APPLICATION Track Poster Presentations

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Thomson Instrument Company	Bridging the Gap in Screening and Scale-Up in Insect Cells, CHO, Hybridoma, HEK293 Cell Lines (Single-Use Optimum Growth Flasks 5 L Flasks)	Sam Ellis
Department of Bioscience Technology, Chung Yuan Christian University, Chungli, Taiwan; Laboratory of Virology, Wageningen University, the Netherlands	Additive Effect of Calreticulin and Translation Initiation Factor eIF4E on Secreted Protein Production in the Baculovirus Expression System	Chao-Yi Teng, Monique M. van Oers, and Tzong-Yuan Wu
The University of Queensland	Improved Secretion of Glycoproteins in the Baculovirus-Insect Cell System	Cindy Chang, Emilyn Tan, and Linda H.L. Lua
European Molecular Biology Laboratory (EMBL) and TAP Biosystems	MultiBac Expression System: Comparison of Growth and Multiprotein Production in Shake Flask and Automated Miniature Bioreactor (ambr™) Cultures	Maxime Chaillet, Frederic Garzoni, Sinyee Yau-Rose, PhD, Barney Zoro, and Imre Berger
Department of Bioscience Technology, Chung Yuan Christian University, Chungli, Taiwan	Isolation and Characterization of a Hybrid Baculovirus with Extended Host Ranges Through Co-Infection of *Autographa californica* Multiple Nucleopolyhedrovirus and *Maruca vitrata* Multiple Nucleopolyhedrovirus	Ming-Hsiang Chen and Tzong-Yuan

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LaDonya Jackson • Center for Integrated BioSystems, Utah State University

Growth and Protein Production of a Recombinant CHO Cell Line Utilizing a Novel Antibody-Free Fetal Bovine Serum

By LaDonya Jackson, Shaun Barnett, Zonda Wang, and Kamal Rashid

ABSTRACT: Bovine sera isolated from calves (calf bovine serum, CBS) and fetuses (fetal bovine serum, FBS) are widely used for growing animal cells in culture both for basic research and for the production of recombinant proteins in the biopharmaceutical industry. It is estimated that 60–70% of all the recombinant biopharmaceuticals, such as therapeutic monoclonal antibodies (MAbs) and injectable vaccines, are produced from mammalian cell culture. A potential problem in using bovine sera is the presence of bovine immunoglobulins (antibodies). Multiple studies have shown that bovine antibodies against a variety of pathogenic and nonpathogenic antigens are present in both FBS and CBS (even before pre-colostrum feeding), indicating that bovine fetuses are exposed to such antigens *in utero* by vertical transmission or compromise of placenta. The presence of antibodies that are positive to a variety of antigens in bovine sera can affect many aspects of mammalian cell culture investigations. The presence of antibodies against *Neospora caninum*, for an example, may bind to antibody producing cells during MAb production. The presence of bovine antibodies can block or compete during screening of hybridoma cells. This may lead to false positive results in diagnostic assays and other biological applications. False negatives will also occur when the antibodies in the serum mask contamination by bovine viruses in vaccines. Due to these intrinsic problems with using bovine sera, it was essential to develop safer bovine sera. Therefore, the primary objective of this study is to determine whether an antibody-free fetal bovine serum derived from bovine immunoglobulin knockout cattle produced by BioDak LLC is superior to the traditional bovine sera especially CBS.

The following variables were selected for this comparative study: cell yield, cell viability, glucose uptake, lactate formation and alkaline phosphatase production. A recombinant CHO (rCHO) cell line (SEAP) that secrets the protein alkaline phosphatase was utilized in these studies. The cells were grown in DMEM supplemented with 10% serum. The T-flasks were incubated under the conditions of 5% CO_2 at 37 °C. The experimental design utilized two separate runs for each serum type for validity and reproducibility. Cell growth and viability were measured utilizing the Countess Automated Cell Counter instrument (Invitrogen). Glucose uptake and lactate production were measured utilizing a YSI 2700 Select Biochemistry Analyzer. The secreted alkaline phosphatase concentrations were monitored utilizing an enzyme assay (AnaSpec, Inc.). These results show that the antibody-free serum is superior to CBS and as good as FBS in terms of growth and productivity. A follow-up study is in the process of being conducted utilizing a Vero cell line with the following variables: cell yield, cell viability, glucose uptake, and lactate formation. Detailed results for each variable will be showcased in this presentation.

BIOGRAPHY: LaDonya Jackson is a senior in Biotechnology at Utah State University. She has worked in Utah State University research laboratories since the start of her undergraduate studies in 2010. She started as an undergraduate researcher in the laboratory of professor Ken White, learning cell culture techniques, *in vitro* maturation, *in vitro* fertilization, as well as molecular cloning, and nuclear transfer techniques. Early in 2012, LaDonya started an undergraduate research project in the laboratory of professor Kamal Rashid, PhD, at the Center of Integrated Biosystems investigating an antibody-free serum and its utility in the growth and productivity of a recombinant CHO cell line. The results of this research project are presented at this conference. LaDonya also helped Dr. Rashid in the laboratory portion of the Cell Culture Methods course offered to seniors in biological sciences at USU, supported graduate student efforts in research on cell growth and optimization studies utilizing different types of bioreactors, and assisted with the different bioprocess oriented, hands-on training courses offered by the center to members of the biopharmaceutical industry. She is also a member of the staff of a BARDA/HHS funded vaccine manufacturing training courses offered at the Center to select developing country vaccine manufacturer scientists. Presently she is finalizing her studies at USU and applying to graduate programs with emphasis on biotechnology and molecular biology.

Sam Ellis • Thomson Instrument Company

Bridging the Gap in Screening and Scale-Up in Insect Cells, CHO, Hybridoma, HEK293 Cell Lines (Single-Use, Optimum Growth 5L Flasks)

By Sam Ellis

ABSTRACT: Optimum Growth Flasks (patented) give excellent growth with space saving capability. By using Optimum Growth Flasks, users are able to grow 2.5 L of cell culture versus the competition's max capacity of 1 L. The Optimum Growth Flasks have replaced expensive, disposable, Fernbach flasks and also small Wave® Bags (5 L and 10 L). The Optimum Growth Flasks also give high viability cultures with great use of space, as shown by our data in insect cells, CHO, hybridoma, HEK293 cell lines. We will show data from GPCR proteins, vaccines, and antibodies.

BIOGRAPHY: Sam is a biochemist and molecular biologist who has worked in industry for 13+ years on process development for different analytical and fermentation processes. Sam Holds more that 15+ patents worldwide and has contributed to development of many techniques within the realm of analytical sciences and biochemistry. He serves as the Vice President of Thomson Instrument Company. He was recently co-chair of a session at Recent Advancement of Fermentation technology (Raft) for the Society of Industrial Microbiology. Thompson Instrument Company's motto is "Solutions at Work."

Chao-Yi Teng, PhD • Department of Bioscience Technology,
Chung Yuan Christian University, Chungli, Taiwan

Additive Effect of Calreticulin and Translation Initiation Factor eIF4E on Secreted Protein Production in the Baculovirus Expression System

By Chao-Yi Teng, Monique M. van Oers[1], and Tzong-Yuan Wu
1. Laboratory of Virology, Wageningen University, the Netherlands

ABSTRACT: The baculovirus expression vector system is widely being used for the over-expression of eukaryotic proteins. However, the yield of membrane-bound or secreted proteins is relatively low compared to intracellular proteins. In a previous study, we have demonstrated that the co-expression of the human chaperones calreticulin (CALR) or β-synuclein (β-syn) increased the production of a secreted protein considerably. A similar effect was seen when co-expressing insect translation initiation factor eIF4E. To further improve secretory protein production, different combinations of these genes were tested (CALR alone, β-syn + CALR, or β-syn + CALR + eIF4E) by assessing the expression level of a recombinant secreted alkaline phosphatase (SEFP). An additional 1.8-fold increment of SEFP production was obtained when cells co-expressed the three "helper" genes, compared to cells, in which only CALR was co-produced with SEFP. Moreover, a time course assay showed that the duration of the SEFP production lasted much longer in cells that co-expressed these three "helper" genes (up to 10 dpi). Utilization of this triple-support vector offers significant advantages when producing secreted proteins and is likely to have useful applications for the production of viral vaccines and other pharmaceutical products.

BIOGRAPHY: Dr. Chao-Yi Teng is a postdoctoral research fellow in the laboratory of Tzong-Yuan Wu at the Chung Yuan Christian University in Taiwan. She received a PhD in bioinformatics and biological structure from National Tsing Hua University, Taiwan, an MS in bioscience technology from Chung Yuan Christian University, Taiwan and a BS in medical chemistry at Chia Nan University of Pharmacy and Science, Taiwan. Her primary research interests are in identifying potential genes for improving expression of secreted proteins in BEVS. Besides, she is experienced with development of IRESes based bi-, tri-, and multiple expression vectors for glycoprotein production. In 2010, she was awarded a 12-month scholarship in Wageningen University (NL), sponsored by National Science Council (NSC) of the R.O.C. During her visiting, she was working on the project of enhanced recombinant protein production by co-expression of helper genes and supervised by Dr. Monique van Oers. Currently, her research is focused on developing multi-locus BEVS with extended host range for multiple proteins expression.

Cindy Chang • The University of Queensland

Improved Secretion of Glycoproteins in the Baculovirus-Insect Cell System

By Cindy Chang, Emilyn Tan, and Linda H.L. Lua

ABSTRACT: The eukaryotic protein processing capabilities of insect cells has led to baculovirus expression vector system (BEVS) as a favourable system for the production of secreted proteins. Low secretion efficiency is one of the challenges for using this system to produce glycoproteins. A common observation is the accumulation of the protein of interest inside the insect cells instead of being secreted into the culture medium. To identify the factor(s) affecting protein expression and secretion using BEVS, the expression of three glycoproteins are tested in two commonly used BEVS (Bac-to-Bac® and *flash*BAC™) with three different signal peptides in two insect cell lines (High Five™ and Sf9) at different culture temperatures. Overall, higher secretion is detected using High Five insect cells. No notable enhanced secretion is observed in the modified *flash*BACGOLD™ system, which contains a dual gene deletion (chitinase chiA and cathepsin v-cath) postulated to improve protein secretion. The effect of the different signal peptides on secretion efficiency varies amongst target proteins. Lowering the culture temperature has an impact on the stability and secretion efficiency. Optimisation of the parameters is imperative to achieve improved secretion of glycoproteins.

BIOGRAPHY: Ms. Cindy Chang has extensive experience on recombinant protein production. Her role as the Operations Manager at the Protein Expression Facility has allowed her to use her knowledge and skills in molecular cloning and *in vivo* expression systems to overcome challenges associated with recombinant protein production. She has contributed to the thousands of constructs and hundreds of proteins generated by the facility. With her in-depth understanding on the baculovirus-insect cell system, Ms. Chang has established a high-throughout, baculovirus-insect cell expression platform in the facility. This streamlines both the cloning process and expression screen for multiple constructs with the aim to determine optimal parameters for large-scale protein production.

APPLICATION Track Poster Presentations

Barney Zoro, PhD • TAP Biosystems
Sinyee Yau-Rose, PhD • TAP Biosystems

MultiBac Expression System: Comparison of Growth and Multiprotein Production in Shake Flask and Automated Miniature Bioreactor (ambr™) Cultures

By Maxime Chaillet, Frederic Garzoni, Sinyee Yau-Rose, PhD, Barney Zoro, and Imre Berger

European Molecular Biology Laboratory (EMBL) and TAP Biosystems

ABSTRACT: The detailed molecular analysis of multiprotein complexes is an emerging focus in the biological sciences. Techniques are needed which can increase both the yield of protein and the number of such complexes which can be expressed. It is anticipated that the application of laboratory automation technology can facilitate medium to high throughput, multicomplex protein expression programs and molecular characterisation studies.

This poster highlights initial work carried out to assess the advanced microscale bioreactor (ambr™) as a new tool for high throughput screening of insect cells lines and viruses for miniature scale (10 – 15 mL) protein expression.

Two insect cell lines (Sf21 and High Five) were tested in the ambr system. The results confirm that the uninfected insect cell lines can be successfully cultured and maintained in ambr, providing similar growth curves to those obtained from conventional shake flask cultures. Subsequent viral infection of the cells using the ambr system resulted in the expression of one protein complex with successful negative controls for viral cross-contamination. This study also demonstrates the capability of the ambr system for parallel cell screening and culture splitting (dilution). The next logical step is to optimize the process to mimic larger scale bioreactor cultures, as has successfully been achieved for mammalian cell cultures.

BIOGRAPHY: Barney's personal and professional goal is to facilitate industry-wide acceptance and use of advanced bioreactor technologies, demonstrating technical capability and scientific value through an evidence-based approach. Barney read Chemical Engineering at Cambridge University and continued his study at University College London (UCL) with a Masters degree and a Doctorate in Biochemical Engineering, with a focus on Tissue Engineering. He has published papers in *Biotechnology and Bioengineering*, presented conference posters, and presentations and lectures periodically at UCL.

BIOGRAPHY: Sinyee is a bioprocess product specialist at TAP Biosystems, with extensive experience on TAP's advanced microscale bioreactor (ambr) system. Through her work at TAP Biosystems, Sinyee has collaborated with many industrial and academic institutions, building strong relationships with key opinion leaders in the biopharmaceutical industry. Sinyee obtained a Masters degree and a PhD in Biochemical Engineering, both from UCL and is published in *Biotechnology and Bioengineering* and has presented a number of conference posters on bioreactor and fermentation technologies.

Ming-Hsiang Chen • Department of Bioscience Technology,
Chung Yuan Christian University, Chungli, Taiwan

Isolation and Characterization of a Hybrid Baculovirus with Extended Host Ranges Through Co-Infection of Autographa californica Multiple Nucleopolyhedrovirus and Maruca vitrata Multiple Nucleopolyhedrovirus

By Ming-Hsiang Chen and Tzong-Yuan
Department of Bioscience Technology, Chung Yuan Christian University, Chungli, Taiwan

ABSTRACT: The host range of baculoviruses usually limits its applications. Here, we isolated EGFP-expressing hybrid viruses, A-M bac, which encompasses the host ranges of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and *Maruca vitrat*a multiple nucleopolyhedrovirus (MaviMNPV) by using a fluorescence-based selection method. The A-M bac virus could accomplish the viral life cycle among *Spodoptera frugiperda* 21 (Sf21) or High-Five (Hi-5) cells which are permissive for AcMNPV, and NTU-MV532 cells which are permissive for MaviMNPV. In addition, infection of these cell lines with A-M bac virus produced higher levels of exogenous EGFP expression, especially in Sf21 and Hi-5 cells, than EGFP-expressing AcMNPV. Analysis of the whole genome sequence of A-M bac, a region nearby the polyhedron locus of AcMNPV was replaced by a 10.2-kb DNA fragment of MaviMNPV and resulted in loss of four ORFs (Ac-ORF603, Ac-bro, Ac-ctx, Ac-Orf12) and one homologous region (Hr1a). Furthermore, we also found some of these conserved ORFs showed relatively lower identities between AcMNPV and MaviMNPV, such as AcOrf4 or Ac-pe38. This hybrid virus might help to identify the host range factors and to elucidate mechanisms of host restriction among various baculoviruses to their insect hosts in the future.

BIOGRAPHY: Ming-Hsiang Chen works as a research assistant in the laboratory of Professor Tzong-Yuan Wu at the Chung Yuan Christian University in Taiwan. Mr. Chen received his master's degree from the department of Microbiology and Immunology in National Cheng Kung University, Taiwan, in 2006. He has research experience for more than four years in two different fields of laboratories. During the first two years, he worked on the evaluation of using mesenchymal stem cells to ameliorate the progression of a neurodegenerative disease, Spinocerebellar ataxia, and his works were published on the *Journal of Biomedical Science* in 2011. From 2010 to present, he has focused on the field of baculovirus, especially on the topic of isolating a hybrid baculovirus with extended host ranges.