

Plenary Symposia

PS-1

Moving from In Vitro to In Vivo RNAi. CHRIS CUNNING. Invitrogen Corporation, Carlsbad, CA 92008. Email: christofer.cunning@invitrogen.com

RNA interference (RNAi) is a powerful tool to inhibit gene expression and has revolutionized biology. The ability to demonstrate phenotypic alterations in animals represents the ultimate confirmation of conclusions arrived at in vitro, making a move to in vivo applications the logical progression for RNAi research. We have developed Stealth™ RNAi, a chemically modified RNAi duplex, which displays increased specificity and enhanced stability in serum compared to standard siRNA. The advantages of Stealth™ RNAi chemistry combined with rational design based on knowledge of the target gene sequence are the ideal reagent for systematic inhibition of gene expression both in vitro and in vivo. Here, we demonstrate the key components for successful RNAi and the experimental steps needed to move RNAi research in vivo. Additionally, our recent data and progress in this area will be highlighted.

PS-2

New Development of MicroRNA Research and Role of miR-34s in p53 Tumor Suppressor Network. CAIFU CHEN. Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404. Email: ChenCX@appliedbiosystems.com

We will present the most recent advances in microRNA (miRNA) research and their impact on cancer research. New development of TaqMan MicroRNA assays including Megaplex RT/PreAmp TaqMan MicroRNA arrays for single cell and FFPE samples will be described. In addition, we will show an example of using TaqMan miRNA assays to discover novel p53 tumor suppressor miRNAs. A global reduction in miRNA levels is often observed in human cancers, suggesting that miRNAs may play an intrinsic role in tumor suppression. To identify miRNA components of tumor suppressor pathways, we compared miRNA expression profiles of wild-type and p53-deficient cells. This analysis revealed a family of miRNAs, miR-34a-c, whose expression reflected p53 status. Precursors of all three miR-

34 family miRNAs are direct transcriptional targets of p53, whose induction by DNA damage and oncogenic stress depends on p53 both in vitro and in vivo. Ectopic expression of miR-34 induces cell cycle arrest in both primary and tumor-derived cell lines, consistent with the observed ability of miR-34 to downregulate a program of genes promoting cell cycle progression. The p53 network suppresses tumor formation through coordinated activation of multiple transcriptional targets, and miR-34 may act in concert with other effectors to inhibit inappropriate cell proliferation.

PS-3

Control of Coleopteran Insect Pests Through RNA Interference. JIM ROBERTS. Monsanto, 800 N. Lindbergh Blvd., St. Louis, MO 63167. Email: james.k.roberts@monsanto.com

Small interfering RNAs (siRNAs) function in important regulatory roles in plants and animals. Double-stranded RNA (dsRNA) has been widely exploited as a precursor to generate siRNA to suppress endogenous genes, whereas there are far fewer examples using dsRNA directed to exogenous RNA. We will present studies that explore the use of dsRNA to control major insect pests in agriculture. Western corn rootworm (WCR, *Diabrotica virgifera virgifera*) larvae exhibit significant stunting and mortality in an artificial diet bioassay when fed a diet containing dsRNAs derived from a variety of WCR genes. Similar results were obtained with two other coleopteran species, *Diabrotica undecimpunctata howardii* (southern corn rootworm, SCR) and *Leptinotarsa decemlineata* (Colorado potato beetle, CPB). Analysis of treated WCR larvae demonstrated an early and specific decrease in the levels of mRNA corresponding to the WCR gene target. Transgenic corn plants engineered to express dsRNA containing WCR sequences show significant reduction in WCR feeding damage in a growth chamber assay. This is the first reported exploitation of the RNAi pathway to control an insect pest via *in planta* expression of a dsRNA.

PS-4

Next Generation Sequencing Technologies, Their Implications, and Prospects for Next-Next Gen Technologies. J. A.

SCHLOSS. Division of Extramural Research, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892-9305. Email: js173g@nih.gov

Not long ago, generating a human reference DNA sequence was considered an almost unimaginable challenge. With that task complete, little time was lost in the quest for new methods to survey, and eventually completely sequence, increasing numbers of human, other animal, plant and microbial genomes to identify and understand functional elements of these genomes, and the contributions of sequence variation to—depending on the beast—health and disease, optimization of food value and commercial potential, virulence, role in a community, etc. With cost a daunting impediment, those new technologies must produce very high quality data for a small fraction of the cost of the reference sequence. NHGRI launched parallel programs in 2004 to reduce the cost initially by two, and eventually by four orders of magnitude. At about \$1,000 per complete human-sized genome sequence, very large human disease association studies would become practical even for rare variation, and sequencing might become a practical tool for individualized medicine. Opportunities abound, similarly, for applications broadly across such endeavors as agriculture, ecology, and energy. Considerable progress has been made toward the initial goal of driving costs toward \$100,000, with successful development of chemistries and instrumentation, and with commercial platforms in testing and use in multiple laboratories. These technologies have the advantage, over previous high-throughput sequencing and genotyping methods, that they produce digital data, with each sequence fragment originating from a single molecule. Thus, rare events, such as rare variants or very low abundance transcripts, are revealed, enabling not only cost reduction but also new biological insights. Emergence of these new technologies is opportune in the context of NIH data generation and discovery projects including ENCODE, the Human Microbiome, the Cancer Genome Atlas, and pursuing promising signals from genome-wide association studies. Yet, substantial challenges remain to achieve optimal data quality and cost reduction. Meanwhile, advances in mechanistic understanding and fabrication capability at the nanoscale offer opportunities for development of a next-next generation of sequencing technologies.

PS-5

Peptidomic Profiling of Endocrine Cell Culture Media for Bioactive Peptide Discovery. S. W. TAYLOR. Amylin Pharmaceuticals, San Diego, CA 92121. Email: staylor@amylin.com

Peptides are an important class of drugs for indications such as diabetes, pain, bone disorders, and as anti-infectives.

Peptide hormones derived from the neuroendocrine system have great therapeutic value because of their high activity, specificity, and low toxicity. Traditionally, characterization of natural peptides has involved collection of a large amount of source material followed by laborious subfractionation following specific activity assays. Mass spectrometric-based techniques, coupled with genomic information and new informatic algorithms, have accelerated the process of peptide characterization from minute quantities. The concept of peptidomics, in contrast to proteomics, and high-resolution mass spectrometric methods for peptide discovery will be presented. Examples will be presented for endocrine cell culture models where media from unlysed basal and forskolin-stimulated cells are harvested and compared. Differentially secreted peptides are subsequently targeted for characterization with minimal sample manipulation on an LC time scale. The work flow involves “top down label-free” relative quantitation after nanoLC separations and subsequent analysis on a hybrid linear ion trap-Fourier transform ion cyclotron resonance mass spectrometer (LTQ-FT). This instrument has a flexible duty cycle, enabling characterization of peptides from <1 to 16 kDa in a single run. Cutting edge informatic tools for identification of endogenous peptides will be presented. Finally, the utility of this analytical approach in identifying intact polypeptides that have undergone novel post-translational modification will be illustrated.

PS-6

Data Management and Extraction of Biological Information from Large Data Sets. DAVID MOUNT. University of Arizona, Department of Molecular and Cellular Biology, PO Box 210106, Tucson, AZ 85721. Email: mount@u.arizona.edu

The success of the human genome project has created unparalleled opportunities for exploring interactions among human genes, nutrition, and disease. Bioinformatics is a relatively new discipline that provides computational support for these investigations, including experimental design, production of web-based databases and analytical tools, and more sophisticated statistical analysis and data modeling tools. A large bioinformatics community trained in biology, statistics, and mathematics is actively involved in developing new tools to provide this support. Bioinformatics support should be sought when planning experiments since good experimental design increases the chance of reaching research goals. Bioinformatics can also implement secure websites for storing and sharing data. Support can also include analytical tools for a variety of experiments such as microarray gene expression data, proteomics, haplotype analysis, RNAi knockdown, chemical and drug screening,

and a variety of other large data collection projects. The outcome should be a useful model for predicting behavior of a biological system, or one or more useful genetic or molecular predictions that can be tested in the laboratory.

PS-7

Animal and Plant Cultures: Production of Biopharmaceuticals and Secondary Metabolites. W. R. CURTIS. Dept. of Chemical Engineering, The Pennsylvania State University, University Park, PA 16802. Email: WRC2@psu.edu

A comparative overview of culture systems for plant and animal cells and tissues as *biomolecule production platforms* will be presented. The ‘maturing’ pharmaceutical industry now faces a global marketplace where the rapidly rising costs of health care highlight the need to examine the costs of production as well as alternative production platforms. Plant-derived biopharmaceuticals have always faced the competition of agronomic production, and established bacterial and mammalian culture protein production platforms; therefore, innovations for cost reduction in plant cell culture technology may have an increasingly important role for biomolecule commercialization. These concepts will be presented in the context of our recent work including production of kilogram quantities of tobacco cells expressing an animal vaccine as well as *Agrobacterium*-mediated transient protein expression at the 50-L scale in plant suspension culture. This work includes our low-cost plastic bag bioreactor technology (US patent 6,245,555) and feed-forward bioreactor control (US patent 6,709,862) that eliminates expensive and unreliable *in situ* probes for typical feed-back bioreactor control. Analogies of organized tissues (roots) and mammalian perfusion culture will be noted. Introducing heterotrophic capabilities into algae culture creates a ‘traditional’ plant tissue culture production platform (e.g., fatty acids; Martek Biosciences Corp.). Axenic/monoculture of algae is effectively large-scale plant tissue culture where the carbon source is omitted in favor of photoautotrophic growth for the production of high value biopharmaceuticals and secondary metabolites (e.g., IGV Ltd., Germany). Driven in large part by our interest in developing CO₂ capture technology for energy-efficient biofuel production, we have developed photobioreactors for ultra high-productivity algae culture. The trade-off of reduced bioreactor costs versus increased bioreactor complexity will be discussed.

PS-8

Process Development for mAb Therapeutic Production in 10,000 L-reactors with CHO cells. CHIKASHI HIRA

SHIMA, Shinya Takuma, and Kenji Wada. Bio-product Technology Research Department, Chugai Pharmaceutical, Kita-ku, Tokyo, 115-8543, JAPAN. Email: hirashimacks@chugai-pharm.co.jp

Chugai Pharmaceutical has been producing Epogin, a recombinant human erythropoietin, and Neutrogin, a recombinant human granulocyte-colony stimulating factor, since 1990 and 1991, respectively, using CHO cells in 2,500 L bioreactors. And recently, Actemra, a humanized anti-human IL-6 receptor monoclonal antibody, was filed for rheumatoid arthritis in the USA and Europe, following the approval for Castleman’s Disease in Japan in 2005. In general, monoclonal antibody therapeutics requires much higher dosage than cytokine therapeutics. Besides, since Actemra showed very promising results with clinical trials on rheumatoid arthritis, Chugai decided to expand the production facility and completed construction of additional six 10,000-l animal cell culture tanks in May 2006. Now, it has antibody drug production facilities with the total capacity of 80,000 l, making it one of the largest antibody production plants in the world. Monoclonal antibody production capacity depends on not only the reactor sizes but also the ability of recombinant production cells and the optimization levels of culture media and fed-batch culture conditions. Furthermore, it is important to scale-up the optimized process at small scale to the production size with good reproducibility between scales as well as between runs. In this presentation, Chugai’s strategic approaches in order to overcome these issues will be addressed with the past experiences in Chugai.

PS-9

Air Lift Balloon Type Bioreactor: Platform for Commercial Production of Plant Based Small Molecules and Tissues. GANAPATHY SIVAKUMAR¹, Maureen C. Dolan^{1,2}, Jose Condori¹, Selester Bennett², Loretta Bacchetta³, and Fabricio Medina-Bolivar^{1,2}. ¹Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401; ²Nature West Inc. Jonesboro, AR 72401; and ³Biotech Genomics, ENEA, Rome, ITALY. Email: sivakumar@astate.edu

In traditional medicine, plant roots are recognized for containing many useful bioactive small-molecules with therapeutic activity. However, this untapped reservoir of secondary metabolites in roots has been challenging to commercialize due to limited cost-effective methods for producing large-scale, disease- and pesticide-free plant root raw material or molecules. To this end, *in vitro* production of both adventitious and hairy root cultures has captured much interest as a sustainable, scalable production system

that can be precisely controlled to reproducibly yield tissues and select groups of plant-derived small molecules for use as specialty chemicals, pharmaceuticals, cosmetics and nutraceuticals. Indeed some success with large-scale commercial production of plant roots from medicinal plants has been established using the airlift balloon-type bioreactor. This bioreactor is one of the simplest reactors to use, offers low shear stress, and has a relatively low capital cost. We have demonstrated the use of this airlift balloon bioreactor technology for cultivating bioactive molecule-rich adventitious and hairy roots. Data will be presented showing several key physical and chemical parameters for optimizing growth of root biomass and production capacity in this bioreactor system for several root culture lines from several different plant species. Some scaling results will be presented along with results on the use of resins to capture plant root secreted metabolites from the medium. The ongoing development of this airlift balloon bioreactor for culturing root tissues offers a promising, pro-growth, high-tech, renewable, platform for producing not only valuable root biomass, but also suites of unique and valued compounds to the specialty chemical, drug discovery, pharmaceutical, nutraceutical, cosmetic, and agrochemical markets.

PS-10

Novel Plant Reactors for Pharmaceutical Production. CHUNZHAO LIU^{1,3} and Pamela J. Weathers^{2,3}. ¹National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, P.R. China; ²Worcester Polytechnic Institute, Worcester, MA 01609; and ³Arkansas Biosciences Institute, Arkansas State University, State University, AR 72467–0639. Email: czliu@home.ipe.ac.cn

Bioreactors are an efficient and promising system for producing pharmaceuticals from plant cell/tissue cultures and propagating disease-free, genetically uniform and massive amounts of plants *in vitro*. Once the availability of plant cell/tissue cultures based on liquid media has been demonstrated, the scale-up of the whole process should be established by an economically feasible technology for their large-scale production in appropriate bioreactors. It is necessary to design a suitable and economical bioreactor configuration that can provide adequate mixing and mass transfer while minimizing the intensity of shear stress and hydrodynamic pressure to plant tissues. The aim of this presentation is to identify the problems related to scaling-up plant bioreactors with emphasis placed on developing novel bioreactors for pharmaceutical production from *in vitro* cell/

tissue cultures (suspended cells, shoots and hairy roots) under controlled environmental conditions.

PS-11

The Impact of Improved Traits and Genetics on Biofuel Production. MICHAEL EDGERTON, Monsanto Company, 800 N. Lindbergh Blvd. St. Louis. MO 63167. Email: mike.edgerton@monsanto.com

We are placing ever increasing demands on agriculture. Growth in global population and income levels is driving demand for the feed products needed to produce meat. Declining and unstable petroleum supplies in the face of increased global petroleum demand are propelling demand for biofuels. At the same time concerns about climate change and other environmental impacts of farming are leading us to look more closely at production methods. Increased productivity and efficiency of production are required if the multiple demands of feed, fuel, and environmental services are to be met. Monsanto's corn technology program has three major areas of focus, all of which help to address the need for increased productivity and efficiency. First and most important are technologies that increase and stabilize yields (productivity) under a variety of environmental and cultural conditions. Second, we have developed and commercialized hybrids that have a genetic predisposition to produce grain with higher ethanol yields (efficiency) when used in dry grind ethanol facilities. Third, we have developed high yielding corn varieties that accumulate >50% more oil than conventional hybrids, increasing energy content for animal diets and oil yield for food grade vegetable oil or biodiesel production in processing facilities. Increased grain yield per acre and ethanol or oil yield per bushel offer the improvements in productivity and efficiency needed to help meet the growing demand for feed and fuel.

PS-12

70 Yr of Lessons on Biofuel Production from Brazil. LUCIANO NASS¹ and DAVID ELLIS². ¹Embrapa Labex-USA Genetic Resources, USDA/ARS/NCGRP and ²USDA-ARS, National Center for Genetic Resources Preservation, 1111 South Mason St., Fort Collins, CO 80521. Email: Luciano.Nass@ARS.USDA.GOV, David.Ellis@ARS.USDA.GOV

Brazil, the largest and most populous country in Latin America, ranks fifth in land area and population in the world. Brazil's location in the tropical and subtropical

zones ensures intense solar radiation and year-round water supply for bioenergy production. In addition, the vast untapped land mass allows new land to be used for bioenergy production without reducing the farm area devoted to food production. Ethanol production in Brazil, based on sugarcane, has a long, interesting, and turbulent history starting the 1930s. The development of flexible-fuel vehicles (FFVs), cars capable of running on gasoline, ethanol, or any combination of both fuels, renewed customer interest in biofuels. In 2006, about 78% of new cars manufactured in Brazil were FFVs. Like ethanol, biodiesel has received increased interest and several oleaginous species are being used and investigated; however, soybean is currently the largest source for biodiesel production. Brazil is currently facing challenges to reach goals established in January 2005, with the government called for a mandatory use of at least 2% (B2) biodiesel by 2008 and 5% (B5) by 2013. Although Brazil has been working with renewable sources for energy production for over 70 years, Brazil's programs are continually updated and reviewed for future needs. Some of these challenges include the development of new cultivars, the agroecological zoning for biofuels crops, the use of biotechnology to introduce traits of interest, and improve industrial processes and products.

PS-13

Biofuel Development in the Caribbean – the Pros and Cons. SYLVIA ADJOA MITCHELL, The Biotechnology Centre, 2 St. John's Close, University of the West Indies, Mona Campus, Kingston 7, Jamaica, WEST INDIES. Email: sylvia.mitchell@uwimona.edu.jm, sylviamitchell.biotech@gmail.com

The island states of the Caribbean who do not have their own oil face a serious dilemma. Jamaica, for example, imports 90% of the energy it uses as oil, which cost the country US \$ 1.8 billion in 2007. But how to reduce the use

of this expensive oil without upsetting the precarious development process and what alternatives are there that will not cause worse dislocations or affect the environment? Biofuel, along with other renewable alternatives, has to be seen in this light. There are promising signs such as the commissioning of ethanol dehydration factories in 2004 and July 2007 (Jamaica Broilers) using hydrous ethanol produced in Brasil. The experience Jamaica Broilers has had in entering the global biofuel market (of which USA and Brasil produce 80%) will be summarized. JB estimates that they invested US \$20 million to build the biofuel factory, which has a 40-gal fuel-grade ethanol yearly capacity with a net operating margin so far of US \$0.10 a gallon. Another biofuel company established in 2004 exported 45 million gallons of ethanol from Jamaica to the USA in 2007. Local use of ethanol could cut the energy bill but would require mandating the use of 10% ethanol to replace MTBE in gas vehicles (biofuel), and this could save Jamaica US \$ 35–40 million per year. It is also proposed to make biodiesel mandatory (20% ethanol to replace B20 in diesel). Further development of biofuel could come from development of local feedstocks such as castor bean and *Jatropha* spp. but for marginal lands only. Other renewables being researched include biodigesters, wind and solar energy. The development of the biofuel industry in Jamaica is very important and is being supported by the Caribbean Basin Initiative (CBI) by the USA whereby certain Caribbean countries (19 so far) are exempt from the 54¢/gal tariff on imported fuel ethanol into the USA. Countries benefiting from the CBI include Jamaica, Costa Rica, El Salvador, and Trinidad & Tobago where ethanol dehydration plants have recently been commissioned. Ethanol entering the USA through the CBI represented 2.7% of the USA market or ~90 million gallons in 2005, a drop in the bucket but of much developmental impact for the Caribbean, and with much promise for the future. The pros and cons of these developments plus a glance into a possible viable future for biofuels in the Caribbean will be discussed.