane proteins. The major advantage provided by insect cell expression is its ability to express cytoplasmic and secreted proteins with post-translational modifications to produce active protein (Table 1).

One of the more significant observations we made during the development of this process is that the signal peptide of most human genes is recognized and cleaved during the secretion from insect cells (Fig. 2). This greatly simplified our cloning strategy, relying on the proteins' resident pre- and pro-regions for secretion and activation.

Expression Vectors

The proliferation of expression vector options has naturally kept pace with the expansion of heterologous expression hosts available for production.[13] In the past, exploiting numerous expression vectors has been limited by the logistics of cloning into numerous expression vectors. The traditional approaches to cloning are very labor intensive and not easily amenable to high-throughput.

Many of the disadvantages encountered in adapting conventional cloning to high-throughput are avoided using Topo® cloning (Invitrogen Corp, Carlsbad, CA).[14] *Vaccinia* DNA topoisomerase-1 covalently joins polymerase chain reaction (PCR)-generated DNA fragments to a suitable vector, providing a ligase-free method of cloning. This is a highly efficient alternative method of cloning, requiring a five-minute room temperature incubation, with no special reaction conditions or additions, and is very amenable to automation.

Topo cloning and development of commercially available vectors designed to exploit recombination cloning have revolutionized the process of moving genes from vector to vector, making it possible to access numerous expression vectors and their accompanying expression systems relatively quickly and easily.[15,16] The development of recombination cloning has effectively eliminated traditional sub-cloning by replacing PCR and restriction enzyme/DNA ligase reactions with site-specific transfer of genes from one vector to another via recombination.

To meet the specific needs of both enzymatic and structural characterization we constructed our own vector, designated pBEV, to avoid some of the drawbacks associated with other vectors. Like other recombination vectors, pBEV relies on recombination events to access alternative expression systems, but unlike other vectors its recombination sites are not part of the coding region of the gene and are therefore not part of the protein expressed. Multisystem vectors like pBEV and pTriEx-1, which include mammalian cells as well as *E. coli* and insect cells as hosts, avoid the generation of fusions and their associated problems in subsequent structural or functional characterization.[17]

pBEV uses a tandem promoter system with the bacteriophage *T7lac* and baculoviral *polh* promoters positioned in series to direct *E. coli* and insect cell expression. Cloned inserts are expressed containing either N- or C-terminal His-tags, resulting in mature protein once cleaved. pBEV has the advantage of one-step cloning, allowing immediate expression in *E. coli*. The disadvantage

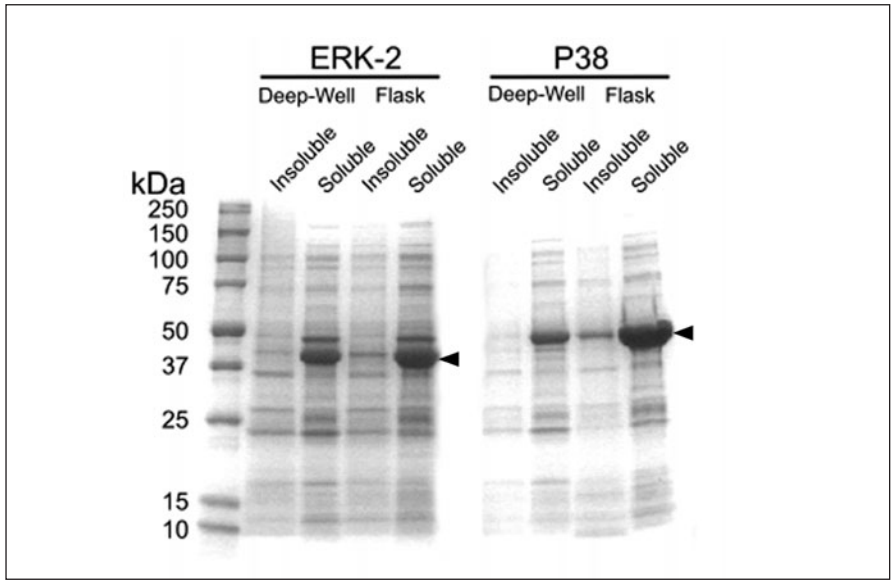


Fig 3. Expression using pBEV expression vector in *E.coli*

Full-length Extracellular signal-regulated kinase 2 (ERK-2) and Mitogen-activated protein kinase p38α (P38) were expressed and purified from 5 ml *E. coli* cultures grown in 24-well blocks and 2l Fernbach flasks (5 ml extracted from a 1-L culture). Automated purification of proteins from insoluble and insoluble fractions was performed using Ni-NTA magnetic agarose beads.

of pBEV is the limited number of expression systems and fusion permutations offered, when compared to other commercially available multi-system vectors. This was viewed as an acceptable compromise in keeping with our desire to pursue just the minimum of expression hosts and a strategic decision to avoid fusion proteins and the additional complexity they add to the process.

Expression Screening

Conventional expression screening is usually initiated with an analysis of protein production in multiple expression vectors and hosts. Further optimization of expression performance would be achieved through exploration and refinement of a number of variables including temperature, induction time, and time-course. The conditions for successful expression would then be scaled accordingly, often to liter volumes, to provide enough biomass to establish expression and purification yields following purification and analysis. Even with our strategy where we have sought simplification using one vector and two hosts, the process as previously described would present logistical problems. Choosing a suitable host for successful expression, which is often protein specific, can be an empirical process requiring time-consuming experimentation to optimize and analyze production. Therefore, the development of a method for screening a large number of proteins expressed in either *E. coli* or insect cells, and screening conditions for maximum expression would be essential to the development of a high-throughput process. Miniaturized and automated protein expression and purification would be required to allow the largest number of clones and conditions to be explored, thereby increasing the probability of a successful outcome. Conventional cell cultivation using shake flasks would be impractical because of the numbers of clones being screened. Alternatively, we were able to establish that deep-well blocks, originally designed for growing bacterial cultures for DNA purification, could act as a surrogate for shake flasks when growing *E. coli* and insect cells for expression.

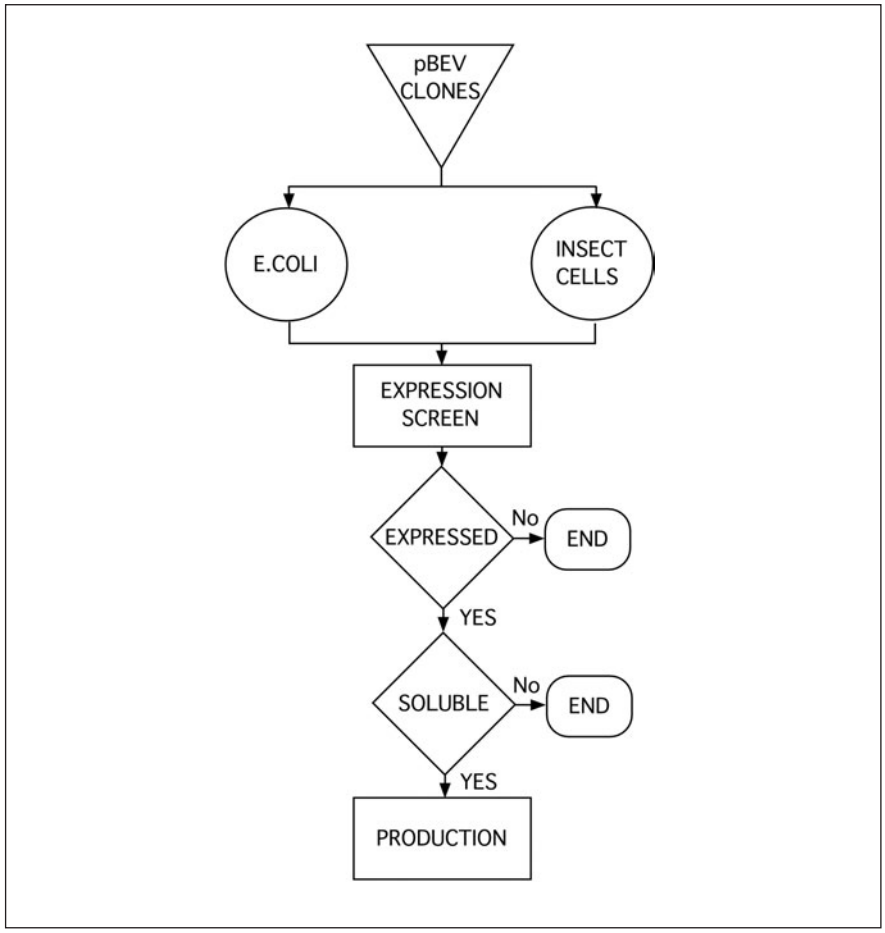


Fig 4. Schematic for high-throughput protein expression strategy

The workflow enabling high-throughput triage of proteins expressed in both *E. coli* and insect cells for the production of proteins for structural and enzymatic analysis.

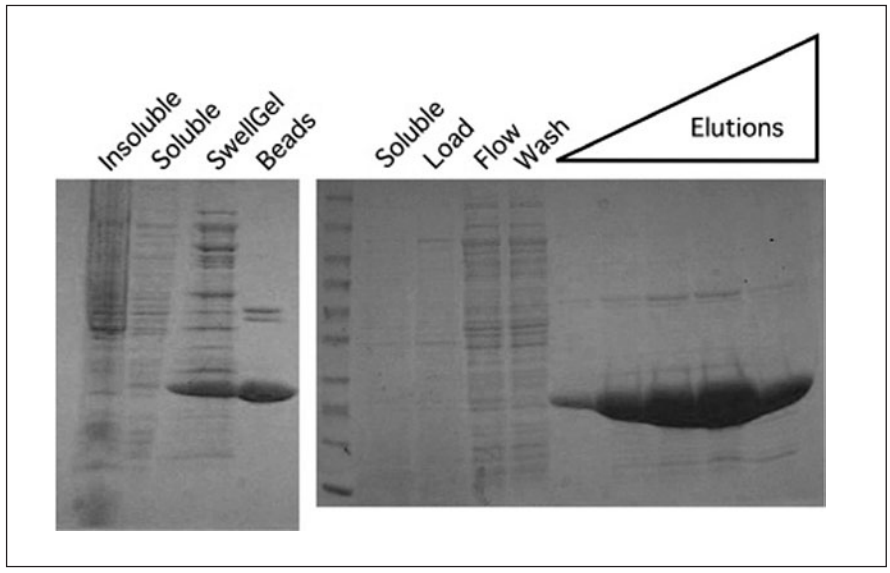


Fig 5. Scale-up from deep-well blocks into 10-L bioreactor

Dual specificity protein phosphatase (DSP) purified from *E. coli* cells grown in deep well blocks, using SwellGel™ and Ni-NTA magnetic agarose beads, and DSP purified using Ni-NTA resin from *E. coli* grown in a 10-L bioreactor.[19]

To screen the products of expression we used the affinity between the His-tag epitope, common to all recombinant protein produced by pBEV, and immobilized divalent metal ions. The high affinity and binding capacity of the Ni-NTA resin allows soluble and insoluble cellular fractions to be screened for recombinant protein. Using Ni-NTA magnetic agarose beads, purification was reduced to a micro-titer plate format and automated using a liquid handling robot. Following fractionation and purification it was possible to analyze soluble and insoluble proteins by SDS-PAGE (Fig. 3) or, depending on the number screened, dot-blot analysis.

In this manner we have used high-throughput expression to screen thousands of proteins identifying those that are soluble and/or over-expressed (Fig. 4). The ability to rapidly identify soluble and insoluble expressed proteins, the latter probably being the greatest impediment in protein purification and characterization, has considerable value when expressing recombinant proteins for production. Proteins that are insoluble or expressed at low levels usually present significant challenges in purification and are problematic in crystallography. Also, protein solubility in aqueous buffer can often be taken as evidence of native like folding and potentially active protein.

Scale-up and Protein Production

The screening process we have described offers a rapid method of identifying the most tractable protein within a population utilizing our optimal conditions. Once identified, the protein and the expression system can then be used for large-scale protein production. We found the expression results achieved in the multi-well format are directly transferable to large-scale (10–100 L) for both *E. coli* and insect cell production (Fig. 5). To achieve high-throughput at micro- and large-scale levels, we purposely pursued a limited set of conditions for expression designed to produce an optimum level of expression for the majority of the proteins expressed. These conditions were defined and derived through literature precedence and in-house experimentation. While this rigid strategy may discriminate against some proteins, it does allow the maximum number of proteins to be expressed in the shortest possible time. Those difficult proteins will then be re-examined and optimized utilizing the full array of conditions and time-consuming techniques available to the protein expressionist.

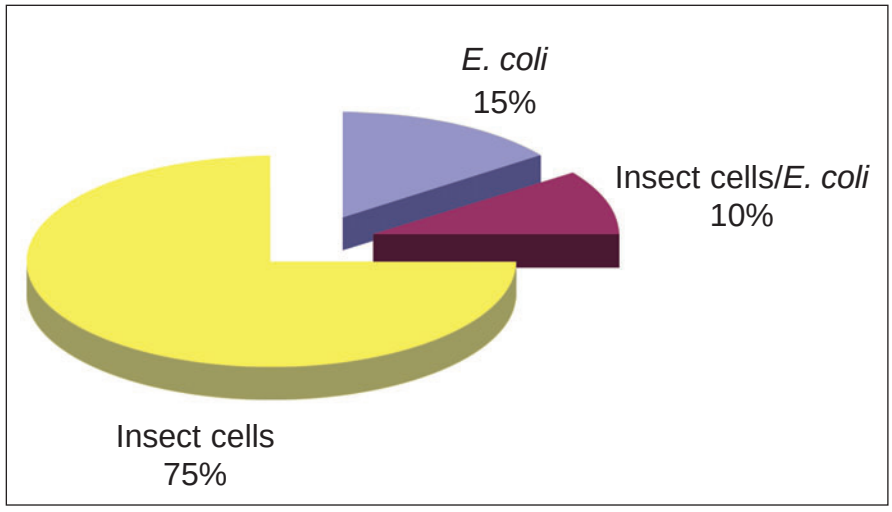


Fig 6. Distribution of recombinant source of kinases using high-throughput expression strategy.

The efficiency of large-scale production was also greatly increased using a Wave Bioreactor for insect cell production.[8] The bioreactor was used with a Cellbag, a single-use pre-sterilized plastic bag, which avoids the time-consuming sterilization and cleaning associated with traditional bioreactors. The Wave Bioreactor only requires temperature and air control, conveniently allowing optimization of expression for production. Using these optimized conditions with the Wave Bioreactor, we have obtained a high degree of consistency in production: greater than 95% of our runs produced protein within a 2–4 hr window around 72 hr and had cell viabilities between 65–75%. Reproducibility of expression, a critical element in maintaining the protein production pipeline, was enhanced with real-time monitoring of cell viability using the Cedex analysis system.

Employing a strategy of parallel expression in *E. coli* and insect cells we have been able to successfully screen and express thousands of proteins from a diverse set of protein families. The vast majority of proteins we have in production are currently being expressed in insect cells. For example, 75% of the human kinases we have produced have been expressed using insect cells (Fig. 6). Also, the fraction of proteins successfully expressed in insect cells increased when producing more biologically and structurally complicated proteins such as proteases and membrane proteins.

Final Comments

At the outset of this work, our goal was to increase throughput and maintain the predictable delivery of recombinant protein for enzymatic and structural analysis. To obtain increased operational efficiency required substantial re-engineering of what has been traditionally been a manual experiment. By standardizing methodology, introducing automation, focusing on a set of highly defined deliverables, and replacing all too often open-ended experiments with the linear time-lines of a process, we were able to achieve significant increases in productivity.

In common with others who have followed this path, data analysis and management was required to facilitate this process.[18] A key component of our operation was "Clone Tracking," Vertex Pharmaceuticals' proprietary laboratory database information management system that allows monitoring of the workflow from clone to protein to pure enzyme and protein crystal. For this

process to work efficiently, a highly integrated network of disciplines has been developed and co-opted into supporting the protein expression platform we have described. We believe this approach has resulted in greater efficiencies and a substantial numerical increase in the supply of high quality proteins for drug design.

ACKNOWLEDGEMENTS

We wish to acknowledge members of the Molecular Cloning group at Vertex Pharmaceuticals for cloning the cDNA used in this study and to thank Sridevi Singamsetti for production assistance. We also wish to thank Ted Fox and Scott Raybuck for valuable discussions and critical reading of the manuscript.

REFERENCES

1. Walsh G. Biopharmaceutical benchmarks. *Nat Biotechnol* 2000;18(8):831–3.
2. Mallik A, Pinkus GS, Sheffer S. Biopharma's capacity crunch; 2002.
3. Arbige AV, Pitcher WH. Industrial enzymology: a look towards the future. *Trends Biotechnol* 1989;7:330–335.
4. Karia R, Lis Y, Walker SR. The Erosion of effective patent life: An international comparison. In: Griffin JP, editor. Medicines: regulation and risk. Belfast: Queens University Press; 1992. p 287–302.
5. Service RF. Structural genomics. Tapping DNA for structures produces a trickle. *Science* 2002;298(5595): 948–50.
6. Caron PR, Mullican MD, Mashal RD et al. Chemogenomic approaches to drug discovery. *Curr Opin Chem Biol* 2001;5(4):464–70.
7. Chambers SP. High-throughput protein expression for the post-genomic era. *Drug Discov Today* 2002; 7(14):759–65.
8. Singh V. Disposable bioreactor for cell culture using wave-induced agitation. *Cytotechnology* 1999;30(1): 149–158.
9. Keefer LM, Piron MA, De Meyts P. Human insulin prepared by recombinant DNA techniques and native human insulin interact identically with insulin receptors. *Proc Natl Acad Sci USA* 1981;78(3):1391–5.
10. Marston FA. The purification of eukaryotic polypeptides synthesized in *Escherichia coli*. *Biochem J* 1986; 240(1):1–12.
11. Chen W, Raybuck SA, Fulghum JR et al. Expression and purification of human interleukin-1 beta converting enzyme from *Trichoplusia ni* insect cells using a baculovirus expression system. *Prot Exp Pur* 1997;9(1):69–75.
12. Wilson KP, Black JA, Thomson JA et al. Structure and mechanism of interleukin-1 beta converting enzyme. *Nature* 1994;370(6487):270–5.
13. Pouwels PH, Enger-Valk BE, Brammar WJ. Cloning vectors: a laboratory manual. New York: Elsevier; 1985.
14. Heyman JA, Cornthwaite J, Foncerrada L et al. Genome-scale cloning and expression of individual open reading frames using topoisomerase I-mediated ligation. *Genome Res* 1999;9(4):383–92.
15. Hartley JL, Temple GF, Brasch MA. DNA cloning using in vitro site-specific recombination. *Genome Res* 2000;10(11):1788–95.
16. Liu Q, Li MZ, Leibham D, Cortez D, Elledge SJ. The univector plasmid-fusion system, a method for rapid construction of recombinant DNA without restriction enzymes. *Curr Biol* 1998;8(24):1300–9.
17. Novy R, Yaeger K, Monsma S et al. pTriEx multisystem vector for protein expression in *E. coli*, mammalian and insect cells. *InNovations* 1999:1–5.
18. Bertone P, Kluger Y, Lan N et al. SPINE: an integrated tracking database and data mining approach for identifying feasible targets in high-throughput structural proteomics. *Nucleic Acids Res* 2001;29(13):2884–98.
19. Draveling C, Ren L, Haney P et al. SwellGel: an affinity chromatography technology for high-capacity and high-throughput purification of recombinant-tagged proteins. *Prot Exp Pur* 2001;22(2):359–66.
20. Vlak JM, Keus RJ. Baculovirus expression vector system for production of viral vaccines. *Adv Biotechnol Processes* 1990;14:91–128.

The Williamsburg BioProcessing Conference

Asia-Pacific 2004

FIRST ANNUAL MEETING

Process Development and Production Issues for Eukaryotic Cell Derived Products

The Four Seasons Hotel Sydney
Sydney, Australia

Antibodies & Recombinant Proteins
June 14–16, 2004

Viral Vectors & Vaccines
June 17–18, 2004

Topics Include:

Antibodies & Recombinant Proteins:

- Product Characterization
- Stability & Storage
- Expression Enhancement
- Regulatory Issues
- Cell Line Development
- Viral Clearance
- Assay Development
- Purification
- Safety Testing
- Conjugation & Formulation

Viral Vectors & Vaccines:

- Vector Development
- Raw Material Screening
- Assay & Media Development
- Viral Yield Optimization
- Downstream Processing
- Storage & Stability
- QA / QC Issues
- Reference Material Issues
- Product Characterizations

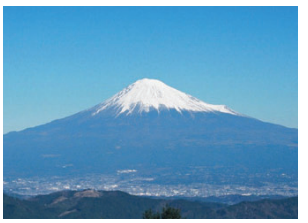
BioProcessing™ JOURNAL

WilBio

Celebrating Our 10th Anniversary WilBio

The Williamsburg BioProcessing Foundation




info@wilbio.com ■ 757.423.8823 ■ www.wilbio.com