Poster Presentations

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GENEWIZ, Inc.	Viral Genome Sequencing on the Ion Torrent PGM: Increased Coverage for a Fraction of the Time and Cost	Jack Yu, Ginger Zhou, Wenying Huang, Natallia Kalinava, Ekaterina Bogdanova, Conrad Leung, Yankai Jia, and Jeffrey Shaman
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Dina Darwis • Nanyang Technological University, Singapore

High-Throughput Recombinant Protein Production in Bacterial and Baculovirus Expression Systems: Efficient Small Scale Screening and Optimized Large-Scale Production

Dina Darwis[1], Martina Nilsson[1], Pär Nordlund[1,2], and Tobias Cornvik[1]

ABSTRACT: Recombinant proteins are widely used for structural and functional studies. High-throughput recombinant protein production is well established in E. coli while it is more difficult and time consuming in baculovirus expression vector system (BEVS). In our lab we have successfully screened 6000 constructs in E. coli and produced 1000 soluble proteins in large scale. As a second tier we have established BEVS expression, which is used in particular for extracellular proteins and the ones that are not soluble in E. coli. In addition, we have successfully used BEVS for membrane protein production. To stream line both the bacterial and baculovirus systems we have adapted ligation independent cloning (LIC), followed by parallel transformations and expression. Using BEVS, we have successfully screened 200 constructs and several of these have been scaled up. The methods for cloning, expression screening and scale up will be presented as well as optimization of the purification procedures.

1. School of Biological Sciences, Nanyang Technological University, Singapore.
2. Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Sweden.

BIOGRAPHY: Dina Darwis is a research associate in Protein Production Platform at Nanyang Technology University. She received a Master's Degree in Science from Illinois State University in 2000. Her work focused on protein-protein interaction with Yeast Two-Hybrid method. In 2008, she started working with baculoviral expression vector system (BEVS) to transiently transduce human embryonic stem cells. She is currently responsible for high-throughput recombinant protein production.

Yamuna Dasarathy, PhD • Pall Life Sciences

High Throughput, Multi-Mode Biophysical Analysis for Early Stage Stability 'Fingerprinting'

ABSTRACT: We present an innovative, high-throughput analytical technology, which allows generation of a comprehensive physical stability 'fingerprint' of a candidate molecule. The technique consumes very little protein sample and is therefore suitable for use very early in the development process. A key feature is the ability to simultaneously record multiple different types of biophysical stability data, which when combined with the high throughput nature of the technique allows thorough mapping of a protein's physical stability behavior. The stability fingerprint can be used in candidate or formulation development, as well as potentially forming part of the process of fully characterizing a molecule.

BIOGRAPHY: Yamuna Dasarathy has spent most of her career in the Biopharmaceutical Industry. Her expertise spans clinical research, downstream process development, technical marketing and business development. Her current role focuses on marketing Pall Life Sciences' chromatography product portfolio and protein characterization instrumentation/ analytical services. Before joining Pall, Yamuna was in the CMO Industry, developing and growing the business through effective marketing of cGMP manufacturing services. Prior to that, she was with Pharmacia/Amersham/GE Healthcare managing the GE lab chromatography product portfolio. Yamuna has a PhD in Biochemistry followed by productive research at Brandeis University and Tufts-New England Medical Center, Boston, MA. She acquired an MBA later from Rutgers State University, NJ.

Poster Presentations

Yamuna Dasarathy, PhD • Pall Life Sciences

Direct Protein Capture and Contaminant Removal from Undiluted Feedstocks

By Sylvio Bengio, Magali Toueille, Jérôme Champagne, and René Gantier

ABSTRACT: Anion exchange chromatography is a standard method for protein capture or impurity removal. Conventional sorbents using Q or DEAE functionalities have low binding capacity for proteins in a high salt medium. This necessitates biological feedstock to be diluted or diafiltered into a buffer aiding direct capture by ion exchange sorbents. In this poster we show that with a novel IEX sorbent, we can directly capture and purify proteins from cell culture, plasma, or other feedstock, with minimal sample treatment. This sorbent has high dynamic binding capacity at short residence time (>100 mg/mL BSA at 1 to 2 min residence time), it maintains binding capacity at conductivities up to 15 mS/cm; therefore, it allows direct feed processing without dilution or ultrafiltration / diafiltration (UF/DF). We discuss several applications including synergistic effect with Protein A sorbents for removal of host cell proteins (CHOP) in monoclonal antibody purification, capture of an acidic protein (pI 3.5) and purification of HSA from undiluted plasma.

Xinping Huang, MD • Emory University

Simple and Effective Method for Producing High Titer Recombinant Adeno-Associated Virus 1, 2, and 5 Using Polyethyleneimine (PEI)

By Xinping Huang, Antja-Voy Hartley, Yishi Yin, and Kerry Ressler

ABSTRACT: The adeno-associated virus (AAV) is one of the most useful viral vectors for gene delivery in vivo and in vitro. Many methods have been established to produce and characterize recombinant AAV (rAAV) vectors. Here we describe a 2-plasmid or 3-plasmid co-transfection approach with 25 kDa linear polyethyleneimine (PEI) in 293T cells. 72 hours post-transfection, supernatant and cells were collected. Virus in the supernatant was precipitated with 40% PEG 8000 in 2.5 M NaCl, combined with the cell lysate, then treated with 10% Deoxycholate and Benzonase. The crude virus was then purified by a discontinuous iodixanol density gradient ultracentrifugation, then dialyzed and concentrated with an Amicon 15 100,000 MWCO concentration unit. The genomic titer of the viral stock, as determined by qPCR with plasmid standards was $2x10^{12}$–$6x10^{13}$ vg/mL. These viral vectors showed high expression in vivo and in vitro.

BIOGRAPHY: Xinping Huang earned her Medical Degree from Nantong Medical College, China in 1987. After distinguishing herself as an Anesthesiologist in an affiliate hospital of Nantong Medical College for 12 years, Dr. Huang later moved to the United States in 2002 where she joined the Emory University's Viral Vector Core as a Research Specialist in the Center for Neurodegenerative Disease. For more than three years, she has been the technical leader for optimizing the Core's lentivirus production protocol as well as developing high through-put production of recombinant adeno-associated virus. She continues to be a resourceful asset to both Emory Investigators and extramural Researchers.

Poster Presentations

Marko Banjac • Manager of Downstream Process Development, BIA Separations

Integration of Monolithic Analytical Columns Into the Biopharmaceutical Manufacturing Process to Enable Fast and Real-Time HPLC Analytical Assay Both Up and Downstream

ABSTRACT: Biomanufacturing of antibodies, therapeutic proteins and vaccines or gene delivery vectors (either DNA or virus based) is a very complicated process where many things can go wrong. This is even more pronounced as the target biomolecules are extremely susceptible to the environmental conditions both during cultivation (upstream processing) as well as during isolation and purification (downstream processing). One can always doubt whether we have enough information about our complex biomolecule samples to consistently develop a safe product by running a robust and efficient purification bioprocess. By using and understanding novel technologies one can design new process analytic technology (PAT) initiatives to overcome some of these problems. Here, we present novel monolithic analytical columns – CIMac columns – that can bridge this gap. In the first example, CIMac columns were applied for monitoring the purification process of virus like particles (VLP), which are used for production of vaccines and as delivery systems in gene therapy. In the second example, the monolithic analytical columns were also applied for monitoring the fermentation process of bacteriophages.

BIOGRAPHY: Marko Banjac is Manager of Downstream Process Development for BIA Separations, GesmbH. Mr. Banjac has more than nine years of hands-on experience in Downstream Process Development for industrial production of pDNA, various viruses and vaccines. He is a co-inventor of a patented process for purification of live attenuated, Vero cell grown, Influenza virus vaccines. Marko has extensive experience in technology transfers to GMP production facilities.

Taylor Hatten and Kamal Rashid, PhD • Utah State University, Center for Integrated BioSystems

Productivity Studies Utilizing Recombinant CHO Cells in Stirred-Tank Bioreactors: A Comparative Study Between Pitched-Blade and Packed-Bed Bioreactor Systems

By Taylor Hatton, Shaun Barnett, Abby Benninghoff, and Kamal Rashid, PhD

ABSTRACT: Traditionally, large-scale production of animal cells in suspension culture requires the use of stirred-tank bioreactors, of which the most common type is the pitched-blade system. The packed-bed basket technology developed by New Brunswick Scientific (an Eppendorf Company) provides a shear free environment for large-scale (up to 100L) production of animal cells. At present, little information is available on the utility of this system for the production of secreted proteins, especially in perfusion mode of operation. The perfusion process provides a homeostatic environment for optimal cell growth similar to that experienced by cells in vivo. In contrast, the batch culture approach does not appropriately model this homeostatic environment due to the depletion of nutrients and accumulation of waste products in the culture system. Thus, the objective of this study was to compare the growth and productivity of alkaline phosphatase (ALKP)-secreting Chinese Hamster Ovary (CHO) cells cultured in these two bioreactor types: pitched-blade bioreactors operated in batch mode versus packed-bed bioreactors operated in perfusion mode. CHO cells cultured in the packed-bed bioreactor operated in perfusion mode produced greater amounts of ALKP compared to cells cultured in the pitched-blade system run in batch mode. These observations suggest that continuous exposure of cells to fresh culture media and the shear-free culture environment provided by the Fibra-Cel disks offered more favorable growth conditions for CHO cells, allowing for either greater cell proliferation (higher density) or greater protein production on a per cell basis. Overall, the results of this comparison study suggest that packed-bed bioreactors provide significant advantages for moderate-scale production of cells; the benefits of this bioreactor system may translate to large-scale cell culture for generating secreted protein products useful in medical applications.

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.

Yankai Jia, PhD • Head of High-Throughput Sequencing, GENEWIZ, Inc.
Jeffrey Shaman, PhD • Associate Manager – Regulatory Services Unit (GLP/cGMP), GENEWIZ, Inc.

Viral Genome Sequencing on the Ion Torrent PGM: Increased Coverage for a Fraction of the Time and Cost

By Jack Yu, Ginger Zhou, Wenying Huang, Natallia Kalinava, Ekaterina Bogdanova, Conrad Leung, Yankai Jia, and Jeffrey Shaman

ABSTRACT: The Ion Torrent PGM provides a fast sequencing solution for small genomes, such as viral and bacterial genomes, and has become increasingly popular for de novo and re-sequencing applications. In this study, we sequenced a 37.5 kb DNA viral genome using Sanger and Ion Torrent technologies to compare the quality, coverage, time, and cost. We performed de novo assembly and sequence alignment with various amounts of genomic coverage. Our data suggest that 20x coverage from the PGM is sufficient for covering 99% of the reference virus genome, whereas 100x genomic coverage is required for de novo assemblies. Our findings further demonstrate that the Ion Torrent proves to be more efficient when compared to the time and cost resources required for shotgun cloning and subsequent Sanger sequencing.

BIOGRAPHY: Dr. Yankai Jia earned his PhD from the University of Paris VI and completed post-doctoral research at UT Southwestern Medical Center. Yankai brings extensive experience in DNA microarray development and high-throughput DNA sequencing to GENEWIZ as the Head of High-Throughput Sequencing. Prior to joining GENEWIZ in 2011, Yankai worked in molecular and cellular biology and product development for organizations including Eli Lilly, Motorola, and Altria. Previously, Yankai was co-founder and Chief Scientific Officer at NJ Pharmatech, Inc., a CRO specializing in genomics and clinical data analysis.

Suresh Daniel • Rocky Mountain Biologicals, Inc.

RMBIO Lipogro®: A Safer and Effective Cholesterol Supplement for Enhancing Productivity of Mammalian Cultures

By Suresh Daniel, David Jackson, and VK Daniel

ABSTRACT: RMBIO Lipogro was developed as a stable, water-soluble concentrate of biologically active cholesterol, phospholipids and essential fatty acids purified from validated raw materials from GBR-1 countries, such as Australia. Providing a balanced profile of key nutritional components in their native forms while ensuring the highest level of safety among similar products, we have shown here that RMBIO Lipogro supports robust cell growth and high cell density, and boosts protein production in cholesterol-dependent and non-dependent mammalian cell lines. In cholesterol-dependent NS0 cells, relative cell specific production in the presence of RMBIO Lipogro is higher in comparison with fetal bovine serum or a competitor cholesterol concentrate. Manufactured in a dedicated facility under Good Manufacturing Practices (GMP) using proprietary serum fractionation processes by Rocky Mountain Biologicals, Inc., RMBIO Lipogro can be customized to suit the needs of the individual user.

BIOGRAPHY: Suresh founded Rocky Mountain Biologicals, Inc. (RMBIO) in 2005. RMBIO is engaged in the technical manufacturing of animal-derived protein fractions that remain critical raw materials for both the diagnostic and biopharmaceutical industries. Adhering to FDA guidance for the sourcing of GBR1 proteins to mitigate any potential BSE risk, RMBIO focused on sourcing its raw material for the production of its serum-free media supplements from Australia and New Zealand. Suresh recently oversaw an expansion of the RMBIO facility in Missoula, Montana which will add capabilities of contract serum and media production. Prior to starting RMBIO, Suresh was the Director of Clinical Research for the Western Montana Clinic, The International Heart Institute of Montana and Tamarack Management. As the president and founder of Rocky Mountain Clinical Research, a company that provided clinical research services to the pharmaceutical and medical device manufacturing industries, he brought extensive knowledge of the clinical research industry and regulatory requirements instituted by the FDA to RMBIO.