Poster Presentations

Organization	Title	Author(s)
InDevR Inc.	Virus Counter: Rapid Virus Quantification for Bioprocessing	Erica D. Dawson and Kathy L. Rowlen
TAP BioSystems Ltd	Facilitating Production of Cell Banks Using an Automated Cryovial Dispenser	Stephen Guy, Dave Thomas, and Miriam Foster
Utah State University, Center for Integrated BioSystems	Integrating Biotechnology Training, Services and Research at Utah State University	Kamal Rashid, PhD CIB Associate Director
Utah State University, Center for Integrated BioSystems	Large-Scale, Developmentally-Synchronous *Caenorhabditis elegans* Liquid Cultures Using a New Tangential-Flow Filtration Method	Jason D. Brown, Mark W. Signs, Jamie Q. White, Sridhar Viamajala, and Kamal A. Rashid
BIA Separations	A Novel Plasmid DNA Purification Process: From Laboratory to Production Scale	F. Smrekar, A. Podgornik, A. Štrancar, M. Peterka
Chesapeake PERL, Inc.	Expression and Characterization of HIV-1 gp41 Protein Using Baculovirus Infection of Whole Insect Larvae	Zhi Liu, Gary R. Matyas, Piet de Dreu, Robert Malone, George Buchman
Helmholtz Centre for Infection Research	New Versatile Donor Vectors for Protein Production in Eukaryotic Systems	Steffen Meyer, Carmen Lorenz, Bahar Baser, Mona Wördehoff, and Joop van den Heuvel
Pall Life Sciences	*Title Pending*	*Author Info Pending*

Erica D. Dawson • InDevR Inc.

Virus Counter: Rapid Virus Quantification for Bioprocessing

By Erica D. Dawson and Kathy L. Rowlen

ABSTRACT: Traditional methods for virus quantification such as plaque assay are time and labor intensive, and can often be a significant bottleneck in the research and development process. We present the results from several case studies comparing a new instrument for virus quantification, the ViroCyt 2100 Virus Counter, to standard methods including viral plaque titer assay and quantitative PCR. The ViroCyt 2100 is a specialized bench top flow cytometer that has been designed to quickly and accurately determine volumetric concentration in real-time for intact virus particles in solution using fluorescence.

In a first case study, serial dilutions of a baculovirus viral stock were provided in a blinded fashion to three different laboratories. Plaque assays were conducted in triplicate at two sites, and several replicate ViroCyt 2100 measurements were made on each dilution by InDevR. The results indicate a linear correlation between the two methods (R^2=0.86), with the ViroCyt 2100 providing greater accuracy and precision in significantly less time. In a second case study using serial dilutions of a 2009 pandemic H1N1 influenza virus strain, ViroCyt 2100 measurements were compared to quantitative PCR measurements. A strong linear correlation between the threshold cycle (Ct) value and the ViroCyt 2100 measurements (R^2=0.96) was observed. The ViroCyt 2100 Virus Counter represents a new, rapid method for virus quantification that can be utilized to monitor bioprocesses in real-time with high accuracy.

Stephen Guy • TAP BioSystems Ltd

Facilitating Production of Cell Banks Using an Automated Cryovial Dispenser

By Stephen Guy, Dave Thomas, and Miriam Foster

ABSTRACT: The rapid aseptic preparation of cryovials is vital for the generation of high quality cell banks. Issues exist with current manual methods as they are prone to variation, place constraints on batch sizes, and repetitive tasks raise operator health concerns. These can be overcome by a simple bench top automated cryovial processing system. Fill-It provides rapid and aseptic automated filling of racks of either: 24, 48 or 96 screw cap cryovials with minimal operator interaction. It fits within standard biological safety cabinets and is suitable for validation in GMP environments.

A comparison of cell suspensions pre- and post-dispense with Fill-It showed no significant loss in cell count or viability. Gravimetric determination of dispense performance showed <5% CV across the volume range with each rack being processed in 135 sec or less. Testing uniformity of cell dispensing with CHO cells and HDF cells demonstrated the system is capable of delivering uniformity across a 480 vial cell bank. Cells from cryovials prepared using Fill-It and cryopreserved in vapour phase liquid N2 and then revived were not significantly different to cryovials prepared manually.

These results demonstrate Fill-It can rapidly, accurately, precisely and uniformly dispense cell suspensions into screw cap cryovials without damaging cells. The faster, more efficient processing offered by Fill-It allows cell banks to be created in a shorter period of time and can also lead to QC savings as larger cell banks can be produced. The production of a cell bank within a single process rather than multiple smaller runs means that cell bank QC costs are reduced so leading to significant cost savings.

Kamal Rashid, PhD • CIB Associate Director, Utah State University, Center for Integrated BioSystems

Integrating Biotechnology Training, Services and Research at Utah State University

By Kamal Rashid and Shaun Barnett
Center for Integrated Biosystems, Utah State University, Logan, Utah

ABSTRACT: The Center for Integrated BioSystems (CIB) at Utah State University represents a novel approach in bringing a broad spectrum of instrumental and intellectual resources to the needs of academic, industrial and governmental entities, both in the U.S. and internationally. The Biotechnology and Bioprocessing Training Program annually offers a series of week-long hands-on courses in the traditional areas of animal cell culture, microbial fermentation, protein purification, plus new courses in proteomics, gene expression analysis, and bioinformatics. Outreach efforts have established collaborative training programs in Singapore, Thailand, and the Dominican Republic.

Built on the foundation of traditional core facility offerings, the CIB offers both bioprocessing capability to the industrial microbiological community and cutting edge analytical services in the fields of genomics, proteomics. metabolomics, and bioinformatics. Using a unique integrated base of laboratory research capability and diverse analytical instrumentation, CIB personnel collaborate with researchers on-campus and around the world to tackle biological questions suited to the Center's suite of expertise. This outreach both strengthens the research efforts at Utah State University and establishes bridges to institutions in other states and countries.

BIOGRAPHY: Dr. Kamal A. Rashid has over thirty years of academic experience in both research and biotechnology educational program development. During his career he has developed, directed and implemented biotechnology training courses at Utah State University, Penn State University and internationally. He joined Utah State University in July 2000 as the Biotechnology Center's Associate Director and Research Professor of Toxicology. During his tenure at Utah State University, he developed and equipped the bioprocess facility at the Center with the most advanced bioreactors and fermentors that are utilized in both research and training programs. From September 2001 to September 2002 he held the position of the Acting Executive Director of the Biotechnology Center.

Prior to joining USU, he was a faculty member at the Department of Biochemistry and Molecular Biology at the Pennsylvania State University. While at Penn State, Dr. Rashid conducted research on the impact of environmental pollutants on human health, developed and taught biotechnology undergraduate courses, developed and directed the Penn State biotechnology training programs, directed the nationally-recognized Summer Symposium in Molecular Biology for ten years and was the key person in the development of the Biotechnology Undergraduate degree and the course curriculum in the department. He has established several cooperative agreements between Penn State, Utah State and several international institutions. Dr. Rashid has delivered numerous lectures and training programs in several countries, including China, Dominican Republic, Egypt, Iraq, Korea, Malaysia, Philippines, Puerto Rico, Thailand, Taiwan, Singapore and US. Dr. Rashid is very well recognized for his continuing education, teaching and international programs. He has received a national Faculty Service Award in 1997 from US University Continuing Education Association for his Meritorious Service to Penn State University.

Dr. Rashid has also a long standing presence in US industrial circles. He co-founded Cogenics, Inc., where he served as Vice President for Research and Development from 1988-1990. He is also the founder of and president of the International Biotechnology Associates that has provided industry with consultations for more than fifteen years. He has established much collaboration with the biotechnology and biopharmaceutical industries during the past twenty years and has trained hundreds of employees of these industries in bioprocess technology. He is an advocate of industry-academia collaborations and has given many presentations at national conferences and published articles in bioprocessing journals to emphasize such collaborations.

Presently he has a multi-year, multimillion dollar grant from the US Department of Health and Human Services to train employees of vaccine manufacturing facilities from eleven countries in the latest advances in cell-based vaccine production with emphasis of Influenza vaccines. These countries include Brazil, Egypt, Korea, India, Indonesia, Mexico, Romania, Russia, Serbia, Thailand and Vietnam.

Kamal Rashid, PhD • CIB Associate Director, Utah State University, Center for Integrated BioSystems

Large-Scale, Developmentally-Synchronous Caenorhabditis elegans Liquid Cultures Using a New Tangential-Flow Filtration Method

By Jason D. Brown[1], Mark W. Signs[2], Jamie Q. White[3], Sridhar Viamajala[4], and Kamal A. Rashid[1]

ABSTRACT: Nematode worms release pheromones to communicate information about food, stress, and sex to other individuals. The nematode species *C. elegans* is a genetic system for studying the biology underlying these pheromone-mediated behaviors. Identifying the chemical structure of *C. elegans* pheromones requires purifying them directly from large quantities of conditioned growth media. Release of pheromones may depend on growth conditions, population density, and developmental stage. However, published studies only report nematode pheromones from developmentally-asynchronous cultures at variable population densities.

Here, we have developed reliable methods for liquid culture of developmentally-synchronous populations of *C. elegans* at controlled population densities from 5,000 to 10,000 worms/mL at the scale of 10 to 100 L. Generating synchronous populations requires harvesting eggs from adults using alkaline bleach. In this study, we developed modified tangential flow filtration (TFF) systems to rapidly bleach adults and recover large quantities of healthy, viable eggs. This method achieved synchronous cultures at scales 2.5 x greater than cultures based on agar plates, and 5 x greater than previously reported for synchronous liquid cultures. We routinely harvested 2.5 x 10^7 animals (total) from a single 5 L large-scale culture. Furthermore, our TFF system effectively recovered pheromone-containing media at all scales tested. This work makes it feasible to isolate other biologically useful secreted molecules from transgenic *C. elegans*.

Jason D. Brown[1]* (jas.d.brown@aggiemail.usu.edu), Researcher I; Mark W. Signs[2] (mws6@psu.edu), Director, Shared Fermentation Facility; Jamie Q. White[3], PhD (jwhite@biology.utah.edu), Postdoctoral Fellow; Sridhar Viamajala[4], PhD (sridhar.viamajala@utoledo.edu), Assistant Professor; and Kamal A. Rashid, PhD[1] (kamal.rashid@usu.edu), Associate Director, Research Professor.

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Poster Presentations

Aleš Štrancar, PhD • BIA Separations

A Novel Plasmid DNA Purification Process: From Laboratory to Production Scale

By Smrekar F, Podgornik A, Štrancar A, Peterka M

ABSTRACT:

1. Introduction

Application of plasmid DNA for gene therapy and vaccination has gained huge interest in last two decades. Downstream processing has major influence on achieving regulatory demands in pDNA production and in order to get optimal purity, different purification techniques have to be included. CIM monolithic chromatographic support, being designed for purification of large molecules and nanoparticles, seems to be a matrix of choice for pDNA purification. The objectives of our work were upgrading existing process with novel hydrophobic ligand and exploring different strategies of pDNA purification process on methacrylate monoliths.

2. Material and methods

Plasmid DNA was extracted from *E.coli* using alkaline lysis followed by precipitation with 0,5M $CaCl_2$. For chromatography, CIM monolithic Hydrophobic interaction and Anion exchange columns were characterized. DBC, separation, recovery and purity were analyzed using different flowrates and mobile phases. To evaluate impurities of pDNA fractions, the analysis for proteins, endotoxins and gDNA was performed.

3. Results

$CaCl_2$ addition to the final concentration of 0,5M was used in lysate prior to chromatographic processing. This removed majority of impurities, but in order to obtain pharmaceutical grade pDNA, further purification is needed. With this sample, it is possible to load more than 6mg of pDNA on 1ml DEAE column. For the next step, new CIM HIC support was developed, allowing, together with removal of impurities, the separation of oc/sc pDNA conformations.

4. Conclusion

Combination of both chromatographic steps, using optimized $CaCl_2$ precipitation, enables pDNA production that satisfies all regulatory requirements. HIC monolith enabled separation of supercoiled pDNA from open circular pDNA, genomic DNA and endotoxins, regardless to flow rates in the range at least up to 380 cm/h. Process was found to be reproducible, scalable, and exhibits high productivity.

Poster Presentations

George W. Buchman III, PhD • Chief Scientific Officer, Chesapeake PERL, Inc.

Expression and Characterization of HIV-1 gp41 Protein Using Baculovirus Infection of Whole Insect Larvae

By Zhi Liu[1], Gary R. Matyas[2], Piet de Dreu[1], Robert Malone[3], George Buchman[1]

ABSTRACT: The Baculovirus Expression Vector System (BEVS) continues to emerge as a critical next generation platform for protein expression for vaccine, diagnostic, therapeutic and research applications. Insect-produced proteins are now licensed in commercial products, such as a vaccine indicated for Human Papilloma Virus, and in a late-stage prostate cancer therapy. Recombinant baculovirus infection of insect cells features high rates of expression of soluble and functional protein, and insect cells possess the vast majority of mammalian post-translational pathways. The PERLXpress system at Chesapeake PERL takes baculovirus infection of insect cells for heterologous protein expression one step further, to whole insects. Mass automated rearing and infection of *Trichoplusia ni* insect larvae via oral administration enables high protein expression (1 – 10 mg/ g tissue) with minimal expression development. Over the past decade of platform development proteins ranging from blood serum proteins, influenza antigens, smallpox proteins, virus-like particles, and lipophilic proteins have been produced in high yield and proper function.

In this report we highlight recent efforts to express full-length HIV gp41 in whole insect larvae. The entire coding region including C-terminal transmembrane (TM) and cytoplasmic tail (CT) regions was cloned and expressed. An engineered gp41 in which the Fusion Peptide (FP) is replaced with insect sequences was further studied. The protein was found to associate with membrane fractions, and was enriched by differential centrifugation. Identity was verified by Western analysis and directed LC/MS. Mice immunized with the gp41 antigen preparation demonstrated a specific anti-gp41 response.

BIOGRAPHY: George W. Buchman, PhD, is a seasoned Biotechnology professional with 23 years of industrial experience. Dr. Buchman has been CSO for Chesapeake Protein Expression and Recovery Laboratories (C-PERL) since 2006. The company produces proteins, using baculovirus infection of whole insects, on a custom basis for Government, Institutional and Commercial clients. Dr. Buchman serves as PI for several projects that include influenza antigen production, smallpox vaccine development, preparation of nerve agent bioscavenger proteins (e.g., butyrylcholinesterase), and a DOE-funded effort in manufacturing of proteins for biofuel production. Prior to C-PERL George was involved with bioinformatics for five years in Business Development and Program Management roles at Gene Logic and Celera Genomics, respectively. He spent nearly thirteen years at Life Technologies, Inc. in several areas including HPV Diagnostics, R&D (DNA Amplification), Manufacturing Management, Fermentation and Technical Manufacturing. George received his PhD degree in Biochemistry from the University of Maryland in College Park. He is an avid runner and pilot.

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1. Chesapeake PERL, Inc., Savage MD
2. Walter Reed Army Institute of Research, US Military HIV Research Program
3. RW Malone MD LLC, Vaccines and Biologics

Steffen Meyer • Helmholtz Centre for Infection Research

New Versatile Donor Vectors for Protein Production in Eukaryotic Systems

By Steffen Meyer, Carmen Lorenz, Bahar Baser, Mona Wördehoff, and Joop van den Heuvel

Recombinant Protein Expression (RPEX), Division of Structural Biology,
Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany

ABSTRACT: The availability of large amounts and high quality of relevant target proteins is a prerequisite for structural analyses. Since many viral and mammalian proteins either require post-translation modification for proper folding and full biological function or have to be coexpressed with binding partners as a multi protein complex for its stability, *E.coli* is often not suitable as an expression system. Generally, protein expression in invertebrate and vertebrate eukaryotic cell lines is more time-consuming and expensive than in bacteria. That is why a profound screening for the best protein expression construct as well as for the most appropriate host regarding both yield and quality of the target protein is essential.

Therefore, we developed a set of novel expression/donor vectors for rapid screening for expressible protein variants and evaluating the optimal expression host. The range of applications of these vectors to optimize the production strategy comprise a variety of highly efficient hosts and expression methods including transient and stable expression combining both early and late viral promoters, elements of the Baculovirus Expression Vector System (BEVS) and a unique recombination mediated cassette exchange system (RMCE) for fast generation of stable CHO cells developed in our Institute. The vectors presented in this work enable fast screening of many protein variants in high throughput and are suitable for direct transfer to large-scale protein production with the optimal expression construct avoiding time consuming recloning steps.

BIOGRAPHY: Steffen Meyer is a PhD student at the Helmholtz-Centre for Infection Research in Braunschweig, a federal research institute of the Helmholtz Association, the largest scientific organization in Germany. He graduated in biotechnology at the Braunschweig University of Technology in 2008. After a 6-month traineeship in process development at Glycotope GmbH in Berlin, he started his PhD work in the group Recombinant Protein Expression in the department for Molecular Structural Biology at the Helmholtz-Centre for Infection Research. His research interests include eukaryotic protein expression, vector design and downstream processing.